

# Flavor preferences conditioned in C57BL/6 mice by intragastric carbohydrate self-infusion

Anthony Sclafani<sup>a,\*</sup>, John I. Glendinning<sup>b</sup>

<sup>a</sup>*Department of Psychology, Brooklyn College and the Graduate School, The City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210-2889, USA*

<sup>b</sup>*Department of Biological Science, Barnard College, Columbia University, New York, NY 10027, USA*

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## Abstract

This study determined the feasibility of conditioning flavor preferences in mice by self-administered intragastric (IG) nutrient infusions. Male C57BL/6J mice were surgically fitted with an IG catheter that was attached by a tether system to an infusion pump. The mice were given ad-libitum access to chow and a flavored solution 23 h/day. Drinking was monitored with a computerized lickometer system that controlled the infusion pumps. In Experiment 1, drinking one flavored solution (CS<sup>+</sup>, e.g., grape-saccharin) was paired with matched infusions of 8% maltodextrin, whereas drinking another solution (CS<sup>-</sup>, e.g., cherry-saccharin) was matched with water infusions across 6 one-bottle training days. During training, the mice drank more CS<sup>+</sup> than CS<sup>-</sup>; this was due to an increase in bout size but not bout frequency. In subsequent two-bottle choice tests, the mice strongly preferred (91%) the CS<sup>+</sup> to the CS<sup>-</sup>. Experiment 2 obtained a significant but less robust (71%) CS<sup>+</sup> preference in mice trained with unsweetened CS solutions. These data indicate that mice, like rats, acquire an increased acceptance and preference for flavors paired with the postingestive actions of nutrients. Our understanding of flavor-nutrient learning can be advanced by studying this process in selected mouse strains and genetically modified animals.

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## 1. Introduction

It is well known that the feeding response of animals is influenced by the orosensory properties and postingestive consequences of foods. The flavor (taste, odor, texture) of food can both stimulate intake and promote overconsumption with potential negative health effects (e.g., obesity and diabetes). Once consumed, the postingestive actions of food have feedback effects that influence ongoing and subsequent food consumption. Most research on postingestive controls has focused on negative feedback, i.e., satiation and satiety [23]. High sugar foods, for example, have osmotic and caloric effects that can lead to rapid satiation. Data from rats demonstrate that nutrients can also have powerful positive feedback effects that can increase ingestion and condition strong food preferences [19,20]. Ultimately, food intake and selection are determined by the interaction of

orosensory stimuli combined with positive and negative postingestive feedback signals, which are integrated with long-term metabolic and adiposity signals.

To understand the precise mechanisms that control food intake, it is necessary to experimentally isolate the independent contributions of oral and postoral stimulation. For example, while it is clear that rat and mouse strains differ in their macronutrient selection and propensity to develop dietary obesity [18,22], little is known about whether the strain differences are related to differential responses to the oral or postoral actions of the macronutrients. Various techniques have been employed in rats to separate oral vs. postoral controls of intake. These include brief-access taste tests and sham-feeding tests, which assess the contribution of oral factors, and intragastric (IG), intraduodenal, and intravenous infusions, which assess the contribution of postoral controls [24]. To investigate the interaction of oral and postoral factors in feeding, Sclafani et al. [2,7,21] adapted “electronic esophagus” preparations used by prior investigators [15,25]. This preparation allows rats to feed and drink normally while self-infusing nutrients through

\* Corresponding author. Tel.: +1-718-951-5606; fax: +1-718-951-4824.

E-mail address: Asclafani@gc.cuny.edu (A. Sclafani).

implanted gastric catheters over several weeks. Studies using the electronic esophagus preparation have revealed that rats learn to prefer flavored solutions that are paired with IG nutrient infusions over flavored solutions that are paired with IG water infusions [19]. This is viewed as a form of Pavlovian learning with the flavor cue representing the conditioned stimulus (CS) and the infused nutrient the unconditioned stimulus (US) [6]. The magnitude of the conditioned preference varies as a function of the nature and intensity of the US (nutrient type and density) as well as the CS flavor quality (e.g., sweetened vs. unsweetened) [19]. In addition to conditioning flavor preferences, nutrient infusions can also produce increases in the total intake of the flavored CS solution (increased acceptance of the CS) [20].

