Sugar and Fat Conditioned Flavor Preferences in C57BL/6J and 129 Mice:
Oral and Postoral Interactions

Anthony Sclafani1 and John I. Glendinning2

1Department of Psychology, Brooklyn College and the Graduate School,
The City University of New York, Brooklyn, NY 11210

2Department of Biological Sciences, Barnard College, Columbia University,
New York, NY 10027, USA

Running Head: Sugar and Fat Conditioning

Contact Information:
Anthony Sclafani, Ph.D.
Department of Psychology
Brooklyn College of CUNY
2900 Bedford Ave.
Brooklyn, NY 11210
Tel: 718-951-5606 Fax: 718-951-4824
E-mail: Asclafani@gc.cuny.edu

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ABSTRACT

Anthony Sclafani and John I. Glendinning. Sugar and Fat Conditioned Flavor Preferences in C57BL/6J and 129 Mice: Oral and Postoral Interactions. Am J Physiol Regul Integr Comp Physiol. C57BL/6J (B6) mice consume more sugar and fat solutions than do 129 mice in 24-h preference tests. Previous studies have attributed this observation to strain differences in taste responsiveness to these nutrients. Here, we tested the hypothesis that differences in postingestive responsiveness contribute to the strain differences. In Experiments 1, B6 and 129 mice were trained to associate consumption of a flavored solution (CS+) with intragastric (IG) infusions of 16% sucrose and a different flavored solution (CS-) with IG water infusions (22 h/day). They were then retrained with new flavors paired with IG infusions of 5.6% soybean oil and water. Although both strains developed preferences for the nutrient-paired CS+ solutions, the B6 mice displayed significantly stronger preferences. The B6 mice consumed more CS+ during training, which may have contributed to their enhanced preference. In a second experiment, training intakes were equated by giving B6 and 129 mice “isosweet” CS solutions prepared with different amounts of sucrose and saccharin. The B6 and 129 mice consumed more of the sugar or fat paired CS+ than the water-paired CS- during training. The two strains also displayed equally strong preferences for the CS+ over CS- in choice tests, indicating that they had similar postingestive responsiveness to the sucrose and soybean oil. We propose that B6 mice consume more sugar and fat than 129 mice because their stronger orosensory response stimulates greater intake, which leads to greater stimulation of postingestive nutrient detectors and further enhancement of consumption.

Keywords:
Sweet taste, sucrose, saccharin, soybean oil, gastric infusions, drinking patterns

Footnote: Address for reprint requests and other correspondence: Anthony Sclafani, Department of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210 USA. e-mail: Asclafani@gc.cuny.edu
INTRODUCTION

Inbred mouse strains differ substantially in their acceptance (total intake) and preference (intake relative to water) for various nutritive and non-nutritive solutions including sugar, maltodextrin, oil, artificial sweeteners, and monosodium glutamate (2-4; 8; 14). This differential intake has been attributed in large part to strain differences in orosensory responsiveness to these substances. In particular, allelic variation in the \textit{Tas}1r3 gene, which encodes a G protein-coupled sweet taste receptor, has been found to play a central role in determining strain differences in taste responsiveness to sugars and artificial sweeteners (15; 20). Less is known about the sensory basis for strain differences in the appetite for oil or high-fat foods. The present study investigated the interaction of oral and postoral factors in the daily intake of sugar and fat solutions in mice using a gastric conditioning procedure.

While strains of mice differ in the intake of noncaloric (saccharin, sucralose, acesulfame K) and caloric (sucrose, glucose, maltose) sweeteners (4; 7; 9; 14), differences in peak sweetener intakes are greater for sugars (4; Glendinning and Sclafani, unpublished data). This may be because sugars are more effective in stimulating sweet receptors than are artificial sweeteners. However, it is also possible that differences in caloric and noncaloric sweetener intakes are due to the postoral actions of sugars. Extensive research on rats fitted with intragastric (IG) catheters demonstrates that co-infusing sugar as the animal drinks a flavored solution increases the intake and preference for that solution (18; 24). We recently reported a similar postoral conditioning effect in C57BL/6J mice (23). On one-bottle training days, the mice consumed almost twice as much of a flavored saccharin solution (the conditioned stimulus, CS+) that was paired with IG infusions of 8% maltodextrin (a glucose polymer) than they did of a differently flavored saccharin solution (the CS-) paired with IG water infusions. In a subsequent choice test the mice displayed a 90% preference for the CS+ solution over the CS- solution. Whether inbred mouse strains differ in their postoral conditioning response to sugars as they do in their oral taste response is not known. This question is of particular interest in view of recent studies reporting the expression of T1R2 and T1R3 sweet receptor proteins, as well as other taste signaling proteins (T1R1, T2Rs, gustducin) in the rodent stomach and
The role, if any, of these gut taste receptor proteins in postoral flavor conditioning is not known.

