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Gingival Taste Bud Papillae Associated with Retromolar Salivary Gland

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Abstract

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Abstract
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Keywords
“salivary gland”
“Von Ebners Gland”
“gustatory papillae”
“gustducin and PLCbeta2”
“retromolar trigone ”
“Tas1rs and Tas2rs”

Introduction

The gustatory papillae and taste buds form an elaborate chemosensory system that is well suited to detect, localize, and respond to chemicals in the oral cavity. In mammals, the gustatory system is comprised of taste buds found in three different types of papillae assembled on specific parts the tongue (Mistretta 1991). The fungiform papillae are distributed on the anterior two-thirds of the tongue and are innervated by a branch of the facial nerve (N.IX), known as the chorda tympani (Whitehead, Frank *et al*. 1987). The CV, the largest of the lingual papillae, and the foliate, located on the posterolateral tongue bilaterally, are localized on the posterior third of the tongue. The CV and foliate papillae contain the majority of the lingual taste buds and are found lining both sides of the lateral trench walls (Tomiyama 1977, Bartoshuk 1991, Jung, Akita *et al*. 2004, Finger 2005, Sohn, Gwon *et al*. 2011). Gustatory information from the CV and foliate papillae is transmitted by the glossopharyngeal nerve (N. IX) to the nucleus of the solitary tract in the brain stem (Witt and Reutter 1997). In rats, about 80 percent of the taste buds are found on the tongue. Among the lingual taste buds, approximately 35 percent are in the CV, 45 percent are in the bilateral foliate, and 20 percent are in the fungiform papillae (Miller 1976, Miller and Smith 1984).

In rodents, each taste bud is composed of 50-80 elongate, mature taste cells divisible into three morphological types: Type I, Type II, and Type III (Finger 2005, Chaudhari and Roper 2010), with each type displaying characteristic molecular and functional features. Type I cells make up about half of the cells in a taste bud. They have narrow irregular shaped nuclei, express enzymes and transporters necessary for elimination of ions and neurotransmitters, and have glia like functions. Type II cells make up one third of the cells in a taste bud. Type II cells are larger than type I cells, have spherical nuclei, and function as chemosensory receptors for sugars, amino acids and/or bitter stimuli. Type II cells express elements of the canonical taste signaling cascade including G-protein couple receptors (GPCR), gustducin (α, β and γ), phospholipase C-beta 2 (PLCβ2), inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), and transient receptor potential cation channel subfamily M member 5 (TrpM5) (McLaughlin *et al*., 1992, Wong *et al*., 1996, Huang *et al*., 1999, Chandrashekar *et al*., 2000, Mueller *et al*., 2005). Lastly, Type III cells are the least prevalent cell type in taste buds, and do not express GPCRs. Type III cells detect sour taste and have dense-cored vesicle prominent synaptic contacts, suggestive of their role in taste signal transmission to the nervous system (Finger 2005, Roper and Chaudhari 2017).

The CV and foliate papillae are associated with excretory ducts of an underlying serous secreting minor salivary gland, von Ebner gland (Gurkan and Bradley 1987). Von Ebner salivary glands possess ducts exiting into the base of the troughs between the foliate and CV papillae (Hand and Frank 2015) and secrete serous contents into the base of the moats around the CV and foliate papillae. This anatomic arrangement serves to enhance the mechanism of taste perception, as well as aid in digestion of food particles. The secretion is thought to serve many functions, including dissolving food particles, facilitating perception of taste molecules through digestive enzymes, and beginning the process of lipid hydrolysis (Tester RF 2006, Hand and Frank 2015).

It has been previously described in the literature that oral mucosa structures other than the CV and foliate papillae contain taste buds associated with salivary glands. Iida *et al*. first described the presence of "taste bud papillae" around the orifice of a "molar gland" of the retromolar mucosa in mice, rats, and hamsters using light and scanning electron microscopy (Iida, Yoshioka *et al*. 1983).

Miller & Smith also described the presence of taste bud papillae on the "buccal wall lateral to the foliate papillae"(Miller and Smith 1984). This retromolar mucosa was later physiologically characterized by Travers and colleagues for its orosensory response capabilities. They observed activation of gustatory neurons rostral division of the nucleus of the solitary tract (rNTS) when stimulating the anterior tongue, foliate papillae, nasoincisor duct, retromolar region, and soft palate. Although they found that stimulation of the retromolar mucosa was the least effective for activating rNTS G neurons, this was the first report of neuronal response from the retromolar taste mucosa (Travers and Norgren 1995). Although these previous studies described both the anatomical location of the retromolar taste buds in association with a salivary gland (Iida, Yoshioka *et al*. 1983, Miller and Smith 1984) and its gustatory response to chemical stimulation (Travers and Norgren 1995), the following questions remain unanswered. First, it is unknown if taste cells are present, what types of taste cells, and which taste receptors and downstream taste signaling elements may be present. Second, the mandibular gland's association with the retromolar taste buds and the composition of its secretion remains undetermined. Finally, the important question about the function of this retromolar sensory/secretory structure remains unknown. Here, we will answer some of these questions and discuss the possible function of the retromolar taste buds and associated salivary gland. We will further characterize the retromolar taste bud cells for the expression of taste receptors and elements of the gustatory signaling cascade. Using transgenic mice expressing green fluorescent protein (GFP) under taste-element specific promoters we visualized and characterized anatomically the retromolar taste buds and the salivary gland and secretory ducts. Moreover, we used immunohistochemistry to characterize the different cell types and signal transduction molecules within the taste buds, and finally semi-quantitative RT-PCR to characterize the taste receptor expression profile. Future studies involving physiology, biochemistry and psychophysics will be necessary to determine the function of the retromolar taste buds, the composition of the retromolar mandibular salivary glands, and the characterization of the retromolar mucosal region for the presence of taste buds and responses to chemical stimulation in humans.

Materials and Methods

Animals

All experimental procedures were approved by the Animal Care and Committees of the Monell Chemical Senses Center. Adult transgenic mice used for this study were the TRPM5-GFP, T1R3-GFP (Clapp, Medler *et al*. 2006), and GAD1-GFP (Chattopadhyaya, Di Cristo *et al*. 2004) mice, all in C57/BL6 background. Expression of GFP in Type II taste cells was validated by Clapp *et al*. (Clapp, Medler *et al*. 2006).

