Chem. Senses 34: 93–99, 2009

doi:10.1093/chemse/bjn059
Advance Access publication October 14, 2008

The Relationship between Fungiform Papillae Density and Detection Threshold for Sucrose in the Young Males

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Abstract

The aim of this study was to investigate the relationship of fungiform papillae density with taste detection thresholds for sucrose of young male adults. One hundred and eighty two subjects aged 18–23 years (mean age: 21.9 ± 1.2 years) were included. The densities of fungiform papillae were recorded with the aid of the digital camera, and the taste detection thresholds for sucrose were detected using a modified forced-choice triangle test. The mean density of papillae within all 170 statistic participants was 92.43 ± 2.64/cm^2, for the 6-mm-diameter stained section of the tongue tip. The average detection threshold was 10.83 ± 0.24 mmol/l, and the highest and lowest detection thresholds were 19.88 ± 1.31 and 5.85 ± 0.43 mmol/l, respectively. Also, an inverse correlation between the fungiform papillae density and the detection threshold was observed.

Key words: fungiform papillae density, sweet detection threshold, young males

Introduction

The dorsal anterior tongue in humans is rich in fungiform papillae, most of which contain multiple taste buds. Each bud is characterized by a single apical pore through which taste cells within a bud extend microvilli that provide a surface for the reception of tastants, transduction of chemical into electrical signals, and activation of taste cells (Segovia et al. 2002). High numbers of fungiform papillae are commonly found in people who are classified as supertasters of the bitter substance 6-*n*-propylthiouracil (PROP and phenylthiocarbamide [PTC] in earlier work) compared with moderate and nontasters (Miller and Reedy 1990b; Bartoshuk et al. 1994; Essick et al. 2003; Shahbake et al. 2005), whereas degeneration or loss of taste following medications or neural damage is accompanied by a decrease in the number of papillae found in individuals (Zuniga et al. 1997; Sollars and Bernstein 2000). These differences are known to possess corresponding genetic differences (Reed et al. 1999; Kim et al. 2003). The PTC gene encodes a specific bitter receptor; other related genes may determine the number of chemoreceptor cells (taste buds and fungiform papillae) on the dorsal surface of the anterior tongue, which also determines taste intensity (Miller and Reedy 1990b; Delwiche et al. 2001). However, there is little information on the relationships between the numbers or densities of fungiform papillae and the sweet detection threshold for the sucrose solution.

Measurement of papillae number or density in living tissue has been achieved using noninvasive video microscopy (Miller and Reedy 1990a; Schiffman et al. 1998). However, although the video microscope is an excellent tool for this purpose, its use is limited to the research laboratory. One disadvantage of the video microscopy is that it is not portable system for outpatient clinics in hospitals and children in school to gain an insight to taste function (Segovia et al. 2002). Another disadvantage of the video microscope is that it requires 30–60 min to obtain high-quality images from an individual to allow counting of papillae. This time period is unacceptable to patients in pain or uncomfortable from clinical treatments or to young children with their limited attention span. So Shahbake et al. (2005) developed a rapid and portable device digital camera, as an alternative method for

obtaining images of taste papillae. They measured the fungiform papillae on the dorsal surface of the anterior tongue of living humans using a digital camera and a video microscope and found that both procedures provided similar results and the camera providing a more rapid, portable, and flexible imaging procedure. Also they discovered that small regions of the anterior tongue which provide reliable measurements of fungiform papillae density that correlate highly with the total number of fungiform papillae on the anterior tongue (Shahbake et al. 2005).

The aim of this study was to investigate the fungiform papillae density on the small region of the most anterior tongue with the aid of the digital camera and the sweet detection threshold for sucrose of young males and to test the hypotheses that subjects with more fungiform papillae density have lower detection threshold for sucrose.

Materials and methods

Subjects

The subjects were 182 young male students aged 18–23 years (mean age: 21.9 ± 1.2 years), from 4 colleges enrolled in the study in our university, who were tested in our sensory science laboratory. All participants did not show any positive responses to the conditions included in the test questionnaire (including some questions, such as chronic sinusitis, chronic obstructive pulmonary disease, diabetes, psychological disorders, and loss of olfactory sense), and they were nonsmokers. All subjects were divided into 13 groups (every group containing 14 participants); 14 participants in one group on one Sunday morning (9:00 to 11:30 AM) were invited to sensory science laboratory to carry out the 2 test programs, including the detection of the sweet detection threshold for sucrose and the measurement of fungiform papillae density, respectively. Every participant had a break for 15 min after completing one test program. So it took 13 Sunday mornings to complete all tests of 13 groups.

