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Is Glycine ''Sweet'' to Mice? Mouse Strain Differences in Perception of Glycine Taste

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Abstract

Glycine is an amino acid tasting sweet to humans. In 2-bottle tests, C57BL/6ByJ (B6) mice strongly prefer glycine solutions, whereas 129P3/J (129) mice do not, suggesting that they differ in perception of glycine taste. We examined this question using the conditioned taste aversion (CTA) generalization technique. CTA was achieved by injecting LiCl after drinking glycine, and next its generalization to 10 taste solutions (glycine, sucrose, saccharin, D-tryptophan, L-tryptophan, L-alanine, L-proline, L-glutamine, NaCl, and HCl) was examined by video recording licking behavior. Both B6 and 129 mice generalized the aversion to sucrose, saccharin, L-alanine, and L-proline and did not generalize it to NaCl, HCl, and L-tryptophan. This indicates that both B6 and 129 mice perceive the sweetness (i.e., a sucrose-like taste) of glycine. Thus, the lack of a glycine preference by 129 mice cannot be explained by their inability to perceive its sweetness. Strain differences were observed for CTA generalization to 2 amino acids: 129 mice generalized aversion to L-glutamine but not D-tryptophan, whereas B6 mice generalized it to D-tryptophan but not L-glutamine. 129.B6-*Tas1r3* congenic mice with 2 genotypes of the *Tas1r3* locus (B6/129 heterozygotes and 129/129 homozygotes) did not differ in aversion generalization, suggesting that the differences between 129 and B6 strains are not attributed to the *Tas1r3* allelic variants and that other, yet unknown, genes are involved in taste perception of amino acids.

Key words: amino acid, D-tryptophan, L-glutamine, *Sac* locus, sweet taste, *Tas1r3* gene

Introduction

Although substantial progress in understanding mechanisms of taste reception has been made in the recent years, many aspects of taste perception of amino acids still remain unclear. In this study, we examined genetic determinants of perception of several amino acids in mouse strains that differ in preferences for glycine. Glycine is an amino acid perceived by humans as sweet (Solms et al. 1965; Schiffman et al. 1981; Hayakawa and Kawai 2003). Similarly, some rodents generalize conditioned taste aversion (CTA) between sucrose and glycine (Nowlis et al. 1980; Pritchard and Scott 1982; Kasahara et al. 1987; Yamamoto et al. 1988; Danilova et al. 1998) indicating that they also perceive its sweet taste (defined here operationally as similar to taste of sucrose, a compound humans find to be almost exclusively sweet). Consistent with this, rodents often prefer glycine in 2-bottle choice tests with water (Iwasaki et al. 1985; Kasahara et al. 1987; Lush et al. 1995; Gilbertson et al. 1997). However, mice exhibit strain differences in glycine preference (Lush et al. 1995; Bachmanov, Tordoff, and Beauchamp 2001) and other taste responses to glycine (Eylam and Spector 2004; Dotson and Spector 2004). Whereas mice from the C57BL/6ByJ (B6) inbred strain strongly prefer glycine solutions over a range of concentrations, mice from the 129P3/J (129) inbred strain do not display such preferences (Bachmanov, Tordoff, and Beauchamp 2001). Indifference to most glycine concentrations in 129 mice could be due to an inability of these mice to perceive glycine sweetness. We examined this hypothesis using CTA generalization technique (Experiment 1).

The 129 and B6 inbred strains carry different alleles of the *Tas1r3* (formerly *Sac*) gene (Bachmanov, Li, et al. 2001; Reed et al. 2004) that encodes one of the proteins (T1R3) forming the sweet taste receptor (Nelson et al. 2001, 2002; Li, Staszewski, et al. 2002). Allelic variants of the *Tas1r3* gene affect sweet taste responses in mice (Inoue, Reed, et al. 2004) and thus may also affect their perception of glycine sweetness. However, B6 and 129 mice have many other

polymorphic genes, which can also underlie their differential glycine preference. In order to find out whether differences in glycine taste perception between B6 and 129 mice are due to the variation of the *Tas1r3* or other genes, we assessed CTA generalization in mice from the 129.B6-*Tas1r3* congenic strain. The 129.B6-*Tas1r3* congenic strain was produced by serial backcrossing of offspring from the 129 × B6 intercross onto the 129 strain and selection of mice carrying a fragment of B6 chromosome 4 including the *Tas1r3* gene (Li et al. 2001). As a result, the congenic mice have genetic background of the 129 strain and a donor chromosomal fragment containing the *Tas1r3* gene from the B6 strain. The size of the donor fragment does not exceed 194 kb and encompasses, besides *Tas1r3*, several other genes that have been excluded as candidates for the *Tas1r3* locus (Bachmanov, Li, et al. 2001). We maintained 129.B6-*Tas1r3* mice as a segregating congenic strain by mating congenic mice that have only one chromosome containing the B6 donor fragment (B6/129 genotype at the *Tas1r3* locus) with 129 inbred mice. As a result, in each backcross generation, we obtained mice with 2 different *Tas1r3* genotypes, B6/129 heterozygotes and 129/129 homozygotes. Because the B6 allele of the *Tas1r3* gene is dominant, B6/129 heterozygotes are phenotypically different from 129/129 homozygotes (Bachmanov, Li, et al. 2001; Li et al. 2001). Congenic littermates with B6/129 and 129/129 *Tas1r3* genotypes were used in this study (Experiment 2).

