

## Sweetener Preference of C57BL/6ByJ and 129P3/J Mice

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### Abstract

Previous studies have shown large differences in taste responses to several sweeteners between mice of the C57BL/6ByJ (B6) and 129P3/J (129) inbred strains. The goal of this study was to compare behavioral responses of B6 and 129 mice to a wider variety of sweeteners. Seventeen sweeteners were tested using two-bottle preference tests with water. Three main patterns of strain differences were evident. First, sucrose, maltose, saccharin, acesulfame-K, sucralose and SC-45647 were preferred by both strains, but the B6 mice had lower preference thresholds and higher solution intakes. Second, the amino acids D-phenylalanine, D-tryptophan, L-proline and glycine were highly preferred by B6 mice, but not by 129 mice. Third, glycyrrhizic acid, neohesperidin dihydrochalcone, thaumatin and cyclamate did not evoke strong preferences in either strain. Aspartame was neutral to all 129 and some B6 mice, but other B6 mice strongly preferred it. Thus, compared with the 129 mice the B6 mice had higher preferences for sugars, sweet tasting amino acids and several but not all non-caloric sweeteners. Glycyrrhizic acid, neohesperidin, thaumatin and cyclamate are not palatable to B6 or 129 mice.

### Introduction

Genetic variation among inbred mouse strains provides a tool to identify genetic loci underlying variable traits (Lush, 1991; Whitney and Harder, 1994). Mouse strains have large differences in consumption of sweeteners (substances that evoke sweet taste sensation in humans). Previous studies using two-bottle choice tests indicate that mice from C57BL/6 strains have high avidity and mice from 129 strains have low avidity for sweeteners, although only a few sweeteners have been examined (Lush, 1989; Belknap *et al.*, 1993; Capeless and Whitney, 1995; Lush *et al.*, 1995). We therefore used mice from the C57BL/6ByJ (B6) and 129P3/J (129, formerly 129/J) inbred strains in this study to represent a range of genetic variation in sweetener preferences among laboratory mice.

Using two-bottle tests we examined a wide range of sweeteners, which were selected based on the following criteria. First, all of them evoke sweet taste sensations in humans. Second, they represent various chemical groups: sugars (sucrose and maltose), sugar alcohols (sorbitol and erythritol), a chlorinated sugar analog (sucralose), amino acids (D-phenylalanine, D-tryptophan, L-proline and glycine), a dipeptide (aspartame), a protein (thaumatin), *N*-sulfonyl amides (saccharin and acesulfame-K), a sulfamate (cyclamate), a guanidinacetic acid-based sweetener (SC-45647), a triterpenoid glycoside (glycyrrhizic acid) and a dihydrochalcone glycoside (neohesperidin dihydrochalcone). Third, previous psychophysical and neurophysiological studies have revealed differences in responses to these

sweeteners (Plata-Salaman *et al.*, 1993; Schiffman and Gatlin, 1993; DuBois, 1995; Naim *et al.*, 1998). Fourth, comparative studies have shown that several of these compounds are sweet to some mammalian species but not to others (Beauchamp *et al.*, 1977; Jakinovich, 1981; Naim *et al.*, 1982; Ferrell, 1984; Schiffman and Gatlin, 1993; Hellekant *et al.*, 1994; Glaser *et al.*, 1995; Nofre *et al.*, 1996; Danilova *et al.*, 1998).

In this paper we describe the results of testing B6 and 129 mice with the sweeteners using two-bottle tests. An accompanying paper (Inoue *et al.*, 2001) describes an electrophysiological study of multiunit chorda tympani responses to lingual application of sweeteners in these two mouse strains.

### Materials and methods

#### Animals

Male mice of the C57BL/6ByJ (B6,  $n = 66$ ) and 129P3/J (129, formerly 129/J,  $n = 72$ ) strains were obtained from The Jackson Laboratory (Bar Harbor, ME). Six groups of B6 and 129 mice were tested with different compounds. Group 1 (8 B6 and 12 129 6.5-month-old mice) was tested with sucrose; group 2 (11 B6 and 12 129 2-month-old mice) with saccharin, D-phenylalanine, glycine, aspartame, L-proline and D-tryptophan; group 3 (12 B6 and 12 129 2-month-old mice) with glycyrrhizic acid, SC-45647,

thaumatin and neohesperidin hydrochalcone; group 4 (12 B6 and 12 129 2-month-old mice) with maltose, cyclamate and sorbitol; group 5 (11 B6 and 12 129 3-month-old mice) with acesulfame-K; group 6 (12 B6 and 12 129 2-month-old mice) with sucralose and erythritol. These compounds were tested in each group in the order listed.