Determination of detection threshold for sucrose

Sucrose was reagent grade, and the serials sweet solutions (2.5×10^{-4} to 0.5 M) were prepared in distilled water. All the successive solutions comprised a total of 10 grades that differed by 0.25 log units of the molar concentration.

The solutions and distilled water, mean volume 15 ml, were presented in 30-ml odorless plastic cups labeled with 3-digit random numbers at constant room temperature (ca., 22 °C). All the stimuli were sipped and expectorated. Panelists were requested not to eat or drink during the 1 h before test. The subject rinsed his mouth twice with distilled water prior to tasting each sample.

The procedure employed to test the sucrose detection threshold was similar to that described previously (Lim and Lawless 2006). Using a modified forced-choice ascending concentration series method of limits described in ASTM E679-91 (ASTM 1997), we obtained the sucrose detection threshold. At each selected sucrose concentration, a triangle sample set consisting of 1 test (stimuli sucrose solution) and 2 blank samples (distilled water) was presented to subjects. Each possible order of presentation within each triangle test (SWW, WSW, and WWS; S, stimuli and W, water) was counterbalanced over sessions as well as subjects. The solution series were presented to the subjects in order of ascending concentrations from the lowest concentration, and the subjects had to indicate which of the 3 samples was different from the other 2. If the subject could not distinguish, he was forced to guess. Another set of triangle test with higher concentration was presented to the same subject after taking an at least 1 min break. The test completed till the subject either finished all sets of scale estimation or made 3 correct discriminations in a series. The best-estimate threshold for each subject was considered to be the geometric mean of the concentration when the last missing occurred and the next higher concentration (Lim and Lawless 2006). After repeating 5 times of the procedure, the sweet detection threshold was determined by using the mean of the 5 best-estimate thresholds.

Labeling of fungiform papillae and recording with digital camera

The procedure employed to measure the fungiform papillae density was similar to that described previously (Shahbake et al. 2005). After testing of taste detection threshold and resting for 10 min, the subjects rinsed their mouth with distill water. Their tongue was dried with a filter paper by the experimenter, and a 6-mm-diameter circular piece of filter paper (No. 1, Shijiazhuang Kelin Filter Paper Co., Ltd, HeBei Province, China) that contained a blue food dye (Robert's Brilliant Blue FCF133) was placed on the tip of the anterior part of the left side of the tongue closest to the midline (Figure 1A) for 3 s. On removal of the filter paper, the tongue was again dried. In the procedure used with the digital camera, to minimize head movement during filming, a subject supported their head by placing their arms on a table and held their head with their hands such that their chin protruded forward. The subject then protruded their tongue and held it steady with their lips (Figure 1A). A 10×3 mm wide piece of filter paper placed on the right side of the anterior tongue provided a scale to calculate the magnification of each image (Figure 1A). Following this, 3 images of the stained area were recorded with a Canon IXUS 75 (7.0 megapixels) camera. The digital images were downloaded to a computer and analyzed in the Adobe Photoshop 7.0 program. The average total time to obtain the images from a subject with the digital camera was about 10 min.

Identification and density counting of fungiform papillae

Fungiform papillae were identified according to earlier criteria (Miller 1995; Shahbake et al. 2005) and readily distinguished from filiform papillae for the coloring stained the