Conditioned taste aversion generalization is a technique commonly used to assess taste quality perception in nonhuman animals. In this study, we used this approach to assess taste perception of glycine in inbred and congenic mice. We conditioned mice to avoid glycine by pairing glycine ingestion with injection of sickness–inducing lithium chloride (LiCl) and then examined suppression of ingestive responses to prototypical taste stimuli representing the main taste qualities (sweet sucrose and saccharin, salty NaCl, sour HCl, and bitter L-tryptophan), as well as several amino acids (D-tryptophan, L-alanine, L-proline, and L-glutamine) with different taste profiles as perceived by humans.

Materials and methods

Housing and animal care, solutions, apparatus, procedure, and data analysis were identical in Experiments 1 and 2. In the Experiment 1, CTA tests were conducted using mice from the 129P3/J (129) and C57BL/6ByJ (B6) inbred strains. In the Experiment 2, these tests were conducted in mice from the 129.B6-*Tas1r3* segregating congenic strain with different genotypes at the *Tas1r3* locus.

Animals

C57BL/6JJCL mice used to validate the lick-counting method were purchased from SLC Japan (Shizuoka, Japan). C57BL/6ByJ (B6) and 129P3/J (129) inbred mice (Experiment 1) were obtained from the Jackson Laboratory (Bar Harbor, ME). 129.B6-*Tas1r3* congenic mice (Experiment 2) were bred at Monell. During the experiments, the mice were housed in individual cages in a temperature-controlled room at 23 °C on a 12:12-h light:dark cycle (7 AM on, 7 PM off). They had free access to pelleted Teklad Rodent Diet 8604 (Harlan, Madison, WI). In each group of mice, water deprivation lasted between 17 and 24 h.

Experiment 1

Male mice from the 129 (n = 15) and B6 (n = 17) strains were divided into 2 groups. Group 1 mice (8 from the 129 strain and 10 from the B6 strain) were injected with LiCl, and group 2 mice (7 from the 129 strain and 7 from the B6 strain) were injected with saline. At the beginning of the experiment, mice were 8- to 10-weeks old and weighed 22.5 ± 1.4 g (129 strain, mean ± SD) or 24.1 ± 2.0 g (B6).

Experiment 2

129.B6-*Tas1r3* congenic mice from the N6F3 generation were used in the experiment. The mice were genotyped for *49O2-T7*, *Tas1r3*, and *D4Mon63* markers that outline the chromosomal donor fragment (genotyping methods are described in Li et al. 2001; Li, Bachmanov, et al. 2002). Fifteen mice heterozygous for the donor fragment (B6/129 *Tas1r3* genotype; 5 males and 10 females) and 12 mice with no donor fragment (129/129 *Tas1r3* genotype; 5 males and 7 females) were divided into 2 groups. Group 1 mice (2 male and 3 female 129/129 homozygotes and 2 male and 5 female 129/B6 heterozygotes) were injected with LiCl, and group 2 mice (3 male and 4 female 129/129 homozygotes and 3 male and 5 female 129/B6 heterozygotes) were injected with saline. At the beginning of the experiment, mice were 13- to 42-weeks old and weighed 17.1 ± 2.1 g (129/129 homozygotes) or 17.9 ± 1.2 g (129/B6 heterozygotes).

Solutions

The following 10 solutions were used as test stimuli: glycine (Gly, 100 mM), L-tryptophan (L-Trp, 30 mM), NaCl (30 mM), L-alanine (L-Ala, 100 mM), D-tryptophan (D-Trp, 30 mM), HCl (1 mM), saccharin (Sac, 2 mM), L-proline (L-Pro, 100 mM), L-glutamine (L-Gln, 100 mM), and sucrose (Suc, 100 mM). These solutions were prepared in deionized water, preserved in a refrigerator, and used within a week. LiCl (0.15 M) and NaCl (0.15 M) for injections were filtered using syringe filter (0.2 μm diameter, Dismic-25 mixed cellulose ester, Advantec, Tokyo, Japan). All chemicals were purchased from Sigma (St Louis, MO).

