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Age-related Alteration of Taste Bud Distribution in the Common Marmoset

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Abstract

Alteration in the number of taste buds on the soft palate (SP), fungiform (FF), foliate (FL) and circumvallate (CV) papillae in the common marmoset at different postnatal ages was examined histologically. After paraffin embedding, complete serial sections at 10 μm thickness were made and stained by HE. Digitized images for each section were examined carefully. The number of FF taste buds at day 1 was 334. While only 20% of all the taste buds at birth possessed a taste pore, 39% of 174 SP taste buds at day 1 possessed a taste pore. The number of taste buds with pores at day 1 was small for the center CV (19 of 59), one side CV (7 of 25), and one side FL (2 of 16). These results suggest that the functional maturation of SP taste buds may precede maturation in other areas of the tongue. The total number of taste buds increased with increasing age, reached a maximum at 2 months of age: FF, 1069; SP, 609; CV-center, 530; CV-side, 390; FL, 201, and decreased thereafter. Almost all taste buds possessed a taste pore after 2 months of age. The decrease in the number of taste buds in the oral cavity with increase in age may change taste sensitivity.

Introduction

Age-related alteration of the number of taste buds in postnatal mammals is one of the most important factors which may affect a change in gustatory function with increasing age. The appearance and maturation of taste buds are different among the subpopulations in the oral cavity (Mistretta, 1972; Hosley and Oakley, 1987; Belecky and Smith, 1990). Recently, we demonstrated that maturation of taste buds within the soft palate (SP) in the rat precedes that within the three types of tongue papillae (fungiform, FF; foliate, FL; circumvallate, CV), and that >100 taste buds appeared and were maintained within the SP in adult rats (Harada *et al.*, 2000). Quantitative examination in a human newborn also revealed the presence of 419 taste buds within the soft palate (Lalonde and Eglitis, 1961). In human adults, however, the number of SP taste buds was reduced compared to both earlier (Nilsson, 1979; Imfeld and Schroeder, 1992) and later (Harada, 1994) stages of life. These results suggest that the number of human SP taste buds rapidly decreases with increasing age. Investigation of human CV and FL papillae showed that the number of taste buds decreased in old age (Arey *et al.*, 1935; Mochizuki, 1937). In contrast, it was reported for adult rhesus monkey that age does not affect the number of taste buds within the FF, CV and FL (Bradley *et al.*, 1985a). Thus, the present investigation was designed to elucidate age-related developmental changes of taste-bud distribution within the SP, FF, CV and FL papillae at different postnatal ages in the common marmoset.

Materials and methods

Animals and tissue processing

Seven common marmosets [*Callithrix jacchus*; longevity 10–16 years (Hearn, 1987)] aged 1–3 days, 2 months, and 2, 7 and 9 years were provided from two institutes (Department of Pharmacology, Faculty of Medicine and Hospital, Kagoshima University; Primate Research Institute, Kyoto University) and one commercial company (Kyudo Co. Ltd). The procedure employed to make tissue blocks embedded in paraffin was similar to that described previously (Harada *et al.*, 2000). Complete serial sections were cut on a rotary microtome at 10 μm and mounted on egg-albumin-coated slides. The sections on slides were stained with Mayer's hematoxylin and counter-stained with 0.25% eosin–alcohol.

Each section was examined with a light microscope (40–200, BH2, Olympus). The image was digitized using a high-resolution digital camera (HC-2000, Fuji-Film) and stored online as a pict on a microcomputer (Power Macintosh 7300/180). The method used to record and process digitized images of individual taste buds was similar to that described previously (Harada *et al.*, 2000). Observing the sequence of photographs of each section made it possible to check and identify each taste bud and its taste pore. The

