SUPPLEMENT

Genetics of Amino Acid Taste and Appetite[1–3]

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ABSTRACT

The consumption of amino acids by animals is controlled by both oral and postoral mechanisms. We used a genetic approach to investigate these mechanisms. Our studies have shown that inbred mouse strains differ in voluntary amino acid consumption, and these differences depend on sensory and nutritive properties of amino acids. Like humans, mice perceive some amino acids as having a sweet (sucrose-like) taste and others as having an umami (glutamate-like) taste. Mouse strain differences in the consumption of some sweet-tasting amino acids (D-phenylalanine, D-tryptophan, and L-proline) are associated with polymorphisms of a taste receptor, type 1, member 3 gene (*Tas1r3*), and involve differential peripheral taste responsiveness. Strain differences in the consumption of some other sweet-tasting amino acids (glycine, L-alanine, L-glutamine, and L-threonine) do not depend on *Tas1r3* polymorphisms and so must be due to allelic variation in other, as yet unknown, genes involved in sweet taste. Strain differences in the consumption of L-glutamate may depend on postingestive rather than taste mechanisms. Thus, genes and physiologic mechanisms responsible for strain differences in the consumption of each amino acid depend on the nature of its taste and postingestive properties. Overall, mouse strain differences in amino acid taste and appetite have a complex genetic architecture. In addition to the *Tas1r3* gene, these differences depend on other genes likely involved in determining the taste and postingestive effects of amino acids. The identification of these genes may lead to the discovery of novel mechanisms that regulate amino acid taste and appetite. *Adv Nutr* 2016;7(Suppl):806S–22S.

Keywords: mouse, inbred strain, behavior, consumption, intake, preference, gustatory nerves, sweet, umami

Introduction

Amino acids are essential components of organisms. They play multiple roles in their free form and as building blocks of proteins. Dietary amino acids and proteins constitute one of the macronutrients. Animals can detect macronutrients in food by using taste. For example, the carbohydrate content of food can be predicted on the basis of the sweet taste of sugars. Similarly, the protein content of food can be predicted on the basis of the taste of amino acids, which are often present in free form in protein-containing foods. Consistent with this, most amino acids have a taste, which makes some of them important as taste-active components in food. In addition to taste, postingestive mechanisms can also guide the choice and consumption of amino acids. Once ingested, amino acids and their metabolites may generate signals that affect appetite and satiety

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TABLE 1 Amino acids used in the reviewed studies[1]

Amino acid	Predominant taste quality[2]	Nutritional value[3]	Metabolism[4]	Experiment[5]							
				B6 and 129 inbred mice (12–18)			B6 × 129 F_2 hybrid mice (19)		129.B6-*Tas1r3* congenic mice (20)		
				2BT	CT	CTA	2BT	CT	2BT	BAT	CT
D-Histidine	Sweet	8.5%	—		+						
D-Phenylalanine	Sweet	51.6%	—	+	+		+	+	+	+	+
D-Tryptophan	Sweet	24.7%	—	+				+	+		+
Glycine	Sweet	Nonessential	Glucogenic	+	+	+	+	+	+	+	+
L-Alanine	Sweet	Nonessential	Glucogenic	+	+			+	+		+
L-Glutamate monoammonium	Umami	Nonessential	Glucogenic		+			+			
L-Glutamate monosodium	Umami	Nonessential	Glucogenic	+	+	+	+	+	+		
L-Glutamine	Sweet	Nonessential	Glucogenic	+				+	+		
L-Histidine	Bitter	Essential	Glucogenic	+							
L-Proline	Sweet	Nonessential	Glucogenic	+	+			+	+		+
L-Serine	Sweet	Nonessential	Glucogenic		+						
L-Threonine	Sweet	Essential	Glucogenic and ketogenic	+	+				+		
L-Tryptophan	Bitter	Essential	Glucogenic and ketogenic			+					
L-Valine	Bitter	Essential	Glucogenic	+							

[1] B6, C57BL/6ByJ inbred mouse strain; BAT, brief-access test; CT, chorda tympani nerve response; CTA; conditioned taste aversion; F_2, hybrids of the second filial generation; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, 129P3/J inbred mouse strain; 2BT, 2-bottle choice test.

[2] Human sensory data are from references 21–27 and AA Bachmanov (unpublished data, 2015). Humans perceive the taste of L-glutamic acid and its salts as umami, but depending on its chemical form, L-glutamate may also have other taste components: for example, salty (for monosodium L-glutamate), bitter (for monoammonium L-glutamate), or sour (for free L-glutamic acid). Although L-histidine and L-valine are described as having a predominantly bitter taste, their bitterness is weak, and L-valine also has a weak sweet taste; consistent with the lack of strong taste to humans, mice did not strongly prefer or avoid these amino acids (Figure 1).

[3] Nutritional values of L-amino acids are described as being essential or nonessential for mice (28). For D-amino acids, we provide nutritional values expressed relative to a corresponding L-form in mice (29). These values were calculated in 14-d growth assays with mice maintained on synthetic diets with variable amounts of L- and D-isomers of the same amino acid and constant amounts of the remaining amino acids (detailed methods are described in references 30 and 31).

[4] Data were available only for L-amino acids and glycine.

[5] The "+" symbols indicate that an amino acid was used in the study for the column; cells are blank for amino acids not used in the study for the column.

(1–6). Dietary amino acid deficiency may activate a specific hunger for the missing amino acid(s) and thereby prevent the pathological consequences of amino acid deficiency (7–11). A better understanding of the mechanisms involved in processing different amino acids by the organism may open new avenues for uses of these amino acids as flavor, nutritive, and therapeutic agents.

This review summarizes our studies of amino acid taste and appetite in mice. Genetic analyses were accompanied by physiologic experiments aimed at understanding the mechanisms responsible for genetic variation in amino acid consumption. We used amino acids that differ with respect to taste quality (e.g., sweet compared with bitter), chemical structure (e.g., D- compared with L-enantiomers), nutritional value (e.g., essential compared with nonessential), or metabolism (e.g., glucogenic compared with ketogenic) (**Table 1**). Humans perceive amino acids and their salts as having ≥1 basic taste qualities: sweet, umami, bitter, salty, and/or sour. Analyses of amino acid taste quality perception in laboratory animals with the use of behavioral and electrophysiologic approaches have shown that it has much in common with human taste perception. For example, if an amino acid tastes sweet to humans, rodents typically perceive it tasting similar to sucrose, show appetitive responses to it, and show activation of the same taste nerve fibers as those activated by sucrose. For brevity, we use in this review human psychophysical descriptors for taste qualities of amino acids, but it is more accurate to describe taste quality perception by nonhuman animals with the use of chemical names of taste stimuli (e.g., "sucrose-like taste" or "NaCl-like taste" for "sweet" or "salty," respectively).

When amino acid–responsive taste bud cells are activated, the signal is transmitted via afferent gustatory nerves to the nucleus of the solitary tract in the brainstem; from this point, the gustatory neuraxis provides input to multiple processing centers in the brain, which evokes the taste perception and modulates ingestive behavior. In addition to taste, amino acid consumption can also be influenced by postoral and motivational factors. To reflect these aspects of processing taste information and ingestive behavior, we used multiple measures of responsiveness to amino acids: taste intensity (based on electrophysiologic recordings of activity in taste nerves), taste quality perception [based on conditioned taste aversion (CTA)[12] generalization], palatability (based on initial licking responses in brief-access tests), and measures of voluntary consumption (intakes and preferences, based on long-term 2-bottle choice tests).

On the basis of our initial surveys of inbred mouse strains (32, 33), we selected 2 strains for detailed genetic and

[12] Abbreviations used: B6, C57BL/6ByJ inbred mouse strain; CTA, conditioned taste aversion; *dpa*, D-phenylalanine aversion locus; F_2, hybrids of the second filial generation; LiCl, lithium chloride; Sac, saccharin preference locus; T1R, proteins from the taste receptor, type 1 family; T1R1, taste receptor, type 1, member 1 protein; T1R2, taste receptor, type 1, member 2 protein; T1R3, taste receptor, type 1, member 3 protein; *Tas1r*, genes from the taste receptor, type 1 family; *Tas1r1*, taste receptor, type 1, member 1 gene; *Tas1r2*, taste receptor, type 1, member 2 gene; *Tas1r3*, taste receptor, type 1, member 3 gene; 129, 129P3/J inbred mouse strain.