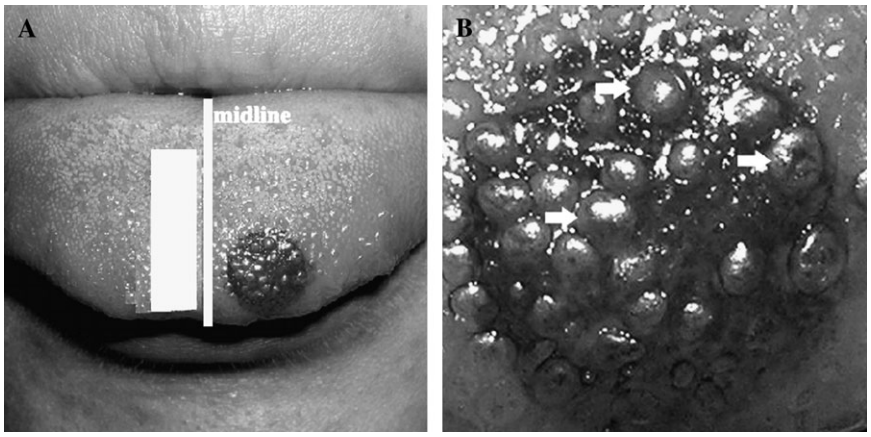

Figure 1 Fungiform papillae in a human tongue. **(A)** Tongue with midline highlighted with a white line and showing the 6-mm-diameter stained area where papilla counts were conducted and the 10 mm scale. **(B)** Image of the stained area obtained with the digital camera, arrow indicates typical fungiform papillae.

filiform papillae blue (dark) while the fungiform papillae remain unstained (light) (Miller and Reedy 1990b) (Figure 1B). The best image from an individual was used for counting papillae. Counting was conducted randomly across subjects with the identity of the subject unknown to the analyst.

Data analysis

Results are presented as means ± standard error of the mean, and the Gaussian fit was conducted on the relationship between the participant frequency and the density of fungiform papillae using Origin 7.0 software (OriginLab, Northampton, MA).

Results

Fungiform papillae density and distribution frequency

Most of fungiform papillae commonly were mushroom-shaped elevated structures consisting of a large head and a thin neck (Figure 1B); however, some were flat with little elevation, short, and cylindrical and some on the tip of the tongue were conical, even some papillae had hairs or hair-like projections.

The lowest and highest density of fungiform papillae were 7.07 ± 0.35/cm^2 (2 participants) and 233.43 ± 0.00/cm^2 (only 1 participant). The mean density of papillae within all 182 participants was 96.96 ± 3.06/cm^2, for the 6-mm-diameter stained section of the tongue tip. Due to the big individual difference of papillae density within all participants (Figure 2), we divided the serial papillae density (from 0 to 233.43) into 19 intervals (Table 1) with the 12.50 increasing step (papillae density); however, there were few participants in some intervals (from 0.00 to 12.50 and from 175.00 to 250.00, Table 1) and were lack of representatives, so we cast off those data and utilized the data of medium 13 intervals (from 12.50 to 175.00 of fungiform papillae density, above 5 participants per interval, all 170 participants) below (Table 1). So the mean density of papillae within all 170 participants was 92.43 ± 2.64/cm^2, for the 6-mm-diameter stained section of the tongue tip, and the relationship between the frequency distribution and the density of fungiform papillae were showen below (Figure 3, Gaussian fit, R^2 = 0.7962; Table 1).

For the medium 9 intervals (above 10 participants per interval), the density of fungiform papillae varied 4-fold (from 37.50 to 150.00/cm^2) and the papillae density of 73% participants (124/170) located from 50.00 to 125.00/cm^2 (Figure 2, Table 1).

Relationship between fungiform papillae density and sweet detection threshold

The anatomical attributes were strongly associated with subject's sweet detection thresholds of the sucrose solutions. Average detection threshold of 170 participants was 10.83 ± 0.24 mmol/l, and the highest and lowest detection thresholds were 19.88 ± 1.31 and 5.85 ± 0.43 mmol/l (average data of 5 times for 1 participant), respectively. As shown in Figure 4, an inverse correlation between the papillae density and the detection threshold was observed, especially within medium 9 points (the papillae density was from 37.50 to 150.00/cm^2).