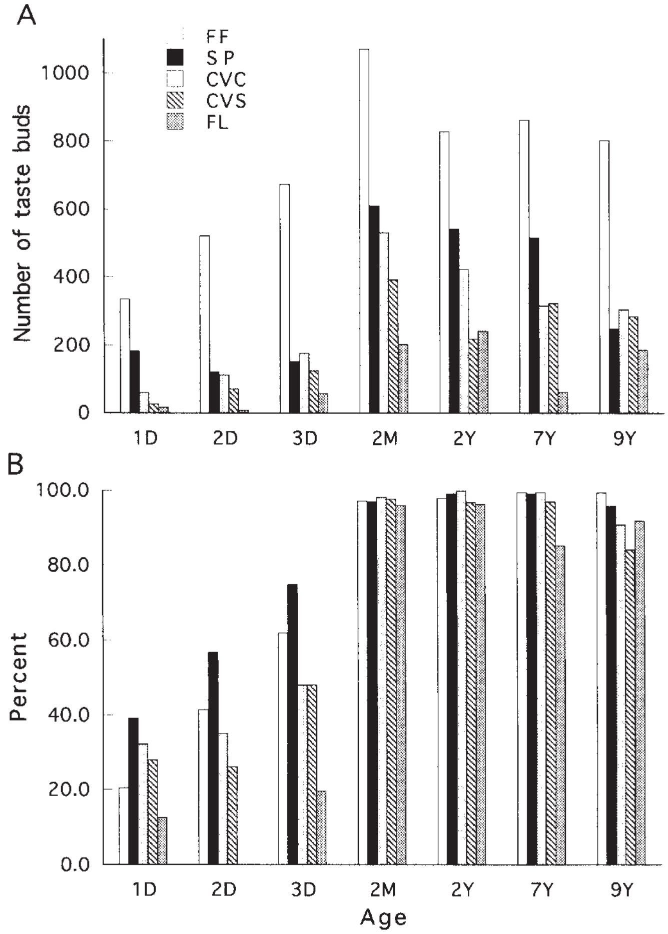

Figure 1 Number of taste buds **(A)** and percentage of the number of taste buds with a pore **(B)** located within the fungiform papillae (FF), soft palate (SP), center circumvallate (CVc), side circumvallate (CVs) and foliate papillae (FL) at different ages in the marmoset. Data are from an animal at each age.

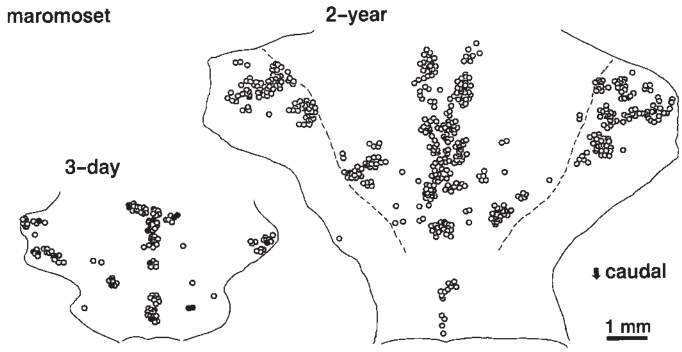

Figure 2 Distribution of taste buds on the soft palate in 3-day-old and 2-year-old marmosets.

Table 1 Number of taste buds observed within fungiform (FF), soft palate (SP), center circumvallate (CVc), side circumvallate (CVs) and folliate (FL) papillae at 1, 2 and 3 days (D), 2 months (M), and 2, 7 and 9 years (Y) of age

Age	FF	SP	CVc	CVs	FL	Total
1D	334	182	59	25	16	657
2D	521	120	111	69	7	904
3D	672	151	175	123	56	1356
2M	1069	609	530	390	201	3390
2Y	826	540	422	217	241	2704
7Y	861	515	315	322	61	2457
9Y	800	248	303	283	185	2287

Data are from a marmoset at each age. The total number of taste buds includes twice that observed on one side for the CVs and FL.

largest extent of the height and width of each taste bud, with or without a taste pore, was measured. The size of the taste bud was calculated by the equation, area = π height width/4, which estimates the cross-section of a taste bud as an ellipse. Also, the ratio of height divided by width was calculated to obtain the general shape of each taste bud.

