

# Flavor Preferences Conditioned by Dietary Glutamate<sup>1–3</sup>

Karen Ackroff\* and Anthony Sclafani

Brooklyn College of the City University of New York, Brooklyn, NY

## ABSTRACT

Our understanding of the molecular basis of umami taste and its appetitive qualities has been greatly aided by studies in laboratory rodents. This review describes methods for testing responses to the prototypical umami substance monosodium glutamate (MSG) in rodents. Two techniques, forced exposure to MSG and 2-bottle choice tests with ascending concentrations, were used to evaluate the responses to the taste of umami itself, and 2 other methods used oral or postoral MSG to modify the responses to other flavors. Intake and preference for MSG are enhanced in mice by experience with MSG and with other nutrients with positive postoral effects. In addition, flavor preferences are enhanced in mice and rats by gastric or intestinal MSG infusions via an associative learning process. Even mice with an impaired or absent ability to taste MSG can learn to prefer a flavor added to an MSG solution, supporting the notion that glutamate acts postorally. The more complex flavor of dashi seasoning, which includes umami substances (inosinate, glutamate), is attractive to rodents, but dashi does not condition flavor preferences. Details of the postoral glutamate detection process and the nature of the signal involved in learned preferences are still uncertain but probably involve gastric or intestinal sensors or both and vagal transmission. Some findings suggest that postoral glutamate effects may enhance food preferences in humans, but this requires further study. *Adv Nutr* 2016;7(Suppl):845S–52S.

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## Introduction

Although the existence of umami as a distinct taste was proposed 100 y ago (1), the confirmation of its status has only come about in the past few decades, when umami taste receptors were identified in rodents. Electrophysiologic data

suggested the existence of receptors, several of which have been confirmed (2). Species and strain differences in sensitivity, as well as the study of knockout mice missing elements of umami receptor systems, have contributed to the information from studies in rats and mice that is fundamental to our understanding of umami taste (3).

Rodents continue to provide information on the appetitive qualities of umami. This review describes the methods for measuring unlearned responses to umami as well as conditioning techniques that reveal an important role of postoral nutrient factors in umami appetite. Umami differs from the other basic tastes, which elicit clear appetitive or aversive responses upon first encounter. As detailed in this review, naive mice are typically indifferent to umami over a range of concentrations, but mice experienced with other tastants will consume substantial amounts of umami solutions and display strong preferences. The common denominator for tastants that provide effective experience appears to be the association with positive postoral outcomes. In addition, umami substances have postoral effects that can condition preferences and increase intake of flavors, including the taste of umami itself. This review focuses on findings from our laboratory, supplemented with relevant data from other laboratories.

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\*To whom correspondence should be addressed. E-mail: kackroff@gc.cuny.edu.

## Methods for Testing Oral and Postoral Effects in Rodents

Our laboratory has well-developed procedures to explore the postoral effects of nutrients and has shown that the postoral effects of carbohydrates, fats, and proteins can condition preferences for flavors in rats and mice (4). We applied these methods to evaluate the postoral reinforcing effects of monosodium glutamate (MSG)<sup>4</sup> and the nucleotide inosine monophosphate (IMP). We also tested dashi, a complex flavoring ingredient that includes umami components. Two basic measures are available: acceptance (the absolute intake of the tastant) and preference (the relative intake of the tastant compared with water, expressed as a percentage of total intake in 2-bottle tests). There are 4 basic methods in these studies in rodents; 2 of these measure changes in response to orally presented solutions of MSG and 2 are more explicit evaluations of the changes in response to flavors paired with MSG.

The effect of experience, or exposure to a solution, on preference for it and for subsequently presented MSG is evaluated with a simple technique. The animals are administered a 2-d, 2-bottle test with a solution compared with water and then administered a single bottle (i.e., forced exposure) of that solution for the next 4 d. Finally, a second 2-d, 2-bottle test is conducted. If forced exposure to the solution alters the animal's evaluation of the solution, it will be apparent as a change in preference relative to water in the second choice test.

A more detailed evaluation of responses to MSG (intake, preference) is obtained with a commonly used concentration series, in which ascending concentrations of MSG are presented in 2-d, 2-bottle tests compared with water. The concentrations span a wide range: 0.1, 1, 10, 100, 150, 300, and 450 mmol MSG/L. This method provides a means of comparing mouse strains that vary in avidity for MSG and also reveals the effects of previous exposure to a tastant when compared with the response curves of naive animals.