The present study examined the hypotheses that the postoral actions of sucrose contribute to its preference and overconsumption by mice and that differences in the responsiveness to these postoral actions mediate, in part, strain differences in sucrose consumption. Mice from two well-studied strains, the sweet “sensitive” C57BL/6J (B6) strain and the sweet “subsensitive” 129 strain, were trained with a CS+ flavored solution (e.g., cherry) paired with co-infusions of 16% sucrose, and another flavored solution (e.g., grape) paired with co-infusions of water. The oral intake and IG infusion were matched in volume so that the sugar concentration in the stomach was diluted to 8%. Previous studies indicate that peak differences in 24 h sucrose intakes between B6 and 129 mice occur at 4 to 8% concentrations (4;21). In Experiment 1, both unsweetened and saccharin-sweetened CS solutions were used because in our prior study IG maltodextrin infusions conditioned a stronger preference and greater acceptance in B6 mice trained with sweet rather than nonsweet solutions (23). Saccharin solutions, however, are much less attractive to 129 mice than to B6 mice which might impair flavor conditioning in 129 mice (4). In Experiment 2, therefore, B6 and 129 mice were trained using “isosweet” sucrose plus saccharin mixtures. Mixtures of dilute sugar and saccharin are very attractive to rats and mice (5;29), and a pilot study determined two mixtures of different concentrations that stimulated solution intakes to the same degree in B6 and 129 mice.

In addition to measuring the conditioning responses to IG sucrose infusions, the responses of the 129 and B6 mice to IG soybean oil infusions were also compared. This was done to determine if any deficit in sucrose conditioning in 129 mice was specific to sugar or represented a more general deficit in postoral nutrient conditioning. Possible strain differences in fat conditioning were of particular interest given the report of reduced preference and acceptance for soybean oil in 129 mice compared to B6 mice (2). Strain differences were observed with dilute (1%, 3%, 10%) but not concentrated (30% or 100%) oil which suggested that they were related to the orosensory perception of the oil rather than to fat appetite,
per se (2). Studies in rats, however, demonstrate that the postoral actions of nutritive oils condition flavor preferences (13). Thus, strain differences in oil preference and acceptance may be related to postoral as well as orosensory factors.

METHODS

Subjects

In Experiment 1 male C57BL/6J (B6; n=11) and 129P3/J (129; n=9) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 7 and 4 weeks of age, respectively. The animals were initially housed in pairs in standard plastic cages in a room maintained at 22 ºC with a 12:12 h light-dark cycle. Ten days prior to surgery, which occurred at 11 weeks of age, the animals were singly housed in infusion test cages. Experiment 2 used male C57BL/6J (n=9) and 129X1/SvJ (n=8) mice obtained from the Jackson Laboratory at 11 weeks of age. The 129X1/SvJ substrain (formerly, 129/SvJ) was used because mice from the 129P3/J substrain were unavailable; both substrains have the same Tas1r3 allelic variant (16;20) and both underconsume saccharin solutions compared to B6 mice (4;27). The animals were housed in pairs in infusion test cages (see below) for 5 days and then singly housed for 8 days prior to surgery at 13 weeks of age. Purina Chow (5001, PMI Nutrition International, Brentwood, MO) and tap water were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at Brooklyn College.

Gastric Surgery

The mice were anesthetized with isoflurane (2%) inhalation and were fitted with a gastric catheter constructed of micro-renathane tubing (0.033 in OD x 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 into the stomach through a small incision in the greater curvature and secured with a purse-string suture (6-0 silk). A polypropylene mesh (7 mm square), which was pre-positioned 2 cm from the catheter tip, was fixed against the stomach and was kept in place by a second silastic collar. 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 2 cm through a hole in the skin. The tip of the catheter was closed with a stainless-steel stylet. The abdominal incision was closed using Nexaband adhesive (Veterinary Products Laboratories, Phoenix, AZ) and the skin incision was sutured closed (5-0 silk) and treated with triple antibiotic ointment. In a second procedure performed 3-7 days later, the mice were briefly (~ 5 min) anesthetized with isoflurane and the gastric catheter was extended with a 27-cm length of micro-renathane tubing. The tubing passed through an infusion harness with a spring tether (CIH62, Instech Laboratories, Plymouth Meeting, PA) that was fitted to the mouse. Following each procedure, the mice were allowed to recover in small, heated plastic containers before being returned to their home cages.

Prior to the surgery, the mice were trained to consume a palatable, wet mash consisting of powdered chow and 8% maltodextrin (3:2). Consumption of this diet by the mice after surgery facilitated recovery.

Apparatus

Testing occurred in custom-made infusion cages (15 x 15 x 32 cm high) constructed of clear plastic with a stainless-steel perforated floor. Fluid was available from one or two stainless steel sipper spouts attached to 50-ml plastic centrifuge tubes. The mice licked the spouts through two slots (5 x 20 mm, 32 mm apart) in a stainless steel plate at the front of the cages. Chow pellets were available from a stainless steel wire mesh tube that entered the back wall of the cage.

After the mouse was fitted with the infusion harness, the spring tether was connected to a swivel on a counterbalanced lever (Instech Laboratories) positioned at the top of the cage. The output port of the swivel was connected to the mouse’s gastric catheter tubing and the input port was connected to a 30-ml plastic syringe mounted in a syringe pump (A-99, Razal Scientific, Stamford, CT). Licking was monitored by an electronic lickometer (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 sec. The pump rate was 0.5 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 26 g 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.