During the experiments the mice were housed in individual cages in a temperature controlled room at 23°C on a 12 h light:12 h dark cycle (7:00 a.m. on, 7:00 p.m. off). They had free access to deionized water and Teklad Rodent Diet 8604 (Harlan Teklad, Madison, WI) (24.5% protein, 50.3% carbohydrate and 4.4% fat; 3.93 kcal/g gross energy; 0.31% sodium, 0.99% potassium and 1.46% calcium).

### Taste solutions

Sweetener solutions were prepared in deionized water. We tested sucrose, maltose, sorbitol, saccharin, D-phenylalanine, D-tryptophan, L-proline, glycine, cyclamate (cyclamic acid, hemicalcium salt), aspartame (Asp-Phe methyl ester), neohesperidin dihydrochalcone (Sigma Chemical Co., St Louis, MO), glycyrrhizic acid monoammonium salt (Aldrich Chemical Co., Milwaukee, WI), acesulfame-K (Hoechst Food Ingredients, Edison, NJ), erythritol (M&C Sweeteners/Mitsubishi Chemical and Cargill, Blair, NE), sucralose (McNeil Specialty, New Brunswick, NJ), SC-45647 (a Nutrasweet compound) (Tinti and Nofre, 1991; DuBois, 1995) and thaumatin (a gift of G. DuBois). Detailed information about most of these sweeteners can be found elsewhere (Schiffman and Gatlin, 1993). The order of testing of the sweeteners within each group of mice is described above.

The range of solution concentrations (Table 1) was selected so that the weakest solutions would be below the human detection threshold and the strongest solutions would be above the human threshold and would approximately match the sweetness of 2–7.5% sucrose in humans (Schiffman and Gatlin, 1993). In some cases limited compound solubility or sweetness potency precluded matching the upper level of sweetness.

### Measurement of fluid intake

Fluid intake was measured using two-bottle preference tests of individually caged mice. Construction of drinking tubes and other experimental details have been described previously (Bachmanov *et al.*, 1996b). The drinking tubes were positioned to the right of the feeder with their tips 15 mm apart and each extended 25 mm into the cage. Each tube had a stainless steel tip with a 3.175 mm diameter hole from which the mice could lick fluids.

The mice were presented with one tube containing a solution and the other tube containing deionized water. The solutions were tested in order of increasing concentration. For most sweeteners the concentrations were increased by approximately half-log steps. Each concentration was tested for 48 h. The positions of the tubes were switched every 24 h

in order to control for side preferences. Daily measurements were made in the middle of the light period by reading fluid volume to the nearest 0.2 ml. There were no breaks between testing different concentrations of the same sweetener, but between testing different sweeteners the mice received deionized water in both drinking tubes for at least 2 days.

### Data analyses

Average daily (24 h) fluid intakes were calculated for each mouse for each solution concentration. Preference scores were calculated as the ratio of the average daily solution intake to average daily total fluid (solution + water) intake, in percent. The B6 mice were heavier than were the 129 mice ( $28.8 \pm 0.3$  and  $24.9 \pm 0.3$  g, respectively, means  $\pm$  standard errors,  $P < 0.0001$ , *t*-test). To account for this, body weights of individual mice were measured before and after each test series, averaged and used to calculate intakes per 30 g body wt (the approximate weight of an adult mouse). The relationship between body weight and fluid intake have been discussed in detail previously (Bachmanov *et al.*, 1998).

The data for each sweetener were analyzed separately using two-way ANOVA with strain as the between-group factor and concentration as the within-group factor. Scheffé *post hoc* tests were used to evaluate differences between individual means. These statistical tests used a two-tailed criterion for significance of  $P < 0.05$ .

The significance of preference or avoidance of a taste solution in the two-bottle tests was determined by comparing the solution and water intakes using paired *t*-tests. Because this comparison was made for each sweetener concentration for each strain, the total number of comparisons was 136. To avoid potential false positives due to multiple testing, we applied a Bonferroni correction, which set the two-tailed critical level of statistical significance at  $P < 0.05/136$ , or 0.000368. Preference or avoidance thresholds were defined as the lowest solution concentrations that were consumed in significantly larger or smaller amounts than water, respectively.

### Results

For most sweeteners, concentration significantly affected solution intakes and preferences [in all cases  $F(3-11, 63-231) \geq 3.9$ ,  $P < 0.01$ , main effect of concentration in two-way ANOVA]. Exceptions were neohesperidin hydrochalcone (ns) and aspartame [effect of concentration was significant for intakes,  $F(5, 105) = 3.2$ ,  $P < 0.05$ , but not for preferences]. Four typical patterns of preference scores as a function of increasing concentration were observed (Figure 1): (i) indifference–preference; (ii) indifference–preference–avoidance; (iii) indifference–avoidance; (iv) only indifference. In some cases the concentration–response curves were similarly shaped in B6 and 129 mice, with 129 preferences being shifted towards the higher concentrations (e.g. sugars, saccharin, acesulfame, sucralose and SC-45647) or with