Discussion

This study had 2 major aims: first, to determine the densities of fungiform papillae and sweet detection thresholds for sucrose of young male adults, respectively; second, to test the hypothesis that higher numbers or densities of fungiform papillae were the likely basis of the higher sensitivity for sucrose

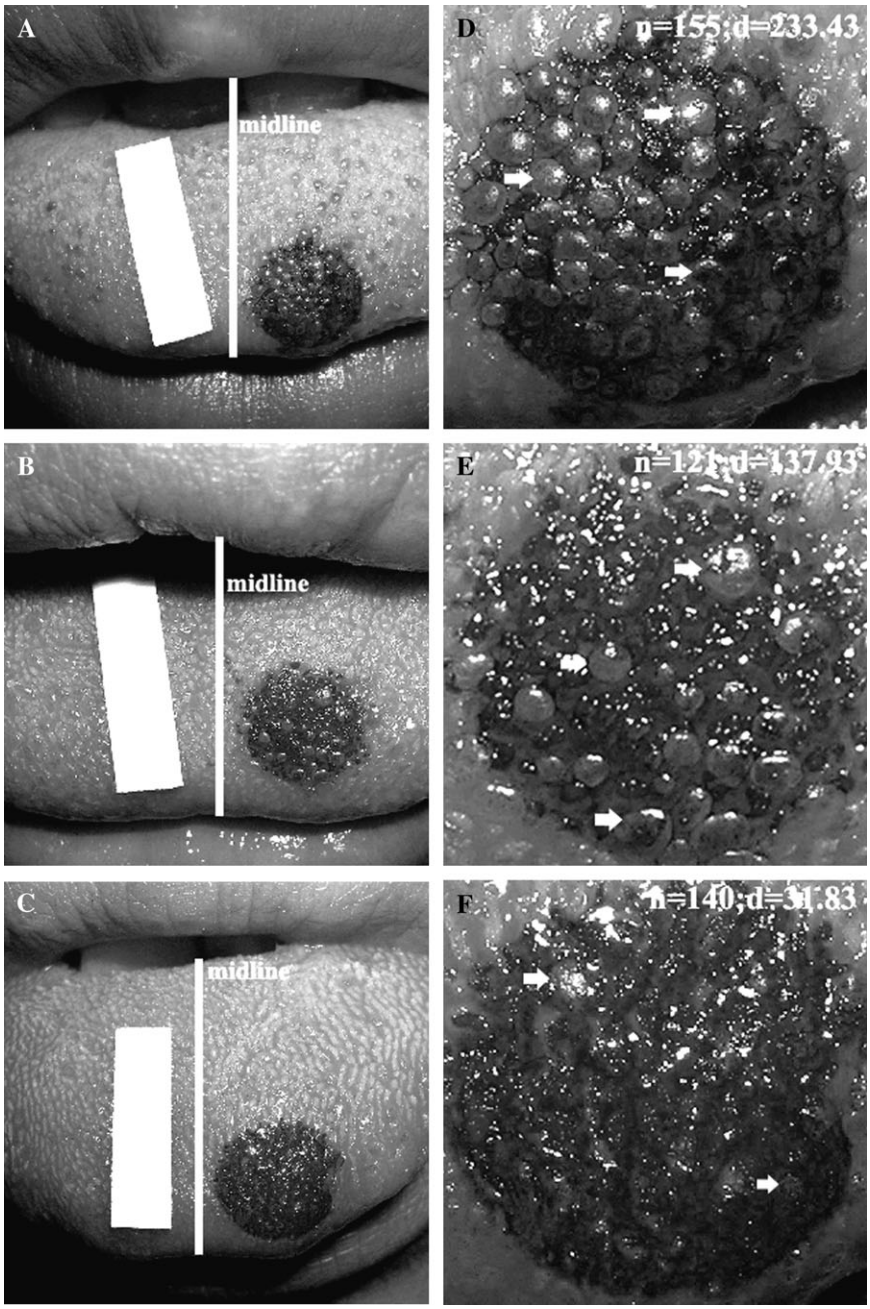

Figure 2 Fungiform papillae in human tongues. **(A**,**B**,**C)** are standards for the tongue with different density of fungiform papillae. **(D**,**E**,**F)** Insets in (A,B,C) viewed at the highest magnification used with the digital camera to count papillae, standard for high, medium, and low papillae density of tongue. Arrows indicated typical fungiform papillae; n and d are serial number of participants and density of papillae, respectively.

in the region of the anterior tongue (Stein et al. 1994; Segovia et al. 2002). The results showed that there is very big individual difference about the average density of fungiform papillae and the detection threshold for sucrose solution, also, there is an inverse correlation between the fungiform papillae density and the sucrose detection threshold.