Test Solutions

In Experiment 1A, the CS solutions contained 0.05% (w/w) cherry or grape unsweetened Kool-Aid Mix (General Foods, White Plains, NY) in tap water. These citric acid-based drink mixes share a “sour” taste but have distinctive odors to humans. The grape and cherry solutions are equally unpreferred relative to plain water by the B6 and 129 mice (unpublished findings). In a second phase of the experiment, the same flavored solutions were sweetened with 0.2% sodium saccharin (Sigma Chemical Co., St. Louis, MO). The IG infusates were water or 16% (w/w) sucrose (Domino Sugar, Inc., Yonkers, NY) in water. For half the mice, cherry was the CS+ flavor paired with IG sucrose infusion, and grape was the CS- flavor paired with water infusion; for the other half of the mice, the flavor-infusate pairs were reversed. Because the orally consumed CS+ solution was mixed with equal volumes of gastrically infused 16% sucrose solution, the final sugar concentration in the gut was 8%.

In Experiment 1B, the CS solutions contained 0.05% orange or lemon-lime Kool-Aid mix added to 0.2% saccharin solutions. The CS+ and CS- solutions were paired with IG infusions of a 6.4% soybean oil emulsion and water, respectively. The 6.4% oil emulsion was isocaloric to the 16% sucrose used in Experiment 1A (6.4 kcal/ml) and was prepared by diluting 20% Intralipid (Baxter Healthcare Corp, Deerfield, IL) with tap water.

In Experiment 2A and 2B the same flavor pairs were used but were presented in isosweet solutions designed to stimulate approximately equal intakes in the B6 and 129 mice. Based on the results of a pilot study, the B6 mice were tested with CS solutions containing 0.4% sucrose and 0.04% saccharin, while the 129 mice were tested with solutions containing 2% sucrose and 0.2% saccharin. The IG infusates were adjusted to account for the different CS solutions presented to the two strains. In Experiment 2A, the 129
mice were infused with 14% sucrose and water, respectively, as they drank the CS+ and CS- solutions. The B6 mice were infused with 15.6% sucrose plus 0.16% saccharin when they consumed the CS+ and 1.6% sucrose plus 0.16% saccharin when they consumed the CS-. Thus, for both strains, the combination of oral CS solution and IG infusate yielded a mixture in the stomach containing 8% sucrose plus 0.1% saccharin on CS+ training days, and a mixture containing 1% sucrose plus 0.1% saccharin on CS- training days. In Experiment 2B, the 129 mice were infused with 5.6% soybean oil when they drank the CS+, and water when they drank the CS-. The B6 mice were infused with a mixture of 5.6% soybean oil, 1.6% sucrose and 0.16% saccharin when they consumed the CS+, and a mixture of 1.6% sucrose plus 0.16% saccharin when they consumed the CS-. Thus, for both strains, the combination of oral CS solution and IG infusate yielded a mixture in the stomach containing 2.8% soybean oil, 1% sucrose, and 0.1% saccharin on CS+ training days, and a mixture containing 1% sucrose plus 0.1% saccharin on CS- training days. Note that these mixtures of oral and IG infusates were isocaloric to those used in Experiment 2A.  

**Procedures and Experiments**

**Experiment 1.** The mice were housed in the test cages with chow and water ad libitum. In addition, 2 g of wet mash was given for 2 days before and after surgery. The mice were adapted to IG infusions by being infused with water as they drank water from the sipper tube for 3-4 days. In Experiment 1A, there were 6 one-bottle training days with the CS+ solution paired with IG infusions of 16% sucrose (days 1, 3, 5) and the CS- solution paired with IG infusions of water (days 2, 4, 6). A two-bottle choice test (the “reinforced test”) was then conducted for 2 days with the CS+ vs. CS-, each paired with the same infusions used during training. The mice were then retrained for 6 days with the same CS+ and CS- solutions now containing saccharin. A reinforced two-bottle test was conducted as above followed by a second test (the “nonreinforced test”) in which intakes of both the CS+ and CS- solutions were paired with IG water infusions for 2 days.  

The mice were given plain water to drink for 2 days prior to the start of Experiment 1B. They were then given 6 one-bottle training days with new saccharin-sweetened CS solutions (orange and lemon-
lime); the CS+ and CS- were paired with IG soybean oil and water infusions, respectively. A two-bottle reinforced test (2 days) was then conducted.

The B6 mice were tested 3 months before the 129 mice. Identical procedures were used so the data are presented together.

**Experiment 2.** The new B6 mice and 129 mice used in the second experiment were tested concurrently. The animals were adapted to the test cages as in the first experiment. In Experiment 2A they were trained for 6 days with the isosweet CS solutions. The CS+ was paired with IG sucrose and the CS- was paired with IG water (129 mice) or the dilute sucrose plus saccharin mixture (B6 mice). A reinforced two-bottle test was then conducted with the CS+ vs. CS- followed by a nonreinforced test for 2 days each. In the nonreinforced test, the CS+ and CS- were both paired with IG water infusions for the 129 mice and with 1.6% sucrose and 0.16% saccharin for the B6 mice. In Experiment 2B the mice were trained and tested with new isosweet CS solutions (orange and lemon-lime); the CS+ was paired with IG soybean oil and the CS- was paired with IG water (129 mice) or the dilute sucrose plus saccharin mixture (B6 mice). Due to an error, training proceeded for 8 rather than 6 days. Reinforced and nonreinforced CS+ vs. CS- tests were then conducted for 2 days each. One 129 mouse died during Experiment 2B and its data were excluded.

