Journal of Gerontology: BIOLOGICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci. 2012 April;67A(4):336–344
doi:10.1093/gerona/glr192

Published by Oxford University Press on behalf of The Gerontological Society of America 2011.
Advance Access published on November 4, 2011

# Age-Related Changes in Mouse Taste Bud Morphology, Hormone Expression, and Taste Responsivity

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Normal aging is a complex process that affects every organ system in the body, including the taste system. Thus, we investigated the effects of the normal aging process on taste bud morphology, function, and taste responsivity in male mice at 2, 10, and 18 months of age. The 18-month-old animals demonstrated a significant reduction in taste bud size and number of taste cells per bud compared with the 2- and 10-month-old animals. The 18-month-old animals exhibited a significant reduction of protein gene product 9.5 and sonic hedgehog immunoreactivity (taste cell markers). The number of taste cells expressing the sweet taste receptor subunit, T1R3, and the sweet taste modulating hormone, glucagon-like peptide-1, were reduced in the 18-month-old mice. Concordant with taste cell alterations, the 18-month-old animals demonstrated reduced sweet taste responsivity compared with the younger animals and the other major taste modalities (salty, sour, and bitter) remained intact.

**Key Words:** Taste buds—Aging—Sweet taste—Glucagon-like peptide-1—T1R3.

*Received May 25, 2011; Accepted September 19, 2011*

*Decision Editor: Rafael de Cabo, PhD*

THE aging process is a dynamic and highly integrative process characterized by the accumulation of multiple complex physiological changes over time. All bodily systems are subject to the aging process, including the gustatory system. The nose and mouth, together with their associated olfactory and gustatory neural systems, serve to detect and assess an individual's chemical environment and produce the complex sensations of smell and taste. Decreased sensory functioning with age is evident in many of the sensory systems. Various studies have confirmed that the functionality of the olfactory and gustatory systems tend to deteriorate with age, thereby impairing overall health, sense of wellbeing, and quality of life (1,2).

Processing of taste begins with molecular events at the surface membranes of modified epithelial-derived taste cells (TCs), which are organized in taste buds within circumvallate, foliate, and fungiform papillae (3,4,5). Mammals have four different types of TCs (types I, II, III, and IV) within their taste buds that exhibit different molecular phenotypes and functional roles. Type I TCs are glial-like cells that play a role in maintaining overall taste bud structure (6). It has also been shown that the salt-sensing channel, epithelial sodium channel (ENaC), is expressed in Type I cells (7). Type II cells play a role in transducing sweet, bitter, and umami stimuli (8), and they utilize a G protein–coupled transduction cascade for signaling (4,5). When taste stimuli activate membrane bound G protein–coupled receptors (GPCRs) in Type II cells, phospholipase Cβ2 (PLCβ2) is activated, leading to increased cytosolic inositol trisphosphate ($IP_3$) levels, which in turn triggers calcium release from intracellular stores (9,10). Disruption of this signaling pathway can impair the ability to detect multiple taste stimuli in mice. Therefore, PLCβ2 is thought to be essential for the transduction of bitter, sweet, and umami stimuli (11). Type II cells typically do not form conventional synapses with nerve terminals in taste buds (12) but are thought to use adenosine triphosphate (ATP) as a transmission signal to primary afferents (13,14). Type III cells, however, do typically synapse directly with afferent nerve fibers from three cranial nerves (15) and can release serotonin (5-hydroxytryptamine; 5-HT) upon depolarization (16). The neural cell adhesion molecule (NCAM), which is expressed on Type III cells, is a membrane-associated glycoprotein that mediates cell–cell adhesive interactions by a homophilic mechanism in a calcium-independent manner. It has been postulated that NCAM expression is required in Type III cells prior to the formation of synaptic contacts with their nerve fibers (17). Additionally, Type III cells also contain polycystic kidney disease 2-like 1 protein (PKD2L1) (18), a transient receptor potential (TRP) ion channel that is necessary for sour transduction. Protein gene product 9.5 (PGP9.5), a neuronal marker that has been found in certain types of paraneurons (19,20), is also expressed in Type III cells as well as in a subset of Type II cells in circumvallate papillae. Finally, Type IV cells (also termed "basal cells") are progenitor cells that can differentiate into the other three TC

types (21). Sonic Hedgehog (Shh) expression is involved in the induction and morphogenesis of early circumvallate papillae (22) and is expressed exclusively in epithelial cells on the basal side (23) as a transient precursor of elongated cells and as a signal center for the proliferation of progenitor cells (21).