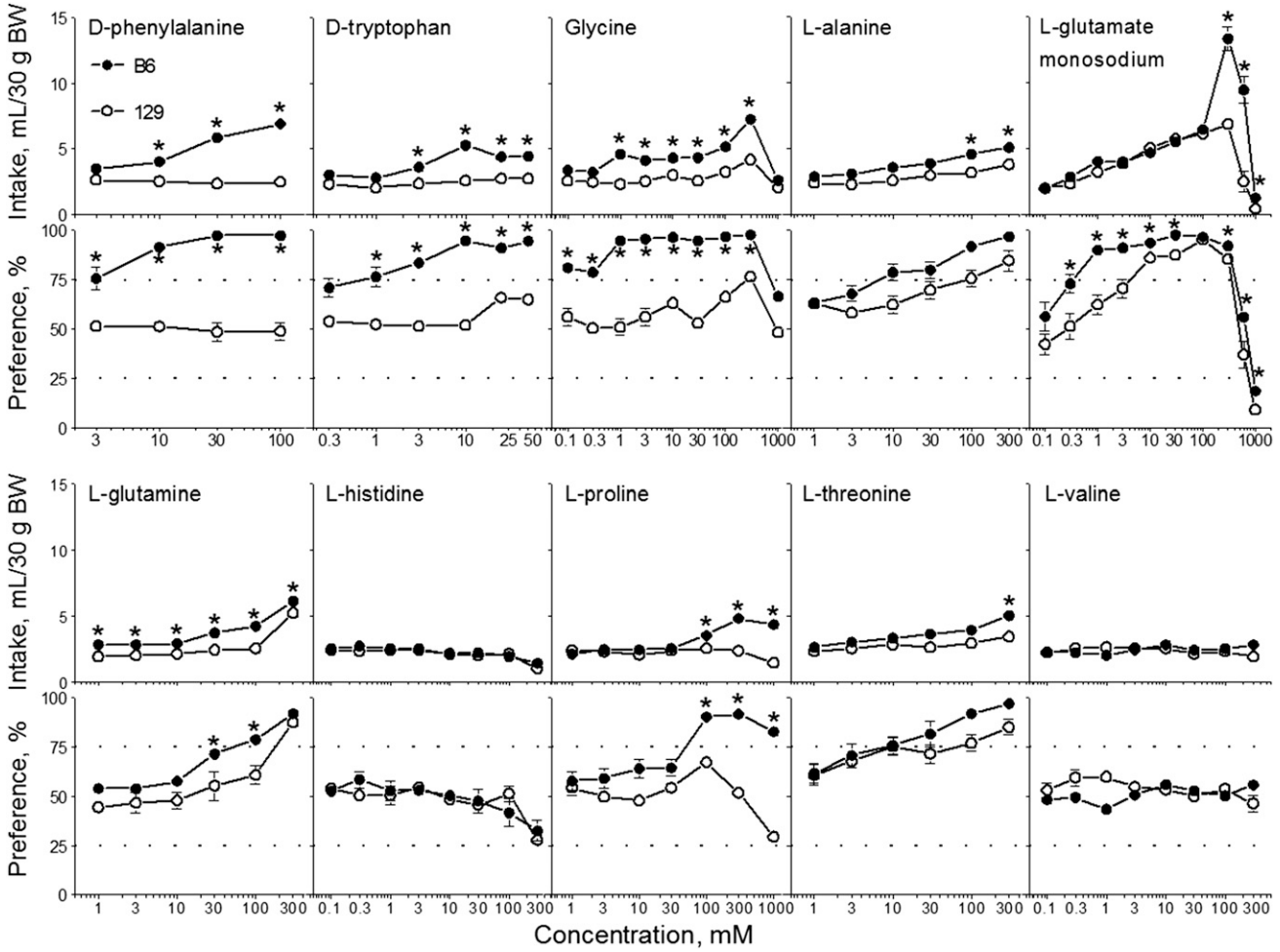

FIGURE 1 Amino acid solution intakes (top) and preference scores (bottom) of B6 and 129 mice in 48-h 2-bottle choice tests with ascending concentrations of amino acid solutions. Values are means ± SEs, n = 7–18. The dotted horizontal lines show thresholds of preference (75%) and avoidance (25%). *Significant difference between B6 and 129 strains at a given concentration, $P < 0.05$ (post hoc or planned comparison tests). B6, C57BL/6ByJ inbred mouse strain; BW, body weight; 129, 129P3/J inbred mouse strain. Adapted from references 12–14 with permission.

physiological analyses: C57BL/6ByJ (B6) and 129P3/J (129). In addition to exhibiting large differences in consumption of amino acids, these strains are well suited for genetic analyses because they have distant genealogy and because their genome sequence is available.

Inbred Strain Differences in Behavioral and Neural Taste Responses to Amino Acids

Two-bottle choice tests

To examine the contribution of taste and nutritive properties to the strain differences in amino acid consumption, we measured voluntary consumption of 10 amino acids in B6 and 129 mice with the use of 48-h 2-bottle tests (**Figure 1**). These amino acids differed with respect to taste quality, chemical structure, nutritive value, and metabolism (Table 1).

Compared with the 129 mice, the B6 mice exhibited higher consumption of (intakes and preferences; Figure 1) and lower preference thresholds (**Table 2**) for sweet-tasting (D-phenylalanine, D-tryptophan, glycine, L-alanine, L-glutamine, L-proline, and L-threonine) and umami-tasting (L-glutamate) amino acids. Mice from the 2 strains did not differ in consumption of L-histidine or L-valine, which have tastes that are neither sweet nor umami.

Both B6 and 129 mice preferred certain concentrations of glycine, L-alanine, monosodium L-glutamate, L-glutamine, and L-threonine, but preference thresholds were lower in B6 mice than in 129 mice. For glycine, preference scores of 129 mice barely reached the preference threshold (75%) and then only at a single, high concentration (300 mM; preference score: 76% ± 3%). Whereas B6 mice preferred certain concentrations of D-phenylalanine, D-tryptophan, and L-proline, 129 mice did not prefer these amino acids at any concentration tested. For L-histidine and L-valine, both B6 and 129 mice were indifferent to all concentrations tested.

These results show that the strain differences in amino acid consumption do not depend on chemical structure,

TABLE 2 Taste preference thresholds (in mM) of B6 and 129 inbred and 129.B6-*Tas1r3* congenic mice in 48-h 2-bottle choice tests with ascending concentrations of amino acid solutions[1]

	Inbred mice		129.B6-*Tas1r3* congenic mice (20)	
Amino acid	**B6 vs. 129**	**Reference**	**B6 vs. 129[2]**	***Tas1r3* effect on consumption[3]**
D-Phenylalanine	3 < ND	(12)	30 < ND	+
D-Tryptophan	1 < ND	(12)	30 < ND	+
Glycine	0.1 < 300	(12)	ND = ND	−
L-Alanine	10 < 100	(13)	ND = ND	−
L-Glutamate monosodium	1 < 10	(14)	ND = ND	−
L-Glutamine	100 < 300	(13)	ND = ND	−
L-Histidine	ND = ND	(13)		
L-Proline	100 < ND	(12)	100 < ND	+
L-Threonine	30 < 100	(13)	ND = ND	−
L-Valine	ND = ND	(13)		

[1] Taste preference threshold was defined as the lowest solution concentration for which animals display preference score ≥75%. Thresholds reported in the original publications (12, 14) were defined using a different criterion, and in some cases differ from threshold values shown in this table. B6, C57BL/6ByJ inbred mouse strain; ND, not determined (the highest concentration tested was below preference threshold); *Tas1r3,* taste receptor, type 1, member 3 gene; 129, 129P3/J inbred mouse strain; < and >, presence and direction of the difference in taste preference threshold between strains or genotypes; =, no difference in threshold; +, significant difference between mice with different *Tas1r3* genotypes; −, lack of significant difference between mice with different *Tas1r3* genotypes.

[2] *Tas1r3* genotypes.

[3] *Tas1r3* effect on consumption as reported in Table 3.

nutritive value, or metabolism. This is because B6 mice had higher consumption of sweet- and umami-tasting amino acids regardless of their chiral, nutritive, or metabolic properties. These results also suggest that B6 and 129 mice do not differ in a generalized amino acid appetite, and that strain differences in amino acid consumption are restricted to only the sweet- and umami-tasting amino acids we tested. We therefore next examined peripheral taste responsiveness to, and taste quality perception of, sweet- and umami-tasting amino acids (described below in the sections entitled "Gustatory nerve electrophysiology" and "Taste quality perception").

The B6 mice consumed remarkably large amounts of 300-mM monosodium L-glutamate: for some B6 mice, daily consumption of monosodium L-glutamate exceeded half of their body weight. This consumption was higher than for any of the other amino acids tested and rivals the amounts of some highly preferred sweet substances ingested by this same strain (12). Although B6 and 129 mice differ in voluntary consumption of NaCl and sweeteners (12, 13, 34–39), the strain differences in monosodium L-glutamate consumption are independent of the strain differences in the consumption of NaCl or sweeteners, as explained below.

Monosodium L-glutamate contains sodium, which contributes a salty component to its taste and also has its own postingestive effects when consumed. Therefore, strain differences in monosodium L-glutamate consumption potentially could be influenced by differential responses to its sodium cation. However, this is unlikely because the direction of the strain differences in monosodium L-glutamate and NaCl consumption is opposite (i.e., B6 mice have lower NaCl intakes and preferences than do the 129 mice) (34–38). Thus, if anything, the sodium will diminish the B6-129 strain difference in monosodium L-glutamate consumption. The point here is that the strain differences in monosodium L-glutamate consumption are most likely due to specific effects of the L-glutamate moiety rather than to the effects of the sodium moiety present in monosodium L-glutamate.

We found that, compared with 129 mice, B6 mice consumed more monosodium L-glutamate and several sweeteners (12, 13, 39). This observation raises the possibility that the elevated consumption of monosodium L-glutamate and sweeteners by B6 mice is due to a common underlying mechanism. However, there was no correlation between monosodium L-glutamate and sweetener preferences in the hybrids of the second filial generation (F_2) derived from the B6 and 129 strains (14), which shows that these 2 traits have an independent genetic determination. Thus, strain differences in the consumption of monosodium L-glutamate depend on mechanisms different from those underlying strain differences in the consumption of sweet-tasting amino acids.

Our observation of strain-specific effects of exposure to monosodium L-glutamate on its subsequent consumption (14) suggested that differences in monosodium L-glutamate consumption between B6 and 129 mice depend on postingestive effects of L-glutamate. During the 96-h 2-bottle tests of B6 and 129 mice with 300 mM monosodium L-glutamate and water, the strain differences in monosodium L-glutamate consumption were larger at the end of the test than they were in the beginning of the test. During this test, monosodium L-glutamate intakes and preferences increased in B6 mice and decreased in 129 mice. This suggests that the postingestive effects of monosodium L-glutamate are rewarding to B6 mice and aversive to 129 mice (14, 40). Similar effects of exposure to monosodium L-glutamate were observed in B6 and 129 mice in subsequent studies (41, 42). Consistent with this, B6 mice acquired flavor preferences conditioned by oral monosodium L-glutamate, whereas 129 mice did not (42). The direct evidence that glutamate can generate postingestive signals that influence appetite comes from reports of flavor preferences conditioned by intragastric infusions of monosodium L-glutamate in rats and mice (4–6, 43). This signal may involve postingestive metabolism of glutamate that differs between B6 and 129 mice (40, 44). Overall, these data

support the possibility that the strain differences in glutamate consumption depend on postingestive mechanisms.

