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Stimulus Processing of Glycine is Dissociable from that of Sucrose and Glucose Based on Behaviorally Measured Taste Signal Detection in Sac 'Taster' and 'Non-taster' Mice

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Abstract

Mouse strains have been divided into 'tasters' and 'non-tasters' based on their relatively high and low preference, respectively, for low concentrations of sucrose and saccharin. These phenotypic differences appear to be due to a polymorphism in the gene at the *Sac* locus encoding for the T1R3 taste receptor selectively affecting the functionality of the T1R2+3 heterodimer. To psychophysically examine whether these phenotypes are due to sensory sensitivity as opposed to hedonic responsiveness, we measured taste signal detection of sucrose, glucose, and glycine by *Sac* taster (C57BL/6J and SWR/J) and non-taster (129P3/J and DBA/2J) strains in an operant conditioning paradigm using a gustometer. The taster mice had lower detection thresholds for sucrose and glucose compared with the non-taster mice. The detection thresholds corresponded well with reported responsiveness to low concentrations of these sugars in two-bottle intake tests suggesting that the *Sac* taster phenotype has a sensory basis and is not simply a matter of strain differences in the hedonic evaluation of weak intensities of the stimuli. Taster status did not entirely account for the strain differences in detection thresholds for glycine, a 'sweet' tasting amino acid. Collapsed across strains, detection thresholds for sucrose and glucose were highly correlated with each other ($r = 0.81$), but only modestly correlated with those for glycine ($r \leq 0.43$). This suggests that stimulus processing of glycine in the perithreshold intensity domain can be dissociated from that of sucrose and glucose. The mechanism underlying this difference may be related to the ability of glycine to bind with the T1R1+3 heterodimer.

Key words: C57BL/6J, DBA/2J, 129P3/J, SWR/J, sweet taste, T1R receptors

Introduction

It has been known for sometime that various inbred mouse strains differ in regard to their intake of saccharin and sucrose. Based on these differences, inbred mouse strains have been classified as 'tasters', such as C57BL/6 (B6) and SWR, and 'non-tasters', such as 129 and DBA/2 (D2) (e.g. Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996). The taster strains ingest a significantly larger amount of and demonstrate a higher preference for low concentrations of sucrose and saccharin than non-taster strains in two-bottle tests.[1] Crosses between mice from taster and non-taster strains have indicated Mendelian inheritance for the two phenotypes with the taster phenotype being dominant and suggest a single locus influencing taste preference for sweeteners (Pelz *et al.*, 1972; Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995). The chromosomal locus implicated in the differences between the phenotypes was originally termed *Sac* (Fuller, 1974; Lush, 1989) and was eventually linked to mouse chromosome 4 (e.g. Lush *et al.*, 1995; Bachmanov *et al.*, 1997; Blizard *et al.*, 1999; Li *et al.*, 2001).

More recently, the molecular basis of the *Sac* taster phenotype in mice was shown to involve the T1R family of G-protein coupled taste receptors. Three receptors in this family have been identified (T1R1, T1R2 and T1R3) and are expressed in subsets of taste bud cells (Hoon *et al.*, 1999; Kitagawa *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001). The gene at the *Sac* locus (*Tas1r3*) was shown to encode the T1R3 receptor protein (Bachmanov *et al.*, 2001b; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001). In addition, these T1R receptors appear to form heterodimers that determine their binding characteristics. Taste transduction of

natural sugars is thought to be mediated through the T1R2+3 heterodimer, as is the case for some preferred D-amino acids, while taste transduction of L-amino acids (but not their D-enantiomers) is thought to be mediated via the T1R1+3 heterodimer (e.g. Nelson *et al.*, 2001, 2002; Li *et al.*, 2002; Zhao *et al.*, 2003). The occurrence of non-taster mouse strains was explained by the finding of allelic variation in the gene encoding for the T1R3 receptor protein (e.g. Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001; Reed *et al.*, 2004) which was shown to selectively affect the T1R2+3 heterodimer (e.g. Li *et al.*, 2002; Nelson *et al.*, 2002) without affecting its ability to dimerize (Nelson *et al.*, 2002) and without creating a 'null' form of the T1R3 receptor (Damak *et al.*, 2003). Moreover, crosses between taster and non-taster strains as well as transgenic introduction of the taster allele into a non-taster's genome eliminated the differences in intake behavior between these strains (e.g. Blizard *et al.*, 1999; Li *et al.*, 2001, 2002; Nelson *et al.*, 2001), further strengthening the hypothesis that the T1R3 receptor was the gene product responsible for the differences observed in sweetener preference across strains.

Interestingly, glycine, an amino acid that is reported as sweet to humans and is preferred by rodents in intake tests (e.g. Schiffman *et al.*, 1981; Pritchard and Scott, 1982; Iwasaki *et al.*, 1985; Lush *et al.*, 1995; Bachmanov *et al.*, 2001a), appears to activate both the T1R1+3 and the T1R2+3 heterodimers in heterologous expression systems (Li *et al.*, 2002; Nelson *et al.*, 2002). This observation is consistent with the generalization between sucrose and glycine in conditioned taste aversion tests in rodents (Nowlis *et al.*, 1980; Pritchard and Scott, 1982; Kasahara *et al.*, 1987; Yamamoto *et al.*, 1988; Danilova *et al.*, 1998; see also Tapper and Halpern, 1968). In addition, glycine preference in mice appears to depend on the taster status of the strain (Lush, 1989; Lush *et al.*, 1995; Bachmanov *et al.*, 2001a), although a recent report calls this dependency into question (Inoue *et al.*, 2004). The putative ability of glycine to activate both types of T1R receptor complexes may owe to its lack of a chiral carbon. Natural sugars such as sucrose and glucose activate the T1R2+3 but are not thought to bind with T1R1+3 heterodimer, as was recently confirmed in T1R knockout mice studies (Zhao *et al.*, 2003). One study, however, reported no significant difference in the two-bottle preference scores for glucose between T1R3 knockout mice and either their B6 wild-type controls or 129 non-taster mice (Damak *et al.*, 2003). These researchers suggested that glucose and perhaps other natural sugars could be engaging other transduction mechanisms that do not depend on the T1R3 receptor. Thus, sucrose, glucose and glycine, all considered sweet-tasting compounds, may not share identical signaling pathways in taste receptor cells.

