

Taste Sensitivities to PROP and PTC Vary Independently in Mice

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Abstract

Mammals use common mechanisms to detect, transduce and process taste stimulus information. For example, they share families of receptors that respond to amino acids, and sweet- and bitter-tasting stimuli. Nonetheless, it is also clear that different species exhibit unique taste sensitivities that may reflect specific genetic variations. In humans, sensitivities to the chemically similar, bitter-tasting compounds 6-*n*-propylthiouracil (PROP) and phenylthiocarbamide (PTC) are heritable and strongly correlated, suggesting a common genetic basis. However, it is unknown whether PROP and PTC taste sensitivities are similarly correlated in mice. Here we report that PROP and PTC taste sensitivities vary independently between two inbred strains of mice. In brief-access taste tests C3HeB/FeJ (C3) and SWR/J (SW) mice possess similar taste sensitivity to PTC, while SW mice are significantly more sensitive to PROP than are C3 mice. In two-bottle preference tests, however, SW mice display greater aversion to both compounds. This discrepancy may be explained by the observation that SW mice consumed taste solutions at a greater rate during the intake test than did C3 mice. Therefore, PTC avoidance is correlated with the amount of PTC consumed in the intake tests rather than the concentration of PTC tested. These findings suggest that post-ingestive factors play a significant role in PTC avoidance during intake tests and highlight an important advantage of brief-access tests over intake tests in resolving the gustatory and post-ingestive contributions to taste-related behaviors. Most strikingly, these results demonstrate that in mice, unlike in humans, PTC and PROP taste sensitivities vary independently, thereby suggesting a subtle functional diversity of bitter-taste mechanisms across mammalian species.

Keywords: bitter, brief-access test, intake test, mouse

Introduction

Several genetic loci critical for normal gustatory function have been defined by behavioral genetics studies in mice. For example, mapping of the saccharin-sensitivity locus (*Sac*) in mice facilitated the identification of a receptor involved in the detection of sugars, sweeteners and amino acids (Bachmanov *et al.*, 2001b; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001), while several loci linked to bitter taste sensitivity in both humans and mice were mapped to chromosomal regions containing putative bitter taste receptor genes (Lush and Holland, 1988; Capeless *et al.*, 1992; Lush *et al.*, 1995; Adler *et al.*, 2000; Matsunami *et al.*, 2000; Bachmanov *et al.*, 2001a). The functions of some of these common genes have been further elucidated by comparisons of human and mouse genomes, and the use of novel functional assays and heterologous protein expression systems (e.g. Chandrashekar *et al.*, 2000; Li *et al.*, 2002;

Nelson *et al.*, 2002). While some of these genes are clearly orthologues, differences in gene number and nucleotide sequences between species may have profound effects on the range of taste stimuli to which an animal is sensitive (Li *et al.*, 2002). Therefore, understanding the range of taste sensitivities present in different individuals and species will facilitate any analysis of the molecular contribution to taste function.

In humans, sensitivities to the bitter-tasting anti-thyroid compounds phenylthiocarbimide (PTC) and 6-*n*-propylthiouracil (PROP) are inherited traits determined by a dominant allele with strong evidence for linkage to a single chromosomal locus (Fox, 1931; Snyder, 1931; Blakeslee, 1932; Bartoshuk *et al.*, 1994; Reed *et al.*, 1999; Drayna *et al.*, 2003; Kim *et al.*, 2003), and may largely reflect the contribution of a single *TAS2R*-type receptor (Kim *et al.*, 2003). PTC and PROP, which are related chemicals, have both been

used in human taste testing and their sensitivity among human subjects is highly correlated (Barnicot *et al.*, 1951; Fisher *et al.*, 1963; Lawless, 1980). More recently, the use of PTC in taste tests has been largely abandoned due to its toxicity and strong odor; PROP is less toxic, and has no apparent odor (Fisher *et al.*, 1963; Lawless, 1980). In mice, the relationship between PTC and PROP aversion is less clear. There is disputed evidence for a single locus determining PTC sensitivity, with phenotypic variation in two-bottle intake (solution versus water) across different strains (Klein and DeFries, 1970; Lush, 1986). However, interpretations of these studies were complicated by observations that mice show a decrease in PTC consumption over the duration of intake tests, a phenomenon that suggests that the toxicity of PTC results in a conditioned taste aversion (Klein, 1969; Lush, 1986; Whitney and Harder, 1986). PROP intake phenotypes also vary among strains, although PROP aversion in mice correlates with sensitivity to quinine or sucrose octaacetate (SOA), while PTC does not (Whitney and Harder, 1994; Boughter and Whitney, 1998; Harder and Whitney, 1998).

Here we present data demonstrating that gustatory sensitivity to PTC and PROP varies independently in mice. We utilized a brief-access taste test that minimizes possible post-ingestive feedback, thereby providing a relatively uncontaminated measure of taste sensitivity. Brief-access tests, which involve repeated presentation of stimuli for short trials, have been used extensively to measure taste responsiveness in rats (Davis, 1973; Krimm *et al.*, 1987; Smith *et al.*, 1992; St John *et al.*, 1994) and mice (Smith *et al.*, 2001; Boughter *et al.*, 2002; Glendinning *et al.*, 2002). We tested two strains of mice, SWR/J (SW) and C3HeB/FeJ (C3), that have been shown to vary in level of aversion to a broad array of bitter-tasting stimuli in two-bottle tests (Boughter and Whitney, 1998). We have used both the taste salient brief-access assay and two-bottle tests, in which the gustatory contributions to bitter avoidance cannot be separated from

post-ingestive effects, to assess the sensitivities of mice from both strains to PTC and PROP, as well as to two other bitter-tasting stimuli, cycloheximide (CYX) and magnesium chloride (MgCl_2). Strain-specific variations in taste sensitivities to these compounds were correlated with relative fluid consumption by each strain and, in the case of PROP and CYX, with the allelic variation of known bitter taste receptor genes.

