Flavor Preferences Conditioned by Oral Monosodium Glutamate in Mice

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Abstract

The prototypic umami substance monosodium glutamate (MSG) reinforces preferences for its own flavor, as well as preferences for flavors associated with it, by conditioning processes. Mice of 3 inbred strains (C57BL/6J (B6), 129P3/J, and FVB/NJ) and 2 taste-knockout (KO) groups derived from the B6 lineage were initially indifferent to 200 mM MSG, but this evaluation was altered by forced exposure to MSG. B6 and KO mice acquired an MSG preference, 129 mice remained indifferent, and FVB mice avoided MSG. The shifts in preference imply a postoral basis for MSG effects, suggesting that it could produce preferences for associated flavors. New mice were trained with a conditioned stimulus (CS+) flavor mixed in 200 mM MSG and a CS− flavor in water. Similar to the parent B6 strain, mice missing the T1r3 element of an umami receptor or the downstream signaling component Trpm5 learned to prefer the CS+ flavor and subsequently showed similar preferences for MSG in an ascending concentration series. Consistent with their responses to forced exposure, the 129 strain did not acquire a significant CS+ preference, and the FVB strain avoided the CS+ flavor. The 129 and FVB strains showed little attraction in the ascending MSG concentration series. Together, these data indicate that the postoral effects of MSG can modulate responses to its own and MSG-paired flavors. The basis for strain differences in the responses to MSG is not certain, but the taste-signaling elements T1r3 and Trpm5, which are also present in the gut, are not required for mediation of this flavor learning.

Key words: 129, C57BL/6, FVB, MSG, T1r3, Trpm5

Introduction

Considerable attention has been focused recently on the nature of the attractive qualities of dietary glutamate. Umami, the savory taste associated with monosodium glutamate (MSG) and some nucleotides, is a primary taste quality with multiple sensors in both oral and postoral loci (Chaudhari et al. 2009; Iwatsuki et al. 2011). MSG and foods naturally rich in glutamate have been used extensively in human cuisine to enhance the flavor of food. Increasing evidence indicates that MSG also has a postoral reward component. When naive C57BL/6J (B6) mice are first exposed to an ascending series of MSG concentrations, their intakes remain low and they do not express preferences for MSG relative to water. However, B6 mice having previous experience with MSG solutions or a variety of other tastant solutions show increased intake and preference across a range of MSG concentrations (Bachmanov et al. 2000; Ackroff et al. 2012).

The enhancement of MSG preference with experience suggests a flavor-conditioning process based on the postoral effects of MSG: its flavor becomes more attractive due to its association with rewarding effects detected in the gut (Ackroff et al. 2012; Sclafani and Ackroff 2012). If this is so, it should be possible to train a preference for a flavor paired with MSG intake, an effect that has been called flavor-nutrient conditioning. This is a Pavlovian conditioning process, in which a conditioned stimulus (CS) is paired with nutrient stimulation. One common technique involves adding a CS+ flavor to an orally consumed solution of the putatively rewarding substance, and alternately adding another flavor, the CS−, to a control solution such as water. Preference for the CS+ over the CS− in 2-bottle choice indicates a reinforcing effect of the nutrient, and this test has also been used to examine differences between nutrients such as different sugars (Sclafani and Ackroff 1994). This procedure also permits the additional action of flavor–taste learning, which is based on the attractive taste of the nutrient, and several methods exist to minimize or eliminate it to isolate the postoral effects. One is to use animals...
that cannot taste the nutrient, as was done with P2X2/P2X3 double-knockout (KO) mice, which lack adenosine triphosphate (ATP) receptors on gustatory nerves and are thus unable to detect signals from taste cells (Finger et al. 2005). After training with distinctive flavors (with an odor component that served as the cue) paired with MSG and water, both the P2X2/P2X3 KO mice and the wild-type (WT) controls preferred the CS+ flavor, indicating that oral detection of MSG was not essential for learning a flavor preference (Stratford and Finger 2011). Another method uses intragastric (IG) infusion of the MSG solution or water as the animal drinks the CS+ and CS− flavor solutions, respectively. A preference for the CS+ after such training indicates that the reinforcing effect occurs postorally, and this effect has been demonstrated in rats (Uematsu et al. 2009; Uematsu et al. 2010; Ackroff and Sclafani 2011) and mice (Ackroff and Sclafani 2013). Studies in humans (Prescott 2004; Yeomans et al. 2008) also support the notion that postoral MSG experience is important in learned preferences for MSG-associated flavors.

The present study used the oral conditioning method to characterize the rewarding effects of MSG by studying mice with different genetic backgrounds. The B6 strain used in our initial study (Ackroff et al. 2012) is more attracted to MSG than the 129 strain (Bachmanov et al. 2000), whereas the FVB mouse avoids concentrated MSG solutions (unpublished data). These 3 inbred strains were compared in Experiment 1. A second experiment compared the responses of the parent B6 strain to those of KO mice missing T1r3 (part of an umami receptor) or Trpm5 (part of a signaling cascade within taste cells). If the reward for flavor learning is mediated by these elements, the T1r3 KO and Trpm5 KO strains should be impaired. Both signaling proteins are also found in the gut, where they mediate detection of amino acids, including glutamate (Bezençon et al. 2007; Daly et al. 2013).