It has been shown that a number of metabolic hormones are functionally localized in subsets of TCs (24), and the mechanisms for modulating taste sensitivity by these hormones are just now being explored. For example, the gut hormone glucagon-like peptide 1 (GLP-1) has recently been demonstrated to be a taste-modulating hormone, and both GLP-1 and its cognate receptor have been shown to be expressed on subsets of Type II and III cells. Mice lacking GLP-1 receptors exhibit significantly reduced sweet taste responsivity and significant hyperresponsivity to umami taste (25,26). Additionally, it has also been shown that diabetic db/db mice (which have a dysfunctional leptin receptor) have greater gustatory neural sensitivities and higher behavioral preferences for various sweet substances than lean control mice (27). It has therefore been postulated that circulating leptin can act as a negative modulator for sweet taste (27–29). Moreover, we recently reported that mice lacking the gut hormone vasoactive intestinal peptide demonstrate enhanced sweet taste responsivity and reduced taste bud leptin receptor expression (30). Furthermore, we have also shown that the stomach-derived hormone ghrelin is produced in TCs and that ghrelin receptor null mice show reduced salt taste responsivity (31). Thus, it is clear that numerous metabolic hormones are expressed in the taste buds of the tongue and that they play important modulatory roles for taste transduction.

At this present time, relatively little is known about how the normal aging process alters overall gustatory function and taste bud morphology and function. Some previous studies have reported changes in taste responses and taste bud numbers with age (32,33), but quantification of each of the four cell types or some of the hormones they contain during aging has not been performed thus far. Therefore, we carried out a study to investigate the effects of the aging process on taste bud morphology, function, and taste responsivity.

## Materials and Methods

### *Animal and Tissue Processing*

All animal testing procedures were approved by the Animal Care and Use Committee of the National Institute on Aging. Male mice on a B6C3F1/J background were employed for our studies and all mice were on a 12-hour light and dark cycle. We investigated mice of three different age groups: 2 months old ($n = 10$), 10 months old ($n = 10$), and 18 months old ($n = 10$). Taste bud size and number of TCs per taste bud were initially analyzed in the different groups ($n = 3$), and based upon these results, taste testing was carried out on the mice that were 10 and 18 months of age ($n = 7$ per group taste testing). After taste testing was completed, animals were anesthetized using isoflurane, and the tongues were carefully collected from each animal. Excised tongues were fixed in 4% paraformaldehyde (Sigma, St. Louis, MO) for 1 hour and then cryoprotected with 20% sucrose in 0.1 M phosphate buffer overnight at 4°C. Serial sections (8–10 μm thickness) were cut from the tissues containing circumvallate papillae using a cryostat (HM 500M; Micron, Laborgerate GmbH, Germany). All tongues were collected from the 10-month-old and 18-month-old animals and were analyzed in depth ($n = 10$ per group) for taste bud size, taste bud number, TCs per taste bud, number of Type I, II, III, and IV cells, and metabolic hormone and receptor expression.

Table 1. Primary Antibodies Used in Immunofluorescence Analyses

| Antigen | Host | Vendor | Dilution |
|---|---|---|---|
| PLCβ2 | Rabbit | Santa-Cruz, Santa Cruz, CA | 1:200 |
| NCAM | Rabbit | Chemicon, Temecula, CA | 1:500 |
| PGP9.5 | Rabbit | Biogenesis, Raleigh, NC | 1:200 |
| Shh | Rabbit | Santa-Cruz, Santa Cruz, CA | 1:100 |
| T1R3 | Rabbit | Santa-Cruz, Santa Cruz, CA | 1:100 |
| GLP-1 | Mouse | US biological, Swampscott, MA | 1:100 |
| Leptin Receptor (Ob-Rb) | Goat | R&D systems, Minneapolis, MN | 1:200 |
| Ghrelin | Goat | Santa-Cruz, Santa Cruz, CA | 1:100 |

*Notes*: GLP-1 = glucagon-like peptide-1; NCAM = neural cell adhesion molecule; PGP9.5 = protein gene product 9.5; PLCβ2 = phospholipase Cβ2; Shh = Sonic Hedgehog.

### *Immunohistochemistry*

After antigen retrieval with 1× citrate buffer (Biogenex, San Ramon, CA) at 98°C for 20 minutes, immunofluorescence (IF) analyses were performed as described previously (25). Cryostat sections were blocked in 5% bovine serum albumin (Sigma) and 0.1% Tween-20 in 1X Tris-buffered saline (pH 7.4) for one hour at room temperature, followed by incubation in a specific primary antibody in 1% bovine serum albumin and 0.1% Tween-20 in Tris-buffered saline (pH 7.4) overnight at 4°C. Sources and dilutions of the applied primary antibodies are listed in Table 1, and the types of TC markers used are summarized in Supplementary Figure S1A. After washing, sections were incubated for 1 hour in fluorescent secondary antibodies (FITC, Rhodamine Red-X [1:1,000 dilution; Jackson ImmunoResearch, West Grove, PA]) along with TO-PRO-3 (1:7,000 dilution; Molecular Probes, Carlsbad, CA), in some cases, for nuclear staining. No fluorescent staining was observed in any sections when the primary antibodies were omitted.

