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Contribution of α-Gustducin to Taste-guided Licking Responses of Mice

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Abstract

We examined the necessity of α-gustducin, a G protein α-subunit expressed in taste cells, to taste-mediated licking responses of mice to sapid stimuli. To this end, we measured licking responses of α-gustducin knock-out (Gus−/−) mice and heterozygotic littermate controls (Gus+/−) to a variety of 'bitter', 'umami', 'sweet', 'salty' and 'sour' taste stimuli. All previous studies of how Gus−/− mice ingest taste stimuli have used long-term (i.e. 48 h) preference tests, which may be confounded by post-ingestive and/or experiential effects of the taste stimuli. We minimized these confounds by using a brief-access taste test, which quantifies immediate lick responses to extremely small volumes of sapid solutions. We found that deleting α-gustducin (i) dramatically reduced the aversiveness of a diverse range of 'bitter' taste stimuli; (ii) moderately decreased appetitive licking to low and intermediate concentrations of an 'umami' taste stimulus (monosodium glutamate in the presence of 100 μM amiloride), but virtually eliminated the normal aversion to high concentrations of the same taste stimulus; (iii) slightly decreased appetitive licking to 'sweet' taste stimuli; and (iv) modestly reduced the aversiveness of high, but not low or intermediate, concentrations of NaCl. There was no significant effect of deleting α-gustducin on licking responses to NH_4Cl or HCl.

Key words: taste, α-gustducin, brief-access taste test, knock-out mice

Introduction

In mammals, the ingestive response to sweeteners, amino acids and many 'bitter' compounds is initiated by the interaction of chemical compounds with G-protein-coupled receptors (GPCRs) on the apical membrane of taste cells (Gilbertson *et al*., 2000; Glendinning *et al*., 2000). Two families of GPCRs are known to mediate this detection process—the T2Rs and T1Rs. A few T2Rs have been shown to respond selectively to compounds that elicit 'bitter' taste sensations in humans (Adler *et al*., 2000; Chandrashekar *et al*., 2000; Bufe *et al*., 2002). The T1Rs, which appear to function predominantly as heterodimers, are activated by amino acids and sweeteners (natural and artificial). The heterodimer of T1R2+T1R3 responds selectively to compounds that elicit 'sweet' taste sensations in humans, whereas that of T1R1+T1R3 responds selectively to compounds that elicit 'umami' taste sensations (Nelson *et al*., 2001; Zhao *et al*., 2003). Once these taste receptors are activated by their respective ligands, they activate one or more G-proteins (gustducin, rod-transducin or Gi2), which in turn stimulate one or more effector systems (adenylyl cyclase, phosphodiesterase or phospholipase C β_2; Huang *et al*., 1999; Clapp *et al*., 2001; Yan *et al*., 2001; Ogura *et al*., 2002). These early transduction steps initiate a cascade of downstream events, including the release of neurotransmitter from the taste cell, generation of action potentials in the postsynaptic afferent neuron, propagation of the action potentials up the gustatory neuraxis, and, ultimately, ingestion or rejection Glendinning *et al*., 2000; Spector, 2000).

There is compelling evidence that the α-subunit of gustducin (α-gustducin) contributes significantly to the transduction of 'bitter', 'sweet' and 'umami' taste stimuli (Wong *et al*., 1996; Ruiz-Avila *et al*., 2001; He *et al*., 2002, 2004; Ruiz *et al*., 2003). Gustducin is co-expressed with T1Rs or T2Rs in some taste cells (Adler *et al*., 2000; Max *et al*., 2001; Li *et al*., 2002; Kim *et al*., 2003) and its subunits have been shown to activate specific components of known gustatory effector systems (e.g. PLC-β_2, PDE and Ca^{2+} store-operated channels) in signaling pathways responsive to 'sweet' and 'bitter' compounds (Huang *et al*., 1999; Clapp *et al*., 2001; Yan *et al*., 2001; Ogura *et al*., 2002). α-Gustducin

knockout mice (i.e. Gus−/− mice) exhibit diminished behavioral and/or gustatory nerve responsiveness to natural and artificial sweeteners (Wong *et al.*, 1996; He *et al.*, 2002), 'bitter' compounds (Ruiz-Avila *et al.*, 2001; Wong *et al.*, 1996; He *et al.*, 2002; Caicedo *et al.*, 2003; Ruiz *et al.*, 2003) and amino acids (Ruiz *et al.*, 2003; He *et al.*, 2004). Transgenic expression of wild-type α-gustducin in Gus−/− mice restores normal behavioral responsiveness to these taste stimuli (Ruiz-Avila *et al.*, 2001).

While there is no doubt that α-gustducin contributes to the transduction of 'sweet', 'bitter' and 'umami' taste stimuli, the following observations indicate that other G proteins are also involved in these signaling pathways. First, in anterior tongue, most taste cells doubly positive for T1R2+T1R3 (i.e. presumed 'sweet' responders) also express α-gustducin (Kim *et al.*, 2003). Yet, in posterior tongue, very few taste cells doubly positive for T1R2+T1R3 also express α-gustducin—instead it is the T2R-positive taste cells that co-express α-gustducin (Kim *et al.*, 2003). Second, Caicedo *et al.* (2003) reported that only half of the mouse circumvallate taste cells that displayed increases in intracellular Ca^{2+} concentration (in response to stimulation with 'bitter' taste stimuli) also expressed gustducin; the other half of the taste cells expressed a different G protein, Gi2. Third, electrophysiological recordings from taste nerves and long-term preference tests reveal that knocking out gustducin diminishes (but does not eliminate) responses to a range of concentrations of sucrose, SC45647 (an artificial sweetener), quinine, denatonium benzoate and MSG (Wong *et al.*, 1996; He *et al.*, 2002, 2004; Ruiz *et al.*, 2003). Fourth, expression of a dominant-negative form of α-gustducin in Gus−/− mice reduced these responses further still (Ruiz-Avila *et al.*, 2001), indicating that another G protein in the α-gustducin-positive taste cells mediated the responses. Fifth, double knockout mice lacking both α-gustducin and α-transducin showed responses to 'umami' taste stimuli that were diminished relative to single knockout mice lacking only one of the G proteins (He *et al.*, 2004).

The purpose of this study was to clarify the necessity of α-gustducin to the taste-mediated ingestive response of mice to a diversity of taste stimuli. Although the molecular, Ca^{2+}-imaging and nerve recording findings discussed above strongly suggest that the peripheral taste system of mice contains both α-gustducin-dependent and α-gustducin-independent pathways for transducing taste stimuli, the nature of the perceptions generated by the gustducin-independent pathways remains to be determined. All previous studies of the taste-related behavioral responses of Gus−/− mice to sapid stimuli have used long-term (i.e. 48 h) preference tests as the behavioral measure. Owing to the protracted nature of these preference tests, the specific contribution of taste to the observed intake may be confounded by post-ingestive feedback and/or experiential effects of the chemical stimuli (Mook, 1963; Rabe and Corbit, 1973; Weingarten and Watson, 1982; Flynn and Grill, 1988; Fregly and Rowland, 1992; Bachmanov *et al.*, 2000; Spector 2000). Here we used a brief-access taste test, in which chemical stimuli are presented during a series of brief trials (i.e. 5 s), to measure unconditioned lick responsiveness to an array of taste stimuli in Gus −/− mice and their heterozygotic littermate controls. Although this test does not assess taste quality *per se*, it does provide an index of the taste-related affective potency of the stimuli, while minimizing the influence of post-ingestive feedback or experiential effects. This approach has been used successfully in the past to analyze taste-mediated ingestive responses of inbred strains of mice (Boughter *et al.*, 2002; Glendinning *et al.*, 2002; Nelson *et al.*, 2003; Dotson and Spector, 2004).

Materials and methods

Subjects

We used genetically engineered Gus−/− mice that were bred at the Mount Sinai School of Medicine. The genetic background of the mice was 99.2% C57BL/6J and 0.8% 129/SvEmsJ (for more husbandry details, see He *et al.*, 2002). All mice were housed individually in standard shoebox cages (27.5 × 17 × 12.5 cm) in a room with automatically controlled temperature, humidity and lighting (12 h:12 h light:dark cycle). All subjects were adult males. The sample sizes for each experiment are indicated in the figure legends. Except where noted otherwise, the mice were maintained *ad libitum* on TestDiet laboratory chow (5012, Purina Mills Inc., Richmond, IN) and water. We tested mice during the light phase of their light–dark cycle, except during experiment 6. In the latter experiment, we tested mice during the dark phase (see below for details).

Lickometry

Each test was conducted in a commercial gustometer (Davis MS160-Mouse; DiLog Instruments, Tallahassee, FL). This device provided each mouse with access to a single sipper tube during successive 5 s trials. Immediately prior to a trial, the computer positioned a sipper tube directly behind a slot (1.5 cm wide, 4.0 cm high) in the back of the testing chamber, and then opened the shutter. The trial 'began' when the mouse took its first lick from the sipper tube, and ended 5 s later when the shutter closed. During the 7.5 s inter-trial interval, a different sipper tube was positioned behind the shutter in preparation for the next trial. Each mouse was offered a range of different concentrations of each taste stimulus during a test session (see below for details). The order of presentation was randomized without replacement in blocks so that every concentration of a taste stimulus and water was presented once before the initiation of a second block. Unconditioned licking responses were recorded for later analysis. Each test session lasted 30 min, during which the mouse could initiate as many 5 s trials as possible.

Training procedure

Before taste testing was conducted, the mice were given 2 days of training with water as the taste stimulus. This served to familiarize the mice with the gustometer and trained them to lick from the sipper tube to obtain fluid. Because the mice were placed on a water-restriction schedule (see below) throughout training, they were highly motivated to lick from the sipper tube. Each training session began when the mouse took its first lick, and lasted 30 min. On training day 1, the mouse could drink freely from a single sipper tube throughout the session as the shutter permanently open. On training day 2, the mouse could only drink from a sipper tube during 5 s trials. All mice adapted readily to these procedures and took >250 licks per training session.

Testing

We used the brief-access taste test described in Glendinning *et al*. (2002). Testing began once training was completed. Multiple concentrations of a single taste stimulus were presented during the 30 min test session. The mouse was permitted to initiate as many trials (and hence, blocks) as possible throughout the 30 min test session. We included water as a solution within each block of taste stimulus concentrations.

Previous work with C57BL/6J mice (Glendinning *et al*., 2002) demonstrated that it is necessary to pool results from three test sessions to obtain reliable estimates of lick responsiveness to aversive taste stimuli. This is because the mice initiate relatively few trials (i.e. 10–25) with aversive taste stimuli. In contrast, one can obtain reliable estimates of lick responsiveness during a single test session with normally preferred taste stimuli because mice initiate relatively large numbers of trials (i.e. 30–75).

Taste stimuli

Taste stimuli were obtained from Sigma-Aldrich (St Louis, MO), unless stated otherwise. Immediately prior to testing, the stimuli were dissolved in deionized water and presented at room temperature. In all experiments, mice were exposed to a range of concentrations of each stimulus. The specific concentrations were selected because they elicited the full dynamic range of response in Gus+/− mice during preliminary trials.