The position of the CS+ and CS- solutions in the infusion cages (i.e, left-right) was counterbalanced throughout training and testing to control for potential side preferences. Chow was available ad libitum throughout the experiment. The CS solutions were available 22 h/day; the infusion cages and equipment were serviced during the remaining 2 h/day.

**Data Analysis**

In all experiments, oral intakes were averaged over one-bottle training and two-bottle test days and were evaluated with analysis of variance (ANOVA) procedures (Strain x CS; Strain x CS x Test). The two-bottle intakes of the individual mice were also expressed as percent CS+ intakes (CS+ intake/total intake x 100). These data were analyzed with t-tests or ANOVA following an arcsine transformation as
recommended by Kirk (12). In Experiment 2A the one-bottle data were analyzed in detail by comparing CS+ and CS- intakes and drinking patterns over the three training days with each solution (Strain x CS x Day). A drinking bout was defined as a period of drinking containing at least 30 licks and with interlick intervals no longer than 5 min [23]. Mean bout size (g) was determined by dividing the total 22-h CS intakes by the number of bouts. Significant interaction effects were evaluated using simple main effects tests (SMET) according to Winer (30); the CRUNCH4 software package (Crunch Software Corp) was used for statistical analysis. Differences between means were considered statistically significant if \( P < 0.05 \).

RESULTS

Experiment 1A: Sucrose conditioned preferences for unsweetened and sweet flavors.

The B6 weighed more than 129 mice at the time of surgery (24.9 vs. 22.0 g, \( t(19) = 4.095, P < 0.001 \)). Prior to the start of training, the B6 mice consumed slightly, but not significantly more water than did the 129 mice (3.8±0.4 and 2.9±0.4 g/day) and were infused with matched amounts of water IG. Figure 1 presents the intakes of the unsweetened CS solutions during one-bottle training and the two-bottle test. Overall, CS training intakes were greater in B6 mice than 129 mice (\( F(1,18) = 7.75, P < 0.05 \)) and CS+ intakes exceeded CS- intakes (\( F(1,18) = 5.24, P < 0.05 \)). There was a trend for a strain by CS interaction (\( F(1,18) = 4.34, P = 0.0504 \)); this stemmed from the fact that only the B6 mice consumed more (\( P < 0.01 \)) CS+ than CS-. The B6 mice also consumed more (\( P < 0.01 \)) CS+ than did the 129 mice but the strains did not differ in CS- intakes. In the two-bottle test, the B6 but not the 129 mice consumed significantly more CS+ than CS-, and the B6 mice consumed more CS+ than the 129 mice (CS x Strain interaction, \( F(1,18) = 8.28, P < 0.01 \)). The percent CS+ intake of the B6 mice also exceeded that of the 129 mice (84% vs. 62%, \( t(18) = 2.86, P < 0.05 \)).

As illustrated in Figure 2, there was considerable variability in the magnitude of conditioned preferences for the CS+ within both strains. Further analysis revealed that the magnitude of the CS+ preferences during the two-bottle test was positively correlated with CS+ intakes during training (\( r^2 \))
In addition, CS+ training intakes were correlated with baseline water intakes \( r^2 = 0.636, P < 0.001 \). Thus, the mice that consumed the most water also consumed the most CS+ during training and developed the strongest conditioned preference for the unsweetened CS+. A logical implication of this analysis is that the B6 mice acquired a stronger preference than the 129 mice for the CS+ simply because they consumed more of the CS+ and were infused with more sucrose during training. Note that while the B6 mice weighed slightly more than the 129 mice at the start of this study, the strain differences in CS training intakes remained essentially unchanged when intakes were analyzed as intakes/30 g body weight (data not shown).

Figure 3 presents intakes of the saccharin-sweetened CS+ and CS- solutions. Overall, B6 mice consumed substantially more of the CS solutions in training than did the 129 mice \( F(1,18) = 105.10, P < 0.001 \); this difference was most pronounced for the CS+ solution \( F(1,18) = 16.00, P < 0.001 \). SMET indicated that both B6 and 129 mice consumed more CS+ than CS- during training \( P < 0.001 \) and \( P < 0.05 \), respectively. Both strains also consumed substantially more of the CS+ than CS- in the reinforced and nonreinforced two-bottle choice tests \( F(1,18) = 179.47, P < 0.001 \).

Overall, the B6 mice consumed more than did the 129 mice during these tests with the difference being greatest for the CS+ solution \( F(1,18) = 67.47, P < 0.001 \). The mice consumed more of the CS+ solution during the reinforced than the nonreinforced choice tests \( F(1,18) = 7.77, P < 0.05 \). Analysis of the percent CS+ intake data indicated no overall difference in CS+ preferences between the two tests or between the strains, although there was a Test x Strain interaction \( F(1,18) = 8.95, P < 0.01 \). The percent CS+ intake of the B6 mice exceeded that of the 129 mice in the nonreinforced test (95% vs. 84%, \( P < 0.05 \)). Further analysis revealed that the magnitude of the CS+ preference in the nonreinforced test was positively correlated with the intake of the sweetened CS+ during one-bottle training \( r^2 = 0.332, P < 0.01 \); the CS+ preference in the reinforced test and training intakes were not significantly correlated.