**Table 1** Sweetener concentrations tested, preferred and avoided by B6 and 129 mice

Solution	Unit	Range tested	Preferred <sup>a</sup>		Avoided <sup>b</sup>	
			B6	129	B6	129
Sucrose	mM	29–935	58–935	234–935	–	–
	%	1–32	2–32	8–32	–	–
Maltose	mM	0.08–833	28–833	83–833	–	–
	%	0.003–30	1–30	3–30	–	–
Sorbitol	mM	1.7–1647	16.5–549	55–549	1647	1098–1647
	%	0.03–30	0.3–10	1–10	30	20–30
Erythritol	mM	2.5–2457	246–491	–	1638–2457	819–2457
	%	0.03–30	3–6	–	20–30	10–30
Saccharin	mM	0.43–255	0.43–85	4.3–85	255	170–255
	g/l	0.1–62	0.1–20.5	1–20.5	62	41–62
Acesulfame-K	mM	0.01–100	0.3–100	10–100	–	–
	g/l	0.002–20	0.06–20	2–20	–	–
Sucralose	mM	0.0008–25	0.25–25	2.5–25	–	–
	mg/l	0.3–10000	0.1–10	1–10	–	–
SC-45647	mM	0.0003–0.9	0.009–0.9	0.09–0.9	–	–
	mg/l	0.1–300	3–300	30–300	–	–
D-Phenylalanine	mM	3–100	10–100	–	–	–
	g/l	0.5–16.5	1.7–17	–	–	–
D-Tryptophan	mM	0.3–50	3–50	–	–	–
	g/l	0.06–10.2	0.6–10	–	–	–
L-Proline	mM	1–1000	100–1000	100	–	1000
	g/l	0.12–115	11.5–115	11.5	–	115
Glycine	mM	0.1–1000	0.1–1000	100–300	–	–
	g/l	0.008–75	0.008–75	7.5–22.5	–	–
Glycyrrhizic acid	mM	0.0011–3.4	–	–	–	–
	mg/l	1–3000	–	–	–	–
Neohesperidin	mM	0.00016–0.16	–	–	–	–
	mg/l	0.1–100	–	–	–	–
Thaumatococcus	mM	0.0000013–0.0045	–	–	–	–
	mg/l	0.03–100	–	–	–	–
Cyclamate	mM	0.15–151	–	0.5	151	–
	g/l	0.03–30	–	0.1	30	–
Aspartame	mM	0.03–10	–	–	–	–
	mg/l	8.8–2943	–	–	–	–

–, no significant preference/avoidance; solution intake did not differ significantly from water intake.

<sup>a</sup>Consumed in significantly greater amounts than water. The lowest concentration in the range represents the preference threshold.

<sup>b</sup>Consumed in significantly smaller amounts than water. The lowest concentration in the range represents the avoidance threshold.

<sup>c</sup>For explanation see Results.

both strains being indifferent (e.g. glycyrrhizic acid, neohesperidin and thaumatococcus). In other cases the two strains had differently shaped concentration–response curves (e.g. amino acids and erythritol).

For most sweeteners strain differences in preferences (except for sorbitol, glycyrrhizic acid and thaumatococcus) and intakes (except for sorbitol, glycyrrhizic acid, neohesperidin hydrochalcone and thaumatococcus) were significant [in all cases  $F(1,18–22) \geq 4.9$ ,  $P < 0.05$ , main effect of strain in two-way ANOVA]. Similarly, strain  $\times$  concentration interaction effects were significant [in all cases  $F(3–11,63–231) \geq 2.2$ ,