Standardization of licking responses

For aversive taste stimuli, we controled for individual differences in lick rate and motivation to lick by calculating a *tastant/water lick ratio.* This involved dividing the average number of licks to each taste stimulus concentration (across all trials with that concentration) by the average number of licks to water alone (across all trials), separately for each mouse. A lick ratio of 1.0 signifies that licks to the taste stimulus equaled licks to water, whereas a lick ratio <1.0 signifies that there were fewer licks to the taste stimulus relative to that for water.

For normally preferred taste stimuli, we controlled for individual differences in lick rate by calculating the standardized lick ratio (SLR; Glendinning *et al*., 2002) separately for each mouse. To this end, the average number of licks per trial for each concentration was divided by that animal's maximum potential lick rate per trial based on the mean of the interlick interval (ILI) distribution measured during training day 1 with water (only ILIs > 50 ms and < 200 ms were used) (see Glendinning *et al*., 2002, for details). A SLR approaching 0 indicates that the taste stimulus elicited only sporadic licking, whereas an SLR near 1.0 indicates that the taste stimulus elicited nearly continuous licking across each 5 s trial. Although the SLR does not necessarily control for variation in the motivational state arising from differential responses to the food and water restriction schedule, it does control for individual differences in local lick rate.

Water-deprivation procedure

To encourage sampling from the sipper tube during training and during tests with aversive taste stimuli (in experiments 1–4), we water-deprived the mice for 22.5 h, subjected the animals to a 30 min test session, and then gave them water *ad libitum* for 1 h. We usually repeated this procedure across 4–5 successive days/week. Under this restricted water-access schedule, all mice maintained their body mass between 85–90% of their baseline values across successive days and showed no overt signs of distress.

Food and water-restriction procedure

To encourage sampling from the sipper tube during tests with normally preferred taste stimuli (i.e. experiments 5–7), we food- and water-restricted each mouse for 23.5 h (see below for details), subjected it to a 30 min test session, and then gave it a recovery day over which it had food and water *ad libitum*. We usually repeated this procedure three times per week. Under these conditions, the mice: (i) maintained their body mass at >80% of their baseline values; (ii) regained 100% of their baseline body mass over each recovery day; (iii) failed to show any overt signs of distress; and (iv) showed virtually no interest in water alone, but exhibited vigorous concentration-dependent increases in lick responsiveness for normally preferred taste stimuli.

The food- and water-restriction procedure involved limiting each mouse to 1 g of laboratory chow (dustless precision 1 g food-pellets; BioServ) and 2 ml of water. This equaled approximately 20 and 30% of the their normal daily food and water intake, respectively (J. Glendinning, unpublished data).

Because each mouse was subjected to multiple test sessions (one for each of the six taste stimuli), it was necessary to interject a recovery day between successive test sessions, during which food and water were available *ad libitum*. After the recovery day, each mouse was once again food- and water-restricted.

Experiment 1: Do Gus−/− mice show attenuated lick responsiveness to aversive taste stimuli?

Based on findings from long-term preference tests, investigators have reported that knocking out α-gustducin diminishes the aversion to a broad range of concentrations of quinine hydrochloride and denatonium benzoate (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002; Ruiz *et al.*, 2003). We asked whether this finding would generalize to a more diverse range of aversive taste stimuli in a brief-access taste test.

Methods

Taste stimuli and test concentrations

The 'bitter' taste stimuli were quinine hydrochloride, QHCl (0.006, 0.01, 0.03, 0.1, 0.3, 1 mM), denatonium benzoate (0.1. 0.3, 1, 3, 10 mM), sparteine (0.1, 0.3, 1, 3, 10 mM), salicin (1, 10, 100 mM), cycloheximide (0.01, 0.03, 0.1, 0.3, 1, 3 μM), caffeine (10, 30, 100 mM), quinacrine (10, 30, 100, 400 μM) and urea (0.3, 1, 3 M). All of these compounds taste bitter to humans (the authors, unpublished data), and all but caffeine and urea have been shown to activate α-gustducin in either an *in vitro* assay involving isolated taste membranes (Ruiz-Avila *et al.*, 2000) or G protein-coupled T2R taste receptors in a heterologous cell reporter system (Chandrashekar *et al.*, 2000; Bufe *et al.*, 2002).

Testing procedure

After training, each mouse was run through three test sessions with each of the following taste stimuli in the following order: QHCl, denatonium benzoate, sparteine, salicin, cycloheximide, caffeine, quinacrine and then urea. The mice were all water-deprived prior to testing (see above for details).

Data analysis

We analyzed the tastant/water lick ratios from each taste stimulus (e.g. the range of QHCl concentrations) with a two-way ANOVA. We treated stimulus concentration as a within factor and genotype as a between factor. To decompose significant interactions, we ran each main effect through a one-way ANOVA. The α level was set at 0.05 for this and all subsequent statistical tests.

We also performed one-sample paired *t*-tests to determine whether the mean tastant/water lick ratios for each QHCl concentration were significantly above or below the indifference point (i.e. 1.0) when the concentration main effect from the ANOVA was significant. To control for the use of multiple paired comparisons on the same data set, we performed a Bonferroni correction (i.e. divided the α level by the number of comparisons that were made).

Results

Gus−/− mice were significantly less responsive than Gus+/− mice to all eight aversive taste stimuli (Figure 1). A two-way

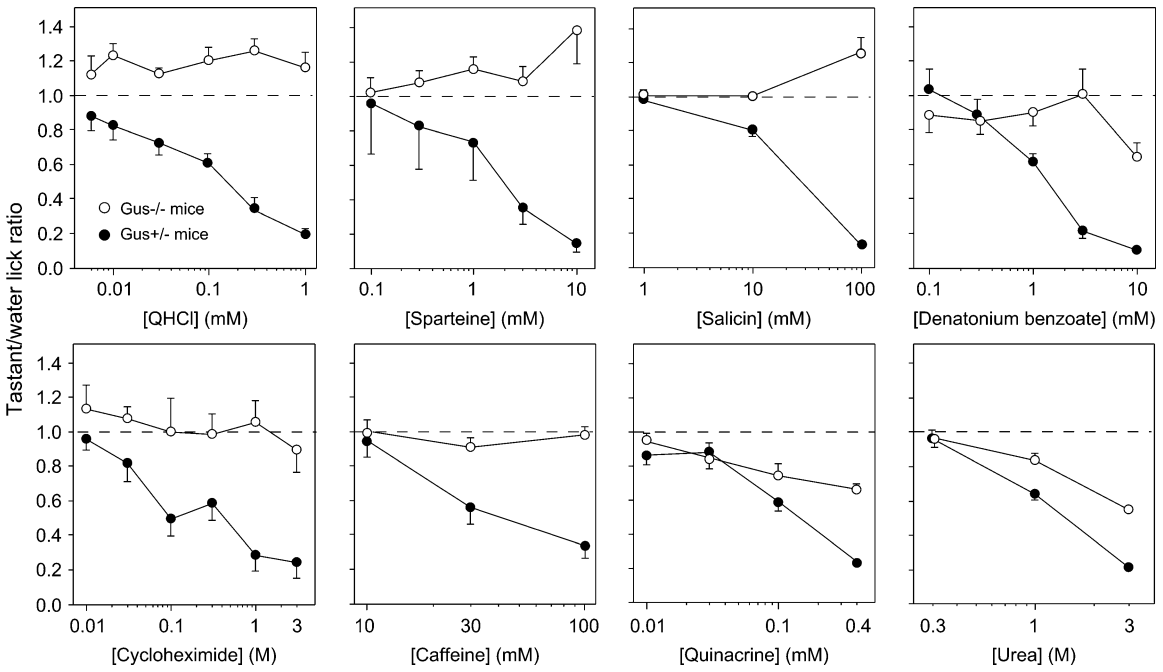

Figure 1 Tastant/water lick ratios of Gus+/− (closed circles) and Gus−/− (open circles) mice for a range of concentrations of eight 'bitter' taste stimuli. See Table 1 for an analysis of the results within each panel. The same mice within each genotype (n = 10–12) were tested with all eight taste stimuli in separate tests. Each mouse was offered the entire range of concentrations of a taste stimulus plus water in blocks of 4–7 stimuli presented randomly without replacement. Responses to each taste stimulus were pooled across three test sessions. Each symbol indicates mean ± SE. These data are from experiment 1.

ANOVA revealed significant main effects of genotype and stimulus concentration for all compounds; there was also a significant interaction of the main effects (Table 1). To explore the nature of the interactions, we further analyzed the main effect of stimulus concentration, separately for each genotype and aversive taste stimulus. Gus+/− mice showed similar responses to all of the aversive taste stimuli—i.e. they all exhibited significant concentration-dependent reductions in lick responsiveness (in all one-way ANOVAs, $P < 0.05$). Gus−/− mice, on the other hand, showed more variable responses to the eight aversive stimuli. For sparteine and salicin, they exhibited indifference followed by *increases* in lick responsiveness at the highest concentration (in both one-way ANOVAs, $P < 0.05$); for QHCl, cycloheximide and caffeine, they failed to show any concentration-dependent changes in lick responsiveness (in all three one-way ANOVAs, $P > 0.05$); and for denatonium benzoate, quinacrine and urea, the mice exhibited concentration-dependent *decreases* in lick responsiveness, although their avoidance curves were right-shifted in comparison to their +/− littermates (in all three one-way ANOVAs, $P < 0.05$). While the concentration–response curve for QHCl was virtually flat, all of the mean values were significantly greater than 1.0 (one-sample paired *t*-test: in all cases, *t*-value ≥ 3.32, df = 9, $P < 0.01$).

Taken together, these results demonstrate that for all eight aversive taste stimuli, Gus−/− mice show significantly less avoidance than do Gus+/− mice. Further, Gus−/− mice actually lick more vigorously from some concentrations of QHCl, sparteine and salicin than from water alone.

Table 1 Analysis of the lick responsiveness of Gus+/− and Gus−/− mice to eight 'bitter' taste stimuli (see data in Figure 1)

Taste stimulus	Main effect	df	*F*-ratio
QHCl	Genotype	1,17	72.1*
	Concentration	5,85	9.2*
	Interaction	5,85	11.9*
Denatonium	Genotype	1,17	11.5*
	Concentration	4,68	42.8*
	Interaction	4,68	7.3*
Sparteine	Genotype	1,17	39.0*
	Concentration	4,68	5.0*
	Interaction	4,68	26.1*
Salicin	Genotype	1,18	185.2*
	Concentration	2,36	40.9*
	Interaction	2,36	146.6*
Cycloheximide	Genotype	1,17	13.8*
	Concentration	5,85	7.4*
	Interaction	5,85	3.1*
Caffeine	Genotype	1,17	13.3*
	Concentration	2,34	19.0*
	Interaction	2,34	14.5*
Quinacrine	Genotype	1,15	42.0*
	Concentration	4,60	24.0*
	Interaction	4,60	55.5*
Urea	Genotype	1,17	15.9*
	Concentration	2,34	196.9*
	Interaction	2,34	15.8*

Two-way ANOVA, performed separately for each taste stimulus; genotype was a between factor and stimulus concentration a within factor. $n = 10–12$ mice/genotype; $*P \leq 0.05$.

