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The Relative Affective Potency of Glycine, L-Serine and Sucrose as Assessed by a Brief-access Taste Test in Inbred Strains of Mice

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Abstract

In general, rodents prefer both sucrose and L-serine relative to water and treat both compounds as possessing a similar taste quality (e.g. 'sweetness') despite that they are believed to bind with different T1R heterodimeric receptors in taste bud cells. We assessed the affective potency of these compounds along with glycine, which is thought to bind with both T1R receptor complexes, using a brief-access taste test in a gustometer. Unconditioned licking responses of two 'taster' strains (C57BL/6J and SWR/J), which display high preference for low concentrations of sucrose, and two 'non-taster' (129P3/J and DBA/2J) strains, which display blunted preference for low concentrations of sucrose, were measured during 5 s trials of varying concentrations of a single compound when mice (n = 10/strain/stimulus) were non-deprived and when access to home-cage water was restricted. In non-deprived mice, sucrose generated monotonically increasing concentration–response curves regardless of strain, whereas glycine was only marginally effective at stimulating licking and L-serine produced relatively flat functions. The profile of responsiveness across strains was more complex than expected. For example, when tested with sucrose in the non-deprived condition, the 129P3/J non-taster strain surpassed the responsiveness of taster mice at mid-range to high concentrations. Under water-restricted conditions, these mice also were significantly more responsive to high concentrations of both sucrose and glycine compared with the other strains when stimulus licking was standardized relative to water. Thus, the affective potency of the stimuli tested here seems to be related to the ability of the compounds to bind with the T1R2+3 receptor complex. However, the profile of strain responsiveness to these tastants in the brief-access test does not appear to be simply explained by the sweetener 'taster' status of the strain.

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

Introduction

An understanding of the neural basis of sweetener[1] and amino acid taste perception has been propelled by remarkable discoveries regarding the molecular biology of transduction processes in the mammalian peripheral gustatory system. Specifically, a gene family has been identified which encodes for three G-protein coupled receptors (T1R1, T1R2 and T1R3) that bind with sugars, synthetic sweeteners and amino acids (Hoon *et al.*, 1999; Bachmanov *et al.*, 2001a; Kitagawa *et al.*, 2001; Li *et al.*, 2001, 2002; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001). The T1R3 receptor has been shown to combine with T1R1 or T1R2 to form functional heterodimers. The T1R2+3 complex is activated by a variety of both natural and synthetic sweeteners as well as 'sweet-tasting' D-amino acids (Nelson *et al.*, 2001; Li *et al.*, 2002; Zhao *et al.*, 2003). Further studies have revealed that the combination of mouse T1R1 and T1R3 gives rise to a heterodimeric receptor that interacts with most of the 20 common L-amino acids (Nelson *et al.*, 2002). In addition, T1R3 is thought to function independently as a low affinity receptor, binding with high concentrations of natural but not synthetic sweeteners (Zhao *et al.*, 2003).

Although it is clear that in a variety of mammalian species many sugars and synthetic compounds possess a perceptual quality in common termed 'sweetness', the taste quality of amino acids appears to be more varied. Researchers have tried to perceptually categorize amino acids in rodents by using the conditioned taste aversion generalization paradigm to quantify the degree to which these compounds are similar to prototypical chemical stimuli thought to represent basic taste qualities (e.g. sucrose, NaCl, citric acid, quinine). Taken together without regard to strain or species differences, results from such experiments demonstrate that a subset of D-amino acids, including D-alanine, D-valine, D-

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methionine, D-tryptophan, D-phenylalanine, D-histidine and D-leucine, and a subset of L-amino acids, including L-alanine, L-proline, L-serine, L-glutamine and glycine (which does not have chiral carbon), are all treated as possessing some degree of a qualitative similarity with the taste of sucrose and are thus considered 'sweet' (e.g. Tapper and Halpern, 1968; Nowlis *et al.*, 1980; Schiffman *et al.*, 1981; Pritchard and Scott, 1982; Ninomiya *et al.*, 1984; Kasahara *et al.*, 1987; Ninomiya and Funakoshi, 1989; Ninomiya and Kajiura, 1993; Stapleton *et al.*, 2002); other amino acids tested fail to fall into this category.