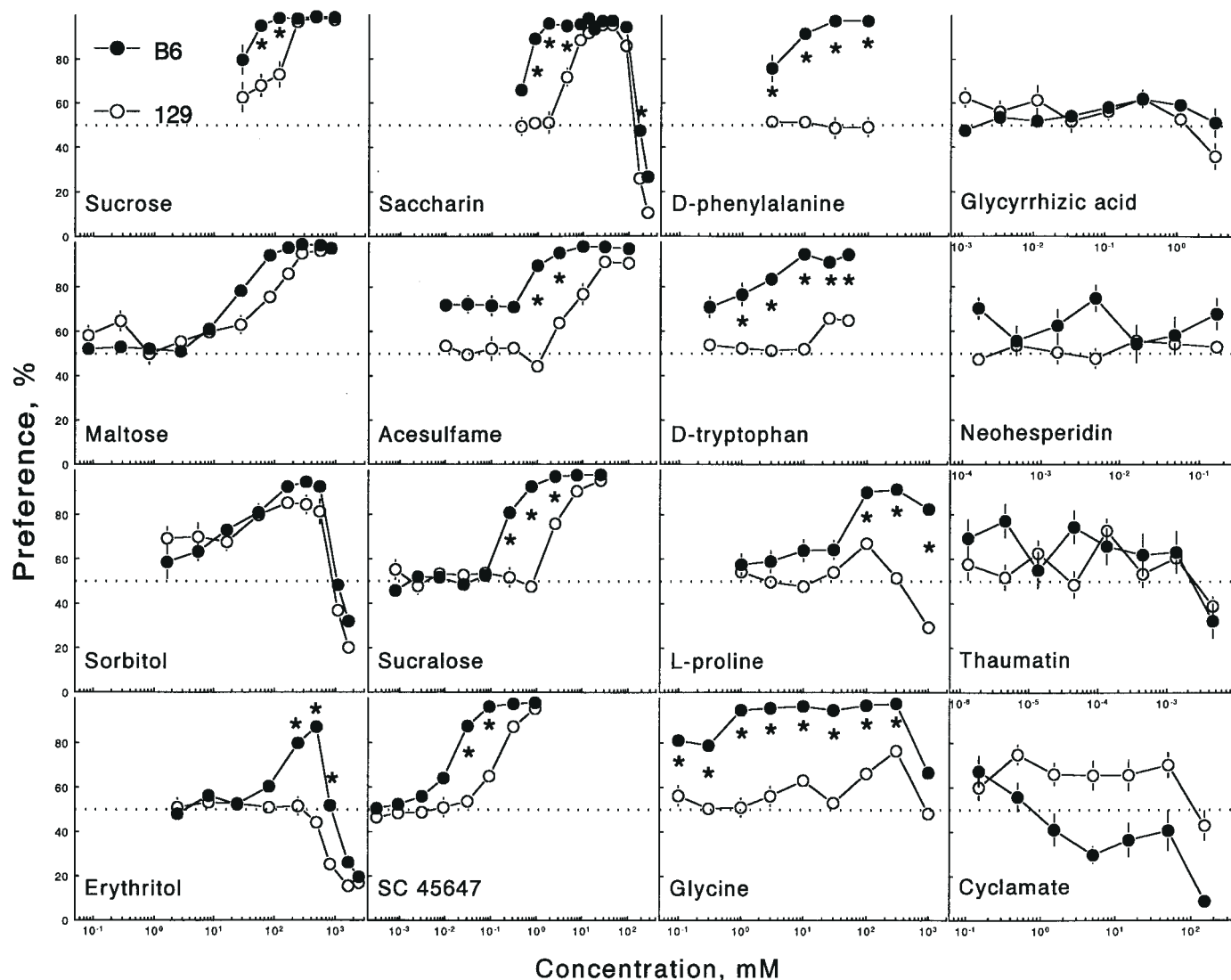
$P < 0.05$ ], except for neohesperidin hydrochalcone and aspartame preferences and intakes.

#### Sucrose

Compared with 129 mice, B6 mice had lower preference thresholds (Table 1), higher preferences for 58 and 117 mM (2 and 4%, respectively) solutions (Figure 1) and drank more of the 117–467 mM (4–16%) solutions (Figure 2).

#### Maltose

Compared with 129 mice, B6 mice had lower preference thresholds and drank more of the 167 and 278 mM (6 and



**Figure 1** Preferences of B6 and 129 mice for 16 sweeteners. Vertical bars represent SE. \*Significant difference between B6 and 129 strains,  $P < 0.05$ , Scheffé *post hoc* tests.

10%) solutions. Maltose preference depended on strain [B6 mice had a higher overall preference than did the 129 mice; effect of strain,  $F(1,22) = 4.9$ ,  $P < 0.05$ , ANOVA] and on a strain  $\times$  concentration interaction [ $F(10,220) = 6.5$ ,  $P < 0.001$ ], however, no significant strain differences for individual concentrations were detected in *post hoc* tests.

#### Sorbitol

Compared with 129 mice, B6 mice had lower preference thresholds and higher avoidance thresholds. Although sorbitol preference and intake depended on a strain  $\times$  concentration interaction [ $F(8,176) \geq 3.0$ ,  $P < 0.01$ ], no significant strain differences for individual concentrations were detected.