Most of fungiform papillae commonly were mushroom-shaped elevated structures consisting of a large head and a thin neck; however, some were flat with little elevation, short, and cylindrical and some on the tip of the tongue were conical, even some papillae had hairs or hair-like projections, as previously described (Kullaa-Mikkonen and Sorvari 1985; Miller 1995; Segovia et al. 2002). The number of fungiform papillae is highest at the tip of the tongue. Miller and Reedy (1990b) mapped the anterior 2–3 cm of the tongue tip in 12 young adult subjects. The surface area of the tongue tip

Table 1 The number of participants, the average fungiform papillae density, and sweet detection threshold of 19 papillae density intervals with the 12.50 increasing step

Intervals	0.00–12.50	12.50–25.00	25.00–37.50	**37.50–50.00**	**50.00–62.50**	**62.50–75.00**	**75.00–87.50**	**87.50–100.00**	**100.00–112.50**	**112.50–125.00**	**125.00–137.50**	**137.50–150.00**	150.00–162.50	162.50–175.00	175.00–187.50	187.50–200.00	200.00–212.50	212.50–225.00	225.00–250.00
Number of participants	2	6	5	**13**	**17**	**27**	**17**	**29**	**17**	**17**	**14**	**14**	6	6	4	1	2	2	1
Density of papillae	8.84 ± 1.77	21.22 ± 1.83	33.95 ± 0.87	**44.62 ± 1.10**	**56.80 ± 0.77**	**68.90 ± 0.64**	**81.56 ± 0.64**	**93.66 ± 0.76**	**105.89 ± 0.64**	**117.96 ± 1.17**	**130.61 ± 0.78**	**143.74 ± 1.21**	155.62 ± 1.58	165.05 ± 1.18	181.26 ± 0.88	190.99 ± 0.00	205.13 ± 0.00	219.28 ± 0.00	233.43 ± 0.00
Detection threshold	19.88 ± 0.00	17.54 ± 1.05	14.27 ± 0.93	**14.30 ± 0.62**	**12.45 ± 0.73**	**10.70 ± 0.54**	**11.35 ± 0.80**	**10.85 ± 0.59**	**10.80 ± 0.63**	**9.15 ± 0.49**	**8.52 ± 0.33**	**8.36 ± 0.52**	6.63 ± 0.49	8.19 ± 0.85	7.61 ± 0.59	8.19 ± 0.00	9.36 ± 1.17	9.36 ± 1.17	8.19 ± 0.00

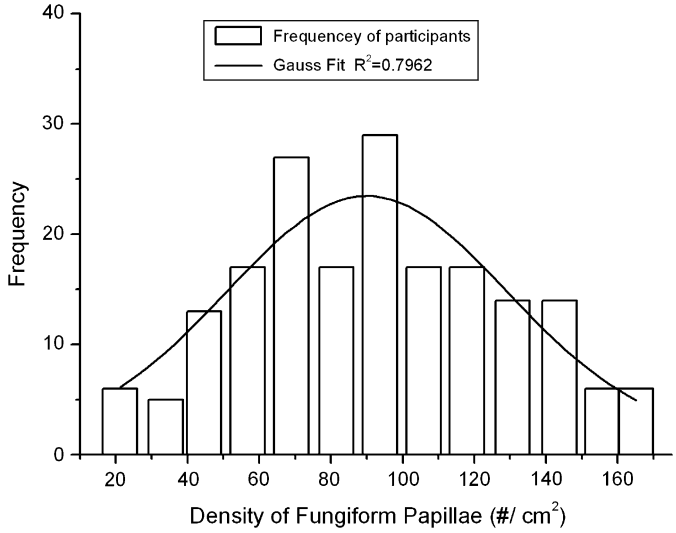

Figure 3 Frequency distribution of 13 density intervals (above 5 participants per interval) of fungiform papillae with 12.50 increasing step from 12.50 to 175.00 (#/cm^2).