Experiment 2: Are high concentrations of QHCl, cycloheximide and sparteine aversive to Gus−/− mice?

In the previous experiment, Gus −/− mice did not exhibit a concentration-dependent decrease in licking in responsiveness to QHCl, sparteine, cycloheximide, caffeine and salicin. Here, we tested the hypothesis that knocking out α-gustducin completely eliminated the aversive taste of all concentrations of these compounds. To this end, we asked whether Gus−/− mice would display lick avoidance to QHCl, cycloheximide and sparteine when offered concentrations higher than those used in experiment 1. We could not test caffeine because concentrations higher than those used in experiment 1 (i.e. 300 mM) produced toxic effects during 30 min test sessions (J. Glendinning, unpublished data). Salicin also had to be excluded because concentrations higher than those used in experiment 1 were insoluble in water.

Methods

We used the same mice and multi-bottle no-choice testing procedure as in experiment 1. The only difference was that we used a higher range of concentrations of QHCl (1, 3 and 10 mM), cycloheximide (3, 10, 20 and 30 μM) and sparteine (10, 30 and 100 mM). The order of testing the different taste stimuli was as follows: QHCl, cycloheximide and then sparteine.

Because of concerns about the toxicity of the high concentrations of each taste stimulus, we calculated two separate tastant/water lick ratios for each taste stimulus. Ratio 1 was calculated based on licking responses during the first block of trials in test session 1. Ratio 2 was based on licking response during all subsequent blocks of trials across test sessions 1–3. If a taste stimulus elicited an aversive response through a post-ingestive toxicity mechanism, then we expected ratio 2 to be substantially lower than ratio 1 across all concentrations of a taste stimulus. On the other hand, if a taste stimulus elicited an aversive response through a gustatory mechanism, then we expected that ratios 1 and 2 would be similar across all concentrations of a taste stimulus.

We tested these predictions with a two-way ANOVA, treating both Ratio and Stimulus Concentration as within factors. We analyzed each taste stimulus separately.

Results

Gus−/− mice displayed robust concentration-dependent inhibition of licking in response to all three bitter taste stimuli during both the first block of trials (in test session 1) and all subsequent blocks of trials (in test sessions 1–3) (Figure 2). For each 'bitter' taste stimulus, the two-way ANOVA revealed a significant effect of stimulus concentration ($P < 0.05$), but no significant effect of trial ($P > 0.05$) or interaction of stimulus concentration×trial ($P > 0.05$).

These findings, together with those from experiment 1, demonstrate that QHCl, cycloheximide and sparteine all inhibit licking in Gus−/− mice, but only at relatively high concentrations. Further, they provide support for the hypothesis that the inhibition of licking at these high concentrations is mediated by taste, and not post-ingestive toxicity. This is because the concentration–response curves derived from the first block of trials were statistically indiscriminable from those derived from all subsequent blocks of trials.

Experiment 3: Do Gus−/− mice show attenuated lick responsiveness to NaCl, HCl and MSG?

In long-term preference tests, Gus−/− mice exhibit normal preference–response curves across a broad range of NaCl and HCl concentrations (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002; Ruiz *et al.*, 2003), but attenuated preferences for midrange concentrations of monosodium glutamate (MSG; 60 – 100 mM; Ruiz *et al.*, 2003; He *et al.*, 2004). The present experiment sought to address two interpretive limitations of these studies. First, because of their long duration, one cannot determine the relative contribution of taste versus postingestive and/or experiential factors to the ingestive response (see e.g. Bachmanov *et al.*, 2000; Ruiz *et al.*, 2003). Second, because MSG was not always presented with an epithelial sodium channel blocker (e.g. amiloride hydrochloride) for the long-term preference studies, it is impossible to determine the extent to which the taste of the Na^+ ion in MSG contributed to the ingestive response. To address these limitations, we examined the short-term lick response of Gus−/− mice to MSG in the presence of varying concentrations of amiloride.

Methods

Taste stimuli and test concentrations

We tested a range of concentrations of NaCl and NH_4Cl (0.01, 0.1, 0.2, 0.3, 0.6, 1.0 M), HCl (0.1, 1.0, 10, 100 mM) and MSG (0.01, 0.03, 0.1, 0.3, 1.0 M). We ran three separate tests with MSG. In these tests, the five concentrations were mixed with water alone, 10 μM amiloride or 100 μM amiloride (test 3). We used 10 μM amiloride because it has been found to reduce the response of the chorda tympani nerve to the Na^+ component of MSG in B6 mice (He *et al.*, 2004). We also used 100 μM amiloride because it has been found to (i) eliminate the response of the chorda tympani nerve to the Na^+ component of MSG in T1R1 and T1R3 knock-out mice (Zhao *et al.*, 2003), (ii) increase the behavioral detection threshold for sodium in C57BL/6 mice (Eylam and Spector, 2003), but (iii) lack a perceptible taste in mice (Eylam *et al.*, 2003). In all test sessions, we included a water control. When amiloride was mixed with the taste stimulus, it was added to all test solutions, including the water control.

Testing procedure

We used the same mice and multi-bottle no-choice testing procedure as in experiment 1. We ran mice through a single test session with NaCl, NH_4Cl and MSG (with and without amiloride), but had to use three test sessions with HCl to obtain reliable estimates of lick responsiveness. Each mouse was tested with the taste stimuli in the following order: NaCl, NH_4Cl, HCl, MSG without amiloride, MSG in 10 μM amiloride, and MSG in 100 μM amiloride.

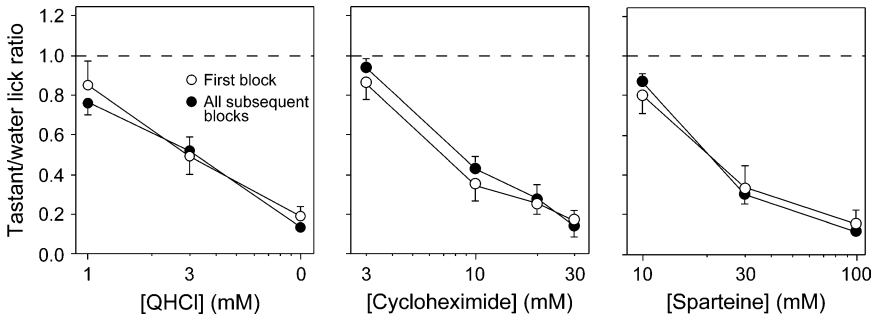

Figure 2 Tastant/water lick ratios of Gus−/− mice for high concentrations of QHCl, cycloheximide and sparteine. Each mouse was offered the entire range of concentrations of a test compound along with water in blocks of 4–5 stimuli presented randomly without replacement. We distinguish licking responses from the first block of taste stimuli during test session 1, and those from all subsequent blocks of taste stimuli across test sessions 1–3. Each symbol represents mean ± SE. These data are from experiment 2.

Data analysis

The tastant/water lick ratios from each taste stimulus were analyzed separately using a two-way ANOVA. Stimulus concentration was a within factor and genotype a between factor. To explore the factors contributing to a significant interaction, we ran unpaired *t*-tests (two-tailed) across genotype at specific MSG concentrations. In addition, to determine whether the mice licked more vigorously from specific concentrations of MSG than water alone, we used a one-sample paired *t*-test (*t*-tailed) to determine whether the tastant/water lick ratios at each MSG concentration differed significantly from 1.0 (i.e. indifference). To control for the use of multiple *t*-tests on a related data set, we used the Bonferroni correction to adjust the α level (i.e. 0.05/number of comparisons).

Results

The Gus−/− and Gus+/− mice both exhibited strong concentration-dependent inhibition of licking for NaCl, NH_4Cl and HCl (Figure 3). Indeed, the analysis of the NH_4Cl and HCl data revealed a significant main effect of concentration, but a non-significant main effect of genotype; the interaction of the main effects was non-significant as well (Table 2). For NaCl, the main effect of genotype was not significant, but the genotype × concentration interaction was significant; this latter finding reflects the fact that 0.6 and 1.0 M NaCl inhibited licking less effectively in the Gus−/− mice (for both concentrations, unpaired *t*-value > 5.4, df = 34, $P < 0.01$). Taken together, these data show that Gus−/− mice exhibit normal lick avoidance responses to NH_4Cl and HCl, but attenuated lick avoidance responses to high concentrations of NaCl (≥300 mM).

When MSG was mixed with 0 or 10 μM amiloride, mice from both genotypes showed indifference at low concentrations and avoided high concentrations (Figure 4, left and middle panels). Indeed, a two-way ANOVA of the data revealed a non-significant main effect of genotype, but a significant main effect of concentration (Table 3). The significant interactions of genotype × MSG concentration are explained by the fact that 1.0 M MSG was less aversive to Gus−/− mice than to Gus+/− mice, when mixed with 0 or 10 μM amiloride (in both cases, $P < 0.05/5$).

When MSG was mixed with 100 μM amiloride, the mice from each genotype showed qualitatively distinct responses. The Gus+/− mice exhibited an appetitive licking response (i.e. a tastant/water lick ratio > 1.2) to MSG concentrations ≤ 0.3 M, but an aversion to 1.0 M MSG (Figure 4). On the other hand, the Gus−/− mice exhibited a weak appetitive response to all MSG concentrations. These inferences are supported by two statistical analyses. First, a one-way ANOVA revealed a significant main effect of concentration on the tastant/water lick ratios for Gus+/− mice [$F(4,40) = 31.1$, $P < 0.05$], but not for Gus−/− mice [$F(4,28_) = 0.8$, $P > 0.05$]. These latter findings indicate that the affective value of

Table 2 Analysis of lick responsiveness of Gus+/− and Gus−/− mice to NaCl, NH_4Cl and HCl (see data in Figure 3)

Taste stimulus	Main effect	df	*F*-ratio
NaCl	Genotype	1,36	0.1
	Concentration	5,180	187.9*
	Interaction	5,180	9.1*
NH_4Cl	Genotype	1,17	<0.1
	Concentration	5,85	151.7*
	Interaction	5,85	1.9
HCl	Genotype	1,17	0.5
	Concentration	2,34	50.0*
	Interaction	2,34	1.7

Two-way ANOVA, performed separately for each taste stimulus; genotype was a between factor and stimulus concentration a within factor. n = 10–12 mice/genotype; *$P \leq 0.05$.