In the first part of each experiment, we established the response of naïve mice to 200 mM MSG, seeking a concentration that was neither preferred nor avoided on initial exposure, so that changes in preference could be assessed after additional experience. In the second part of each experiment, we determined whether the mice would acquire a preference for a CS+ flavor added to a 200 mM MSG solution and, afterward, evaluated their preference for a range of MSG solutions without an added flavor. New mice of each strain were given 200 mM MSG as the unconditioned stimulus (US) in flavor conditioning, by mixing a CS+ flavor into the MSG solution and a CS− flavor in water. To the extent that the postoral effects of the MSG are rewarding, a preference for the CS+ flavor will develop. This process parallels the development of preference for the flavor of MSG itself, which is attributable to the postoral effects of concentrated MSG.

**Experiment 1**

Our previous report on the effects of MSG experience on preference examined B6 mice. Bachmanov et al. have conducted several comparisons of B6 mice with the 129 strain, which is less attracted to MSG (Bachmanov et al. 2000; Inoue et al. 2004a; Ji and Bachmanov 2007; Murata et al. 2009). These strains also differ in response to sweet taste and sugars, which has been attributed to different alleles of the T1r3 component of the T1r2+T1r3 sweet receptor. The B6 has the “sweet sensitive” allele associated with greater attraction to sweet taste than the “sweet subsensitive” allele found in the 129 strain (Max et al. 2001; Nelson et al. 2001). T1r3 also combines with T1r1 to form one of the umami receptors, found both orally and postorally. Analysis of hybrids of B6 and 129 strains indicated that variation in T1r3 does not alter the response to umami substances (Shigemura et al. 2009), but the mice were tested after experience with sweet substances, including sucrose, which has been shown to alter behavioral responses to MSG (Ackroff et al. 2012). Other glutamate taste receptors, such as the metabotropic glutamate receptors mGluR1 and mGluR4, also exist in mice (Nakashima et al. 2012; Yasumatsu et al. 2012), but their variations among mouse strains have not been characterized.

Another strain of interest is FVB, which has a variant of the “sweet sensitive” T1r3, different from that of the B6 mouse (Max et al. 2001; Nelson et al. 2001). This strain is even more attracted to sugars and noncaloric sweeteners than are B6 mice (Pothion et al. 2004; Reed et al. 2004; Glendinning et al. 2005b, 2010; Blednov et al. 2010). The response of FVB mice to MSG has not been reported, but preliminary findings indicated that FVB mice are indifferent to MSG at low concentrations and avoid it at high concentrations (unpublished data). To examine this apparent negative response to MSG in more detail, we included FVB mice in the present study. Persistent avoidance of MSG in this strain could be useful in establishing the basis for MSG responsiveness because it may be found to differ during the postoral handling of umami substances.

**Experiment 1A: response to oral MSG in naïve mice**

Our previous study of oral responses to MSG (Ackroff et al. 2012) showed that naïve B6 mice that initially avoided 300 mM MSG could be trained to prefer it by forced exposure, providing MSG solution as the sole source of fluid. This suggested that the MSG flavor was not initially attractive, but association with postoral MSG effects converted the flavor to a preferred one. This effect was dependent on MSG concentration: mice given 10 mM MSG were indifferent before and after training, and mice given 100 mM preferred it from the outset. However, the latter animals did not show as strong a preference as the 300 mM group in subsequent tests with a range of MSG concentrations vs. water. This indicated that a relatively concentrated solution, which provided a stronger postoral stimulus, might be important in the development of the preference. However, we sought an initially neutral concentration for conditioning. In another experiment in the same report, we found peak preference for
MSG vs. water in experienced mice at 200 mM. These mice were given flavor preference conditioning with the 200 mM concentration and they developed strong preferences for the MSG-paired flavor (unpublished data). One goal of the present studies was to establish that this concentration would condition preferences in naïve mice.

Experiment 1A was conducted (i) to establish that 200 mM MSG was initially not preferred when orally consumed in a test vs. water and (ii) to measure its preference following forced exposure. The goal was to determine whether this concentration was suitable as a flavor-conditioning agent for subsequent work. By using a concentration that was neither attractive nor avoided by the mice, we could attribute shifts in preference to the postoral effects of MSG.

**Materials and methods**

**Animals.**

Male C57BL/6J (B6) (N = 7), 129P3/J (N = 8), and FVB/NJ (N = 7) mice were derived from individuals obtained from Jackson Laboratories. The mice were singly housed in plastic tub cages with ad libitum access to chow (5001, PMI Nutrition) and deionized water in a room maintained at 22 degrees C with a 12:12 light–dark cycle (lights on at 0900h). Experimental protocols were approved by the institutional animal care and use committee at Brooklyn College and were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health.

**Apparatus.**

Solutions were prepared daily using monosodium glutamate (Sigma Chemical) and deionized water (hereafter, water). The tests were conducted in the animal's home cage. Fluid was available through sipper spouts attached to 50-ml plastic tubes that were placed on top of the cage. The sipper spouts were inserted through holes positioned 3.7 cm apart in a stainless steel plate positioned to the right of the food bin, and the drinking tubes were fixed in place with clips. Fluid intakes were measured to the nearest 0.1 g by weighing the drinking bottles on an electronic balance interfaced to a laptop computer. Daily fluid spillage was estimated by recording the change in weight of 2 bottles that were placed on an empty cage, and intake measures were corrected by this amount.

**Procedure.**

For 2 days, the animals were given 2 bottles of water. For the next 2 days, they were given a preference test with 200 mM MSG and water, with the positions of the bottles reversed from the first to the second day. This was followed immediately by 4 days of 1-bottle access to 200 mM MSG, with the left–right position alternated daily. For the next 4 days, the preference test was repeated, with choice between 200 mM MSG and water. Fluid intakes were averaged as 2-day blocks and evaluated with analysis of variance (comparing groups in 1-bottle tests and Group × Fluid for 2-bottle tests). Percentage MSG intakes were calculated as MSG intake divided by total fluid intake × 100.