Statistical analysis

A two-way analysis of variance (group versus age) was used to analyze differences in taste bud number and characteristics for each of the developmental changes. An independent measures *t*-test was used to compare two means.

Results

The number and distribution of taste buds

Only 20% of 334 FF taste buds in a newborn marmoset at day 1 possessed a taste pore (Figure 1B). Although the number of taste buds within the SP at day 1 was 182, half of that for the FF (Figure 1A), 39% of SP taste buds possessed taste pores which was twice that of FF taste buds (Figure 1B). The SP taste buds were densely gathered together into several groups, and the fundamental pattern of their distribution was established at birth (Figure 2). At birth (day 1), the numbers of taste buds within the FF and SP were 31.2 and 29.9%, respectively, of maximum observed at 2 months of age (Table 1). The percentage of taste buds of maximum was smaller for the center CV (CVc, 11.1%), side CV (CVs, 6.4%) and FL (6.6%). The percentage of taste buds containing a pore in each subpopulation of the CVc (32.2%), CVs (28.0%) and FL (12.5%) was smaller than that for the SP (Figure 1B).

The total number of taste buds in each subpopulation increased with increasing age and reached a maximum at 2 months of age (Figure 1A), where ~100% of taste buds in each subpopulation possessed a taste pore (Figure 1B). After 2 months, the numbers of taste buds for each subpopulation, especially for the SP, decreased significantly (two-way ANOVA, P = 0.0189, F = 4.895, d.f. = 3) with increasing age (Figure 1A). Also, the percentage of taste

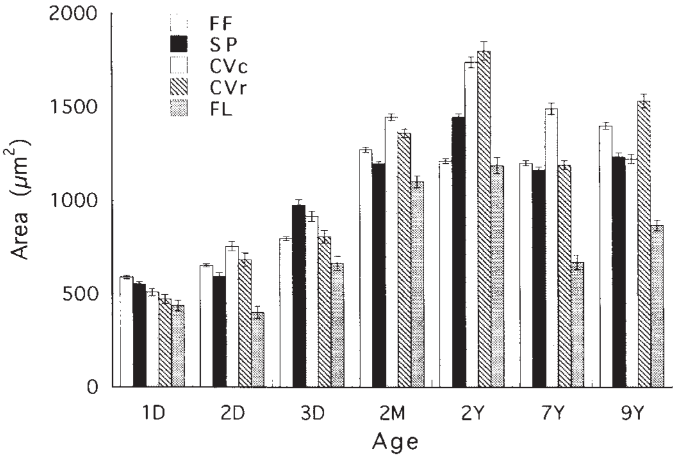

Figure 3 Postnatal development area of FF, SP, CV and FL taste buds after birth. The area of each taste bud was calculated by the formula ($\pi \times$ height $\times$ width/4). Data are from an animal at each age. Error bars depict SE.

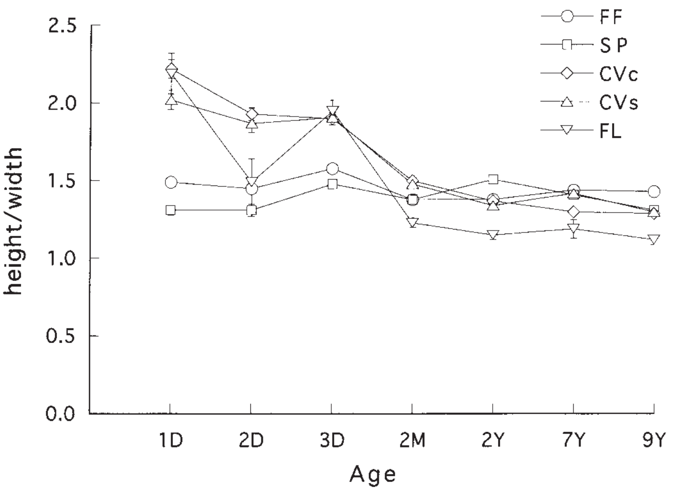

Figure 4 Postnatal development of the ratio of height/width of FF, SP, CV and FL taste buds after birth. Data are from an animal at each age. Error bars depict SD.

buds with taste pores tended to decrease after 7 years of age (Figure 1B).