### *Quantification of Immunoreactive TCs*

In order to obtain a systematic sample without bias throughout the papillae, each papilla was systematically sectioned and every tenth section was saved onto a slide. As taste buds

are approximately 80–100 μm in length, sampling every tenth section ensured that no two sections were collected from the same taste bud. Confocal images were collected using an LSM-710 confocal microscope (Carl Zeiss MicroImaging, Thornwood, NY) in single planes. Approximately 100–120 taste buds per group were analyzed, as described previously (31). Cells were scored as immunoreactive only if a nuclear profile was present within the cell. The total number of cells in the section was determined by counting the number of TO-PRO-3 stained nuclei present in each taste bud. Finally, the percentage of immunoreactive TCs was calculated by dividing the number of immunoreactive TCs by the total number of the TCs in each taste bud. All data were collected in a blinded fashion. One of the investigators scored samples that had ID numbers representing animals, and after quantification was complete, the animal IDs were matched to their phenotypes.

*Quantification of Taste Bud Size and TC Numbers Per Taste Bud*

To calculate taste bud size, the perimeter of the taste bud (from every tenth section) was outlined and the corresponding area was computed using the Zeiss LSM Image Browser. To count the number of cells in a single taste bud, 20 taste buds were randomly selected at different regions on each tongue section, where one nucleus corresponded to one cell on the section. TC numbers were then calculated by counting the average number of TCs per taste bud using a calibrated graticule.

*Taste Behavioral Tests and Data Analysis*

The taste behavioral testing was performed as previously described (30,34; Supplementary Figure S1B). All taste testing took place during daylight hours, and all mice were habituated to the laboratory environment for 30 minutes each day prior to the initiation of taste testing. All tastants were prepared with purified water from the National Institute on Aging animal facility and reagent grade chemicals and were presented to the animals at room temperature. Test stimuli consisted of various concentrations of sucrose (25, 75, 150, 200, 300, 600, and 1,000 mM; Fisher Scientific, Atlanta, GA), sodium chloride (NaCl: 15, 100, 300, 500, 600, and 1,000 mM; Sigma-Aldrich, St. Louis, MO), denatonium benzoate (DB: 0.001, 0.01, 0.1, 0.3, 1, and 3 mM; Sigma-Aldrich), and citric acid (CA: 0.1, 0.5, 1, 3, 10, 30, and 100 mM; Fisher Scientific). Brief-access taste testing took place in a Davis MS-160 gustometer (DiLog Instruments, Tallahassee, FL) as previously described (35–39). Brief-access procedures minimize postingestive effects that may confound other assays, such as intake tests or 2-bottle taste tests (35). Mice accessed the taste stimuli (presented as a concentration range) or water in sipper bottles through a small opening in the mouse chamber. Before taste testing was initiated, mice were trained to lick a stationary tube of water in the gustometer after being placed on a 16-hour (5:00 PM–9:00 AM) restricted water-access schedule. Unconditioned licking responses were recorded for later analyses in 25-minute brief-access test sessions, during which mice could initiate as many trials as possible in this period. Stimulus presentation order was randomized within blocks. The duration of each trial (5 seconds) was regulated by a computer-controlled shutter that allowed access to the sipper tube. There was a 7.5 second interpresentation interval, during which time a stepper motor moved one of up to seven tubes (containing water or a specific concentration of tastant) in front of the shuttered opening. Two different testing protocols were used: one for normally preferred stimuli (sucrose) and one for normally avoided stimuli (NaCl, DB, and CA). For sucrose, animals received 5 days of testing using the five stimulus concentrations and purified National Institute on Aging animal facility water. Prior to each day of sucrose testing, animals were placed on a