Apparatus

The CTA tests were conducted in test cages made of clear polycarbonate with dimensions 175 × 245 × 125 mm or 125 × 200 × 110 mm (Figure 1). A 15-mm diameter hole was drilled on one side of the cage. A 25-mm long metal cylinder was firmly inserted in the hole. A 1.5-ml plastic tube with a 2-mm diameter hole drilled in the tip was filled with

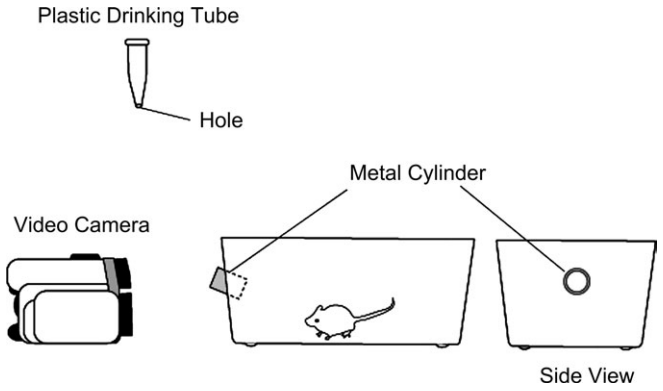

Figure 1 Test cage.

a fluid and placed inside the metal cylinder. Mice can lick solutions from this tube, with each leak delivering approximately 25 µl. Figure 2A shows a mouse in a state of close mouth (left) and making a lick (right). Mouse licking behavior was videotaped from the bottom of the cage, and the numbers of licks were counted by a trained experimenter while watching the video recordings with the real-time speed. Before counting licks in real time, the experimenter was trained by comparing lick numbers counted in real time with corresponding lick numbers based on analysis of still images. The training took a few hours and was completed after achieving consistency between lick counts based on the real-time video and still images. Numbers of licks for each trial of each mouse were counted at least twice. If the first 2 counts produced an identical number of licks, this number was used for data analysis. If the first 2 counts produced different lick numbers, they were counted again until 2 identical lick numbers were obtained; this number then was used for data analysis. In most cases, lick numbers were identical during the first 2 counts. If this was not the case, no more than 4 counts were needed to obtain 2 identical numbers. Validation of this technique (see Results) has demonstrated excellent correspondence between the actual number of licks (counted based on still images) and those estimated from watching the videotape in real time.

Procedure

The mice were trained, conditioned, and tested under a restricted water access schedule (Table 1). The training and testing were conducted between 7 AM and 9 PM.

Lick training

The water bottles were removed from the home cages 17–24 h before the first training session. On the first 3 days of the experiment, a mouse was placed in a test cage, and deionized water was presented in the plastic drinking tube 12 times during 6-s periods separated by 4-s intertrial intervals. Fifteen minutes after the beginning of the session, each mouse was returned to the home cage, given access to a water bottle for 15 min and then deprived of water again.

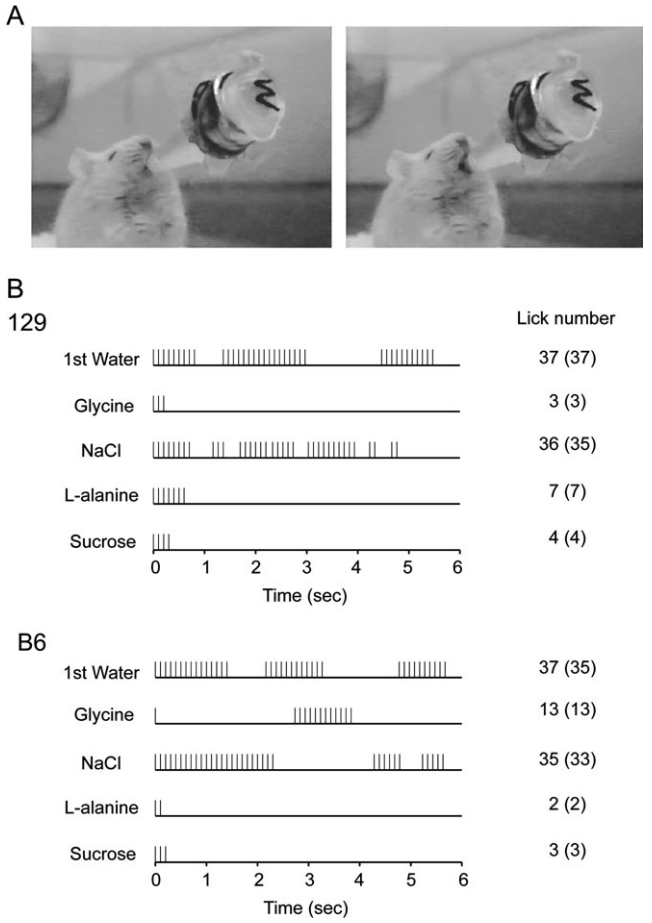

Figure 2 **(A)** Sequential still images showing different phases of mouse lick movement. **(B)** Recordings of licking water, glycine, NaCl, L-alanine, and sucrose by a 129 mouse (top) and a B6 mouse (bottom) conditioned to avoid glycine. Time "0" on the horizontal axis corresponds to the first mouse lick. The vertical lines indicate the frames with the mouse tongue protruded, which corresponds to the time of a lick. To the right, the number of licks counted based on still frames and counted while watching a video recording in real-time speed (in parenthesis) are shown.