**Results and discussion**

The data are shown in Figure 1. In the initial 2-bottle test, intakes of MSG and water did not differ. Overall, intakes were lower in the 129 mice than in the B6 and FVB strains: F(2,19) = 9.8, P < 0.01. Percentage MSG intakes also did not significantly differ among groups: 52%, 44%, and 55% intakes of 200 mM MSG by the B6, 129, and FVB groups. During the 1-bottle period, average daily intakes of the B6 mice exceeded those of the 129 and FVB mice: F(2,19) = 11.2, P < 0.001. Comparison of the 1-bottle MSG intakes to the water baseline found a Group × Test interaction (F(2,19) = 12.4, P < 0.001). Simple main effects indicated that the strains did not differ in baseline water intake (3.6, 3.6, and 5.1 g/day for the B6, 129, and FVB mice) and that only the B6 mice consumed more MSG than water (F(1,19) = 49.2, P < 0.0001).

In the preference test that followed the 1-bottle MSG exposure, B6 mice drank more fluid than the other strains (F(2,19) = 5.7, P < 0.05), and the Group × Fluid interaction (F(2,19) = 7.5, P < 0.01) reflected greater intake of MSG than water by the B6 group. The 78% MSG intake of the B6 mice exceeded the 54% and 29% intakes of the 129 and FVB groups: F(2,19) = 15.2, P = 0.0001. Comparison of the initial and final percentage intakes confirmed that the B6 percentage intakes exceeded those of the other strains: F(2,19) = 9.8, P < 0.01. The Group × Test interaction (F(2,19) = 15.4, P < 0.0001) and simple main effects showed that B6 mice increased their percentage MSG intake, FVB mice reduced theirs, and the 129 mice did not change.

This experiment established that all 3 strains were initially indifferent to 200 mM MSG. In 1-bottle exposure, the B6 mice consumed more MSG than the other strains, consistent with previous work showing greater avidity than the 129 mice (Bachmanov et al. 2000). In addition, only the B6 strain subsequently preferred MSG to water. The 129 mice remained indifferent to 200 mM MSG, which is consistent with a previous report of 129 strain’s indifference to 200 mM MSG in an ascending concentration series (Ruiz et al. 2003). In contrast, the FVB mice drank more water than MSG after forced exposure, which is a novel finding.

**Experiment 1B: oral flavor preference conditioning in B6, 129, and FVB mice**

Experiment 1B next sought to extend the comparisons among the 3 strains to the acquisition of a preference for a flavor added to MSG. In an MSG-experienced group reported previously (Ackroff et al. 2012), we conducted pilot oral flavor
conditioning with flavors mixed in 200 mM MSG, a concentration that had been avidly consumed and preferred. These mice showed sustained preferences (71–79%) across four 2-day test blocks. However, these results may have depended on the animals’ substantial previous experience with MSG. To determine whether previous exposure to MSG was important to the flavor-learning process, Experiment 2 used the same flavor-training procedure in MSG-naïve mice. The general procedure was similar to that of Stratford and Finger (2011), with alternating 1-bottle training days followed by 2-bottle tests between the CS+ (without MSG) and CS− flavors. This nonreinforced testing was conducted for 8 days to test the persistence of preference for the CS+ solution. The simple prediction was that a strain’s response to the added flavor should be in the same direction as its response to MSG in Experiment 1A.

Following the flavor-conditioning tests, the mice were given a test with 200 mM MSG vs. water to determine whether their experience with flavored MSG altered their preference for unflavored MSG. This was followed by 2-bottle tests with a range of ascending MSG concentrations vs. water. Previous work with B6 mice suggested that they would prefer a range of concentrations more strongly than the 129 mice (Bachmanov et al. 2000; Ackroff et al. 2012). The response of the FVB mice was less certain, though their avoidance of MSG following forced exposure in Experiment 1A suggested that they were more likely to avoid MSG than the other strains.

Materials and methods

Animals.

Male B6 (N = 9), 129 (N = 9), and FVB (N = 10) mice were derived from mice obtained from Jackson Laboratories. The mice were 8–11 weeks old (B6: 58–60 days; 129: 55–69 days; FVB: 66–75 days) at the start of CS training. Housing and data collection were the same as in Experiment 1A.

Test solutions.

The concentration of the MSG solution was 200 mM, to which 0.05% w/w cherry or grape Kool-Aid (unsweetened mix; Kraft Foods) was added to make the CS+MSG training solution. The alternate CS− solution was the other Kool-Aid flavor in water; half the mice had grape as the CS+ and cherry as the CS−; the flavors were reversed for the remaining animals. For the tests, both the CS+ and CS− flavors were presented in water.

Procedure.

In the first week, the mice were given access to 2 bottles of water. This was followed by 6 days of alternating 1-bottle exposures to CS− (days 1, 3, and 5) and CS+MSG (days 2, Figure 1  Mean (±SEM) intakes of 200 mM MSG and water in Experiment 1A. Intakes are shown for B6 (top), 129 (center), and FVB (bottom) strains of mice during a 2-day initial 2-bottle preference test (Pre), 4 days of 1-bottle MSG, and a 4-day posttraining 2-bottle preference test (Post). Percentage intakes of MSG are shown atop the columns for the 2-bottle tests. Asterisk indicates significant (P < 0.05) difference between MSG and water intakes.
After 1 day of water intake, the mice were given 8 days of 2-bottle tests with the CS− and CS+ flavors offered in water. After another day of water, the mice were given a 2-day 2-bottle test with 200 mM MSG vs. water.