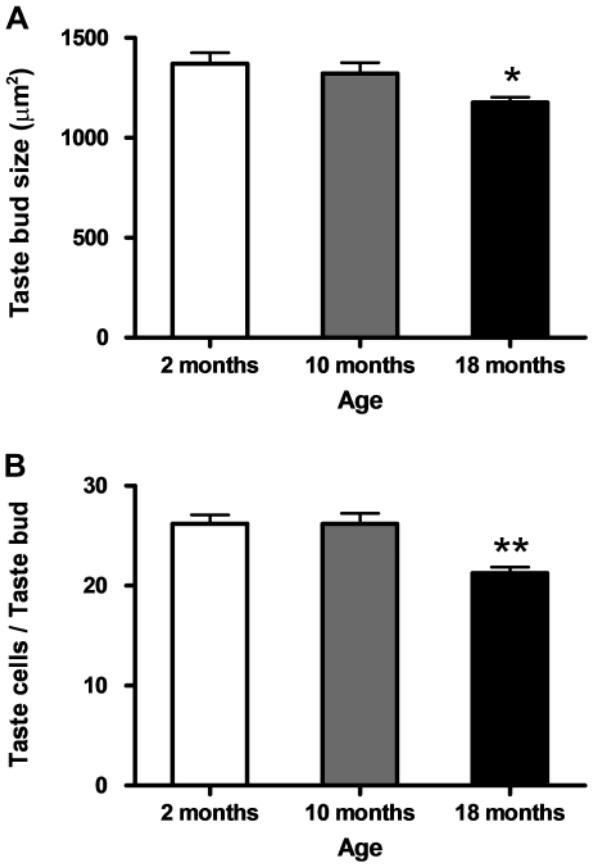


Figure 1. Taste bud size and taste cell (TC) numbers per taste bud in 2-month-, 10-month- and 18-month-old mice. (**A**) To calculate taste bud size, the perimeter of the taste bud (from every tenth tongue section) was outlined and the corresponding area was computed using a Zeiss LSM Image Browser. Values are expressed as means ± *SEM*. (**B**) The total number of cells in the section was determined by counting the number of TO-PRO-3–stained nuclei present in each taste bud. The percentage of immunoreactive TCs was calculated by dividing the number of immunoreactive TCs by the total number of the TCs in each taste bud. Approximately 100–120 taste buds per mouse were analyzed. Values are expressed as means ± *SEM*. *$p < .05$; **$p < .01$, $n = 10$ per group.

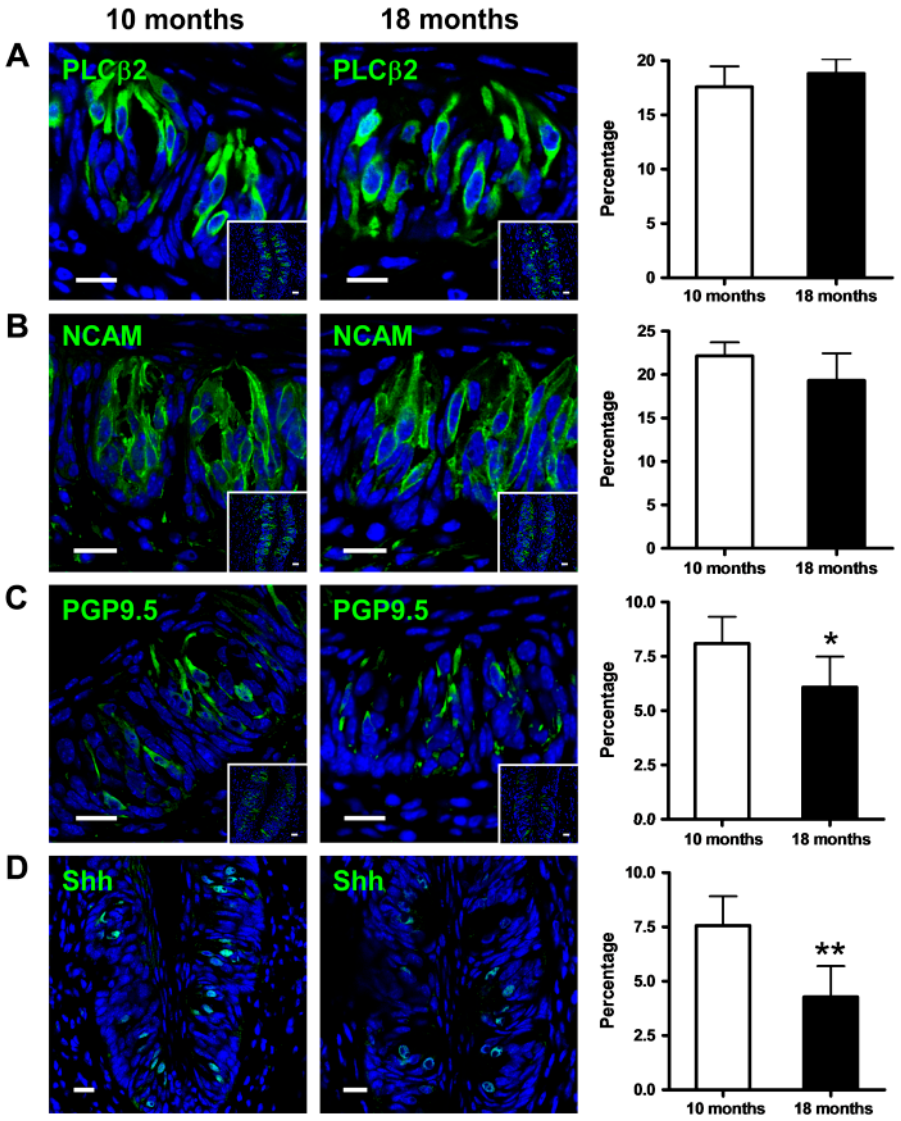


Figure 2. Expression of taste cell (TC) makers in circumvallate papillae TCs of 10-month- and 18-month-old mice. (**A**), (**B**), (**C**), and (**D**) are high magnification representative fluorescent images of the different TC markers (PLCβ2, NCAM, PGP9.5, and Shh). The inset boxes in each image indicate a low magnification field view of the whole taste bud highlighted. The histograms associated with each taste bud figure represent the percentage of cells containing each marker out of the total number of cells in each taste bud. Scale bars, 20 µm. Blue is TO-PRO-3 nuclear stain. Values are expressed as means ± *SEM*. *$p < .05$; **$p < .01$, $n = 10$ per group.