The behavioral method used to initially identify these strain variations in responsiveness to sweeteners was the two-bottle intake test. With some exceptions, this is also the behavioral procedure that was used to corroborate some of the molecular findings. Although this method is simple to execute and provides some insight into taste-guided ingestive behavior, intake can be influenced by other factors, most notably postingestive events (see Spector, 2003). Moreover, at low concentrations certain stimuli may be detectable but affectively neutral (i.e. neither preferred nor avoided). Indeed, this seems to be the case when behavioral responses to NaCl are measured in B6 mice (Eylam and Spector, 2002). Here the taste stimuli served as discriminative cues in an operant conditioning paradigm. Thus, with the aid of a gustometer we were able to measure immediate responses to small volumes of taste stimuli increasing our confidence that the behavior was guided by oral sensory stimulation. We used this procedure as part of a signal detection task in which animals were trained to discriminate between water and a given taste compound irrespective of the hedonic value of the stimulus. By varying concentration, psychophysical sensitivity functions were derived for sucrose, glucose, and glycine in *Sac* taster and non-taster mice. Accordingly, we were able to test whether the higher preferences for low concentrations of 'sweeteners' such as sucrose reported in taster mice are linked to sensory sensitivity and thus provide a more comprehensive behavioral characterization of these phenotypes. Additionally, if taste detectability of these compounds is dependent on the T1R2+3 heterodimer then taster mice should have lower thresholds than the non-taster mice in this task.

Materials and methods

Subjects

Twelve C57BL/6J (B6), 12 SWR/J (SWR), 12 129P3/J (129) and 12 DBA/2J (D2) naïve adult (7 weeks ± 2 days of age) male mice (Jackson Laboratories, Bar Harbor, ME) with mean body masses of 21.85 g (±0.41), 18.30 g (±0.41), 20.22 g (±0.40) and 20.52 g (±0.76), respectively, upon arrival, served as subjects.

The mice were housed individually in polycarbonate shoebox cages in a colony room where the temperature, humidity and lighting (12 h light/12 h dark) were controlled automatically. Subjects had free access to pellets of laboratory chow (LabDiet 5001; PMI Nutrition International Inc., Brentwood, MO) and distilled water. One week after their arrival, the mice were put on a restricted water-access schedule where fluid was available only during the training and testing sessions on Monday–Friday. On weekends the mice received *ad libitum* water and food with the water bottles replaced on the home cage after the last session on Friday and removed again on Sunday, no more than 24 h before testing. While on the water-restriction schedule, mice that dropped below 85% of their body mass relative to that measured during *ad libitum* water access received supplemental water [1–2 ml depending on their weight gain after supplementation; mice from the SWR strain progressively increased their need for supplementation (up to 12 ml) due

to their known development of diabetes insipidus with age (see Kutscher *et al.*, 1975; Kutscher and Schmalbach, 1975)] immediately following their respective daily testing session. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Taste stimuli

All taste solutions were prepared daily with distilled water and reagent grade chemicals and presented at room temperature. The sucrose (Fisher Scientific, Atlanta, GA) concentrations used for testing were 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 M. The same glycine (Sigma, St Louis, MO) concentrations with the addition of 0.0125 M were used. The glucose (Fisher Scientific, Atlanta, GA) concentrations used were 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 M. Initially, the ranges of concentrations used were selected to include clearly detectable concentrations. Lower concentrations were then added until the performance of the mice approached chance levels.

Apparatus

Animals were trained and tested in a specially designed testing apparatus referred to as a gustometer (modified from Spector *et al.*, 1990; see also Eylam and Spector, 2002, 2003). All fluid deliveries were computer-controlled and occurred through a centrally positioned sample spout. The animal received access to this spout by extending its tongue through a slot in the side wall of the test chamber. When tongue contact was made, an electric circuit was completed (<50 nA). The initial lick, after the animal completed an 'attending' response (see below), triggered the filling of the spout shaft and subsequent licks caused ~1.6 µl of fluid to be deposited into the fluid column. Water was delivered from two stationary 'reinforcement' spouts horizontally oriented and flanking the access slot. Contact with the correct reinforcement spout during the appropriate stage of each trial resulted in the delivery of water (~1.6 µl/lick). This apparatus allowed us to deliver small volumes of stimuli and measure immediate responses, increasing our confidence that the behavior was under orosensory control.

Trial structure

The trial structure was described in detail by Eylam and Spector (2002, 2003). Briefly, the mice were tested in daily 25 min sessions during which they were allowed to complete as many trials as possible. To initiate a trial, the mouse was required to perform an attending response by licking the spout twice within 250 ms. This helped ensure that the mouse was engaged in licking when the stimulus was delivered. Once a trial was initiated, the stimulus was delivered and the mouse was allowed five licks or 2 s (whichever came first) on the sample spout. Then, the sample spout rotated away from the animal's reach. At this time, the house lights were turned off and the cue lights above the reinforcement spouts were lit. The mouse was given 10 s (limited hold) to lick one of the two reinforcement spouts. A correct choice resulted in a 4 s period in which the animal could lick up to 15 licks of the water reinforcer. If an incorrect choice was made or no response was initiated within the allocated time, the mouse received a 30 s time-out during which all lights were turned off and no fluid was available. When 15 licks were taken, 30 s had passed, or when a time-out was completed, the sample spout was rotated over a funnel, rinsed with distilled water and dried with pressurized air and then rotated back into position in front of the slot. Some of these parameters varied during training (see below).