In Pavlovian flavor-conditioning methods, we used 2 conditioned stimuli (CS): the CS+ flavor associated with MSG and the CS− flavor associated with water. The technique for pairing the stimuli distinguishes the 2 forms of flavor preference conditioning: oral and intragastric. In oral conditioning, animals are trained with a CS− flavor (e.g., grape) in water and a CS+ flavor (e.g., cherry) in an MSG solution. The solutions are presented alternately for 6 d, followed by an 8-d, 2-bottle test with the CS+ and CS− flavors both presented in water, which allows us to evaluate the strength and persistence of MSG reinforcement. This method combines oral and postoral stimulation by MSG because the animal experiences the taste of the MSG as well as its postoral actions. The study of

knockout mice missing components of the taste system allows some dissociation of these factors with the use of the oral method.

The second flavor preference method, intragastric conditioning, explicitly studies the postoral effects of MSG by pairing the ingestion of a flavored solution with the gastric infusion of MSG in rats and mice. The animals are implanted with gastric catheters and placed in infusion cages during conditioning sessions. In our standard postoral conditioning method, the animals' licks on drinking spouts containing the CS flavors are detected by a computerized system that activates infusion pumps to infuse a matched amount of fluid to the gut. The animals thus control the volume of infusion by their drinking behavior. The orally ingested fluid combines in the stomach with the infused MSG so that the net concentration is half that of the infused concentration. One-bottle training sessions alternate between a CS+ flavor paired with an MSG infusion and a CS− flavor paired with a water infusion for a total of 6 or 8 sessions. The standard grape and cherry flavors are sweetened with saccharin in some experiments. Two-bottle preference tests are conducted for 2–6 d with the CS+ and CS− both paired with water infusions or no infusions (extinction test) or with MSG and water infusions, respectively (reinforced test).

## Oral Responses to MSG: Effect of Exposure to Sapid Solutions

In an early study of preference response to MSG solutions in C57BL/6 (B6) mice, Bachmanov et al. (5) reported a robust increase in intake during a concentration series; intake peaked at 300 mmol/L, with a >90% preference in the range of 1–300 mmol MSG/L. We conducted a replication in naive B6 mice (6), which yielded surprising results. MSG intakes were rather low and the strongest preferences, at 100 and 150 mmol/L, were only ~60% and neither differed significantly from indifference. A closer reading of the Bachmanov et al. (5) study revealed that the strong MSG preferences were shown only in B6 mice that had previous experience with MSG or other tastants; naive B6 mice did not prefer 300 mmol MSG/L to water in initial tests. We therefore determined if forced 1-bottle exposure to MSG would enhance MSG preference. After a 4-d exposure to a single bottle of 300 mmol MSG/L, in which intakes were substantial, the mice strongly preferred this concentration to water in a 2-d, 2-bottle test. We therefore administered the same mice a second concentration series. We observed increased acceptance for 10–450-mmol/L concentrations and near-total preferences for MSG over water at concentrations from 10 to 300 mmol/L, replicating the experienced B6 data in the Bachmanov et al. (5) study. To control for the effects of exposure to the first concentration series, a separate group of naive mice was administered 2 concentration series without an intervening forced exposure to MSG and did not show an increased MSG preference or intake in the second series. Together, these data showed that a period of forced exposure to a concentrated solution of MSG could enhance

<sup>4</sup> Abbreviations used: B6, C57BL/6 mouse strain; CS, conditioned stimulus/stimuli; CS+, flavor paired with MSG; CS−, flavor paired with water; IMP, inosine monophosphate; MSG, monosodium glutamate; P2X2/P2X3, P2X purinoceptor 2 and P2X purinoceptor 3; Trpm5, Transient receptor potential cation channel subfamily M member 5; T1r, taste receptor type 1 family.

subsequent preference for low to high MSG concentrations. We interpret this as a postoral effect in which MSG reinforces the preference for its umami flavor. Because these findings indicated that experience associating flavors and postoral effects had a large impact on MSG stimulus strength, we conducted additional experiments to explore this factor.