16-hour (5:00 PM–9:00 AM) restricted food and water-access schedule (1 g of food and 2.5 mL of water) in order to maintain motivation to drink and thus increasing the number of stimulus presentations taken during testing (38,39). In a similar manner for the other tastants, NaCl, CA, and DB, animals received 5 days of testing with the five stimulus concentrations and with purified animal facility water. Similarly to the testing performed with sucrose, the mice were water restricted during NaCl, DB, and CA testing in order to increase the number of stimulus presentations taken. Additionally, a water rinse presentation (1 second) was interposed between the test trials for NaCl, DB, and CA to help control for any potential tastant carryover effects.

*Data Analysis and Statistical Methods for Behavioral Testing*

The average number of licks per trial for each stimulus concentration was divided by the average number of water licks per trial, yielding a tastant/water lick ratio. This ratio controls for individual differences in motivational state (37). The ratios were analyzed with standard ANOVA and Student's *t* test using GraphPad Prism (v5.0). The conventional $p \leq .05$ was applied as the statistical rejection criterion. Tastant concentration–lick ratio response curves were fitted to the mean data for each group using a classical four parameter logistic sigmoidal dose–response equation in the nonlinear regression suite of GraphPad Prism (v5.0).

Table 2. Immunocytochemical Marker-Positive TCs in Mouse Circumvallate Papillae TCs

| Marker | Average marker-positive TCs per taste bud (cell numbers, mean ± *SEM*) | | |
|---|---|---|---|
| | 10 months (average 26.2 TCs per bud) | 18 months (average 21.3 TCs per bud) | *p* Value |
| PLCβ2 | 4.6 ± 0.5 | 3.9 ± 0.3 | .0942 |
| NCAM | 5.8 ± 0.4 | 4.1 ± 0.7 | .0754 |
| PGP9.5 | 2.1 ± 0.3 | 1.3 ± 0.3 | .0379 |
| Shh | 1.9 ± 0.4 | 0.9 ± 0.3 | .0062 |
| T1R3 | 1.7 ± 0.3 | 1.0 ± 0.2 | .0214 |
| GLP-1 | 3.9 ± 0.6 | 1.6 ± 0.3 | .0032 |
| Leptin receptor | 2.3 ± 0.5 | 1.8 ± 0.2 | .1288 |
| Ghrelin | 2.4 ± 0.2 | 1.1 ± 0.5 | .0361 |

*Notes*: GLP-1 = glucagon-like peptide-1; NCAM = neural cell adhesion molecule; PGP9.5 = protein gene product 9.5; PLCβ2 = phospholipase Cβ2; Shh = Sonic Hedgehog; TC = taste cell.

## Results

### *Age-Related Alterations in Taste Bud Size and Number of TCs Per Taste Bud*

We first determined whether aging caused any alterations in the number of taste buds per papilla, taste bud size, or TC numbers per taste bud. We found no significant difference in the number of taste buds between the three different ages (2, 10, and 18 months). However, there was a significant reduction in taste bud size in the 18-month-old animals compared with the 2- and 10-month-old animals ($p \leq .05$; Figure 1A). Additionally, there was also a significant reduction in the number of TCs per taste bud in the 18-month-old animals compared with the 2- and 10-month-old animals ($p \leq .01$; Figure 1B).

We next investigated the qualitative nature of the different TC types by staining for the classical TC immunohistochemical markers. As we had detected significant differences in taste bud size and the number of TCs per taste bud longitudinally between the 10-month-old animals and the 18-month-old animals, we next investigated these two ages in more depth. Details of the antibodies used and the specific TC markers used are summarized in Table 1. Briefly, we employed PLCβ2as a Type II cell marker and NCAM as a Type III cell marker, PGP9.5 was used as both a Type II and Type III cell marker, and Shh was used as a Type IV cell marker. For both PLCβ2- and NCAM-expressing cells, there was no significant difference between the 18-month-old and 10-month-old animals (Figure 2A and B; PLCβ2 $p = .0942$ and NCAM $p = .0754$; Table 2). For both PGP9.5- and Shh-expressing cells, there was a significant reduction in the 18-month-old animals compared with the 10-month-old animals (Figure 2C and D; PGP9.5 $p = .0379$ and Shh $p = .0062$; Table 2). Immunocytochemical analyses were also performed on the 2-month-old mice, however, we found no significant differences between the 2-month-old and 10-month-old animals for the expression of TC markers and the metabolic hormones investigated (data not shown). Therefore, for this study, we focused on the aging-related differences in gustation between the 10-month-old and 18-month-old animals.