The molecular biology pertaining to the transduction of both sugars and synthetic sweeteners as well as 'sweet-tasting' D-amino acids is consistent with the electrophysiological and behavioral phenotypes expressed by different inbred strains of mice, but such a correspondence regarding sweet-tasting L-amino acids (and glycine) is less straightforward. It has been known for many years that mouse strains can be differentiated according to their intake of and nerve responsiveness to sweeteners. In general, 'taster' mice have lower preference thresholds for sweeteners in two-bottle tests and their chorda tympani nerves (CT) are more responsive to sucrose, saccharin, and various 'sweet-tasting' D-amino acids (especially D-phenylalanine) when compared with 'non-taster' mice (e.g. Capretta, 1970; Pelz *et al.*, 1973; Fuller, 1974; Ninomiya *et al.*, 1984; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996; Frank and Blizard, 1999; Inoue *et al.*, 2001; Zhao *et al.*, 2003).[2] These taster/non-taster phenotypes in mice were genetically linked to a single chromosomal locus referred to as *Sac* that was later discovered to encode for the T1R3 receptor (e.g. Fuller, 1974; Ramirez and Fuller, 1976; Lush, 1989; Capeless and Whitney, 1995; Lush *et al.*, 1995). Taster and non-taster mouse strains have different alleles of the *Tas1r3* gene that give rise to receptors with slightly different amino acid sequences (Bachmanov *et al.*, 2001a; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001). Interestingly, the taster and non-taster allele of *Tas1r3* generates receptors that are functionally similar when combined with T1R1, but the non-taster form of the T1R3 receptor displays impaired binding when combined with T1R2 (Nelson *et al.*, 2002; Damak *et al.*, 2003). Thus non-taster mouse strains possess a dysfunctional T1R2+3, but an apparently normal T1R1+3, heterodimer complex. Indeed, there is evidence that L-amino acids, which bind with the T1R1+3 receptor, stimulate the CT comparably in both taster and non-taster mice, with the possible exception of L-proline (Ninomiya *et al.*, 1984; Inoue *et al.*, 2001). Yet, two-bottle preference for 'sweet-tasting' L-amino acids and glycine appears to depend on the 'taster' status of the mouse strain based on testing with sugars (Iwasaki *et al.*, 1985; Lush, 1989; Capeless and Whitney, 1995; Lush *et al.*, 1995; Bachmanov *et al.*, 2001b). These behavioral findings are curious considering that L-amino acids are believed to bind primarily with the T1R1+3 receptor which, as noted above, is thought to display similar binding properties in both taster and non-taster mice (Nelson *et al.*, 2002).

In light of the apparent tension between the predicted behavior of mouse strains based on the molecular biology of amino acid taste transduction and the observed behavior seen in the two-bottle preference test, we examined the relative effectiveness of sucrose, glycine and L-serine to stimulate licking in C57BL/6J (B6), SWR/J (SWR), DBA/2J (D2) and 129P3/J (129) mice in a brief-access taste test. As noted above, inbred mice vary in their preference for all three of these compounds as assessed in two-bottle intake tests, and there is evidence that these compounds possess some common perceptual properties with respect to taste quality (i.e. 'sweet') in at least some rodents. If glycine and L-serine generate concentration–response functions that emulate sucrose, then it would suggest that these compounds are similar in their affective potency.

In addition, we sought to examine the generality of the response profiles generated by these compounds by including taster (B6 and SWR) and non-taster (129 and D2) mouse strains in the experimental design allowing us to make inferences regarding the effect of the non-taster form of the *Tas1r3* allele on taste-guided behavior (Capretta, 1970; Pelz *et al.*, 1973; Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996; Max *et al.*, 2001; Nelson *et al.*, 2001). With some notable exceptions (Glendinning *et al.*, 2002, 2003; Zhang *et al.*, 2003; Zhao *et al.*, 2003), most of the work conducted to date involving strain comparisons of unconditioned behavioral responsiveness to these compounds has been based on two-bottle intake tests (water versus taste compound). Although taste certainly influences the behavior in that test paradigm, postingestive events can also influence intake. The brief-access taste test involves the measurement of licking during very short trials with a sapid solution increasing the confidence that the responses are based on the oral sensory features of the stimulus. Many trials of various concentrations of the taste stimulus are presented during a session and concentration–response functions are derived. The taste solutions are delivered in randomized blocks to minimize systematic carry-over effects and to mitigate the influence of postingestive factors on the response to a given stimulus in the set.

Materials and methods

Subjects

A total of 120 male naive mice (Jackson Laboratories, Bar Harbor, ME) from four different strains, C57BL/6J (B6), SWR/J (SWR), 129P3/J (129) and DBA/2J (D2), served as subjects (n = 30/strain). Within each strain, animals were randomly assigned to one of three stimulus groups (n = 10/group). The mice were housed individually in polycarbonate shoebox cages in a colony room where the temperature and lighting were controlled automatically (12 h:12 h). Testing

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and training took place during the lights-on phase. Mice were habituated to the laboratory environment for 7 days before testing and were ~8 weeks of age at the start of testing. During this time, food and water were available *ad libitum*. During periods when the animals were placed on a water-restriction schedule, mice that dropped below 80% of their free-feeding weight received 1 ml supplemental water 2 h after the end of the testing session. We tested all the animals over 7 weeks and only had to provide supplemental water on 24 occasions.

Taste stimuli

All solutions were prepared daily with purified water (Elix 10; Millipore, Billerica, MA) and reagent grade chemicals, and were presented at room temperature. Test stimuli consisted of five concentrations of sucrose (0.0625, 0.125, 0.25, 0.5 and 1.0 M; Fisher Scientific, Atlanta, GA), L-serine (0.25, 0.5, 0.75, 1.0 and 1.5 M; Sigma-Aldrich, St Louis, MO), glycine (0.25, 0.5, 0.75, 1.0 and 1.5 M; Sigma-Aldrich, St Louis, MO) and purified water. Sucrose was chosen because (i) it is a prototypical sweetener that is commonly used in taste experiments, (ii) it has been used to differentiate taster (B6 and SWR) from non-taster (D2 and 129) mice in two-bottle preference tests and (iii) it binds with the T1R2+3, but not the T1R1+3, receptor complex. L-serine and glycine were chosen because (i) there is evidence that at least in some rodents these compounds share a perceptual quality with sucrose, (ii) they are preferred by some rodents at mid-range concentrations in two-bottle preference tests and (iii) they appear to bind primarily with the T1R1+3, but only modestly, if at all, with the T1R2+3 receptor complex.

