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Peripheral Taste System in Inbred Mouse Strains

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PERIPHERAL TASTE SYSTEM IN INBRED MOUSE STRAINS

A Thesis
Presented for
The Graduate Studies Council
The University of Tennessee
Health Science Center

In Partial Fulfillment
Of the Requirements for the Degree
Master of Dental Science
From The University of Tennessee

By
William Wes Shelton, D.D.S.
May 2010

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DEDICATION

I dedicate this thesis to my wife, the love of my life and my best friend, Ella Shelton, and to my family for their everlasting love and support.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and thanks to Dr. Christopher Nosrat for being a fantastic mentor throughout this master's thesis project and for allowing me to be part of his ongoing studies. I can't thank him enough for his time and effort spent with me in order to make this project possible.

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ABSTRACT

Taster and non-taster mice strains have been used extensively in animal taste research. Inbred mouse strains *C57* and *FVB* have been proposed to be tasters, where as strains *129* and *Balb/C* are classified as non-tasters. Therefore, evaluating the peripheral taste system in these mice is of great importance to determine if there are morphological differences that might explain the physiological classification.

The purpose of the present study was to determine if inbred mice that have been shown to be tasters have a larger number of fungiform papillae, have larger fungiform papillae and have larger taste buds.

My work in this study involved counting the number of fungiform papillae from male and female of each strain, utilizing green food dye under light microscopy. I also utilized a scanning electron microscope after tongues were dehydrated in alcohol and Hexamethyldisilazane (HMDS) and sputter coated with gold to evaluate size and shape of fungiform papillae. I used antibodies to troma -1, utilizing indirect immunohistochemistry on tissue sections to evaluate size and shape of taste buds within each strain.

Unpaired t-test was used to analyze the results. There was a significant difference in the number of fungiform papillae as seen under light microscopy in the non-taster *129* strain compared to the other strains. Data generated by scanning electron microscope also suggest that the size of the fungiform papillae is significantly smaller in the non-taster *Balb/C* and *129* strains.

Moreover, the *129* strain had the lowest number of fungiform papillae whereas *Balb/C* mice had the smallest fungiform surface area among the strains studied. We also evaluated whether multiplying fungiform papillae number by the papillary surface area might also be used as an indicator for the size of the receptor field in different mouse strains. Using this method, we showed that non-taster strains had a smaller receptor field area than taster strains. In summary, our study shows that the taster/non-taster phenotype is reflected in the tongue surface morphology among the strains studied.

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CHAPTER 1. INTRODUCTION

It has been shown that there are differences in taste sensitivity in humans and animals. Based on this physiological sensitivity, the classification of taster and non-taster has been given in humans and in animals. Taste behavioral research has been done based on these classifications. In humans, taster and non-taster classification is based on an individual's ability to detect/taste phylthiocarbamide (PTC). In mice, the taster and non-taster classification is based on the contrasting preference for sucrose octaacetate (SOA) and gylcine.

In tasters, some populations, whether human or mice, have low detection thresholds to individual tastants. It is just the opposite in non-tasters, in which it takes high concentration of tastants to elicit a response. The difference between tasters and non-tasters is not clearly understood, but it is believed there is a genetic component. Whether or not genetic background is reflected in the morphology and number of fungiform papillae is not clear. It has been suggested that there is a subset of tasters in humans that are termed supertasters (Bartoshuk and Duffy 1994; Reedy *et al,* 1993). Supertasters were shown to have a larger number of fungiform papillae and a larger number of taste pores than were seen in non-tasters. It was shown in humans that with increased number of stimulated taste buds and fungiform papillae, there was an increase in taste sensitivity and more profound taste intensity (Miller and Whitney 1989)**.** It has also been show in humans and rats that there is no decrease in taste buds or significant difference in taste bud diameter when comparing age, health or gender (Miller and Whitney 1989; Mistretta 1989). Miller and Whitney (1989) reported there were an increased number of taste buds in the vallate papillae in taster mice compared to non-taster mice. They did not test whether there were a greater number of fungiform papillae in taster and non-taster mice.

The aim of this study is to test the hypotheses that taste sensitivity in different strains of mice is related to tongue surface morphology. We studied tongue surface morphology by examining fungiform papillae and the taste buds contained inside the papillae. Fungiform papillae are the arch prototype for gustatory papillae due to the containment of generally a single taste bud. Fungiform papillae are mainly located on the anterior tongue in both rodents and humans. Better understanding of the number of fungiform papillae and the size of not only the papillae, but also the taste buds, will assist us to a better understand the interrelationship between the peripheral taste system morphology and taste physiology.

It is our hypothesis that the taster mouse strains will have a higher number of fungiform papillae and the fungiform papillae, and their taste buds will be larger compared to non-taster strains. We set out to test our hypothesis by utilizing two taster strains *(C57BL/J* and *FVB)* and two non-taster strains *(Balb/C* and *129)*. We utilized six male and six female for each strain. We counted the fungiform papillae under light microscopy and utilize SEM imaging to determine size of the fungiform papillae. Indirect immunohistochemistry was utilized to evaluate size of the taste buds.

CHAPTER 2. REVIEW OF LITERATURE

Tongue Morphology

Three key terms connected with tongue morphology: (1) *Lingual papilla* are specialized structures with an epithelium over a connective tissue core with a well-defined shape that rises above the level of the dorsal tongue surface and can include a mature taste bud. (2) *Gustatory organ* refers to an embryonic or mature papilla. (3) *Taste bud* is a sensory organ that transduces gustatory stimuli into neural signals; it is composed of a collection of dozens of cells that span the depth of the epithelium, are oriented perpendicularly to the epithelium and are in synaptic contact with sensory nerve fibers or in functional association with nerve fibers (Mistretta 1989).

The dorsal surface of the mammalian tongue is covered by specialized structures called lingual papillae. There are four lingual papillae; fungiform, circumvallate, foliate and filiform papillae (**Figure 1** and **Figure 2**). The first three papillae are considered gustatory, i.e. contain taste buds. Taste buds not only occur in distinct papillae, but also in the epithelium of the palate, oropharynx, larynx and the upper esophagus. There is a rather similar distribution of taste buds over the tongue in humans and rodents. Fungiform papillae are mushroom shaped, enlarged heads with a slender neck; these are mainly located on the anterior part of tongue. As early as 1875, Hoffman concluded that taste perception is dependent of the number of taste buds on a particular location (1875). In humans there are around 4,600 total taste buds in all papillae; vallate papillae comprise about 48%, foliates about 28% and fungiform about 24% (Miller and Reedy 1990a). Humans have on average nine vallate papillae, which are located on the posterior tongue. The circumvallate papilla in rodents is a single taste bud containing papilla along the posterior midline. The fungiform papillae will contain one or more taste bud depending on species. There is generally a single taste bud on the apical epithelium of the fungiform papillae in rodents. In humans it is estimated there are 3.5 taste buds per papilla. Miller and Reedy quantified taste in terms of tongue surface area, which they referred to as "taste bud density," or number of taste buds per cm^2. They found 320 fungiform papillae per tongue and multiplied by 3.5 taste buds for a total of 1120 fungiform taste buds (Miller and Reedy, 1990a; 1990b). The fungiform papilla is a gustatory organ that is composed of: taste epithelium, non-taste epithelium and connective tissue. Taste epithelium which surrounds non-taste epithelium is made of up taste bud cells and cells competent to differentiate to taste cells. The sensory fibers that innervate papillae and taste buds cells are encompassed by the connective tissue core (Mistretta and Liu 2006). The fungiform papillae emerge histologically in mice at embryonic day (E) 11.5- E12 (Kaufman 1992). Foliate papillae are located at the lateral edges of the tongue and contain multiple taste buds. Filiform are the most numerous but do not contain taste buds. There is a certain number and location of taste buds with the three papilla types that will differ depending on the species (Mistretta, *et al*. 1999). The location and number of gustatory papillae and taste buds form a patterned organ system that is well suited to detect chemicals or tastants on the tongue (Mistretta and Liu 2006).