Table 1 Conditioned taste aversion test procedure[a]

	Lick training phase			Conditioning phase	Testing	
Day	1st	2nd	3rd	4th	5th	6th

[a]Mice had restricted access to water throughout all 6 days of the test (see text).

Conditioning

On the 4th day, each mouse was presented with a 100-mM glycine solution from the same plastic drinking tube in the test cage during 15 min, and immediately after this exposure, each mouse was injected intraperitoneally with 0.7 ml/10 g body weight of either 0.15 M LiCl (0.45 g/kg body weight;

Group 1) or 0.15 M NaCl (0.61 g/kg body weight; Group 2). Fifteen minutes after the injection, each mouse was returned to the home cage, given access to a water bottle for 15 min, and then deprived of water again.

Testing

On the 5th and 6th days, test stimuli and deionized water were presented to mice on the same schedule as on days 1–3, and the licks were recorded with a video camera. Each mouse was presented with the same 12 stimuli in the same order: deionized water, glycine, L-tryptophan, NaCl, L-alanine, deionized water, D-tryptophan, HCl, saccharin, L-proline, L-glutamine, and sucrose. We presented deionized water twice to monitor drinking motivation. The lick rates were similar during both water presentations (Tables 2 and 3) indicating that there was no change in motivation to lick throughout the experiment. In the beginning of the testing session (on day 5 of the experiment), we found that 2 mice did not avoid glycine after the first LiCl injection. Further testing of these 2 mice was stopped, and they were injected with LiCl again immediately after access to glycine. Fifteen minutes after the injection, these 2 mice were returned to the home cages, given access to a water bottle for 15 min and then deprived of water again. After the second LiCl injection, these mice avoided glycine, and therefore, their testing was resumed.

Data analysis

There were no systematic differences in lick rates on testing days 1 and 2. Therefore, we calculated the average numbers of licks during the 6-s period for each mouse for each solution based on 2 values obtained on 2 testing days. These averages were analyzed using 3-way analysis of variance (ANOVA) with solution as a within-subject factor and strain and treatment as between-subject factors. When there were significant effects of treatment or strain, post hoc comparisons using *t*-tests were used to evaluate differences between means for individual solutions.

Individual lick ratios were calculated for each mouse from the experimental group as the ratio of the individual average lick rate for a solution to the mean lick rate of the control group for this solution. Next, we calculated group means and standard errors for the lick ratios (shown in Figures 4 and 5). The ratio "0" indicates complete aversion, and "1" indicates lick rate similar to that in the control group.

Results

The reliability of lick counting while watching a videotape

Because video recording is not commonly used to measure mouse licking, we confirmed the validity of this method. First, we compared the number of licks counted while watching a videotape and those counted based on a number of still images showing the mouse tongue protruded (Figure 2B). This was done for one 129 mouse (top) and one B6 mouse (bottom) conditioned with LiCl to avoid glycine and presented with water, glycine, NaCl, L-alanine, and sucrose. The mice licked in bouts of 0.09- to 2.1-s duration with interlick intervals of 90–120 ms. The lick numbers counted while watching a videotape in real time and those counted using

Table 2 Lick rates (licks/6 s) of taste stimuli in 129 and B6 mice (means ± standard errors)

Solution	Concentration (mM)	129 Strain		B6 Strain	
		LiCl (n = 8)	NaCl (n = 7)	LiCl (n = 10)	NaCl (n = 7)
1st Water		27.3 ± 3	30.1 ± 2	32.6 ± 1.2	31.1 ± 1.4
Glycine	100	9.5 ± 2.1[a]	29.9 ± 2.7	8.6 ± 1.5[a]	33.9 ± 0.9
L-Tryptophan	30	25.7 ± 5.1	27.4 ± 4.8	29 ± 2.7	31.2 ± 2.2
NaCl	30	27.3 ± 4	31.5 ± 2.5	32.1 ± 1.6	30.5 ± 2.1
L-Alanine	100	14.8 ± 3.3[a]	33.2 ± 1.7	20 ± 3.6[a]	32.4 ± 2.1
2nd Water		32 ± 3.6	33.7 ± 1.5	33.3 ± 0.9	31.9 ± 1.8
D-Tryptophan	30	20.4 ± 4.8[b]	28.1 ± 3.8	7.5 ± 0.8[ab]	32.7 ± 2.5
HCl	1	25.6 ± 4.5	28.8 ± 2.6	29.4 ± 2	26.9 ± 3
Saccharin	2	6.5 ± 2.1[a]	30.1 ± 3.5	6.5 ± 1.2[a]	32.6 ± 1
L-Proline	100	13.1 ± 4.1[a]	26.6 ± 3.2	20.5 ± 3.8[a]	30.6 ± 1.5
L-Glutamine	100	9.7 ± 2.4[ab]	29.1 ± 2.9	25.9 ± 2.9[b]	30.6 ± 3.2
Sucrose	100	6.4 ± 2.7[a]	22.3 ± 3.1	9.4 ± 2.1[a]	34.1 ± 0.7

[a]Significant difference between NaCl- and LiCl-treated groups, $P < 0.05$ (post hoc *t*-test).
[b]Significant difference between 129 and B6 strains, $P < 0.05$ (post hoc *t*-test).