Immunohistochemistry

Mice were perfusion-fixed in 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB). Tissues were post-fixed (4°C, 60 min) and cryoprotected in 20% sucrose-phosphate buffer (4°C, overnight). Cryostat sections (14-16μm) were collected and dried onto Superfrost Plus slides (Fisher Scientific). Slides were rinsed in 0.1M phosphate-buffered saline (PBS:150mM sodium chloride, 25mM sodium phosphate dibasic anhydrous, 75mM sodium phosphate monobasic monohydrate; pH 7.2) and nonspecific binding was blocked for 1h in blocking solution (2% normal

goat or donkey serum, 1% bovine serum albumin, 0.3% Triton in PBS). Next, the slides were incubated overnight with primary antibodies in blocking solution (Table 1). Following incubation with the primary antibodies, the tissue samples were rinsed with PBS (3x20min) and incubated for 2h with fluorescent secondary antibodies (Table 2). The slides then were washed 2×10min in 0.1M PBS and one time for 10min in 0.05M PB before cover-slipping slides with Fluromount G (Southern Biotechnology Associates). All images were collected with a Leica TCS SP2 Laser Scanning Confocal Microscope (Leica Microsystem) using UV, Ar, GeNe, and HeNe lasers, as well as appropriate excitation spectra. Scanware software (Leica Microsystems) was used to acquire z-series stacks captured at a step size of 0.25–0.35μm. For each image, channels were merged to produce the composite image using the native acquisition software for each device. Brightness, contrast and gamma were adjusted in Adobe Photoshop (Adobe Systems) to approximate the appearance of the original histological samples.

Retromolar Tissue Biopsy and RT-PCR

Invitrogen RNAseZap (Waltham, MA) was used on all instruments and surfaces prior to biopsy removal to avoid RNAse contamination. Tissue containing the retromolar taste buds and CV papillae were isolated from C57BL/6 wild type mice after being anesthetized using isoflurane and sacrificed by cervical dislocation. CV papillae and retromolar epithelium were removed using a scalpel and smooth grip forceps under microscopic visualization. The specimens were immediately placed in fresh lysis buffer containing 1% β-mercaptoethanol on ice. Sample lysis, homogenization, and RNA purification were completed according to the manufacturer's instructions using the Invitrogen PureLink RNA Mini Kit (Waltham, MA). RNA from mouse CV papillae and retromolar tissue was extracted according to the manufacturer's instructions using the Invitrogen PureLink RNA Mini Kit (Waltham, MA). To validate the integrity of the extracted RNA, the concentration and quality of the RNA samples was analyzed in the Agilent 2200 Bioanalyzer (Santa Clara, CA) and an RT-PCR for β-actin was performed for both CV papillae and retromolar samples. DNase I treatment and reverse transcription (cDNA synthesis) were performed using Invitrogen SuperScript IV Vilo Master Mix with ezDNASE (Waltham, MA). Reactions were set up in which the reverse transcriptase enzyme was omitted as a control to detect for possible genomic DNA contamination. To validate the PCR, we included cDNA from mouse CV papillae, since all 35 TAS2R receptors and their downstream signally effectors are known to be present in the CV papillae (Lossow, Hubner *et al*. 2016, Yoshida, Takai *et al*. 2018). The following PCR conditions were used: 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds, and concluding with a 72°C final extension for 7 minutes. Amplified sequences were visualized by gel electrophoresis in 2% agarose gels. RT-PCR primer sequences and annealing temperatures are located in Table 3.

Results

Consistent with previous reports (Iida, Yoshioka *et al*. 1983, Miller and Smith 1984), our data confirmed the presence of taste buds in the retromolar mucosa surrounding two orifices of glandular salivary ducts, lateral to the foliate papillae on the tongue. Examination of the tongue and surrounding buccal tissue from our whole mount tissue preparation from transgenic mice expressing GFP under the TrpM5 promoter (Clapp, Medler *et al*. 2006) show the presence of GFP-

positive taste buds (Fig. 1B, C) clustered in groups of 1-3 positioned around two orifices underneath ducts from a minor secretory gland. The orifice of the gland is comprised of epithelium arranged in concentric circles. These nonlingual taste bud clusters and glandular orifices were seen bilaterally at the rear of the mandible, distal to the last molar and anterior to the ascending ramus (Figure 1).

The CV and the foliate papillae contain highly organized taste buds situated within troughs of the papillae. Each taste bud possesses a taste pore, with the apical region oriented toward the trough (Choi, Lee *et al*. 2015, Roper and Chaudhari 2017). The retromolar taste buds, by contrast, appear to be randomly distributed in the epithelium surrounding the orifice of the retromolar salivary gland duct (Figure 1, 2), with apical regions oriented toward the oral cavity perpendicular to the epithelium (Fig. 1B). Each side of the mandible contains retromolar taste "papillae" with an average number of taste buds of 18.25+/-2.9 (sd).

Since gustatory papillae and minor salivary glands form a close association with one another to facilitate the perception of taste (Hand 1970, Gurkan and Bradley 1987, Sbarbati, Crescimanno *et al*. 1999, Hand and Frank 2015), we explored whether retromolar taste bud papillae are also associated with salivary glands. To characterize the morphology and spatial 3D organization of the retromolar taste bud papillae and associated salivary gland, we performed serial sectioning of oral cavity tissue from TrpM5-GFP mice that included the posterior tongue and the retromolar portion of the mandibular gingival epithelium. Tissue sections were cut on the coronal plane and collected starting distal to the last mandibular molar and extending through the posterior part of the tongue (Fig. 2). Taste buds, represented by endogenously expressed GFP, can be seen situated in the superficial gingival epithelium anterior to the ductal structure (Figure 2, A-I, red arrows) and surrounding the ductal orifice (Figure 2, D-I). Posterior to the orifice, the secretory duct connects to a glandular structure that extends in the retromolar trigone (Figure 2, D-L). The acini of the gland contain serous/mucus cells located on the basal membrane of the gland. The terminal secretory sections pass into the narrow glandular tubules, which form the excretory duct. The gland and the excretory duct that opens on the surface epithelium between the taste buds of the retromolar papillae, are placed between muscle fiber bundles under the mucous membrane of the mandibular gingiva (Fig. 2 D-L). This glandular structure morphologically resembles von Ebners' salivary glands associated with the CV and foliate papillae (Hand 1970). Sections also show that the retromolar taste buds are located more anteriorly on the gingiva (Figure 2 A-I) than the foliate papillae on the tongue (Figure 2 K-L), but the two chemosensory structures are both in close proximity to food during mastication.