Methods

Mice

A total of 144 male mice (*Mus musculus*) were used in these experiments: 72 from each inbred strain (SW and C3). Table 1 lists the number of mice tested in each experiment. All mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed individually in plastic cages ($28 \times 17.5 \times 13$ cm) with stainless steel wire lids. Food was available *ad libitum*. Mice were naive and 52–100 days old at the start of each experiment, and were age-matched across strain. Immediately prior to testing, the mean weight of SW mice was 22.0 g and the mean weight of C3 mice was 27.1 g.

Solutions

The taste stimuli used in these experiments were made from reagent-grade chemicals: PTC, PROP, MgCl_2 and CYX (Sigma Aldrich Corp., St Louis, MO). Multiple concentrations of each solution were made fresh daily using distilled water, and all taste stimuli were presented at room temperature (concentrations are listed in Table 1).

Preference tests

Forty-eight-hour, two-bottle preference tests were used to measure the consumption of a taste stimulus relative to distilled water. This methodology has been described in detail elsewhere (Boughter and Whitney, 1998). Mice were tested individually in plastic home cages with stainless-steel

Table 1 Test compounds, concentrations and number of subjects

Compounds	Concentrations (mM)	No. of mice tested ^a			
		Brief access		Preference test	
		SW	C3	SW	C3
PTC	0.03, 0.1, 0.3, 1, 3	7	8	7	8
MgCl_2	10, 30, 100, 300, 1000	7	8	8	8
PROP	0.1, 0.3, 1, 3, 10	8	8	8	8
CYX	0.0001, 0.0003, 0.001, 0.003, 0.01	8	8	8	6
PTC	0.3, 1, 3, 10, 30	6	6		

^aOne SW/brief-access mouse died before any data could be collected for PTC or MgCl_2 . One SW mouse was removed from the PTC preference test, due to lack of water consumption. Two C3 mice died mid-way through the CYX preference test. Data collected prior to their deaths were included. An additional two data points reflecting no drinking during single 48 h periods were removed from the preference test data set.

wire lids, and two graduated drinking cylinders were placed on either side of the lids; bottles were switched after 24 h to control for any possible side preference. The time spent switching the bottles was negligible. Eight mice per strain were tested with an ascending five-concentration series of a particular stimulus, and mice were tested on only one compound. Prior to the first day of testing with taste solutions, mice were placed in the testing cages with two cylinders of distilled water to familiarize the mice with the setup. Therefore, it took 11 days to complete testing for each compound.

Brief-access tests

All brief-access tests were conducted in the Davis MS-160 computer-controlled gustometer (DiLog Instruments, Inc., Tallahassee FL). Mice were placed in a test cage (30 × 14.5 × 16 cm) with a stainless-steel mesh floor, and could access taste stimuli or water via a small opening at the front of the chamber. A trial began when a shutter opened to allow access to a stainless steel drinking tube, and ended after a defined time period when the shutter closed. A gentle airstream was directed across the shutter opening to help disperse potential odors of the taste solutions. In between trials, a stepping motor placed one of up to 16 drinking tubes in front of the access opening. Licks at each sipper tube were counted via a high-frequency AC contact circuit.

Brief-access testing procedures were based on those recently described by Boughter and colleagues (Boughter *et al.*, 2002). Water-deprived mice were first trained to lick water in the gustometer and then tested with a five-concentration series of a taste stimulus. Mice were water-deprived for 24 h prior to the first day of the experiment, and were subsequently restricted to water consumed during the testing session (~1.5 ml per session). On the first training day, mice were placed in the test chamber and given access to distilled water for 30 min. On the second training day, access was restricted to 5 s trials. Mice initiated a trial with a single lick on the tube; after 5 s the shutter closed. The shutter would reopen after a 10 s interval and the mouse could initiate a subsequent trial. If a mouse did not lick within 120 s of the shutter opening, the shutter was closed and the next trial begun. Mice could initiate up to 20 trials during the training session. Mice failed to initiate a trial within the 120 s window ~6% of the time.

Testing occurred on days 3–5. The trials were 5 s in length, with an inter-trial interval of 10 s, and a time limit of 120 s. There were two types of trials: water rinse trials and test trials. Each test trial was preceded by a water-rinse trial, to minimize potential carry-over effects. Test trials consisted of the presentation of either water (water test trial) or one of five concentrations of stimulus. An individual mouse was presented with each concentration twice a day for the 3 days, thereby creating six observations for each concentration. The presentation order of test stimuli was randomized (thus it differed between any two mice) in two blocks of six: with

rinse trials, the mouse could then initiate up to 24 test trials per day. After testing on day 5, mice received water in their home cages *ad libitum* for 48 h, followed by a second 24 h water deprivation prior to a second week of testing with another compound. The second week of testing was identical to the first week, to ensure that water deprivation and body weights for the second compound tested closely matched those for the first. Prior to the final day of testing body weight had dropped, on average, to 78% of the initial weight (the body weight of all mice declined significantly during the week, but importantly, there was not a significant difference between strains).

SW and C3 mice were tested with the brief-access procedure using the same four compounds and concentrations as in the two-bottle tests (Table 1). The total mice were divided into four ‘squads’ of eight mice each (four from each strain). A brief-access test lasted 2 weeks, during which one squad was tested with two compounds, one in the first week and the other in the following week. The compounds tested were varied such that each appeared once in the first week, and once in the second week. The order of testing was as follows: squad 1, week 1 PTC, week 2 MgCl₂; squad 2, week 1 PROP, week 2 CYX; squad 3, week 1 MgCl₂, week 2 PROP; squad 4, week 1 CYX, week 2 PTC. Results between the first and second week for each stimuli were similar, and the data were combined for analysis. An additional brief-access test, using a higher range of PTC concentrations, was conducted during a one-week period with a group of 12 naive mice.