The size and shape of taste buds

Although taste buds within each population increased in overall size and reached a maximum at 2 months of age, there was no significant difference (ANOVA, $P > 0.05$) in the overall size (area) of the taste buds for the different populations (Figure 3). The ratios of height/width of SP and FF buds ranged between 1.31 and 1.58 from birth to adulthood, indicating that the shape for these subpopulations of taste buds was nearly spherical (Figure 4). In contrast, the ratios of height/width for CVc, CVs and FL taste buds were 2.22, 2.02 and 2.19, respectively, at age 1 day, showing that taste buds were elongated along their long axis to the apical surface. The elongation rapidly

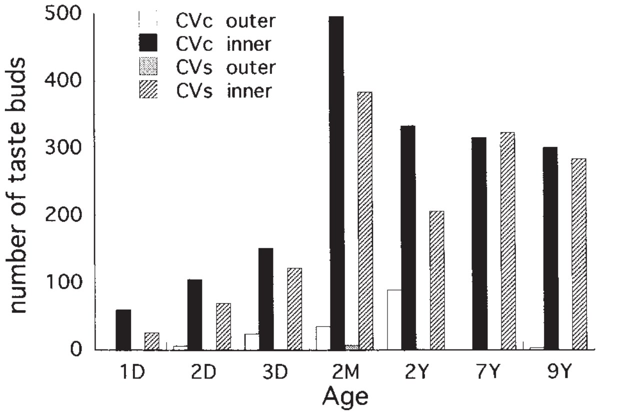

Figure 5 Numbers of taste buds located within the inner and outer walls of the CVc and CVs at different ages in the marmoset. Data are from an animal at each age.

decreased with increase in age and reached a plateau at 2 months of age similar to that for the FF and SP taste buds (Figure 4).

Distribution of taste buds within the CV

In the marmoset, few taste buds were distributed within the outer wall of the CVc (Figure 6, top). This uneven distribution between the inner and outer walls of the CVc or CVs existed throughout the life of the marmoset (Figure 5). The lowest percentage of the total number of taste buds occurring on the inner wall of the CV was 69.1% (CVc, 2 years).

Discussion

In the newborn rat, taste buds within the SP and FF papillae are few and immature (Harada *et al.*, 2000). Although the gestational period of the marmoset (144 days) was seven times longer than that for the rat (21 days), the number of taste buds within the FF and SP in the marmoset was 30% of maximum number observed at 2 months of age, and the percentages (of maximum number) were <10% for the CVc, CVs and FL. Both the percentage of the number of marmoset taste buds with a taste pore and the size of the taste buds increased from birth until 2 months of age. Thus, taste buds in the marmoset are also immature at birth and develop during the lactation period (Hearn, 1987). Since gustatory stimulation is effective in inducing normal development in the nucleus tractus solitarius (Lasiter, 1995), the present results suggest that gustatory stimulation during the lactation period might accelerate the development of taste buds and that the gustatory system may mature during the weaning period in mammals.