### *Age-Related Alterations in Sweet Taste Receptor Expression and Gustation-Controlling Metabolic Hormones*

We next investigated sweet taste receptor expression as well as the lingual expression of metabolic hormones involved in gustation. The T1R3 subunit is a critical component of the sweet taste receptor (T1R2/T1R3) and is necessary for sweet taste receptor function. It has been demonstrated that mice that normally prefer sweet tastants, but are genetically altered so that they lack the T1R3 subunit (T1R3 knockout), display significant reductions in sweet taste responsivity (40,41). We found that the number of T1R3-expressing cells were significantly reduced in the 18-month-old animals compared with the 10-month-old animals (Figure 3A; $p = .0214$, Table 2). Similarly to T1R3, we also found that there was a significant reduction in the number of GLP-1-expressing cells in the 18-month-old animals compared with the 10-month-old animals (Figure 3B; $p = .0032$, Table 2). Interestingly, we found no significant alteration in leptin receptor expression between the 18-month- and 10-month-old animals (Figure 3C; $p = .1288$, Table 2). Both GLP-1 (produced in TCs) and leptin receptor activation play a role in modulating sweet taste responsivity (25,29). GLP-1 enhances sweet taste responsivity and leptin receptor activation decreases sweet taste sensitivity (25,29). Recently, we reported that ghrelin is expressed in TCs and that ghrelin signaling plays a role in modulating salt taste. Ghrelin expression was found to be significantly reduced in the 18-month-old animals compared with the 10-month-old animals (Figure 3D; $p = .0361$, Table 2).

### *Age-Related Alteration in Taste Responsivity*

As we had detected significant alterations in taste bud size, TC number, sweet taste receptor expression, and metabolic hormone expression in the 18-month-old animals compared with the 10-month-old animals, we next investigated potential differences in their taste perception. We tested the ability of both the 10-month- and 18-month-old groups to detect four prototypic tastants, that is, sweet (sucrose), salty (sodium chloride, NaCl), sour (citric acid, CA), and bitter (denatonium benzoate, DB, Figure 4). We employed a standardized computer-controlled gustometer, which uses a brief access procedure that minimizes postingestive effects (42; Supplementary Figure S1B). For the aversive stimuli, CA, NaCl, and DB, we found that there were no significant changes in taste responsivity between the 10-month-old and the 18-month-old animals (Figure 4B–D). However, for sucrose, we found that the 18-month-old animals exhibited significantly reduced taste responsivity compared with the 10-month-old animals (Figure 4A, $p \leq .05$ and $p \leq .01$). This alteration in taste behavior in the oldest mice (18 months)