To characterize the chemosensory profile of the retromolar taste buds, we used immunohistochemistry on tissue sections obtained from TrpM5- , T1R3-, or GAD1-GFP mice. Tissue sections were stained with primary antibodies specific for the taste chemosensory markers PLC□2 or GNAT3 (gustducin). Our experiments reveal GFP-positive retromolar taste bud cells in both the TrpM5- and T1R3-GFP mice immunoreactive for PLCβ2 and Gnat3, with morphological features of elongated cells with large round nuclei consistent with Type II taste cells (Figure 3A-C). The staining patterns observed are consistent with staining patterns that have been previously described in Type II taste cells of the CV and foliate papillae (Tizzano, Dvoryanchikov *et al*. 2008). In addition, tissue sections from GAD1-GFP mice (Chattopadhyaya, Di Cristo *et al*. 2004) stained with GNAT3 antibody showed the presence of Type III taste cells with endogenously expressed

GFP not colocalizing with GNAT3 in the retromolar taste buds (Figure 3E). Furthermore, we characterized the retromolar taste buds for their association to sensory innervation with a primary antibody specific against calcitonin gene-related peptide (CGRP) from TrpM5-GFP mouse retromolar tissue. There is a clear association between retromolar taste buds and peptidergic polymodal nocioceptive sensory innervations. Immunoreactive fibers for CGRP were observed in the retromolar tissue wrapping around TrpM5-GFP positive taste buds (Figure 3D-D'), consistent with what was observed in previous studies where CGRP immunoreactive nerve fibers were densely distributed in the connective tissue core of the CV and foliate papillae (Montavon and Lindstrand 1991, Kusakabe, Matsuda *et al*. 1998).

Finally, based on previous studies showing that retromolar taste buds are able to detect a mixture of sucrose, quinine hydrochloride, NaCl and HCl (Travers and Norgren 1995), we explored whether these taste buds express sweet and bitter taste receptors and the canonical taste signaling cascade genes *gnat3*, *plcb2* and *trpm5*. Using semi-quantitative RT-PCR from dissected retromolar tissue from wildtype mice, we determined that retromolar taste buds express all 3 *Tas1Rs* as well as the taste transduction signaling genes *gnat3, plcb2* and *trpm5* (Figure 4), which confirm our immunohistochemistry results (Figure 3). Unlike the CV papillae, not all the *tas2r* genes are expressed in the retromolar taste buds. Of the 35 mouse Tas2Rs (Lossow, Hubner *et al*. 2016, Yoshida, Takai *et al*. 2018)), only 26 *tas2r* genes are expressed in the retromolar taste buds (Figure 4).

Discussion

We have closely characterized a previously described chemosensory structure in the rodent mandible, using an approach that utilizes transgenic mouse models, biochemistry, and molecular biology techniques. We confirmed the presence of retromolar taste buds that were previously described in the 80s and 90s using light and electron microscopy (Iida, Yoshioka *et al*. 1983, Miller and Smith 1984). The number of taste buds we found, 18.25+/-2.9 (sd) on each side of the retromolar mandible, is comparable with the approximated 15 mandibular taste buds reported by Iida and colleagues. The reported total number of 42 +/-6 retromolar taste buds in mice (Iida, Yoshioka *et al*. 1983) suggests that taste buds may be present in the maxillary retromolar region as well. The retromolar taste buds function as an accessory chemosensory organ in the oral cavity as shown either by previous electrophysiology studies (Travers and Norgren 1995) and by our immunohistochemistry data obtained from T1R3-, TrpM5-, and Gad1-GFP mice. Our results show that retromolar taste buds contain Type II and III taste cells, suggesting that they are indeed chemosensory cells. While the overall cellular morphology of the retromolar chemosensory taste buds is similar to the CV and foliate papillae taste buds (Hand 1970, Hand and Frank 2015), the retromolar taste buds are not contained within troughs of the papillae. Rather the buds are positioned on the surface of the retromolar epithelium, with the apical surface and taste pores oriented toward the oral cavity. This orientation bears resemblance to the taste buds found on the palate (Miller 1977), pharynx, and larynx (Travers and Nicklas 1990), with taste pores oriented toward the oral cavity perpendicularly to the epithelium.

While the Von Ebner's glands associated with CV and foliate papillae secrete serous fluid containing digestive enzymes into the papillae troughs (Hand 1970, Hand and Frank 2015), the composition of the retromolar gland secretions warrant further investigation. Since the retromolar salivary gland ducts open directly onto the surface of the epithelium posterior to the third molars, the contents of the secretions may serve to lubricate the retromolar area as well as augment the preliminary digestion of lipids and proteins. Future experiments involving the extraction of contents from the retromolar salivary gland is necessary to determine its physiological function and significance.

Travers and Norgen demonstrated that the rat retromolar region is responsive to gustatory stimulation. When a cocktail comprised of sucrose, sodium chloride, hydrogen chloride, and quinine hydrochloride was applied to the retromolar region, activation of the gustatory neurons of the nucleus of the solitary tract was observed (Travers and Norgren 1995). To support this data, we investigated which taste receptors are expressed in the retromolar region. Our RT-PCR analysis revealed that all 3 tas1r and 26 of the 35 tas2r genes and their downstream signaling mediators (gnat3, plcb2, and trpm5) are expressed in the retromolar taste bud tissue. T2R receptors are believed to serve as a sensory warning against the ingestion of toxic food compounds, including bitter and bacterial substances (Behrens, Foerster *et al*. 2007), while T1Rs are used for food intake and energy balance (Jiang, Cui *et al*. 2005). The expression of sweet, umami and bitter taste receptors on retromolar taste buds may augment their function in other regions of the oral cavity. However, the full complement of tas2r genes are not expressed in the retromolar taste buds, so they may serve a more specialized function than the T2Rs of the CV, foliate papillae, and other oral taste buds.