At the end of this phase, the mice were given 2 bottles of water for 2 days. Then they received an ascending series of 2-day 2-bottle tests with MSG vs. water as in a previous study (Ackroff et al. 2012). The MSG concentrations were 0.1, 1, 10, 100, 150, 300, and 450 mM. Solutions were available 23 h/day, and the bottles were weighed and refilled during the remaining hour. Throughout testing, the left–right positions of the MSG and water bottles were alternated from the first to the second day of each test to control for side preferences.

Data analysis.

Training intakes were averaged across days and evaluated with analysis of variance (Group × CS (CS+ vs. CS−)). Fluid intakes were averaged as 2-day blocks for the CS+ vs. CS− tests (Tests 1–4) and the MSG vs. water test. Preferences were also expressed as percentage intakes (CS+ or MSG solution intake/total intake × 100). The CS intakes were evaluated with analysis of variance (Group × Test × CS). Significant interaction effects were evaluated using simple main effects tests. Percentages of CS+ intakes were compared with analysis of variance (Group × Test). The significance of the CS+ preference in each test block was evaluated within each group by comparing CS+ and CS− intake using paired t-tests with Bonferroni correction for multiple comparisons. With correction for 4 test blocks, differences were significant when $P < 0.0125$.

The absolute and percentage intakes of MSG solution were each evaluated with 2-way analysis of variance (Group × Concentration). Significant interaction effects were evaluated using simple main effects tests. The significance of the solution preference at each concentration was evaluated by comparing MSG intake and water intake using paired t-tests with Bonferroni correction for multiple comparisons. With correction for 7 concentrations, differences were significant when $P < 0.0071$.

Results and discussion

The training intakes are shown in Figure 2. Overall, the mice consumed more CS+MSG than CS− solution: $F(1,25) = 35.2$, $P < 0.0001$. The B6 mice consumed more of the solutions than did the 129 and FVB mice: $F(2,25) = 9.5$, $P < 0.001$. The Group × CS interaction ($F(2,25) = 11.1$, $P < 0.01$) showed that B6 and 129, but not FVB, mice consumed more CS+MSG than CS− and that the strains differed in CS+MSG but not in CS− intakes.

The 2-bottle test intakes of the CS flavors presented in water are shown in Figure 2. The main effect of Test reflected greater intake in Test 1 than in Tests 2–4 ($F(3,75) = 10.7$, $P < 0.001$).
Percentage MSG intakes of the B6 mice exceeded those of the other strains at 100, 300, and 450 mM. Percentage MSG intakes of the 129 mice exceeded those of the FVB mice at 300 and 450 mM. The B6 mice had the highest percentage intakes at 100–300 mM MSG, lower intakes at 10 and 450 mM, and lowest intakes at 0.1 and 1 mM. The percentage MSG intake of the 129 mice was significantly lower at 450 mM than that at 100–300 mM. The FVB mice had the lowest percentage MSG intake at 450 mM, followed by that at 300 mM, and their highest percentage intakes were at 10–150 mM.

The 3 strains showed clear differences in their responses to MSG exposure and flavor preference training, ranging from...
preference to indifference to avoidance. The implications of these differences are examined further in the General Discussion. The B6 strain treated MSG quite positively, which allowed us to examine the contributions of components of the umami taste system in Experiment 2 using B6-derived taste-KO mice.

Experiment 2

Experiment 1 showed that B6 mice, though initially indifferent to 200 mM MSG, began to prefer it and also learned to prefer added flavors. To probe the basis of these learned preferences, we studied mice that lack components of the umami taste system that are normally present in both oral and postoral loci. In addition to the parent B6 strain, we studied KO mice lacking Tas1r3 (which codes for part of an umami sensor) or Trpm5 (which codes for a Ca2+-activated cation channel that is part of a signaling cascade within taste cells). The T1r1+T1r3 receptor is a major umami detector, with a signaling pathway mediated by Trpm5; so, if the reward for flavor learning is mediated by this receptor, both KOs should be impaired. Both signaling proteins are also found in the gut, where they may mediate amino acid detection (Bezençon et al. 2007). T1r3 KO mice do not respond to MSG in brief licking tests (Zhao et al. 2003), but KO mice generated by another laboratory behaved like WT mice in 24-h intake tests (Damak et al. 2003). These differences may be due to the nature of the test or the variation in the KO methodology. Trpm5 KO mice do not respond to MSG in brief taste tests and when given low concentrations in 24-h tests, but they preferred MSG at 100–300 mM and avoided it at 1 M in 24-h tests, presumably due to postoral effects (Damak et al. 2003, 2006). The residual detection of MSG by Trpm5 KO mice reflects Trpm5-independent detection by mGluR receptors (Jotaki et al. 2009; Yasumatsu et al. 2012). Although neither T1r3 nor Trpm5 is essential to learning about the postoral effects of sugars (Sclafani and Ackroff 2012), little is known about the contribution of these signaling elements in the gut to postoral learning about MSG.

Experiment 2A: response to oral MSG in naïve mice

This experiment was conducted using the same procedure as Experiment 1A, with the same goals: to establish the initial responses to 200 mM MSG in a test vs. water and then to retest the preference following forced exposure to MSG. Mice of the 2 KO strains were compared with the B6 group of Experiment 1A.

Procedure

Male mice were born in the laboratory in separate colony rooms. Trpm5 KO mice (N=8) were derived from individuals developed on a B6 background (Damak et al. 2006). The targeted mutation for the Trpm5 KO is on the iso-genic B6 background, generated in C57BL6 embryonic stem (Bruce 4) cells (Damak et al. 2006). T1r3 KO mice (N=8) were derived from individuals that were produced by homologous recombination in C57BL/6j embryonic stem cells and maintained on this background (Damak et al. 2003). The mice were 10 weeks old at the start of training. Details of housing and procedure were the same as in Experiment 1A.