Training

The training schedule was the same as described previously (Eylam and Spector, 2003). Mice were trained to respond to the presentation of sucrose by licking one reinforcement spout and to respond to the presentation of water by licking the other reinforcement spout (side counterbalanced between mice within strains). Training started with 3 days of spout introduction, during which only one spout was available to the animal; the others were retracted (sample spout) or covered (reinforcement spout). In the next phase of training, a given reinforcement spout was associated with a given stimulus. One reinforcement spout was retracted and its access hole in the side-wall was covered. The animal was required to first lick a single taste stimulus (0.6 M sucrose or water) from the sample spout and then contact the available reinforcement spout to receive water. This procedure was repeated the next day with the initially covered reinforcement spout exposed and the other one covered. The alternative stimulus (e.g. water, if 0.6 M sucrose was presented first) was delivered through the sample spout. This two-session sequence was repeated three times for a total of six sessions in this phase during which no time-out was instituted and the limited hold was set to 180 s. After this, both reinforcement spouts and both sample fluids were made available during the session with both reinforcement spouts exposed. A correction procedure was used in which a stimulus (water or sucrose) was repeatedly presented until a criterion number of correct responses was made, upon which the alternative stimulus was presented. This criterion was decreased when the animal reached a performance level of 75% correct or higher. The criterion was changed from 6 to 4, to 2 and then to 1, ending with a randomized presentation of 0.6 M sucrose and water in blocks of two trials. During this criterion phase the time-out was set to 10 s and then 20 s while the limited-hold was shortened to 15 s and then 10 s. Finally, two more concentrations of sucrose were introduced (0.2 and 0.4 M) for another week of testing with a time-out of 30 s and a limited-hold of 10 s. These stimuli were presented in blocks of six (three sucrose concentrations and three water). Within each block, the stimulus presentation was randomized without replacement.

Testing

All the mice were tested daily in 25 min sessions. During each session, half of the reservoirs were filled with different concentrations of a taste stimulus and the other half, as well as the two reservoirs connected to the reinforcement spouts, were filled with distilled water. Stimuli were delivered in randomized blocks of 10 (without replacement) so that the probability of a taste stimulus presentation was 0.5.

Mice were first tested with a range of sucrose concentrations (0.0125–0.8 M) for 5 weeks (Suc I). They were initially tested with the training range of concentrations and these concentrations were varied weekly according to the overall performance of mice from all four strains. Lower concentrations were introduced weekly until performance approached its nadir. However, one or two clearly detectable concentrations were always included to maintain and measure stimulus control. Due to some technical problems with the sample spouts, which were replaced immediately following this phase of testing, we were not confident with the reliability of the data (although they were generally consistent with the results from later tests) from this phase, and thus they will not be discussed in this paper.

To assure stimulus control after replacement of the spouts, the mice were retrained on the sucrose detection task for 10 sessions. In this phase, the five highest concentrations of sucrose were presented in randomized blocks (five sucrose and five water in each block in a randomized order) as in the Sucrose I phase. Two mice from the D2 strain and one mouse from the 129 strain were removed from the experiment at this time since they did not re-learn the task during this training phase and their performance was not adequate. Subsequently, the mice were trained with 0.1 M D-phenylalanine (D-Phe) until they reached a 75% criterion of performance. The mice that reached this criterion were tested with a range of D-Phe concentrations (0.00625–0.1 M) for 5 weeks. However, only a few of the 129 mice and none of the D2 mice reached the aforementioned performance criterion and, thus, could not be further tested. The concentration of D-Phe at maximum solubility in water is 0.1 M. Due to this solubility constraint, we cannot be sure whether the lack of training in the non-taster strains was due to their inability to detect D-Phe at any concentration or because they simply had a shifted sensitivity curve. Thus, these data will not be presented here either.

After the end of the D-Phe testing, the mice were given free access to food and water again for 24 days. Approximately 24 h prior to the following testing phase, the water bottles were removed and the mice were subsequently retrained for 10 days on the sucrose detection task as before the D-Phe testing. Following that, the procedure described for the Suc I phase was repeated (Suc II). This phase lasted 5 weeks in order to obtain sucrose detectability data using the new sample spouts. Following the Suc II phase, the mice were given free access to food and water again for ~2 weeks and then were placed on the water restriction schedule and trained for five sessions with 0.6 M glycine followed by another five sessions with 0.2, 0.4 and 0.6 M glycine. After this training period, a glycine detectability function was derived over 6 weeks of testing. Next, the mice were given free access to food and water again for 1 week and then were placed on the water-restriction schedule and trained for four sessions with 1.0 M glucose followed by another two sessions with 0.8, 1.0 and 1.2 M glucose. After this training period, a glucose detectability function was derived over 5 weeks of testing. Lastly, the mice were tested again with sucrose (Suc III) for 5 weeks with the same concentrations as in Suc I and II. This phase was conducted to examine whether sucrose detectability changed with time and experience. In addition to the three mice eliminated from the experiment before the start of the D-Phe phase, three mice died during the course of the experiment (one D2, one SWR and one 129 mouse). The data from these mice were excluded for the phases they did not complete as well as from the calculation of the performance averaged over the two sucrose phases.

Water control test

At the termination of testing, immediately following the Suc III phase, the animals were given a water control test in which all the reservoirs were filled with distilled water. Half of the reservoirs were arbitrarily assigned to the left and half were assigned to the right reinforcement spout. Mice were tested for two consecutive days to examine whether they could perform competently without chemical cues in the fluids.

Data analysis

The proportion correct on stimulus trials was adjusted for false alarm (FA) rate using the following formula:

$$\text{Corrected hit rate} = P(\text{hit})_c = \frac{P(\text{hit}) - P(\text{FA})}{1 - P(\text{FA})} \times 100 \quad (1)$$

where $P(\text{hit})$ is the proportion of correct responses on taste stimulus trials (responses on the taste stimulus side) and $P(\text{FA})$ is the proportion of incorrect responses on water trials (responses on the taste stimulus side). Only trials with a response were included in this measure.