Procedure

We used a brief-access procedure similar to that described by Glendinning *et al.* (2002). Testing took place in a lickometer referred to as the Davis rig (Davis MS-160; DiLog Instruments, Tallahassee, FL; see Smith, 2001). This device allowed the mouse access to a single tube containing a taste stimulus for a brief period of time (5 s) and then after a 7.5 s inter-presentation interval, a different tube was offered. The stimulus array for each compound tested included the five different concentrations detailed above and purified water contained in separate bottles. A given trial started after the first lick. Presentation order was randomized without replacement in blocks so that every concentration of a stimulus and water was presented exactly once before the initiation of the subsequent block. Unconditioned licking responses were recorded for later analysis. Sessions were 30 min in duration during which mice could initiate as many trials as possible. The animals were first trained to lick a stationary tube of water for 30 min in the Davis rig after being placed on ~23.5 h restricted water access schedule. Animals then received 2 days of testing with five stimulus concentrations and purified water while maintained on the water-restriction schedule. This was done to familiarize the animals with the stimulus array. The water bottles were then replaced on the home cages and the mice were tested for three days non-deprived.

Data analysis

For deprived days, a Tastant/Water Lick Ratio was calculated. This ratio was derived by taking the average number of licks per trial for each concentration and dividing it by the average licks per trial when water was delivered. This ratio controls for individual differences in lick rates and for differences in motivational state. The Tastant/Water Lick Ratio is useful for analyzing responses of animals highly motivated to lick due to the restricted water access schedule. In the non-deprived condition, the average number of licks per trial for each concentration was collapsed across test sessions and divided by that animal's maximum potential lick rate per trial based on the mean of the inter-lick interval (ILI) distribution measured during training (only inter-lick intervals >50 and <200 ms were used), yielding a Standardized Lick Ratio (see Glendinning *et al.*, 2002). Five mice out of 120 did not sample on the stationary water training day, three were included in the analysis of the data, so the ILI value used to standardize their data was taken from the first day of water-deprived testing (the correlation between ILIs measured during the stationary water training day and the first day of water deprived testing for the remaining 115 mice was $r = 0.852$). Standardizing the licking response in this fashion controls for individual differences in characteristic local lick rates.

The ratio scores were analyzed with two-way strain × concentration analyses of variance (ANOVAs). When a strain main effect or a strain × concentration interaction was significant, one-way ANOVAs were conducted to test for simple effects. Differences between strains at each concentration were evaluated using Tukey's honestly significant difference test. Differences between Standardized Lick Ratio scores in response to a given concentration and those measured for water were tested with matched *t*-tests. The conventional $P \leq 0.05$ was applied as the statistical rejection criterion. Only mice that had at least one trial at every concentration were included in the analysis of a given stimulus.

Results

Standardization data

Because there can be within-strain and between-strain differences in the local lick rate as well as in the motivational response to the water restriction schedule, it is important to account for these factors in any licking measure of taste responsiveness. As recommended by Glendinning *et al.* (2002), the Tastant/Water Lick Ratio was calculated for animals tested when under the water-restriction schedule and the Standardized Lick Ratio was calculated for animals tested when non-deprived to statistically control for non-

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taste influences in licking. Table 1 contains the means of the individual values representing licks during water trials used in the calculation of the Tastant/Water Lick Ratio for the various strains and compounds. A two-way ANOVA on water licks revealed a significant main effect of strain [$F(3,107) = 40.7$, $P < 0.001$] and test solution [$F(2,107) = 9.15$, $P < 0.001$] as well as a significant interaction [$F(6,107) = 5.31$, $P < 0.001$]. One-way ANOVAs were conducted within each taste compound to test for strain differences in water licks. There was a significant main effect of strain on the mean number of licks to water when mice were tested with sucrose [$F(3,36) = 15.6$, $P < 0.001$], L-serine [$F(3,36) = 15.4$, $P < 0.001$] and glycine [$F(3,36) = 19.6$, $P < 0.001$] in the water restriction condition. Interestingly, one-way ANOVAs conducted within each strain to test for differences in water licks to the stimuli revealed that the non-taster strains increased licks to water when tested with L-serine in the deprived condition relative to licks taken in the other stimulus conditions (Ps ≤ 0.001). The taster strains did not significantly differ in their responses to water across stimulus conditions.

Table 2 contains the means of the individual values representing the ILI observed when water-restricted animals were licking water from a stationary spout. These means exclude the mice that were not included in the analysis of responses under non-deprived conditions ($n = 93$). The reciprocal of these values were multiplied by 5000 to derive the estimated maximum possible licks during a 5 s trial and used in the calculation of the Standardized Lick Ratio for various strains and compounds. As expected, a two-way ANOVA revealed a significant effect of strain [$F(3,81) = 52.1$, $P < 0.001$] but no significant stimulus effect [$F(2,81) = 0.1$, $P = 0.909$] or interaction [$F(6,81) = 0.7$, $P = 0.657$]. Collectively, the results from these analyses confirm the necessity for standardizing the licking data across animals and strains.