Data analysis of behavioral tests

Data from the two-bottle tests is reported in the form of preference ratios (PR; amount solution consumed/total amount consumed). For example, a PR of 0.5 indicates no preference between the taste solution and water. PRs were determined for each 48 h period per mouse, and then averaged together per strain. For the brief-access tests the number of licks for each stimulus trial (each concentration being presented twice per mouse per session), plus water test trials, were averaged across the three test sessions for each individual mouse. These data are reported as lick ratios (LR: average number of licks to stimulus/average number of licks during water test trial). For example, an LR of 1.0 indicates that mice delivered an equal number of licks to sipper tubes dispensing water or taste solution. Water rinse trials were not used in calculations of LRs. Data from both two-bottle tests and brief-access tests were analyzed using a general linear model: repeated measures (concentration) with a between-subjects (strain) factor, plus planned comparisons based on the expectation of strain differences (Statistica software, StatSoft, inc., Tulsa, OK). Additional comparisons between averaged strain data, and between mean preference scores and 0.5 or between mean lick ratios and 1.0, were made with a Student’s *t*-test. The statistical rejection criterion (α) for all tests was set *a priori* at the 0.05 level.

Identification of Tas2R alleles

Oligonucleotides were designed based on the *Tas2r5* and *Tas2r8* cDNA sequences reported by Adler *et al.* (2000) and synthesized by the University of Maryland School of Medicine Biopolymer Core. Oligonucleotides were used to amplify the entire coding regions of each gene by polymerase chain reaction from SW or C3 genomic DNA isolated from mouse livers using the general protocol outlined by Blin and Stafford (1976). Amplified products were subcloned into pGemT-Easy (Promega, Madison, WI) and sequenced by the University of Maryland School of Medicine Biopolymer Core. Each gene was independently amplified, subcloned and sequenced at least twice to verify the fidelity of the sequence. The SW and C3 sequences were aligned with the published sequences using ClustalW.

Results

Preference tests

We first asked whether C3 and SW mice differed in their preference for each bitter compound (PTC, PROP, MgCl₂ and CYX) in standard 48 h, two-bottle preference tests. Both strains avoided each bitter compound in a concentration-dependent fashion (Figure 1). The strain differences for PTC and PROP were in the same direction, with SW mice displaying a greater level of aversion to both compounds [for PTC, $F(1,13) = 9.7$, $P < 0.01$; for PROP, $F(1,14) = 58.3$, $P < 0.001$]. For PTC, significant differences between the two strains were observed only at intermediate concentrations. SW mice were also more sensitive than C3 mice to MgCl₂ [$F(1,14) = 42.0$, $P < 0.001$]. Interestingly, C3 mice displayed