Sigmoidal three-parameter logistic curves were fit to the corrected hit rates for strain means as well as for individual mice using the following formula:

$$f(x) = \frac{a}{1 + 10^{(\log(x) - c) \times b}} \quad (2)$$

where x represents stimulus concentration, a represents the maximum asymptote of performance, b represents the slope of the function and c represents the $\log_{10}$ taste stimulus concentration at half-asymptotic performance which was operationally defined as the detection threshold for the corrected hit rate analysis.

A two-way ANOVA (strain × concentration) with concentration treated as a within factor was used to evaluate the differences in performance measures between strains and across concentrations. In addition, the threshold values (i.e. *c*-parameter) for individual animals, calculated from the curves fit to individual corrected hit rate data, were compared across strains using a one-way ANOVA followed by Tukey *post-hoc* paired comparisons. The degree of relationship between the sensitivity of the mice to the three taste compounds was examined by correlating the respective individual *c*-parameters collapsed across strain.

Our choice of threshold measurement (the *c*-parameter of the curve fit) is somewhat arbitrary and was selected to allow us to compare a specific point of sensitivity in the heart of the dynamic range of detectability while adjusting for the maximal performance of each animal. In order to be able to compare the level of sensitivity between the results from our operant conditioning procedure with those published from two-bottle intake tests, we also determined the concentration at which the uncorrected hit rate [*P*(hit)] was significantly different (*t*-tests) from the false alarm rate [*P*(FA)]. This measure [*P*(Hit) > *P*(FA)] allowed us to find the lower limit of detection under our testing conditions.

Finally, performance on the water control test was individually analyzed for statistically significant differences from chance (i.e. 50%) using a one-tailed version of the normal approximation of the binomial distribution. The rejection criterion was $P < 0.05$ for this and all other statistical tests.

Results

Mice from all strains took many trials and did well in the operant discrimination task, as was reflected by the asymptotic performance values at high concentrations of all stimuli (see *a*-values in Table 1) and the concentration dependence of the responses. Performance to each stimulus concentration across compounds was based on at least 14 trials with a response, usually many more (as high as 117), with the caveat that low concentrations were tested for fewer sessions in order to maintain stimulus control.

Sucrose

A comparison of the corrected hit rates between Suc II and III revealed a significant main effect of strain [$F(3,38) = 45.60$, $P < 0.001$], phase [$F(1,38) = 13.09$, $P = 0.001$] and concentration [$F(8,304) = 1046.21$, $P < 0.001$], as well as significant interactions between phase and concentration [$F(8,304) = 9.45$, $P < 0.001$] and strain and concentration [$F(24,304) = 13.98$, $P < 0.001$]. Importantly, there was no significant interaction between phase and strain [$F(3,38) = 1.71$, $P = 0.18$] and the triple interaction was not significant either [$F(24,304) = 0.70$, $P = 0.85$]. Moreover, the *c*-parameters (defined as threshold) from the curve fits did not significantly differ between the Suc II and Suc III phases, but the distributions for the latter did appear tighter (Figure 1, Tables 1 and 2). A strain × phase ANOVA of the *c*-parameters (threshold) revealed a significant main effect of strain [$F(3,37) = 44.27$, $P < 0.001$], but not of phase [$F(1,37) = 2.58$, $P = 0.12$] nor an interaction [$F(3,37) = 0.73$, $P = 0.54$). Because Suc II and Suc III served as 'bookends' to the glucose and glycine tests, coupled with the lack of a significant difference in the mean thresholds between the two phases, the curve parameters from both phases were collapsed together. This was done by calculating means for each curve parameter across individuals (excluding animals that did not complete both phases; see Materials and methods section) and these new mean parameters (Table 1) were used for the remaining analyses. Also, curves were fit to the mean of the corrected hit rate data from Suc II and Suc III for ease of comparison with the other stimuli (Figure 2a).

The mean psychophysical functions representing sucrose sensitivity for the B6 and SWR taster strains were similar, whereas the functions for the 129 and D2 non-taster strains were shifted to the right (Figure 2a). A two-way ANOVA of the corrected hit rate data indicated significant main effects of strain [$F(3,38) = 45.60$, $P < 0.001$] and concentration

Table 1 Mean (±SE) individual curve parameters across phases (corrected hit rate)

Phase	B6 mice			SWR mice			129 mice			D2 Mice		
	a	*b*	*c**	*a*	*b*	*c**	*a*	*b*	*c**	*a*	*b*	*c**
Sucrose II (Suc II)	80.77 (±2.77)[a]	–2.701 (±0.47)[a]	–1.168 (±0.06)[a]	87.27 (±0.91)[a]	–3.009 (±0.23)[a]	–1.128 (±0.03)[a]	75.04 (±2.71)[b]	–4.153 (±0.71)[b]	–0.809 (±0.05)[b]	69.56 (±3.91)[b]	–6.342 (±3.17)[b]	–0.739 (±0.07)[b]
Sucrose III (Suc III)	86.69 (±1.93)[a]	–2.034 (±0.13)[a]	–1.158 (±0.03)[a]	93.89 (±1.55)[c]	–2.226 (±0.13)[c]	–1.029 (±0.02)[c]	89.48 (±2.68)[d]	–2.181 (±0.56)[d]	–0.784 (±0.03)[d]	82.51 (±3.55)[c]	–3.083 (±0.63)[e]	–0.693 (±0.02)[e]
Mean sucrose (M-Suc)	83.73 (±2.15)[a]	–2.368 (±0.25)[a]	–1.163 (±0.04)[a]	90.33 (±0.96)[c]	–2.633 (±0.15)[c]	–1.079 (±0.02)[c]	81.41 (±2.47)[d]	–3.375 (±0.56)[d]	–0.803 (±0.03)[d]	77.44 (±2.69)[e]	–4.496 (±1.37)[e]	–0.695 (±0.02)[e]
Glucose (Glu)	84.54 (±2.52)[a]	–5.149 (±1.91)[a]	–0.610 (±0.03)[a]	94.47 (±1.27)[a]	–3.550 (±0.40)[a]	–0.471 (±0.03)[a]	93.40 (±2.36)[c]	–3.357 (±0.29)[c]	–0.253 (±0.03)[c]	89.08 (±3.23)[e]	–2.886 (±0.36)[e]	–0.187 (±0.07)[e]
Glycine (Gly)	84.09 (±3.18)[a]	–1.152 (±0.11)[a]	–1.380 (±0.05)[a]	92.12 (±2.95)[a]	–1.514 (±0.14)[a]	–1.179 (±0.06)[a]	92.91 (±3.34)[c]	–0.952 (±0.15)[c]	–1.063 (±0.08)[c]	78.35 (±7.38)[e]	–0.896 (±0.14)[e]	–1.170 (±0.01)[e]

[a]$n = 12$ (all mice); [b]$n = 8$; [c]$n = 11$; [d]$n = 10$; [e]$n = 9$.
*c-Values are expressed in $\log_{10}$(molar) units, while a-values are in % units.