Sucrose

In the deprived condition, mice took between 12 and 72 trials per session (mean ± SE = 39.81 ± 1.42). A two-way ANOVA of the Tastant/Water Lick Ratios revealed a significant main effect of strain [$F(3,36) = 18.1$, $P < 0.001$], a significant main effect of concentration [$F(4,144) = 5.9$, $P < 0.001$] and a significant interaction [$F(12\ 144) = 10.4$, $P < 0.001$]. Strain differences at each concentration are delineated in Table 3. Confirming what is apparent in Figure 1, separate one-way ANOVAs for each strain revealed that only the 129 mice showed a significant monotonically increasing concentration–response function [$F(4,36) = 11.9$, $P < 0.001$]. Although we did not expect to find meaningful results in the water-restriction condition considering that mice will usually lick water at a maximal rate making it difficult to ascertain a response to appetitive stimuli and we did not expect to see an aversive response profile elicited by these 'sweet-tasting' compounds, it appears that the 129 mice did suppress licking to water relative to the other strains and, as a result, increased their Tastant/Water Lick Ratio to the stimulus [$F(3,36) = 15.6$, $P < 0.001$]. There were some significant concentration-dependent effects on the Tastant/Water Lick Ratio for the other three strains (All Fs > 3.0, all Ps < 0.05), but it is obvious that these functions were relatively flat and generally equal to or below a value of 1.0. The 129 mice had significantly higher ratios at all five concentrations compared with the B6 and D2 mice and at the four highest concentrations compared with the SWR mice (all P-values <0.05); the latter three strains did not differ.

In the non-deprived condition, mice took between 0 and 72 trials per session (mean ± SE = 21.99 ± 1.21). All strains clearly showed a concentration dependent increase in licking to sucrose [see Figure 2; $F(5,170) = 531.9$, $P < 0.001$], but their concentration–response functions significantly differed [strain × concentration interaction: $F(15,170) = 10.9$, $P < 0.001$]. Strain differences at each concentration are delineated in Table 4. The SWR mice were significantly more responsive to lower sucrose concentrations compared with D2 and 129 mice. At the lowest concentration tested (0.0625 M), the Standardized Lick Ratio was significantly greater than that for water in the SWR and B6 (both ts > 2.2,

Table 1 Mean number of licks to water ± SE taken by each of the 12 groups when tested under the water-restricted condition

	Sucrose	L-serine	Glycine
B6	32.15 ± 1.437[b]	30.56 ± 2.33[a]	32.3 ± 2.167[b]
SWR	41.75 ± 2.164[a]	44.14 ± 1.949[b]	48.33 ± 1.5[a]
129	22.93 ± 1.683[c]	34.05 ± 1.855[a*]	24.8 ± 2.255[b]
D2	34.79 ± 2.439[b]	47.14 ± 1.895[b*]	32.78 ± 2.868[b]

See text for overall ANOVA results. Simple effects were tested with one-way ANOVAs within strain or within test stimulus followed by Tukey's honestly significant difference tests. Within each strain, an asterisk indicates a significant difference of the values for a test stimulus. Within each test stimulus, values with the same superscripted letter are not significantly different.

Table 2 Mean of the inter-lick interval (ILI) distribution ± SE observed in the 12 groups when licking water from a stationary spout (see text for exceptions)

	Sucrose	L-serine	Glycine
B6	121.273 ± 1.1[a]	121.951 ± 1.462[a]	121.343 ± 1.87[a]
SWR	97.971 ± 1.241[b]	98.627 ± 1.448[b]	94.357 ± 1.044[b]
129	108.26 ± 1.149[c]	109.819 ± 0.816[c]	108.564 ± 1.69[c]
D2	104.418 ± 2.318[d]	105.945 ± 1.767[d]	104.676 ± 2.788[d]

See text for overall ANOVA results. Simple effects were tested with one-way ANOVAs within strain or within test stimulus followed by Tukey's honestly significant difference tests. Within each strain, an asterisk indicates a significant difference of the values for a test stimulus. Within each test stimulus, values with the same superscripted letter are not significantly different.

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Table 3 Strains listed in order of mean Tastant/Water Lick Ratio, for sucrose, L-serine and glycine in the deprived condition

Sucrose[1]							
0.0625 M:	129	≥	SWR	=	D2	=	B6
0.125 M:	129	>	SWR	=	D2	=	B6
0.25 M:	129	>	SWR	=	D2	=	B6
0.5 M:	129	>	SWR	=	D2	=	B6
1.0 M:	129	>	SWR	=	D2	=	B6

L-Serine[1]							
0.25 M:	129	=	B6	=	SWR	=	D2
0.5 M:	SWR	≥	129	=	B6	=	D2
0.75 M:	129	≥	SWR	=	B6	=	D2
1.0 M:	129	=	B6	=	SWR	>	D2
1.5 M:	SWR	=	129	=	B6	>	D2

Glycine[1]							
0.25 M:	129	=	B6	=	SWR	=	D2
0.5 M:	129	≥	SWR	=	B6	=	D2
0.75 M:	129	>	B6	=	SWR	=	D2
1.0 M:	129	>	D2	=	B6	=	SWR
1.5 M:	129	>	D2	=	SWR	=	B6

[1]At concentrations at which the ANOVA detected a significant strain effect, strains falling under the same line did not significantly differ in Tukey's HSD *post hoc* comparisons ($P < 0.05$).