The experimental techniques described above were developed for rats and further progress in our understanding of ingestive behavior can be achieved if these techniques are adapted for use in mice. This is because of the large number of inbred mouse strains and genetically altered mice that are available to study the orosensory, neurobehavioral, and metabolic controls of food intake and energy balance [8,11,12,14]. Recently, a brief-access taste test has been introduced to study orosensory determinants of ingestion in mice [10]. The present study determined the feasibility of investigating nutrient-conditioned flavor preference and acceptance in mice using an electronic esophagus system.

To this end, two experiments were conducted to examine flavor conditioning in mice of a well-studied strain (C57BL/6J) that were fitted with chronic IG catheters. In the first experiment, they were offered ad-libitum access to food and a saccharin-sweetened flavored solution (e.g., grape-saccharin, the CS+) that was paired with matched IG infusions of 8% maltodextrin (a glucose polymer derived from starch). On alternate days, a different flavored solution (e.g., cherry-saccharin, CS-) was paired with water infusions. After six training days, flavor preferences were then evaluated in two-bottle CS+ vs. CS- choice tests (22 h/day). In the second experiment, new mice were subjected to the same conditioning procedure except that unsweetened CS flavors were used. This was of interest because of the well-documented differences in sweet taste sensitivity among various strains of inbred mice [8,14]. To determine whether these strains also differ in their conditioning response to the postingestive actions of nutrients, sweet taste must be eliminated as a conditioning factor.

## 2. Experiment 1

### 2.1. Method

#### 2.1.1. Subjects

Male C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 7 weeks of age. The nine mice that completed the study weighed 21–26 g at surgery. The animals were initially housed in pairs in standard plastic

mouse cages in a room maintained at 22 °C with a 12:12 h light–dark cycle. Purina Chow (5001, PMI Nutrition International, Brentwood, MO) and tap water were available as noted.

#### 2.1.2. Surgery

The mice were anesthetized with a ketamine (63 mg/kg) and xylazine (9.4 mg/kg) mixture and were fitted with an IG catheter constructed of microrenathane tubing (0.033 in OD × 0.014 in ID; Braintree Scientific, Braintree, MA). The tip of the tubing was heat flanged and fitted with a small silastic collar that served as an anchor to keep the tube in the stomach. The tube and collar were inserted 1 mm into the stomach through a small incision in the greater curvature and secured with a purse-string suture (7–0 silk) and polypropylene mesh which was fixed to the stomach wall with Nexaband adhesive (Veterinary Products Laboratories, Phoenix, AZ). The distal end of the catheter passed through an incision in the abdominal muscle, was routed under the skin to the back of the neck, and passed through a hole in the skin to an infusion harness and spring tether (CIH62, Instech Laboratories, Plymouth Meeting, PA). The abdominal and skin openings were closed using Nexaband adhesive and treated with triple antibiotic ointment. The mouse was then allowed to recover in a small, heated tub cage before being returned to the infusion cage.