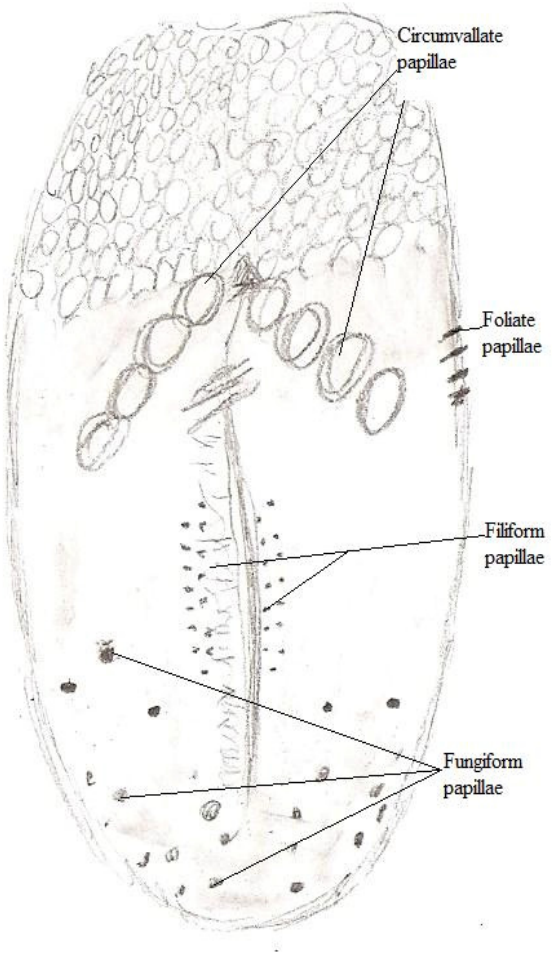

Figure 1. Human tongue

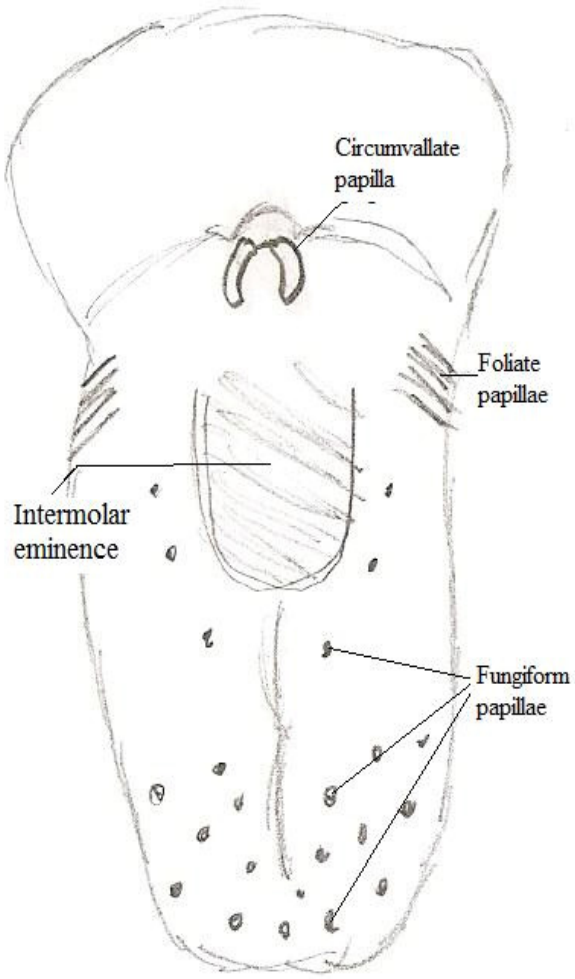

Figure 2. Mouse tongue

Taste Bud Morphology

Taste buds are multicellular, pear-shaped sensory organs. Although there is a variation in the morphology of taste buds, it is now believed that there are distinct populations of cells in mammalian taste buds, including receptor cells and cells with synapses. Histological identification was first utilized to describe these distinct cells, later identified as Types I, II, III, IV cells by electron microscopy and immunohistochemistry (Roper 2007) (**Figure 3**). Type I cells are the most frequent cells found in a taste bud (Murray 1986). Type I cells share some common features with glial cells (Roper, Finger, Barbel 2004). Type I cells might have a secretory and phagocytotic function and possibly produce the amorphous material of the taste pore (Farbman 1965; Menco 1989; Ohmura, Horimoto *et al*. 1989; Witt 1996). Type II cells contain the G-protein coupled receptors that detect bitter, sweet and umami tastes, indicating that Type II cells are sensory receptor cells (Roper 2007). These cells are located on the periphery of the taste buds but synapses are not observed (Kinnamon, Taylor *et al*. 1985; Kinnamon, Sherman *et al*. 1988; Royer and Kinnamon 1988). Type III cells are the only cells that posses features of typical synapses, and have been termed presynaptic cells by some investigators (Roper 2007). They make up 5-7% of the cells in a taste bud and due to the synaptic property are considered gustatory relay cells. Type IV cells are small undifferentiated cells that appear at the base of the taste buds, and do not form processes that reach the pore (Royer and Kinnamon 1991). They are considered to be stem/progenitor taste cells.

Taste buds are clusters of receptor cells found inside the papillae and have fingerlike projections called microvilli that extend into the oral environment through an opening called the taste pore. Chemicals from food (tastants) bind to the cell surface taste receptor molecules or activate specific ion channels. This reaction causes electrical changes in the taste cells which then send this message to the brain through taste nerves (Smith and Margolskee 2001). The ability to be able to distinguish between tastes is an important part of survival. The brain via a series of chemical reactions interprets the following as basic tastes: salty, sour, sweet, bitter and umami (Smith and Margolskee, 2001). Salt is critical to survival for ion and water homeostasis. Due to this fact, salt in appropriate concentrations is favorable taste for mammals but in high concentrations is aversive. Sour foods tend to have a negative taste in large quantities, and are also aversive at high concentrations. Taste also has a protective quality, for most spoiled meats and fruits will have a sour taste and the body will respond appropriately as to not ingest what it feels is harmful food. Alkaloids and toxins are usually bitter and thus avoided. The human body has actually managed to overcome the bitter taste, for such flavors as caffeine and nicotine. Sweet taste tends to relate to carbohydrates, again essential in energy use and energy storage. Umami is considered a savory taste relating to amino-acids in proteins, thus encouraging the ingestion of proteins. Proteins are used in the human body to build muscle and transport molecules. The ability to differentiate between these tastes is essential for survival whether we are referring to humans or animals.

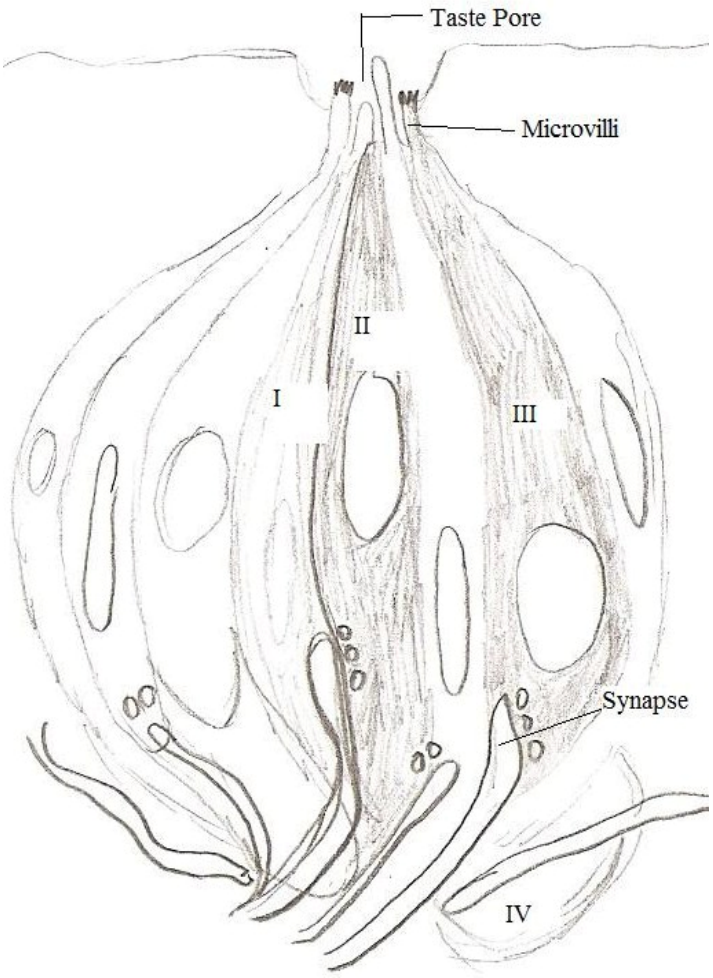

Figure 3. Taste bud

Innervation

Taste buds are innervated by branches of the facial, glossopharyngeal and vagal nerves. The majority of taste buds are located within taste papillae (Smith and Margolskee 2001). We will focus mainly on fungiform and circumvallate papillae. Fungiform papillae are innervated by a sensory branch of the facial nerve, the chorda tympani. The chorda tympani courses through the facial canal and exits the skull through the petrotympanic fissure where it joins the mandibular division of the trigeminal nerve; the lingual nerve. The combo chorda-lingual nerve will now enter the anterior tongue and make its way to the connective tissue of fungiform papillae; also it innervates apical papilla epithelium, where taste bud differentiation will take place (Mistretta and Liu, 2006). It has been shown that only 25% of nerves entering the fungiform papillae originated from the chorda tympani while most nerve fibers originated from trigeminal/lingual nerve (Farbman and Hellekant, 1978). The glossopharyngeal nerve supplies not only taste fibers to taste buds in the circumvallate and foliate papillae, but it also provides general somatosensory to the papillae. The glossopharyngeal is also responsible for the taste buds in the pharynx, whereas the vagus nerve innervates taste buds of the epiglottis, larynx and esophagus.

The development of taste buds was once thought to be directly related to its innervations, the so called "neural induction theory." It was thought that the facial, glossopharyngeal and vagus nerves invade the mesenchmye of the tongue and induce thickenings in the lingual epithelium. These thickenings are termed "placodes." These placodes would continue to differentiate until nerve fibers reached the inner surface of the epithelium and induced the formation of taste bud primordia. Differentiation would continue until the taste cells finally gained access to the external environment through the formation of a taste pore (Northcutt 2004). This theory was taken to task when it was shown that taste buds developed in the absence of innervations in aquatic salamanders in what is known as the "early specification model" (Northcutt 2004). The gustatory epithelium is clearly predefined and expresses growth factors and morphogens long before the arrival of pioneering gustatory nerves (Hall and Bryan 1981; Nosrat, Blomlof *et al*. 1997). This theory proposes that during gastrulation in axolotl, cells within the orophayngeal endoderm can signal one another, and some cells become taste bud progenitors, while the remaining cells will be general epithelium. A chemical is produced by the orophayngeal epithelium which attracts nerve fibers. In mammals, it has been shown to be brain-derived neurotrophic factor (Nosrat, Blomlof *et al*. 1997). Once the nerve fibers reach the epithelium, they form contacts within the taste bud primordia. At this point the differentiation of the taste bud primordia will continue and taste cells form synapses with afferent fibers (Northcutt 2004).

Genetics

Inbred mice have been shown to have genetic differences related to sweet and bitter taste. Glycine, an amino acid, in solution has been shown to taste bitter and sweet in different strains of mice, and there are differences between strains in their ability to

detect each taste (Lush and Holland 1988). Taste sensitivity, aversion and preference are tested using a two-bottle preference test. In a two-bottle preference test, mice are given a choice between compounds dissolved in water, and based on their choice they are classified as taster or non-taster. If a group of mice avoid drinking the solution they are referred to as tasters, the other group has a slight tendency to avoid the solution and are referred to as non-tasters. There is another category that has been shown in humans but not in mice; supertasters. In a study by Bartoshuk *et al.* (1994), supertasters were identified as perceiving intense bitter tastes from concentrated PROP (6-n-propylthiouracil). Supertasters were shown to have a larger number of fungiform papillae, had a large number of taste pores (indicating a functional taste bud associated with the taste pore), and had rings of tissue that were not seen on the non-tasters' Fungiform papillae (Bartoshuk, Duffy *et al.* 1994). The difference between the tasters and non-tasters in this instance could be due to a single gene, *Glb (*glycine bitterness) (Lush and Holland 1988). Detection of another bitter tasting compound, sucrose octaacetate (SOA), has been shown to differ between tasters and non-tasters (Boughter and Whitney 1997).

When looking at sweet compounds, the interaction of a sweetener with a G protein-coupled taste receptor (GPCR) gives a sweet taste perception on the apical ends of taste receptor cells. Hoon *et al.* (1999) were able to identify two taste-specific GPCRs, T1R1 and T1R2. Hoon *et al.*(1999) and Roper (2007) speculated that based on the protein expression pattern on the tongue, T1R1 was a sweet receptor. Several groups have searched for genes related to T1R1 and T1R2 (Roper 2007). Their search resulted in finding the location of the gene that has been shown to be prominent in regulating differences in sweet preferences among mouse strains; the saccharin preference (*Sac*) locus on distal chromosome 4 (Bachmanov, Tordoff *et al*. 2001). The *Sac* locus gene may be the gene that encodes a sweet taste receptor; this receptor is T1R3 of the T1R family of taste receptors (Bachmanov, Tordoff *et al*. 2001). Having allelic variation for the *T1R3* can make a mouse either sweet sensitive or subsensitive (Sclafani 2006). Besides the sweet taste, T1R receptor family members have also been identified as candidates for umami receptors (Roper 2007). Maruyarna *et al.* (2006) and Roper (2007) showed there was a diminished response to sweet taste in T1R3-null mice, but many did respond to umami-taste stimuli. These findings indicated that the umami taste is likely to be transduced by T1R1 + T1R3 dimers as well as other receptors, some of which have yet to be identified (Roper 2007). A family of GPCRs that recognizes bitter tastes was identified in 2000 (Adler, Hoon *et al*. 2000; Matsunami, Montmayeur *et al*. 2000; Roper 2007). This family was named T2Rs (Roper 2007). Their role as bitter receptors was confirmed when mouse and human cells transfected with T2Rs responded to denatonium and cycloheximide, two intensely bitter compounds (Chandrashekar, Mueller *et al*. 2000; Roper 2007).