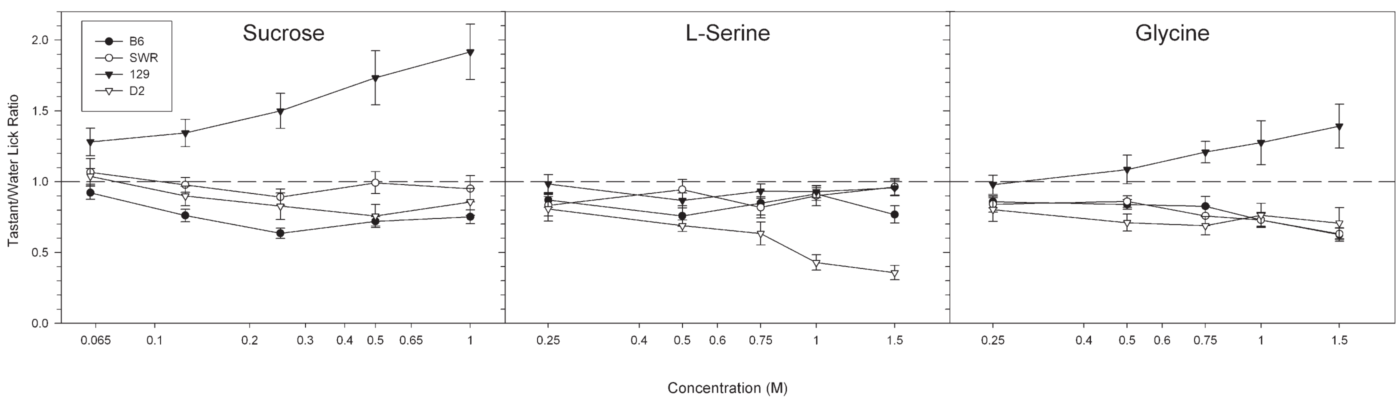

Figure 1 Mean (± SE) Tastant/Water Lick Ratio as a function of sucrose, L-serine and glycine concentration for four different inbred strains of mice (n = 10/stimulus/strain). The Tastant/Water Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the average licks to water. The dashed line on the graph represents a Tastant/Water Lick Ratio of 1.0, which indicates licking to the taste stimulus was equivalent to licking to water. This ratio controls for differences in oral motor competence and physiological state. These animals were tested in two consecutive sessions while on a 23.5 h water-restriction schedule.

*P*s < 0.05), but not the D2 and 129, strains (both *t*s < –0.2, *P*s > 0.7). As the sucrose concentration was raised, however, D2 and 129 mice steeply increased their responsiveness to sucrose and eventually equaled or surpassed the licking in SWR mice. B6 mice had a concentration response profile somewhat in between the SWR and the 129 and D2 mice.

L-Serine

In the deprived condition, mice took between 0 and 45 trials per session (mean ± SE = 23.54 ± 0.97). There was a significant strain effect [$F(3,36) = 9.8$, $P < 0.001$] on the Tastant/Water Lick Ratio and a significant strain × concentration interaction [see Figure 2; $F(12,144) = 6.6$, $P < 0.001$]. Surprisingly, the D2 mice actually decreased their lick rate as the L-serine concentration was raised [Figure 1; $F(4,36) = 15.3$, $P < 0.001$], whereas the other strains displayed relatively flat functions. Strain differences at each concentration are shown in Table 3.

In the non-deprived condition, mice took between 0 and 50 trials per session (mean ± SE = 8.87 ± 1.05). There was no significant difference in the Standardized Lick Ratio between the strains [$F(3,21) = 0.1$, $P = 0.9$], but there was a significant effect of concentration [$F(5,105) = 4.2$, $P = 0.002$], though the magnitude of this effect was relatively minor; there was no significant strain × concentration interaction (see Figure 2).

Glycine

In the deprived condition, mice took between 0 and 62 trials per session (mean ± SE = 31.21 ± 1.2). There was a significant strain effect [$F(3, 36) = 10.6$, $P < 0.001$] on the Tastant/Water Lick Ratio and a significant strain × concentration interaction [$F(12,140) = 5.7$, $P < 0.001$]. Strain differences at each concentration are delineated in Table 3. As was the case with sucrose, separate one-way ANOVAs indicated that only the 129 mice increased their Tastant/Water Lick Ratio monotonically as a function of concentration [$F(4,36) = 7.1$, $P < 0.001$; see Figure 1]. This increase in licking was first significantly greater than 1.0 at the 0.75 M concentration ($P = 0.022$). There were some significant concentration-

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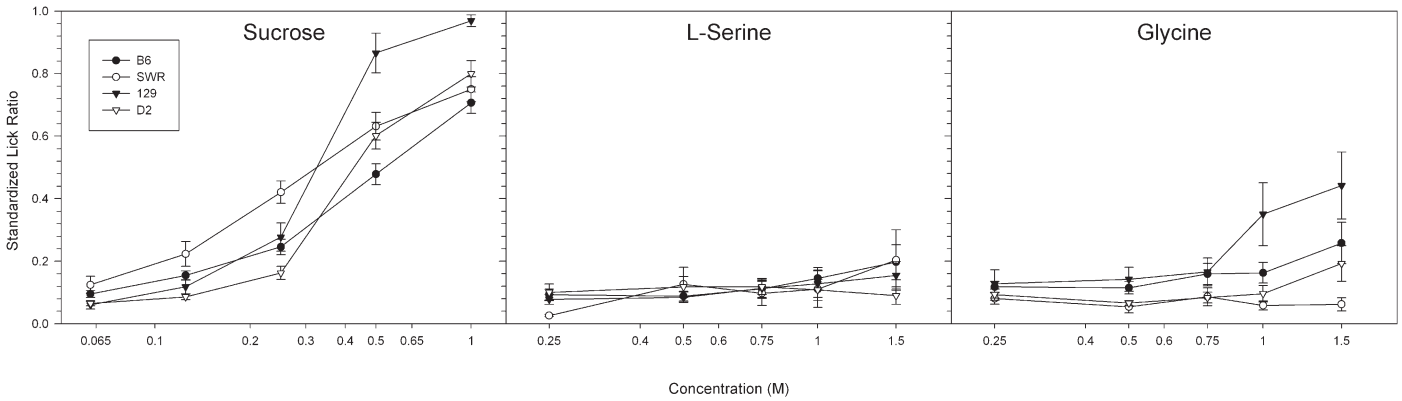