#### 2.1.3. Apparatus

The mice were tested in infusion cages (10 × 23.5 × 27 cm) with plastic walls and a stainless steel grid floor. Above the cage, plastic tubing from a syringe pump (A-99, Razel Scientific, Stamford, CT) was connected to the input port of a light-weight swivel on a counterbalanced lever (Instech Laboratories). The output port of the swivel was connected to the mouse's IG catheter tubing that was protected by a stainless steel spring connecting the swivel and the tether fitted on the mouse. One or two drinking bottles (50 ml plastic centrifuge tubes) with stainless steel spouts were mounted on the front wall of the cage. The mice licked the spouts through slots in a stainless steel plate fixed on the wall of the cage. Licking was monitored by an electronic drinkometer (Med Electronics, St. Albans, VT) and a microcomputer, which controlled the syringe pumps. The computer software accumulated licks and turned the infusion pumps on or off, as required, every 3 s. The pump rate was 0.1 ml/min and the oral intake/infusion ratio was maintained at approximately 1:1 by adjusting a lick/pump activation parameter. In two-bottle tests, two infusion pumps were attached via a Y-connector to the input port of the swivel. Intakes were measured to the nearest 0.1 g and IG infusions were recorded to the nearest 0.5 ml. The lick data were stored in 6-s bins on disk for offline analysis of drinking patterns.

#### 2.1.4. Test solutions

The CS solutions contained 0.05% (w/w) sodium saccharin (Sigma Chemical, St. Louis, MO) and were flavored

with 0.05% (w/w) cherry or grape Kool-Aid Mix (General Foods, White Plains, NY). The IG infusates were water and 8% (w/w) maltodextrin (Maltrin QD 580, Grain Processing, Muscatine, IA). For half the mice, cherry–saccharin was the CS+ paired with IG maltodextrin infusion, and grape–saccharin was the CS– paired with water infusion; the flavor–infusate pairs were reversed for the remainder of the animals. Note that orally consumed flavored saccharin solution mixed with the IG maltodextrin solution in the stomach so that the carbohydrate concentration in the gut was 4%. The mice were adapted to drinking unflavored 0.05% saccharin prior to flavor conditioning. The mice were also adapted to consume a palatable mash consisting of powdered chow and 8% maltodextrin (3:2). This diet was used to facilitate postsurgical recovery.

### 2.1.5. Procedure

Prior to surgery, the mice were singly housed in the infusion cages for 3–4 days with ad-libitum access to 0.05% saccharin solution and chow; they also had 1 g/day of mash diet. Following surgery, they were returned to the cage with their tether and IG catheter attached to the swivel system and given, in addition to ad-libitum chow and saccharin solution, mash diet (1 g) for 3 days. After several days (2–6) of postsurgery recovery, the animals were infused with water intragastrically as they drank unflavored saccharin solution 22 h/day. They were then given 6 one-bottle training days with the CS+ solution paired with IG infusions of maltodextrin (Days 1, 3, 5) and the CS– solution paired with IG infusions of water (Days 2, 4, 6). This was followed by a reinforced two-bottle choice test with the CS+ vs. CS– for 2 days; the intakes of the CS+ and CS– remained paired with IG infusions of maltodextrin and water, respectively. A nonreinforced two-bottle test was then conducted for 2 days with both the CS+ and CS– now paired with IG water infusions. The left–right position of the CS+ and CS– solutions was counterbalanced throughout training and testing. Chow was available ad libitum throughout the experiment.

### 2.1.6. Data analysis

Oral intakes and bout pattern data during one-bottle training were analyzed using repeated-measures analyses of variance procedures (CS  $\times$  Days). Drinking patterns were evaluated with a bout defined as a period of drinking containing at least 30 licks and with interlick intervals no longer than 5 min [9]. Mean bout size (g) was determined by dividing the total 22-h CS intakes by the number of bouts. Two-bottle intakes were averaged over the 2 days of the reinforced and nonreinforced tests and evaluated with analysis of variance (CS  $\times$  Test).

## 2.2. Results

In the 2 days prior to CS training, the mice consumed 4.2 g/day of unflavored saccharin solution and were infused

with a similar amount of water IG. Fig. 1 presents the oral intakes and bout patterns of the CS+ and CS– solutions during the 6 one-bottle training days. Overall, the mice consumed more CS+ than CS– during training [ $F(1,8) = 8.253, P < .05$ ]. Intake of the CS+ tended to increase over days compared to the CS– but the CS  $\times$  Day interaction just failed to be significant [ $F(1,8) = 3.475, P = .056$ ]. Analysis of the drinking bout data revealed that mean bout sizes were greater on CS+ training days than on CS– training days [ $F(1,8) = 7.857, P < .05$ ]; the difference tended to increase over days but the CS  $\times$  Day interaction was not significant. In contrast, CS+ and CS– bout numbers did not differ during training.