To test the MSG experience effect in detail, we administered separate groups of naive mice 2-bottle MSG or water tests before and after forced 1-bottle exposure (4 d) to different MSG concentrations [0 (control), 10, 100, or 300 mmol/L]; the mice then received a concentration series with MSG (0.1–450 mmol/L) or water. Mice administered the 10-mmol/L concentration neither preferred MSG nor increased their preference after the 1-bottle exposure. Mice who received the 100-mmol/L concentration showed an initial 70% preference, which was not significantly enhanced by the 1-bottle experience (74% in the second test). The most profound effect was observed in the 300-mmol/L group. These mice initially avoided the 300 mmol MSG/L (26%) but drank large amounts during the 1-bottle exposure and subsequently preferred 300 mmol MSG/L to water (69%). In the subsequent concentration series, only the 300-mmol/L group showed enhanced acceptance (greater intakes than the other groups at 100–450 mmol/L) and preference (stronger preferences than the other groups at 10–450 mmol/L). Thus, the 1-bottle experience with 300 mmol MSG/L had a profound effect on the subsequent preference of B6 mice for a wide range of MSG concentrations.

The primary oral umami and sweet taste receptors are heterodimers of taste receptor type 1 family (T1r) components and share a common element: the umami receptor is T1r1+T1r3 and the sweet receptor is T1r2+T1r3. Because of this commonality, and the previous experience of some of the mice with sweet tastants in the Bachmanov et al. study, we tested the effects of experience with 2 sources of sweet taste, sucrose and nonnutritive sucralose, which is highly preferred by B6 mice (7). Groups of mice were exposed to sucrose or sucralose before the standard MSG concentration series. The sucrose group, but not the sucralose group, showed enhanced MSG intake and preference that were very similar to the 300-mmol MSG/L exposure (6). This outcome was consistent with that of the Bachmanov et al. (5) study and supports the notion that previous experience with sugar solutions can enhance subsequent MSG intake and preference. The ineffective sucralose experience suggests that only nutritive sugars promote positive responses to MSG.

We next tested other nutrients for their ability to enhance subsequent MSG preference. Polycose (Abbott Laboratories), a nonsweet, nutritive carbohydrate, is of interest because, unlike sucrose and MSG, Polycose preference in mice is not dependent on the T1r2+T1r3 taste receptor (8, 9). Fat experience was provided with Intralipid (Baxter), a soybean oil emulsion that is attractive to mice. We also tested the soluble protein casein hydrolysate, which is of interest

because of the hypothesis that glutamate detection is biologically important as a taste representation of protein in food (10). Separate groups of mice were pre-exposed to isocaloric 8% Polycose solution, 3.2% Intralipid, or 8% casein hydrolysate (6). All these nutrients enhanced subsequent MSG preference, although to different degrees. Overall, the largest increases in MSG intake and preference occurred after experience with MSG, followed by sucrose and Polycose, with Intralipid and casein exposure having a lesser effect. These effects were not related to the amount of energy consumed during exposure.

We expanded our examination of experience effects to another umami substance, the nucleotide IMP. Mice were exposed to an initial IMP concentration series (0.1, 0.3, 1, 3, 10, 30, or 100 mmol/L) compared with water (6). Intakes of IMP did not differ from water, and total fluid intakes were not stimulated above those for water alone. The mice were then administered 1-bottle 300 mmol MSG/L over 4 d, followed by 300 mmol MSG/L or water. MSG was preferred (78% of total intake) in the second of these 2-d tests. In a second IMP concentration series, the mice preferred IMP at all but the lowest concentration, and total fluid intake was stimulated at 10–100 mmol/L. The IMP results are comparable to those obtained with MSG and show that naive B6 mice are not naturally attracted to umami compounds but develop a strong preference after experiencing MSG.