Table 3 Lick rates (licks/6 s) of taste stimuli in 129.B6-*Tas1r3* congenic mice with different genotypes at the *Tas1r3* locus (means ± standard errors)

Solution	Concentration (mM)	129/129 Homozygote		129/B6 Heterozygote	
		LiCl (n = 5)	NaCl (n = 7)	LiCl (n = 7)	NaCl (n = 8)
1st Water		34.4 ± 2.4	37.3 ± 2	30.4 ± 2.4	32.5 ± 2.5
Glycine	100	6.6 ± 1.8*	38 ± 1.5	7.3 ± 2*	34.7 ± 1.7
L-Tryptophan	30	22.8 ± 3.9	34.6 ± 3	26.6 ± 3.4	20.9 ± 2.6
NaCl	30	29.2 ± 3.2*	36.7 ± 1.9	25.9 ± 4.4*	35.1 ± 2.2
L-Alanine	100	12.5 ± 4.4*	36.3 ± 2.5	16.7 ± 4.3*	33.4 ± 2.2
2nd Water		28.7 ± 3.9	33.1 ± 2.9	29.9 ± 4.3	22.4 ± 3.6
D-Tryptophan	30	10.6 ± 3.2*	35.6 ± 3.3	6.6 ± 1.9*	36.4 ± 1.8
HCl	1	17.6 ± 6.5	33.1 ± 3.9	23.3 ± 3.7	23.3 ± 4.7
Saccharin	2	8.4 ± 4.3*	34.6 ± 3.3	8.7 ± 1.9*	33.2 ± 3.9
L-Proline	100	11.3 ± 5*	27.2 ± 5.2	8.7 ± 3.8*	13.9 ± 2.3
L-Glutamine	100	6.8 ± 2.4*	26.3 ± 4.5	5.6 ± 1.5*	18.7 ± 2.3
Sucrose	100	13.7 ± 7.5*	20.6 ± 2.3	3.3 ± 1.1*	20.9 ± 4.4

*Significant difference between NaCl- and LiCl-treated groups, $P < 0.05$ (post hoc t-test).

still images were either identical or differed in no more than 2 out of 35–37 licks (≤6%). There was a strong positive correlation between the lick numbers counted while watching a videotape in real time and those counted using still images (correlation coefficient > 0.99).

Second, we compared the number of mouse licks and the amount of fluid consumed during presentation of water. Two male C57BL/6JJCL mice were trained to lick water using a procedure described in the Materials and Methods. A trained mouse was placed in a test cage, deionized water was presented in the plastic drinking tube for approximately 15 min, and mouse licking behavior was videotaped. Approximately every 5–120 s, weight of the water tube was measured to calculate cumulative water intake. Cumulative numbers of licks for corresponding periods were calculated based on lick counting while watching a videotape. The Figure 3 shows that there was a strong positive correlation between the number of licks and water intake. These results demonstrate that the mouse licks can be reliably measured by watching a videotape. Thus, we used this method in subsequent experiments.

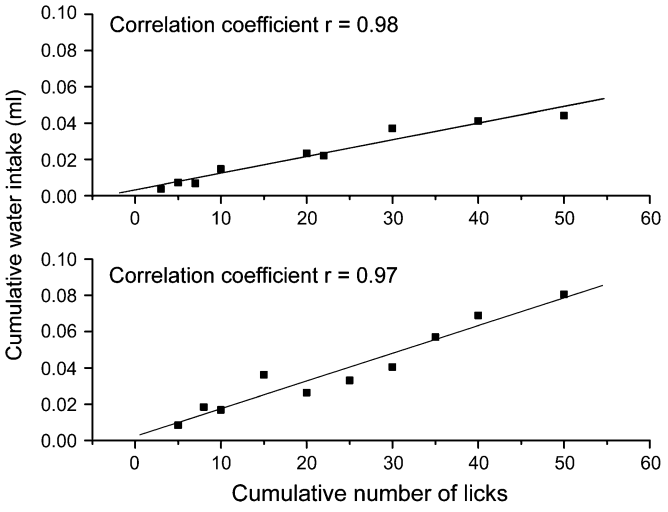

Figure 3 The relationship between lick rate and fluid intake in 2 water-deprived C57BL/6JJCL mice. Upper panel: mouse nr. 1 (body weight 35.53 g); lower panel: mouse nr. 2 (body weight 27.55 g). The horizontal axis shows the cumulative number of licks, and the vertical axis shows the cumulative water intake during corresponding periods. Each dot represents cumulative lick and intake values for a particular period.