CHAPTER 3. RESEARCH OBJECTIVES

Hypothesis

The peripheral taste system morphology in inbred mice is reflective of the classification of taster/non-taster. Three specific aims for this hypothesis follow.

Specific Aim 1

We hypothesized that the number of fungiform papillae would correspond to the taster/non-taster classification.

Procedure: We aimed to quantify the number of fungiform papillae on each tongue by staining the tongue with green food dye, which would allow the fungiform papillae to be visible under light microscopy. Each tongue was counted by two independent researchers trained in counting tongue papillae under light microscopy.

Specific Aim 2

We hypothesized that the morphology and size of the fungiform papillae would correlate to the taster/ non-taster classification.

Procedure: Scanning electron microscope was used to evaluate the morphology and size of fungiform papillae. Three tongues from each strain were used, 2 males and 1 female. Computer software was employed to measure the size and for analysis.

Specific Aim 3

We hypothesized that fungiform taste bud size would correlate to the taster/ non-taster classification.

Procedure: Immunohistochemistry with specific antibodies was performed on sagittally and frontally cryo-sectioned tongue tissue. Taste bud size was evaluated using Image J software version 1.40g (Wayne Rasband, National Institute of Health, USA).

CHAPTER 4. METHODS AND MATERIALS

Materials and Methods

The mice were obtained from the Jackson Laboratory (Jackson, Michigan). Animal use has been approved by IACUC. Four strains of mice were purchased, two taster strains *(FVB, C57)* and two nontaster strains *(Balb/C, 129)*. Six male and six female of each strain of mice were evaluated. At eight weeks of age the mice were euthanized by CO2 asphyxiation and perfused with 4% paraformaldehyde in phosphate buffered saline (PBS) through the ascending aorta. Tongues were removed, post-fixed, rinsed and cryopreserved in 10% sucrose in PBS. All animal procedures were in compliance with approved institutional animal care and use protocols and according to National Institutes of Health guidelines.

Papilla Count

For the initial count of the fungiform papillae number, before cryopreservation, the papillae of intact tongues were visualized using green food coloring and counted under a Nikon SMZ-1500 steriomicroscope. Count was completed by two experienced researchers; one researcher counted twice on two separate occasions, counts were averaged together. Count was completed for all four strains of inbred mice including six female and six male of each strain. Average counts were placed in computer software Instat and data was analyzed using unpaired t-tests.

Scanning Electron Microscopy

Tongues for scanning electron microscopy procedure were dehydrated in series of alcohol and a final step of HMDS, mounted on aluminum stubs and sputter-coated with gold and observed in a Joel JJM-5510 SEM. Three male animals of each strain were used for scanning electron microscopy. For the dehydration of tongues the following steps were followed:

- Incubate tongue in 70% alcohol for 2 hours
- Incubate tongue in 95% alcohol for 2 hours
- Incubate tongue in 100% alcohol overnight

The SEM operator was allowed to make decision on the best magnification per sample based on operator expertise. Length, Width and Area measurements were taken on SEM pictures utilizing computer software Image J. The SEM picture chosen for analysis was the picture that had exactly or at least six identifiable fungiform papillae. Image J software was used to set scale on each picture to measure the length, width and area on six fungiform papillae per species. Measurements were completed by same examiner on two separate occasions. Then the numbers were averaged and placed in computer software Instat for statistical analysis using unpaired t-tests.

Immunohistochemistry

Three female animals of each strain were used to prepare tongues for sectioning; in one instance, three (*129)* males were also used. Tongues were sectioned into three sections: anterior, middle and posterior. Since the anterior and middle portions contain fungiform papilla and the posterior section contains circumvallate papillae. Tongues were frozen and sectioned (14 μm) and mounted onto gelatin coated slides. Slides were washed in PBS (10min x 2). Slides were then placed in acetic acid for five minutes, followed by washing in PBS (5 min x 5). We then placed 100ml per slide of antibodies to Troma-1 (taste cell marker) at a ratio of 80:1 with triton PBS. Slides were placed in storage containers lined with absorbent sheets soaked in PBS and incubated overnight in refrigeration. After twenty-four hours, slides were washed with PBS (5min x 3). 100ml of secondary antibody Alexfluor 594 at a ratio 100:1 with triton PBS was placed on slides. Slides were then evaluated under a microscope and fixed with 1:1 mix glycerin/PBS and covered with glass cover slips. Using indirect immunohistochemistry, taste buds and their innervation were visualized under a fluorescent microscope (Nikon 80i). Digital images were taken of fungiform papillae/taste buds and circumvallate papillae/taste buds using Nikon DXM-1200 digital camera. Pictures were placed in Image J software and measurements were taken for length, width and area of taste buds. An O.1mm ruler was used to set the scale and all measurements were completed during one session. Due to difference in number of quality sections obtained from each strain, 10 of the most representative tastes buds from all sections were used. These measurements were averaged to gain a mean number which was inserted into computer software Instat for statistical analysis using unpaired t-tests.

CHAPTER 5. RESULTS

Papillae Count

All fungiform papillae were counted on surface stained tongues from six male and six female transgenic mice, utilizing four different strains *(Balb/C, 129, FVB, C57).* The mean papillae count was 83.5 in *Balb/C*, 65.9 in *129*, 85.3 in *FVB* and 90.1 in *C57* (**Table 1**). Unpaired t-tests showed a significant difference between strain *129* and all other strains ($p<0.001$). There was also a significant difference between strains *Balb/C* and *C57* ($p<0.05$). Not only was the number of fungiform papillae decreased in non-taster strains, but papillae distribution was different for strain *129*. Strain *129* presented with almost all fungiform papillae in the anterior portion, whereas the other strains showed papillae as we moved posteriorly. **Figure 4** (error bar represents standard error of mean, or SEM), demonstrates the significant difference between strain *129* and strains: *FVB, C57 and Balb/C*. There were no statistical difference between male and female within each strain.

Scanning Electron Microscope

Pictures of three male transgenic mice of each species *(Balb/C, 129, FVB, C57)* were examined after having scanning electron microscopy completed. Length, width and area were measured on pictures utilizing Image J software. *Balb/C* was measured on picture with 200x magnification. *129* were measured on picture with 90x magnification. *C57* was measured on picture at 100x magnification, and *FVB* was measured on picture with 150x magnification. Due to lack of fungiform papillae in the posterior section, all pictures and measurements were taken from anterior tongue. The mean length was 0.060 mm for *Balb/C*, 0.066 mm for *129*, 0.085 mm for *FVB* and 0.063 mm for *C57*. There was significant difference when comparing length of all species to *FVB* after running unpaired t-tests ($p<0.01$). When looking at width the mean was 0.055 mm for *Balb/C*, 0.057 mm for *129*, 0.065 mm for *FVB* and 0.061 mm for *C57*. After unpaired t-tests were run, there was significant difference between *FVB* and *129* ($p<0.05$). There was a significant difference between *FVB* and *Balb/C* ($p<0.01$). When looking at area, the mean was 2.9 mm for *Balb/C*, 3.2 mm for 129, 4.8 mm for *FVB* and 3.4 mm for *C57* (**Table 2**). When unpaired t-tests were run there was a significant difference between *FVB* and all other species ($p<0.001$). **Figures 5, 6, 7** (error bar represents standard error of mean, or SEM), show the difference when fungiform papillae were measured on SEM pictures utilizing Image J software. **Figure 8** shows the 200x magnification SEM picture of fungiform papillae from male strain *Balb/C* used for measurement. **Figure 9** shows the 90x magnification SEM picture of fungiform papillae from male strain *129* used for measurements. **Figure 10** shows the 100x magnification SEM picture of fungiform papillae from male strain *C57* used for measurements. **Figure 11** shows the 150x magnification SEM picture of fungiform papillae from male strain *FVB* used for measurements. Pictures are labeled with which fungiform papillae were measured one to six. Six fungiform papillae were measured from each strain using Image J software, setting a scale on each picture based on the given length bar.

Table 1. Mean papillae count

Measurement	Balb/C	129	FVB	C57
Papillae count	83.53	65.97	85.33	90.11

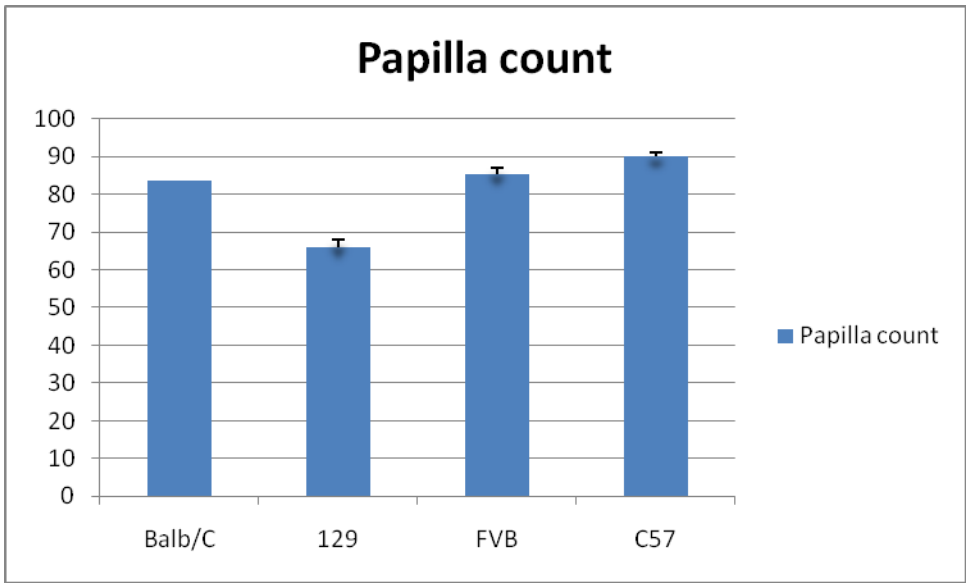

Figure 4. Mean papillae count

Table 2. Mean SEM measurements in mm

Measurement	Balb/C	129	FVB	C57
Area	2.9	3.2	4.8	3.4
Length	0.060	0.066	0.085	0.063
Width	0.055	0.057	0.065	0.061