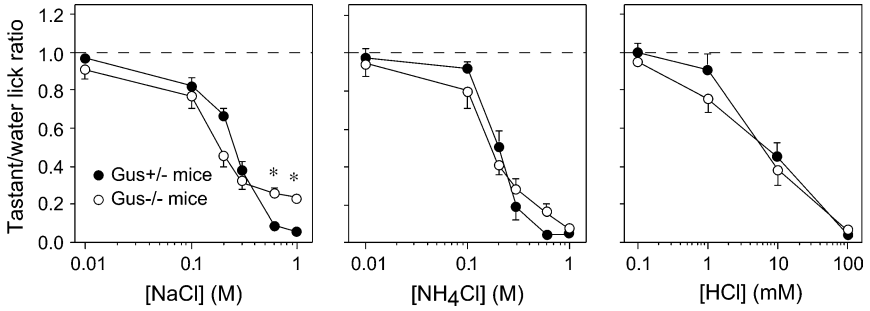

Figure 3 Tastant/water lick ratios of Gus+/− (closed circles) and Gus−/− (open circles) mice across a range of NaCl, NH_4Cl and HCl concentrations. Each mouse was offered the entire range of concentrations of one test compound plus water in blocks of 5–7 stimuli presented randomly without replacement. Mice were tested with each compound in separate sessions. See Table 2 for the results of a two-way ANOVA, performed separately on each panel. Because the interaction of concentration × genotype was significant for NaCl in Table 2, we compared the lick ratios across genotype with the unpaired *t*-test, specifically at the 0.6 and 1.0 M concentrations (*$P < 0.025$). Each symbol represents mean ± SE; n = 9–19 mice/genotype. See Figure 1 for additional details. These data are from experiment 3.

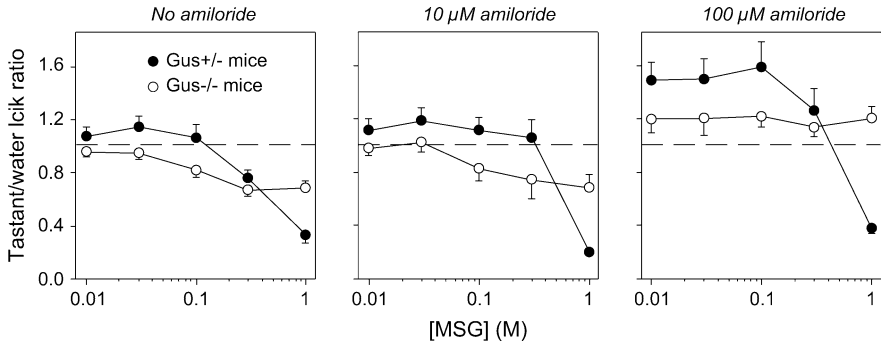

Figure 4 Tastant/water lick ratios of Gus+/– (closed circles) and Gus–/– (open circles) mice for monosodium glutamate (MSG), following 23 h of water deprivation. Each mouse was offered five concentrations of MSG plus a solvent control solution in blocks of six stimuli presented randomly without replacement during a single test session. We dissolved the MSG in one of three amiloride concentrations: 0 μM (left panel), 10 μM (middle panel) or 100 μM (right panel). When amiloride was used, it was added to all test solutions, including the solvent control. See Table 3 for an analysis of the results within each panel. Each symbol represents mean ± SE; n = 8–19 mice/genotype. See Figure 1 for additional details. These data are from experiment 3.

MSG, when mixed with 100 μM amiloride, decreases with increasing concentrations (>100 mM) in Gus+/– mice, but remains stable in Gus–/– mice. Second, a series of one-sample paired t-tests revealed that Gus+/– mice licked significantly more vigorously from the MSG concentrations ≤0.3 M than they did from water alone, but significantly less vigorously from 1 M MSG (all t-values > 3.4; df = 10; P < 0.01). Gus–/– mice, on the other hand, did not exhibit a concentration-dependent change in affective response to MSG; for all concentrations of MSG, except 0.3 M, they licked at slightly higher rates than they did from water alone (all t-values > 3.6; df = 7; P < 0.01).

Taken together, these findings indicate that it is necessary to present MSG in amiloride concentrations of 100 μM to observe robust genotypic differences in lick responsiveness. Under these conditions, Gus–/– mice show weak appetitive responses to a broad range of MSG concentrations (0.01–1.0 M). In contrast, Gus+/– mice show strong appetitive responses to MSG concentrations ranging from 0.01 to 0.1 M, and strong aversive responses to 1.0 M MSG.

Experiment 4: Does knocking out α-gustducin selectively eliminate the aversive taste of MSG?

The results of experiment 3 indicate that knocking out gustducin eliminates the aversive response to 1.0 M MSG, when mixed with 100 μM amiloride. This finding contrasts with earlier reports involving long-term preference tests, which indicated that Gus–/– show normal aversions to 1.0 M MSG, when mixed with 10 μM amiloride (He *et al.*, 2004). In the present experiment, we tested the hypothesis that the results from experiment 3 were an artifact of the water-deprivation procedure, which was used to motivate stimulus-sampling in the gustometer. To this end, we examined the lick response of Gus+/– and Gus–/– mice to MSG when they were in a different physiological state—i.e. one that strongly motivates the mice to ingest nutrients, but not water. This physiological state is produced by 23 h of food- and water-restriction (see below for details; Glendinning *et al.*, 2002).

Table 3 Analysis of the lick responsiveness of Gus+/– and Gus–/– mice to MSG in 0, 10 or 100 μM amiloride (see data in Figure 4)

Taste stimulus	Main effect	df	*F*-ratio
MSG +0 μM amiloride	Genotype	1,33	1.1
	Concentration	4,132	45.9*
	Interaction	4,132	10.6*
MSG + 10 μM amiloride	Genotype	1,18	0.2
	Concentration	4,72	35.8*
	Interaction	4,72	11.8*
MSG + 100 μM amiloride	Genotype	1,17	1.9
	Concentration	4,68	19.9*
	Interaction	4,68	18.2*

Two-way ANOVA on the results, performed separately for each taste stimulus. Genotype was a between factor and taste stimulus concentration was a within factor. *P ≤ 0.05.

Methods

We used mice that were naïve to the taste stimuli. Following training, the mice were subjected to the same multi-bottle no-choice testing procedure as in experiment 3, except that they were food- and water-restricted over the 23 h period immediately prior to testing (see above for details). Each mouse was exposed to five different concentrations of MSG (0.01, 0.03, 0.1, 0.3, 1 M), all mixed with 100 μM amiloride.

Data analysis

We treated the SLR of each mouse to the control solution (i.e. water plus 100 μM amiloride) as a baseline response,

and then sought to determine the lowest concentration of MSG that elicited a significantly elevated (or depressed) SLR. To this end, we used the paired *t*-test, separately for each concentration. We controlled for the use of multiple *t*-tests with a Bonferroni correction.

Results

The Gus−/− and Gus+/− mice both showed concentration-dependent increases in SLR for the lower range of MSG concentrations (Figure 5). The lowest concentration that elicited a SLR significantly above the baseline response was 0.03 M for Gus+/− mice and 0.1 M for Gus−/− mice. The most notable genotypic difference occurred at the high MSG concentrations (Figure 5). While the SLR of Gus−/− mice increased linearly with MSG concentration, that of Gus+/− mice showed a much more shallow increase reaching a maximum at 0.1 M and then plummeted to a value significantly below baseline at 1 M.

These findings corroborate those from the previous experiment, and thus demonstrate that knocking out α-gustducin selectively eliminates the aversive taste of 1.0 M MSG when mixed with 100 μM amiloride.

Experiment 5: Do Gus−/− mice show attenuated lick responsiveness to natural and artificial sweeteners?

Previous studies have shown that knocking out α-gustducin markedly attenuates the preference of mice for a broad range of concentrations of sucrose and SC45647 (an artificial sweetener) in 48 h preference tests (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002; Ruiz *et al.*, 2003). Here, we

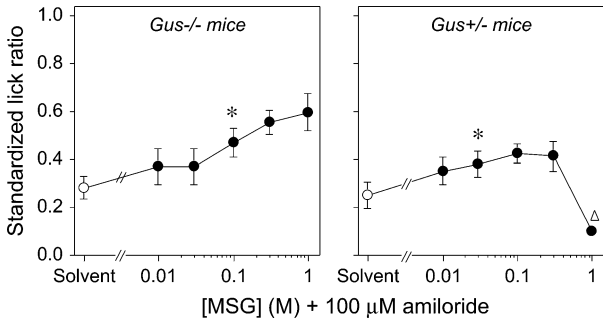

Figure 5 Standardized lick ratios (SLRs) for Gus−/− (left panel) and Gus+/− (right panel) mice across a range of MSG concentrations. To minimize the contribution of the sodium ion to the taste of glutamate, we added 100 μM amiloride to all solutions. We compared the mean SLR for dH_2O (plus 100 μM amiloride) with that to each successively higher concentration of MSG (separately for each panel), using the Wilcoxon matched-pairs signed-rank test. We indicate the lowest MSG concentration that elicited a significantly elevated (*) or reduced (△) SLR relative to water. (α = 0.05/5 according to a Bonferroni correction). Each mouse was offered the entire range of concentrations of MSG along with water in blocks of six stimuli presented randomly without replacement during a single test session. Each symbol represents mean ± SE; *n* = 8–19 mice/genotype. These data are from experiment 4.

examined the lick response of Gus+/− and Gus−/− mice to a broad range of concentrations of sucrose, maltose, fructose, SC45647 and two mixtures of varying-length glucose polymers (Polycose and maltooligosaccharide).

Polycose and maltooligosaccharide are highly palatable to rodents (Davis and Breslin, 2000; Bachmanov *et al.*, 2001; Ramirez, 1994), but not to humans (Feigin *et al.*, 1987). Polycose is a mixture of glucose polymers, ranging from glucose (G1) to G30 or higher; ~9% of Polycose is glucose and maltose. Maltooligosaccharide is a more uniform mixture of glucose polymers, ranging from G2 to G8; only 1.5% is G2. Behavioral and electrophysiological studies indicate that the taste quality of Polycose differs from that of sucrose in rats and hamsters (Nissenbaum and Sclafani, 1987; Sako *et al.*, 1994; Rehnberg *et al.*, 1996), but its taste quality has not been investigated in mice.

Methods

The test stimuli were as follows: sucrose (0.03, 0.1, 0.2, 0.3, 0.6 and 1 M), SC45647 (0.01, 0.03, 0.1, 0.3, 1 and 3 mM), maltose (0.03, 0.1, 0.2, 0.3, 0.6 and 1 M), fructose (0.03, 0.1, 0.2, 0.3, 0.6 and 1 M), Polycose (0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 M) and maltooligosaccharide (14, 28, 55, 111, 221, 442 mM). We determined the molar concentrations of Polycose based on an average molecular weight of 1000 daltons (provided by the manufacturer), and that of maltooligosaccharide based on an average molecular weight of 724 daltons (using the distribution of polymer lengths provided by the manufacturer). The SC45647 was obtained as a gift from Goran Hellekant (University of Wisconsin), the Polycose was purchased from Abbott Laboratories (Columbus, OH), and the maltooligosaccharide was purchased from Pfanstiehl Laboratories (Waukegan, IL).

Testing procedure

We tested the same mice, and used the same multi-bottle no-choice testing procedure, as in experiment 4. Each mouse was tested with all six taste stimuli, in the following order: sucrose, SC45647, maltose, fructose, Polycose and then maltooligosaccharide.

Data analysis

The SLR from each taste stimulus was analyzed separately using a two-way ANOVA; stimulus concentration was a within factor and genotype a between factor.