The B6 and 129 mice consumed more of the sweetened CS solutions during training than they did of the unsweetened CS solutions in the initial part of the study \( F(18) = 74.45, P < 0.001 \). This difference
was greater for the B6 mice than for the 129 mice ($F(18) = 51.05, P < 0.001$) and for the CS+ solution than for the CS- solution ($F(18) = 59.73, P < 0.001$).

***** Insert Figures 1, 2, and 3 About Here *****

**Experiment 1B: Soybean oil conditioned preferences for sweet flavors.**

When trained with new sweetened flavors (orange or lemon-lime) paired with IG soybean oil or water infusions, the B6 consumed more than did the 129 mice ($F(1,18) = 36.48, P < 0.001$; Figure 4). Overall, CS+ training intakes exceeded CS- intakes ($F(1,18) = 43.66, P < 0.001$), although this difference was significant only in the B6 mice (CS x Strain, $F(1,18) = 14.72, P < 0.01$). In the two-bottle choice test, both strains consumed substantially more CS+ than CS- ($F(1,18) = 58.88, P < 0.001$). Overall, the B6 mice consumed more CS solution during the test than did the 129 mice ($F(1,18) = 27.93, P < 0.001$) with the difference being greatest for the CS+ (CS x Strain, $F(1,18) = 20.28, P < 0.001$). The percent CS+ intake of the B6 mice exceeded that of the 129 mice but this difference just failed to reach significance (96% vs. 80%, $t(18) = 2.04, p< 0.056$). The magnitude of the CS+ preference in the choice test, however, was positively correlated with the intake of the sweetened CS+ during one-bottle training ($r^2 = .385, P < 0.01$).

***** Insert Figure 4 About Here *****

**Experiment 2A: Sucrose conditioned preferences for isosweet flavors.**

The body weights of the B6 and 129 mice were similar (25.4 and 23.8 g) at the time of surgery as were their water intakes (4.1±0.3 and 3.6±0.1 g/day) prior to training. Figure 5 presents the intakes of the isosweet CS solutions during one-bottle training and two-bottle testing. The B6 and 129 mice consumed similar amounts of solution during training and both strains drank significantly more CS+ than CS- ($F(1,15) = 53.83, P < 0.001$). Both strains also consumed substantially more CS+ than CS- during the reinforced and nonreinforced two-bottle choice tests ($F(1,15) = 237.51, P < 0.001$). The CS intakes of the two strains were similar in the reinforced test, but differed in the nonreinforced test (CS x Strain, $F(1,15) = 7.96, P < 0.05$); the 129 mice consumed more CS+ than did B6 mice ($P < 0.01$). Analysis of the percent
CS+ intakes also indicated the two strains showed similar, robust CS+ preferences in the reinforced test (98% and 96%), but the CS+ preference of the 129 mice exceeded ($P < 0.05$) that of the B6 mice in the nonreinforced test (99% vs. 91%; CS x Strain, $F(1,15) = 4.73, P < 0.05$).

Although the strains did not differ in their CS+ training intakes or CS+ preference in the reinforced test, there was some within-strain variability in the conditioning response. Further analysis indicated that the training intake of the isosweet CS+ solutions was positively correlated with the CS+ preference in the reinforced test ($r^2 = 0.398, P < 0.001$) and, to a lesser degree, in the nonreinforced test ($r^2 = 0.249, P < 0.05$).

A detailed analysis of the one-bottle training data revealed that CS intakes changed over days and that the two strains differed in their drinking patterns (Figure 6). In both strains, intakes of the CS+ but not the CS- increased from the first to last training day (CS x Day, $F(2,30) = 50.58, P < 0.001$). This trend was most pronounced in the 129 mice (Strain x CS x Day, $F(2,30) = 3.48, P < 0.05$) although, as noted above, the strains did not differ in their mean CS+ intakes during training or testing. Mean bout number ($F(1,15) = 50.97, P < 0.001$) and, to a lesser extent, mean bout size ($F(1,15) = 5.18, P < 0.05$) were greater on CS+ than CS- training days, and these differences increased over training (CS x Days, number: $F(2,15) = 11.20, P < 0.001$; size: $F(2,15) = 5.65, P < 0.01$). Overall, CS+ and CS- bout numbers were lower ($F(1,15) = 7.77, P < 0.05$) and bout sizes were greater ($F(1,15) = 5.84, P < 0.05$) in 129 mice than B6 mice. An analysis of the drinking patterns on the two water baseline days that preceded CS training revealed a similar pattern of strain differences. Water bout size was larger while bout number were smaller in 129 mice than in B6 mice (size: 0.19 vs. 0.15 g/bout, $t(15) = 2.99, P < 0.01$; number: 21.5 vs. 30.1 bouts/day, $t(15) = 4.82, P < 0.01$).

Note that the 129 mice in Experiment 2A consumed significantly more of the 2% sucrose plus 0.2% saccharin CS solutions during training than the 129 mice in Experiment 1A consumed of the 0.2% saccharin CS solutions ($F(1,16) = 83.67, P < 0.001$), with the difference being greatest for the CS+ solutions (CS x Experiment interaction, $F(1,16) = 13.42, P < 0.01$). On the other hand, the B6 mice in the
two experiments did not differ in their intakes of the 0.2% saccharin and 0.4% sucrose plus 0.04% saccharin CS solutions.

Experiment 2B: Soybean oil conditioned preferences for isosweet flavors.