Gustatory nerve electrophysiology

In long-term 2-bottle choice tests, B6 mice consumed more sweet- and umami-tasting amino acids than did 129 mice (Figure 1). The consumption of taste solutions in long-term tests can be influenced not only by perception of their sensory attributes but also by their postingestive effects. Because some amino acids have nutritive value to mice (see Table 1), postingestive factors may contribute to the strain differences in their consumption, in addition to their taste properties. We assessed the possible role of afferent gustatory input in these strain differences by measuring integrated responses to lingual application of solutions of 9 different amino acids in the mouse chorda tympani nerve, one of the nerves that carry afferent gustatory signals (**Figure 2**).

All amino acids tested evoked responses in the chorda tympani nerve at some concentrations. Compared with 129 mice, B6 mice had a greater response magnitude to, and lower response threshold for, L-proline. Responses to the other 8 amino acids did not differ significantly between the B6 and 129 strains. In tests with monosodium L-glutamate, possible strain differences in responsiveness to the L-glutamate moiety may be masked by differences in responsiveness to the associated sodium moiety. That is, if B6 mice have stronger responses to the L-glutamate moiety but weaker responses to the sodium moiety, the net response to monosodium L-glutamate could be similar in B6 and 129 mice. However, this was not the case because mice from both strains had similar responses to NaCl (15, 16). Thus, the lack of strain differences in responses to monosodium L-glutamate and NaCl implies that the 2 strains have similar responsiveness to the L-glutamate moiety of its sodium salt. This is further supported by similar chorda tympani responses of B6 and 129 mice to monoammonium L-glutamate.

Although B6 mice had a greater preference for D-phenylalanine, glycine, L-alanine, monosodium L-glutamate, L-proline, and L-threonine than did 129 mice (Figure 1), only L-proline evoked significantly larger whole-nerve chorda tympani responses in B6 mice. Thus, variation between the B6 and 129 strains in peripheral taste responsiveness may contribute to strain differences in the consumption of at least some sweet-tasting amino acids, exemplified by L-proline. It is possible, however, that the strains actually differ in peripheral taste responsiveness to these sweet-tasting amino acids, but these differences were not detected because of the following reasons: *1*) whole-nerve rather than single-fiber recording was used, *2*) the differences may reside in gustatory nerves other than the chorda tympani, or *3*) low signal-to-noise ratio of responses to these amino acids could have led to a false-negative finding. The latter possibility is supported by genetic variation in chorda tympani responses to D-phenylalanine in

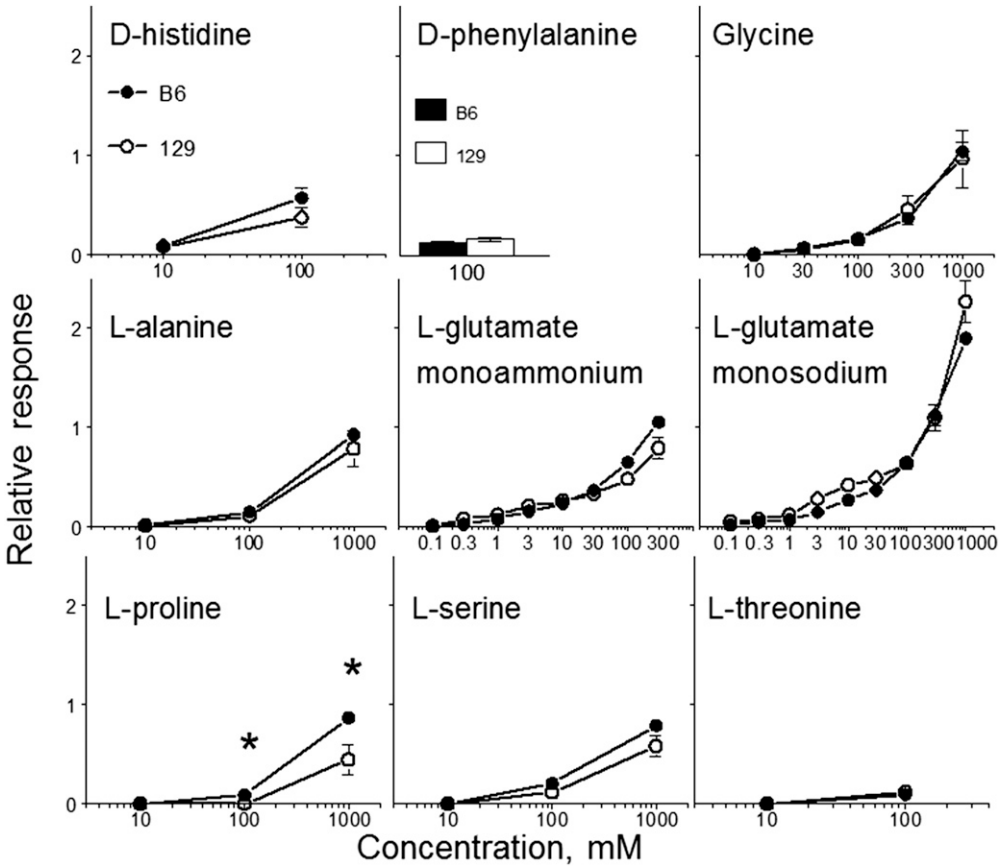

FIGURE 2 Chorda tympani nerve responses to lingual stimulation with amino acids (relative to 100 mM NH_4Cl) in B6 and 129 mice. Values are means ± SEs, $n = 5–7$. *Significant difference between B6 and 129 strains at a given concentration, $P < 0.05$ (*t* test). B6, C57BL/6ByJ inbred mouse strain; 129, 129P3/J inbred mouse strain. Adapted from references 15 and 16 with permission.

TABLE 3 Strain differences and effects of allelic variations in the *Tas1r3* gene on taste responsiveness to amino acids in mice[1]

	Inbred mice[2]				*Tas1r3* effect[3]				
	2BT[4]		CT[5]		B6 × 129 F_2 hybrid mice (19)		129.B6-*Tas1r3* congenic mice (20)		
Amino acid	Strain difference	Reference	Strain difference	Reference	2BT[4]	CT[5]	2BT[4]	BAT[6]	CT[5]
D-Phenylalanine	+	(12)	−	(16)	+	+	+	+	−
D-Tryptophan	+	(12)				+	+		+
Glycine	+	(12)	−	(16)	−	−	−	−	−
L-Alanine	+	(13)	−	(16)		−	−		−
L-Glutamate monosodium	+	(14)	−	(15)	−	−	−		
L-Glutamine	+	(13)				−	−		
L-Proline	+	(12)	+	(16)		−	+		+
L-Threonine	+	(13)	−	(16)			−		

[1] Results for individual amino acids are generally consistent among measures and experimental populations. Minor discrepancies in effects of *Tas1r3* genotype on responses to D-phenylalanine and L-proline are probably due to weak CTs to these stimuli, which lower signal-to-noise ratios and decrease statistical power to detect genetic differences. As a result, we might not have been able to detect the effect of *Tas1r3* genotype on CTs to D-phenylalanine in inbred and 129.B6-*Tas1r3* congenic mice and to L-proline in F_2 hybrids. Blank cells indicate that there are no data for a particular test and taste stimulus. B6, C57BL/6ByJ inbred mouse strain; BAT, brief-access test; CT, chorda tympani nerve response; F_2, hybrids of the second filial generation; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, 129P3/J inbred mouse strain; 2BT, two-bottle choice tests.

[2] The "+" and "−" symbols indicate a significant difference or lack of a significant difference between B6 and 129 strains. If there was significant strain difference, B6 mice always had higher taste responsiveness than did 129 mice.

[3] The effect of *Tas1r3* allelic variation is shown as a significant difference (+) or lack of significant difference (−) between mice with different *Tas1r3* genotypes. If there was an effect of the *Tas1r3* allelic variation, the B6 allele always increased taste responsiveness.

[4] Long-term 2BTs; differences in taste solution intakes, preference scores, and/or preference thresholds.

[5] Electrophysiologic recordings of integrated whole-nerve CTs to lingual application of taste stimuli; differences in response magnitude and/or response thresholds.

[6] Initial licking responses in BATs; differences in standardized lick ratios.

B6 × 129 hybrids (see **Table 3**) and by a larger response to D-phenylalanine in the nucleus of the solitary tract of B6 mice than in 129 mice (45, 46), which shows that the strain differences in preferences for D-phenylalanine may also be related to gustatory mechanisms. Consistent with the chorda tympani results, however, B6 and 129 mice had similar responses in the nucleus of the solitary tract to glycine (45, 46). It is unlikely that strain differences in the consumption of sweet-tasting amino acids are influenced by their postingestive effects, because these amino acids differ in their nutritive properties (see Table 1). However, postingestive effects are likely involved in strain differences in the consumption of higher concentrations of monosodium L-glutamate (discussed in the previous section, "Two-bottle choice tests").

Taste quality perception

The results of 2-bottle choice tests showed that B6 and 129 mice differed in their consumption of sweet- and umami-tasting amino acids. We examined whether these strain differences in taste preferences are due to genetic variation in taste quality perception by using the CTA generalization paradigm, a technique commonly used to assess taste quality perception in nonhuman animals (47). We used glycine and monosodium L-glutamate as representative sweet- and umami-tasting amino acids, respectively. We conditioned mice to avoid glycine or monosodium L-glutamate (conditioned stimuli) by pairing its ingestion with an injection of nausea-inducing lithium chloride (unconditioned stimulus), and then examined the suppression of licking responses in brief-access tests to prototypical taste stimuli representing the main taste qualities (sweet, salty, sour, and bitter).