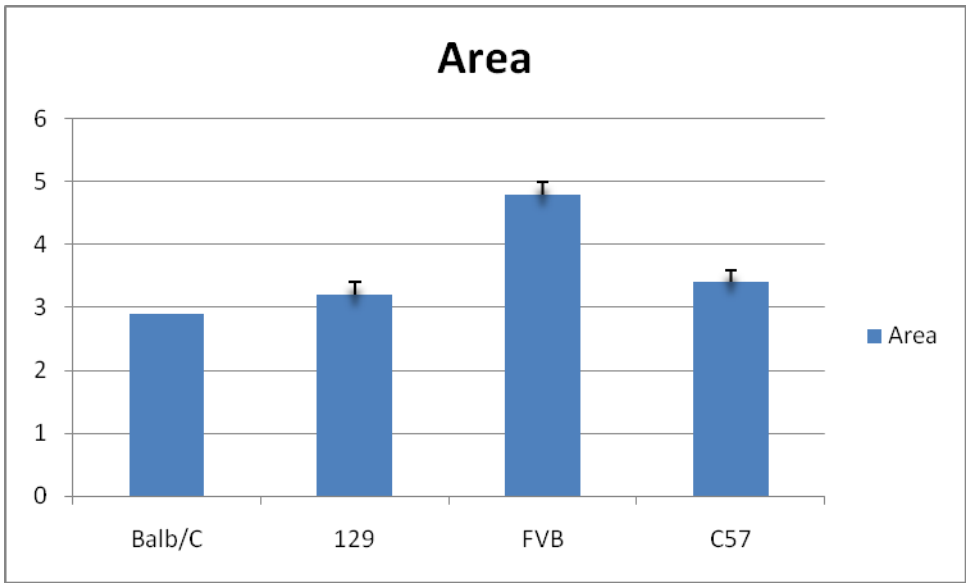

Figure 5. Mean fungiform papillae area measured from SEM pictures in mm

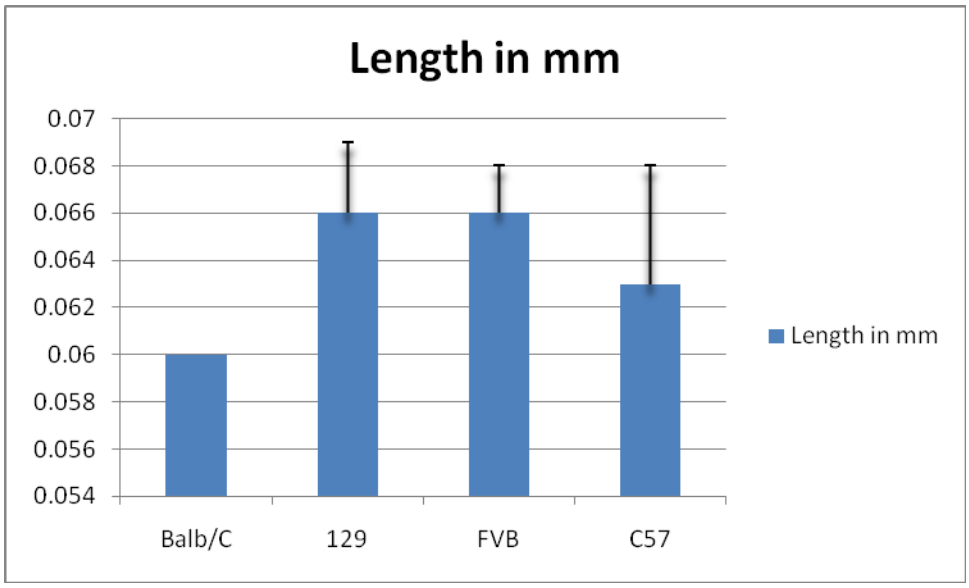

Figure 6. Mean fungiform papillae length measured from SEM pictures in mm

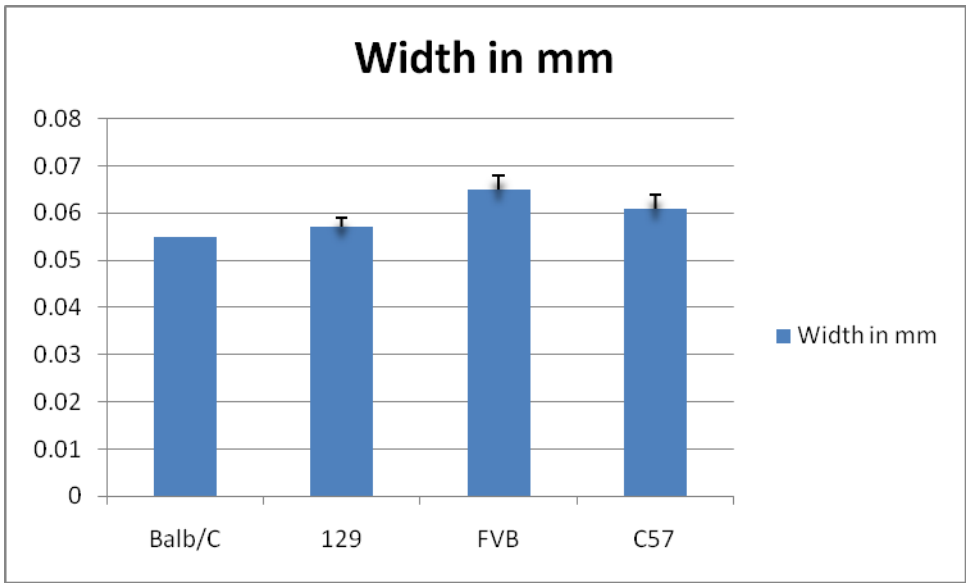

Figure 7. Mean fungiform papillae width from SEM pictures in mm

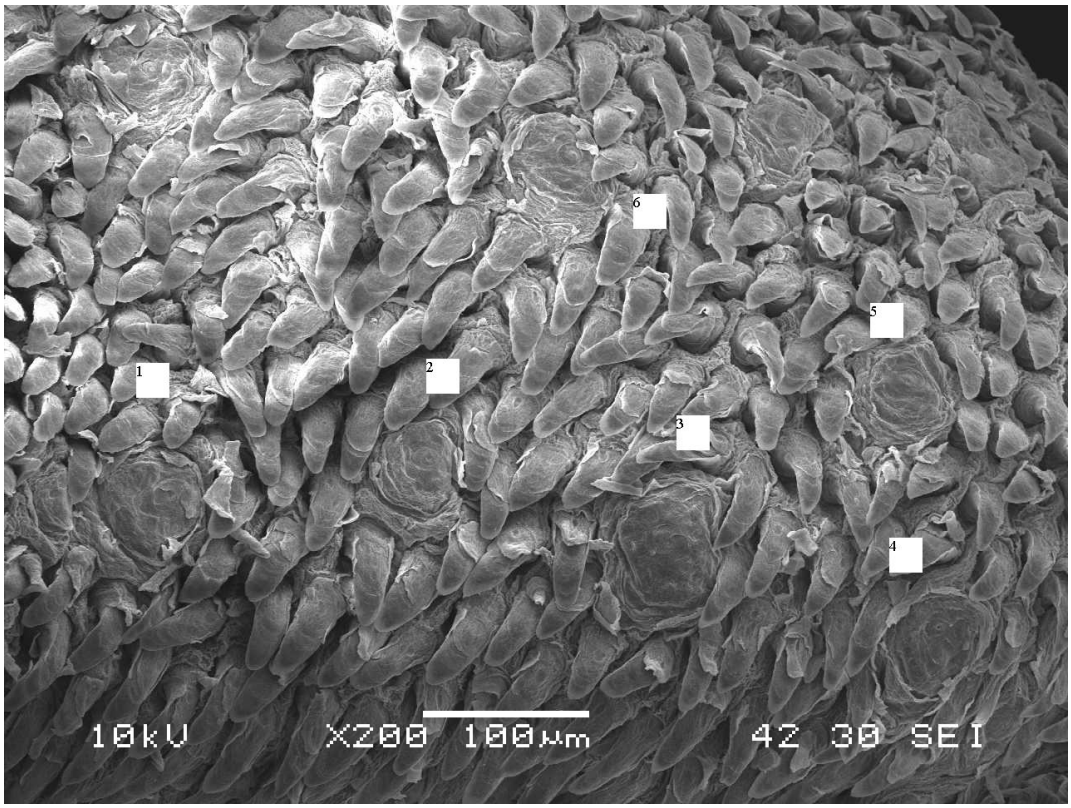

Figure 8. SEM picture of fungiform papillae strain *Balb/C* 200x magnification

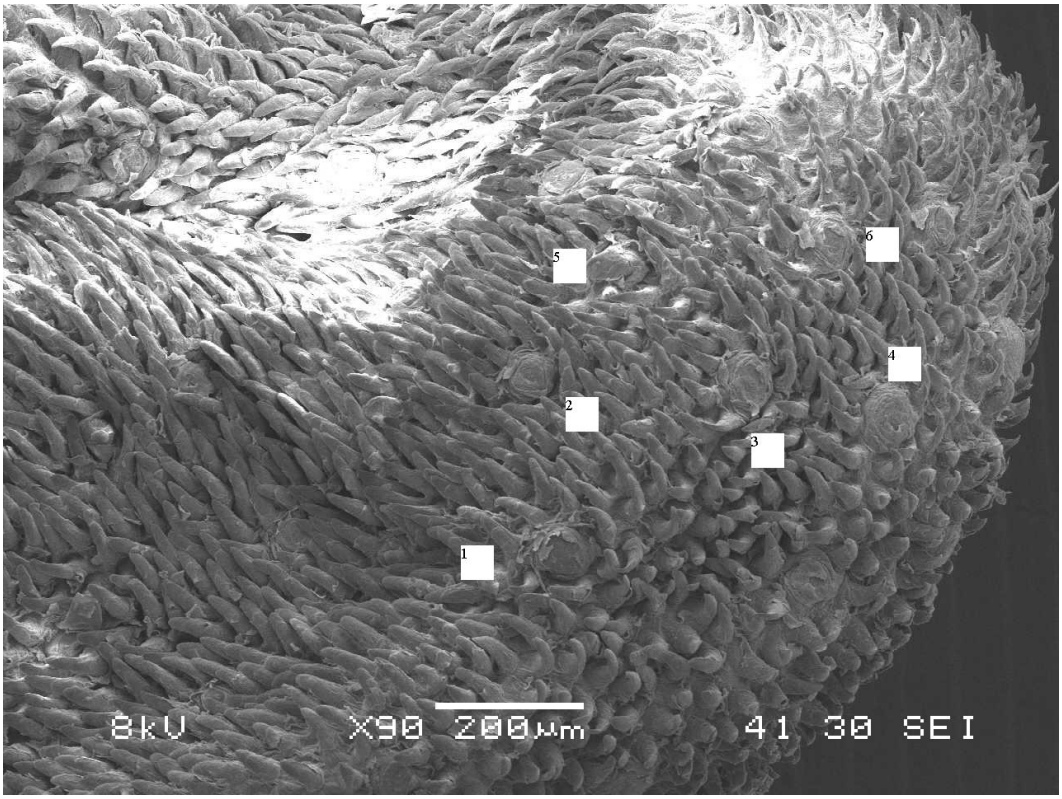

Figure 9. SEM picture of fungiform papillae strain *129* 90x magnification

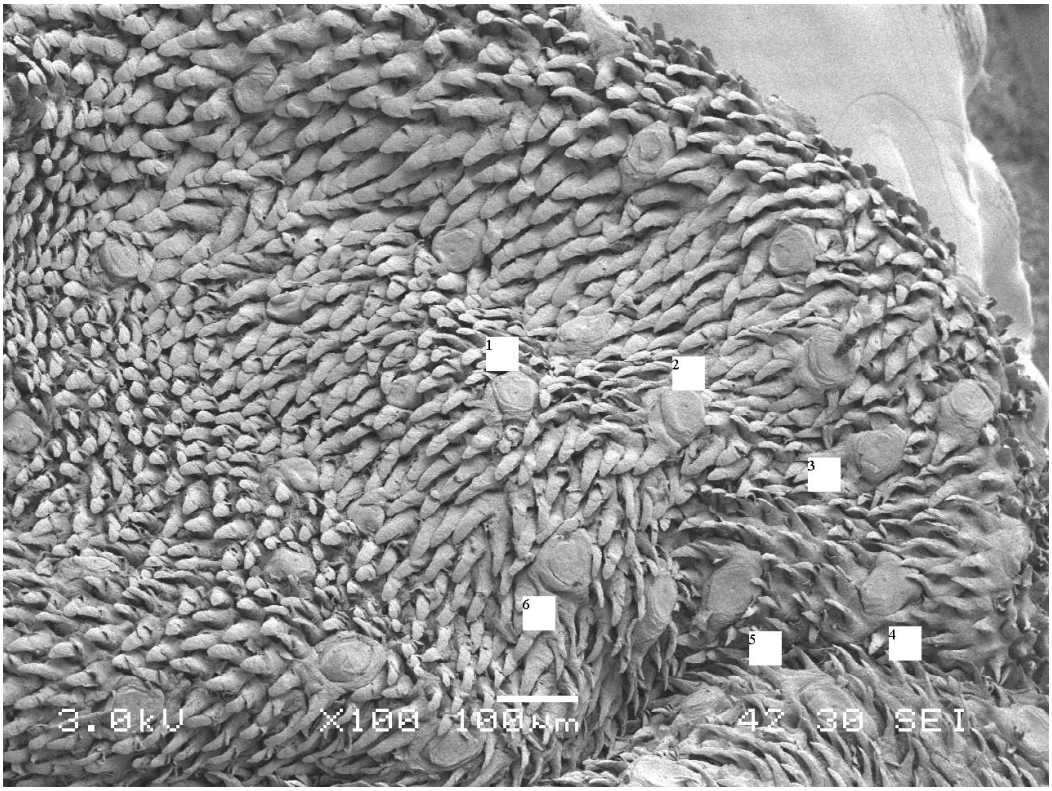

Figure 10. SEM picture of fungiform papillae strain *C57* 100x magnification

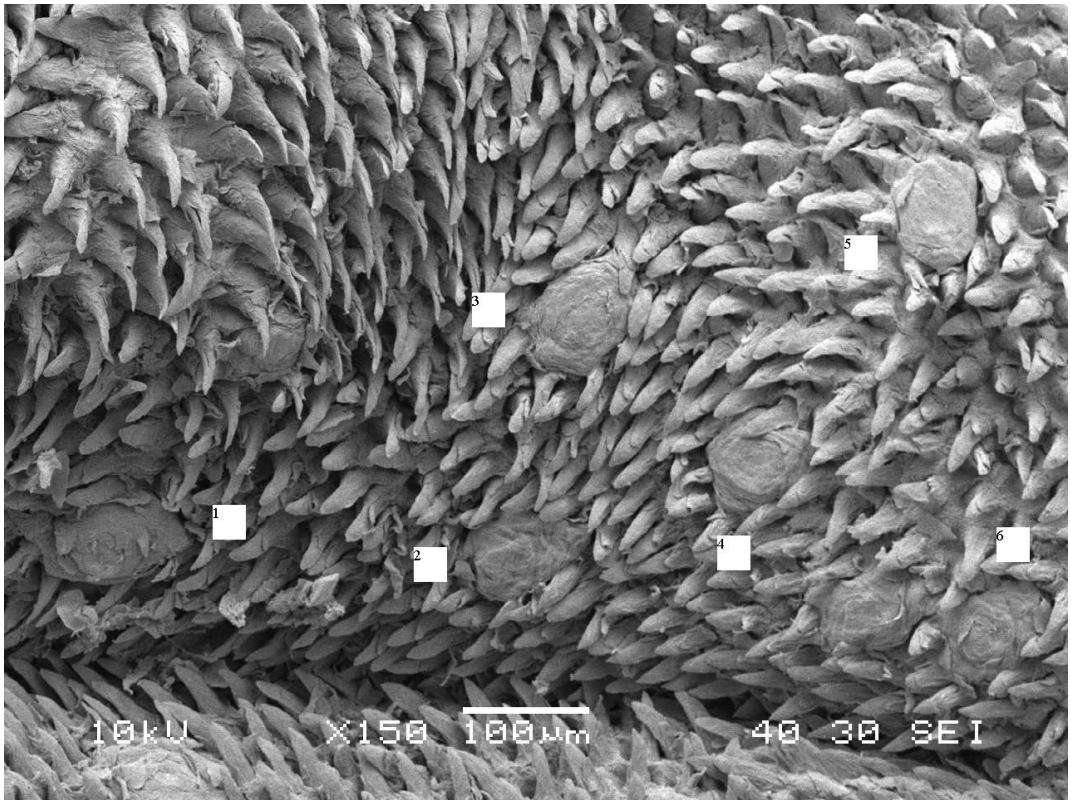

Figure 11. SEM picture of fungiform papillae strain *FVB* 150x magnification

Immunohistochemistry

Three female of each strain were used for analysis in indirect immunohistochemistry. Due to improper sectioning, strain *129* was deemed not appropriate for data collection. At this time, three male *129* strains were used in indirect immunohistochemistry. Due to lack of fungiform papillae in posterior section, all pictures and measurements were taken from the anterior tongue. Length, width and area were measured using Image J software. Measurements were combined to determine a mean value. The mean values for length were 0.043 mm for *Balb/C*, 0.045 mm for *129*, 0.042 mm for *FVB* and 0.045 