Figure 7 summarizes the results of the soybean oil conditioning experiment conducted with the isosweet orange and lemon-lime flavors. The mice consumed significantly more CS+ than CS- during one-bottle training \( (F(1,14) = 103.19, P < 0.001) \) and there were no strain differences in intake. The B6 and 129 mice also consumed substantially more CS+ than CS- during both the reinforced and nonreinforced two-bottle tests \( (F(1,14) = 103.09, P < 0.001) \) and the strains did not differ in their CS intakes. Overall, the mice drank more CS+ in the reinforced test than in the nonreinforced test \( (CS \times Test, F(1,14) = 13.52, P < 0.01) \). The percent CS+ intakes were somewhat higher in the 129 mice than B6 mice, and in the reinforced test than nonreinforced tests, but these differences were not significant. In addition, CS+ preferences in the reinforced and nonreinforced tests were not correlated with CS+ intakes during one-bottle training.

***** Insert Figures 5, 6 and 7 About Here *****

DISCUSSION

The present findings demonstrate that intragastric infusions of sucrose condition flavor preference and acceptance in both B6 and 129 mouse strains, and that the magnitude of the effect is strongly influenced both by the sweetness of the conditioning flavors and by the quantity of CS+ consumed and IG sucrose infused during training. IG soybean oil infusions also conditioned strong preferences for sweetened flavors in both strains. The results for each type of nutrient are discussed in turn.

Sucrose Conditioning

Similar to our prior conditioning results obtained with IG maltodextrin infusions (23), B6 mice learned to prefer flavors paired with IG sucrose infusions, with the preference being stronger for sweetened CS (96-98%) than unsweetened CS (88%) flavors. The 129 mice also learned to prefer sucrose-paired flavors, but in this case a significant effect was obtained only with sweetened CS solutions. In Experiment 1, the 129 mice displayed weaker preferences than did the B6 mice for both the unsweetened CS+ (62%
vs. 88%) and saccharin-sweetened CS+ (88% vs. 98%) solutions paired with IG sucrose infusions. However, overall training intakes of the 129 mice were lower than those of the B6 mice. This indicates that reduced exposure to the CS+ and IG sucrose during training rather than a reduced responsiveness to the postoral effects of sucrose accounts for the lower CS+ preferences of the 129 mice. Consistent with this view, the magnitude of two-bottle preference for the unsweetened CS+ was correlated positively with the amount of CS+ consumed, and therefore IG sucrose infused, during one-bottle training for both the 129 and B6 mice, which confirms prior B6 results (23). Note that the 88% preference for the unsweetened CS+ displayed by the B6 mice in Experiment 1A was greater than the 70% preference observed in our prior study (23) in which the CS+ was paired with infusions of 8% rather than 16% carbohydrate. This is further evidence that flavor preference conditioning is influenced by amount of nutrient reinforcement received during training. There was also a positive correlation between training intake of the sweetened CS+ and CS+ preference, although this was significant only for the nonreinforced test data.

The finding that the 129 mice consumed less of the saccharin-sweetened CS solutions than did the B6 mice confirms the many prior studies documenting their reduced responsiveness to low concentrations of this artificial sweetener (4;7;11). On the other hand, the finding that 129 mice consumed less than B6 mice of the unsweetened CS solutions, which had a mildly “sour” taste, cannot be attributed to strain differences in the preference for “sour” solutions in general (1), or unsweetened Kool-Aid solution in particular (Sclafani and Glendinning, unpublished observations). Rather, it appears to reflect the general tendency of 129 mice to consume less fluid, including water, than do B6 mice (28; Sclafani, unpublished data). Baseline water intakes were, in fact, correlated with the training intakes of the unsweetened CS+.

In Experiment 2A the problem of differential training intakes was eliminated by offering the 129 and B6 mice “isosweet” CS solutions containing different concentrations of sucrose and saccharin. The two strains consumed very similar amounts of the CS solutions during one-bottle training. Furthermore, both strains consumed about twice as much CS+ than CS- during training which demonstrates that the IG sucrose infusions produced increased flavor acceptance. The fluid intakes on CS+ training days were
extraordinary; total intakes (oral plus IG infusion) of the B6 and 129 mice (34.4 and 37.2 g/day) exceeded the animals’ body weights at the time (26.3 and 24.7 g). Following training, the B6 and 129 mice both displayed extremely high (96-98%) preferences for the sucrose-paired CS+ in the two-bottle choice test.

A detailed analysis of the Experiment 2A training data revealed that CS+ but not CS- intakes increased over days, which confirms prior results obtained with B6 mice (23) and is indicative of a conditioning process (18). The 129 mice increased their CS+ intake more than did the B6 mice, which suggests a stronger conditioning response although the CS+ intakes of the two strains did not differ in the subsequent two-bottle reinforced test. The strains significantly differed in their drinking patterns, with the 129 mice consuming larger and less frequent CS bouts than did the B6 mice. Importantly, the strains displayed similar drinking pattern differences during the water baseline period which indicates that the CS bout pattern data were probably not due to the different sucrose and saccharin solutions used to train the 129 and B6 mice. The bout data revealed that the mice overconsumed the CS+, relative to the CS- and water, by increasing daily bout number and, to a smaller extent, bout size. This contrasts with our earlier report of increased bout size but not bout number in B6 mice trained with a CS+ paired with IG infusions of 8% maltodextrin (23). It may be that the greater energy density and/or osmolarity of the 16% sucrose infusions used in the present study limited the degree to which the mice could increase their bout size on CS+ training days. Taken together, the present and prior data indicate that IG carbohydrate infusions condition increased flavor acceptance in mice, that the animals accomplish this by increasing their bout size and/or number, and that B6 and 129 mice differ in their drinking patterns.