We also discovered, after these studies were conducted, that the expression of MSG preference in mice can be affected by a seemingly small methodologic difference. In a study seeking to use MSG as an attractant to induce ethanol intake (11), naive B6 mice were reported to prefer 25–400 mmol MSG/L to water, in contrast with the indifference shown by naive mice in our experiments. We noted several differences from our procedure, including a smaller series of ascending concentrations given for 4 d each and separated by 3-d water-only periods and a reversed light-dark cycle. However, an unreported procedural difference proved to be most critical. In the previous study the MSG and water spouts were separated by 16 cm, whereas the spouts were separated by 3.7 cm in our experiments. We repeated these series variables and tested the effects of reversing the light cycle and presenting the spouts farther apart (6). The greater spout distance yielded initial preferences for MSG in both standard and reversed lighting, whereas spouts presented closer together produced our typical finding of initial indifference to MSG. This finding suggests that mice tend to alternate drinking at 2 spouts when they are close together. Furthermore, the oral mixing of 2 solutions has been observed for various tastants, including MSG (12–14). Presenting the spouts farther apart may discourage oral mixing and lead to greater MSG preference measures. Importantly, this method did not produce any increases in MSG intakes, only in preferences. Tastant-naive Sprague-Dawley rats are also reported to show preferences for MSG solutions over a range of concentrations (2.7–53 mmol/L) in 24-h MSG compared with water tests

(15). Whether this preference is dependent on sipper tube position is not known.

### Flavor Preferences Conditioned by Oral MSG

To extend our study on the effects of orally consumed MSG and to test whether mice would learn preferences for arbitrary flavors associated with MSG, we used 200 mmol MSG/L, which preliminary work indicated was neither preferred nor avoided by naive mice (16).

In addition to B6 mice, we examined 2 other strains of inbred mice, 129 and FVB, and 2 taste knockout strains that were missing umami detection elements. The 129 mice have been compared with B6 mice and have consistently shown lesser avidity for MSG (5, 17–19). The B6 and FVB strains have the “sweet sensitive” allele of the T1r3 receptor element associated with greater attraction to various sweeteners than the “sweet subsensitive” allele found in the 129 strain (20, 21). We also tested 2 taste knockout strains derived from B6 stock. T1r3 knockout mice lack part of the T1r1+T1r3 umami receptor, and transient receptor potential cation channel subfamily M member 5 (Trpm5) knockout mice lack the ion channel that aids transduction in T1r cells. All 5 strains were initially indifferent to 200 mmol MSG/L, and 1-bottle forced exposure had variable effects on subsequent preference. The B6 mice and the knockout strains developed preferences for 200 mmol MSG/L (71–78%), the 129 mice remained indifferent (54%), and the FVB mice avoided MSG (29%).

New groups of all 5 strains were administered 1-bottle flavor training with CS+ and CS– flavors (grape and cherry) added to 200 mmol MSG/L and water (16). The subsequent 2-bottle test with the CS+ and CS– flavors in water was extended to 8 d to measure the persistence of the preference. During training, the B6 mice and knockout strains consumed more CS+MSG than CS– solution, and in testing they showed significant and lasting preferences (64–81%) for the CS+ flavor in water. The 129 and FVB mice drank similar amounts of CS+MSG and CS– in training, and in the flavor test the 129 mice were indifferent and the FVB mice actually preferred the CS– flavor (percentage intake as CS+ was only 22%). The mice were then administered a 2-d, 2-bottle test with plain 200 mmol MSG/L or water. Preferences for unflavored 200 mmol MSG/L were largely consistent with responses to the CS+ flavors: the B6 and knockout strains strongly preferred MSG (78–97%) and the 129 mice did not (64%, NS). Interestingly, the FVB mice did not avoid MSG in this test (47%, NS), unlike those in the exposure study, suggesting that their avoidance was focused on the CS+ flavor rather than on MSG itself. These data showed that strains differed markedly in flavor conditioning based on MSG and that the oral response to the unflavored MSG solution in an exposure method is a good predictor of the conditioning response to added flavors. The conditioned flavor aversion induced by MSG in FVB mice is a novel finding, to our knowledge, and MSG metabolism in this strain requires study. The learned preference for the added CS flavor as well as the experiential enhancement

of MSG intake and preference in B6 and knockout strains show a positive postoral effect of MSG. Although the knockout strains would retain some umami detection via the alternate metabotropic glutamate receptor pathways (22, 23), and thus might have been able to taste MSG, there is additional evidence that oral umami detection is not required for flavor conditioning. Mice that are missing the gustatory nerve P2X purinoceptor 2 and P2X purinoceptor 3 (P2X2/P2X3) receptor, which do not respond to any taste stimuli, were studied in a similar procedure, with grape and cherry flavors added to 150 mmol MSG/L and water (24). These double-knockout mice consumed more flavored MSG than control mice from the start, and both groups showed strong preferences for the MSG-paired flavor. Without oral taste ability, the only way these mice could learn about MSG was via postoral sensing.