Results

The mice showed robust concentration-dependent increases in lick responsiveness to the taste stimuli (Figure 6). For all stimuli, except sucrose, there was a significant main effect of concentration, but a non-significant main effect of genotype and a non-significant interaction of concentration × genotype (Table 4). The non-significant main effect of genotype indicates that both genotypes had equal lick responsiveness

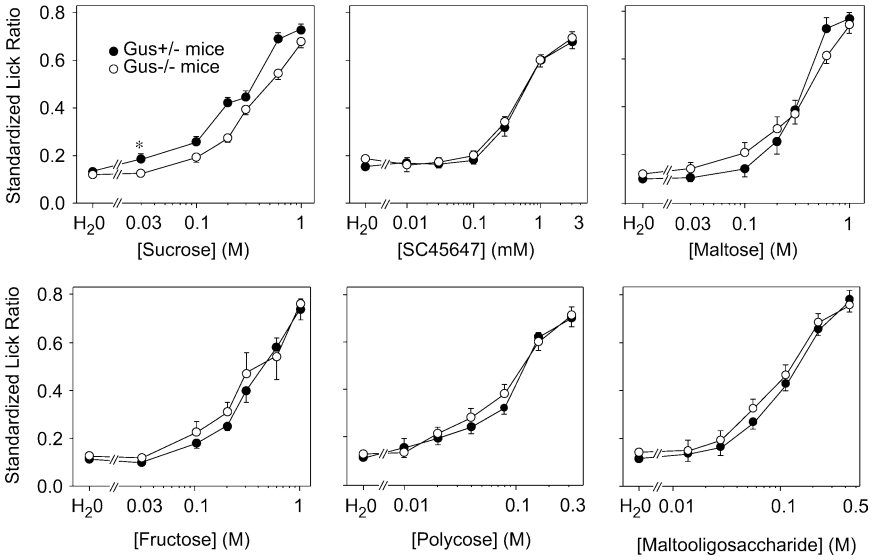

Figure 6 Standardized lick ratios for Gus−/− (open circles) and Gus+/− (closed circles) mice across a range of concentrations of sucrose, SC45647, maltose, fructose, Polycose and maltooligosaccharide. All mice had been food- and water-restricted prior to testing. Each mouse was offered the entire range of concentrations of a sweetener plus water in blocks of seven stimuli presented randomly without replacement during a single test session. See Table 4 for an analysis of the results within each panel. We indicate (with an asterisk) the lowest concentration of sucrose that elicited a SLR that was significantly higher in Gus+/− than in the Gus−/− mice (unpaired t-test; $P < 0.05$). Each symbol represents mean ± SE; n = 8–17 mice/genotype. These data are from experiment 5.

to a broad range of natural and artificial sweeteners. For sucrose, on the other hand, there was a significant main effect of concentration *and* genotype (Table 4). Further, the interaction of concentration × genotype was significant.

Taken together, the results of this assay indicate that knocking out α-gustducin has little impact on lick responsiveness of mice to a structurally diverse array of natural and artificial sweeteners. The only exception was sucrose—Gus−/− mice exhibited a small, but significant, attenuation in lick responsiveness to this natural sweetener.

Experiment 6: Is lick responsiveness of Gus−/− mice to sucrose and SC45647 influenced by water- and food-restriction?

Taste nerve recordings and long-tem preference tests indicate that Gus−/− mice are substantially less responsive than Gus+/− mice to sucrose and SC45647 (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002, 2004; Ruiz *et al.*, 2003). In contrast, the results of experiment 5 indicate that Gus−/− mice show only moderately attenuated lick responsiveness to sucrose and normal lick responsiveness to SC45647. To explain these contradictory findings, we hypothesized that the food- and water-restriction procedure (used in experiment 5) disproportionately elevated lick responsiveness of the Gus−/− mice to sweeteners. If so, then we predicted that non-restricted Gus−/− mice should generate results more consistent with those reported in taste-nerve recordings and long-tem preference tests—i.e. they should lick substantially less vigorously than Gus+/− mice across a range of sucrose and SC45647 concentrations.

Methods

Training procedure

We used the same mice as in experiment 5, but trained them to perform in the gustometer in a non-deprived state. At the beginning of the dark cycle, we removed the mouse from its home cage and placed it in a gustometer. The mouse was given 60 min to acclimate to the gustometer prior to each training session. During training days 1 and 2, we presented a 0.3 M Polycose solution in a stationary spout over a 60 min test session. We used the Polycose solution because it stimulates licking, but was different than the test stimuli used in the experimental phase of testing. During training day 3, the 0.3 M polycose solution was presented during successive 5 s trials, separated by a 7.5 s inter-trial interval (as described in experiment 1). The mouse could initiate as many trials as it wanted over the 60 min session. By the end of training day 3, all mice had learned to drink vigorously from the sipper tubes. Parenthetically, we had to illuminate the room containing the gustometer with a 40 W red light bulb as testing was conducted in the dark phase of the light:dark cycle.

Testing procedure

We used the same mice, multi-bottle no-choice testing procedure, and concentrations of sucrose and SC45647 as in experiment 5. The only difference was that we tested the mice on two successive days and pooled together the results for the analysis; this was necessary because the non-deprived mice initiated fewer numbers of trials. To control for any effects of stimulus testing order, we used a counter-balanced design

Table 4 Analysis of the lick responsiveness of Gus+/– and Gus–/– mice to sucrose, SC45647, maltose, fructose, Polycose and maltooligosaccharide (see data in Figure 6)

Taste stimulus	Main effect	df	*F*-ratio
Sucrose	Genotype	1,37	21.1*
	Concentration	5,185	235.7*
	Interaction	5,185	2.0
SC45647	Genotype	1,37	0.9
	Concentration	5,185	161.6*
	Interaction	5,185	0.2
Maltose	Genotype	1,25	< 0.1
	Concentration	5,125	126.8*
	Interaction	5,125	2.2
Fructose	Genotype	1,15	0.5
	Concentration	5,75	60.7*
	Interaction	5,75	0.4
Polycose	Genotype	1,37	0.1
	Concentration	5,185	134.9*
	Interaction	5,185	0.6
Maltooligosaccharide	Genotype	1,37	0.4
	Concentration	5,185	231.1*
	Interaction	5,185	0.6

Two-way ANOVA, performed separately for each taste stimulus, on the response to six concentrations of each sweetener; the response to water alone was excluded from the analysis. Genotype was a between factor and stimulus concentration was a within factor. *$P \leq 0.05$.

(i.e. half of the mice were tested initially with sucrose, and the other half with SC45647). Because the mice were not food- and water-restricted, we could test them on successive days.

Data analysis

We ran a two-way ANOVA on the SLRs, with genotype as a between factor and concentration as a within factor. The ANOVAs were conducted separately for each taste stimulus.

Results

The Gus +/– and Gus –/– mice both exhibited strong concentration-dependent increases in SLR for sucrose and SC45647 (Figure 7). For sucrose, there was a significant main effect of genotype and concentration, and a significant interaction of genotype × concentration (Table 5). The significant interaction reflects the fact that lick responsiveness of both genotypes was identical at low concentrations but diverged at the midrange and high concentrations, with the Gus+/– mice licking slightly more vigorously. For SC45647, there was a significant main effect of concentration, but no significant main effect of genotype or interaction of genotype × concentration (Table 5).

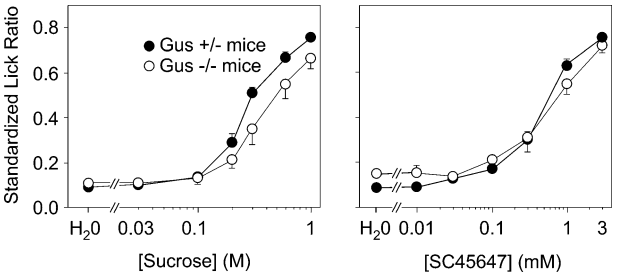

Figure 7 Standardized lick ratios of non-restricted Gus+/– (closed circles) and Gus–/– (open circles) mice across a range of concentrations of sucrose (left panel) and SC45647 (right panel). Each mouse was offered six concentrations of a sweetener plus water in blocks of seven stimuli presented randomly without replacement during a single test session. See Table 5 for an analysis of the results within each panel. We indicate mean ± SE; n = 9–20 mice/panel. These data are from experiment 6.

Taken together, these results contradict the hypothesis that that the food- and water-restriction procedure disproportionately increased the lick responsiveness of Gus–/– mice. This is because the results from non-deprived mice in this experiment are qualitatively and quantitatively similar those from food- and water-restricted mice in the previous experiment.

Experiment 7: Is lick responsiveness of Gus–/– mice to natural and aritificial sweeteners influenced by the stimulus presentation method?

When Gus+/– and Gus–/– mice were examined using long-term preference tests with sucrose and SC45647 (Wong *et al.*, 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002; Ruiz *et al.*, 2003), they were (i) offered a choice between water and a taste stimulus over a 48 h test session, and (ii) tested with different concentrations of each taste stimulus during separate 48 h tests, in an ascending concentration series. In contrast, when Gus+/– and Gus–/– mice were tested with the brief-access procedure in experiments 5 and 6, they were offered a broad range of concentrations of each specific taste stimulus during a single 30 min test session (in a randomized block design).

Here, we tested the hypothesis that the different methods of stimulus presentation contributed to the contradictory results generated by long-term preference tests and brief-access taste tests. To this end, we redesigned the brief-access taste test so that it used a method of stimulus presentation that more closely approximated that of the two-bottle preference test. We predicted that Gus–/– mice would be substantially less responsive than Gus+/– mice to sucrose, SC45647 and maltose.

Methods

We used the same mice as in experiments 4–6, but subjected them to a two-bottle no-choice testing procedure. That is, we

Table 5 Analysis of the lick responsiveness of non-restricted Gus+/− and Gus−/− mice to a range of concentrations of sucrose and SC45647 (see data in Figure 7)

Taste stimulus	Main effect	df	*F*-ratio
Sucrose	Genotype	1,15	5.5*
	Concentration	5,75	180.9*
	Interaction	5,75	3.4*
SC45647	Genotype	1,15	0.6
	Concentration	5,75	175.4*
	Interaction	5,75	0.5

Two-way ANOVA, performed separately for each taste stimulus, on the response to six concentrations of each sweetener; the response to water alone was excluded from the analysis. Genotype was a between factor and stimulus concentration a within factor. *$P \leq 0.05$.

presented each mouse with two solutions per 30 min test session—water and a specific sweetener concentration. To control for potential order effects across trials within the 30 min test session, we treated the two test solutions as a block, and randomized (without replacement) the presentation sequence of each solution within each block. The mouse was permitted to initiate as many trials (and hence, blocks) as possible throughout the test session.

We tested three sweeteners in the following order: sucrose, SC45647 and then maltose. To mimic the stimulus presentation sequence used in the long-term preference tests, we presented six concentrations of each taste stimulus in an ascending series (across successive test sessions). We used the same concentrations of each sweetener as in experiment 5.