Results and discussion

The data are shown in Figure 4. In the initial 2-bottle test, intakes of MSG and water did not differ, and this was true for all groups of mice. Preferences for MSG also did not differ among groups: 52%, 47%, and 44% of total intakes as 200 mM MSG by the B6, T1r3 KO, and Trpm5 KO mice. During the 1-bottle period, average daily intakes (11.4, 8.5, and 11.1 g/day) did not differ significantly in the 3 groups of mice. Comparison of the 1-bottle MSG intakes to the water baseline found only a Test effect (F(1,20) = 55.1, P < 0.001), indicating that the groups did not differ in baseline water intake (3.6, 4.6, and 5.3 g/day for the B6, T1R3 KO, and Trpm5 KO groups).

In the preference test that followed the 1-bottle exposure to MSG, the mice drank more MSG than water (F(1,20) = 22.5, P < 0.0001), and there were no significant group differences. Preferences for MSG were 78%, 71%, and 74% for B6, T1r3 KO, and Trpm5 KO groups. The responses of the KO mice to MSG were similar to those of naïve female mice, given the same series of tests with isomolar glucose (unpublished data). That is, naïve KO mice did not significantly prefer 200 mM glucose to water in an initial test. The T1r3 KO and Trpm5 KO mice displayed significant glucose preferences (83 and 84%, respectively) following 1-bottle training. In contrast, naïve B6 mice initially displayed a significant 76% preference for glucose, which increased to 96% following 1-bottle glucose training.

These data show that sensing via the T1r1+T1r3 umami receptor and transduction via the Trpm5 channel have little impact on the process that converts indifference to MSG into preference. This may reflect a residual ability to detect MSG via Trpm5-independent mGluR1 and/or mGluR4 pathways (Yasumatsu et al. 2012). In addition, the KO mice retain the ability to taste salt, so they could in theory be responding to the sodium ion of MSG. However, exposure to sodium does not appear to contribute to the acquisition of preference for MSG, as reviewed in the General Discussion. Furthermore, the parallel data for glucose show that mice need not be able to taste a reinforcing substance to learn to prefer it based on postoral effects. In the next experiment, the 3 strains were compared in a flavor-conditioning procedure to test the importance of the MSG taste components to MSG conditioned preferences.
As in Experiment 1B, naïve KO mice were given flavor preference conditioning. For the KO mice, with their reduced ability to detect oral MSG, the added flavor might be more salient than the MSG flavor, so they might show strong preferences for the CS+. Furthermore, as in Experiment 1B, the mice were given 2-bottle tests with unflavored MSG vs. water.

Procedure

Male Trpm5 KO (N=10) and T1r3 KO (N=9) mice were of the same origin as the animals in Experiment 2A. The mice were 8–11 weeks old (T1r3 KO: 63–72 days; Trpm5 KO: 62–79 days) at the start of CS training. Housing, data collection, solutions, and methods were the same as in Experiment 1B. The data were compared with those of the B6 mice from Experiment 1B.

Results and discussion

The training intakes are shown in Figure 5. Overall, the mice drank more of the CS+MSG than the CS− solution ($F(1,25) = 92.3, P < 0.0001$), and the Trpm5 KO mice consumed more of the solutions than did the B6 and T1r3 KO groups ($F(2,25) = 6.2, P < 0.01$). The interaction of Group × CS ($F(2,25) = 8.2, P < 0.0001$) and simple main effects showed that all 3 groups consumed more CS+MSG than CS− and that they differed in CS+MSG but not in CS− intakes.

The data for the tests with the CS flavors presented in water are shown in Figure 5. During testing, CS+ was preferred to CS− in all tests ($F(1,25) = 91.6, P < 0.0001$). The only other significant effects were a greater intake in Test 1 than in Tests 2–4 ($F(3,75) = 12.7, P < 0.0001$) and a marginal interaction of Test × Group ($F(6,75) = 2.2, P = 0.056$). Mean percentage CS+ intake of the Trpm5 KO group (81%) exceeded that of the B6 (64%), but not the T1r3 KO (76%), mice ($F(2,25) = 4.0, P < 0.05$).

In the test of unflavored 200 mM MSG vs. water, the Trpm5 KO mice drank more than the other strains ($F(2,25) = 23.5, P < 0.0001$), and overall MSG intake exceeded that of water ($F(1,25) = 179.2, P < 0.0001$). The interaction of Fluid × Group ($F(2,25) = 20.4, P < 0.0001$) and simple main effects showed that all 3 strains drank more MSG than water. MSG intakes of the Trpm5 KO mice exceeded those of the B6 and T1r3 KO mice (14.2 > 8.0, 5.9 g), but water intakes did not differ (0.4–1.4 g). The preference for MSG in the Trpm5 KO mice (97%) exceeded that of the T1r3 KO mice (78%) but not that of the B6 mice (86%; $F(2,25) = 4.3, P < 0.05$).