#### Erythritol

Unlike B6 mice, 129 mice did not prefer any erythritol

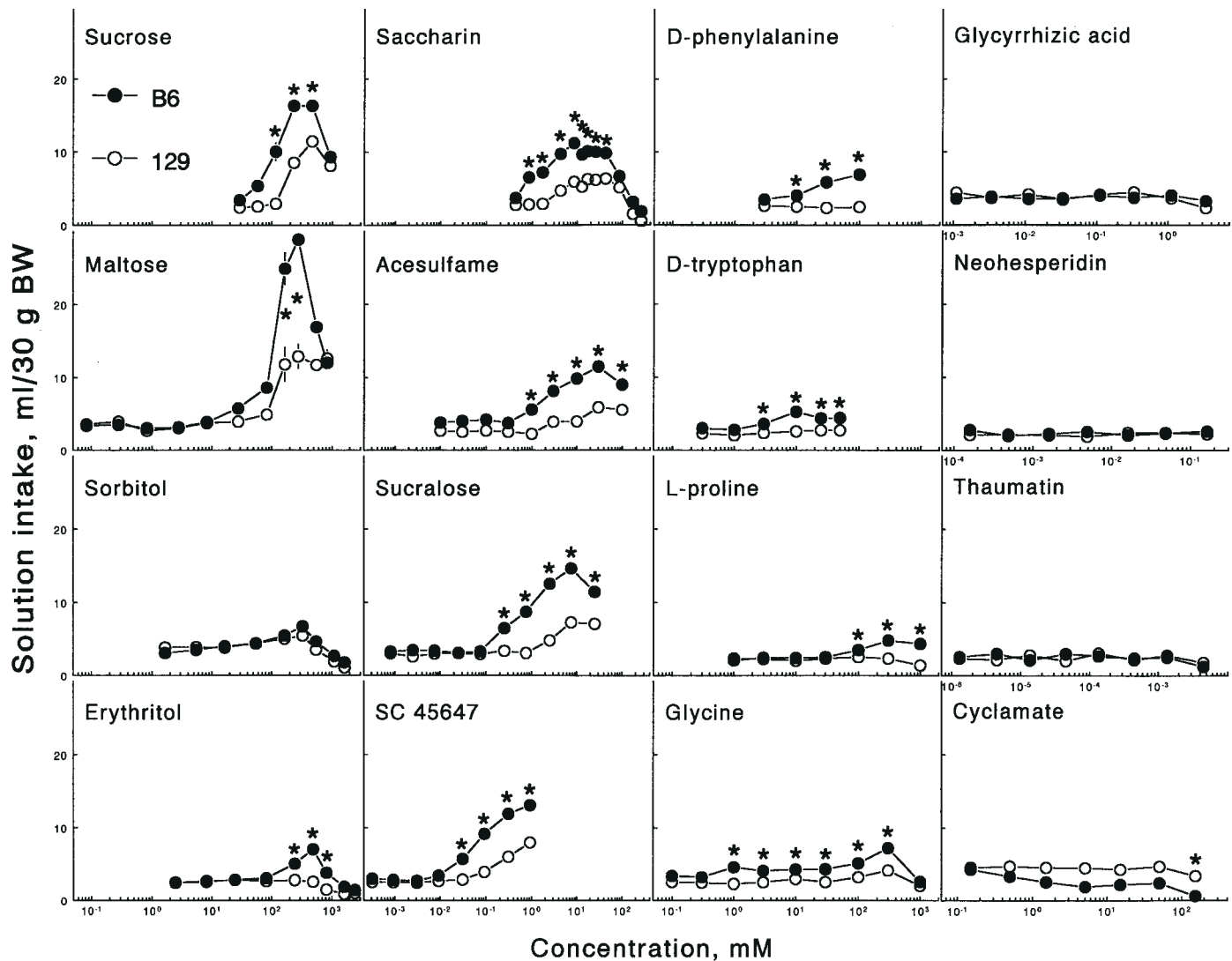
concentration. B6 mice had higher erythritol avoidance thresholds than did 129 mice. Compared with 129 mice, B6 mice had higher preferences for and intakes of 246–819 mM (3–10%) erythritol.

#### Saccharin

Compared with 129 mice, B6 mice had lower preference and higher avoidance thresholds, higher preference scores for 0.85–4.3 mM (0.2–1 g/l) and 170 mM (41 g/l) solutions and they drank more of the 0.85–43 mM (0.2–10.3 g/l) solutions.

#### Acesulfame-K

B6 mice preferred 0.01, 0.03 and 0.3–100 mM solutions relative to water. Because preferences for 0.01–0.3 mM solutions by B6 mice fluctuated near the threshold of statistical significance and may have exceeded it by chance, a more conservative estimation of preference threshold for the B6



**Figure 2** Consumption of 16 sweeteners by B6 and 129 mice. Explanations are the same as in Figure 1.

strain would be 0.3–1 mM. Compared with 129 mice, B6 mice had lower preference thresholds, higher preferences for 1 and 3 mM (0.2 and 0.6 g/l) solutions and they drank more of the 1–100 mM (0.2–20 g/l) solutions.

#### *Sucralose*

Compared with 129 mice, B6 mice had lower preference thresholds, higher preferences for 0.25–2.5 mM (0.1–1 g/l) solutions and they drank more of the 0.25–25 mM (0.1–10 g/l) solutions.

#### *SC-45647*

Compared with 129 mice, B6 mice had lower preference thresholds, higher preferences for 0.03 and 0.09 mM (10 and 30 mg/l) solutions and they drank more of the 0.03–0.9 mM (10–300 mg/l) solutions.