Taste buds are almost equally distributed on the inner and outer wall of the CV groove in the rat (Fish *et al.*, 1944; Hosley and Oakley, 1987), and there was no significant difference ($P > 0.05$) in the number of taste buds between the two walls (inner wall, 297.3 ± 27.3; outer wall,

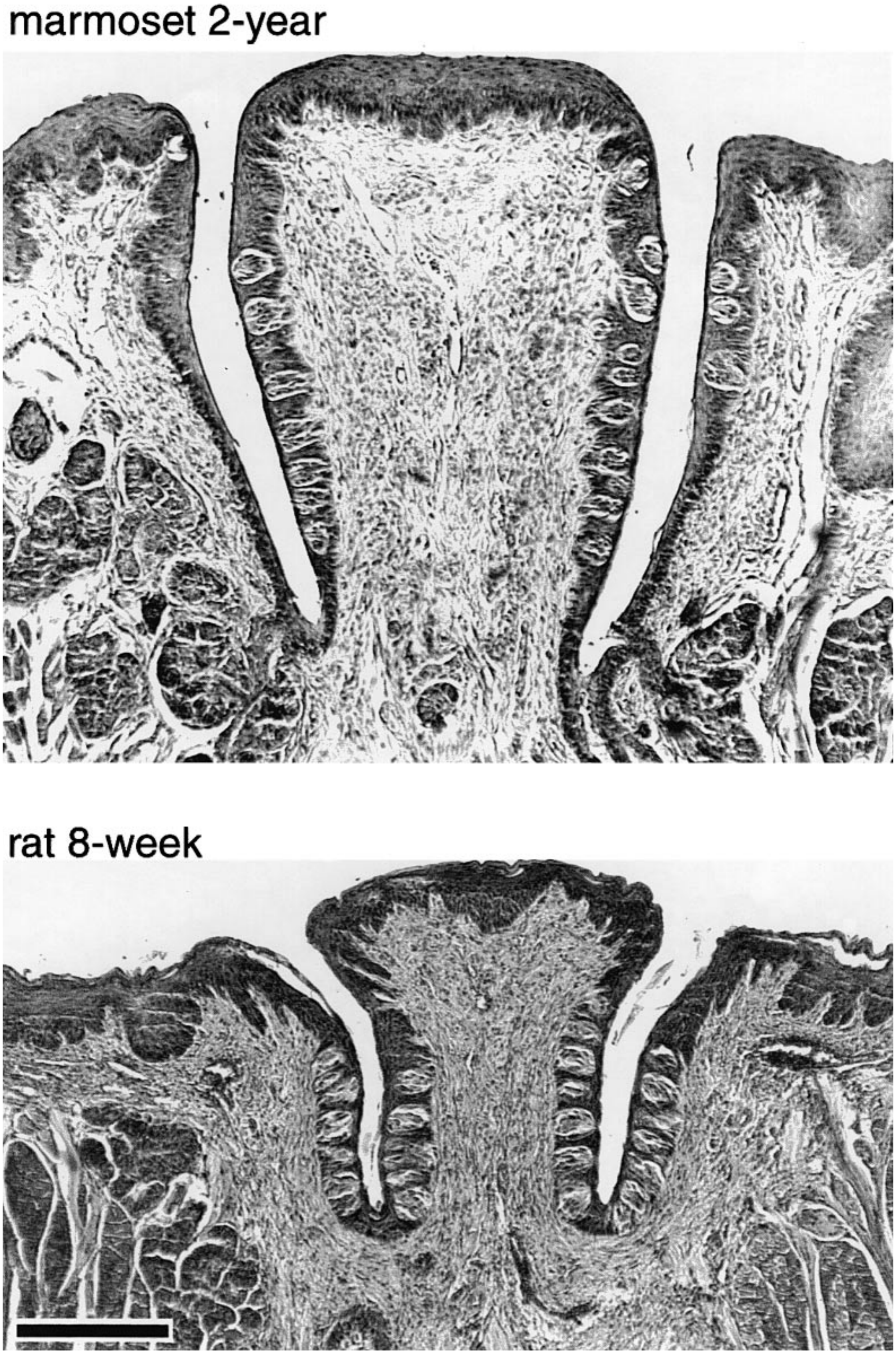

Figure 6 Taste buds on the papillae in a 2-year-old marmoset and an 8-week-old rat. Only a four taste were observed within the outer wall in the marmoset CV. Vertical bars indicate 200 m.