Since the retromolar taste buds are located on the surface of the epithelium adjacent to the last molar, they may have greater exposure to food particles than the buds on the CV and foliate papillae. Interestingly, the distribution of oral taste buds in the posterior portion of the oral cavity resembles a V-like shape: proximal-laterally the retromolar chemosensory structure, followed by the foliate, and converging more distal-centrally with the CV papillae. This distribution would allow for more efficient detection of food during mastication in the retromolar area. As food is masticated by the molars, it is pushed posteriorly into the retromolar trigone. The retromolar taste buds and associated gland may then augment the function of the gustatory papillae and Von Ebner secretory glands in facilitating taste perception and digestion, as well as evaluating the chemical composition of potential toxins before swallowing a bolus of food.

Interestingly, taste changes (dysgeusia) and dry mouth post third molar extraction in humans have been reported in the literature (Shafer, Frank *et al*. 1999, Akal, Kucukyavuz *et al*. 2004, Ridaura-Ruiz, Figueiredo *et al*. 2012). During third molar extraction, the retromolar tissue is often disturbed to efficiently remove the tooth. It is possible that damage to the retromolar taste bud structure and innervation or the associated gland could occur during the extraction procedure. Since taste abnormalities can lead to decrease in quality of life, weight loss, malnutrition, and certain diseases and conditions (Bromley 2000), further studies are needed to determine if this structure is present in humans and if it plays a role in gustatory perception. Future studies involving physiology, biochemistry, and psychophysics will be necessary to determine the function of the retromolar taste buds, the composition of the retromolar mandibular salivary glands, and the characterization

of the retromolar mucosal region for the presence of taste buds and responses to chemical stimulation in the mouse model and humans.

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Figure Legends

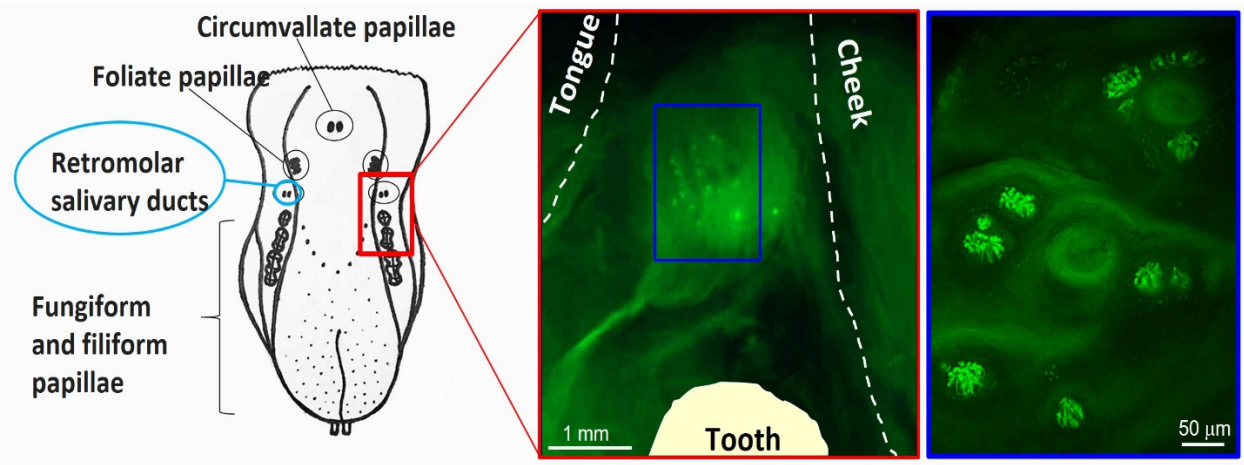

Figure 1. (A) Semi-schematic drawing of mouse tongue showing location of retromolar taste buds and salivary ducts. (B) Whole mount of oral mucosa showing retromolar taste buds and salivary ducts (red inset). (C) Magnified retromolar taste buds and salivary ducts.

Table 1. Primary antisera and antibodies

Antisera against	Marker for	Company, Catalog No., Lot No.	Host; dilution
GNAT3 (α-gustducin)	G-protein subunit in Type II taste cells	Santa Cruz Biotechnology, Cat #sc-395, Lot #J0615	Rabbit; 1:1000
PLCβ2	Transduction component in Type II taste cells	Santa Cruz Biotechnology, Cat #sc-206, Lot #L0715	Rabbit; 1:500
CGRP	Peptidergic nocioceptor	Sigma-Aldrich; Ref # C8198 Lot #066M4836V	Rabbit; 1:5000

Table 1. List of the primary antibodies used in the immunohistochemistry experiments.

Table 2. Secondary antisera

Primary antibody	Secondary antibody	Antisera against
GNAT3 (α-gustducin)	Alexa 568	Donkey anti-rabbit
PLCβ2	Alexa568	Donkey anti-rabbit
CGRP	Alexa 568	Donkey anti-rabbit

Table 2. Secondary antisera.