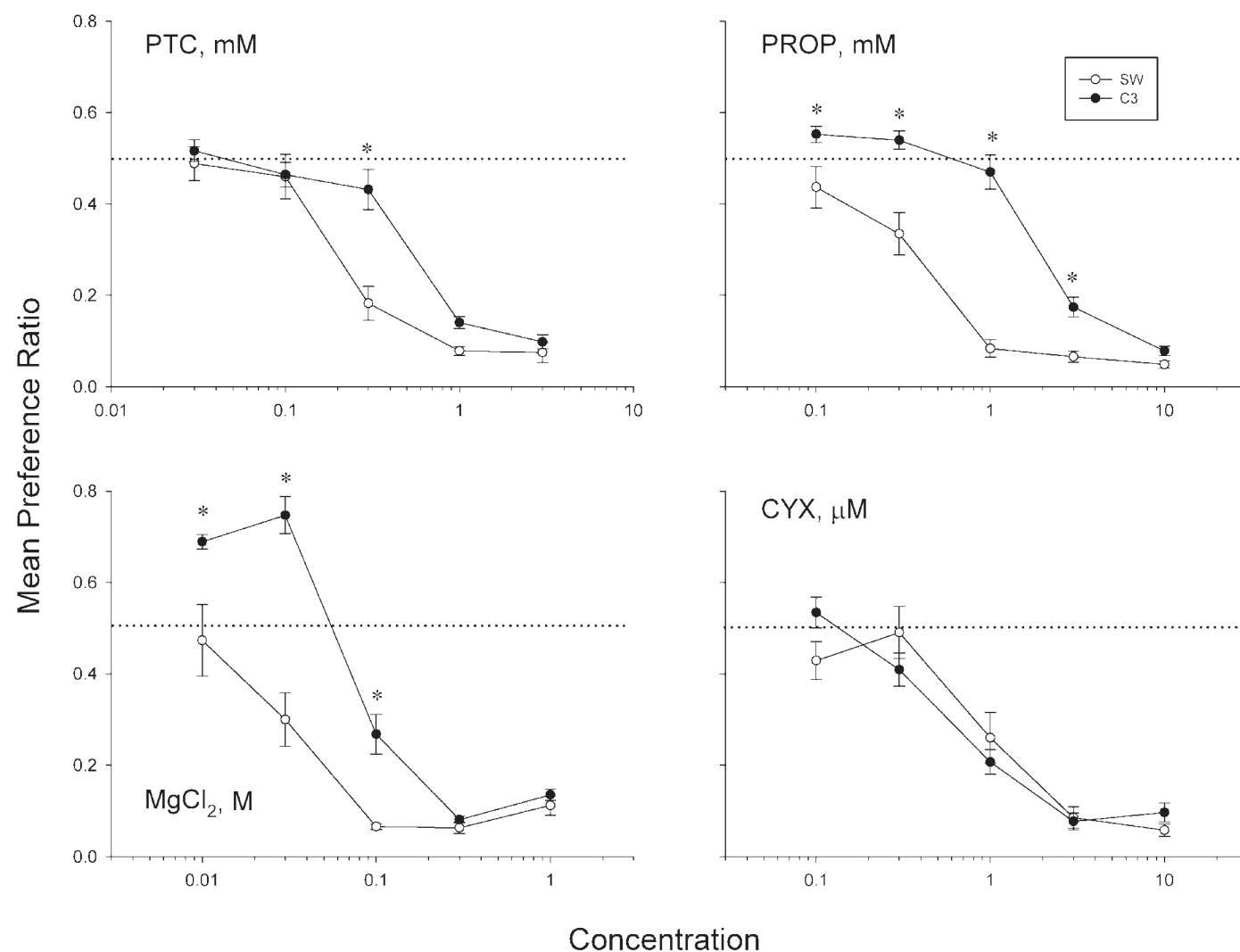


Figure 1 Preference ratios (mean \pm SE) for SW and C3 mice to concentration series of PTC, PROP, MgCl₂ and CYX. The dotted lines on each graph represent a preference score of 0.5, which indicates equivalent consumption from solution and water tubes. Asterisks identify significant strain effects at particular concentrations, as indicated by planned comparisons ($P < 0.05$). Preference ratios for each strain decreased with increasing concentration; SW mice showed greater aversion to PTC, PROP and MgCl₂, but not CYX.

a significant preference for 0.01 and 0.03 M MgCl_2 ($t = 11.4$, and 6.1, respectively; $P < 0.001$). Both strains strongly avoided 1–10 μM cycloheximide, but did not differ from one another in their level of aversion.

Brief-access tests

The gustatory contributions of taste stimuli cannot be easily separated from post-ingestive effects in standard intake tests. To control for such post-ingestive effects, as well as any potentially confounding olfactory cues, we tested the same mouse strains using a brief-access test (Boughter *et al.*, 2002). Mice avoided PROP, MgCl_2 and CYX in a concentration-dependent fashion in the brief-access test (Figure 2). However, mice displayed only weak avoidance of PTC. SW mice had a mean lick ratio = 0.81 to the highest concentration (3 mM) of PTC, the only concentration licked at a

significantly different rate than water ($t = 2.8$, $P < 0.05$). In contrast, SW mice strongly and significantly avoided 0.3–3 mM PTC in the preference test (preference ratios < 0.19 ; $t > 8.5$, $P < 0.001$; see Figure 1). Surprisingly, C3 mice were somewhat more sensitive than SW mice to PTC in the brief-access test, although this difference across all concentrations was not significant [$F(1,13) = 3.5$, $P > 0.05$]. In contrast, results of the brief-access test with PROP were consistent with the two-bottle test: SW mice showed greater aversion than C3 mice [$F(1,14) = 7.0$, $P < 0.05$]. The strains did not differ in their level of aversion to MgCl_2 , while C3 mice possessed a stronger level of aversion to CYX [$F(1,14) = 6.6$, $P < 0.05$].

We asked if olfactory cues might contribute to avoidance in the brief-access test. We measured the latency to the first lick for each compound, because concentration-dependent