In the ascending series of 2-bottle tests (Figure 6), all groups significantly preferred MSG to water at 100–300 mM, and the B6 and T1r3 KO mice also preferred 450 mM. MSG
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intake differed across concentrations \((F(6,150) = 146.3, P < 0.0001)\) and Trpm5 KO mice drank more than B6 but not more than T1r3 KO mice \((F(2,25) = 4.7, P = 0.018)\). The Group × Fluid interaction \((F(12, 150) = 6.7, P < 0.0001)\) and simple main effects showed that the Trpm5 KO mice consumed more MSG than the other strains at 100 and 150 mM and consumed less MSG than the T1r3 KO mice at 450 mM. Intakes at 0.1–10 mM did not differ and were lower than those of the other concentrations. Percentage MSG intakes (Figure 6) differed across concentrations \((F(6,150) = 122.1, P < 0.0001)\), with significantly greater percentage intakes at 100–300 mM concentrations. Although the main effect of Group was not significant, an interaction of Group × Concentration \((F(12,150) = 2.9, P < 0.01)\) reflected a group difference at 450 mM: the preference of Trpm5 KO mice

Figure 5 Mean (±SEM) intakes of CS+MSG and CS− during 1-bottle training and of CS+ and CS− in 4 consecutive 2-day 2-bottle preference tests of B6 (top), T1r3 KO (center), and Trpm5 KO (bottom) mice in Experiment 2B. Percentage intakes of CS+ are shown atop the columns for the 2-bottle tests. Asterisks indicate significant \((P < 0.05)\) differences between CS+ and CS− intakes.

Figure 6 Mean (±SEM) MSG solution intake (top panel) and percentage MSG intake relative to total intake (MSG + water; bottom panel) in Experiment 2B. Each point is the mean of 2 test days. Asterisks indicate significant \((P < 0.05)\) differences between the Trpm5-KO group and the other strains. The solid horizontal lines between the panels show the concentration ranges of significant MSG preference relative to water for the B6, T1r3 KO, and Trpm5 KO groups.
Experiment 2B showed that B6 mice and B6-derived KO mice with missing components of the taste system displayed increased oral acceptance during flavor training and sustained preference for the CS+ across 8 days of testing in the absence of MSG. They also preferred unflavored 200 mM MSG relative to water and preferred a range of MSG concentrations to water in an ascending test. The similarity of most measures in the 3 groups suggests that the oral effects of MSG are less important than the postoral effects in conditioning the preferences for MSG and associated flavors and that postoral detection does not rely on T1r3 or Trpm5 in the gut.

In our previous experiments on the effects of MSG exposure on MSG preferences in B6 mice (Ackroff et al. 2012), we found enhanced intake and preference starting at 10 mM and extending up to 300 mM; although percentage preferences for 450 mM were sometimes greater than those of less-experienced mice, 450 mM MSG was not significantly preferred relative to water. In the present study, we did not see preferences at 10 mM but did find them in B6 and T1r3 KO mice at 450 mM. The lack of effect at 10 mM may be the result of overshadowing by the flavor initially added to MSG so that the mice did not subsequently recognize the flavor of plain MSG until it was sufficiently concentrated at 100 mM. The significant preference for 450 mM could reflect a learned tolerance of mild hyperosmotic effects during the flavor-training period with 200 mM MSG.

Trpm5 KO mice drank more flavored MSG during training and twice as much 200 mM MSG in the initial preference test than the other groups; furthermore, they drank more than the other strains at 100 and 150 mM during the ascending series. Yet, they did not prefer 450 mM MSG to water and drank less of it than the other strains. These differences could reflect the disturbed osmotic responses that have been observed in Trpm5 KO mice (Kokrashvili et al. 2009).

General discussion

The present data establish that 200 mM MSG, though initially a neutral stimulus in the tested mouse strains, can condition preferences for MSG and MSG-paired flavors. The data also demonstrate large strain differences in MSG intake, preference, and flavor conditioning. As in previous work, B6 mice acquired preferences for MSG, whereas the 129 mice were markedly less responsive (Bachmanov et al. 2000; Ackroff et al. 2012). FVB mice, which—similar to B6 mice—are strongly attracted to sweet tastes (Glendinning et al. 2010), had the opposite response to MSG and strongly avoided it. These differences predicted their response to an added CS+ flavor, with sustained CS+ preference in B6 mice, indifference in 129 mice, and avoidance by FVB mice. The same pattern was seen in the higher concentrations of the ascending MSG series, with B6 mice drinking and preferring MSG more than the other strains, 129 mice being indifferent, and FVB mice avoiding the MSG. The responses of KO mice missing components of the MSG taste system were surprisingly similar to those of the parent B6 strain, though those of the Trpm5 KO strain were generally exaggerated due to larger intakes.

The concentration of 200 mM MSG proved to be an optimal concentration for conditioning MSG preferences in B6 mice. Because all the tested strains of mice were initially indifferent to it, changes in their responses would not reflect an unconditioned positive or negative reaction to MSG but rather would show how the postoral effects of MSG altered its evaluation. Moreover, 200 mM MSG was both initially neutral and capable of positive conditioning. In contrast, our previous study of the effects of MSG exposure in B6 mice (Ackroff et al. 2012) compared other concentrations that did not have this pair of properties: 10 mM was unpreferred but ineffective, 100 mM was initially preferred and had moderate positive effects on subsequent MSG preference, and 300 mM was initially avoided but had strong positive conditioning effects. Because 1-bottle exposure to 200 mM MSG increased subsequent intake and preference of B6 mice in 2-bottle tests, this concentration was used for tests of MSG’s ability to confer preferences for added flavors.

Inbred strains

The B6 mice drank more CS+MSG than CS− in training and showed sustained preference for the CS+ flavor in testing. They also preferred plain 200 mM MSG, indicating that their 1-bottle experience with added CS flavor enhanced preference for the taste of MSG in a manner similar to the effects of unflavored MSG in Experiment 1A. When given the ascending series of MSG concentrations, the absolute and percentage intakes of MSG by B6 mice resembled those of other B6 groups with previous MSG experience (Ackroff et al. 2012). Animals of the B6 strain treated MSG as a rewarding stimulus.