Name	Sequence (5'-3')	#BP RNA	Name	Sequence (5'-3')	#BP RNA	Name	Sequence (5'-3')	#BP RNA
Gnat3_131F	GAGAGCAAGGAATCAGCCAG	121	**Tas2r114_7F**	AGCACAATGGAAGGTGTCCT	615	**Tas2r129_671F**	TTGCAGATGCCCACATCAGA	147
Gnat3_252R	GTGCTTTTCCCAGATTCACC		**Tas2r114_622R**	GCCTGCGATGTCTCCAAAGT		**Tas2r129_818R**	GCTGCAACAATCTCGCAGAA	
Trpm5_952F	TTCCCCAGCGAGTGTTTCTC	269	**Tas2r115_692F**	AGACTGTGGTTGCCTTCCTC	230	**Tas2r130_37F**	GCTGTTGGTGAGGCCTTAGT	509
Trpm5_1221R	CCATTCCACGTCCCCATTGA		**Tas2r115_922R**	AGGTTTTCTCACGCTTGCAC		**Tas2r130_546R**	GACAGAGGCATGTCCAGCTT	
Plcb2_1954F	CCTGGAGGTGACAGCTTATGA	124	**Tas2r116_569F**	TTGCTGTGTCACTGGTCACT	115	**Tas2r131_314F**	CCCACATTTCCCATCCCCTT	304
Plcb2_2078R	GCTCCGTGAAGGAAGAGACA		**Tas2r116_684R**	TCTGATGTGGGCCTTAGTGC		**Tas2r131_618R**	GTCAAGGCTTCGGGAGTGTT	
Tas2r102_26F	AGGCGACGCTGTTATATGCC	328	**Tas2r117_92F**	ATGGGTTCATGGTCCTGGTC	468	**Tas2r134_457F**	ATGGCGGCCTGTGAAAACTA	206
Tas2r102_354R	AAGCCAGAGGCTGAAGTGAC		**Tas2r117_560R**	AACACCTGCCTGTGACACTT		**Tas2r134_663R**	GTGAGCCTGGGTGCTGTAAT	
Tas2r103_565F	ACCCCATTCGCTGTGTCTTT	312	**Tas2r118_127F**	TCACCGGTGGAGACGATTCT	229	**Tas2r135_543F**	GAGTGGCCATCAACCTTGGA	287
Tas2r103_877R	AGGCTTGCCTCAGCTTACTG		**Tas2r118_356R**	CTCAGCCAGAGGAAGATGGG		**Tas2r135_830R**	GCAGAACTGAGTACCAGCGT	
Tas2r104_434F	TTCCGCTAGCTGTGAAGGTC	447	**Tas2r119_276F**	TCTGGTTTGCCACATGGCTT	382	**Tas2r136_712F**	CCCAGTGCTTCAACCCACAT	251
Tas2r104_881R	AGTGCCCTCATAGTGGCTTG		**Tas2r119_658R**	GGCATGTCTGCTAGGTTCCC		**Tas2r136_963R**	CCAGAACCTTGCTCTCACCT	
Tas2r105_294F	GTTTGCCACCAGCCTAAGCA	212	**Tas2r120_282F**	CACTTGGCTGGGGACCATAC	387	**Tas2r137_19F**	ACAAGCAAGGATCAGGGTGG	638
Tas2r105_506R	TCCCAGTACATCTCCGAGGTC		**Tas2r120_669R**	GTGGACCATGGTGCTCTGAT		**Tas2r137_657R**	CAGAAGGTAGGCAACCAGGG	
Tas2r106_2F	TGCTGACTGTAGCAGAAGGA	132	**Tas2r121_658F**	CGAGACCCCAGCACTAAAGC	230	**Tas2r138_618F**	AGCTTTCCTGGTTTCCTCGG	365
Tas2r106_134R	AAGCCAGCTGTGGAGAACTT		**Tas2r121_888R**	CATCACCCAAAGACTGGCTTG		**Tas2r138_983R**	GGAGGAACCTTGTGGACTGG	
Tas2r107_119F	GCTCGGAGTTTTAGGGGACA	754	**Tas2r122_181F**	CAACAATTGCTGGTGCCTCT	590	**Tas2r139_3F**	GGCTCAACCCAGCAACTACT	429
Tas2r107_873R	AGAGGCATGTGGCTGTCAAA		**Tas2r122_771R**	GGAGCTTGCCACAATAAGCA		**Tas2r139_432R**	CCACAGAAGCCAGGGCATTA	
Tas2r108_112F	AGTCGCAGAATTGCCTCTCC	576	**Tas2r123_102F**	AGTGAACATCATGGACTGGGT	147	**Tas2r140_665F**	CCAGCACCACAGCCCATATT	182
Tas2r108_688R	GCCTCATAGCACCCATGTGA		**Tas2r123_249R**	TCTCCTAGGCAAATGTGGGC		**Tas2r140_847R**	TTAGGACACAAGAGTGGCCC	
Tas2r109_587F	CTGTCCCCGTTGTTTTGTCC	328	**Tas2r124_393F**	GCTTCTGGGAAGCTTGGTGT	288	**Tas2r143_99F**	AGAGTGGATGAGGAACCGGA	584
Tas2r109_915R	CAACACAGAGAGAGAGGCGT		**Tas2r124_681R**	ATTTCTGTGGGCCGTAGCAC		**Tas2r143_683R**	GCCATGGTATGTGCCTGAGT	
Tas2r110_700F	CAGGTCAATGCCAAACCACC	269	**Tas2r125_666F**	CACCACCACAGCTGCACATA	269	**Tas2r144_651F**	CTCACTCAAGAGGCACACCC	106
Tas2r110_969R	GCACCTCAGACAATGCAACA		**Tas2r125_935R**	CAGGGAACCAACATCCGTACA		**Tas2r144_757R**	TGAGAGAGTGGCTGGTCGAT	
Tas2r113_632F	ATATGCAGCACACCGCCAAA	179	**Tas2r126_140F**	TCCTCTTCAGTTTGGGCACC	284	**Actb_251F (β-actin)**	GGTCAGAAGGACTCCTATGTGG	102
Tas2r113_811R	CCAGAGCCCAGACAAACAAA		**Tas2r126_424R**	CGGACACCAAGATAGAGCCC		**Actb_353R (β-actin)**	TGTCGTCCCAGTTGGTAACA	

Table 3. RT-PCR primers for Tas2R bitter taste receptors, downstream signaling effectors (GNAT3, PLCB2, Trpm5), and control (Beta actin).