mm for *C57*. These values were non-significant in the differences. The mean values for width were 0.027 mm for *Balb/C*, 0.026 mm for *129,* 0.027 mm for *FVB* and 0.023 mm for *C57*. There was a significant difference between the widths of *Balb/C* and *C57* ($p<0.001$). The mean values for area were 0.001099mm2 for *Balb/C*, 0.001054mm2 for *129*, 0.001131mm2 for *FVB* and 0.000928mm2 for *C57 (***Table 3***)*. No significant difference is seen between the means for area between the tested species. **Figures 12, 13, 14 (**error bar represents standard error of mean, or SEM), show the difference when taste buds were measured after indirect immunohistochemistry pictures utilizing Image J software. **Figure 15** is an example of a taste bud following indirect immunhistochemisty from female strain *Balb/C*. **Figure 16** is an example of a taste bud following indirect immunohistochemistry from male strain *129*. **Figure 17** is an example of a taste bud following indirect immunohistochemistry from female strain *FVB*. **Figure 18** is an example of a taste bud following indirect immunohistochemistry from female strain *C57*. Length, width and area were measured on each taste bud utilizing Image J software.

Table 3. Mean taste bud size area measurements in mm², length and width in mm

Measurement	Balb/C	129	FVB	C57
Area	0.001099	0.001054	0.001131	0.000928
Length	0.043	0.045	0.042	0.045
Width	0.027	0.026	0.027	0.023