Data analysis

To make our data comparable to those of the long-term preference tests, we calculated a preference ratio, separately for each sweetener concentration and mouse. We first calculated the mean number of licks across all trials with each sweetener solution and the mean number of licks across all trials with each corresponding water solution. Then, we divided the mean number of licks from the sweetener solution by the mean total number of licks from both the sweetener solution and water. A preference ratio of 0.5 indicates indifference to the sweetener solution, whereas a preference ratio of 1.0 indicates a strong preference for the sweetener solution. We subjected the preference ratio data to a two-way ANOVA, treating genotype as a between factor and concentration as a within factor. To help clarify significant interaction, we also made comparisons across genotypes with an unpaired *t*-test, separately for each sweetener concentration. To control for the use of multiple *t*-tests, we corrected the α level with a Bonferroni correction (i.e. α level = 0.05/number of comparisons).

To calculate a preference threshold, we calculated the lowest concentration that generated a preference ratio that was significantly above 0.5, separately for each genotype and taste stimulus, using a one-sample paired *t*-test (α level = 0.05, before Bonferroni correction).

To examine lick responsiveness, we initially calculated SLRs for the responses to each sweetener solution and to water. Then, we analyzed the ratios in two ways. First, we determined the lowest sweetener concentration that elicited a SLR significantly greater than that elicited by water alone, separately for each genotype and sweetener. This was accomplished with a paired *t*-test.. Second, we asked whether the SLRs were higher in Gus+/− mice that in Gus−/− mice, using separate two-way ANOVAs for each sweetener. We treated genotype as a between factor and sweetener concentration as a within factor.

Results

We found a significant main effect of concentration on the preference ratios for all three sweeteners, illustrating that both genotypes showed strong concentration-dependent increases in preference (Figure. 8a, Table 6). The significant main effect of genotype for all three sweeteners (Table 6) reveals, however, that the preferences were generally lower in Gus−/− mice. This genotypic difference was not apparent at all sweetener concentrations, as indicated by the significant interaction of concentration and genotype (Table 6). In fact, the preference ratios of Gus−/− mice were significantly lower than those of Gus+/− mice (i) at all concentrations of sucrose except 1 M; (ii) at all concentrations of SC45647 except 0.1 and 3 mM; and (iii) at only one concentration of maltose, 0.2 M (in all cases, *t*-value > 3.05, df = 17, $P < 0.05/6$).

The analyses of the SLRs corroborated those of the preference ratios (Table 6). There was a significant main effect of concentration for all three sweeteners, showing that the Gus+/− and Gus−/− mice both exhibited strong concentration-dependent increases in lick responsiveness (Figure 8b). There was also a significant main effect of genotype for sucrose and SC45647 (but not maltose), illustrating that the concentration-dependent increase in licking was less marked in the Gus−/− mice, at least for two of the sweeteners. The effect of knocking out gustducin on the maltose response was revealed by the significant interaction between concentration and genotype. This interaction shows that knocking out gustducin selectively attenuated lick responsiveness to mid-range concentrations of maltose. This latter finding is consistent with the significant genotypic differences in preference ratios described above for 0.2 M maltose.

We ran two additional analyses to determine the specific effect of knocking out gustducin on lick responsiveness to low concentrations of the three sweeteners. First, the preference thresholds of Gus+/− mice were lower than those of Gus−/− mice for sucrose and SC45647, but not for maltose (Figure 8a). Second, the lowest concentration of sucrose and

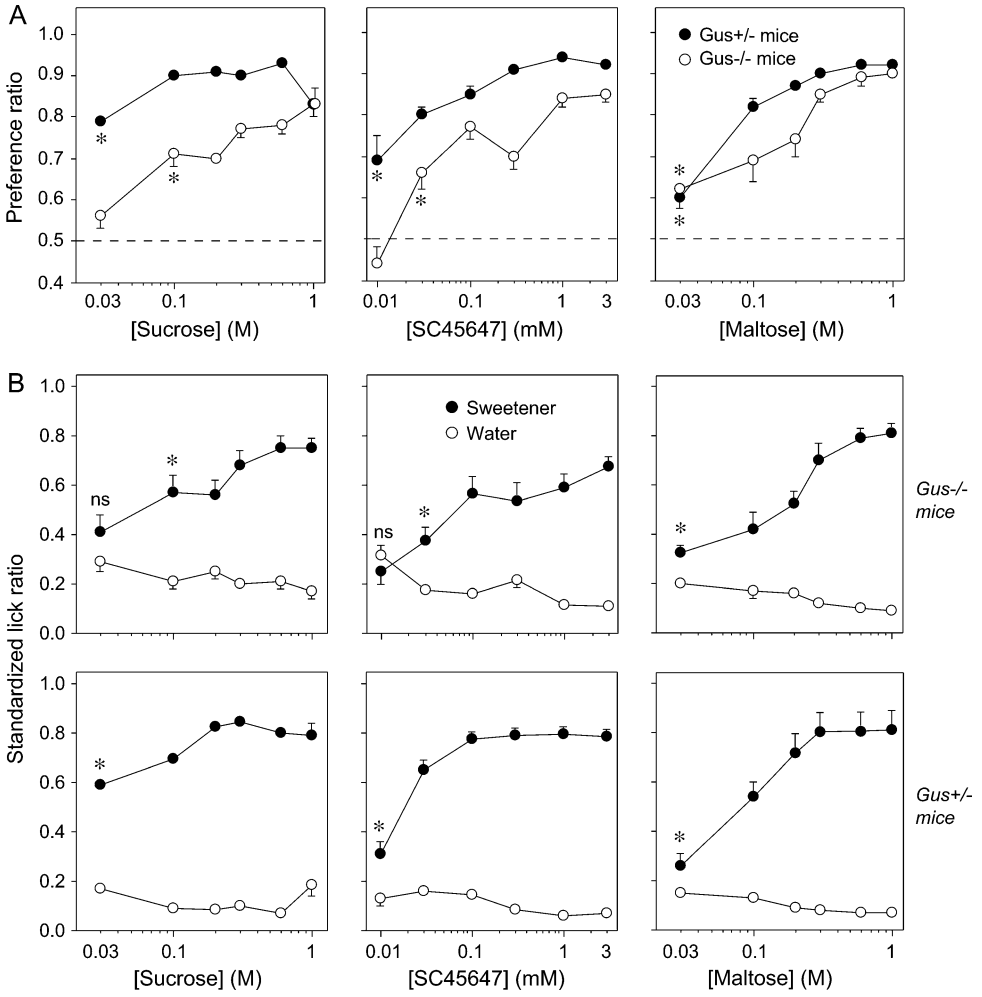

Figure 8 Lick responsiveness of Gus+/– and Gus–/– mice to a range of concentrations of natural (sucrose and maltose) and artificial (SC45647) sweeteners. In each test session, a mouse received blocks of two fluid stimuli presented randomly without replacement: one stimulus contained a specific sweetener solution (e.g. 0.03 M sucrose) and the other contained water. **(A)** Preference ratios from both genotypes, calculated separately for each sweetener. In each panel, we indicate the preference threshold concentration with an asterisk; the preference threshold is the lowest concentration that elicited a preference ratio significantly above 0.5 (one-sample *t*-test, $P \leq 0.05$). **(B)** Standardized lick ratios for each sweetener concentration and water, presented separately for Gus–/– mice (top row of panels) and Gus+/– mice (bottom row of panels). We indicate with an asterisk the lowest concentration of each sweetener that elicited a ratio significantly higher than did water alone (paired *t*-test, $P \leq 0.05$). See Table 6 for an analysis of the results within each panel. We show mean ± SE; $n = 9$–11 mice/panel. These data are from experiment 7.

SC45647 both elicited significantly greater SLRs than water alone in the Gus+/– mice, but not in the Gus–/– mice; the lowest concentration of maltose, however, elicited statistically similar SLRs in both genotypes (Figure 8b).

Taken together, these results demonstrate that the method of stimulus presentation influences lick responsiveness of mice to natural and artificial sweeteners. When we offered multiple concentrations of a sweetener during a test session (in experiment 5), there was a small but significant genotypic difference in lick responsiveness to sucrose, but not to SC45647 and maltose. In contrast, when we offered one concentration of a sweetener during a test session (in this experiment), there was a significant genotypic difference in lick responsiveness to all three sweeteners. This latter finding reflects the fact that the concentration–response curves for Gus–/– mice were all right-shifted in comparison to their +/– littermates.

Discussion

'Bitter' compounds

Our results demonstrate that α-gustducin plays a key role in the taste-mediated aversion of mice to 'bitter' taste stimuli. While previous studies have drawn the same inference (Wong *et al*., 1996; He *et al*., 2002; Caicedo *et al*., 2003; Ruiz *et al*., 2003), the generality of those conclusions was limited because the previous studies tested only two 'bitter' taste

Table 6 Analysis of the lick responsiveness of Gus+/− and Gus−/− mice to a range of concentrations of sucrose, SC45647 and maltose (see data in Figure 8)

Dependent measure	Taste stimulus	Main effect	df	F-ratio
Preference ratio	Sucrose	Genotype	1,18	22.4*
		Concentration	5,90	20.6*
		Interaction	5,90	8.0*
	SC45647	Genotype	1,17	39.3*
		Concentration	5,85	34.1*
		Interaction	5,85	3.7*
	Maltose	Genotype	1,17	12.5*
		Concentration	5,85	49.7*
		Interaction	5,85	2.5*
Standardized lick ratio	Sucrose	Genotype	1,18	4.9*
		Concentration	5,90	32.8*
		Interaction	5,90	4.7*
	SC45647	Genotype	1,17	9.3*
		Concentration	5,85	78.9*
		Interaction	5,85	4.7*
	Maltose	Genotype	1,17	2.2
		Concentration	5,85	83.5*
		Interaction	5,85	4.4*

Two dependent measures—preference ratio and standardized lick ratio—were analyzed. For the latter measure, we analyzed the lick responses to the sweetener solutions (and ignored the responses to water alone). We performed a separate two-way ANOVA for each dependent measure and sweetener. Genotype was a between factor, and taste stimulus concentration a within factor. *$P \leq 0.05$.

stimuli (quinine and denatonium). We tested a structurally diverse battery of 'bitter' taste stimuli, and found that deleting the α-gustducin gene significantly attenuated lick responsiveness to all eight taste stimuli, although to varying degrees. For instance, the concentration–response curve for quinacrine and urea was shifted to the right ~0.5 log unit, whereas that for the other six bitter taste stimuli was shifted to the right >1.0 log unit. A recent study by Caceido and Roper (2003), which examined the response of mouse circumvallate taste cells to aversive taste stimuli using *in situ* Ca^{2+} imaging, offers the most likely explanation for our findings. Caicedo and Roper found that α-gustducin null mice displayed a diminished peripheral gustatory response to quinine and denatonium by reducing both the number of quinine- and denatonium-sensitive taste cells and the sensitivity of individual taste cells to quinine and denatonium. We propose that knocking out α-gustducin has a similar effect on the peripheral response to the other aversive taste stimuli tested herein, and that this reduced peripheral responsiveness is what shifted the concentration–avoidance curves to the right.