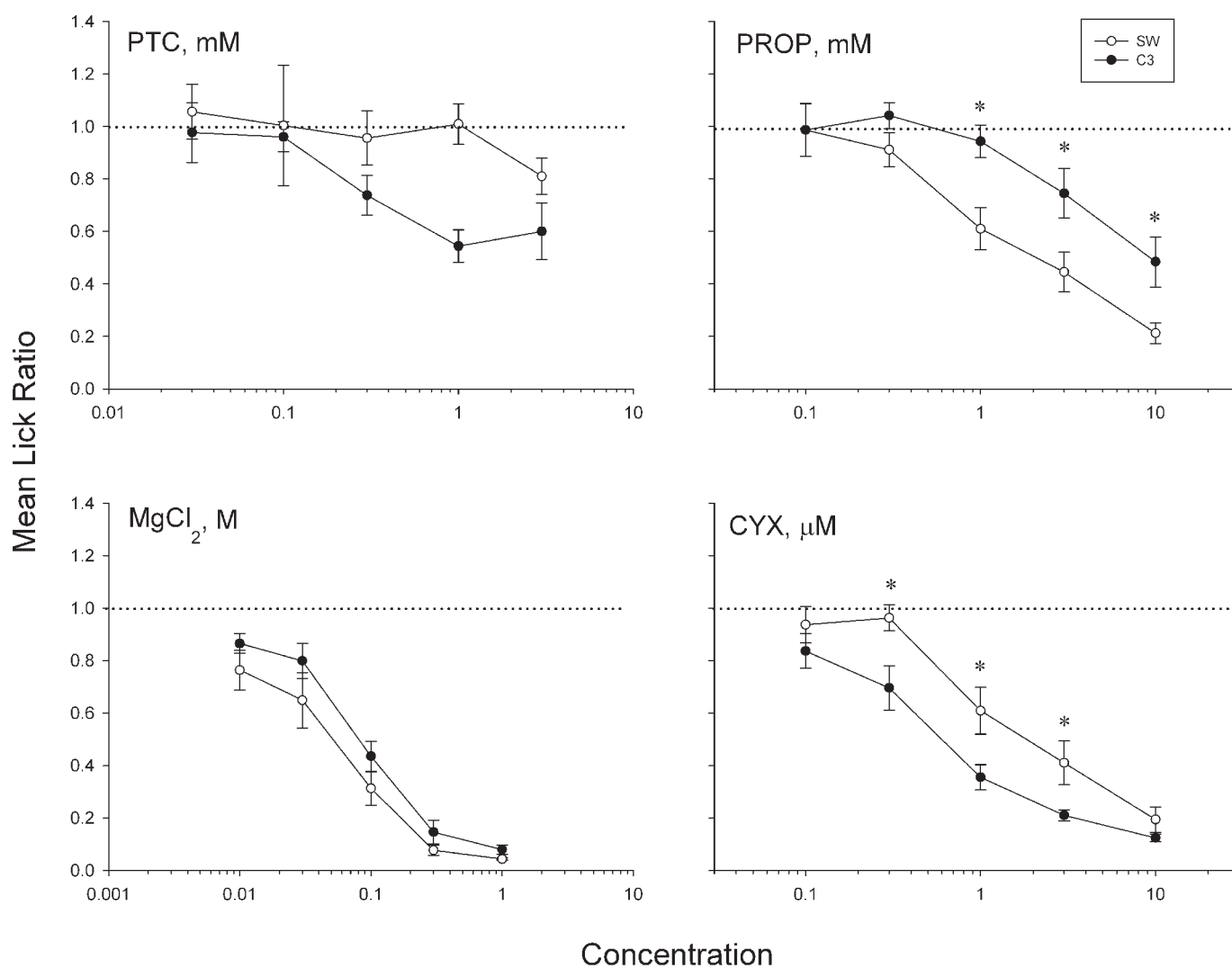


Figure 2 Lick ratios (mean \pm SE) for SW and C3 mice to concentration series of PTC, PROP, MgCl_2 and CYX. The dotted lines on each graph represent a ratio score of 1.0, which indicates a lick rate equal to that of water. Asterisks identify significant strain effects at particular concentrations, as indicated by planned comparisons ($P < 0.05$). Lick ratios for each strain decreased with increasing concentration; SW mice made fewer licks than C3 mice to PROP at high concentrations, while C3 mice were more sensitive to CYX. The strains did not differ significantly for the other compounds.

changes in latency have been shown previously to be indicative of olfactory contributions (Rhinehart-Doty *et al.*, 1994). For both strains, no significant differences were seen in the latency to the initiation of licking for any concentration of PTC or PROP (Figure 3). For MgCl_2 , SW mice had a greater mean latency [$F(1,13) = 8.5$, $P < 0.05$]. For cycloheximide, there was no strain difference, but an effect of concentration [$F(5,70) = 2.6$, $P < 0.05$]. However, it is evident that the latencies for the five concentrations of cycloheximide are roughly the same, indicating that the mice could not distinguish stimulus concentration with a non-taste cue.

To determine if either strain displayed strong avoidance to higher concentrations of PTC in the brief-access procedure, we tested additional mice with 0.3–30 mM (Figure 4). Both

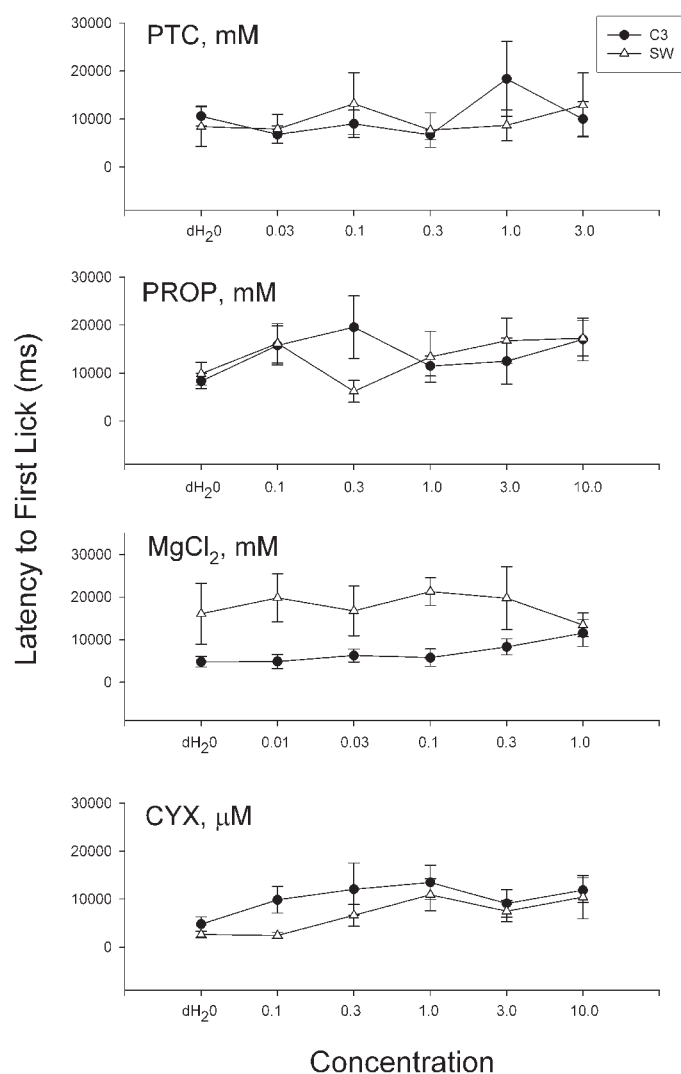


Figure 3 Latency to first lick (mean \pm SE) for SW and C3 mice to water and concentrations of stimuli in the brief-access experiment. The strains did not differ in latency to either PTC, PROP or CYX, but did to MgCl_2 . The only effect of concentration found was for CYX.

strains avoided licking 10 and 30 mM PTC, although the strains did not differ significantly in their level of aversion.

PTC and water consumption

We hypothesized that the differences between results from the preference and brief-access tests for PTC were due to post-ingestive effects associated with consumption, as suggested by Whitney and Harder (1986). In the PTC preference test, SW mice displayed aversion at a lower concentration (and therefore on an earlier day) than did the C3 mice (Figure 1). Figure 5 shows mean consumption of PTC and water by each strain during each 48 h period with each concentration. SW mice consume more water throughout the test (Figure 5a), and also consume more (per body weight) of 0.03 and 0.1 mM PTC than C3 mice, but then consume less of 0.3 or 1 mM (Figure 5b). Aversion to PTC began after day 5 for SW mice (no PTC was given on day 1), with an average consumption of 0.76 μmol of PTC (unadjusted) over the 96 h period. C3 mice, which did not display aversion to the compound through day 5, had consumed on average 0.64 μmol of PTC. Aversion to PTC did begin for the C3 mice after day 6 of testing, at which point these mice had an average consumption of 1.48 μmol of PTC. Additionally, we confirmed that SW mice normally consume more fluid than C3 mice in an intake test by testing both strains with distilled water only using the two-bottle paradigm over a 10 day period. On average, SW mice consumed 16.7 ml water/30g body wt per 48 h period, as opposed to 12.5 ml/30g for C3 mice ($t = 5.89$; $P < 0.001$).