The results of the two-bottle choice tests are presented in Fig. 2. Overall, the mice consumed substantially more CS+

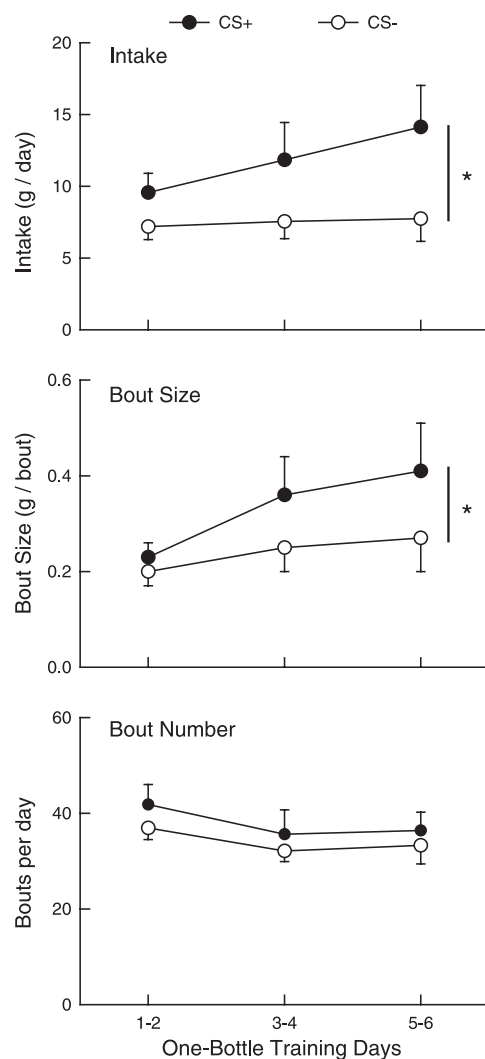


Fig. 1. Mean ( $\pm$ S.E.) daily intake (top), bout size (middle), and bout number (bottom) of CS solutions (saccharin–sweetened grape or cherry) during one-bottle training days in Experiment 1. As the mice drank the CS+ solution on Days 1, 3, and 5 they were infused intragastrically with 8% maltodextrin, and as they drank the CS– solution on Days 2, 4 and 6 they were infused with water. The asterisk denotes an overall significant ( $P < .05$ ) difference between CS+ and CS– intakes.

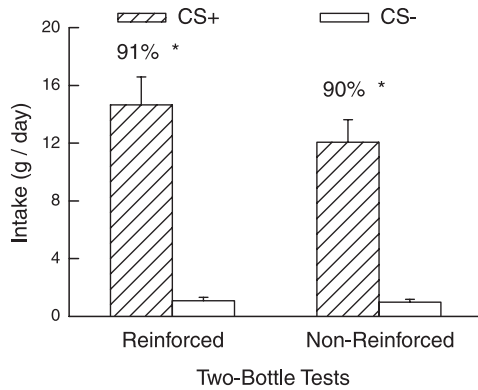


Fig. 2. Mean ( $\pm$ S.E.) intake of CS+ and CS- solutions during two-bottle tests of Experiment 1. As the mice drank the CS+ and CS- solutions (saccharin-sweetened grape or cherry) during the reinforced test they were infused intragastrically with 8% maltodextrin and water, respectively. During the nonreinforced test, intakes of both CS+ and CS- were paired with IG water infusions. Numbers atop bars represent the mean percentage of CS+ intake (calculated separately for each mouse). The asterisk denotes a significant ( $P < .01$ ) difference between CS+ and CS- intakes.

