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# MSG intake and preference in mice are influenced by prior testing experience

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## HIGHLIGHTS

- C57BL/6 mice naïve to sapid solutions were indifferent to MSG solutions.
- Forced exposure to MSG converted indifference to preference.
- Experience with nutritive solutions also enhanced MSG preference and intake.
- Experiential and procedural variables can influence solution preferences in mice.

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## ABSTRACT

Monosodium glutamate (MSG), the prototypical umami substance, is used as a flavor enhancer in many foods, but when presented alone is often only weakly attractive. Yet with experience mice will develop strong preferences for MSG solution over water. The present experiments explored the conditions that change indifference to preference for MSG. C57BL/6J mice were given a series of 2-day two-bottle tests with water vs. an ascending series of MSG concentrations (0.1–450 mM) to assess preference and intake. Naive mice were indifferent to all concentrations, but following forced one-bottle exposure to 300 mM MSG they preferred most concentrations and consumed more MSG. Exposure to 100 mM MSG also increased subsequent MSG preference but not intake. Experience with other nutritive solutions (8% sucrose, 8% Polycose, 8% casein hydrolysate, and isocaloric 3.5% soybean oil emulsion) also enhanced subsequent MSG preference and intake. Polycose and sucrose experience were almost as effective as MSG experience. However, not all sapid solutions were effective; 0.8% sucralose and 10 mM MSG exposure did not alter subsequent MSG preference. The generality of the preexposure effect was tested by offering an ascending series (0.1–100 mM) of inosine monophosphate (IMP), another umami substance; initial indifference was converted to preference after forced exposure to 300 mM MSG. Together these results suggest that a combination of oral and post-oral effects may be responsible for the experience effect, with MSG itself the most potent stimulus. A final experiment revealed that MSG preference in naïve mice is enhanced by presenting the MSG and water drinking spouts far apart rather than side by side. Thus the preferences for umami solutions in mice are subject to influence from prior tastant experience as well spout position, which should be taken into account when studying acceptance of taste solutions in mice.



## 1. Introduction

The prevailing notion in the taste literature is that umami, the savory quality characterized in particular by monosodium glutamate (MSG), is a preferred taste that, along with salty and sweet tastes, signifies the presence of nutrients in foods, e.g., [12,32]. However, MSG alone does not have a clearly positive or negative initial value. For example, neither human adults [55] nor infants [11] prefer MSG solution. Human infants do, however, show positive responses to MSG in soup [11,43], and adults increase preference for the flavors of foods with added MSG [35,58]. MSG enhancement of food preference and intake has been found in other species, e.g., dog [34] and sheep [20], that do not prefer MSG in solution. This effect could be due to taste or post-oral effects of MSG, and some data suggest that ingestion rather than mere tasting is required [35]. The post-oral reinforcing property of MSG has been confirmed in several studies of flavor preference conditioning with intragastric infusion of MSG in rats [4,53,54].

In C57BL/6 (B6) mice, high intakes and strong preferences for MSG over water were reported by Bachmanov et al. [8] in two-bottle tests with ascending MSG concentrations. These mice were compared with the 129 strain, which showed weaker responses to concentrated MSG. The strains differed most strongly at 300 mM MSG, with B6 mice consuming amounts comparable to their substantial intake of preferred saccharin and sucrose solutions, while the 129 mice drank only about half as much [8]. The strong attraction to MSG in B6 mice was not, however, seen in all the tests conducted. Specifically, naive B6 mice did not drink more 300 mM MSG than water (40% MSG preference), although

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mice that were first exposed to 1 mM MSG subsequently displayed a mild preference for 300 mM (61%) [8]. This lack of preference in naïve mice is not widely appreciated, and supports the idea that postingestive effects of MSG contribute to its preference. Other B6 mice given 4-day exposure to 300 mM MSG strongly preferred it during the first 2 days, and in the second half of the test they increased their MSG intakes and maintained their strong (~90%) preferences. This strong initial preference, as well as the strong preferences observed in the ascending series even at low concentrations [8], is difficult to reconcile with the weak or absent preferences in naïve mice. A potentially important difference between the tests is that the strong preferences were observed in mice with extensive prior experience with other tastants, including sucrose and Polycose.

The Bachmanov et al. study suggests that experience with other tastants, like experience with MSG, may promote subsequent MSG preference, but because the animals had been tested with multiple tastants it is not clear which ones might operate in this way. For example, some of the mice had prior experience with sucrose, artificial sweeteners and/or Polycose before receiving MSG, and the effect might have been due to only one of the substances. Polycose is a maltodextrin that has an attractive flavor to mice distinct from sweet taste [51,52,59]. Besides these carbohydrates, the other major nutrient classes, fat and protein, are also attractive to mice [17,38,40]. Protein is of particular interest because dietary glutamate has been hypothesized to signal the presence of protein in food [27], so exposure to the flavor of protein might enhance subsequent response to MSG.