Glycine. Although B6 mice strongly prefer glycine solutions at specific concentrations, 129 mice are indifferent to the same concentrations (Figure 1). We used the CTA generalization technique to examine whether this lack of robust glycine preference in 129 mice could be explained by their inability to perceive glycine sweetness. After conditioning B6 and 129 mice to avoid 100-mM glycine, both strains suppressed licking responses to sweet saccharin and sucrose but not to salty NaCl, sour hydrochloric acid, or bitter L-tryptophan

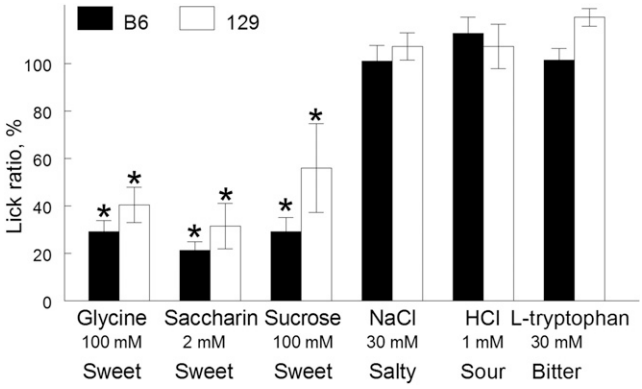

FIGURE 3 CTA generalization in B6 and 129 mice conditioned to avoid 100-mM glycine. Lick ratios were calculated by dividing lick numbers of individual conditioned (LiCl-treated) mice by the mean lick rate of the control (NaCl-treated) group. Values are means ± SEs, n = 7–10. *Significant decrease in lick rate in conditioned mice relative to control mice, $P < 0.05$ (post hoc test). B6, C57BL/6ByJ inbred mouse strain; CTA, conditioned taste aversion; LiCl, lithium chloride; 129, 129P3/J inbred mouse strain. Adapted from reference 17 with permission.

(**Figure 3**). The fact that B6 and 129 mice both generalized the glycine CTA to sweet (saccharin and sucrose) but not other basic taste stimuli indicates that mice from these strains perceive glycine taste as predominantly sweet. This agrees with analyses of taste-evoked activity in the nucleus of the solitary tract, which showed that in both B6 and 129 mice *1*) the across-neuron profile of responses to glycine was most highly correlated with that to sucrose and *2*) glycine-evoked taste responses were most similar to those of other sweeteners on the basis of multidimensional scaling analyses (45).

Our data are consistent with previous reports. CTA generalization between glycine and sucrose was also reported in previous studies in rodents (48–52). Perception of the sucrose-like taste of glycine by rodents is consistent with their appetitive responses to glycine (Figure 1) (50, 53–56) and the perception of glycine as tasting sweet by humans (Table 1).

Thus, the lack of a robust glycine preference by 129 mice is unlikely to be explained by their inability to perceive the sweetness of glycine. Other aspects of taste perception likely underlie the strain differences in glycine preference. For example, it is possible that glycine is a more potent sweet-taste stimulus for B6 mice than for 129 mice, and that although 129 mice detect its sweet taste, the sweetness perception is not intense enough to drive behavior in 2-bottle choice tests.

L-Glutamate. B6 mice consumed more monosodium L-glutamate than did 129 mice in 2-bottle choice tests (Figure 1). We examined whether this strain difference in intake is due to differences in the taste quality perception of monosodium L-glutamate. After conditioning mice to avoid 100-mM monosodium L-glutamate, both B6 and 129 mice suppressed licking responses to salty NaCl but not to sweet sucrose, sour citric acid, or bitter quinine (**Figure 4**). Therefore, both B6 and 129 mice generalized CTA from monosodium L-glutamate to salty but not to other basic taste stimuli, which indicates that mice from both strains perceive monosodium L-glutamate taste as predominantly umami and salty.

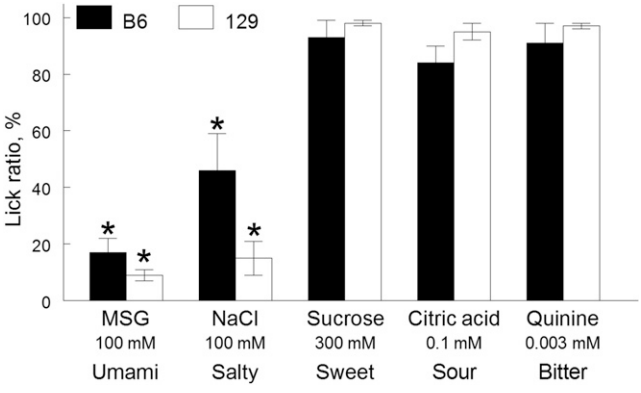

FIGURE 4 CTA generalization in B6 and 129 mice conditioned to avoid 100-mM monosodium L-glutamate. Lick ratios were calculated by dividing lick numbers of individual conditioned (LiCl-treated) mice by the mean lick rate of the control (NaCl-treated) group. Values are means ± SEs, n = 6–10. *Significant decrease in lick rate in conditioned mice relative to control mice, $P < 0.05$ (post hoc test). B6, C57BL/6ByJ inbred mouse strain; CTA, conditioned taste aversion; LiCl, lithium chloride; MSG, monosodium L-glutamate; 129, 129P3/J inbred mouse strain. Adapted from reference 18 with permission.

These results show that both B6 and 129 mice perceive monosodium L-glutamate as a complex taste stimulus, which corresponds to results of other studies in humans and in other animals. These studies showed that mice perceive a unique taste of monosodium L-glutamate (57), which is probably equivalent to human perception of umami taste (Table 1). Humans and rodents also perceive a salty (NaCl-like) taste component of monosodium L-glutamate (Table 1) (21–23, 51, 57, 58), which is likely due to the presence of sodium. Consistent with this, monosodium L-glutamate and NaCl evoked similar patterns of responses in single fibers of the chorda tympani gustatory nerve in rodents (51, 59). Interestingly, rats conditioned to avoid a mixture of monosodium L-glutamate with amiloride (a blocker of the epithelial sodium channel, ENaC, which suppresses an amiloride-sensitive component of taste responses to sodium salts in rodents) generalized the CTA to sucrose (60–63). The lack of CTA generalization between monosodium L-glutamate and sucrose in our experiments may reflect the use of monosodium L-glutamate without amiloride as the conditioned stimulus in our study or species differences between mice and rats in taste quality perception of monosodium L-glutamate.

The similar patterns of monosodium L-glutamate CTA generalization in the B6 and 129 strains indicate that these 2 strains do not differ in their perception of the taste quality of monosodium L-glutamate (at least at the 100-mM concentration used as a conditioned stimulus). These CTA results are consistent with the similar gustatory nerve responses to glutamate salts in the B6 and 129 strains (Figure 2). Together, these data suggest that the strain differences in monosodium L-glutamate consumption are not due to differences in perception of the taste quality of monosodium L-glutamate or in peripheral taste responsiveness to monosodium L-glutamate.

Effect of the Polymorphisms in the taste receptor, type 1, member 3 gene (*Tas1r3*) Taste Receptor Gene on Behavioral and Neural Taste Responses to Amino Acids

The *Tas1r3* gene encodes the taste receptor, type 1, member 3 protein (T1R3) that participates in forming both sweet [taste receptor, type 1, member 2 protein (T1R2)+T1R3] and umami [taste receptor, type 1, member 1 protein (T1R1)+T1R3] receptor dimers. In heterologous expression systems, the mouse T1R2+T1R3 receptor is activated by sweet-tasting D-amino acids, the mouse T1R1+T1R3 receptor is activated by L-amino acids, and both of these receptors are activated by the nonchiral glycine (see the section entitled "Interactions of the Proteins from the Taste Receptor, Type 1 Family (T1R) with Amino Acids").

Inbred mouse strains differ in sequences of the *Tas1r3* gene. Some of these sequence variants, or polymorphisms, are associated with sweet-taste responsiveness. This genotype-phenotype association was a basis for positional cloning of

the mouse saccharin preference locus (*Sac*) as the *Tas1r3* gene (64). B6 mice carry a *Tas1r3* allele that encodes a more sensitive receptor, and 129 mice carry a *Tas1r3* allele for a less sensitive receptor (33, 64). These *Tas1r3* polymorphisms may be responsible for strain differences in amino acid taste responses (see Figures 1 and 2). However, many other genes are also polymorphic between B6 and 129 mice, and allelic variations in these other genes may also influence strain differences in taste responses to amino acids. To assess the contribution of allelic variation in the *Tas1r3* gene to strain differences in amino acid taste responses, we conducted experiments in hybrid and congenic mice.

Analyses of hybrid mice

We performed the following: *1*) produced F_2 hybrids between the B6 and 129 inbred mouse strains; *2*) measured the consumption of amino acid solutions presented in the 2-bottle choice tests; *3*) recorded integrated responses of the chorda tympani gustatory nerve to lingual application of amino acids; *4*) determined the genotypes of markers on chromosome 4, where the *Tas1r3* gene resides; and *5*) conducted linkage analyses. For some, but not all, amino acids, we detected linkages to a distal (subtelomeric) region of chromosome 4, with linkage peaks in the vicinity of the *Tas1r3* gene.