In Experiments 1A and 2A, the mice displayed significant preferences for the CS+ when it was paired with IG sucrose (reinforced test) or IG water (nonreinforced test). This indicates that the mice had acquired a true preference for the CS+ flavor and were not selecting it because of a direct response to the concurrent IG sugar infusions. Nevertheless, absolute CS+ intakes tended to decline in the nonreinforced test and this replicates prior findings obtained in mice and rats (17;23). The reduced CS+ intake reflects a partial loss of the conditioned acceptance response to the CS+ flavor which, according to earlier studies,
extinguishes more rapidly than does the conditioned preference for the CS+ (17). In Experiment 2A, CS+ preference and acceptance declined only in the B6 mice during the nonreinforced test. Presumably, with extended nonreinforced testing CS+ intakes would have also declined in the 129 mice. Nevertheless, their resistance to extinction, relative to the B6 mice, combined with their greater increase in CS+ intake over training days suggests that the 129 mice had acquired a stronger attraction to the sucrose-paired CS+ flavor. This would appear incompatible with the characterization of 129 mice as being less sensitive to sweeteners. However, a recent study investigating sugar motivation using a progressive ratio licking task revealed that 129 mice licked more for 16% sucrose reinforcements than did B6 mice (22).

The results of this study confirm the hypothesis that the postoral actions of sucrose enhance sweetener preference and acceptance in mice. The mice consumed more of the sweetened CS+ that was paired with IG sugar solutions than the water-paired CS- during one-bottle training and two-bottle testing. The findings also support the hypothesis that these postoral actions contribute to strain differences in sugar intake. In particular, the B6 mice consumed more than did the 129 mice of the saccharin-sweetened CS solutions in Experiment 1, and this difference was most pronounced for the CS+ solution paired with concurrent sucrose infusions. Yet, the results of the second experiment indicate that the 129 and B6 mice did not differ in their sensitivity to the postoral actions of sucrose when tested with isosweet CS solutions. Taken together, the data suggest that, relative to 129 mice, B6 mice overconsume intermediate concentrations of sucrose because their stronger taste responsiveness stimulates greater sugar intake, leading to greater stimulation of postoral nutrient detectors which, in turn, promotes further intake. With dilute sugar solutions and artificial sweeteners, postoral actions are minimal or absent and differential taste responsiveness alone can account for the strain differences in sweetener preference and acceptance. Note that B6 and 129 mice do not differ in their acceptance and preference for concentrated (32%) sugar solutions (4). In this case, strain differences in sweet taste responsiveness may be overridden by postoral factors, including unconditioned satiety and conditioned preference effects of sucrose.

Current evidence indicates that the gustatory response to sweeteners is mediated by the combined
action of T1R2 and T1R3 taste receptor proteins and that variations in the T1R3 receptor account for strain differences in sweetener preference and acceptance (15;20). Much less is known about the viscerosensory system that mediates the postoral effects of sugars. Recent reports of the expression of T1R2 and T1R3 sweet receptor proteins, as well as other taste signaling proteins (α-gustducin, T2Rs receptor proteins) in the gut raise the possibility that oral and visceral sensation may involve similar chemoreceptors (6;10;31). This issue requires further research, but the present data do not support a primary role for postoral T1R3 receptors in sucrose conditioning. That is, whereas B6 and 129 mice significantly differ in their oral ingestive response to 8% sucrose (4), Experiment 2 indicates that the two strains are similar in their postoral conditioning response to 8% sucrose. Their postoral response to sucrose at other concentrations, however, remains to be established.

Soybean oil Conditioning

Anticipating that the 129 and B6 mice might differ in their conditioned response to IG sucrose, the mice were also evaluated for IG fat conditioning. Overall, the pattern of strain differences obtained with soybean oil infusions was similar to those observed with sucrose infusions. In Experiment 1B, IG soybean oil induced a greater one-bottle acceptance and two-bottle preference for the saccharin-sweetened CS+ in B6 mice than in 129 mice. However, with the isosweet CS solutions in Experiment 2B, the two strains displayed similar conditioning responses to the soybean oil infusions. Together with the sucrose data, the soybean oil data indicate that B6 and 129 mice do not differ fundamentally in their ability to associate flavors with postingestive nutrient consequences. Bachmanov et al. (2) reported that, compared to B6 mice, 129 mice consumed less soybean oil at 1%, 3% and 10%, but not at 30% or 100% concentrations. They suggested that the reduced preference for dilute oil displayed by the 129 mice was likely due to oral rather than postoral factors. The present findings agree with this interpretation. This issue can be further examined by comparing the short-term licking response of B6 and 129 mice to oil as has been done with SWR/J and AKR/J mouse strains (26). Interestingly, of these four strains, the B6 and SWR/J are the most responsive to sweeteners and to oil suggesting a possible relationship between the T1R3 sweet receptor
and oil preference. On the other hand, a study examining macronutrient intake patterns in 13 inbred mouse strains revealed substantial differences in fat selection that appeared unrelated to known allelic variations in the T1R3 receptor (25). Much remains to be learned about the orosensory and viscerosensory determinants of fat preference and long-term fat selection.