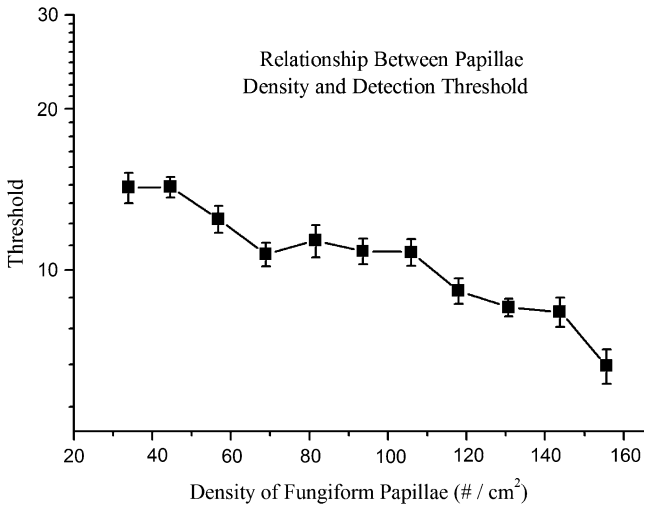

Figure 4 Relationship between fungiform papillae densities and sweet detection thresholds.

contained an average total of 166.7 ± 51.6 (standard deviation) fungiform papillae. Shahbake et al. (2005) analyzed the density of fungiform papillae on the left side of the tongue in 30 adults aged 20–24 years, which revealed an average of 156 ± 5.8 papillae. The average density of papillae within all 182 participants in our study was 96.96 ± 3.06, which is somewhat lower than the number of papillae found in previous reports (Miller and Reedy 1990b; Shahbake et al. 2005). One reason for this difference may be the fact that the tongues of only 12 and 30 study subjects have been examined by Miller and Reedy (1990b) and Shahbake et al. (2005),

respectively. Females have, on average, more fungiform papillae and taste pores than males (Bartoshuk et al. 1994; Prutkin et al. 2000), all male subjects were conducted in our studies. Age also seems to affect this number. Although the number and diameter of fungiform papillae did not differ with age during the study conducted by Kullaa-Mikkonen et al. (1987) and fungiform papillae are regarded as relatively stable anatomical structures (Bartoshuk 2000), Segovia et al. (2002), on the other hand, found a significantly higher density of fungiform papillae in children than in adults, which leads to the conclusion that fungiform papillae atrophy with age. We exclude an age effect in our sample because the groups did not differ significantly with age and we could not find significant correlations between age and number of fungiform papillae.

Negative correlation was found between the density of fungiform papillae and the sweet detection threshold for sucrose in this study. There has been no prior study on such item; however, many studies were carried out to elucidate the relationship between the numbers of fungiform papillae and the bitter substance (PROP and PTC). High numbers of fungiform papillae are commonly found in people who are classified as supertasters of the bitter substance (PROP or PTC) compared with moderate and nontasters (Miller and Reedy 1990b; Bartoshuk et al. 1994; Essick et al. 2003; Shahbake et al. 2005), whereas degeneration or loss of taste following medications or neural damage is accompanied by a decrease in the number of papillae found in individuals (Zuniga et al. 1997; Sollars and Bernstein 2000). These differences are known to possess corresponding genetic differences (Reed et al. 1999; Kim et al. 2003). However, it is unclear why genetic expression of a particular bitter taste receptor (i.e., *TAS2R38*) should be related to the sensitivity to other taste stimuli that are mediated by different receptors. The very first study that investigated the association between fungiform papillae density and perceived taste intensity found a stronger relationship for sucrose and NaCl than for PROP (Miller and Reedy 1990b). More recently, 2 studies that directly assessed the relationship between papillae density and variation in the *TAS2R38* genotype found no significant association (Duffy et al. 2004; Hayes and Duffy 2007).

Limitations to this study include the fact that only adolescent young males have been examined, and it is therefore not possible to include age- and gender-related differences. In addition, with the quantification of the fungiform papillae, there is also no information on the frequency in the occurrence of taste buds, as the number of taste buds per papillae may vary. Also, the correlation between the density of the fungiform papillae, food preferences, and taste intensity in subjects with different ages or genders should be the focus of interest in future studies.

Funding

National Natural Science Foundation of China (30770536); Higher Education Natural Science Foundation of Jiangsu Province in China (07KJD150002).

Acknowledgements

The authors wish to thank the participants for their assistance.

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Accepted September 15, 2008