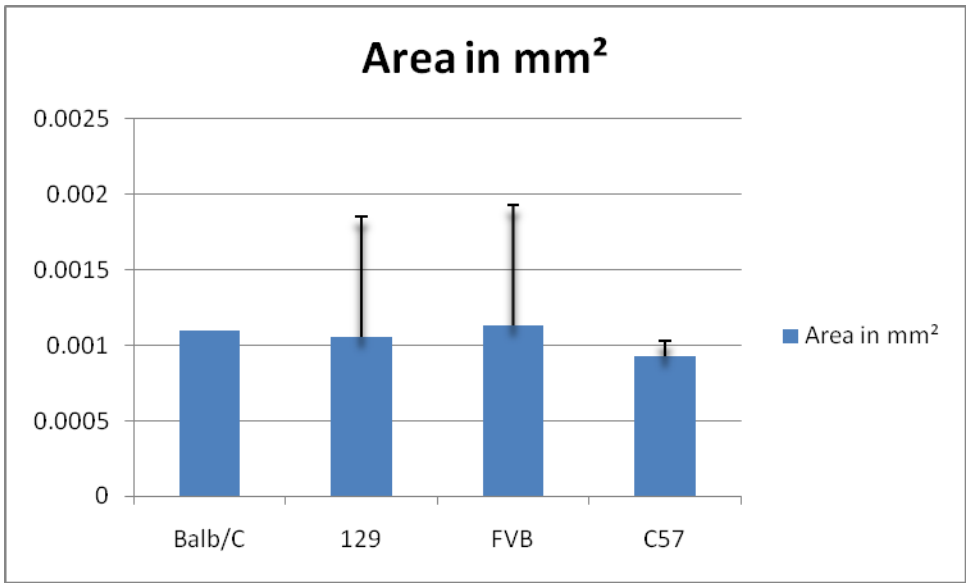

Figure 12. Mean taste bud area in mm²

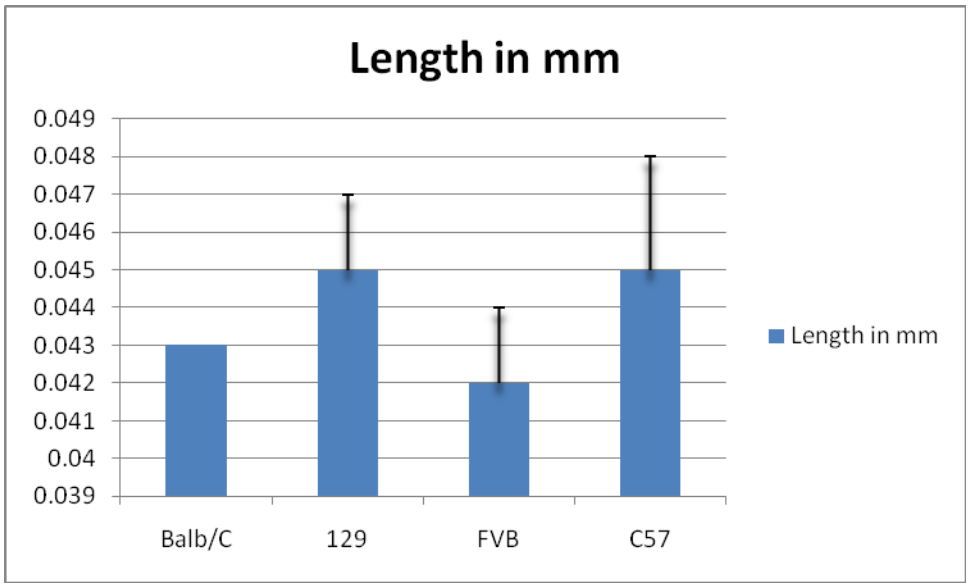

Figure 13. Mean taste bud length in mm

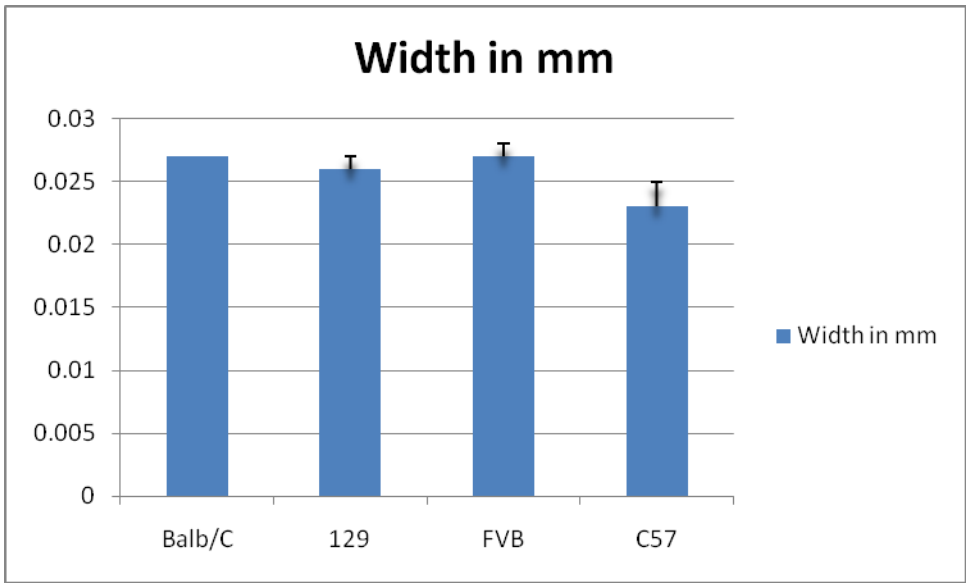

Figure 14. Mean taste bud width in mm

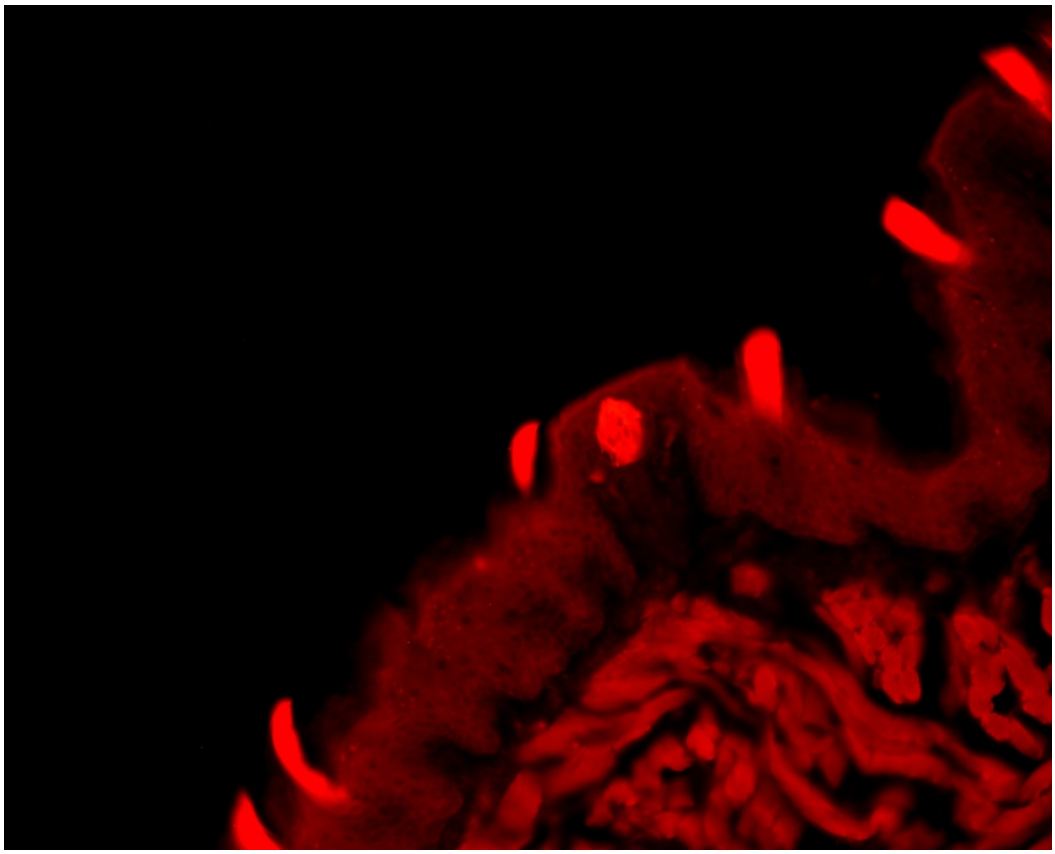

Figure 15. Immunohistochemistry picture of taste bud strain *Balb/C*

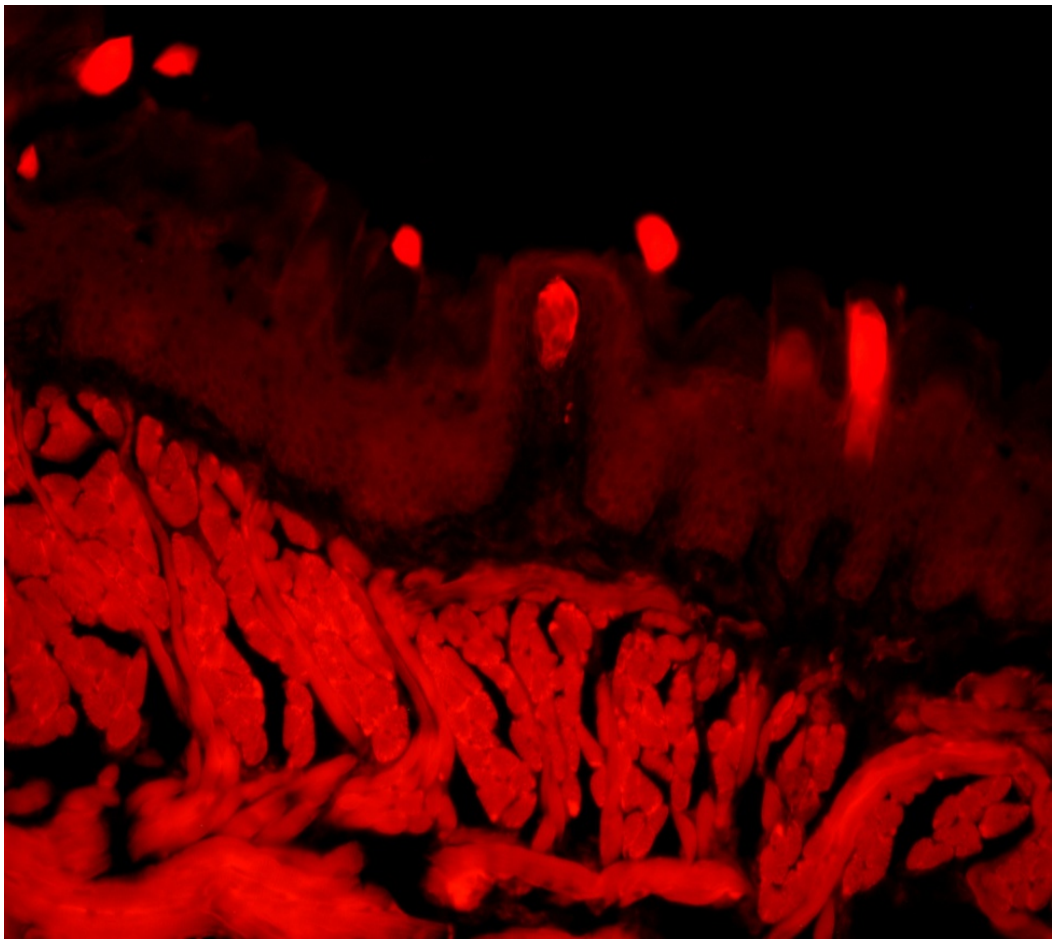

Figure 16. Immunohistochemistry picture of taste bud strain *129*

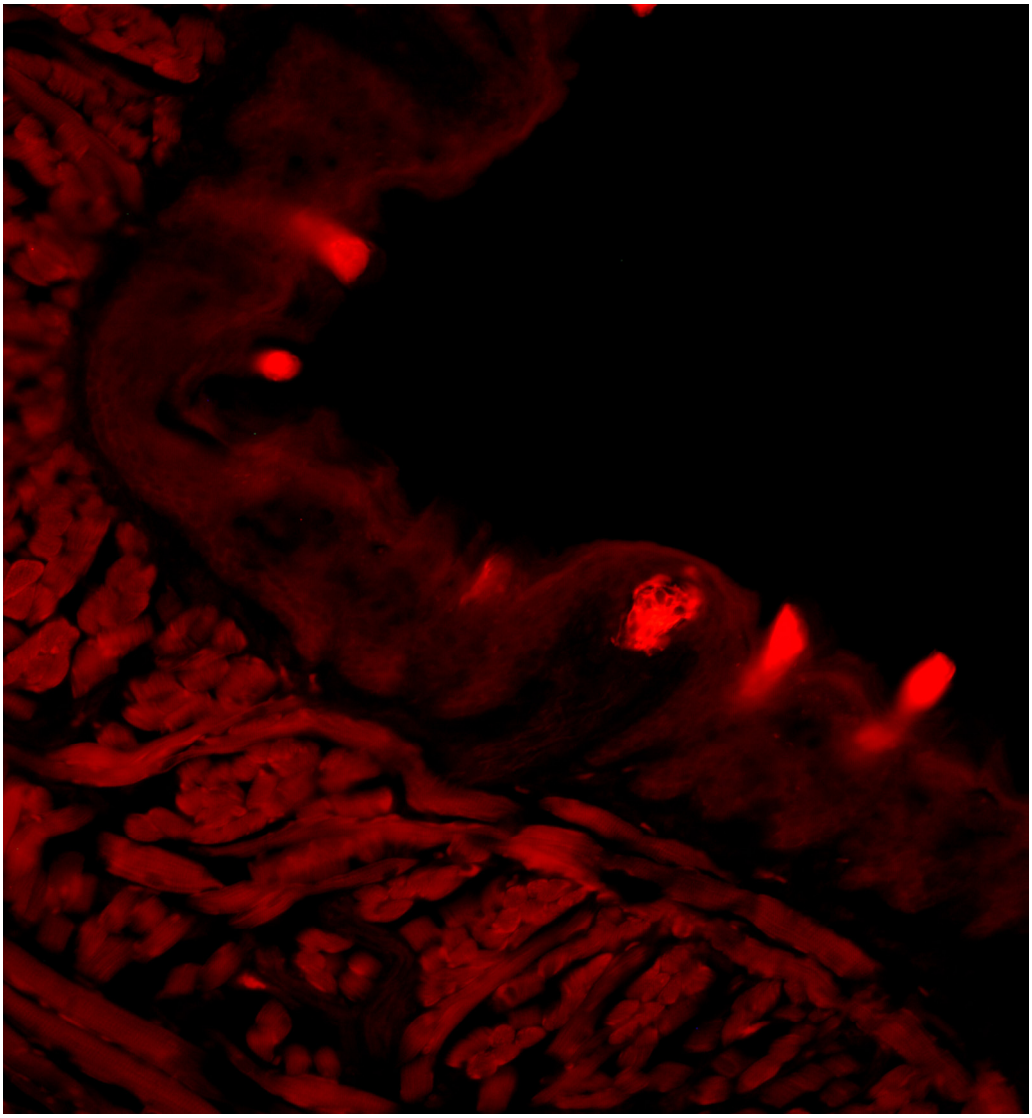

Figure 17. Immunohistochemistry picture of taste bud strain *FVB*

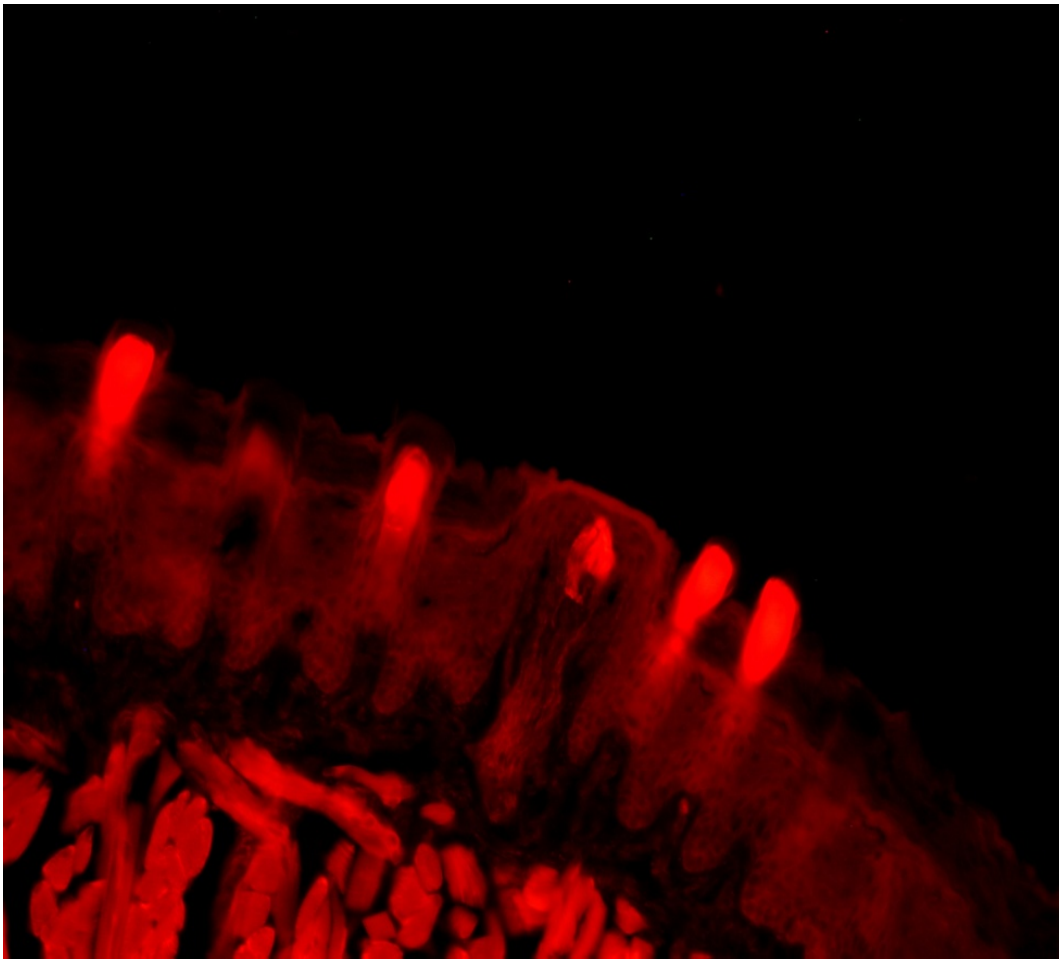

Figure 18. Immunohistochemistry picture of taste bud strain *C57*

CHAPTER 6. DISCUSSION

Two bottle preference tests have been the standard test for assessing taste solution in animals since the 1930's. A two bottle preference test is a simple test where animals are presented two drinking options, one bottle containing water and the other a taste solution. Different solutions are used when testing different taste qualities. Tests between deionized water and sodium saccharin are used to test for sweet, citric acid solution is used to test for sour, quinine hydrochloride (QHCL) and sucrose octaacetate (SOA) are used to test for bitter, NaCl is used to test salty taste preference, whereas inosine monophosphate (IMP) is used to test umami (Tordoff 2007). In the two bottle preference test, animals are given 48 hours with deionized water and a tastants solution. After 48 hours, the solutions are measured to determine how much liquid was consumed of each solution. With sweet/umami solutions, tasters will consume more than non-tasters due to enjoying the taste. With bitter/salty solution, tasters avoid the solution more than non-tasters due to the bitterness taste. The two bottle preference test results have had conflicting data even between inbred strains. This may be due to altered gustatory functioning within the strain (Boughter 2002). Different preference testing methods including utilizing more or fewer bottles have been proposed, all having advantages and disadvantages (Batsell and Best; 1993; Dragoin *et al.*, 1971; Elkins, 1973; Grote and Brown, 1971; Klein *et al.*, 1975). A study by Tordoff and Bachmanov (2003) showed a three bottle preference test has the greatest sensitivity when compared to a two bottle preference test. They also state that their results reinforce the fact that preferences observed in the laboratory may have little or no effect on preferences observed in real life. Since preference testing can have its flaws, our study will help explain strain-based differences in taste preference.

There are different reasons that fungiform papillae may have different sizes and different number in mice strains. Aside from the inbred mouse strains tested here, different knockout mice can have an effect on gustatory function. Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that regulates neuronal development and function (Lewin and Barde, 1996), and has been shown to have an effect on the support of gustatory function (Nosrat I.V., 2000). When BDNF knockout mice are tested for number and size of fungiform papillae, there is a 35% decrease in number of fungiform papillae, and the papillae that remain are smaller in size (Nosrat *et al.*, 1997). These finding correlates with the findings of Mistretta *et al.* (1999), that BDNF null mutant mice had fewer fungiform papillae's, but the remaining papillae's were on the tip of the tongue and absent on the posterior segments.