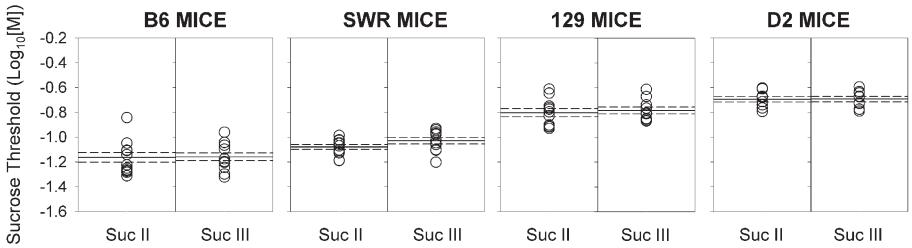

Figure 1 A comparison in the threshold distribution of the second and thirrd sucrose phases for individual animals (open symbols) and their mean (solid lines) and SE (dashed lines) in the B6 (left), the SWR (middle left), the 129 (middle right) and the D2 (right) mouse strains.

Table 2 A comparison of thresholds (in molar concentration) between two data analysis methods

Method	B6 mice			SWR mice			129 mice			D2 mice		
	Suc	Glu	Gly	Suc	Glu	Gly	Suc	Glu	Gly	Suc	Glu	Gly
Corrected hit rate[a]	0.069	0.245	0.042	0.083	0.338	0.066	0.157	0.558	0.086	0.202	0.650	0.068
P(hit) > P(FA)[b]	0.025	0.2	<0.006	0.037	0.2	0.0125	0.075	0.2	<0.006	0.1	0.4	0.0125

[a]Mean *c*-parameters based on the antilog of the values in Table 1 (in molar units).
[b]Calculated as the lowest concentration for which the hit rate significantly differed from the false alarm rate (in molar units).

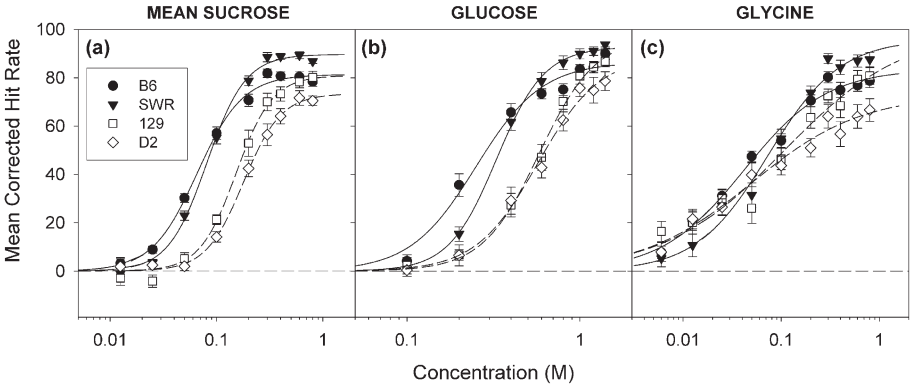

Figure 2 The mean curves fit to mean corrected hit rate data of the four mouse strains: the taster mouse strains in solid symbols (B6 in circles, SWR in triangles) and solid lines and the non-taster strains in open symbols (129 in squares and D2 in diamonds) and hatched line for the two collapsed sucrose phases **(a)**, glucose **(b)** and glycine **(c)**.

[$F(8,304) = 1046.21$, $P < 0.001$], as well as a significant interaction [$F(24,304) = 13.98$, $P < 0.001$]. To assess the differences in sensitivity between strains, a one-way ANOVA was conducted on the mean *c*-parameters (Tables 1 and 2), which revealed a significant effect of strain [$F(3,38) = 52.40$, $P < 0.001$). Tukey *post hoc* comparisons revealed that sucrose thresholds (i.e. *c*-parameters) for the B6 and SWR mice did not significantly differ ($P = 0.23$) but were roughly between two and three times lower than those for the non-taster 129 and D2 strains (both Ps < 0.001) which in turn did not differ from each other ($P = 0.17$).

The slopes (i.e. *b*-parameters) for the sucrose functions did not significantly differ across strains [$F(3,37) = 2.31$, $P = 0.09$], but the asymptotes (i.e. *a*-parameters) did [$F(3,37) = 9.39$, $P < 0.001$]. Tukey *post hoc* comparisons indicated that the SWR taster mice had significantly higher asymptotes compared with those from both non-taster strains (both Ps ≤ 0.03), but did not significantly differ from those of the taster B6 mice ($P = 0.06$). The B6 mice had significantly higher asymptotes than the D2 non-taster mice ($P = 0.03$), but not the 129 non-taster mice ($P = 0.96$) and the latter two strains did not differ on this measure ($P = 0.09$). The *a*-parameter reflects asymptotic performance at the high concentration range and as such represents a measure of both competence in the task and degree of stimulus control. There are several factors that could impact upon this measure, but one is sensitivity. It is possible that for some non-taster mice the task was more difficult because they were less sensitive to the lower concentrations and this may have compromised stimulus control at clearly detectable concentrations. This seems to have been more evident on Suc II, when the mice had relatively less experience, than on

Suc III. It should be stressed, however, that the *c*-parameter takes maximal performance into account for each mouse.