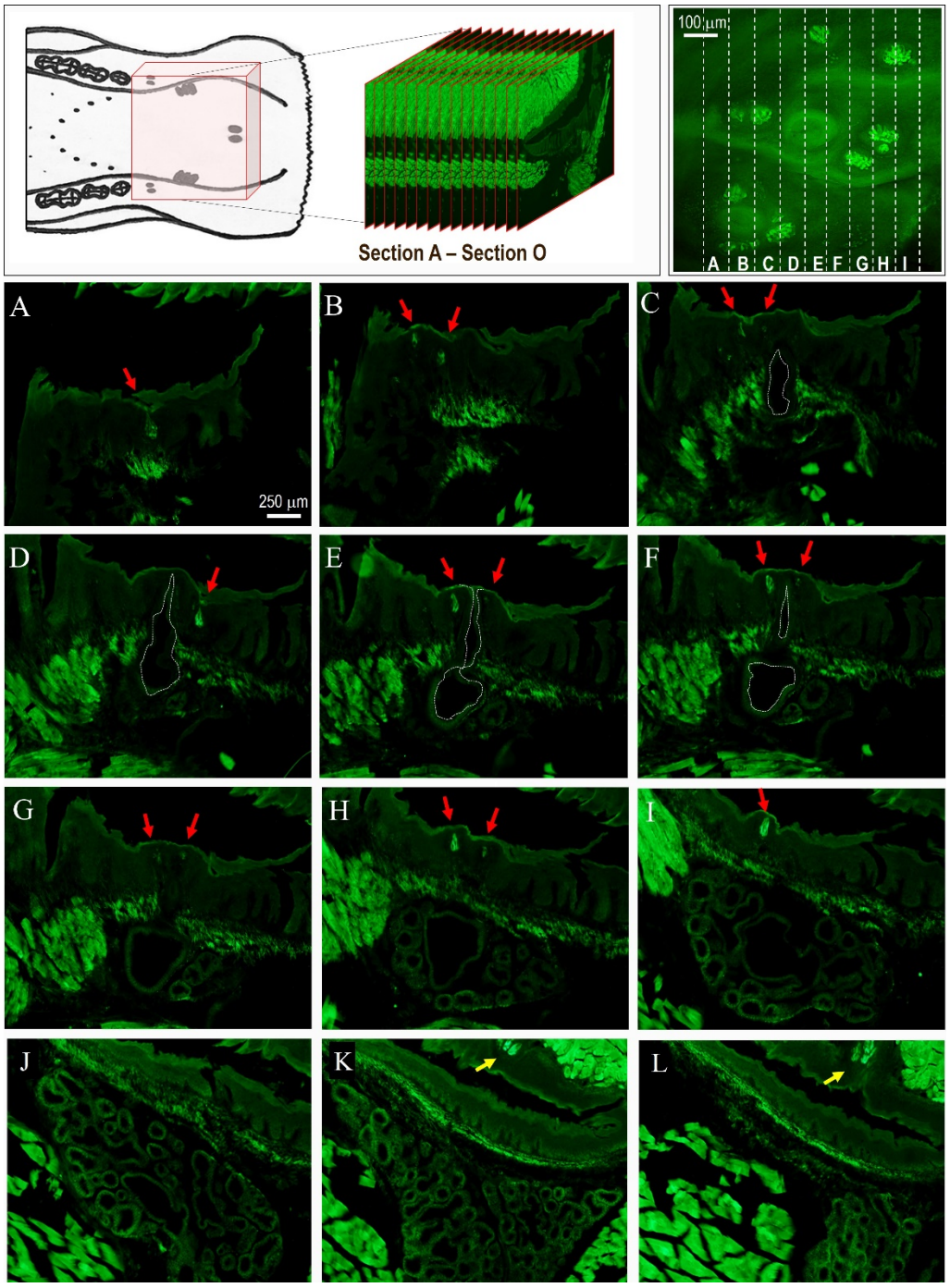

Figure 2. Coronal sections of tongue and surrounding buccal tissue starting from the distal molar and extending through the circumvallate papillae. Coronal sections of tongue and surrounding buccal tissue show taste buds in the gingival epithelium (A-I) on either side of a ductal orifice (E-F). The orifice of the duct connects to a glandular structure below the epithelium (D-F). This glandular structure increases in size and extends posteriorly in the retromolar trigone (J-L, representative sections). The foliate papillae can be appreciated adjacent to the retromolar glandular tissue (K-L). Red arrows represent taste buds and yellow arrow indicate the foliate papilla.