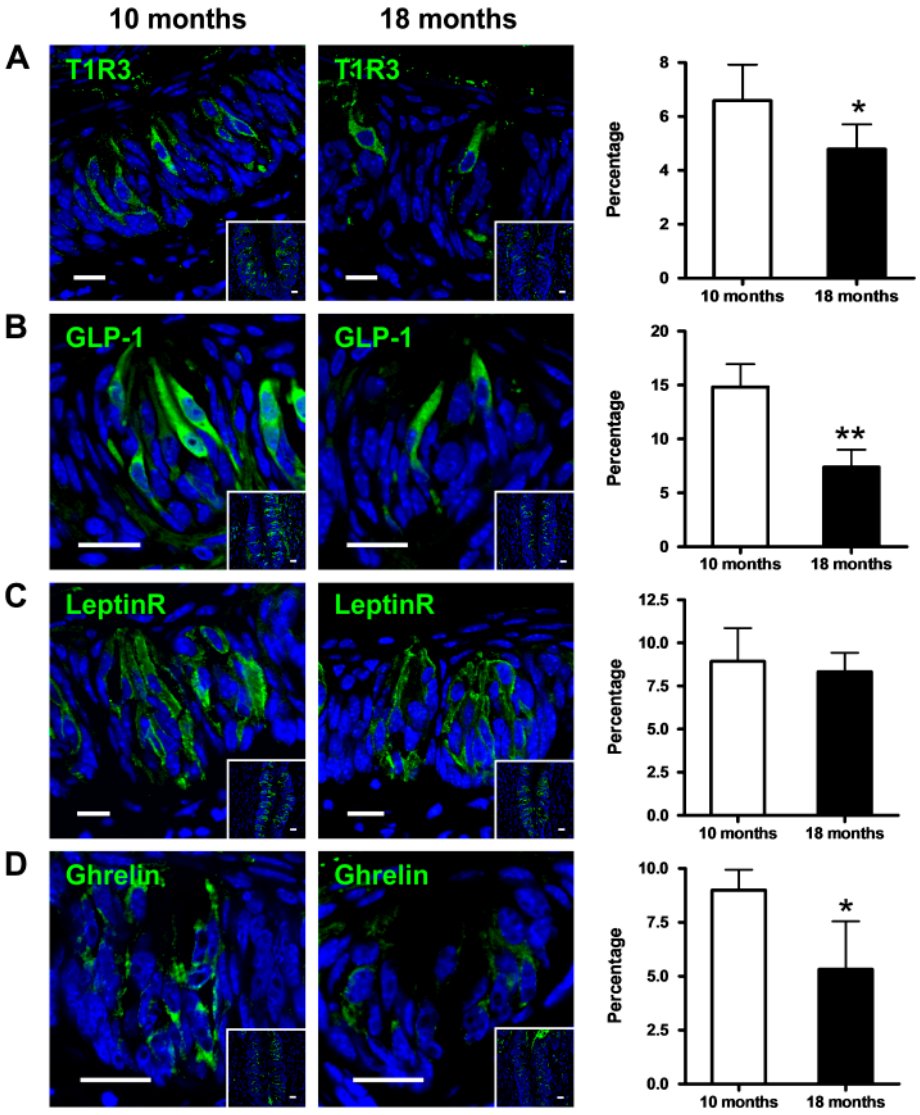


Figure 3. Expression of taste-modulating factors in circumvallate papillae TCs of 10-month- and 18-month-old mice. (**A**), (**B**), (**C**), and (**D**) are representative high magnification fluorescent images of the cell markers (T1R3, GLP-1, leptin receptor (R), and ghrelin). The inset boxes in each image indicate a low magnification field view of the whole taste bud highlighted. The histograms associated with each taste bud figure represent the percentage of the cells containing each marker out of the total number of cells in each taste bud. Scale bars, 20 µm. Blue is TO-PRO-3 nuclear stain. Values are expressed as means ± *SEM*. *$p < .05$; **$p < .01$, $n = 10$ per group.

closely correlates to the alterations in taste bud morphology and metabolic hormone expression that was observed in this group.

## Discussion

In this study, we investigated the effects of advanced aging upon taste bud number, size, function, and taste responsivity. We found that the total number of taste buds did not significantly change between the different age groups, but the taste bud size and the number of TCs per taste bud were significantly reduced in the 18-month-old animals compared with the 10-month- and 2-month-old animals. Eighteen-month-old mice were used in this study to represent an elderly animal, as they have reached more than 80% of their average lifespan, but at this age, they are still relatively free from many aging-related diseases, such as type 2 diabetes, metabolic dysfunction, obesity, and tumors. We have shown previously that alterations in peripheral metabolic health can significantly affect taste responsivity (30). Thus, using even older animals (20–22 months) would be challenging as they would likely be suffering from health problems that could alter their taste responsivity, hence making it difficult to tease out the specific effects of the aging process from the effects due to aging-related diseases.

As differences in taste bud size and TC number per taste bud were detected, we analyzed the abundance of the different TC markers on the TCs. TCs assemble within an onion-shaped

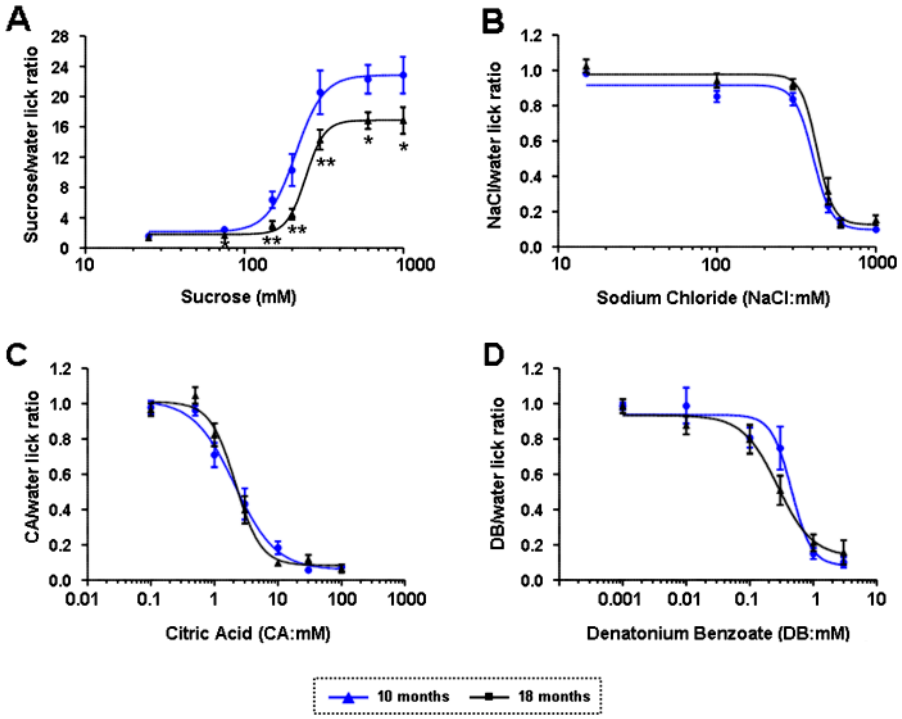


Figure 4. Altered sweet taste responsivity in 18-month-old compared with 10-month-old mice. Taste responses, expressed as tastant/water lick ratios and as a function of stimulus concentration, to sucrose (**A**), NaCl (**B**), citric acid (CA; **C**) and denatonium benzoate (DB; **D**). Datapoints are expressed as means ± *SEM*. Response curves were fit as described in the Materials and Methods section. *$p < .05$; **$p < .01$, $n = 7$ per group.