In the brief-access tests, SW mice also licked more water than C3 mice: water rinse trials and water test trials were averaged for each individual across 2 weeks of testing, and then means for each strain were computed. SW mice

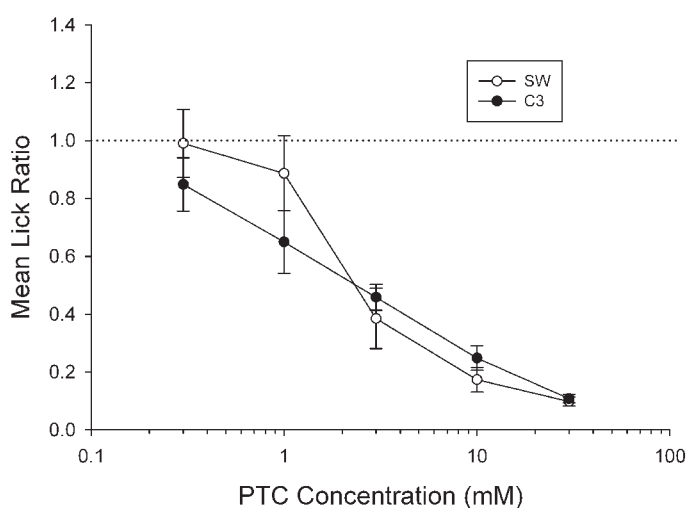


Figure 4 Lick ratios (mean \pm SE) for SW and C3 mice to concentration series of PTC. The dotted line on the graph indicates a lick rate equal to that for water. Mice from both strains demonstrated avoidance of high concentrations of PTC; there was not a significant strain difference.

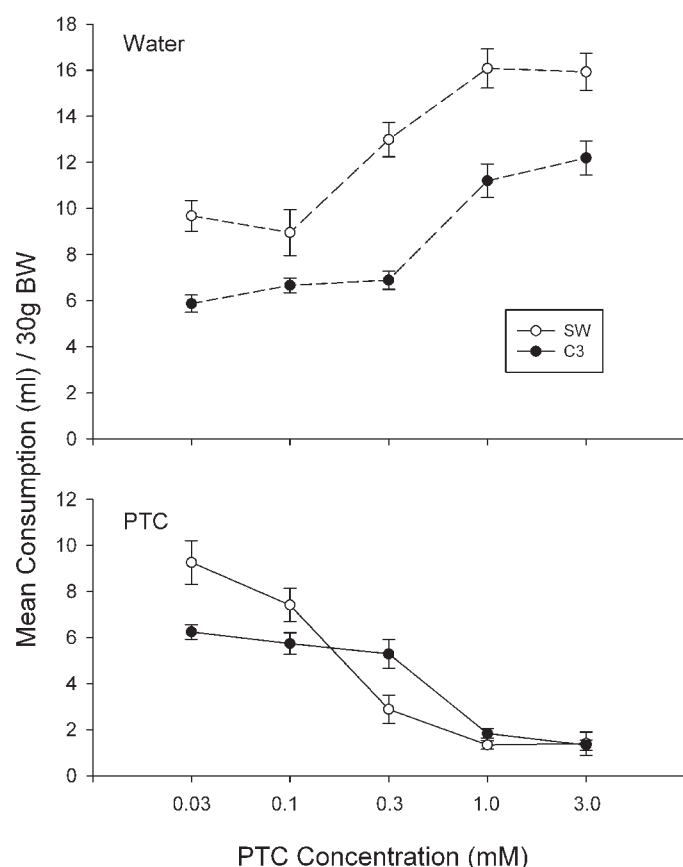


Figure 5 Mean consumption (expressed in ml) of PTC and water for SW and C3 mice during the two-bottle test. Data are expressed in terms of body weight. SW mice drank more PTC than C3 mice during the 48 h tests with 0.03 and 0.1 mM PTC, but less with 0.3 mM PTC. SW mice drank more water throughout the experiment.

consumed water at a rate of 48.1 licks/5 s trial, while C3 mice licked 42.1/5 s ($t = 4.2$, $P < 0.001$). SW mice consumed more PTC than C3 mice during the brief-access experiment (data not shown), although the level of intake was far less than during the two-bottle tests. We have measured licking and intake simultaneously in 20 min periods over several days and concluded that both SW and C3 mice consume $\sim 1.4 \mu\text{l}$ of fluid per lick during a brief-access trial (unpublished findings). Using this estimate we calculated that SW mice, on average, consumed $1.05 \mu\text{mol}$ of PTC during the 3 day period of brief-access testing, while C3 mice consumed $0.68 \mu\text{mol}$. However, SW mice had only consumed $0.63 \mu\text{mol}$ of PTC after 2 days of brief-access testing. It would prove interesting to extend the brief-access test to 4 days or more as the amount of PTC consumed by SW mice by the end of the 3 day brief-access test corresponds to a level at which aversion was observed in the two-bottle preference test.