than CS- in both the reinforced and nonreinforced tests [ $F(1,8) = 45.208$ ,  $P < .01$ ]. Percentage of CS+ intakes ranged from 66% to 96% and exceeded 90% in eight of nine mice; preferences were similar in the mice trained with cherry and grape as the CS+ (94% and 87%). The mice drank less CS+ in the nonreinforced test than in the reinforced test (CS  $\times$  Test interaction) [ $F(1,8) = 6.767$ ,  $P < .05$ ] although the percentages of CS+ intakes were similar in the two tests (91% vs. 90%). CS+ intake during the nonreinforced test exceeded CS- intake during one-bottle training, although both flavors were paired with IG water infusions [12.1 vs. 7.5 g/day,  $t(8) = 3.89$ ,  $P < .01$ ]. The mice took many more CS+ than CS- bouts during the two-bottle tests [32.2 vs. 5.1 bouts/day,  $F(1,8) = 73.625$ ,  $P < .05$ ]. Mean CS+ bout size tended to be larger than CS- bout size (0.45 vs. 0.26 g/bout) but this difference was not significant. (Note that bout size is more variable in two-bottle tests than in one-bottle tests because of the low number of CS- bouts.)

### 3. Experiment 2

#### 3.1. Method

Male C57BL/6J mice ( $n = 9$ ) were trained as in Experiment 1 except that the CS+ and CS- were unsweetened solutions (0.05% grape and cherry Kool-Aid in plain water). The mice were also presented with plain water rather than unflavored saccharin prior to the onset of flavor conditioning. As explained in the Results, following the two-bottle tests, the mice were given four additional days of one-bottle testing with the CS+ and CS- presented on alternate days and paired with IG maltodextrin and water, respectively.

Chow was available ad libitum throughout training and testing.

#### 3.2. Results

As illustrated in Fig. 3, the mice consumed comparable amounts of the CS+ and CS- solutions during one-bottle training sessions. The mean drinking bout size and number also did not differ on CS+ and CS- training days. In the two-bottle choice tests, however, the mice consumed significantly more CS+ than CS- [ $F(1,8) = 6.603$ ,  $P < .05$ ; Fig. 4]. CS intakes and preferences were similar in the reinforced and nonreinforced tests and percentages of CS+ intakes were 70–71% in the two tests. Percentage of CS+ intakes in individual mice ranged from 44% to 96% and the strength of the preference was correlated with the amount of