Two-bottle choice tests. Three amino acids were tested in the 2-bottle choice tests: D-phenylalanine (30 mM), glycine (30 mM), and monosodium L-glutamate (1 and 300 mM). Significant linkages to *Tas1r3* were found for D-phenylalanine but not for glycine or monosodium L-glutamate (**Figure 5**). Consistent with these interval mapping results, D-phenylalanine intakes and preferences were lower in mice homozygous for the 129 allele of *Tas1r3* than in mice homozygous for the B6 allele of *Tas1r3* or in 129/B6 *Tas1r3* heterozygotes (**Figure 6**); this shows dominance of the B6 allele of the *Tas1r3* gene over its 129 allele. F_2 mice with different *Tas1r3* genotypes did not differ significantly in their consumption of glycine or monosodium L-glutamate (Figure 6).

Gustatory nerve electrophysiology. The chorda tympani nerve responses of the hybrid mice were measured for series of concentrations of 7 amino acids. Significant linkages to *Tas1r3* were found for 100-mM D-phenylalanine and 30-mM D-tryptophan but not for glycine (**Figure 7**), L-alanine, monosodium L-glutamate, monoammonium L-glutamate, L-glutamine, or L-proline (data not shown). Consistent with these interval mapping results, chorda tympani responses to 100-mM D-phenylalanine and 30-mM D-tryptophan were lower in mice with the 129 alleles of *Tas1r3* than in mice with the B6 alleles (**Figure 8**). F_2 mice with different *Tas1r3* genotypes did not differ significantly in chorda tympani responses to glycine, L-alanine, monoammonium L-glutamate, monosodium L-glutamate, L-glutamine, or L-proline (Figure 8). The results of these linkage analyses were consistent between behavioral and neural responses: both types of responses to D-phenylalanine were linked to *Tas1r3*, and both types of responses to glycine and monosodium L-glutamate were not linked to this region.

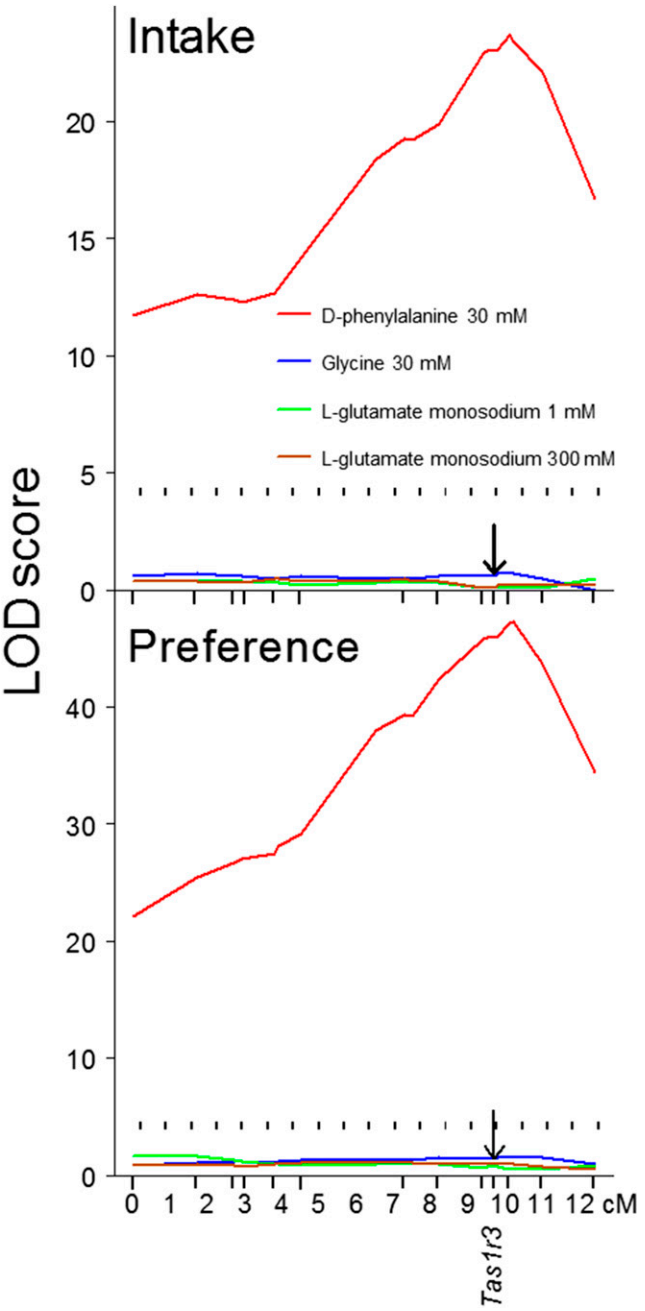

FIGURE 5 Distal chromosome 4 interval mapping of amino acid intakes (in mL/mouse; top) and preference scores (in %; bottom) measured in 96-h 2-bottle choice tests, n = 450–455. The x axis shows distances between chromosomal markers in cM estimated by using MAPMAKER/EXP (http://www.broadinstitute.org/genome_software). Marks on the x axis show marker positions. The arrow indicates the position of the *Tas1r3* gene. The curves trace the LOD scores calculated under an unconstrained (free) model by using MAPMAKER/QTL. The dotted horizontal lines show the threshold for significant (LOD: 4.3) linkage. cM, centimorgan; LOD, logarithm of the odds; *Tas1r3,* taste receptor, type 1, member 3 gene. Adapted from reference 19 with permission.

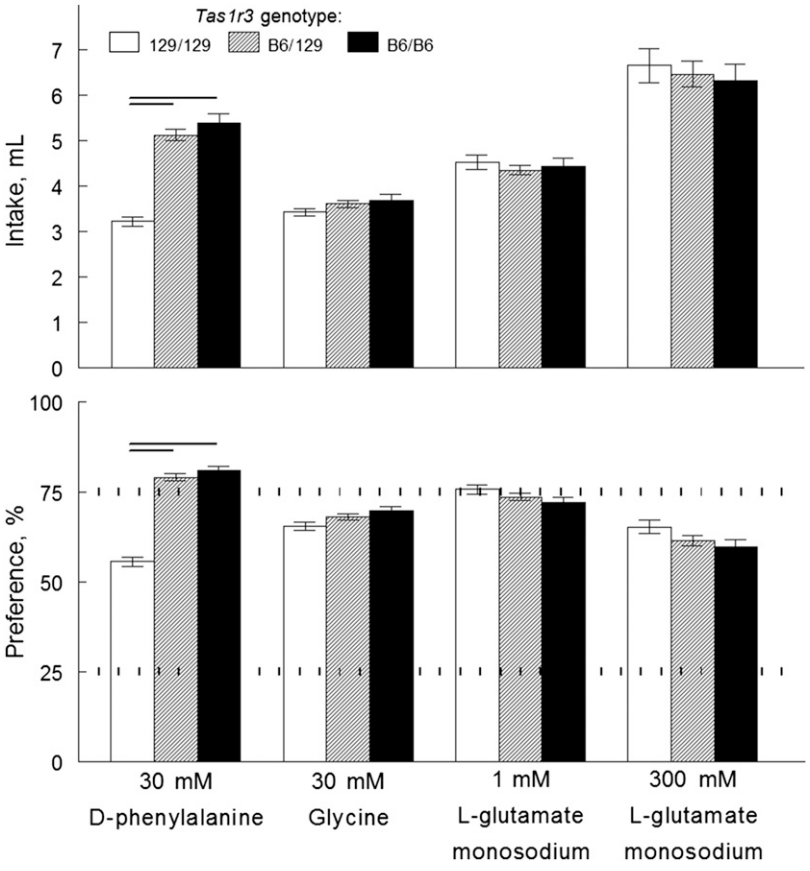

FIGURE 6 Amino acid solution intakes (top) and preference scores (bottom) of F_2 mice with different *Tas1r3* genotypes in 96-h 2-bottle choice tests. Values are means ± SEs, n = 110–219. The dotted horizontal lines show thresholds of preference (75%) and avoidance (25%). The solid horizontal lines indicate significant differences between genotypes, $P <$ 0.01 (planned comparison test). B6, genotype of the C57BL/6ByJ inbred mouse strain; F_2, hybrids of the second filial generation; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, genotype of the 129P3/J inbred mouse strain. Adapted from reference 19 with permission.

Analyses of congenic mice

To confirm and expand our finding that *Tas1r3* polymorphisms affect taste responses to some but not all amino acids in B6 × 129 F_2 hybrids (Figures 5–8), we analyzed taste responses to amino acids in 129.B6-*Tas1r3* congenic mice. We used 3 different measures: consumption in 48-h 2-bottle choice tests, initial licking responses, and responses of the chorda tympani nerve.