T2R polymorphisms

Recently, the T2R family of G protein-coupled receptors has been implicated in the detection of bitter compounds

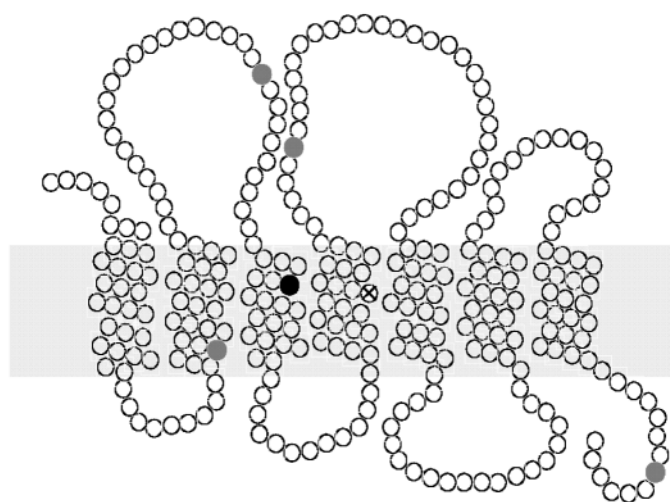


Figure 6 SW and C3 mice express different alleles of the putative CYX receptor, *Tas2r5*. The predicted structure for T2R5 is shown (after Chandrashekar *et al.*, 2000). Filled circles (both gray and black) represent previously identified polymorphisms between CYX 'non-taster' strains (e.g. C57BL/6J) and 'taster' strains (e.g. DBA/2J). The C3 allele displays the 'taster' versions of four of these residues (I44, V85, D155 and R294; gray circles) while the SW allele is identical to other 'nontasters' at all five (T44, I85, T101, G155 and L294). However, the C3 allele exhibited two polymorphisms that were not found in other 'taster' strains: amino acid 101 (black circle) is a Thr in both C3 'taster' mice and all 'nontasters,' but an Ala in other 'taster' mice; amino acid 139 (crosshatched circle) is an Ala in C3 T2R5 but a Thr in all other 'taster' and 'nontaster' alleles.

(Adler *et al.*, 2000; Chandrashekar *et al.*, 2000). Two of these receptors, mouse T2R5 and T2R8, have been shown to interact with CYX and PROP, respectively, in heterologous expression assays (Chandrashekar *et al.*, 2000). We asked whether the different sensitivities to CYX and to PROP exhibited by SW and C3 mice might reflect polymorphisms in these receptors. We amplified the complete coding sequences of *Tas2r5* and *Tas2r8* from SW and C3 genomic DNA, and compared their nucleotide and deduced amino acid sequences to the published *mTas2r5* and *mTas2r8* sequences (Adler *et al.*, 2000). Both the C3 and SW alleles of *Tas2r8* [GenBank accession numbers AY364474 (C3) and AY364475 (SW)] were identical to the published sequence from 129/SvJ and C57BL/6J (GenBank accession number AF227148) (data not shown). However, different *Tas2r5* alleles were identified for C3 and SW mice. The C3 *Tas2r5* allele (GenBank accession number AY364472) was identical to that of DBA/2J, another CYX 'taster' strain (Adler *et al.*, 2000), at all positions but two: amino acid 101 is an Ala in DBA/2J and a Thr in C3 mice, while amino acid 139 is a Thr in DBA/2J and an Ala in C3 mice (Figure 6). SW *Tas2r5* (GenBank accession number AY364473) was identical to that of the C57BL/6J 'non-taster' strain (Adler *et al.*, 2000), including all amino acids that varied between the C57BL/6J and DBA/2J alleles (I44T, V85I, A101T, D155G and R294L; Figure 6). No silent mutations were observed.

Discussion

Bitter stimuli comprise an exceptionally diverse set of compounds that vary greatly in toxicity (Spielman *et al.*, 1992; Glendinning, 1994), and it is not known whether the greater general bitter sensitivity of SW mice (relative to C3) evident in intake tests is based entirely on immediate sensory (gustatory) cues. Results from these experiments indicate that only the strain difference for PROP aversion was consistent in both two-bottle and brief-access tests. As the brief-access procedure is considerably more taste-salient than the two-bottle test for mice (Boughter *et al.*, 2002; Glendinning *et al.*, 2002), it is evident that the general difference in aversion to bitter stimuli between these strains as measured by intake tests may not be simply ascribed to a unitary difference in taste receptor or transduction processes.

In 48 hour, two-bottle preference tests, SW mice displayed a greater level of aversion toward concentration series of the bitter-tasting compounds PTC, PROP and MgCl_2 than did C3 mice. This finding generally agrees with a previous study where SW mice displayed greater aversion than C3 mice to all of 10 bitter stimuli tested (Boughter and Whitney, 1998). In the current experiment, the SW–C3 strain difference was most robust for PROP, although SW mice displayed a stronger aversion than C3 mice for several intermediate concentrations of both PTC and MgCl_2 .

Strikingly, in the brief-access test both strains of mice displayed little avoidance of concentrations of PTC that were strongly avoided in the two-bottle test (Figure 2), while for PROP, the concentration-response functions for both methods matched closely. If the aversions displayed in the two-bottle tests were based purely on gustatory cues, then the functions from both procedures should be fairly similar, as was the case for PROP or cycloheximide. For example, close correspondence between brief-access functions and preference functions was found for sucrose octaacetate, a non-toxic, non-odorous bitter stimulus (Boughter *et al.*, 2002). Strong aversion to PTC in the brief-access test was only realized at 10 mM (Figure 3), a log-step higher than in the preference test. Previous studies have indicated that in two-bottle preference tests, aversion to PTC develops over a period of days: each of five strains, including C3 and SW, were indifferent to 0.1 mM PTC during the initial 48 h period, but completely avoided the same concentration after 8 days of continuous consumption (Whitney and Harder, 1986). At a higher concentration (0.3 mM), aversion develops even faster, by day 4 (D.B. Harder, personal communication). In our two-bottle results, it appears that a correlation may exist between the number of moles of PTC consumed and avoidance. PTC is a highly toxic substance, with an oral LD₅₀ in mouse equal to 10 mg/kg.