In contrast, the 129 and FVB mice showed little indication that they were rewarded by MSG. Neither strain differed in CS+MSG and CS− intakes in training, which is consistent with their lower attraction to MSG than B6 mice in Experiment 1A. The 129 mice were indifferent to the CS flavors in testing and, afterward, did not drink more 200 mM MSG than water. The lack of stimulation to drink MSG in the 129 mice is also clear in the ascending series, with no increase in intake with concentration and preference for MSG only at 100–150 mM. This curve contrasts markedly with that of the 129 mice that had previous experience with sweet solutions (Bachmanov et al. 2000); these animals showed gradually increasing intakes as concentration increased and >80% preference for 10–300 mM MSG.

Although the FVB mice resembled the 129 mice during flavor training, they differed during testing, avoiding the CS+ flavor. In the next test, the FVB mice showed no preference
for unflavored 200 mM MSG. This postconditioning indifference to the taste of MSG differs from that of the FVB mice in Experiment 1A, which avoided MSG after experience, and suggests that the flavor-conditioned mice focused their avoidance on the CS flavor rather than the MSG taste. In the ascending series, they resembled the 129 mice from concentrations of 0.1 to 150 mM but avoided 300 mM, and especially 450 mM. The strong avoidance of concentrated MSG and associated flavors by FVB mice cannot be explained with the present data, but given their initial indifference to 200 mM MSG, the basis for avoidance is likely to be a postoral effect. The existence of an avoiding strain will be useful in further work in determining why MSG is attractive to other strains.

Comparisons between the B6 and 129 mice have shown many differences in responses to sapid solutions. For example, B6 males consumed more ethanol, sucrose, and citric acid and less NaCl than 129 males (Bachmanov et al. 1996), and B6 mice are more strongly attracted to a variety of sweeteners than the 129 mice (Bachmanov et al. 2001). The 129 mice generally consume less of concentrated MSG solutions than B6 mice and show lower preferences (Bachmanov et al. 2000). However, this difference appears to depend on previous experience: naïve 129 mice consumed the same amount of 300 mM MSG as B6 mice (Bachmanov et al. 2000), and MSG intake and preference following experience with multiple sweet substances did not differ between the strains (Inoue et al. 2004b).

The similarity of B6 and 129 gustatory nerve responses to umami stimuli (Inoue et al. 2004a) indicate that their differences lie beyond orosensory detection. Their differences in MSG intake have been attributed to strain differences in the metabolic disposition of MSG (Ji and Bachmanov 2007). After gavage of a concentrated MSG solution, B6 mice showed elevated blood glucose levels, but the 129 mice did not. The authors suggest that the glucose elevation corresponds to a rewarding effect of MSG for the B6 mice, but this explanation is complicated by their large and rapidly administered dose, which does not correspond to the time course of mouse-controlled intake and thus may not be representative of normal responses. In the FVB strain, neither gustatory nerve responses nor metabolic effects of MSG have been examined. The strains differ in multiple measures of glucose metabolism (Berglund et al. 2008), but their relationship to the present data is not clear.

In a previous comparison of the B6 and 129 strains (Sclafani and Glendinning 2005), we conducted intragastric flavor conditioning, in which intake of saccharin-sweetened CS solutions was paired with IG infusion of nutrients (sucrose, soybean oil) or water. The B6 mice expressed stronger flavor preferences than the 129 mice, but they also consumed more of the CS solutions during training and thus were more exposed to the nutrient effects. When we controlled for differences in the intake-stimulating effects of sweet taste, by giving the “sweet subsensitive” 129 mice CS solutions containing more sweetener than the CS solutions given to the B6 mice to equate their intakes, both B6 and 129 mice developed similarly strong flavor preferences (Sclafani and Glendinning 2005). Perhaps the use of a parallel IG procedure, using sweet tastes matched in intensity for the strains, would induce the 129 mice to consume enough MSG to experience its rewarding effects, so that the mild, nonsignificant preferences observed with oral conditioning in Experiment 1B might be enhanced to resemble those of B6 mice. The same might be done for the FVB mice, which also drink so little concentrated MSG that they may not detect reward. If their avoidance of MSG is based on oral dislike, they may still be rewarded postorally and acquire a preference for an MSG-paired flavor.

**Taste KO mice**

Our flavor-conditioning procedure had many elements in common with a previous study of taste KO mice by Stratford and Finger (2011). P2X2/P2X3 double KO mice lack ATP receptors on gustatory nerves and are thus unable to detect signals from taste cells (Stratford and Finger 2011). These mice retain olfactory ability, and so they could distinguish the Kool-Aid CS flavors with distinct odors, which were added to 150 mM MSG and water during training sessions for P2X2/P2X3 KO and WT mice. The double KO and WT mice consumed similar amounts on CS− (water) training days but increased their CS+MSG intake over days. Both P2X2/P2X3 KO and WT controls learned to prefer the CS+ flavor that had been added to MSG. The mice were given 2 more days of training than that in our study, and the training intakes of the WT mice were quite similar to those of our study. In preference tests with the flavors in water, initial preferences for the CS+ were strong (81 and 89% on the first 2 days). The subset of mice given extended nonreinforced tests lost their preference by the sixth day of testing, whereas our B6 mice still preferred the CS+ on day 8 despite fewer training days. Reasons for the difference are not certain but could reflect the lower MSG concentration (150 mM) that they used. Their mild water restriction (18 h access/day) seems unlikely to affect the outcome, although we did find in rats that a preference conditioned by intragastric infusions of MSG extinguished more rapidly in rats maintained on water restriction (Ackroff and Sclafani 2011). In any case, these data indicated that oral detection of MSG was not essential for learning a flavor preference, supporting the idea that MSG reward is based on postoral detection. The Stratford study did not test P2X2/P2X3 KO or WT mice with unflavored MSG following training, and so it is unknown whether these strains, similar to those tested here, also acquired a preference for unflavored MSG.