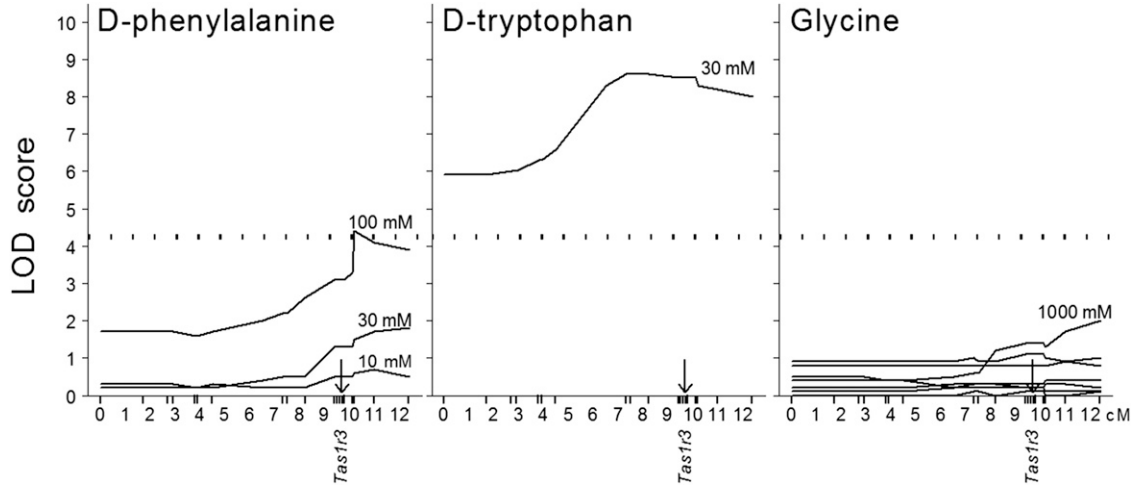

FIGURE 7 Distal chromosome 4 interval mapping of chorda tympani responses to oral stimulation with amino acids, n = 42–58. Concentrations are shown next to the corresponding curves, with the exception of 1- to 300-mM glycine with no significant linkages to this chromosomal region. The x axis shows distances between chromosomal markers in cM estimated by using MAPMAKER/EXP. Marks on the x axis show marker positions. The arrows indicate the position of the *Tas1r3* gene. The curves trace the LOD scores calculated under an unconstrained (free) model by using MAPMAKER/QTL. The dotted horizontal lines show the threshold for significant (LOD: 4.3) linkage. cM, centimorgan; LOD, logarithm of the odds; *Tas1r3,* taste receptor, type 1, member 3 gene. Adapted from reference 19 with permission.

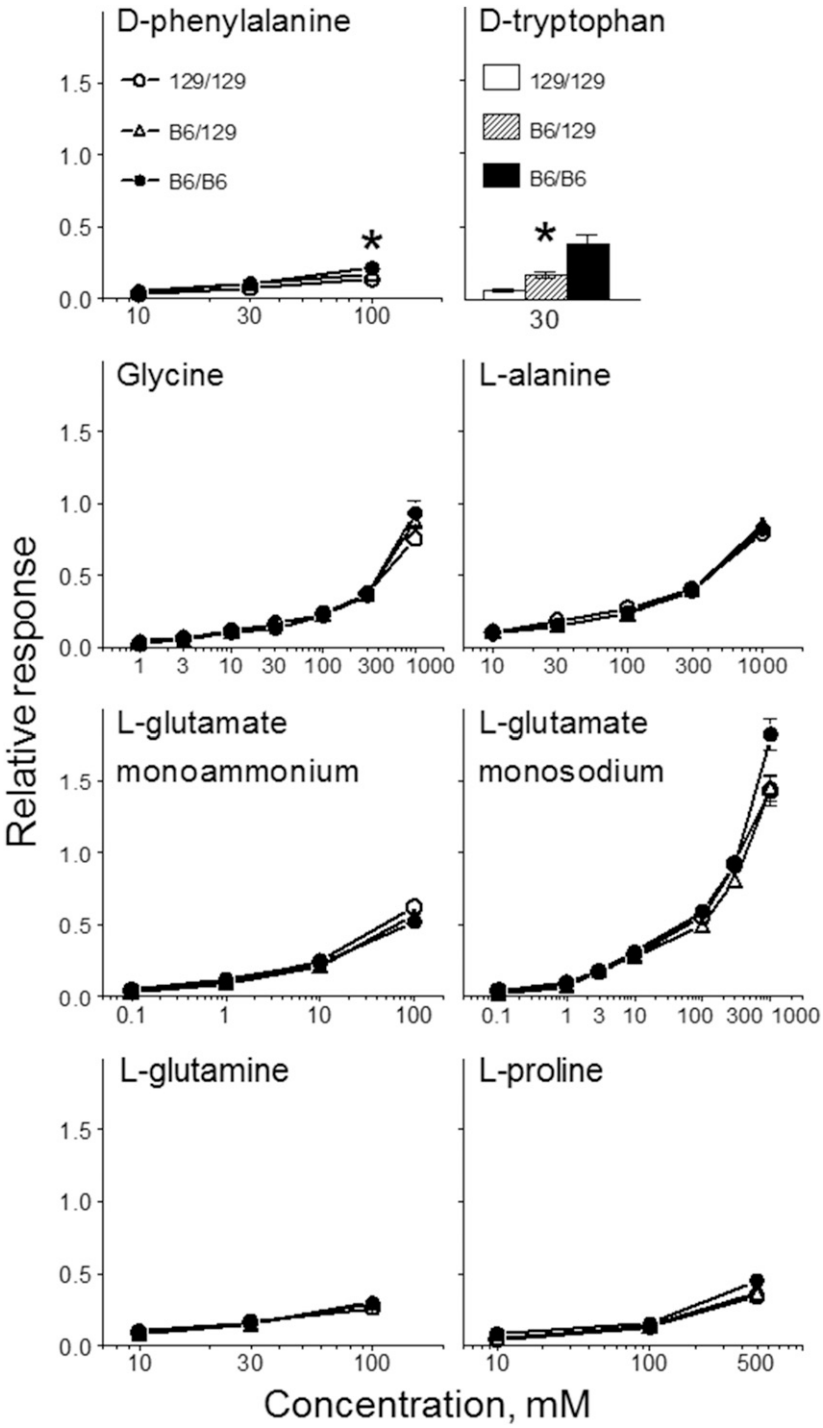

FIGURE 8 Chorda tympani nerve responses to lingual stimulation with amino acids (relative to 100-mM NH_4Cl) in F_2 mice with different *Tas1r3* genotypes. Values are means ± SEs, n = 13–23. *Significant main effect of genotype at a given concentration, $P < 0.01$ (1-factor ANOVA). B6, genotype of the C57BL/6ByJ inbred mouse strain; F_2, hybrids of the second filial generation; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, genotype of the 129P3/J inbred mouse strain. Adapted from reference 19 with permission.

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 (65). As a result, the congenic mice had the genetic background of the 129 strain and a small (<194 kb) donor chromosomal fragment containing the *Tas1r3* gene from the B6 strain (64). We maintained 129.B6-*Tas1r3* mice as a segregating congenic strain by mating congenic mice that had 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. Congenic littermates with B6/129 and 129/129 *Tas1r3* genotypes were used in this study.

2-bottle choice tests. We tested concentration series of 8 amino acids. Compared with 129/129 mice, B6/129 congenic mice had higher intakes and preferences (**Figure 9**) and lower

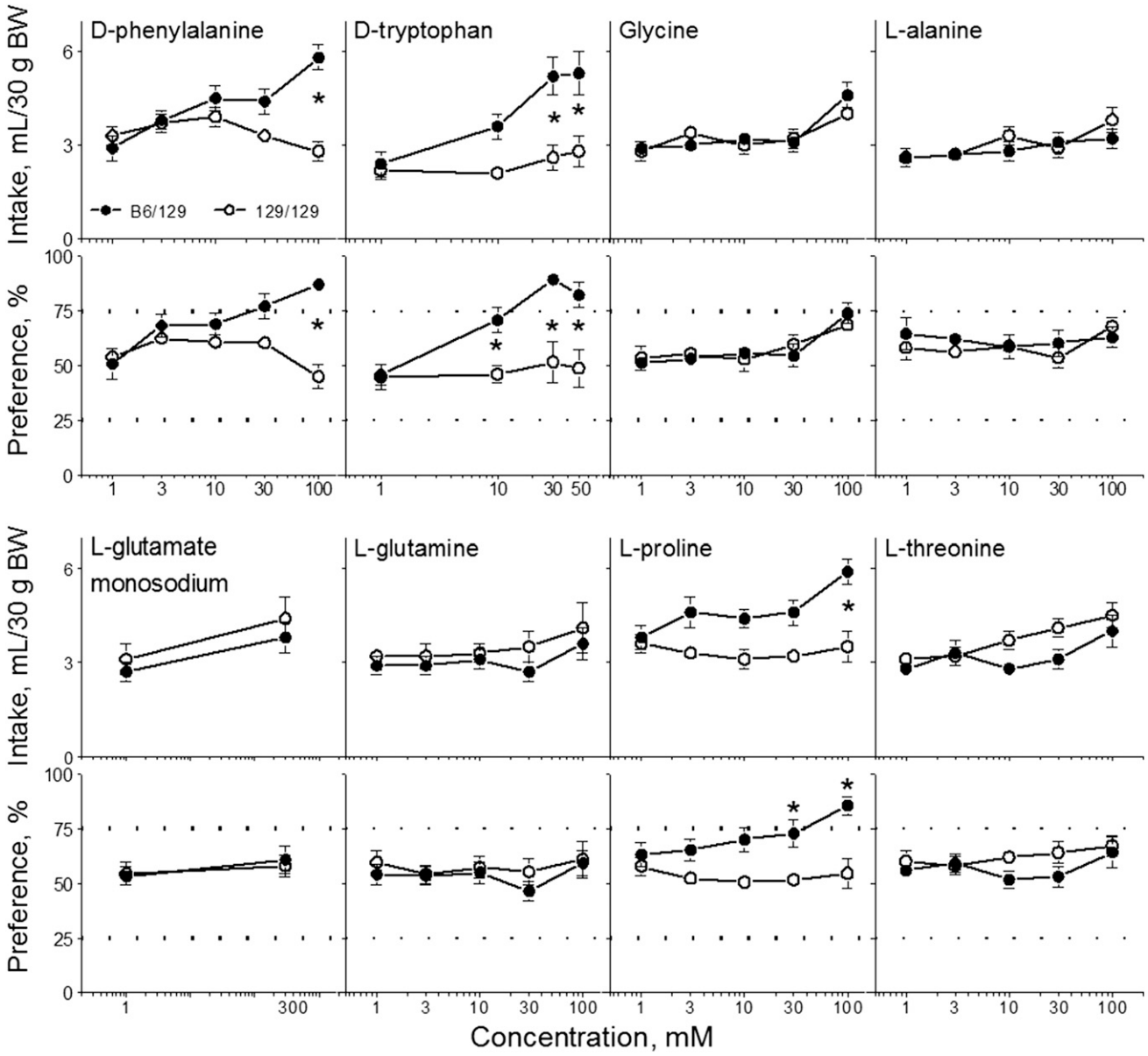

FIGURE 9 Amino acid solution intakes (top) and preference scores (bottom) of congenic mice with different *Tas1r3* genotypes in 48-h 2-bottle choice tests with ascending concentrations of amino acid solutions. Values are means ± SEs, n = 10–14. The dotted horizontal lines show thresholds of preference (75%) and avoidance (25%). *Significant difference between mice with B6/129 and 129/129 *Tas1r3* genotypes at a given concentration, $P < 0.05$ (planned comparison tests). B6, genotype of the C57BL/6ByJ inbred mouse strain; BW, body weight; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, genotype of the 129P3/J inbred mouse strain. Adapted from reference 20 with permission.

preference thresholds (Table 2) for D-phenylalanine, D-tryptophan, and L-proline. B6/129 and 129/129 mice had similar intakes and preferences for glycine, L-alanine, monosodium L-glutamate, L-glutamine, and L-threonine (Figure 9); preference scores for these 5 amino acids did not exceed preference thresholds in mice of both genotypes (Table 2).