Experiment 1

Table 2 shows the average lick numbers for test stimuli and deionized water in 129 and B6 mice. A significant Solution × Treatment interaction effect ($F(11, 308) = 16.46$, $P < 0.001$, 3-way ANOVA) indicates that the LiCl-induced CTA to glycine selectively affected lick rates of examined taste stimuli. A significant Solution × Strain × Treatment interaction ($F(11, 308) = 3.208$, $P < 0.01$) indicates that B6 and 129 mice differed in patterns of CTA generalization. For 129 mice, post hoc t-tests revealed significant ($P < 0.05$) suppression of licks in the LiCl-treated group compared with the NaCl-treated group for glycine, L-alanine, saccharin, L-proline, L-glutamine, and sucrose. For B6 mice, significant lick suppression was observed for glycine, L-alanine, D-tryptophan, saccharin, L-proline, and sucrose. LiCl-treated 129 and B6 mice significantly differed in lick rates for D-tryptophan

($P < 0.05$) and L-glutamine ($P < 0.005$). This strain difference in patterns of CTA generalization is shown in Figure 4.

Experiment 2

In the Experiment 2, we assessed whether differences between the 129 and B6 strains in generalization of CTA to glycine are attributed to the *Tas1r3* gene. Because there were no significant differences between males and females, we combined data for both genders.

Table 3 shows the average lick number for test stimuli and deionized water in 129.B6-*Tas1r3* congenic mice with different genotypes of the *Tas1r3* locus (129/129 homozygotes and 129/B6 heterozygotes). A significant Solution × Treatment interaction effect (F (11, 253) = 12.748, $P < 0.001$) indicates that LiCl-induced CTA to glycine selectively affected lick rates of examined taste stimuli. An absence of a significant Solution × Genotype × Treatment interaction (F (11, 253) = 2.097, $P > 0.05$) indicates that patterns of CTA generalization were similar in the congenic mice with different *Tas1r3* genotypes. Consistent with this, in mice of both genotypes, post hoc *t*-tests revealed significant suppression of licks in the LiCl-treated group compared with the NaCl-treated group for glycine, NaCl, L-alanine, D-tryptophan, saccharin, L-proline, L-glutamine, and sucrose ($P < 0.05$). Patterns of CTA generalization in the congenic mice are shown in Figure 5.

Discussion

In our experiments, we video recorded mouse licking behavior and counted the number of licks while watching a videotape. Because this approach is not commonly used to measure mouse licking, we have verified its reliability. The number of licks counted while watching a videotape was nearly identical to the number of licks counted by analyzing sequential images for the same period (Figure 2B). There was a strong positive correlation between the number of licks counted while watching a videotape and amount of fluid consumed during the same period (Figure 3). These results demonstrate that the number of licks can be reliably counted while watching a videotape. This method has some advantages over conventional methods using automated electric or optical sensors: the same video recording can be analyzed repeatedly to confirm results, there are no artifacts typical for automated measurements (e.g., counting a single lick more than once or counting any contact with a sensor as a lick), and the experimental setup is inexpensive and is not susceptible to malfunction. Although interlick intervals observed in this study (90–120 ms; Figure 2B) were similar to results of a previous study (around 100 ms, Figure 2B in Hayar et al. 2006), lick rates in our study (~5 licks/s) were lower than those obtained using a lickometer in a previous study (~8 licks/s, Ninomiya et al. 1984). The lower lick rate in our experiments may be due to differences in water deprivation regimen and larger drinking spout orifice size (2-mm diameter in our study and 1.5-mm diameter in the study of Ninomiya et al. 1984). Negative correlation between drinking spout orifice size and lick rate was described by Dotson and Spector (2005).

How does glycine taste to mice? All mice used in this study generalized CTA of glycine to prototypical sweet compounds, sucrose and saccharin, and did not generalize it to HCl (sour) and L-tryptophan (bitter). Although B6 and 129 inbred mice did not generalize CTA between glycine and NaCl, in congenic mice, the NaCl lick rate of conditioned mice was significantly lower than that of control mice. However, this NaCl lick suppression was small (23% of control level) and could be attributed to a type I error