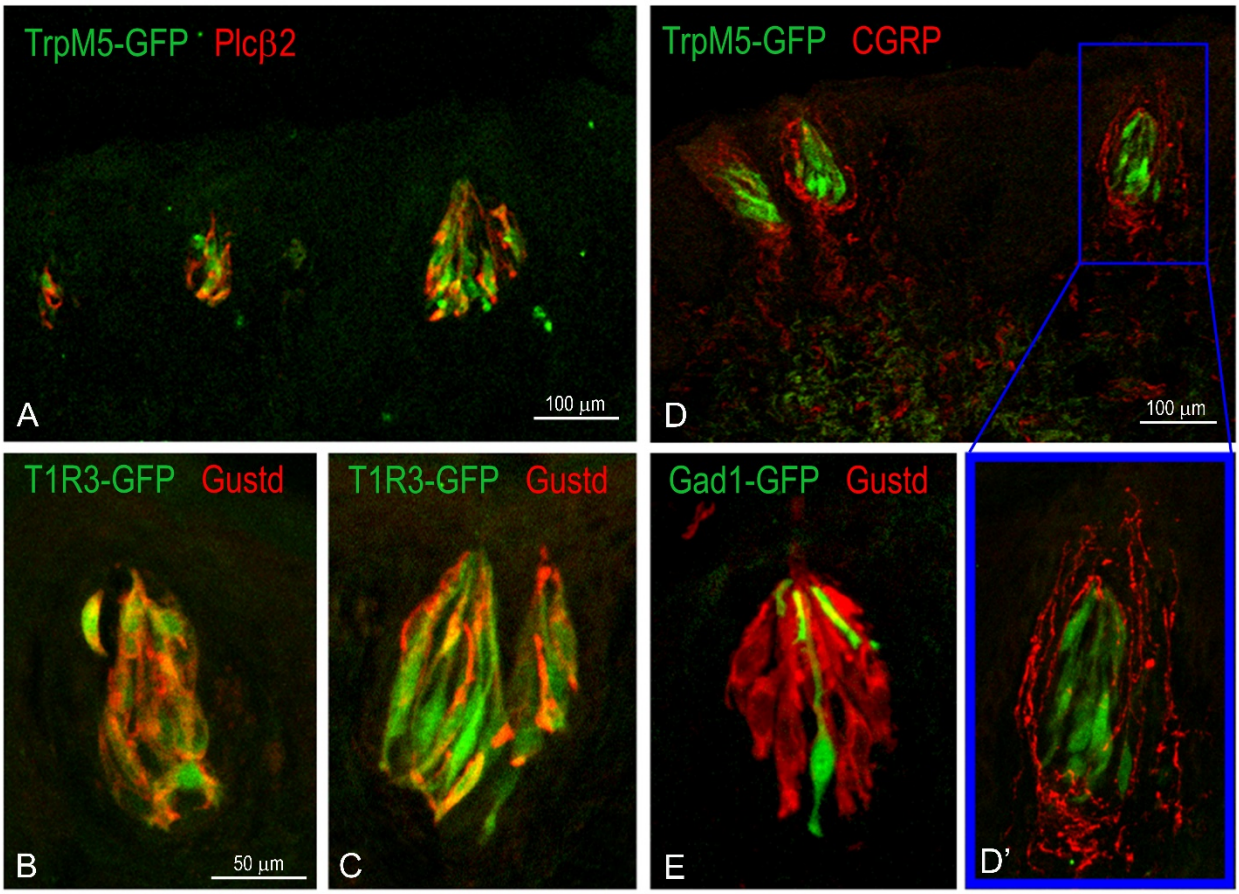

Figure 3. Immunohistochemistry of retromolar taste buds. Tissue was obtained from TrpM5-, Gad1- and T1R3-GFP mice. Plcb2-immunoreactivity (in red) is visible in TrpM5-GFP positive TB cells (A). Gustducin (Gustd, in red) stains most of the T1R3-GFP positive cells (B-C) but does not colocalize with Gad1-GFP positive cells (E). Retromolar TrpM5-GFP positive taste buds show perigemmal CGRP-immunoreactive (in red) peptidergic innervations (D) (magnified in the blue D' inset).

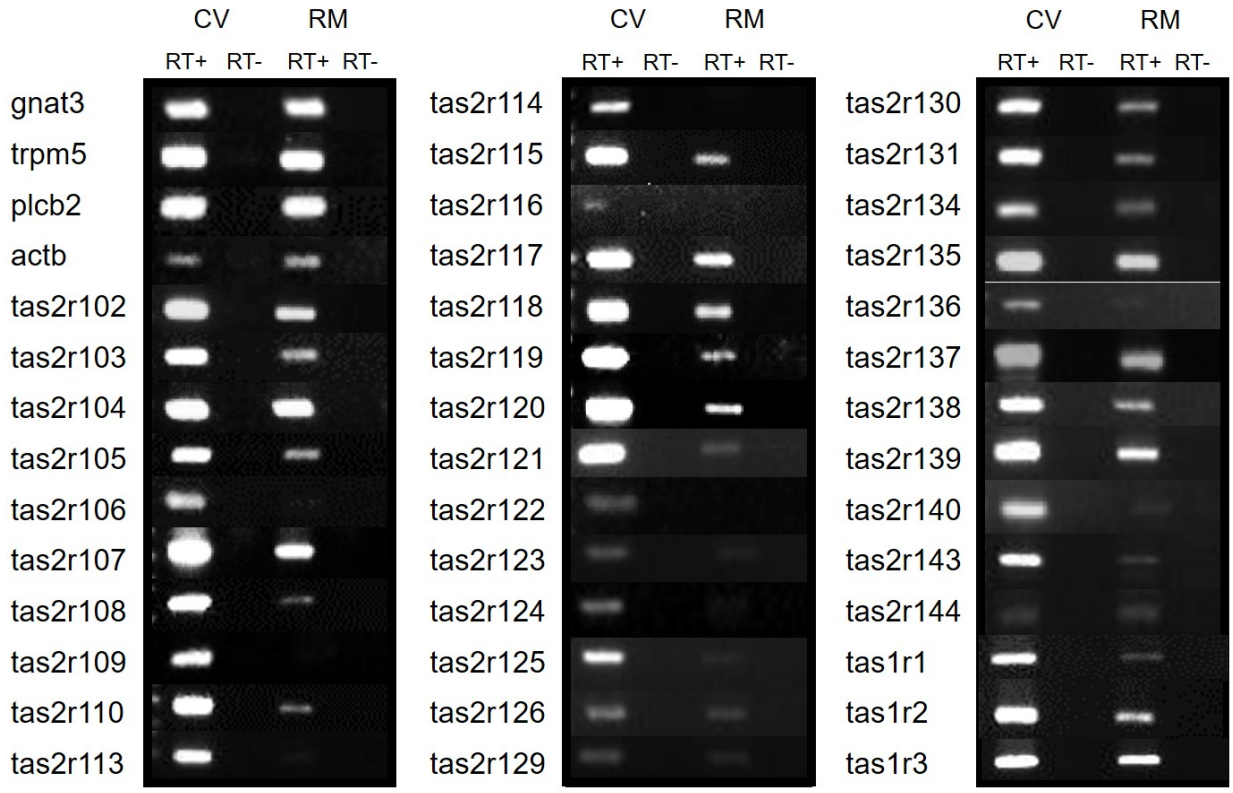

Figure 4. RT-PCR for taste receptor and downstream signally effector genes from mouse circumvallate papillae (CV) and retromolar taste buds tissue (RM). For negative controls, reverse transcriptase was omitted from the reactions. RT+ and RT- represent samples of RNA that were transcribed in the presence or absence respectively or reverse transcription enzyme, respectively. β-actin (actb) was the housekeeping gene of choice.

References

Akal, U. K., Z. Kucukyavuz, R. Nalcaci and T. Yilmaz (2004). "Evaluation of gustatory function after third molar removal." Int J Oral Maxillofac Surg **33**(6): 564-568.

Bartoshuk, L. (1991). "Losses of taste in regions of the tongue's surface." Appetite **17**(1): 69.

Behrens, M., S. Foerster, F. Staehler, J. D. Raguse and W. Meyerhof (2007). "Gustatory expression pattern of the human TAS2R bitter receptor gene family reveals a heterogenous population of bitter responsive taste receptor cells." J Neurosci **27**(46): 12630-12640.

Bromley, S. M. (2000). "Smell and taste disorders: a primary care approach." Am Fam Physician **61**(2): 427-436, 438.

Chattopadhyaya, B., G. Di Cristo, H. Higashiyama, G. W. Knott, S. J. Kuhlman, E. Welker and Z. J. Huang (2004). "Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period." J Neurosci **24**(43): 9598-9611.

Chaudhari, N. and S. D. Roper (2010). "The cell biology of taste." J Cell Biol **190**(3): 285-296.