### Flavor Preferences Conditioned by Intragastric MSG

After successful oral flavor conditioning with the use of 200 mmol MSG/L, we used the intragastric conditioning method to eliminate the oral contact with MSG in B6 mice and to focus on postoral reinforcement (25). This study tested the influence of CS flavor quality on the postoral MSG-based flavor preference. CS flavors were unsweetened or sweetened with 0.05% saccharin for 2 groups of mice. The mice were administered 6 d of 22-h sessions, with alternating exposure to the CS–, with coinfusion of water, and the CS+, with coinfusion of 400 mmol MSG/L (diluted to 200 mmol/L by the ingested CS+ solution). They were then administered 4 d of reinforced 2-bottle tests, followed by 6 d of 2-bottle tests under extinction conditions (intake of both CS solutions paired with water infusion). The group that received unsweetened flavors consumed more CS+ than CS– during training, and this difference increased over days, indicating a positive effect of the infused MSG. Sweet taste generated greater CS intakes in training so that there was less difference between CS+ and CS– intakes overall. In reinforced tests, both groups preferred the CS+, and that percentage of preference was somewhat greater with sweet CS flavors (70–72%) than with unsweetened flavors (59–64%). In extinction tests the mice consumed more CS+ than CS–, but this was significant only in the first 2-d block; therefore, the preference weakened at the same rate in the sweet and unsweetened groups. After this test period, the mice received exposure testing with 200 mmol MSG/L. The sweet group preferred MSG at the outset (71%), whereas the unsweetened group was indifferent, consistent with the known effects of previous exposure to sweet taste associated with positive postoral effects. Preferences increased to 89% and 77%, respectively, after the 1-bottle experience with 200 mmol MSG/L. This experiment showed that sweet taste enhanced training intakes and initial preference for the MSG-paired CS+. However, the CS flavor quality had no effect on the rapid extinction of the flavor preference, which contrasts with the persistent preferences seen in oral conditioning.

When we began our investigations of umami flavor conditioning in rodents, Uematsu and coworkers (26, 27) and Tsurugizawa and Torii (28) were also studying this issue and were focused on discovering the brain areas active during postoral MSG stimulation and flavor preference learning in rats. Because we had established methods to study peripheral mechanisms in rats, we conducted several experiments using rats in modified versions of the mouse protocol.

In the Uematsu et al. (26) conditioning experiment, water-restricted rats were trained with CS+ flavored water paired with intragastric infusions of 60 mmol MSG/L and CS- flavored water paired with intragastric water during eight 30-min training trials. In the subsequent 2-bottle test without infusions the rats showed a 70% preference for CS+. We first conducted studies that varied the training variables of this procedure (29). We replicated the Uematsu et al. procedure in a group of water-restricted rats. A similar procedure was used in a second group of rats, except that they were food-restricted and trained with flavored solutions sweetened with saccharin. During training, CS intakes increased, and the CS+ intake exceeded CS- intake in sessions 5–8. The training intakes of the water-restricted and food-restricted groups did not differ. Both groups showed a significant CS+ preference in the 2-bottle test without infusions, with a nonsignificantly greater preference in the water-restricted rats (78% compared with 68%). The training and test results closely matched those reported in the Uematsu et al. (26) study.

The rats were then given a second training-test cycle to determine if their CS+ preference would improve with additional experience. After training, the water-restricted and food-restricted rats again preferred the CS+ (70% and 64%, respectively), but they differed in the final three 2-d tests. The food-restricted group continued to show a significant CS+ preference (66–76%), but the water-restricted group's CS+ preference weakened (56–61%). Thus, although food restriction training tended to produce a weaker preference initially, the preference was more resistant to extinction than that observed in water-restricted rats. This is not too surprising because both CS+ and CS- solutions would rehydrate the water-restricted rats. We also determined if higher MSG concentrations (120 or 240 mmol/L) would increase the magnitude of the preference, but this proved not to be the case (29).