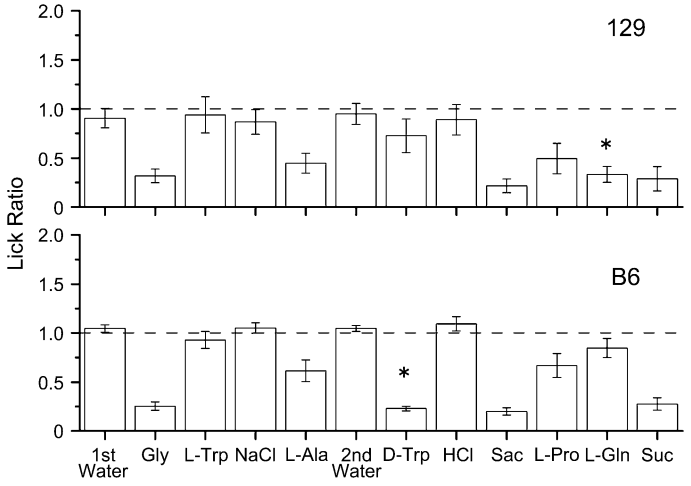

Figure 4 Lick ratios (means ± standard error of mean) of 129 and B6 mice. Lick ratios were calculated by dividing lick numbers of individual LiCl-treated mice by mean lick rate of the NaCl-treated group. When there was a significant difference between 129 and B6 strains, a lower lick ratio is indicated by the asterisk. Gly: glycine; L-Trp: L-tryptophan; L-Ala: L-alanine; D-Trp: D-tryptophan; Sac: saccharin; L-Pro: L-proline; L-Gln: L-glutamine; and Suc: sucrose.

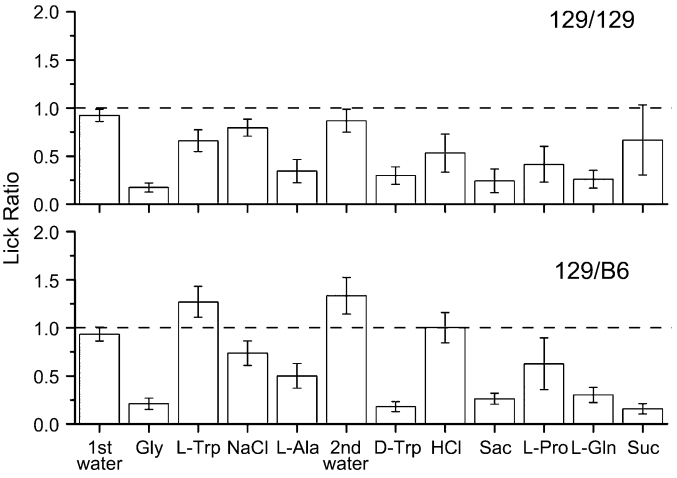

Figure 5 Lick ratios (means ± standard error of mean) of 129.B6-*Tas1r3* congenic mice with different *Tas1r3* genotypes. Explanations are the same as in Figure 4.

(false positive). Overall, these data indicate that mice perceive glycine taste as predominantly sweet. This is consistent with results of other studies (Pritchard and Scott 1982; Iwasaki et al. 1985; Schiffman et al. 1992; Lush et al. 1995).

Amino acids evoke a variety of taste sensations, and humans may perceive the taste of a single amino acid as a mixture of different consensus basic taste qualities. To assess genetic differences in amino acid taste perception in mice, we have examined generalization of CTA of glycine to several amino acids with known taste perception by humans (Hayakawa and Kawai 2003; Kawai and Hayakawa 2005): D-tryptophan and L-alanine (predominantly sweet), L-proline (sweet and bitter), L-glutamine (sweet and umami), and L-tryptophan (bitter). All mice used in this study generalized CTA of glycine to L-alanine and L-proline and did not generalize it to L-tryptophan. This suggests that glycine shares the same taste quality (most likely sweetness) with L-alanine and L-proline and lacks taste quality possessed by L-tryptophan (most likely bitterness). Generalization of CTA of glycine to D-tryptophan and L-glutamine varied among mice with different genotypes. The CTA generalized to D-tryptophan in B6 and congenic mice (of both *Tas1r3* genotypes) but not in 129 mice. The CTA generalized to L-glutamine in 129 and congenic mice (of both *Tas1r3* genotypes) but not in B6 mice. Generalization of CTA of glycine to L-glutamine has also been previously found in ICR mice (Ninomiya and Funakoshi 1989).

In 2-bottle preference tests, B6 mice strongly prefer glycine to water, whereas 129 mice do not (Lush et al. 1995; Bachmanov, Tordoff, and Beauchamp 2001). We hypothesized that the indifference to glycine in 129 mice could be due to an inability of these mice to perceive glycine sweetness and examined this hypothesis using CTA generalization. Our data indicate that both B6 and 129 mice generalize CTA of glycine to sucrose and saccharin and thus must perceive a common taste quality for these stimuli (most likely sweetness). Thus, the absence of glycine preference by 129 mice cannot be explained by their inability to perceive its sweetness.