The fact that Gus−/− mice avoided high concentrations of QHCl, sparteine and cycloheximide solutions could be interpreted in two ways. First, it is possible that these solutions elicited a weak taste that was not aversive, but as a result of repeatedly ingesting them across numerous trials, they began to associate their toxic post-ingestive consequences with the weak taste and thus developed a conditioned taste aversion (CTA) to them. We were able to reject this CTA hypothesis by showing that Gus−/− mice displayed robust lick avoidance of high concentrations of QHCl, sparteine and cycloheximide solutions during their initial encounter with them (i.e. during the first 5 s trial). It is unlikely that all three compounds could have elicited toxic effects so rapidly. A second, and more likely explanation is that high concentrations of QHCl, sparteine and cycloheximide elicited a taste sensation that was aversive to the mice. The logical implication of this finding is that α-gustducin is necessary for the taste-mediated aversion to low concentrations of bitter taste stimuli but not for the aversion to high concentrations. In support of this inference, other investigators have observed α-gustducin-independent signaling pathways for bitter taste stimuli, which appear to use Gi2 (Ruiz-Avila *et al.*, 2001; He *et al.*, 2002, 2004; Caicedo *et al.*, 2003).

Finally, it is notable that Gus−/− mice (but not the Gus+/− mice) licked several concentrations of sparteine, salicin and QHCl significantly more rapidly than water alone in experiment 1 (as indicated by a tastant/water lick ratio > 1.0). This finding indicates that deletion of the α-gustducin gene not only diminished the aversiveness of some bitter taste stimuli, but also changed the hedonic polarity of the ingestive response from aversive to slightly appetitive. It should be noted, however, the Gus−/− mice failed to show appetitive licking responses to QHCl, and sparteine in experiment 2, when we presented a higher range of concentrations of both taste stimuli. The discrepancy between the results of experiments 1 and 2 indicate that the appetitive licking response of Gus−/− mice to 'bitter' taste stimuli is displayed only under specific testing conditions—when highly aversive concentrations of a 'bitter' taste stimulus are excluded from the stimulus array presented during a test session.

Salts and acids

The Gus−/− and Gus+/− mice were equally responsive to a broad range of concentrations of NaCl, NH_4Cl and HCl, confirming previous findings (Wong *et al.*, 1996; He *et al.*, 2002; Ruiz *et al.*, 2003). The only exception to this general pattern was that the magnitude of the aversive response to NaCl concentrations >300 mM were slightly, but significantly, attenuated in Gus−/− mice. We believe that this latter finding is real given our relatively large sample size (19–20 mice/genotype) and the low amount of variability

across mice within each genotype. More work is needed to understand the mechanistic basis of this finding because current models of sodium taste transduction do not involve G proteins (Glendinning *et al.*, 2000). It is notable that deleting the *PLCβ2* gene, which encodes an intermediary enzyme that is critical in G protein-coupled taste transduction, also attenuates lick responsiveness to 1 M NaCl (Dotson *et al.*, 2005).

MSG

The results of the brief-access test with MSG were complex. The magnitude and hedonic valence of the response depended on the presence or absence of the epithelial sodium channel blocker amiloride in the solution, the concentration of MSG and the deprivation schedule before the test. Perhaps most important with respect to the current study, genotype also had an effect. When the animals were tested under the water-restriction condition (Figure 4), and the stimuli were mixed with 100 μM amiloride to minimize sodium taste, the Gus+/− mice licked low concentrations of MSG at rates greater than water, but then displayed significant avoidance at the highest concentration. In contrast, the Gus−/− mice displayed a flat concentration–response function that was slightly, but significantly, elevated from the baseline licking rate for water. When the animals were tested under food- and water-restriction (Figure 5), the Gus+/− mice displayed a modest but monotonic increase in licking as a function of MSG concentration up to 0.3 M, but at 1.0 M they precipitously dropped their lick rate to levels lower than with water. In contrast, the Gus−/− mice displayed a modest monotonic increase in licking as a function of MSG concentration, without avoidance at even the highest MSG concentration tested. It would appear, therefore, that α-gustducin is necessary for the avoidance response to 1.0 M MSG in the presence of 100 μM amiloride. Further, the fact that Gus−/− mice displayed concentration-dependent increases in licking of MSG (when mixed with 100 μM amiloride), at least when they were tested under partial food and water restriction, indicates that α-gustducin is not necessary for appetitive responses to this stimulus to be expressed.

The ingestive responses of Gus+/− mice to MSG in our brief-access taste test differed from those reported previously in long-term preference tests. Most notably, Gus+/− and Gus+/+ mice have been found to prefer midrange concentrations of MSG (i.e. 0.06–0.1 M) when mixed with 0 μM (Ruiz *et al.*, 2003) or 10 μM (He *et al.*, 2004) amiloride. In the brief-access taste test, Gus+/− mice did not show an increase in lick responsiveness to any concentration of MSG, with the possible exception of 30 mM, when mixed with 0 or 10 μM amiloride. We can propose three explanations for this discrepancy. First, because the mice have access to laboratory chow during the 48 h preference test, a positive taste synergy could occur between the MSG and the laboratory chow, enhancing consumption of the MSG solution (see e.g. Okiyama and Beauchamp, 1998). No food was available during our brief-access taste test. Second, positive post-ingestive feedback and/or experiential effects of MSG appear to enhance subsequent MSG consumption when mice are subjected to successive 24 h preference tests with an ascending series of MSG concentrations (Ruiz *et al.*, 2003). These experiential effects would not manifest themselves in the brief-access taste test because all MSG concentrations are tested within a single 30 min test session. Third, the water-deprivation schedule that we used in experiment 3 could have caused the mice to lick at their maximal rate from all aqueous taste stimuli, owing to their high level of thirst. If so, then this would have created a ceiling effect with our tastant/water lick ratio, impairing our ability to detect a positive hedonic effect of MSG in water-deprived mice. This latter possibility seems unlikely, however, given that water-deprived Gus +/− mice exhibited a positive (and significant) hedonic response to concentrations of MSG ≤ 0.1 M when mixed with 100 μM amiloride (see Figure 4, right panel).

As a caveat in the interpretation of the behavioral results discussed here, it is important to recognize that even the addition of amiloride does not necessarily eliminate the possibility that the sodium ion was influencing responses to the glutamate. Although amiloride is virtually tasteless to rodents (Markison and Spector, 1995; Eylam *et al.*, 2003) and effectively blocks the selective transcellular sodium transduction pathway, it does not remove the sodium cation from the stimulus. Consequently, we cannot entirely rule out that Na^+ was transduced through amiloride-insensitive pathways or exerted general osmotic or ionic effects on taste receptor cell function especially at the higher concentrations. Thus, the sodium ion renders MSG a physically and experimentally complex stimulus for studying G-protein-related transduction processes, and is likely complicit in the complicated behavioral responses elicited by this stimulus.

Natural and artificial sweeteners

In experiment 5, the Gus−/− and Gus+/− mice exhibited statistically indiscriminable lick responsiveness to a broad range of concentrations of SC45647, maltose, fructose, Polycose and maltooligosaccharide (Figure 6). The only compound to which the two genotypes differed was sucrose—the Gus−/− mice showed a small but significant attenuation in lick responsiveness. These findings contrast with prior studies, which reported that Gus−/− mice show highly attenuated (i) preferences for sweeteners in long-term preference tests, and (ii) responsiveness of the chorda tympani and glossopharyngeal nerves to lingual stimulation with sweeteners (Wong *et al.* 1996; Ruiz-Avila *et al.*, 2001; He *et al.*, 2002, 2004).

One explanation for this discrepancy is that the brief-access taste test under water- and food-restriction (used in

experiment 5) has limited power for detecting behavioral effects of knocking out proteins involved in the transduction of sweeteners. Two lines of evidence argue against this explanation, however. First, others have reported dramatically attenuated lick responsiveness to sweeteners in mice with null mutations for T1R2, T1R3, T1R2+T1R3 and TRPM5, using the same testing procedure as described in experiment 5 (Zhang *et al.*, 2003; Zhao *et al.*, 2003). Second, we demonstrated in experiment 6 that our use of a food- and water-restriction procedure did not alter lick responsiveness to sweeteners in either genotype.

Although we were able to observe significant reductions in lick responsiveness of Gus−/− mice to a battery of sweeteners in experiment 7, it required the use of testing procedures that optimized the power of the brief-access taste test. Thus, knocking out gustducin does reduce lick responsiveness to sweeteners, but the magnitude of this reduction is substantially less than that produced by knocking out, for instance, TRPM5 (Zhang *et al.*, 2003). More work is needed to explain why knocking out gustducin produces only modest deficits in lick responsiveness to sweeteners during short-term lick tests, but large deficits during long-term preference tests. It may be that the afferent input from the peripheral taste system of Gus−/− mice is sufficient to drive vigorous licking from sweeteners in short-term lick tests, but not in long-term preference tests.

The results of experiment 7 also highlight a contextual effect that may confound interpretation of results from a brief-access taste test—its ability to detect deficits in lick responsiveness to sweeteners varies as a function of the size of the stimulus array presented during a test session. One can increase the test's power simply by reducing the stimulus array from seven (i.e. water and six concentrations of sweetener) to two (i.e. water and one concentration of sweetener). This finding reveals that investigators should be cautious about concluding that a specific genetic manipulation does not alter lick responsiveness to sweeteners if they present a large stimulus array during a test session. This contextual effect most likely involves a type of *simultaneous contrast effect*, in which the reward value of a low sweetener concentration is depreciated by the presence of a higher sweetener concentration (Grigson *et al.*, 1993; Flaherty *et al.*, 1995; Flaherty and Mitchell, 1999). Such simultaneous contrast effects could explain why both Gus−/− and Gus+/− mice showed negligible lick responsiveness to low and intermediate concentrations of sucrose, SC45647 and maltose in Figure 6, but robust lick responsiveness to the same concentrations of the same sweeteners in Figure 8b.

Finally, we should note that as a result of testing the same mice with caloric sweeteners in experiments 5–7, the mice could have formed positive associations between the weak orosensory properties of the caloric sweeteners and their post-ingestive rewarding properties (e.g. Sclafani and Glendinning, 2003). Such an associative learning process could have inflated the affective value of low and intermediate concentrations of sweeteners (caloric and noncaloric). While further tests are needed to evaluate this possibility, we have shown elsewhere (Glendinning *et al.*, 2002) that concentration–response curves for sucrose, generated during brief-access taste tests with C57BL/6J mice, are highly stable across three consecutive test sessions.

Conclusion

This study constitutes the first comprehensive assessment of short-term lick responsiveness of Gus−/− mice to a variety of taste stimuli. While our findings generally agree with those reported from 48 h preference tests and nerve recordings, they offer several novel insights. First, knocking out α-gustducin strongly attenuated lick responsiveness to a structurally diverse battery of 'bitter' taste stimuli, indicating that it plays a key role in the taste-mediated aversion to this class of taste stimuli. The fact that Gus−/− mice displayed significant decreases in lick responsiveness to high concentrations of some 'bitter' compounds shows that the taste-mediated aversion to high concentrations of this class of taste stimuli does not depend solely on α-gustducin. Second, the deletion of α-gustducin completely eliminated the avoidance response to 1.0 M MSG (when mixed with 100 μM amiloride), but had more modest effects on the appetitive response to low and intermediate concentrations of MSG (when mixed with 100 μM amiloride). This suggests that high concentrations of MSG might engage an α-gustducin-dependent transduction pathway, which leads to the generation of an aversive taste. Third, knocking out α-gustducin weakly attenuated lick responsiveness to several sweeteners, but only under specific testing conditions.