These experiments replicate and extend the original report of flavor conditioning by intragastric MSG infusions in water-restricted rats (26). The methods introduced several differences between the rat and mouse intragastric studies. Sprague-Dawley rats were trained and tested with food or water restriction in 30-min sessions, in contrast to the mouse studies that used nonrestricted mice in 24-h/d sessions. Furthermore, the rats were infused with 60 mmol MSG/L. We designed a mouse study to parallel the rat procedure using water-restricted B6 mice trained and tested in 30-min sessions with unsweetened CS+ flavor paired with a 60-mmol MSG/L infusion. The mice showed no CS+

preference (51%) in 2-bottle tests without infusions. In a second cycle with new flavors, we infused 300 mmol MSG/L, but the mice still showed no preference (49%) in the 2-bottle test. Potential reasons for the lack of response in mice include the water restriction, which may have made the mice indifferent to fluid source in testing, and the short sessions, in which they may not have self-infused enough MSG for postoral effects. We did not test other mouse strains, but given the variability in strain responses to MSG, it is possible that other strains might acquire flavor preferences with lower MSG concentrations, similar to rats.

The rat conditioning studies (26, 29) did not identify the source of the reinforcing signal from MSG: it could be from the stomach or it might be intestinal, as we found for sugar conditioning (30). Neither energy nor sodium explains the results, because 60 mmol glucose or NaCl/L was ineffective (26). Vagal responses to gut MSG have been obtained from both gastric and intestinal sites (10, 31–33). To test the idea that MSG detection might be beyond the stomach, rats were infused intraduodenally, bypassing the stomach (29). The CS+ and CS- training intakes did not differ, but average CS intakes increased during training. The rats consumed more CS+ than CS- (63% CS+ preference) in the 2-bottle extinction test. In a second training-test cycle, CS+ preferences remained low but persistent (60–62%). Although these preferences were somewhat weaker than those of gastrically infused animals, they indicate that the intestinal detection of MSG is adequate for flavor preference conditioning.

Uematsu et al. (27) showed that rats that had a total abdominal vagotomy did not learn to prefer a flavor paired with intragastric MSG. Consistent with this observation, vagotomy also altered the neural response to intragastric MSG in the amygdala and lateral hypothalamus, which was taken as evidence that it blocked the coding of positive postingestive actions of glutamate (34). This contrasts with our findings that vagal surgical or chemical denervation does not block postoral carbohydrate or fat conditioning (35), which, in turn, is consistent with the lack of change in forebrain response to glucose after vagotomy (36). A noteworthy difference between umami and the other postorally reinforcing stimuli is that umami is effective at much lower concentrations in the 30-min sessions that are common in rat studies. Successful glucose conditioning has used 444-mmol/L (8%) infusions, 7 times the effective concentration of 60 mmol MSG/L (27, 30).

Unlike orosensory nerve responses, the gastric vagal response to MSG is not enhanced by mononucleotides (37). Although these data are not strongly supportive of postoral umami synergy, the target brain areas responsive to intragastric MSG and nucleotides suggest that synergy could occur (38). We tested for possible enhancement of flavor conditioning by the addition of IMP to the MSG infusate (29). Food-restricted rats were trained and tested as in previous intragastric experiments. One group was infused with 60 mmol MSG/L + 2 mmol IMP/L and the other with 60 mmol MSG/L alone. The logic was that if flavor conditioning by MSG can be

enhanced synergistically, the MSG+IMP infusate should condition a stronger flavor preference. However, the groups did not differ significantly in their preferences for the CS+ solution during testing (58–63% for MSG, 59–70% for MSG+IMP). The single IMP concentration tested may not have adequately stimulated postoral receptors. The numerically greater preferences in the MSG+IMP group suggest that other concentrations should be evaluated.

### Flavor Conditioning by Dashi

We also investigated the oral and postoral basis for the preference for dashi, a fish stock with umami and other flavor components. Rats generalize the complex flavor of dashi to MSG as well as to sweet, sour, bitter, and salty primary tastes, and to some amino acids (39). Our interest in dashi was stimulated by reports (40, 41) that rats and mice prefer dashi stock to water and that exposure enhanced this preference, which suggests the involvement of postoral influences on dashi preference.

A solution of dried-bonito dashi was preferred by rodents at concentrations of 25–50% (of the commercial stock solution) (41) and the degree of preference increased with experience. We tested B6 mice in the oral conditioning procedure (42), adding grape and cherry flavors to 50% dashi and water. Training intakes of CS+dashi exceeded those of CS–water, but the 2-d CS+ compared with the CS– test with flavored water showed no preference (55%). The same mice preferred unflavored dashi to water (74%) and the CS+dashi to CS– (79%). The finding that the mice preferred dashi but not a CS+ mixed into dashi indicates that the flavor of dashi is not an effective unconditioned stimulus to support CS+ preference conditioning.