We have found that B6 and 129 mice differ in generalization of CTA for glycine to D-tryptophan and L-glutamine. This may indicate that mice from these 2 strains are not identical in how they perceive glycine taste. These differences in glycine perception could contribute to strain differences in glycine preference, but exact mechanism for this possible effect is unclear. These strain differences in patterns of CTA generalization can also be explained by identical perception of glycine taste by B6 and 129 mice and their differential perception of D-tryptophan and L-glutamine taste. Therefore, factors other than qualitative taste perception most likely underlie the strain differences in glycine preference. For example, quantitative differences in taste perception may be involved: 129 mice may perceive only a weak sweetness of glycine that is not sufficient to drive preference behavior, or glycine may induce aversive sensations in 129 mice that may offset sweetness-induced palatability (this aversive component does not seem to be bitterness but could, e.g., be an odor). The similar response magnitude in the chorda tympani nerve to lingual application of glycine in B6 and 129 mice (Inoue et al. 2001) could result from a combination of a weaker activation of sweet-responsive units and a greater activation of units transmitting an aversive taste. For example, in the hamster chorda tympani nerve, single fibers responding to a high concentration of glycine are classified not only as sucrose-best fibers but also as NaCl-best and citric acid–best fibers (Danilova et al. 1998), suggesting that glycine elicits multiple taste sensations. In addition, postingestive effects of glycine consumed in the long-term 2-bottle tests could also affect preference. Further studies are needed to understand the basis for these differences in glycine preference.

Differences between B6 and 129 mice in generalization of CTA for glycine to D-tryptophan and L-glutamine must have genetic basis. The B6 and 129 strains have different alleles of the *Tas1r3* taste receptor gene, but they also have different alleles of many other genes. To distinguish whether these strain differences in CTA generalization are due to the *Tas1r3* or other genes polymorphic between B6 and 129 strains, we tested 129.B6-*Tas1r3* congenic mice. The hypothesis was that if congenic mice with B6 allele of the *Tas1r3* gene are similar to B6 mice, then the strain difference must be attributed to the *Tas1r3* genotype. However, if congenic mice with B6 allele of the *Tas1r3* gene are similar to 129 mice, then the strain difference must be attributed to genes other than *Tas1r3*. As a control, we used congenic littermates lacking B6 donor fragment (129/129 homozygotes at the *Tas1r3* locus). Genetically, these mice are almost identical to inbred 129 mice (after 6 backcrosses onto the 129 strain, they retained less than 2% of B6 genetic material unlinked to the *Tas1r3*-containing donor chromosomal fragment). Thus, we assumed that congenic mice without the donor fragment would be phenotypically similar to the inbred 129 mice.

Our results have shown that congenic mice with different *Tas1r3* genotypes (B6/129 heterozygotes and 129/129 homozygotes) and inbred 129 mice generalized the CTA between glycine and L-glutamine, whereas B6 mice did not. This indicates that the similarity of taste perception of glycine and L-glutamine does not depend on the *Tas1r3* gene and must be due to other genes polymorphic between the B6 and 129 strains. Such genes remain to be identified.

The B6 and 129 mice also differed in CTA generalization between glycine and D-tryptophan. Congenic mice with both *Tas1r3* genotypes (B6/129 heterozygotes and 129/129 homozygotes) and B6 mice generalized CTA between glycine and D-tryptophan, whereas 129 mice did not. This strain difference could be due to a polymorphic gene residing within the less than 2% of residual B6 genetic material retained in the congenic strain and unlinked to the *Tas1r3*-containing donor fragment. However, this is unlikely. Lack of significant suppression of licking D-tryptophan in LiCl-conditioned

129 mice may also be a type II error (false negative): the conditioned 129 mice had lick rate 27% lower than control, but this difference did not reach the level of significance ($P = 0.24$ and 0.12 for 2-tailed and 1-tailed t-tests, respectively). Correspondingly, difference between conditioned B6 and 129 mice in D-tryptophan lick rates may be a false positive. Additional studies are needed to confirm the strain differences in CTA generalization among glycine, L-glutamine, and D-tryptophan.

The lack of the effect of *Tas1r3* genotype on taste perception of glycine is consistent with the lack of linkage of behavioral and neural responses to glycine to the *Tas1r3* locus in a cross between B6 and 129 mice (Inoue, Reed, et al. 2004). Furthermore, none of the other *Tas1r* genes, all of which reside in the distal part of the mouse chromosome 4, were linked to glycine taste responses (Inoue, Reed, et al. 2004). Although this does not preclude that glycine activates T1R receptors, it demonstrates that strain differences in behavioral responses to glycine depend on some other genes, which still await their identification.

In summary, we have found that glycine evokes taste similar to that of sucrose, saccharin, L-alanine, and L-proline in inbred (B6 and 129) and congenic (129.B6-*Tas1r3*) mice. However, the patterns of generalization of CTA for glycine to L-glutamine and D-tryptophan differed among these strains. These differences are unrelated to allelic variation of the *Tas1r3* gene, suggesting existence of other, yet unknown, genes involved in taste perception of amino acids.

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