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References

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J.P. and **Zucker, C.S.** (2000) *A novel family of mammalian bitter taste receptors*. Cell, 100, 693–702.

Bachmanov, A.A., Tordoff, M.G. and **Beauchamp, G.K.** (2000) *Intake of umami-tasting solutions by mice: a genetic analysis*. J. Nutr., 130, 935S–941S.

Bachmanov, A.A., Tordoff, M.G. and **Beauchamp, G.K.** (2001) *Sweetener preference of C57BL/6ByJ and 129P3/J mice*. Chem. Senses. 26, 905–913.

Boughter, J.D., St. John, S.J., Noel, D.T., Ndubuizu, O. and **Smith, D.V.** (2002) *A brief-access test for bitter taste in mice*. Chem. Senses, 27, 133–142.

Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J.-D. and **Meyerhof, W.** (2002) *The human TAS2R16 receptor mediates bitter taste in response to b-glucopyranosides*. Nature Gen., 32, 397–401.

Caicedo, A., Pereira, E., Margolskee, R.F. and **Roper, S.D.** (2003) *Role of the G-protein subunit alpha-gustducin in taste cell responses to bitter stimuli*. J. Neurosci., 23, 9947–9952.

Chandrashekar, J., Mueller, J., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zucker, C.S. and **Ryba, N.J.P.** (2000) *T2Rs function as bitter taste receptors*. Cell, 100, 703–711.

Clapp, T.R., Stone, L.M., Margolskee, R.F. and **Kinnamon, S.C.** (2001) *Immunocytochemical evidence for co-expression of Type III IP_3 receptor with signaling components of bitter taste transduction*. BMC Neurosci., 2, 6.

Davis, J.D. and **Breslin P.A.** (2000) *A behavioral analysis of the ingestion of glucose, maltose and maltooligosaccharide by rats*. Physiol. Behav., 69, 477–485.

Dotson, C.D. and **Spector, A.C.** (2004) *The relative affective potency of glycine, L-serine, and sucrose as assessed by a brief-access taste test in inbred strains of mice*. Chem. Senses, 29, 489–498.

Dotson, C.D., Richter, T.A., Roper, S.D. and **Spector, A.C.** (2005) *PLC2 knock-out mice display lick avoidance to high concentrations of quinine and denatonium*. Chem. Senses, in press.

Eylam, S. and **Spector, A.C.** (2003) *Oral amiloride treatment decreases taste sensitivity to sodium salts in C57BL/6J and DBA/2J mice*. Chem. Senses, 28, 447–458.

Eylam, S., Tracy, T., Garcea, M. and **Spector, A.C.** (2003) *Amiloride is an ineffective conditioned stimulus in taste aversion learning in C57BL/6J and DBA/2J mice*. Chem. Senses, 28, 681–689.

Feigin, M.B., Sclafani, A. and **Sunday, S.R.** (1987) *Species differences in polysaccharide and sugar taste preferences*. Neurosci. Biobehav. Rev., 11, 231–240.

Flaherty, C.F. and **Mitchell, C.** (1999) *Absolute and relative rewarding properties of fructose, glucose, and saccharin mixtures as reflected in anticipatory contrast*. Physiol. Behav., 66, 841–853.

Flaherty, C.F., Coppotelli, C., Grigson, P.S., Mitchell, C. and **Flaherty, J.E.** (1995) *Investigation of the devaluation intepretation of anticipatory negative contrast*. J. Exp. Psychol. Anim. Behav. Proc., 21, 229–247.

Flynn, F.W. and **Grill, H.J.** (1988) *Effects of concentration presentation order and intaoral delivery on sucrose intake*. Behav. Neurosci., 102, 992–994.

Fregly, M.J. and **Rowland, N.** (1992) *Comparison of preference thresholds for NaCl solution in rats of the Sprague–Dawley and Long–Evans strains*. Physiol. Behav., 51, 915–918.

Gilbertson, T.A., Damak S. and **Margolskee R.F.** (2000) *The molecular physiology of taste transduction*. Curr. Opin. Neurobiol., 10, 519–527.

Glendinning, J.I., Chaudhari, N. and **Kinnamon, S.C.** (2000) *Taste transduction and molecular biology*. In: T. Finger, W.L. Silver and D. Restrepo (eds), The Neurobiology of Taste and Smell, 2nd edn. Wiley-Liss, New York, pp. 315–351.

Glendinning, J.I., Gresack, J. and **Spector, A.C.** (2002) *A high-throughput screening procedure for identifying mice with aberrant taste and oromotor function*. Chem. Senses, 27, 461–474.

Grigson, P.S., Spector, A.C. and **Norgren, R.** (1993) *Microstructural analysis of successive negative contrast in free-feeding and deprived rats*. Physiol. Behav., 54, 909–916.

He, W., Danilova V., Zou, S., Hellekant, G., Max, M., Margolskee, R.F. and **Damak, S.** (2002) *Partial rescue of taste responses of a-gustducin null mice by transgenic expression of a-transducin*. Chem. Senses, 27, 719–727.

He, W., Yasumatsu, K., Varadarajan, V., Yamada, A., Lem, J., Ninomiya, Y., Margolskee, R.F. and **Damak, S.** (2004) Umami *taste responses are mediated by a-transducin and a-gustducin*. J. Neurosci., 24, 7674–7680.

Huang, L., Shanker, Y.G., Dubauskaite, J., Zheng, J.Z., Yan, W., Rosenzweig, S., Spielman, A.I., Max, M. and **Margolskee, R.F.** (1999) *Gg13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium*. Nat. Neurosci., 2, 1055–1062.

Kim, M.-R., Kusakabe, Y., Miura, H., Shindo, Y., Ninomiya, Y. and **Hino, A.** (2003) *Regional expression patterns of taste receptors and gustducin in the mouse tongue*. Biochem. Biophys. Res. Commun., 312, 500–506.

Li, X., Staszewski, L., Xu H., Durick, K., Zoller, M. and **Adler, E.** (2002) *Human receptors for sweet and umami taste*. Proc. Natl. Acad. Sci. USA, 99, 4692–4696.

Markison, S. and **Spector, A.C.** (1995) *Amiloride is an ineffective conditioned stimulus in taste aversion learning*. Chem. Senses, 20, 559–563.

Max, M., Shankar, Y.G., Huang, L., Rong, M., Liu, Z., Campagne, F., Weinstein, H., Damak, S. and **Margolskee, R.F.** (2001) *Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac*. Nature Gen., 28, 58–63.

Mook, D.G. (1963) *Oral and postingestional determinants of the intake of various solutions in rats with esophageal fistulas*. J. Comp. Physiol. Psychol., 56, 645–659.

Nelson, G., Hoon, M.A., Chadrashekar, J., Zhang, Y., Ryba, N.J. and **Zucker, C.S.** (2001) *Mammalian sweet receptors*. Cell, 106, 381–390.

Nelson, T.M., Munger, S.D. and **Munger, J.D., Jr** (2003) *Taste sensitivities to PROP and PTC vary independently in mice*. Chem. Senses, 28, 695–704.

Nissenbaum, J.W. and **Sclafani, A.** (1987) *Sham-feeding response of rats to polycose and sucrose*. Neurosci. Biobehav. Rev., 11, 215–222.

Ogura, T., Margolskee R.F. and **Kinnamon S.C.** (2002) *Taste receptor cell responses to the bitter stimulus denatonium involve Ca^{2+} influx via store-operated channels*. J. Neurophysiol., 87, 3152–3155.

Okiyama, A. and **Beauchamp, G.K.** (1998) *Taste dimensions of monosodium glutamate (MSG) in a food system: role of glutamate in young American subjects*. Physiol. Behav., 65, 177–181.

Rabe, E.F. and **Corbit, J.D.** (1973) *Postingestional control of sodium chloride solution drinking in the rat*. J. Comp. Physiol. Psychol., 84, 268–274.

Ramirez, I. (1994) *Glucose polymer taste is not unitary for rats*. Physiol. Behav., 55, 355–360.

Rehnberg, B.G., Mackinnon, B.I., Hettinger, T.P. and **Frank, M.E.** (1996) *Analysis of polysaccharide taste in hamsters: behavioral and neural studies*. Physiol. Behav., 59, 505–516.

Ruiz, C.J., Wray, K., Delay, E., Margolskee, R.F. and **Kinnamon, S.C.** (2003) *Behavioral evidence for a role of alpha-gustducin in glutamate taste*. Chem. Senses, 28, 573–579.

Ruiz-Avila, L., Ming, D. and **Margolskee, R.F.** (2000) *An in vitro assay useful to determine the potency of several bitter compounds*. Chem. Senses, 25, 361–368.

Ruiz-Avila, L., Wong, G., Damak, S. and **Margolskee, R.F.** (2001) *Dominant loss of responsiveness to sweet and bitter compounds caused*

by a single mutation in a-gustducin. Proc. Natl. Acad. Sci. USA., 98, 8868–8873.

Sako, N., Shimura, T., Komure, M., Mochizuki, R., Matsuo, R. and **Yamamoto, T.** (1994) *Differences in taste responses to Polycose and common sugars in the rat as revealed by behavioral and electrophysiological studies*. Physiol. Behav., 56, 741–745.

Sclafani, A. and **Glendinning, J.I.** (2003) *Flavor preferences conditioned in C57BL/6 mice by intragastric carbohydrate self-infusion*. Physiol. Behav., 79, 783–788.

Spector, A.C. (2000) *Linking gustatory neurobiology to behavior in vertebrates*. Neurosci. Biobehav. Rev., 24, 391–416.

Weingarten, H.P. and **Watson, S.D.** (1982) *Sham feeding as a procedure for assessing the influence of diet palatability on food intake*. Physiol. Behav. 28, 401–407.

Wong, G.T., Gannon, K.S. and **Margolskee, R.F.** (1996) *Transduction of bitter and sweet taste by gustducin*. Nature, 381, 796–800.

Yan, W., Sunavala, G., Rosenzweig, S., Dasso, M., Brand, J.G. and **Spielman, A.** (2001) *Bitter taste transduced by PLC-beta(2)-dependent rise in IP(3) and alpha-gustducin-dependent fall in cyclic nucleotides*. Am. J. Physiol. Cell Physiol., 280, C754–C751.

Zhang, Y., M. A. Hoon, Chandrashekar J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S. and **Ryba, N.J.** (2003) *Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways*. Cell, 112, 293–301.

Zhao, G.Q., Zhang, Y., Hoon, M.A., Chandrashekar, J., Erlenbach, I., Ryba, N.J.P. and **Zuker, C.S.** (2003) *The receptors for mammalian sweet and umami taste*. Cell, 115, 255–266.

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