#### *D-Phenylalanine*

Unlike B6 mice, 129 mice did not prefer any D-phenylalanine

concentration. Compared with 129 mice, B6 mice had higher preferences for 3–100 mM (0.5–17 g/l) solutions and intakes of 10–100 mM (1.7–17 g/l) solutions.

#### *D-Tryptophan*

Unlike B6 mice, 129 mice did not prefer any D-tryptophan concentration. Compared with 129 mice, B6 mice had higher preferences for 1–50 mM (0.2–10 g/l) solutions and intakes of 3–50 mM (0.6–10 g/l) solutions.

#### *L-Proline*

The preference threshold concentration was 100 mM (11.5 g/l) for both B6 and 129 mice, but preference scores of 129 mice for this solution were lower and did not exceed 70%. Compared with 129 mice, B6 mice had higher preferences for and intakes of 100–1000 mM (11.5–115 g/l) solutions.

#### *Glycine*

B6 mice had lower preference thresholds than did the 129



mice. Although 129 mice preferred 100 and 300 mM solutions over water, their preference scores for these solutions were lower than those of B6 mice and did not exceed 80%. Compared with 129 mice, B6 mice had higher preferences for 0.1–300 mM (0.008–22.5 g/l) solutions and intakes of 1–300 mM (0.08–22.5 g/l) solutions.

#### *Glycyrrhizic acid*

Both B6 and 129 mice were indifferent to all concentrations tested. Although preference scores and intakes depended on a strain  $\times$  concentration interaction [ $F(7,154) \geq 2.7$ ,  $P < 0.05$ ], *post hoc* tests did not detect strain differences for individual concentrations.

#### *Neohesperidin dihydrochalcone*

Both B6 and 129 mice were indifferent to all concentrations tested. The only significant effect found was a strain difference in neohesperidin preference scores [ $F(1,22) = 8.1$ ,  $P < 0.01$ ]: they were higher overall in B6 mice than in 129 mice, but they did not exceed 75% in either strain.

#### *Thaumatococin*

Both B6 and 129 mice were indifferent to all concentrations tested. Although preference scores and intakes depended on a strain  $\times$  concentration interaction [ $F(7,154) \geq 2.2$ ,  $P < 0.05$ ], *post hoc* tests did not detect strain differences for individual concentrations.

#### *Cyclamate*

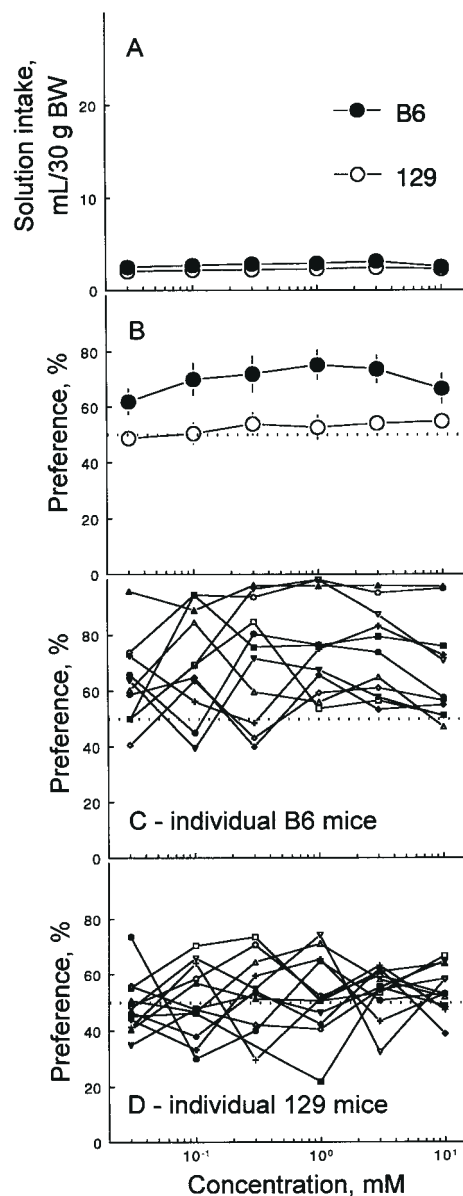
Mice from 129 strain preferred 0.5 mM cyclamate solution over water but were indifferent to other concentrations. B6 mice avoided the highest (151 mM) cyclamate concentration but were indifferent to other concentrations. The preference scores did not exceed 75% in either strain. The preference scores depended on strain [B6 mice had lower preferences than did 129 mice,  $F(1,22) = 25.4$ ,  $P < 0.001$ ] and on a strain  $\times$  concentration interaction [ $F(6,132) = 3.6$ ,  $P < 0.01$ ], but *post hoc* tests did not detect strain differences for individual concentrations. B6 mice had lower 151 mM (30 g/l) cyclamate intakes than did 129 mice.