Figure 2 Mean (± SE) Standardized Lick Ratio as a function of sucrose, L-serine and glycine concentration for four different inbred strains of mice. The Standardized Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the maximum potential licks in a 5 s trial, derived from that animal's previously measured inter-lick interval distribution. This score is used for normally preferred stimuli and controls for differences in characteristic local lick rates. A score of 1.0 reflects licking to the taste stimulus that was at the maximum possible rate. These animals were tested non-deprived in three consecutive sessions. Only mice that had at least one trial at every concentration were included in the analysis of a given stimulus [sucrose—B6 (n = 10), SWR (n = 8), 129 (n = 10), D2 (n = 10); L-serine—B6 (n = 9), SWR (n = 4), 129 (n = 7), D2 (n = 5); glycine—B6 (n = 9), SWR (n = 7), 129 (n = 5), D2 (n = 9)].

Table 4 Strain listed in order of mean Standardized Lick Ratio, for sucrose, L-serine, and glycine in the non-deprived condition

	Sucrose[1]							L-Serine[2]							Glycine[1]						
Water:	SWR	=	B6	=	D2	=	129	**Water:**	B6	=	SWR	=	D2	=	129	**Water:**	SWR	=	B6	=	D2	=	129
0.0625 M:	SWR	≥	B6	=	D2	=	129	**0.25 M:**	129	=	B6	=	D2	=	SWR	**0.25 M:**	B6	=	D2	=	129	=	SWR
0.125 M:	SWR	≥	B6	=	129	=	D2	**0.5 M:**	D2	=	SWR	=	B6	=	129	**0.5 M:**	B6	=	129	=	D2	=	SWR
0.25 M:	SWR	≥	129	≥	B6	>	D2	**0.75 M:**	B6	=	129	=	D2	=	SWR	**0.75 M:**	B6	=	129	=	SWR	=	D2
0.5 M:	129	>	SWR	=	D2	=	B6	**1.0 M:**	B6	=	129	=	SWR	=	D2	**1.0 M:**	129	≥	B6	=	D2	=	SWR
1.0 M:	129	>	D2	=	SWR	=	B6	**1.5 M:**	B6	=	129	=	SWR	=	D2	**1.5 M:**	129	≥	B6	=	D2	=	SWR

[1]For sucrose and glycine, at concentrations at which the ANOVA detected a significant strain effect, strains falling under the same line did not significantly differ in Tukey's HSD *post hoc* comparisons ($P < 0.05$).
[2]There were no significant strain effects for L-serine.

dependent effects on the Tastant/Water Lick Ratio for B6 and SWR mice (All Fs > 4.6, all Ps < 0.01), but it is obvious that the functions for these strains as well as for the D2 mice were relatively flat and generally below a value of 1.0.

In the non-deprived condition, mice took between 0 and 50 trials per session (mean ± SE = 10.64 ± 1.24). There was a significant effect of strain [$F(3,26) = 5.8$, $P = 0.004$] on the Standardized Lick Ratio and a significant Strain × Concentration interaction [$F(15,130) = 2.9$, $P = 0.001$]. Strain differences at each concentration are delineated in Table 4. Separate one-way ANOVAs of the Standardized Lick Ratios for each strain revealed that 129 [$F(5,20) = 8.1$, $P < 0.001$], B6 [$F(5,40) = 3.3$, $P < 0.05$] and D2 [$F(5,40) = 2.5$, $P < 0.05$] mice changed their lick rate as a function of concentration, but the modest increases were apparently limited to higher concentrations (see Figure 2). For example, matched t-tests indicated that the 129 strain did not display significantly elevated licking relative to water until the glycine concentration reached 1.5 M ($P < 0.05$). For B6 and D2 mice, no concentration significantly differed from water. The SWR mice did not significantly change their licking as a function of concentration [$F(5,30) = 0.6$, $P = 0.678$].

Discussion

Overall, as assessed by the brief-access taste test, the amino acids, L-serine and glycine, paled in comparison to sucrose in their ability to generate licking in the mouse strains examined. Collapsed across strain, non-deprived animals licked L-serine and glycine at a mean rate of only 15.4 and 21.4%, respectively, of the maximum possible in the 5 s trial at the highest concentration tested (1.5 M). In striking contrast, 1.0 M sucrose (the highest concentration tested) elicited an average licking rate, collapsed across strain, that was more than five times higher than that seen for L-serine and nearly four times higher than that seen for glycine. The relatively

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broad concentration range used in this study weakens the possibility that the design failed to capture the dynamic range of responsiveness. Thus, the results presented here suggest that the taste-related affective potency of sucrose is far superior to that of glycine or L-serine.