293 ± 34.6; $n = 3$; unpublished data, see Figure 6, bottom). In contrast, the distribution of taste buds in the marmoset was unevenly dispersed on the inner wall of the CVc and CVs throughout life. Such an uneven distribution of taste buds within the CV in primates was shown in the rhesus monkey (Bradley *et al.*, 1985b), in other primates

(Schneider, 1958) and in humans (Stern, 1980; Han and Holmstedt, 1981). These result suggest that innervation within the CV is different among mammalian species, since the nerve supply is essential for the existence of taste buds (Farbman, 1965). It was reported recently that amphibian taste buds develop autonomously from endoderm without induction by the neural crest (Barlow and Northcutt, 1997). If this is also applicable in mammals, another factor, such as the difference of formation of the CV groove between rodents and primates, may be a reason for the different distribution of CV taste buds between these mammals.

In spite of the immaturity of taste buds in the oral cavity, the ability of neonatal mammals to discriminate taste solutions representing different taste qualities has been demonstrated (Steiner, 1973, 1987; Ganchrow *et al.*, 1986; Johanson and Shapiro, 1986). Among the subpopulations of taste buds in the marmoset, the percentages of SP taste buds with a taste pore were greater than those for other papillae. Also, the distribution of taste buds on the SP was already completed at birth, indicating that maturation of SP taste buds was earlier than taste buds in other gustatory regions. However, total number of FF taste buds was twice that of SP taste buds at birth. Compared to the number and maturation of SP taste buds in the rat (Harada *et al.*, 2000), FF taste buds in neonatal marmoset play a more important role in the detection of nutrients than those in the rat.

The present investigation showed that the number of marmoset taste buds within the CVc increased after birth, reached maximum at 2 months and then decreased until 9 years of age. This result is highly similar to that reported in humans, where taste buds per papillae increased until 4–20 years of age, then decreased to one-third of maximum at 74–85 years (Arey *et al.*, 1935). The mean number of taste buds within the human CV was 234 at 21–60 years, and decreased to 140 at 61–90 years of age (Mochizuki, 1937). Although the total number of taste buds in the rhesus monkey was reported to be maximal (10 650) at 8 years of age, then to decrease with increasing age and reach 70% of maximum at 31 years of age, there was no significant difference in the number of taste buds as a function of age (Bradley *et al.*, 1985a). It is possible that the effect of aging on taste bud number may be different among individuals and does not simply depend on increasing age; i.e. senescence does not linearly relate to age. Therefore, an index for expressing the extent of senescence should be employed to elucidate aging effects.

The IXth nerve innervates taste buds in the CV and FL papillae, and is predominantly responsive to QHCl and HCl in several mammalian species, including rats (Frank, 1975), gerbils (Oakley *et al.*, 1979) and mice (Shingai and Beidler, 1985). Similarly, in humans, sensitivity for bitter taste is greater in the area of the tongue comprising CV and FL papillae than in other areas within the oral cavity (Kiesow, 1894). It was also reported that sensitivity to sour and bitter stimuli is greatest on the human palate (Henkin and Christiansen, 1966; Collings, 1974). In contrast, aging generally appears to decrease sensitivity to bitter stimuli (Cooper *et al.*, 1959; Glanville *et al.*, 1964; Kaplan *et al.*, 1965; Cowart, 1989; Cowart *et al.*, 1994). The decline in sensitivity for bitter substances may be correlated with the age-related decrease in the number of CV and SP taste buds.

In conclusion, the present results suggest that the functional maturation of SP taste buds may precede maturation in taste areas on the marmoset tongue, and that the decrease in the number of taste buds in the oral cavity with age might change taste sensitivity.

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