Glucose

The pattern of glucose detectability across strains was very similar to that seen for sucrose. The psychophysical functions for the 129 and D2 mice overlapped and were both shifted to the right compared with those from the B6 and SWR mice (Figure 2b). An ANOVA of the corrected hit rate data revealed a main effect of strain [$F(3,40) = 18.4$, $P < 0.001$] and concentration [$F(7,280) = 535.96$, $P < 0.001$], as well as an interaction [$F(21,280) = 9.62$, $P < 0.001$]. A one-way ANOVA for the thresholds (i.e. *c*-parameters) of each strain (Tables 1 and 2) indicated a significant strain effect [$F(3,40) = 24.05$, $P < 0.001$]. Tukey *post hoc* comparisons revealed that glucose thresholds for the B6 and SWR mice did not significantly differ ($P = 0.06$) but were between 1.7 and 2.7 times lower than those for the non-taster 129 and D2 strains (both Ps < 0.002) which in turn did not differ from each other ($P = 0.68$; Figure 3b). Overall, the thresholds of glucose were higher than those for sucrose in all mice [$t(41) = 27.12$, $P < 0.001$]. A two-way ANOVA for the *c*-parameters of these two sugars revealed a main effect of strain [$F(3,38) = 48.42$, $P < 0.001$] and stimulus [$F(1,38) = 718.0$, $P < 0.001$], but no interaction [$F(3,38) = 0.95$, $P = 0.425$].

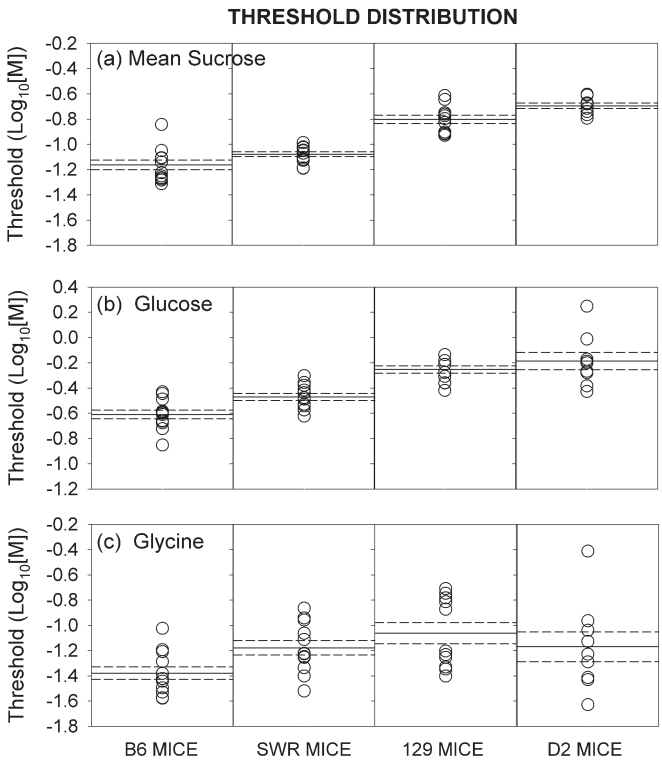

Figure 3 The threshold distribution of individual animals (open symbols) and their mean (solid lines) and SE (dashed lines), in the B6 (left), the SWR (middle left), the 129 (middle right) and the D2 (right) mouse strains for mean sucrose **(a)**, glucose **(b)** and glycine **(c)**.

When analyzing each strain separately, a significant main effect of stimulus was found in all strains (all Fs ≥ 44.35; all Ps < 0.001).

The slopes (i.e. *b*-parameters) for the glucose functions did not significantly differ across strain [$F(3,40) = 0.825$, $P = 0.49$], but the asymptotes (i.e. *a*-parameters) did [$F(3,40) = 4.01$, $P = 0.01$]. Tukey *post-hoc* comparisons indicated that the B6 taster mice had significantly lower asymptotes compared with those from the SWR and 129 strains (both Ps ≤ 0.04), which, in turn, did not differ from each other ($P = 0.99$). The asymptotes for the D2 mice did not significantly differ from any of the other strains (all Ps > 0.4).

Glycine

The pattern of glycine sensitivity across the strains was notably different from that seen for the two sugars. In fact, the strains were much more closely aligned although some modest differences were evident. As was the case for sucrose and glucose, a two-way ANOVA of the corrected hit rate data revealed a main effect of strain [$F(3,40) = 3.57$, $P = 0.02$] and concentration [$F(9,360) = 313.45$, $P < 0.001$], as well as a significant interaction [$F(27,360) = 5.47$, $P < 0.001$]. A one-way ANOVA of thresholds (Table 1) indicated a significant strain effect [$F(3,40) = 3.25$, $P = 0.03$]. Tukey *post-hoc* paired comparisons revealed a significant difference only between the 129 and B6 strains in threshold ($P = 0.02$). Interestingly, the thresholds for the 129 mice were bimodally distributed with half of the animals falling well within B6 distribution (Figure 3c).

The *a*-parameters from the fits for the glycine curves did not significantly differ across strains [$F(3,40) = 2.56$, $P = 0.07$]. There was, however, a significant strain effect for the *b*-parameters [$F(3,40) = 4.23$, $P = 0.01$]. Tukey *post-hoc* paired comparisons revealed that the SWR mice had significantly steeper slopes compared with both non-taster strains (both Ps < 0.025) but the slope of the B6 mice did not differ from those of the other strains (all Ps ≥ 0.22), nor did the slope of the 129 mice differ from that of the D2 mice ($P = 0.99$).

Threshold correlation between stimuli

A strong correlation was found between the thresholds (i.e. *c*-parameters) for sucrose and those for glucose (Figure 4a; $r = 0.81$, $P < 0.001$), while the correlation between the thresholds for sucrose and glycine, although significant, was considerably less impressive (Figure 4b; $r = 0.43$, $P = 0.004$). The same was true for the correlation between glucose and glycine (Figure 4c; $r = 0.40$, $P = 0.007$).