It is crucial to recognize that a mouse must be able to discriminate between a taste solution and distilled water in order to avoid that solution. Even though the toxicity of the PTC leads to aversion, the mice must use sensory cues to

recognize the stimulus. In the two-bottle test it is possible that mice utilize either gustatory, odor or position cues, or all of these. In the brief-access test, we minimized odor cues via a gentle airstream, leaving a function representing only weak avoidance based on immediate gustatory cues. Because intake of PTC is less in the brief-access study, strong aversion did not develop in either strain.

Cycloheximide is also a toxic compound, although not as toxic to mice as PTC (oral LD₅₀ is 133 mg/kg). As was the case with PTC, SW mice drank a larger quantity of both water and cycloheximide during the two-bottle test (data not shown), yet did not differ from C3 mice in avoidance of this compound. Unlike the PTC results, however, the brief-access tests clearly show both strains of mice reduce licking of the stronger (1–10 μM) concentrations of cycloheximide, suggesting avoidance levels in the two-bottle test were primarily taste-based. Furthermore, the brief-access test revealed that C3 mice actually had possessed greater aversion to CYX. Polymorphisms in the mouse *Tas2r5* bitter-taste receptor gene correlate with strain-specific differences in CYX sensitivity in two-bottle preference tests and with T2R5 receptor responses in heterologous expression systems (Chandrashekar *et al.*, 2000). The SW allele of *Tas2r5* contains all four of the polymorphisms that consistently vary between two other CYX ‘nontaster’ strains (C57BL/6 and 129/Sv) and five ‘taster’ strains [C3HeB/FeJ (the C3 strain used in this study), DBA/2J, CBA/Ca, BALB/c and C3H/He]. Interestingly, C3HeB/FeJ mice, which are phenotypically ‘tasters’, have the ‘nontaster’ Thr residue at position 101 (Figure 6), suggesting that this amino acid may be unimportant for interactions with CYX. This reasoning would also suggest that some or all of the four polymorphic residues that consistently vary between ‘taster’ and ‘nontaster’ strains are critical for high affinity interactions with CYX. Structure–function analyses of T2R5 and CYX are needed to test this possibility.

The results for MgCl_2 did not match closely across the two types of test, with no apparent strain difference in the brief-access test. MgCl_2 is the least toxic chemical we used (the mouse oral LD₅₀ is 7600 mg/kg), and therefore the greater apparent SW aversion in the two-bottle test is unlikely to be due to toxicity associated with consumption. However, the strain difference is most prominent at the weaker concentrations (0.01–0.1 mM) and may be due to the preference of the C3 mice at these concentrations (in our study, the MgCl_2 preference was not evident in the brief-access test). Glendinning (1993) previously showed that some *Peromyscus* mice preferred low concentrations of quinine hydrochloride in two-bottle tests. Presumably, water-deprived mice will lick water or weak concentrations of stimuli at a maximal rate, making an appetitive or preferential response difficult to discern in a brief-access test.

These data indicate that in mice, unlike in humans, taste thresholds for solutions of PTC and PROP are not highly correlated. In humans, taste sensitivities to both PTC and

PROP appear to be tightly linked to a single chromosomal locus (Reed *et al.*, 1999; Drayna *et al.*, 2003; Kim *et al.*, 2003). In contrast, SW and C3 mice did not differentially avoid PTC as they did PROP in brief-access tests. Similar strain distribution patterns for PROP, quinine and denatonium aversion were evident in a study with SW, C3 and C3.SW-*Soa*^d congenic SOA taster mice (Boughter and Whitney, 1998). In contrast, PTC aversion did not correlate with quinine aversion in an outbred strain (Whitney and Harder, 1994). In humans, there is ample evidence that PTC/PROP taste sensitivity is not correlated with that for quinine (Fischer and Griffin, 1964; Frank and Korchmar, 1985; Mela, 1989; Schifferstein and Fritjers, 1991; Yokomukai *et al.*, 1993; Delwiche *et al.*, 2001), further underlining the species difference. The stimulus sensitivity has only been determined for a few of the T2R bitter taste receptors, but a suitable candidate for a PROP receptor in mice may be the mT2R8 bitter taste receptor. This receptor was found in heterologous expression assays to respond specifically to only PROP (at relatively high concentrations) and denatonium among a battery of bitter-tasting stimuli, including PTC (Chandrashekar *et al.*, 2000). Although SW and C3 mice clearly differ in their sensitivity to PROP, they share the same *Tas2r8* coding sequence. This observation suggests that a second *Tas2r* may be involved in PROP sensitivity. This is consistent with previous behavioral genetic studies which linked one locus of PROP sensitivity to distal chromosome 6 (Harder and Whitney, 1998), the site of a cluster of *Tas2r* genes (Adler *et al.*, 2000).

The results of this study clearly illustrate the need for reliable taste testing procedures for use in mice. Our results demonstrate that the two-bottle test and the brief-access test provide different measures of behavior in response to the same stimulus. Because the brief-access trials were only 5 s in duration, post-ingestive factors were less likely to influence taste sensitivity. It should also be acknowledged that further insight into the relationship between behavioral sensitivity and taste receptors could be gained using other behavioral techniques, such as detection thresholds or two-stimulus discrimination (St John and Spector, 1996; Spector and Kopka, 2002; Eylam and Spector, 2003). Significantly, detection thresholds measured for quinine in rats tend to occur at concentrations that are not strongly avoided in brief-access tests (e.g. St John *et al.*, 1994; St John and Spector, 1996), likely due to the motivating effects of water deprivation overcoming the aversive qualities of otherwise detectable stimuli. It is possible that a relationship between PTC and PROP in mice could be found using such a procedure. In any case, the fairly large group of bitter taste receptors will require extensive behavioral and physiological characterization in mice and humans. Taste-salient studies will be necessary to correlate receptor function with behavioral sensitivity.

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