#### *Aspartame*

On average, neither the B6 nor 129 strain preferred or avoided any concentration tested (Table 1), but B6 mice had higher overall aspartame preference scores and intakes [effect of strain,  $F(1,21) \geq 5.7$ ,  $P < 0.05$ ; Figure 3A,B]. Within the B6 strain there was a substantial individual variation in aspartame preferences: some B6 mice displayed strong preferences over the range of aspartame concentrations, whereas other B6 mice were indifferent (Figure 3C). No 129 mice showed a strong preference for or avoidance of aspartame (Figure 3D).

## Discussion

In this study we used two-bottle tests to characterize taste responses to 17 sweeteners by B6 and 129 mice. High preference scores and large sweetener solution intakes



**Figure 3** Aspartame solution intakes and preferences by B6 and 129 mice. (A) Solution intakes, strain means  $\pm$  SE. (B) Solution preferences, strain means  $\pm$  SE. (C) Solution preferences by individual B6 mice. (D) Solution preferences by individual 129 mice.

suggest that many of these sweeteners are highly palatable to mice. Although the two-bottle tests do not characterize taste quality perception directly, such high preferences and intakes in replete animals are usually observed only in tests with sweeteners. In addition, several of these compounds have been characterized using conditioned taste aversion tests or single fiber recordings from gustatory nerves in mice (Ninomiya *et al.*, 1984a,b) and other species (Hellekant *et al.*, 1997; Danilova *et al.*, 1998) and they were shown to have sensory properties similar to those of sucrose.

We observed three main patterns of strain differences in taste responses to sweeteners. First, sucrose, maltose,

saccharin, acesulfame-K, sucralose and SC-45647 were preferred by both strains, but B6 mice had lower preference thresholds and higher intakes. Second, the amino acids D-phenylalanine, D-tryptophan, L-proline and glycine were highly preferred by B6 mice, but not by 129 mice. Third, glycyrrhizic acid, neohesperidin, thaumatin, cyclamate and aspartame did not evoke strong preferences in either strain. In the last group of sweeteners, the responses to cyclamate and aspartame had some unique features. Cyclamate was more aversive to B6 mice than to 129 mice, which were relatively indifferent to it. Whereas all 129 mice and some B6 mice were indifferent to aspartame, other B6 mice strongly preferred it. Sugar alcohols produced a somewhat different pattern of strain differences compared with sugars, despite their chemical similarity. Lower concentrations of sorbitol were preferred by mice from both the B6 and 129 strains. Lower concentrations of erythritol were preferred by B6 mice, but were neutral to 129 mice. Mice from both strains avoided high ( $\geq 800$  mM or 10%) concentrations of sugar alcohols, whereas they strongly preferred similar concentrations of sugars.

Although properties of sweetener solutions other than sweetness (e.g. bitterness or post-ingestive effects) probably affected the results of our tests, we believe that peripheral perception of sweet taste was a major determinant of the strain differences. First, compared with 129 mice, preferences and intakes of B6 mice were higher for a wide variety of sweet compounds with different non-sweet sensory and/or post-ingestive properties. This suggests that the pattern of strain differences is explained by a common attribute of these solutions (i.e. sweetness), rather than by variable attributes (e.g. bitterness or post-ingestive effects). Second, greater sweetener consumption by B6 mice is genetically related to larger responses of their gustatory nerves to sweeteners (Bachmanov *et al.*, 1997a,b; Li *et al.*, 2001), suggesting that differences in peripheral sensory input are involved in the genetic variation in behavioral responses to sweeteners.

Nevertheless, non-sweet sensory (e.g. bitterness, viscosity, osmolality or coolness resulting from endothermic reactions with saliva) and post-ingestive factors probably affected the results for some sweeteners. In particular, avoidance of concentrated solutions of saccharin, L-proline and cyclamate may be due to their predominant bitter taste at these concentrations [see also Dess (Dess, 1993)]. Cyclamate probably does not taste sweet to either mouse strain, as was found in other species (Hellekant *et al.*, 1997; Danilova *et al.*, 1998), but B6 mice may be more sensitive to its bitterness compared with 129 mice, resulting in a stronger avoidance by B6 mice. B6 and 129 mice differ in behavioral taste responses to bitter, salty, sour and umami solutions, although these differences are genetically unrelated to their differential sweetener responsiveness (Bachmanov *et al.*, 1996a,b, 2000). The caloric value of the sugars tested (sucrose and maltose) may account for their higher consumption

compared with the non-nutritive sweeteners (e.g. saccharin, acesulfame, sucralose and SC-45647) in both strains. Sugar alcohols are metabolized differently than sugars and may also cause discomfort because of their intestinal osmotic effects (Schiffman and Gatlin, 1993). This may have inhibited the mice from consuming large volumes of these solutions and resulted in avoidance of the more concentrated solutions. It is possible that the B6 and 129 mice differ not only in perceived sweetness of the sugar alcohols, but also in their post-ingestive handling (e.g. intestinal absorption, metabolism or excretion). Thus, the strain differences in consumption of the sugar alcohols may depend on an interaction between perception of their sweetness and their post-ingestive properties.