Brief-access tests. Both B6/129 and 129/129 congenic mice showed concentration-dependent increases in licking responses to D-phenylalanine and glycine (**Figure 10**). B6/129 congenic mice had higher licking responses to 10–100-mM D-phenylalanine than did 129/129 mice. B6/129 and 129/129 congenic mice had similar licking responses for glycine at all concentrations tested. Thus, *Tas1r3* genotype affected licking responses to D-phenylalanine but not glycine.

Gustatory nerve electrophysiology. We tested a series of concentrations of 5 amino acids (**Figure 11**). Compared with 129/129 mice, B6/129 congenic mice had higher chorda tympani responses to D-tryptophan and L-proline. Responses to D-phenylalanine, glycine, and L-alanine were similar in B6/129 and 129/129 congenic mice.

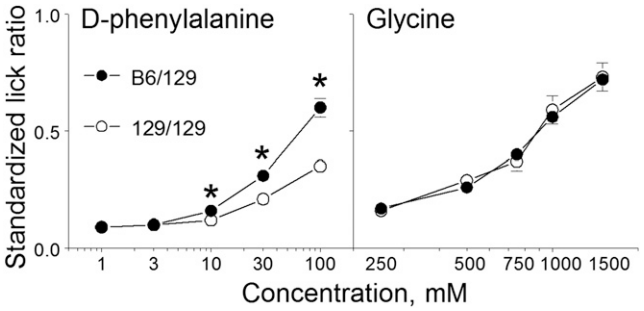

FIGURE 10 Initial licking responses to a range of concentrations of amino acid solutions in brief-access tests of congenic mice with different *Tas1r3* genotypes. The *y* axis shows the standardized lick ratios calculated by dividing the mean number of licks taken per trial by the maximum number of licks that the same mouse could potentially take across a 5-s trial. Values are means ± SEs, $n = 17–20$. *Significant difference between mice with B6/129 and 129/129 *Tas1r3* genotypes at a given concentration, $P < 0.05$ (planned comparison test). B6, genotype of the C57BL/6ByJ inbred mouse strain; *Tas1r3,* taste receptor, type 1, member 3 gene; 129, genotype of the 129P3/J inbred mouse strain. Adapted from reference 20 with permission.

To summarize, taste responses of 129.B6-*Tas1r3* congenic mice to amino acids were analyzed by using 3 different measures: responses of the chorda tympani nerve, initial licking responses, and sweetener consumption in the 48-h 2-bottle choice tests. The results were generally consistent across the 3 measures of taste responsiveness (summarized in Tables 2 and 3). That is, the *Tas1r3* genotype influenced responses to D-phenylalanine, D-tryptophan, and L-proline. The B6 allele of the *Tas1r3* gene was associated with higher sweet-taste responsiveness and sensitivity. The *Tas1r3* genotype did not affect responses to glycine, L-alanine, monosodium L-glutamate, L-glutamine, or L-threonine. This pattern of results fits a model in which changes in properties of the T1R3 taste receptor protein affect afferent activity in sensory gustatory nerves evoked by some amino acids, which, in turn, influences consumption behavior toward these amino acids. The results are also generally consistent between experiments with hybrid and congenic mice (Table 3) and show that allelic variation of the *Tas1r3* gene affects taste responses to some but not all amino acids.

Interactions of the Proteins from the Taste Receptor, Type 1 Family (T1R) with Amino Acids

Traditionally, T1R receptor-ligand interactions have been characterized in vitro by using heterologous expression experiments in which responses to taste stimuli in cells transfected with T1Rs were analyzed. Our data show that the in vivo approach can also be used to understand the function and specificity of taste receptors and to validate the findings of in vitro studies (66). The in vivo experiments examined the effects of the alleles of genes from the taste receptor, type 1 family (*Tas1r*) on taste responses in mice. Two types of gene variation were studied in vivo: targeted mutations disrupting a gene (67–69) and natural allelic variation (19, 20) (**Table 4**). The premise of analyses of receptor-ligand interactions based on the in vivo data is that if a taste response (either behavioral or neural) to an amino acid is affected by the *Tas1r* genotype, then that amino acid must interact with a taste receptor involving T1R.

When T1R3 is co-expressed in a heterologous system with T1R2, it functions as a broad-spectrum sweet-taste receptor and is activated by sweet-tasting D-amino acids and glycine (Table 4). In contrast, a heterodimer of T1R1 and T1R3 proteins functions as an umami taste receptor in humans and is more broadly tuned in rodents to respond to L-amino acids and glycine. Thus, the T1R3 protein is involved in transduction of both sweet and umami tastes, and correspondingly, a disruption of the *Tas1r3* gene in knockout mice diminishes taste responses to both sweet- and umami-taste stimuli (67, 68). Consistent with the in vitro results, taste receptor, type 1, member 1 gene (*Tas1r1*) knockout mice have impaired taste responses to L-amino acids and umami stimuli, and taste receptor, type 1, member 2 gene (*Tas1r2*) knockout mice have impaired taste responses to sweeteners (including sweet-tasting D-amino acids) (68). A more recent study with a different strain of *Tas1r1* knockout mice confirmed a reduction in responses to umami-tasting compounds in the chorda tympani nerve

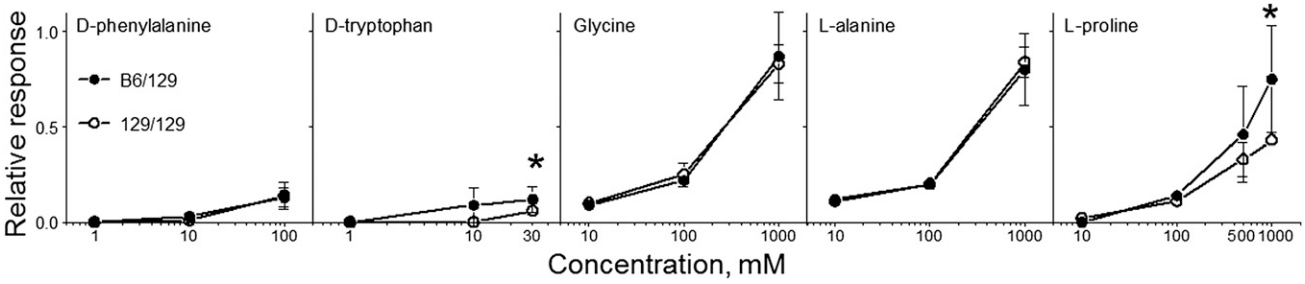

FIGURE 11 Chorda tympani nerve responses to lingual stimulation with amino acids (relative to 100-mM NH_4Cl) in congenic mice with different *Tas1r3* genotypes. Values are medians ± median absolute deviations, $n = 10–14$. *Significant difference between mice with B6/129 and 129/129 *Tas1r3* genotypes at a given concentration, $P < 0.05$ (Mann-Whitney *U* test). B6, genotype of the C57BL/6ByJ inbred mouse strain; *Tas1r3*, taste receptor, type 1, member 3 gene; 129, genotype of the 129P3/J inbred mouse strain. Adapted from reference 20 with permission.