In the present study, the mice displayed similar conditioning responses to the soybean oil and sucrose infusions. This contrasts with prior reports that corn oil infusions have weaker effects on flavor acceptance and preference in rats than do isocaloric carbohydrate infusions (13;19). Differences in training procedures and nutrient sources may account for these discrepant findings although it is also possible that rats and mice differ in their responsiveness to carbohydrates and fats.

Summary

The intake and selection of foods and fluids are greatly influenced by flavor cues, i.e., taste, smell and texture. Sweet taste, in particular, can stimulate ingestion in a variety of species. Genetic variations in sweet taste receptors significantly influence the consumption of caloric and noncaloric sweeteners as revealed by studies of inbred mouse strains. The present study of C57BL/6J and 129 mice confirmed this finding but also revealed that postoral nutrient actions can greatly amplify the stimulatory effect of flavor cues on ingestion. The mice learned to prefer and increase their acceptance of the flavored solutions that were associated with intragastric sugar or oil infusions. The animals discriminated the solutions based primarily on their odors (e.g., cherry vs. grape) but the magnitude of the conditioned response was greater when the odors were associated with a sweet taste. Thus, fluid choice and acceptance were determined by the integration of taste, odor, and postoral stimuli. Although the B6 and 129 strains differ in their sweet taste responsivity, when offered isosweet solutions they do not appear to differ in their response to the postoral actions of sugar or fat or their ability to integrate oral and postoral stimuli.
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Figure Captions

Figure 1. Consumption (in g/day) of the unsweetened CS+ and CS- solutions (grape or cherry) by C57BL/6J (n=11; left panel) and 129P3/J (n=9; right panel) mice when they were paired with IG infusions of 16% sucrose or water. Mean (+S.E.) Daily intakes of the CS+ and CS- solutions are shown for one-bottle training sessions and two-bottle preference test. Numbers atop bars represent mean percentage of CS+ consumed during the preference test calculated separately for each mouse. Data are from Experiment 1A.

Figure 2. Preference for unsweetened CS+ during two-bottle preference test plotted as a function of CS+ intake during one-bottle training sessions of C57BL/6J (n=11) and 129P3/J (n=9) mice. The CS+ solution (grape or cherry) was paired with IG infusions of 16% sucrose. Data are from Experiment 1A.

Figure 3. Consumption (in g/day) of the sweetened CS+ and CS- solutions (grape or cherry) by C57BL/6J (n=11; left panel) and 129P3/J (n=9; right panel) mice when they were paired with IG infusions of 16% sucrose or water. Mean (+S.E.) Daily intakes of the CS+ and CS- solutions are shown for one-bottle training sessions and two-bottle reinforced (Rein) and nonreinforced (NR) preference tests (see text for details). Numbers atop bars represent mean percentage of CS+ consumed during the preference test calculated separately for each mouse. Data are from Experiment 1A.

Figure 4. Consumption (in g/day) of the sweetened CS+ and CS- solutions (orange or lemon-lime) by C57BL/6J (n=11; left panel) and 129P3/J (n=9; right panel) mice when they were paired with IG infusions of 5.6% soybean oil or water. Mean (+S.E.) Daily intakes of the CS+ and CS- solutions are shown for one-bottle training sessions and two-bottle preference test. Numbers atop bars represent mean percentage of CS+ consumed during the preference test calculated separately for each mouse. Data are from Experiment 1B.

Figure 5. Consumption (in g/day) of the isosweet CS+ and CS- solutions (grape or cherry) by C57BL/6J (n=9; left panel) and 129X1/SvJ (n=8; right panel) mice when they were paired with IG infusions of sucrose or water. Mean (+S.E.) Daily intakes of the CS+ and CS- solutions are shown for
one-bottle training sessions and two-bottle reinforced (Rein) and nonreinforced (NR) preference tests (see text for details). Numbers atop bars represent mean percentage of CS+ consumed during the preference test calculated separately for each mouse. Data are from Experiment 2A.

Figure 6. Intakes and drinking bout patterns of the isosweet CS+ and CS- solutions (grape or cherry) by C57BL/6J (n=9; left panel) and 129X1/SvJ (n=8; right panel) mice over the three one-bottle training days with each solution. Mean (±S.E.) The dash line represents the mean water intake and bout data for the two days preceding the start of CS training. Data are from Experiment 2A.

Figure 7. Consumption (in g/day) of the isosweet CS+ and CS- solutions (orange or lemon-lime) by C57BL/6J (n=9; left panel) and 129X1/SvJ (n=7; right panel) mice when they were paired with IG infusions of soybean oil or water. Mean (+S.E.) Daily intakes of the CS+ and CS- solutions are shown for one-bottle training sessions and two-bottle reinforced (Rein) and nonreinforced (NR) preference tests (see text for details). Numbers atop bars represent mean percentage of CS+ consumed during the preference test calculated separately for each mouse. Data are from Experiment 2B.
Figure 1

Figure 2
Figure 3

Figure 4
Figure 7