Water control test

In the water control test only one animal (from the 129 mouse strain) responded significantly different from chance (50%) out of the 42 mice tested (all *P*-values except one >0.05), with a performance average of 45.9 ± 1.3% for the B6 mice, 49.8 ± 0.7% for the SWR mice, 52.8 ± 1.8% for the 129

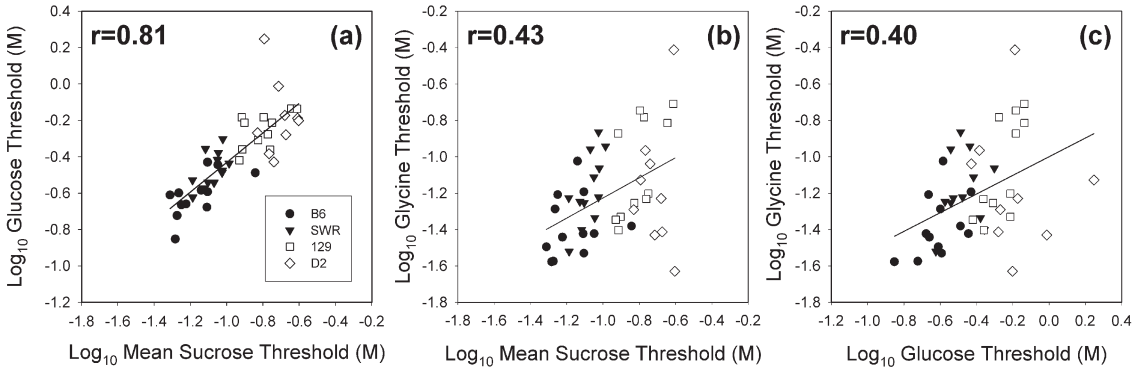

Figure 4 Correlation between individual thresholds of mice of all strains collapsed together (B6 in circles, SWR in triangles, 129 in squares and D2 in diamonds) for sucrose versus glucose **(a)**, sucrose versus glycine **(b)**, and glucose versus glycine **(c)**.

mice and 47.4 ± 2.1% for the D2 mice. The one mouse that responded significantly above chance nonetheless performed poorly (61.8%) and displayed concentration-dependent responses during threshold testing. Moreover, a Bonferroni correction eliminates the statistical significance of this animal's performance. These results confirm that the mice were not guided by any extraneous cues, but rather responded on the basis of the chemical nature of the stimulus.

Discussion

The *Sac* taster/non-taster phenotypes previously established for the B6, SWR, 129 and D2 inbred mouse strains (e.g. Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996) corresponded with the relative sensitivity of these strains to low concentrations of the two sugars tested in the operant taste detection task used here. The taster B6 and SWR mice had significantly lower sucrose thresholds compared with the non-taster 129 and D2 mice. Like sucrose, the glucose thresholds of mice from the taster strains were significantly lower than those of mice from the non-taster strains, supporting the hypothesis raised by recent molecular findings suggesting that these two natural sugars activate similar transduction pathways (Zhao *et al.*, 2003). Given that the non-taster phenotype is thought to be due to a polymorphism in the gene encoding for the T1R3 receptor causing a modification of the amino acid sequence and affecting its binding characteristics only when dimerized with the T1R2, but not with the T1R1 receptor (e.g. Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001; Li *et al.*, 2002; Nelson *et al.*, 2002; Reed *et al.*, 2004), it appears that the detectability of sucrose and glucose depends on which form of the T1R3 receptor is combined with the T1R2 receptor.

Moreover, all four strains had significantly higher thresholds for glucose than for sucrose. The reduced sensitivity to glucose in comparison with sucrose has been well documented, both by mouse CT recordings (Ninomiya *et al.*, 1984) and by two-bottle intake tests (Stockton and Whitney, 1974), as well as by results from various behavioral tests of other mammalian species, including humans (e.g. Cameron, 1947; Guttman, 1954; Hagstrom and Pfaffman, 1959; Morrison, 1969; Stone and Oliver, 1969). Collectively, these results suggest that the T1R2+3 receptor has a greater affinity for sucrose relative to glucose.

Unlike for sucrose and glucose, the taster status of the strains did not entirely account for the glycine results. On the one hand, the taster B6 mice were more sensitive than the non-taster 129 mice and this is consistent with two-bottle preference thresholds (Lush, 1989; Bachmanov *et al.*, 2001a). On the other hand, the SWR taster and D2 non-taster strains did not differ from any of the other strains. In fact, even though the 129 strain was on average less sensitive to glycine compared with the B6 mice, the threshold distribution for this non-taster strain was decidedly bimodal with the lower half falling well within the range of the B6 thresholds, consistent with the recent evidence that taster status is not associated with glycine preference (Inoue *et al.*, 2004). Thus, the taster status, as determined by detection or preference thresholds to sucrose, does not seem to explain, in any simple fashion, the phenotypic variance in sensitivity to glycine seen in the strains examined here. This conclusion is further supported by the correlational analysis of taste thresholds between pairs of compounds. When collapsed across strain, glucose and sucrose thresholds were highly correlated, confirming the taster/non-taster phenotypes and suggesting that the taste detectability of these sugars is heavily dependent on a normal functioning T1R2+3 heterodimeric receptor. In contrast, although thresholds for each sugar significantly correlated with glycine thresholds, the degree of the relationship was relatively modest. This strongly suggests that while the perithreshold intensity processing of glycine taste signals in the gustatory system might overlap somewhat with that of the sugar stimuli tested here, it is far from identical. This implication is consistent with the finding that glycine binds with both the T1R1+3 and the T1R2+3 receptors (Li *et al.*, 2002; Nelson *et al.*, 2002), whereas sucrose and glucose bind only with the latter

heterodimer in heterologous expression systems (e.g. Nelson *et al.*, 2001; Li *et al.*, 2002; Zhao *et al.*, 2003). Thus, the underwhelming degree of covariance between glycine and sugar thresholds is likely due to the additional contribution of the T1R1+3 receptor to glycine detection selectively in this case.