TABLE 4 Amino acid ligands of the T1R receptors[1]

	In vitro										In vivo												
	T1R1+T1R3			T1R2+T1R3							*Tas1r1* KO				*Tas1r2* KO		*Tas1r3* KO					*Tas1r3* polymorphisms[2]	
	Human	Rat	Mouse	Human				Mouse			Behavior[3]	CT			Behavior[3]		Behavior[3]		CT			Behavior[3]	CT
Amino acids	(70)	(70)	(71)	(70)	(72–75)	(76)	Rat (70)	(71)	(72–74)		(68)	(68)	(69)	GL (69)	(68)	CT (68)	(68)	(67)	(68)	(67)	GL (67)	(19, 20)	(19, 20)
D-Alanine			+*			–		+					–	–		+			+				
D-Asparagine			−**					+							+		+						
D-Aspartate	−**																						
D-Glutamate	−**	−**																					
D-Glutamine			−**					+															
D-Histidine			−**			+		+															
D-Isoleucine						+																	
D-Leucine						+																	
D-Phenylalanine			−**			+		+					–	–	+	+	+		+			+	+/−
D-Threonine						–																	
D-Tryptophan	−**		−**	+	+	+	+	+	+				+	–	+	+	+		+	+		+	+
D-Valine						+																	
Glycine	−**		+	+		+	+	+			+		+	–								–	–
L-Alanine			+			–		–			+	+*	+	–			+		+*			–	–
L-Arginine			+								+*						+*						
L-Asparagine			+					–			+*						+*						
L-Aspartate	+*	+*	+*								+*						+*						
L-Cysteine			+																				
L-Glutamate	+	+	+*	–				–			+	+*	+	–			+	+	+*	+	−**	–	–
L-Glutamine	−**		+																			–	–
L-Histidine	−**		+			–																	
L-Isoleucine			+*			–																	
L-Leucine	−**		+*			–																	
L-Lysine	−**		+*																				
L-Methionine			+																				
L-Phenylalanine			+*			–		–															
L-Proline	−**		+*					–					–	–								+	+/−
L-Serine	−**		+					–			+	+*					+		+*				
L-Threonine			+			–		–														–	
L-Tryptophan			−**	–		–	–																
L-Tyrosine	−**																						
L-Valine			+			–																	

[1] References are shown in parentheses. "+" Under "In vitro" indicates a response to a taste stimulus and under "In vivo" indicates an effect on response to a taste stimulus; "−" under "In vitro" indicates a lack of response to a taste stimulus and under "In vivo" indicates a lack of effect on response to a taste stimulus; "*" under "In vitro" indicates a response only when the taste stimulus is applied with IMP but no response without IMP and under "In vivo" indicates an effect on a response to a taste stimulus mixed with IMP. **No response with or without IMP. B6, C57BL/6ByJ inbred mouse strain; CT, chorda tympani nerve response; F_2, hybrids of the second filial generation; GL, glossopharyngeal nerve response; IMP, inosine-5′-monophosphate; KO, knockout; T1R1, taste receptor, type 1, member 1 protein; T1R2, taste receptor, type 1, member 2 protein; T1R3, taste receptor, type 1, member 3 protein; *Tas1r1*, taste receptor, type 1, member 1 gene; *Tas1r2*, taste receptor, type 1, member 2 gene; *Tas1r3*, taste receptor, type 1, member 3 gene; 129, 129P3/J inbred mouse strain.

[2] Effect of *Tas1r3* polymorphisms in B6 × 129 F_2 hybrid and 129.B6-*Tas1r3* congenic mice (see Table 3). The most likely functional *Tas1r3* polymorphism encodes an amino acid substitution of isoleucine to threonine at position 60 (33, 77).

[3] Behavioral responses in 2-bottle choice tests and/or brief-access tests.

(69). Surprisingly, this study also reported that *Tas1r1* knockout mice have reduced chorda tympani nerve responses to sweeteners; the mechanisms responsible for this effect are not clear (69).

The effects of naturally occurring *Tas1r3* polymorphisms in inbred mouse strains (19, 20) in some, but not all, cases were similar to the effects of targeted mutations of the *Tas1r3* gene. *Tas1r3* genotype influenced taste responses to D-phenylalanine, D-tryptophan, and L-proline, but not to glycine, L-alanine, monosodium L-glutamate, L-glutamine, or L-threonine (Tables 3 and 4). For D-phenylalanine and D-tryptophan, effects of the *Tas1r3* allelic variation and gene knockout were consistent: in both cases, *Tas1r3* genotype influenced taste responses to these amino acids. However, the effects of *Tas1r3* genotype on taste responses to L-alanine and L-glutamate were discrepant: whereas targeted mutations of *Tas1r3* affected responses to these amino acids, *Tas1r3* polymorphisms did not affect the responses to them. Taste responses to glycine, L-glutamine, L-proline, and L-threonine were not tested in *Tas1r3* knockout mice; however, because these amino acids activate T1R1 +T1R3 and/or T1R2+T1R3 in vitro, it is likely that *Tas1r3* knockout mice are deficient in taste responsiveness to them.

Why are responses to some amino acids not affected by *Tas1r3* polymorphisms, despite the fact that these amino acids interact with taste receptor(s) involving T1R3? This could be explained by the fact that these amino acids bind to the following: *1*) the T1R3 protein at a site that is not affected by the *Tas1r3* polymorphisms, *2*) a partner protein (T1R1 or T1R2) of the heterodimeric T1R receptor, or *3*) an additional, non-T1R, taste receptor. Several studies suggested that the taste of L-glutamate is transduced not only by the T1R-dependent mechanism but also by alternative transduction mechanisms (69, 78–83). Similarly, T1R-independent mechanisms may be involved in taste transduction of the other amino acids, for which taste responses are not affected by *Tas1r3* allelic variation. This is consistent with residual sweet-taste responsiveness in *Tas1r3* knockout mice (67), which also indicates the presence of T1R-independent taste transduction mechanisms. Such mechanisms may involve glucose transporters and metabolic sensors implicated in sugar taste (84) and an ability of amphiphilic sweeteners to permeate cell membrane and directly interact with intracellular targets (85–87).

Our data show that the in vivo approach can be used to understand the function and specificity of taste receptors and to validate the findings of in vitro studies. Experiments involving the expression of taste receptors in heterologous systems require substantial modification of the conditions that exist in vivo. This includes, for example, modification of receptors to traffic them to the cell membrane, use of variable components of intracellular transduction, and an absence of regulatory influences existing in vivo. Thus, the in vivo and in vitro approaches complement each other by revealing functional characteristics of taste receptors.

Genetic Architecture of Amino Acid Taste and Appetite: Complex Genetics and Multiple Underlying Physiologic Mechanisms

We have shown that B6 and 129 mice differ in behavioral and neural taste responsiveness to several amino acids (Figures 1 and 2, Table 2; summarized in Table 3). The strain differences in responses to D-phenylalanine, D-tryptophan, and L-proline depend on *Tas1r3* polymorphisms. However, *Tas1r3* polymorphisms do not affect responses to glycine, L-alanine, L-glutamate, L-glutamine, or L-threonine. Therefore, strain differences in responses to these other amino acids must depend on genes other than *Tas1r3*. What are these other genes and what are the physiologic mechanisms that mediate their effects on amino acid taste and appetite?

It is unlikely that the other 2 members of the *Tas1r* gene family, *Tas1r1* and *Tas1r2*, are responsible for *Tas1r3*-independent variation in responses to amino acids. All 3 members of the *Tas1r* gene family cluster in distal chromosome 4 (64, 65, 88). The lack of linkages to this region for taste responses to some amino acids (see Figures 5 and 7) shows that none of the known T1R receptors has allelic variants associated with this strain variation.

One of the genetic loci affecting sweet-taste responses is D-phenylalanine aversion locus (*dpa*). This locus affects the ability of mice to generalize a CTA between D-phenylalanine and sucrose, which indicates that *dpa* affects perception of the sweetness of D-phenylalanine. The *dpa* locus also affects responses of sucrose-sensitive fibers of the chorda tympani nerve to D-phenylalanine. The *dpa* locus was mapped to proximal chromosome 4, a region distinct from the subtelomeric chromosome 4 harboring the *Tas1r* genes (89–92). However, it is unlikely that the *dpa* locus mediates differences in taste responses to amino acids between the B6 and 129 strains because we did not detect linkages to the *dpa* chromosomal region in B6 × 129 hybrids (data not shown).

There is evidence that *Tas1r3*-independent strain differences in voluntary consumption of sweet-tasting (glycine, L-alanine, L-glutamine, and L-threonine) and umami-tasting (L-glutamate) amino acids depend on gustatory and postingestive mechanisms, respectively. The fact that the 4 sweet-tasting amino acids all elicit sweet taste but differ in their nutritive properties (see Table 1) suggests that *Tas1r3*-independent genetic variation in sweet taste is a more likely mechanism underlying strain differences than postingestive effects of these amino acids [e.g., see (93)].

In contrast, the difference between B6 and 129 mice in their consumption of concentrated monosodium L-glutamate (Figure 1) is likely mediated by genetic variation in the postingestive effects of L-glutamate. Indeed, B6 and 129 mice have similar peripheral taste responses to L-glutamate salts (Figure 2) and similar perception of the taste quality of monosodium L-glutamate (Figure 4). Together, these findings suggest that gustatory mechanisms are not involved in differential intake of monosodium L-glutamate. In contrast, several lines of evidence indicate that strain-specific postingestive effects of glutamate can affect its intake (14, 40, 42, 44).

Conclusions

We used the mouse as a model organism to study genetics of amino acid taste and appetite. These studies show that the voluntary consumption of amino acids is a trait with complex genetics and multiple underlying physiologic mechanisms. Appetitive responses to some amino acids seem to be determined primarily by their sweet (sucrose-like) taste and are influenced by genetic variation in peripheral taste responsiveness. For some of these sweet-tasting amino acids, genetic differences in taste responsiveness depend on *Tas1r3* polymorphisms; others are *Tas1r3*-independent. Appetitive responses to L-glutamate depend on genetic variation in its postoral rewarding properties.

Because taste responses to some sweet-tasting amino acids are affected by the *Tas1r3* genotype, these amino acids must interact with a taste receptor involving T1R3. These data show the in vivo approach to characterize ligand specificity of the T1R3 taste receptor, which validates the findings of in vitro studies and shows that both the in vivo and in vitro approaches complement each other in the characterization of taste receptors.

Our results indicate that *Tas1r3* is not the only gene underlying strain differences in taste responsiveness to sweet amino acids. The identification of these as yet unknown genes may lead to the discovery of new mechanisms of sweet-taste transduction.

Specific appetites—for example, sodium appetite—are known to be either need-induced or need-free (94, 95). There is evidence that amino acid appetite can also be need-induced or need-free. An example of the need-induced amino acid appetite is a response to deficiency of particular amino acids, which involves learning to reject a deficient diet and to choose a more adequate diet (8). Our studies show that voluntary L-glutamate consumption is an example of a need-free specialized amino acid appetite exhibited by replete animals. Genes responsible for this variation may unveil new mechanisms that regulate appetite and satiety.

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