Choi, M., W. M. Lee and S. H. Yun (2015). "Intravital microscopic interrogation of peripheral taste sensation." Sci Rep **5**: 8661.

Clapp, T. R., K. F. Medler, S. Damak, R. F. Margolskee and S. C. Kinnamon (2006). "Mouse taste cells with G protein-coupled taste receptors lack voltage-gated calcium channels and SNAP-25." BMC Biol **4**: 7.

Finger, T. E. (2005). "Cell types and lineages in taste buds." Chem Senses **30 Suppl 1**: i54-55.

Gurkan, S. and R. M. Bradley (1987). "Autonomic control of von Ebner's lingual salivary glands and implications for taste sensation." Brain Res **419**(1-2): 287-293.

Hand, A. R. (1970). "The fine structure of von Ebner's gland of the rat." J Cell Biol **44**(2): 340-353.

Hand, A. R. and M. E. Frank (2015). Fundamentals of Oral Histology and Physiology, John Wiley & Sons.

Iida, M., I. Yoshioka and H. Muto (1983). "Taste bud papillae on the retromolar mucosa of the rat, mouse and golden hamster." Acta Anat (Basel) **117**(4): 374-381.

Jiang, P., M. Cui, B. Zhao, L. A. Snyder, L. M. Benard, R. Osman, M. Max and R. F. Margolskee (2005). "Identification of the cyclamate interaction site within the transmembrane domain of the human sweet taste receptor subunit T1R3." J Biol Chem **280**(40): 34296-34305.

Jung, H. S., K. Akita and J. Y. Kim (2004). "Spacing patterns on tongue surface-gustatory papilla." Int J Dev Biol **48**(2-3): 157-161.

Klasser, G. D., R. Utsman and J. B. Epstein (2008). "Taste change associated with a dental procedure: case report and review of the literature." J Can Dent Assoc **74**(5): 455-461.

Kusakabe, T., H. Matsuda, Y. Gono, M. Furukawa, H. Hiruma, T. Kawakami, M. Tsukuda and T. Takenaka (1998). "Immunohistochemical localisation of regulatory neuropeptides in human circumvallate papillae." J Anat **192 (Pt 4)**: 557-564.

Lossow, K., S. Hubner, N. Roudnitzky, J. P. Slack, F. Pollastro, M. Behrens and W. Meyerhof (2016). "Comprehensive Analysis of Mouse Bitter Taste Receptors Reveals Different Molecular Receptive Ranges for Orthologous Receptors in Mice and Humans." J Biol Chem **291**(29): 15358-15377.

Miller, I. J., Jr. (1976). "Taste bud distribution and regional responsiveness on the anterior tongue of the rat." Physiol Behav **16**(4): 439-444.

Miller, I. J., Jr. (1977). Gustatory receptors of the palate. Food Intake and Chemical Senses. M. S. Y. Katsuki, S. Takagi, nd Y. Oomura. Tokyo, Japan, Jpn. Sci. Soc.**:** 173-185.
Miller, I. J., Jr. and D. V. Smith (1984). "Quantitative taste bud distribution in the hamster." Physiol Behav **32**(2): 275-285.
Mistretta, C. M. (1991). Developmental Neurobiology of the Taste System. Taste and Smell in Health and Disease. T. V. Getchell. New York, Raven Press**:** 29.
Montavon, P. and K. Lindstrand (1991). "Immunohistochemical localization of neuron-specific enolase and calcitonin gene-related peptide in rat taste papillae." Regul Pept **36**(2): 219-233.
Ridaura-Ruiz, L., R. Figueiredo, E. Valmaseda-Castellon, L. Berini-Aytes and C. Gay-Escoda (2012). "Sensibility and taste alterations after impacted lower third molar extractions. A prospective cohort study." Med Oral Patol Oral Cir Bucal **17**(5): e759-764.
Roper, S. D. and N. Chaudhari (2017). "Taste buds: cells, signals and synapses." Nat Rev Neurosci **18**(8): 485-497.
Sbarbati, A., C. Crescimanno and F. Osculati (1999). "The anatomy and functional role of the circumvallate papilla/von Ebner gland complex." Med Hypotheses **53**(1): 40-44.
Shafer, D. M., M. E. Frank, J. F. Gent and M. E. Fischer (1999). "Gustatory function after third molar extraction." Oral Surg Oral Med Oral Pathol Oral Radiol Endod **87**(4): 419-428.
Sohn, W. J., G. J. Gwon, C. H. An, C. Moon, Y. C. Bae, H. Yamamoto, S. Lee and J. Y. Kim (2011). "Morphological evidences in circumvallate papilla and von Ebners' gland development in mice." Anat Cell Biol **44**(4): 274-283.
Tester RF, Q. X., Karkalas J. (2006). "Hydrolysis of native starches with amylases." Animal Feed Science and Technology **130**(1-2): 16.
Tizzano, M., G. Dvoryanchikov, J. K. Barrows, S. Kim, N. Chaudhari and T. E. Finger (2008). "Expression of Galpha14 in sweet-transducing taste cells of the posterior tongue." BMC Neurosci **9**: 110.
Tomiyama, H. (1977). "A quantitative evaluation of the area and the number of the somata in the human circumvallate taste bud by electron microscope (author's transl)." Nihon Jibiinkoka Gakkai Kaiho **80**(11): 1352-1358.
Travers, S. P. and K. Nicklas (1990). "Taste bud distribution in the rat pharynx and larynx." Anat Rec **227**(3): 373-379.
Travers, S. P. and R. Norgren (1995). "Organization of orosensory responses in the nucleus of the solitary tract of rat." J Neurophysiol **73**(6): 2144-2162.
Whitehead, M. C., M. E. Frank, T. P. Hettinger, L. T. Hou and H. D. Nah (1987). "Persistence of taste buds in denervated fungiform papillae." Brain Res **405**(1): 192-195.
Witt, M. and K. Reutter (1997). "Scanning electron microscopical studies of developing gustatory papillae in humans." Chem Senses **22**(6): 601-612.
Yoshida, R., S. Takai, K. Sanematsu, R. F. Margolskee, N. Shigemura and Y. Ninomiya (2018). "Bitter Taste Responses of Gustducin-positive Taste Cells in Mouse Fungiform and Circumvallate Papillae." Neuroscience **369**: 29-39.