Because we tested several different sweeteners in the same groups of animals, the results of our tests might have been affected by 'carry-over' effects from testing previous compounds. Although we cannot exclude them completely, their possible contribution was most likely very small. First, the mice were given only water to drink for at least 2 days between testing different sweeteners. Second, taste responses clearly depended on solution concentration within a series of a particular sweetener, rather than being correlated with responses to a previously tested compound. There were no instances when strain differences were present for the first (weakest) concentration tested but then disappeared. Third, in this study we tested some of the sweeteners used in other published (Bachmanov *et al.*, 1996a,b, 1997a,b, 2000; Li *et al.*, 2001) and unpublished experiments and found similar results.

A distinctive feature of aspartame preference was a substantial variation among B6 mice, which is consistent with previous data from rats (Sclafani and Abrams, 1986; De Francisco and Dess, 1998). A few B6 mice consistently preferred aspartame to water over the range of concentrations tested, whereas some other B6 mice did not.

The sweet tasting amino acids were strongly preferred by B6 mice at some concentrations, but 129 mice were generally neutral to them and did not display preferences  $>80\%$  at any concentration tested. This contrasted with responses to sugars and several other sweeteners, which at certain concentrations were strongly preferred by both B6 and 129 mice. Electrophysiological experiments also show different patterns for these two groups of sweeteners (Inoue *et al.*, 2001). B6 mice had greater chorda tympani nerve responses to sucrose, maltose, saccharin, sorbitol, acesulfame-K, SC-45647 and sucralose. However, among the amino acids tested only L-proline evoked stronger responses in the chorda tympani nerve of B6 mice, whereas responses to D-phenylalanine and glycine were similar in the two strains [see discussion in Inoue *et al.* (Inoue *et al.*, 2001)].

The indifference of 129 mice to the amino acids may be explained by a non-functional mechanism for detecting amino acid sweetness in this strain. However, it can also be explained by other reasons, for example by different

sweetness potency of the stimuli. Sweetness might not have reached threshold level for the 129 strain even at the highest concentrations of the amino acids tested (limited by solubility), so that if it was possible to test higher concentrations, they might be preferred by 129 mice. This can be illustrated by comparing intakes of solutions of the amino acids and other sweeteners. When the highest intakes of the amino acids by B6 mice were matched with intakes of other sweeteners, the corresponding concentrations of these other sweeteners were below preference thresholds for 129 mice (compare the two middle columns in Figure 2). Another possible reason for the indifference of 129 mice to the amino acids may be that they are more sensitive to their unpleasant sensory or post-ingestive properties compared with B6 mice.

The differences between the B6 and 129 strains in behavioral taste responses to at least some sweeteners are due to allelic variation of a few genes (Bachmanov *et al.*, 1996a). The largest contribution to the mouse strain variation in responsiveness to sweeteners originates from the *Sac* locus (Fuller, 1974; Lush, 1989; Phillips *et al.*, 1994; Lush *et al.*, 1995; Bachmanov *et al.*, 1997a,b; Blizard *et al.*, 1999; Li *et al.*, 2001). Other genetic loci, including *dpa*, also affect mouse strain variation in sweetener responsiveness (Ninomiya *et al.*, 1987, 1991; Phillips *et al.*, 1994; Capeless and Whitney, 1995) and possibly affect the differences between the B6 and 129 strains (Bachmanov *et al.*, 1996a, 1997b).

In this study we tested mice with compounds that taste sweet to humans. Mice (at least from the B6 strain), as well as many other animals, including humans, show high avidity for sugars, sugar alcohols, amino acids and some artificial sweeteners (*N*-sulfonyl amides, a chlorinated sugar analog and a guanidinacetic acid-based sweetener). However, some compounds (cyclamate, aspartame, thaumatin and the glycosides) that taste sweet to humans and other Old World simians (Glaser *et al.*, 1995; Hellekant *et al.*, 1996; Nofre *et al.*, 1996) are evidently not sweet to mice and some other species (Jakinovich, 1981; Naim *et al.*, 1982; Sclafani and Abrams, 1986; Hellekant *et al.*, 1994, 1997; Danilova *et al.*, 1998; De Francisco and Dess, 1998). The absence of a consistent preference for aspartame and thaumatin by B6 and 129 mice corresponds to equally weak chorda tympani responses to them in both strains (Inoue *et al.*, 2001).

In summary, this study shows that compared with 129 mice, B6 mice have higher preferences for and intakes of several compounds tasting sweet to humans. Cyclamate, aspartame, thaumatin, glycyrrhizic acid and neohesperidin dihydrochalcone are not palatable to mice.

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