We studied umami taste-impaired mice to test the possibility that oral dashi taste interfered with the acquisition of a preference for added flavor (42). *Trpm5* knockout mice, which are impaired in umami detection (but can taste other dashi components that are salty and sour), unlike B6 wild-type mice, did not prefer 50% dashi to water before or after the 1-bottle forced exposure to the dashi. New *Trpm5* knockout mice were tested to see if dashi could serve as a CS flavor in oral conditioning. The mice were trained to drink dashi containing 8% glucose, which has a postoral conditioning action in mice. The knockout mice consumed substantially more dashi+glucose than water during 1-bottle training and then strongly preferred (95%) dashi to water in a 2-bottle test. Thus, dashi exposure alone does not condition a dashi preference in *Trpm5* knockout mice, but the knockout mice develop a strong dashi preference on the basis of its association with the postoral actions of glucose.

We also tested the reinforcing effect of postoral dashi without oral dashi contact using intragastric conditioning (42). B6 mice were given saccharin-sweetened CS+ and CS– flavors paired with intragastric infusions of 100% dashi and water, respectively. In the 2-bottle CS+ compared with the CS– test the mice were indifferent to the CS+ flavor. Yet, in a subsequent oral test, the same mice preferred 50% dashi to water (79%). This intragastric study indicated that

the postoral actions of dashi do not reinforce a flavor preference, which is consistent with our oral conditioning results in B6 mice and with the lack of place preference conditioning with dashi in ICR mice reported by other investigators (43).

Thus, unlike MSG, dashi has an inherently attractive flavor to mice but does not have postoral reinforcing effects. This unusual combination may result from its complex mixture of umami and other compounds.

### Flavor Conditioning by Glutamate in Humans

Some findings suggest that postoral factors may contribute to the preferences shown by humans for umami-tasting foods. Humans offered 2 soups, one with and one without added 0.5% MSG, reported an increased liking for the MSG soup but only if they consumed the soups during training trials (44). If the soup was only tasted and not consumed, then a conditioned liking response was not obtained, suggesting the possibility that postgestional factors were “primarily responsible for the enhanced liking” of the MSG soup (44). The findings are not conclusive, however, because the subjects who consumed the soup also had more extensive orosensory exposure than the subjects who only tasted the soup. In another study, a group who repeatedly consumed 0.5% MSG soup increased both their liking and intake of the soup, whereas a group given the same soup without added MSG did not alter intake (45). A recent study found an increased intake after training with a soup with 5% MSG, although liking for the soup was not improved, perhaps due to its very salty taste (46). In all 3 studies, the soup provided in the pre- and posttests did not contain MSG, so the changes in liking and intake cannot be attributed to direct effects of MSG in the test. Together, these studies suggest that postoral MSG could contribute to the enhancement of preferences for MSG-containing foods by humans. Testing for purely postoral effects in humans has thus far been elusive, but established methods can examine this issue effectively in animals.

The existence of entirely postoral positive effects of MSG shows that umami enhancement of the appetite for foods is not limited to oral stimulation. It is possible to study postoral effects without oral stimulation in humans. One approach is to train subjects with different soups paired with orally consumed but untasted MSG capsules and control capsules (47). This same procedure was recently proposed (48) to study the potential postoral satiating actions of MSG in humans (49).

### Conclusions

The study of rats and mice, which detect umami substances with receptors analogous to those of humans, allows us to explore the mechanisms of umami appetite. The existence of species differences in the range of effective concentrations reflects a pattern seen with other materials (e.g., ethanol), and the existence of within-species differences shows the importance of genetic variation (a situation likely reflected in human differences in the appreciation of umami). The oral studies show the enormous influence of previous experience with flavored

solutions on intake of and preferences for MSG, which can convert indifference to preference in umami-naive mice.

A recent review noted that, even when concentrated, umami is not a “profound taste” and concluded that “umami harmonizes other tastes in foods and brings about mildness and deliciousness” (2). The appetitive responses of animals indicate that some of umami’s positive effects occur beyond the mouth, reinforcing further intake and preference for foods that include umami among their flavors.

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