Nerve transection of the chorda tympani nerve can cause degeneration of taste buds in fungiform papillae while cutting the superior laryngeal nerve has this effect on vallate taste buds. The nerve transection, like BDNF, has a larger effect on the posterior and lateral tongue than on the anterior tongue. This finding shows that taste buds on the anterior tongue are influenced less by neurotrophic factors than the posterior tongue (Guagliardo and Hill, 2007). Dystonin (Bullous Pemphigoid Antigen 1) is a protein that is considered to play a role in cytoskeleton organization during axonogenesis (Dalpe *et al.*, 1998; Leung *et al.*, 1999). Like BDNF, the loss of dystonin causes a reduction in the

density of fungiform papillae and taste buds. Unlike BDNF null mutant mice, the dystonin mutated mice had a 67% reduction in number of fungiform papillae and the loss was mainly in the anterior tongue (Ichikawa H., 2007).

The current study shows that differences in taster and non-taster mice are observed and correlate well in terms of the morphology and number of fungiform papillae. Strain *129,* considered a non-taster strain had 21% fewer fungiform papillae than its non-taster counterpart *Balb/C.* When compared to the taster strains, *129* had 23% fewer fungiform papillae than strain *FVB* and 27% fewer fungiform papillae than strain *C57.* When measuring the area of fungiform papillae on SEM pictures, non-taster strain *Balb/C* had the smallest area, measuring 2.9mm, whereas taster strain *FVB* had the largest area measuring 4.8mm. Taster strain *FVB* was 40% larger than non-taster strain *Balb/C*, 34% larger than non-taster strain *129* and 30% larger than taster strain *C57*. By taking the number of fungiform papillae and multiplying it by the area obtained from SEM measurements of the fungiform papillae, the result should be a number that represents the size of the receptor field per tongue (**Table 4, Figure 19**). Taster strain *FVB* has the largest surface area at 409.58 mm, strain *FVB* has 48% and 41% more surface area for tasting than non-taster strains *129* (211.10 mm) and *Balb/C* (242.24 mm) respectively. Taster strain *C57* has a 26% smaller tasting surface area than *FVB* at 306.37 mm. *C57* was 31% larger than strain *129* and 21% larger than strain *Balb/C.*

Genetic composition in different mouse strains predicts the taster/non-taster properties in mice. However, whether or not genetic background is reflected in the morphology and number of taste buds and papillae is not clear. Due to the extensive use of transgenic mice in developmental and biological studies of the peripheral taste system, it is imperative to have better understanding of possible variations in the peripheral taste system in different background strains. The majority of the transgenic mice using homologous recombination in the past were generated using 129 mouse embryonic stem cells.

This study has described the morphological differences between taster and non-taster mouse strains. We know the ability to taste can influence food ingestion and palatability in humans. It has been shown that taster status has an effect on the preference for dairy products in children, where nontasters tend to like cheddar cheese more than tasters. This might have to do with the ability to detect bitter tastes by the tasters. There are individuals that have a higher affinity for sweets, usually in the non-taster group, due to the tasters perceiving greater sweetness and not liking it (Bartoshuk *et al.*, 1994). This perception could explain some of the weight issues that may be genetically predisposing people.