Although neither amino acid was remarkably effective at stimulating licking in non-deprived mice relative to sucrose, glycine generated concentration-dependent increases in licking in water-restricted 129 mice, whereas L-serine did not. For the D2 non-taster mice, we actually observed a concentration-dependent decrease in the Tastant/Water Lick Ratio in response to L-serine in the water-deprived condition. Given that L-serine is thought to possess a sucrose-like taste quality, this finding was unexpected and suggests that L-serine may also bind with other receptors that lead to aversive responses (e.g. T2Rs), at least in the D2 strain. Other researchers have reported higher levels of L-serine licking relative to water by B6, 129X1/SvJ and CB6 (BALB/c × B6 hybrids) mice in a brief-access test (Zhang *et al.*, 2003; Zhao *et al.*, 2003). These discrepancies, in part, are likely the result of methodological differences between the studies. More specifically, in the prior work, both food and water intake was limited in a controlled fashion, based on procedures described by Glendinning *et al.* (2002), to achieve a motivational state that would promote stimulus sampling but would not lead to the asymptotic lick rates generally observed under 24 h water deprivation regimens. Based on the present results, it appears that without the additional effects of nutrient restriction, the gustatory properties of L-serine and glycine alone stimulate only slight, if any, licking behavior, under non-deprived conditions, in the mouse strains tested here.

The profile of strain differences in responsiveness to the compounds tested here was more complex than previously reported. When mice were tested with sucrose in the non-deprived condition, in general the 'taster' strains (B6 and SWR) were modestly more responsive at lower concentrations compared with the 'non-taster' mice (129 and D2), but even this difference failed to reach significance for the B6 strain. As the concentration was progressively raised, the responsiveness of SWR and B6 taster mice converged with that seen in the D2 non-taster mice. Notably, the 129 non-taster mice licked the two highest concentrations of sucrose significantly more than did all three of the other strains. In general, these results are consistent with findings obtained by other researchers (i.e. Glendinning *et al.*, 2003).

When tested in the deprived condition, sucrose, as expected, produced licking rates comparable to water in all strains except for the 129 mice. The 129 mice, in fact, nearly doubled their rate of stimulus responsiveness relative to water at 1.0 M. This same pattern was seen with glycine in the deprived condition, with the 129 mice responding to the compound at nearly 1.5 times the rate of water at 1.5 M. Interestingly, the D2 non-taster mice displayed concentration-dependent decreases in their L-serine Tastant/Water Lick Ratio when water-deprived, whereas the other strains had relatively flat curves. It appears when mice were water-deprived the non-taster strains were less motivated to lick L-serine relative to sucrose and glycine, whereas all three stimuli were treated similarly by the taster strains. The findings from the non-deprived and deprived conditions collectively suggest that the phenotypic descriptors 'taster' and 'non-taster' do not necessarily apply to the responsiveness seen at higher concentrations of putative sweeteners, at least in the brief-access test.

The taster and non-taster classification is based on the preference behavior of various mouse strains to low concentrations of sweeteners in long-term two-bottle intake tests. The brief-access taste test differs from the two-bottle intake test in interpretively important ways. In the brief-access test, immediate responses to small volumes of stimuli are measured raising the confidence that the behavior is driven by taste (see Spector, 2003). Indeed, Spector *et al.* (1996) demonstrated that when rats are deprived of gustatory input from the 7th and 9th cranial nerves innervating the oral cavity, they show essentially flat concentration–response curves for sucrose when tested using a brief-access paradigm providing further evidence that behavior measured using a brief-access procedure is taste-guided. In contrast, in the two-bottle test, intake is usually measured 24 h after stimulus presentation allowing for postingestive factors to influence the outcome. Moreover, differences in stimulus preference at high concentrations are difficult to detect with the two-bottle preference test because of ceiling effects. Typically, preference ratios approach an asymptotic value of 1.0 at very low concentrations for normally preferred stimuli, after which differences are difficult to discern. Other researches have used a shorter-term one-bottle intake test (e.g. 6 h) where ceiling effects and position preferences are avoided or at least minimized (e.g. Blizard *et al.*, 1999). But while the results obtained using the one-bottle test are consistent with those seen when using the two-bottle intake procedure, neither test avoids the confounding effects of viscerosensory input. On the other hand, the brief-access taste test does not appear to be as sensitive to changes in behavior at low concentrations, at least when several higher concentrations are available during the session. Thus, these various procedures have different dynamic ranges of sensitivity. Accordingly, it would appear that, behaviorally speaking, the taster/non-taster distinction is limited to low concentrations of sweeteners. This is consistent with sucrose and glucose detection thresholds measured with an operant procedure in which the hedonic value of the taste stimulus is rendered irrelevant (Eylam and Spector, 2003). Interestingly, in the Eylam and Spector study, the threshold values for glycine measured with the same procedure in the same mice did not distinguish taster and non-taster strains in as straightforward a manner. That is, non-taster 129 mice had significantly higher glycine thresholds relative to B6 mice. However, the glycine thresholds for non-taster D2 mice did

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not differ from those for the taster B6 and SWR mice. In stark contrast, in our study, at the higher concentrations, the 129 mice were the most responsive strain tested in this report. These findings further highlight the difference between suprathreshold responsiveness and threshold sensitivity (cf. Bachmanov *et al.*, 1997).