The P2X2/P2X3 KO mice were created in a mixed B6 and 129 strain (Stratford and Finger 2011), and hybrids of these strains were originally said to resemble the 129 strain with respect to MSG, with lower intake and preference for...
l and 300 mM MSG than the B6 strain (Bachmanov et al. 2000). However, a later analysis showed no differences in intake or preference among the parental strains and the hybrids (Inoue et al. 2004b). As in other studies from the Bachmanov laboratory, the animals were tested with a number of sweet tastants before the MSG tests, which can profoundly alter animals’ response to MSG (Ackroff et al. 2012).

One current formulation to explain the functions of multiple umami receptors in the mouse invokes a kind of division of labor. The T1r1+T1r3 receptor is thought to be involved in preference for MSG and other umami substances and it mediates umami synergy with ribonucleotides, whereas the mGluR1 and mGluR4 receptors provide information specific to umami taste quality and discrimination of umami from other tastes (Tokita et al. 2012; Yasumatsu et al. 2012; Kusuhara et al. 2013). This distinction is based in part on the reduced preference for umami (and sweet) in T1r3 KO and Trpm5 KO mice (Damak et al. 2003; Zhao et al. 2003), which retain the ability to discriminate between these taste qualities (Delay et al. 2006). By this analysis, these KO mice should not be attracted to MSG despite the ability to taste it via mGluRs but should be able to learn to prefer it based on positive postoral effects. Drawing from the parallel to the T1r2+T1r3 dimer involved in sweet taste, a recent article (Tokita et al. 2012) suggested that T1r1+T1r3 “…is simply a sweet-taste receptor that is activated by L-type amino acids.” This implies that mice missing mGluR should be attracted to MSG, though they might not readily distinguish it from other tastes. Indeed, mGluR4 KO mice, which had intact T1r1+T1r3 receptors, have been reported to show stronger preferences for MSG than WT mice (Roper et al. 1997; Chaudhari and Roper 1998; Chaudhari et al. 2009), though, thus far, these data have only been presented in summary form. More data are needed to substantiate this possibility.

Trpm5 KO mice displayed a stronger preference for the MSG-paired CS+ flavor than B6 mice. In another study, we also observed stronger preferences for a glucose-paired CS+ flavor in Trpm5 KO mice than in B6 mice (unpublished data). B6 mice may acquire a weaker preference for the MSG and sugar-paired flavors because the MSG and sugar tastes compete with (overshadow) the CS+ flavors in the formation of a flavor–postoral effect association (Dwyer et al. 2011). The Trpm5 KO mice presumably experience less competition from the tastes of MSG and glucose and thus can form stronger associations between the flavor of the CS+ and postoral US actions. Experiment 2 suggests that the difference among strains in Experiment 1 could involve mGluRs and may not reflect differences in T1r3 or Trpm5 activity. Recent work shows that some variants of mGluR receptors in taste buds have high affinity for umami compounds (Nakashima et al. 2012). If the studied strains differed in mGluR variants, this might contribute to their varying responses to MSG.

Sodium taste

MSG has a salt taste component due to its sodium ion, and some studies of MSG taste sensitivity have incorporated the sodium channel blocker amiloride to remove the influence of salt taste (Glendinning et al. 2005a; Damak et al. 2006). This was not done in the present studies, raising the question of relative salt sensitivity and preference among the strains. However, responses to sodium do not predict the present results. Several studies have found that the differences in MSG responses in the B6 and 129 strains are not correlated with their differences in response to NaCl taste. At intermediate NaCl concentrations, 129 mice consumed more NaCl than B6 mice and preferred it to water, although at high concentrations, both strains avoided NaCl (Bachmanov et al. 1998; Inoue et al. 2004b; Bachmanov et al. 2009). Their gustatory nerve responses to MSG are similar, as are their responses to NaCl (Inoue et al. 2004a). Both strains generalize a conditioned aversion to MSG to NaCl but not to other basic tastes (Murata et al. 2009). The responses of FVB mice to MSG and NaCl have not been compared, but they generally have stronger preferences for NaCl than B6 mice (Mccaughey et al. 2007; Tordoff et al. 2007), suggesting that sodium avoidance is not an explanation for their MSG avoidance. The sodium component of MSG is conceivably more salient for the KO mice because their oral detection of glutamate is diminished, whereas their response to salt is intact (Damak et al. 2003; Zhang et al. 2003). The 24-h preference tests of Trpm5 KO mice, which included amiloride in the MSG, still found responses to concentrated MSG (Damak et al. 2006), but this presumably reflected postoral conditioning.

These experiments show that MSG can condition preferences for associated flavors, which makes sense in view of its usage in human cuisine. In humans, preferences for glutamate-paired flavors may have both oral and postoral bases (Prescott 2004; Yeomans et al. 2008). In mice, the rewarding effects of MSG may be largely postoral, given the limited range of concentrations that are preferred by naive animals. Because mice that cannot use the T1r1+T1r3 umami receptor or its downstream signaling element Trpm5 are still responsive to MSG in the present experiments, there may be an important role for other glutamate receptors such as, perhaps, the mGluR receptors that are found in the gastrointestinal tract and the oral cavity (Akiba and Kaunitz 2011).

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References


Delay ER, Hernandez NP, Bromley K, Margolskee RF. 2006. Sucrose and monosodium glutamate taste thresholds and discrimination ability of T1R3 knockout mice. Chem Senses. 31(4):351–357.