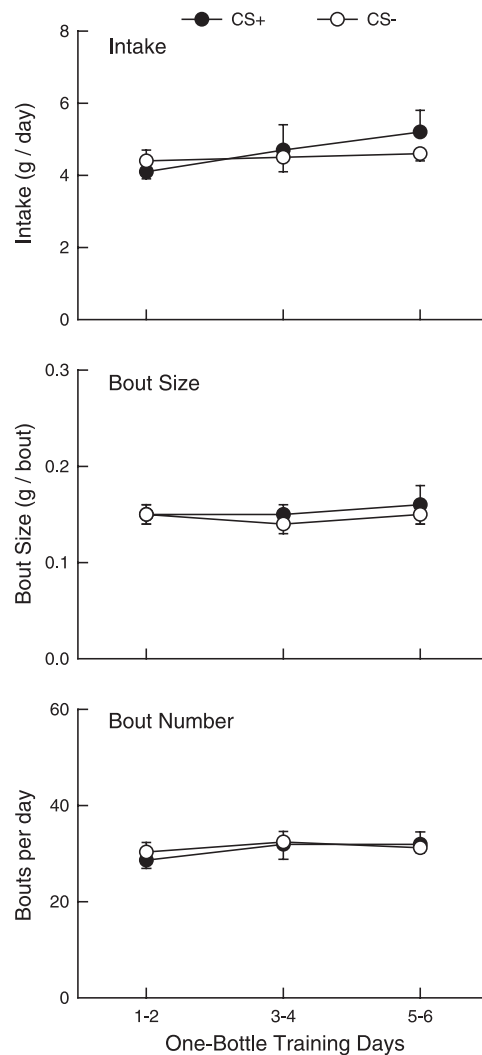


Fig. 3. Mean ( $\pm$ S.E.) daily intake (top), bout size (middle), and bout number (bottom) of CS solutions (unsweetened grape or cherry) during one-bottle training days in Experiment 1. As the mice drank the CS+ solution on days 1, 3, and 5 they were infused intragastrically with 8% maltodextrin, and as they drank the CS- solution on days 2, 4 and 6 they were infused with water.

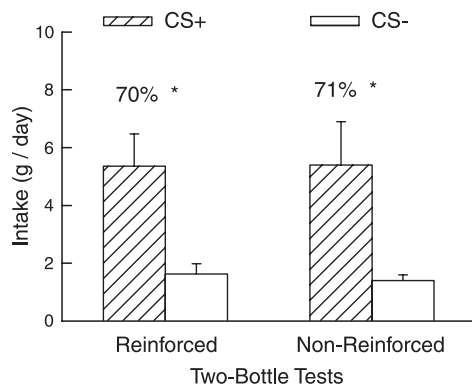


Fig. 4. Mean (+S.E.) intake of CS+ and CS- solutions during two-bottle tests of Experiment 2. As the mice drank the CS+ and CS- solutions (unsweetened grape or cherry) during the reinforced test, they were infused intragastrically with 8% maltodextrin and water, respectively. During the nonreinforced test, intakes of both CS+ and CS- were paired with IG water infusions. Numbers atop bars represent the mean percentage of CS+ intake (calculated separately for each mouse). The asterisk denotes a significant ( $P < .05$ ) difference between CS+ and CS- intakes.

maltodextrin the animals self-infused during one-bottle training ( $r^2 = .567$ ,  $P < .05$ ). The preferences were similar in the mice trained with cherry and grape as the CS+ (69% and 71%). The mice took many more CS+ than CS- bouts during the two-bottle tests [23.2 vs. 9.6 g/day,  $F(1,10) = 6.269$ ,  $P < .05$ ]. Mean CS+ bout size tended to be larger than CS- bout size (0.18 vs. 0.13 g/bout) but this difference was not significant.

During the original one-bottle training trials, there was a trend for CS+ intakes to increase over days and this continued during the reinforced two-bottle tests. The mice were therefore given four additional one-bottle reinforced trials (CS+, CS-, CS+, CS-) following the two-bottle tests. In these trials, they consumed significantly more CS+ than CS- [6.7 vs. 5.4 g/day,  $t(8) = 2.81$ ,  $P < .05$ ]. This difference was due to an elevated CS+ bout size, relative to CS- bout size [0.20 vs. 0.18 g/bout;  $t(8) = 3.03$ ,  $P < .05$ ] and bout number (32.3 vs. 30.1 bouts/day), although the difference in bout number was not significant.

#### 4. Discussion

The present results demonstrate the feasibility of studying flavor-nutrient learning in mice using a long-term IG self-infusion system. The C57BL/6J mice trained 22 h/day with ad-libitum access to food acquired a significant preference for a flavored solution that was paired with IG carbohydrate infusions. In general, the results are comparable to those obtained with rats trained and tested under similar conditions.

Preferences were obtained with sweetened and unsweetened CS solutions in Experiments 1 and 2, although the CS+ preference was stronger with sweetened flavors compared to unsweetened flavors (91% vs. 71%,  $P < .05$ ). This presum-

ably resulted, at least in part, because the saccharin-sweetened CS solutions stimulated greater intakes and thus the mice were reinforced with more maltodextrin on CS+ days when trained with sweetened rather than unsweetened CS solutions. Consistent with this interpretation, the preference for the unsweetened CS+ in Experiment 2 was correlated with the amount of maltodextrin self-infused in Experiment 2. This suggests that pairing an unsweetened CS+ with infusions of a more concentrated maltodextrin solution would enhance flavor conditioning. In rats, preference conditioning is enhanced by increasing maltodextrin concentration from 1% to 16% but then declines somewhat at a 32% concentration [1,13].