If the T1R family of receptors mediates 'behavioral attraction,' as postulated by some (Zhao *et al.*, 2003), then activation of either receptor complex should elicit appetitive behavior. However, compounds that bind with the T1R2+3 complex are apparently much more effective, at least as measured by the assay used in our study. Sucrose, which was shown to stimulate the T1R2+3 complex in a heterologous expression system (HEK 293), generated licking at rates at least four times higher than any other compound tested. Partial support for this dissociation comes from the fact that glycine, which was also shown to stimulate the T1R2+3 complex, but to a lesser extent, in general elicited slight increases in licking at high concentrations resembling its modest ability to bind with the receptor (see Nelson *et al.*, 2002), at least in those mice that sampled all of the concentrations. We found little evidence that L-serine, a compound that binds with the T1R1+3 heterodimer, but not with the T1R2+3, is an effective behavioral stimulus in the brief-access test in non-deprived mice. As noted above, there is evidence that L-amino acids can stimulate significant degrees of licking in mice that have restricted food and water access. Thus, it would appear that the affective value of stimuli that bind with the T1R1+3 receptor depends upon the nutritional/physiological status of the animal, whereas stimuli that bind with the T1R2+3 receptor do act like general 'attractants'.

The behavioral results presented here do not relate to the electrophysiological response properties of the CT nerve in an obvious way (Frank and Blizard, 1999; Inoue *et al.*, 2001). While all three stimuli used in our study reportedly evoke very clear concentration-related increases in CT responsiveness in B6 and 129 mice, the concentration–response functions for glycine and L-serine in non-deprived mice from these strains in the brief-access test had very shallow slopes. Moreover, while the magnitude of CT responses to sucrose is greater in B6 compared with 129 mice even at high concentrations, the 129 mice displayed more vigorous sucrose licking than the B6 mice at the 0.5 and 1.0 M concentrations in the brief-access test. It is conceivable that a subclass of CT fibers might display a better correspondence with the hedonic value of these stimuli and this relationship might be obscured in whole-nerve analyses (cf. Frank and Pfaffmann, 1969). However, it is likely that the affective potency of these stimuli is based on more than just input from the CT. Input from other peripheral nerves and the central neural circuits that translate those signals into behavior must be considered. Thus, while non-taster strains might have an impaired peripheral signal for certain sweeteners that stimulate the T1R2+3 receptor complex, the way that input is interpreted by the brain can also differ from taster strains in a manner that could augment behavior. Likewise, a robust peripheral signal for glycine or L-serine or any taste stimulus does not guarantee that a given behavioral response will be generated.

In summary, we found that sucrose was the most effective compound tested, followed by glycine, and lastly L-serine in generating licking in the brief-access taste test. The order of affective potency seems to be related to the ability of the stimulus to activate the T1R2+3 heterodimeric receptor complex. Furthermore, strain differences in responsiveness to these compounds suggest that the current understanding of 'sweet-tasting' ligand transduction is insufficient in entirely explaining the observed response profiles. For example, the fact that the 129 mice licked at rates greater than the D2, B6 and SWR mice to the higher concentrations of sucrose would not have been predicted by the current molecular biological findings or CT nerve recordings. Apparently, the taster/non-taster distinction which has been shown to be dependent on the polymorphism of the *Tas1r3* gene encoding for the T1R3 receptor is limited to low concentrations of sucrose, whereas responsiveness to higher concentrations of the sugar is related, at least in part, to other genes that might affect stimulus processing anywhere along the gustatory neuraxis. It would be instructive to repeat the behavioral tests conducted here in congenic, transgenic and/or knock-out mice in which the *Tas1r3* gene has been manipulated keeping the genetic background constant to examine the explicit role of the T1R3 variants in behavioral responsiveness to mid-range and high concentrations of sugars, synthetic compounds and amino acids. The results of our study also call into question the very nature of the perceptual quality elicited by the amino acids tested here. As noted above, there is evidence from conditioned taste aversion generalization experiments that rodents treat glycine and L-serine as possessing a sucrose-like taste quality (Nowlis *et al.*, 1980; Pritchard and Scott, 1982; Kasahara *et al.*, 1987; Ninomiya and Funakoshi, 1989). Yet, in the brief-access test with non-deprived mice, the responses to sucrose were discernibly different than those to the amino acids. Thus, it would appear that while the perception evoked by glycine and L-serine might share some qualitative characteristic with sucrose, these amino acids might also generate additional qualities that impact upon their affective value at least in certain species and strains. For example, saccharin is both 'sweet' and 'bitter' tasting to humans depending on concentration (Bartoshuk, 1979; Schiffman *et al.*, 1979). Experiments designed explicitly to test the ability of these mice to distinguish between sucrose, glycine, L-serine, and other L-amino acids and sugars in operant taste discrimination tasks, in addition to a more comprehensive examination of conditioned taste aversion generalization profiles should help refine the characterization of the qualitative similarities and differences of these taste stimuli. Such behavioral experiments can provide a functional context to guide the

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interpretation of findings from more molecular levels of analysis.

Notes

1. We use the term 'sweetener' to denote natural and synthetic chemical compounds which are reported as 'sweet'-tasting by humans.
2. The phenotypic descriptors 'taster' and 'non-taster' may at first glance seem to denote ageusic versus non-ageusic strains; however, this nomenclature is commonly used in the literature to categorize mouse strains with varying degrees of responsiveness to compounds such as sucrose and/or sodium saccharin as assessed behaviorally or electrophysiologically.

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