Although there is evidence that two-bottle test preference thresholds do not always conform to psychophysical functions measured in operant taste detection tasks (e.g. Eylam and Spector, 2002), based on the results here, the correspondence between the two types of measures appears to be reasonable. Sucrose thresholds based on the *c*-parameter for B6 and 129 were similar to preference thresholds reported in the literature (Bachmanov *et al.*, 2001a). The same was true for glycine thresholds in 129 mice. It is true that the glycine detection threshold based on the *c*-parameter in the present study was higher than the glycine preference threshold reported in the literature for B6 mice (Bachmanov *et al.*, 2001a), but this disparity could be an artifact of the specific definition of threshold applied. In our study, threshold was defined as the concentration at which the corrected hit rate was one-half of its asymptotic value. This was done because it effectively represents shifts in the location of the psychophysical function at its steepest locus of rise while taking into account the maximal performance reached in the task for an individual. In contrast, preference thresholds are generally derived by determining the stimulus concentration from a tested set at which intake is significantly (e.g. $P < 0.05$) greater than the intake of water. If we similarly define threshold as the point at which performance on taste trials was not significantly different from that on water trials (see Table 2), the values obtained are lower and in better agreement with the preference thresholds reported in the literature for glycine at least for B6 mice (e.g. Bachmanov *et al.*, 2001a). Although performing a similar exercise with our sucrose data results in detection thresholds that are somewhat lower than the preference thresholds reported by Bachmanov *et al.* (2001a), a circumstance that was also true for glycine thresholds in the 129 strain, the latter study used a very conservative statistical rejection criterion (i.e. α) to protect against false positives from the multiple comparisons they were conducting. Given these analytical and statistical considerations, the results regarding threshold sensitivity to sucrose and glycine as assessed by the signal detection task presented here and two-bottle preference tests are not grossly inconsistent. Therefore, it is likely that at low concentrations the postingestive effects of these compounds are minimal and thus do not impact upon the behavior, although it should be stressed that such influences could occur due to prior experience with higher concentrations (or with other compounds) depending on the order of testing for an animal in a two-bottle test (Flynn and Grill, 1988; Fregly and Rowland, 1992; Bachmanov *et al.*, 1998). The fact that psychophysical functions derived from operant signal detection tasks do not always lead to the same conclusions regarding taste sensitivity as preference-aversion functions derived from two-bottle tests suggests that the two measures are not quantifying exactly the same phenomenon (e.g. Eylam and Spector, 2002). A primary difference between the two procedures is that the hedonic value of the taste solutions drives the preference behavior in a two-bottle test, whereas the operant responses to taste samples in a signal detection task are driven by reinforcement contingencies irrespective of the affective features of the stimulus. The relatively good match found between the two types of measures with respect to the taste compounds tested here suggests that the affective value of these stimuli rises sharply once they are detected.

There were no clear phenotypic patterns that emerged in the analysis of the asymptote (*a*) and slope (*b*) parameters of the psychophysical functions. The asymptote is a measure of general competence in the task and serves to standardize the analysis of sensitivity. Although the taster mice tended to have higher asymptotes compared with the non-taster mice in the analysis of mean sucrose performance, this pattern broke down when glucose was the stimulus and there were no significant strain differences in the asymptotes of the glycine functions. The slope is a measure of the sharpness of the change in detectability. Theoretically, a step function would be the steepest slope possible and represents an idealized view of a threshold in which a subject can always detect a stimulus past a certain concentration and never detect it when the stimulus is below this critical value. For sucrose and glucose, there were no significant strain differences in the slopes of the functions. There were some strain differences in the slope of the glycine functions. The functions for the SWR mice had significantly steeper slopes than those for the non-taster mice and did not differ from those for the B6 strain. However, the slopes for the B6 taster strain did not differ from those for the 129 and D2 non-taster strains. Thus, while the asymptote and slope parameters of the psychophysical functions are important because they contribute to a comprehensive depiction of a given subject's performance, the threshold parameter (*c*), which quantifies the location of the function along the concentration continuum, appears to have been the most theoretically informative from the standpoint of strain differences in sensitivity to the taste compounds.

The use of inbred and genetically altered strains of mice is a very powerful tool for unraveling the neurobiological basis of gustatory function. The success of such approaches depends on accurate assessments of phenotypes. Arguably from the standpoint of gustatory function, appropriate and comprehensive measures of taste-guided behavior are critical. It is only through the behavior of the animal that inferences can be made about perception. Because behavior is the output of the system, however, variability at any one of the many stages of taste signal processing can affect the outcome of phenotyping experiments. In the present case, the *Sac* taster/non-taster phenotypes do not entirely account

for the inter-strain variance in sensitivity and responsiveness to all sweeteners. For example, glycine, an amino acid that appears to share some qualitative similarities with sucrose based on conditioned taste aversion generalization experiments in rodents (Nowlis *et al.*, 1980; Pritchard and Scott, 1982; Kasahara *et al.*, 1987; Yamamoto *et al.*, 1988; Danilova *et al.*, 1998; see also Tapper and Halpern, 1968), is just as detectable to non-taster D2 mice as to SWR and B6 taster mice. Moreover, although *Sac* taster mice are more sensitive to low concentrations of sucrose compared with non-taster mice, some non-taster strains (e.g. 129) can display higher levels of unconditioned responsiveness to high concentrations of sucrose compared with taster mice (Dotson and Spector, 2004; Glendinning *et al.*, 2003). The genetic basis of these strain differences in responsiveness to 'sweeteners' that do not entirely correspond with the *Sac* taster phenotype remains to be determined, but can potentially involve processes anywhere along the gustatory neuraxis.

Notes

1. The 'taster/non-taster' nomenclature is somewhat misleading because the non-taster phenotype does not represent aguesia to sucrose and other 'sweet' compounds, but merely reduced responsiveness at low concentrations. Nevertheless, we have adopted this common terminology in this paper to be consistent with the precedent established in the literature.

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