Increased flavor acceptance was also conditioned by the IG maltodextrin infusions and this effect was most pronounced with the sweetened CS+ flavors. That is, the mice consumed more CS+ than CS- during one-bottle training sessions. This elevated CS+ intake was much more pronounced with the sweet CS solutions in Experiment 1 than with the unsweetened CS solutions in Experiment 2. The substantial increase in CS+ acceptance observed in the first experiment was due to an increase in bout size rather than bout number. Sweetened flavors also promote a greater increase in CS+ acceptance than do unsweetened flavors in rats [1,3,17].

In both experiments, the mice displayed similar preferences in the reinforced and nonreinforced two-bottle tests. This demonstrates that the animals had acquired a true preference for the CS+ flavor and were not selecting it as a direct response to the IG maltodextrin infusions. Nevertheless, in Experiment 1 the mice consumed less of the CS+ in the nonreinforced test than in reinforced test even though their CS+ preference remained strong at 90%. Their absolute intake of the CS+ might have declined further if nonreinforced testing had been extended. Rat studies indicate that whereas CS+ preference (as measured in two-bottle tests) is very resistant to extinction training, CS+ acceptance declines more rapidly [16]. The reason for the differential effect of nonreinforcement training on conditioned flavor preference and acceptance remains to be determined. Note that increased flavor acceptance is less readily conditioned than increased flavor preference in rats [20]. This may be because different processes are involved in acceptance and preference conditioning. In addition, the conditioned increase in CS intake produced by nutrient infusions must counteract the unconditioned satiating actions of the nutrients.

As noted above, the mice consumed more of the sweetened CSs than of the unsweetened CS. This is not surprising because C57BL/6 mice are known to be sweet "tasters" that prefer low concentrations of many sweeteners [5,8,14]. While the mice consumed more sweet CS- than unsweet CS- during one-bottle training (peak intakes: 7.7 vs. 4.6 g/day), they overconsumed the sweet CS+ much more than the unsweet CS+ (peak intakes 14.1 vs. 5.2 g/day). Prior work indicates that C57BL/6 also overconsume nutritive

sweeteners more than nonnutritive sweeteners [5]. This may result in part because nutritive sweeteners have a more preferred taste than do artificial sweeteners. However, the present data also suggest that the postingestive actions of carbohydrates contribute to their overconsumption. Note in particular, that the mice in Experiment 1 drank twice as much CS+ as CS− by the end of one-bottle training although the two CS solutions were both sweetened with 0.05% saccharin. Furthermore, their total fluid intake (oral + IG) on the last CS+ training day was 27 g which compares to a peak intake of about 25 ml/day in mice offered maltodextrin solutions (0.003–30%) to drink by mouth [4]. These findings raise the possibility that the different intakes of caloric sweeteners reported in sweet “taster” and “nontaster” mouse strains may be due, in part, to strain differences in the postingestive reinforcing actions of carbohydrates. This can be evaluated by comparing the ability of IG carbohydrate infusions to condition CS+ preference and acceptance in taster and nontaster mouse strains.

The conditioned CS+ preference and acceptance produced by the IG maltodextrin infusions in this study are consistent with the results of many rat studies. Together, these findings demonstrate that in addition to the well-characterized postoral satiating actions of nutrients, nutrients can have potent postingestive reinforcing actions that increase bout size, total daily intake, and flavor preference [17,19,20]. Relatively little is known about the physiological processes and pathways that mediate this nutrient reinforcing effect. The study of flavor-nutrient conditioning in different inbred mouse strains and genetically altered mice may enhance our understanding of the postingestive controls of food selection and flavor preferences.

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