There is mounting interest towards the genetic involvement of taste stimuli and taste modifiers for humans and other animals. In humans, the interest mainly involves making food and drinks healthier without sacrificing taste, and also making oral medications more acceptable/palatable to patients. As we have seen from the many artificial sweeteners to be introduced to the market, there is a demand for sweet and umami compounds, enhancers of salty, blockers of bitter taste and improving the sensory

Table 4. Receptor field in mm

Measurement	Balb/C	129	FVB	C57
Receptor field	242.24	211.10	409.58	306.37

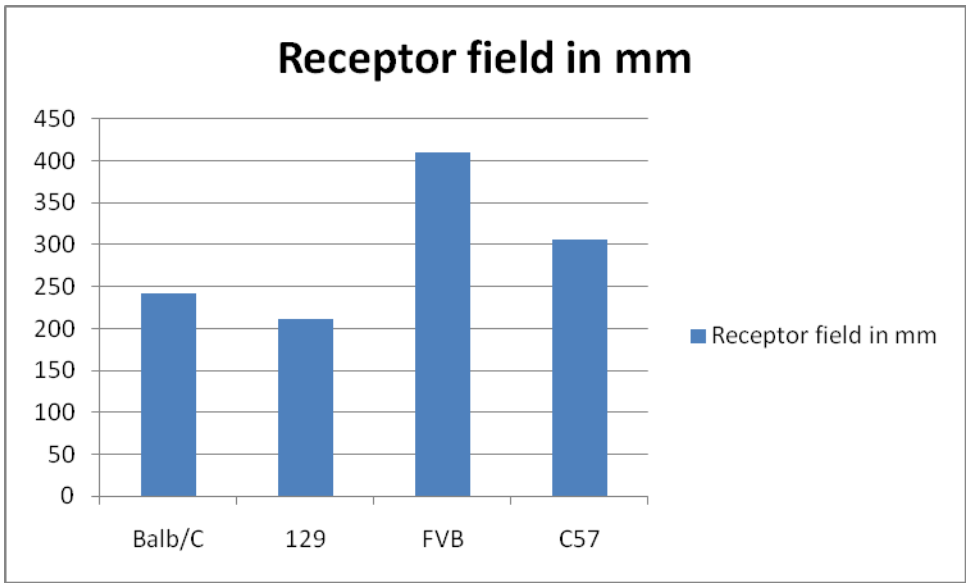

Figure 19. Receptor field in mm

properties of pharmaceuticals. Variation in taste receptors in humans can affect not only what we eat, but how much we eat. The idea that taste choices based on allelic variations in taste receptors could lead to life style choices even lead to risk factors for disease (Bachmanov and Beauchamp, 2007). Just like in two bottle testing for mice, humans that have higher sensitivity to ethanol bitterness may protect against excessive alcohol consumption (Duffy *et al.*, 2004). This study will add to the knowledge related to taste sensation in mice and hopefully will be able to relate to human research.

CHAPTER 7. CONCLUSION

The results of the present study suggest:

1. There is a significant difference between the numbers of fungiform papillae in taster strains *FVB* and *C57* and non-taster strain *Balb/C* compared to a non-taster strain *129.*

2. There is significant difference in the length and area of fungiform papillae in taster strain *FVB* compared to the other taster strain *C57* and non-taster strains *Balb/C* and *129*.

3. Strain *FVB* has a 48% and 41% larger surface receptor field than non-taster strains *129* and *Balb/C* respectively.

4. There is not a significant difference between the taster and non-taster mice strains and the size of taste buds as measured after immunohistochemistry sectioning.

LIST OF REFERENCES

1. Adler, E., *et al.* (2000). "A novel family of mammalian taste receptors." Cell. **100**(6): 693-702.

2. Bachmanov, A.A., *et al*. (2002). "Genetics of sweet taste preferences." Pure Appl Chem. **74**(7): 1135-1140.

3. Bachmanov, A.A. and G.K. Beauchamp. (2007). "Taste receptor genes." Annu Rev Netr. **27**: 389-414.

4. Bachmanov, A.A., *et al*. (2001). "Sweetener preference of C57BL/6ByJ and 129P3/J mice." Chem Senses. **26**(7): 905-913.

5. Bartoshuk, L.M. *et al*. (1994). "PTC/PROP tasting: anatomy, psychophysics, and sex effects." Physiol Behav. **56**(6): 1165-1171.

6. Batsell, W.R. and M.R. Best. (1993). "One bottle too many? Method of testing determines the detection of overshadowing and retention of taste aversions." Anim Learn Behav. **21**: 154-158.

7. Boughter, J.D. and G. Whitney (1997). "Behavioral specificity of the bitter taste gene Soa." Physiol Behav. **63**(1): 101-108.

8. Chandrashekar, J., *et al.* (2000). "T2Rs function as bitter taste receptors." Cell. **100**(6): 703-711.

9. Dalpe, G., *et al.* (1998). "Dystonin is essential for maintain neuronal cytoskeleton organization." Mol Cell Neurosci. **10**: 243-257.

10. Dragoin, W., *et al*. (1971). "A comparison of two methods of measuring conditioned taste aversion." Behav Res Meth Instrum. **3**: 309-310.

11. Duffy, V.B., *et al.* (2004). "Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake." Alcohol Clin Exp Res. **28**: 1629-1637.

12. Elkins, R.L. (1973). "Individual differences in bait shyness: effects of drug dose and measurement technique." Psychol Record. **23**: 349-358.

13. Farbman, A.I. (1965). "Fine Structure of the taste bud." J Ultrastruct Res. **12**: 328-350.

14. Farbman, A.I. and G. Hellekant. (1978). "Quantitative analyses of the fiber population in rat chorda typani nerves and fungiform papillae." Am J Anat. **153**: 509-521.

15. Guagliardo, N.A. and D.L. Hill. (2007). “Fungiform taste bud regeneration in C57/6J mice following chorda-lingual nerve transection.” J Comp Neurol. **504**: 206-216.

16. Grote, F.W. and R.T. Brown. (1971). “Conditioned taste aversions: two-stimulus tests are more sensitive than one-stimulus tests.” Behav Res Methods Instrum. **3**: 311-312.

17. Hall, W.G. and T.E. Bryan (1981). "The ontogeny of feeding in rats: IV. Taste development as measured by intake and behavioral responses to oral infusions of sucrose and quinine." J Comp Physiol Psychol. **95**(2): 240-251.

18. Hoffmamm. A. (1875). “Ueber die verbreitung der geschmacksknospen beim menshech.” Arch Pathol Anat Physiol Klin Med. **62**: 516-530.

19. Hoon, M.A., *et al*. (1999). "Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity." Cell. **96**(4): 541-551.

20. Ichikawa, H., *et al*. (2007). “Dystonin defiency reduces taste buds and fungiform papillae in the anterior part of the tongue.” Brain Research. **1129**: 142-146.

21. Kaufman M.H., (1992). “The atlas of mouse development.” San Diego: Academic Press: 422-423.

22. Kinnamon, J.C., *et al*. (1988). "Ultrastructure of mouse vallate taste buds: III. Patterns of synaptic connectivity." J Comp Neurol. **270**(1): 1-10, 56-57.

23. Kinnamon, J.C., *et al*. (1985). "Ultrastructure of mouse vallate taste buds. I. Taste cells and their associated synapses." J Comp Neurol. **235**(1): 48-60.

24. Klein, S.B., *et al.* (1975). “Acquisition of a conditioned aversion as a function of age and measurement technique.” Physiol Psychol. **3**: 379-384.

25. Leung, C.L., *et al.* (1999). “The intermediate filament protein peripherin is the specific interaction partner of mouse BPAG1-n (dystonin) in neurons.” J Cell Biol. **144**: 435-446.

26. Lewin, G.R. and Y.A. Barde. (1989). “Physiology of the neurotrophins.” Annu Rev Neurosci. **19**: 289-317.

27. Lush, I.E. and G. Holland. (1988). "The genetics of tasting in mice. V. Glycine and cycloheximide." Genet Res. **52**(3): 207-212.

28. Maruyama, Y. *et al.* (2006). "Umami responses in mouse taste cells indicate more than one receptor." J Neurosci. **26**(8): 2227-2234.

29. Matsunami, H., *et al*. (2000). "A family of candidate taste receptors in human and mouse." Nature. **404**(6778): 601-604.

30. Menco, B.P. (1989). "Olfactory and nasal respiratory epithelia, and foliate taste buds visualized with rapid-freeze freeze-substitution and Lowicryl K11M embedding. Ultrastructural and initial cytochemical studies." Scanning Microsc. **3**(1): 257-272.

31. Miller, I.J. and F.E. Reedy. (1990a). "Variations in human taste bud density and taste intensity perception." Physiol Behav.**47**: 1213-1219.

32. Miller, I.J. and F.E. Reedy. (1990b). "Quantification of fungiform papillae and taste pores in living human subjects." Chem Senses. **15**: 281-294.

33. Miller, I.J. and G. Whitney. (1989). "Sucrose octaacetate-taster mice have more vallate taste buds than non-tasters." Neuroscience Letters. **360**: 271-275.

34. Mistretta, C.M. (1989). "Anatomy and neurophysiology of the taste system in aged animals." Ann NY Acad Sci. **561**: 277-290.

35. Mistretta, C.M., *et al*. (1999). "Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs." J Comp Neurol. **409**: 13-24.

36. Mistretta, C.M. and L. Hong-Xiang. (2006). "Development of fungiform papillae: patterned lingual gustatory organs." Arch Histol Cytol. **69**(4): 199-208.

37. Murray, R.G. (1986). "The mammalian taste bud type III cell: a critical analysis." J Ultrastruct Mol Struct Res. **95**(1-3): 175-188.

38. Northcutt, R.G. (2004). "Taste buds: development and evolution." Brain Behav Evol. **64**(3): 198-206.

39. Nosrat, C. A., *et al*. (1997). "Lingual deficits in BDNF and NT3 mutant mice leading to gustatory and somatosensory disturbances, respectively." Development. **124**(7): 1333-1342.

40. Nosrat, I.V., *et al.* (2000). "Lingual BDNF and NT-3 mRNA expression patterns and their relation to innervation in the human tongue: Similarities and differences compared with rodents." J Comp Neurol. **417**: 133-152.

41. Ohmura, S., *et al*. (1989). "Lectin cytochemistry of the dark granules in the type 1 cells of Syrian hamster circumvallate taste buds." Arch Oral Biol. **34**(3): 161-166.

42. Reedy, F.E., *et al*. (1993). "Relationships among papillae, taste pores, and 6-n-propylthiouracil (PROP) suprathreshold taste sensitivity." Chem Senses. **18**: 618-619.

43. Roper, S.D. (2007). "Signal transduction and information processing in mammalian taste buds." Pflugers Arch. **454**(5): 759-776.

44. Royer, S.M. and J.C. Kinnamon. (1988). "Ultrastructure of mouse foliate taste buds: synaptic and nonsynaptic interactions between taste cells and nerve fibers." J Comp Neurol. **270**(1): 11-24, 58-59.

45. Royer, S.M. and J.C. Kinnamon. (1991). "HVEM serial-section analysis of rabbit foliate taste buds: I. Type III cells and their synapses." J Comp Neurol. **306**(1): 49-72.

46. Sclafani, A. (2006). "Sucrose motivation in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice measured by progressive ratio licking." Physiol Behav. **87**(4): 734-744.

47. Smith, D.V. and R.F. Margolskee. (2001). "Making sense of taste." Sci Am. **284**(3): 32-39.

48. Tordoff, M. (2007). "Taste solution preferences of C57BL/6J and 129X1/SvJ mice: Influence of age, sex, and diet." Chem Senses. **32**: 655-671.

49. Tordoff, M. and A. Bachmanov. (2003). "Mouse taste preference tests: Why only two bottles". Chem Senses. **28**: 315-324.

50. Witt, M. (1996). "Carbohydrate histochemistry of vertebrate taste organs." Prog Histochem Cytochem. **30**(4): 1-168.

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