The present work examines the effect of prior experience with MSG and with different palatable solutions on subsequent preference for MSG. By adopting standard 48-h two-bottle tests with an ascending series of MSG concentrations, we generated data comparable to those of past studies. The first experiment began by testing naïve B6 mice, which showed no preference for MSG at concentrations from 0.1 to 450 mM. Experience with 300 mM MSG was sufficient to produce strong MSG preferences similar to those reported by Bachmanov et al. [8]. Experiment 2 sought the minimum effective concentration of MSG exposure that would increase subsequent MSG preference. Experiments 3 and 4 tested the possibility that exposure to other solutions (sucrose, sucralose, Polycose, protein, or fat) could also serve to enhance the response to MSG. Experiment 5 expanded testing to include the effects of MSG exposure on the preference for another umami substance, inosine 5′-monophosphate.

A second goal of the present work was to examine another procedural variable that appeared to have robust effects on MSG preference and intake. Experiment 6 explored the basis for MSG preference in naïve mice tested with an alternate procedure. A recently published study [29] focused on finding an alternative to the sweet taste often used to induce ethanol intake in rodents, and sought an MSG concentration that might be substituted for sweetness in mouse strains with reduced attraction to sweet taste. The immediate preferences they obtained for 25–400 mM MSG in naïve B6 mice contrasted with the lack of preference with our procedure. Primary differences from the methods in the first 5 experiments were the number of days of MSG exposure at each concentration, the time of day when the session began and the physical distance between the spouts. Experiment 6 repeated the alternate procedure with variations of time of day and spout distance to determine which of these seemingly innocuous variables accounted for the difference in MSG preference.

## 2. Experiment 1

This experiment was conducted to evaluate MSG preference in B6 mice naïve to sapid solutions. In the Bachmanov et al. [8] study, tastant-experienced B6 mice increased MSG solution intake as the concentration increased from 0.1 to 100 mM, then increased substantially at the next concentration of 300 mM. The MSG preference was reduced at 600 mM and switched to avoidance at 1000 mM. The peak intake at 300 mM was quite striking, so we included some concentrations from the Bachmanov series and added concentrations on either side of 300 mM (150 and 450 mM) to characterize the animals' attraction to these intermediate values. We did not test higher concentrations, which are typically avoided by mice. Each concentration was presented for 2 consecutive days, in ascending order. Because the responses of naïve mice differed markedly from those of the prior study, the mice in Experiment 1A were next given one-bottle access to the "peak" 300 mM concentration. The test series was then repeated to determine whether this exposure had affected the animals' responses to MSG. Experiment 1B was conducted to determine whether mice, without intervening forced exposure, would display large enhancements of MSG preference when simply retested.

### 2.1. Materials and method

#### 2.1.1. Animals

For Experiment 1A, ten naïve male C57BL/6J (B6) mice were born in the laboratory from stock purchased from Jackson Laboratories (Bar Harbor, ME); they were 7 weeks old at the start of testing. For Experiment 1B, ten naïve male B6 mice were purchased from Jackson Laboratories and were 8 weeks old at testing. The mice were singly housed in plastic tub cages with ad libitum access to chow (5001) and deionized water in a room maintained at 22 degrees C with a 12:12 light–dark cycle (lights on 0900 h). Data collection occurred at 1100 h daily.

#### 2.1.2. Test solutions

Solutions were prepared using deionized water and monosodium glutamate (Sigma Chemical, St. Louis, MO) at concentrations of 0.1, 1, 10, 100, 300, 150, and 450 mM.

#### 2.1.3. Apparatus

The two-bottle 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 two bottles that were placed on an empty cage, and intake measures were corrected by this amount.

#### 2.1.4. Method

*2.1.4.1. Experiment 1A.* For the first week, the mice were given access to two bottles of water. Then they received an ascending series (0.1–450 mM) of 2-day two-bottle tests with MSG vs. water. Following this series, there were 4 days of two-bottle access to water only. For the next 4 days, the mice were given one bottle of 300 mM MSG only. Then they were given 4 consecutive days of two-bottle tests with 300 mM MSG vs. water. Following a 4-day period of two-bottle access to water, the ascending series of MSG vs. water tests was repeated. 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.

*2.1.4.2. Experiment 1B.* The mice were first given the same ascending series of two-bottle tests as in Experiment 1A. Following 4 days of two-bottle access to water only, the ascending series was repeated.

#### 2.1.5. Statistical analysis

Fluid intakes were averaged over the two days at each solution concentration. Preferences were also expressed as percent intakes (MSG solution intake/total intake × 100) to facilitate comparison of test series.