taste bud and are constantly being replenished (every 7–10 days) through differentiation from basal Shh-positive cells at the base of the taste buds (43–45). We found that there was a significant reduction in PGP9.5- and Shh-expressing cells within the taste buds of the 18-month-old animals. As Shh-immunopositive cells are Type IV cells, this suggests that there was reduced Type IV cell turnover, which could account for the reduction in TC numbers per taste bud that was observed in the 18-month-old mice. Consistent with our findings, it has been reported that aged mice demonstrate delayed cell renewal and a highly vacuolated cytoplasm in their taste bud compared with young adult mice (46). These alterations may further lead to a decrease in TC numbers and taste bud size (47–50). Additionally, we also detected significant reductions in sweet taste receptor subunit T1R3 expression and in GLP-1 expression. This alteration in staining patterns for these two sweet taste modulatory factors may explain why the 18-month-old mice showed reduced sucrose responsivity compared with the 10-month-old mice. The exact role of PGP9.5-containing cells is presently unclear, but based upon our data, it would be interesting to speculate that PGP9.5 cells could be intermediary cells that can differentiate into T1R3- and/or GLP-1-containing cells. It is also plausible that PGP9.5 is coexpressed with T1R3 in a subset of differentiated type II cells (51).

Despite the importance of age-related alterations in chemical sensing, there is currently a scarcity of data on the effects of aging upon gustation. It has been reported that taste responses and thresholds change with age in both rats and humans, and taste studies in older human participants have indicated that the average loss at threshold level for a range of various taste compounds is 4.7, that is, the aged required 4.7 times more molecules or ions to detect and recognize a tastant (32). In rats, neural response magnitudes from the whole chorda tympani nerve, which transmits taste signaling to the brain, were expressed as ratios relative to the NaCl (0.1 M) response (33). Response ratios for NaCl and sucrose increased significantly with age; but ratios for $MgCl_2$ and citric acid decreased. The shapes of response/concentration functions for NaCl also differed with age. However, the magnitudes of all age-related differences were small and neural recordings demonstrate that the peripheral taste system functioned well in old rats (33).

Even though some taste responses have been shown to be altered during the aging process, the anatomical analyses have often been contradictory. In human studies, there was found to be no significant correlation between age and the number of taste buds (52). Additionally, Miller and colleagues (53,54) also reported no significant alterations in taste bud density with advancing age in humans. However, a recent histomorphometric study has shown a decrease in taste bud size and TC density in older participants compared with younger groups (55). Bradley and colleagues (56) quantified taste bud number in fungiform, circumvallate, and foliate papillae in rhesus monkeys and found that the

numbers of taste buds in each papilla were not altered with advancing age, which is similar to our findings for mice. Bradley and colleagues (56) also did not find any significant alterations in the relative distribution of taste buds on the tongue with aging.

We found that sweet taste responsivity was significantly reduced in the 18-month-old animals compared with the 10-month-old animals. Interestingly, we did not detect any significant alterations in the other taste modalities (NaCl, CA, and DB). Sour and bitter taste evolved to avoid fermenting and toxic foods, and as these modalities are critical for survival, it is interesting to speculate that evolutionary pressure may have prevented any alterations in those modalities. Diminished functional sweet taste responsivity may explain the commonly reported loss of sweet perception in the aged (57). Research of 10-year trends into dietary intake of free-living elderly people has also shown that aging leads to increases in consumption of sweet-tasting foods. As we demonstrated a decrease in the number of T1R3-expressing cells and the number of GLP-1-expressing cells decreases with advancing age, this may offer an explanation for the reported alteration in sweet perception with age in humans. Even though we detected a significant reduction in ghrelin expression in the 18-month-old animals compared with the 10-month-old animals, we failed to detect any significant alterations in salty or sour taste responsivity. We have previously reported that ghrelin in TCs plays a role in modulating salty and sour taste responsivity (31). Similarly to sweet taste, which has been shown to be modulated by numerous factors (T1R2/T1R3, GLP-1, glucagon, vasoactive intestinal peptide, and leptin receptor [25,29,30,40,58]), salty and sour taste may also be modulated not only by ghrelin but also by numerous—as of yet unknown—factors. It is plausible that one of the many other putative salty and sour modulating factors compensated for the reduction in ghrelin expression, resulting in the maintenance of these two taste modalities with advanced age.

It is clear that there are alterations in both taste bud function and taste responsivity during the normal aging process, with sweet taste being the most affected. In future studies, we also plan to expand upon our current study by including investigations of the age-related effects upon umami taste responsivity. We have previously demonstrated that gustatory function may act as a bridge between physiological dysglycemias and dietary preferences (30). Therefore, our observed alterations of sweet taste sensitivity may indeed be linked with the typical alterations in glycemic control seen in aging animal and human populations.

Further research is needed to determine alterations in gustatory function across life span, and we are planning future studies in which we will investigate taste responsivity in a broader number of age groups (ie prepubertal and postpubertal) and also investigate the potential for sexual dimorphism in the aging-related alterations in TC function and taste responsivity. Gaining a greater understanding of how gustatory function is altered during aging may pave the way for the development of novel strategies to maintain these important sensory processes in the aged.

Funding

This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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