Rapid post-oral stimulation of intake and flavor conditioning by glucose and fat in the mouse

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ABSTRACT

Although widely assumed to have only satiating actions, nutrients in the gut can also condition increases in intake in some cases. Here we studied the time-course of post-oral nutrient stimulation of ingestion in food-restricted C57BL/6J mice. In experiment 1, mice adapted to drink a 0.8% sucralose solution 1 h/day rapidly increased their rate of licking (within 4-6 min) when first tested with an 8% glucose solution and even more so in tests 2 and 3. Other mice decreased their licking rate when switched from sucralose to 8% fructose, a sugar that is sweet like glucose but lacks positive post-oral effects in mice. The glucose-stimulated drinking is due to the sugar’s post-oral rather than taste properties because sucralose is highly preferred to glucose and fructose in brief choice tests. A second experiment showed that the glucose-stimulated ingestion is associated with a conditioned flavor preference in both intact and capsaicin-treated mice. This indicates that the post-oral stimulatory action of glucose is not mediated by capsaicin-sensitive visceral afferents. In a third experiment, mice consumed flavored saccharin solutions as they self-infused water or glucose via an intragastric (IG) catheter. The glucose self-infusion stimulated ingestion within 13-15 min in test 1 and produced a conditioned increase in licking that was apparent in the initial min of tests 2 and 3. Experiment 4 revealed that IG self-infusions of a fat emulsion also resulted in post-oral stimulation of licking in test 1 and conditioned increases in tests 2 and 3. These findings indicate that glucose and fat can generate stimulatory post-oral signals early in a feeding session that increase ongoing ingestion and condition increases in flavor acceptance and preference revealed in subsequent feeding sessions. The test procedures developed here can be used to investigate the peripheral and central processes involved in stimulation of intake by post-oral nutrients.

Keywords: Conditioned flavor acceptance and preference; fructose; sucralose; capsaicin deafferentation; intragastric infusion
INTRODUCTION

The orosensory and post–oral nutritional properties of foods are important determinants of food intake and preference. The flavors of palatable foods, i.e., their taste, odor and texture, stimulate feeding whereas post-oral signals are often assumed to have only inhibitory action, via satiation signals that terminate a feeding bout and satiety signals that suppress post-meal eating (10; 24; 56). However, there is extensive evidence that nutrients can have other post-oral effects that condition food preferences and, in some cases, increase intake (42). This has been demonstrated, for example, by studies in which rodents are trained to drink a flavored non-nutritive solution paired with intragastric (IG) self-infusion of a nutrient. Typically, animals are given multiple training trials with one flavor (the conditioned stimulus or CS+) paired with a concurrent IG nutrient self-infusion and a second flavor (CS-) paired with IG water self-infusion on alternate training days. The conditioned preference is revealed in a subsequent two-bottle choice test with the CS+ vs. CS-. Conditioned flavor preferences have also been reported after only one brief (10 or 30 min) training session each with the CS+ and CS- flavors paired with IG glucose and water, respectively (2; 31). In these studies it was not clear how rapidly the infused nutrient generated the positive post-oral signal that conditions the flavor preference, i.e., within the training session or sometime after the session ends, because the critical CS+ vs. CS- choice test was not conducted until 24 h or more after the last training session.

One early study posited that IG nutrient infusions can rapidly generate signals from upper gastrointestinal receptors that condition flavor preferences (35). This was based on the finding that rats learned to prefer a CS+ paired with an IG milk self-infusion over a CS- paired with IG saline self-infusion during the first 10-min training session in which both CS flavors were available (35). However, in a subsequent study using a similar procedure, the CS+ flavor preference did not emerge until the fifth training session (64). In addition, the 10-min concurrent conditioning paradigm failed to condition a preference for a CS+ paired with an IG glucose infusion (35), which is surprising given that glucose, unlike milk, does not require digestion and is a very effective unconditioned stimulus in other studies (2; 31). We have observed “one-trial” glucose conditioning in rats given concurrent access to CS+ and CS-
flavors, but the training trials were 20-23 h in duration, which allowed the animals multiple discrete bouts
with the CS+ and its paired IG infusion (4; 17). On the other hand, rats given daily 30-min concurrent
training sessions failed to learn to prefer the CS+ flavor paired with IG glucose over 6 training trials
(Sclafani & Ackroff, unpublished data). To the extent that animals sample both CS+ and CS- flavors
during short training sessions, the post-oral nutrient feedback would have to be near-instantaneous for
them to learn which flavor produced the positive nutrient feedback, at least in the first two-bottle test.

The present study adopted a different approach to evaluate the time course over which ingested
nutrients generate positive post-oral feedback signals. In this case, animals were given daily one-bottle
tests with non-nutrient and nutrient solutions. IG nutrient infusions can increase the absolute intake of a
flavored solution in one-bottle tests, a process referred to as acceptance conditioning. Like conditioned
preference, conditioned acceptance involves a learned enhancement in the reward value of the flavor, as
indicated by the finding that intake remains elevated even when the flavored solution is no longer paired
with the IG nutrient infusion (42). Nutrient-conditioned flavor acceptance in rats given short (20-30 min)
training sessions was not evident until after multiple sessions (5 or more) (11; 32; 54). Preliminary
experiments in our laboratory led to the development of new test paradigms that revealed rapid (within 15
min) stimulation of ingestion by the post-oral actions of glucose in C57BL/6J (B6) mice. In the present
study we used these paradigms and lick rate analyses to investigate the post-oral nutrient stimulation of
intake and conditioned flavor acceptance within and between test sessions. The mice in Experiment 1
were adapted to drink a non-nutritive sucralose solution 1 h/day and were then switched to a less
preferred glucose or fructose solution. Fructose was of interest because it is sweet like glucose but has a
much weaker post-oral conditioning effect (4; 46; 47). Therefore, if glucose promoted greater
overdrinking than fructose this would implicate a post-oral stimulatory action. The second experiment
investigated whether glucose-stimulated drinking is associated with a conditioned flavor preference and if
the stimulated drinking and flavor preference requires visceral neural feedback. A third experiment more
directly measured post-oral stimulation of ingestion by glucose by having mice self-infuse the sugar IG as
they drank a non-nutritive saccharin solution. The fourth experiment used the IG self-infusion procedure to determine if the post-oral action of fat can also stimulate ingestion and condition flavor acceptance.

The results of these experiments revealed that glucose and fat produced post-oral stimulation of ingestion within 15 min in the first test session and conditioned increases in flavor acceptance and preference which were evident in subsequent test sessions. The test paradigms used here provide a time window on the stimulating post-oral effects of nutrients that can facilitate the study of gut nutrient signaling and central reward processing that promote food intake.

**Experiment 1. Glucose but not fructose stimulation of intake**

We recently observed that B6 mice equally preferred 8% glucose and 8% fructose solutions in 1-min two-bottle tests but strongly preferred an 0.8% sucralose solution to both sugar solutions (Zukerman and Sclafani, unpublished). Thus, at these concentrations, the two sugars appear to be equally palatable but less palatable than the non-nutritive sucralose. In 24-h choice tests the mice also preferred sucralose to fructose but drank substantially more glucose than sucralose. The opposite preferences observed with glucose and fructose in the 24-h tests are explained by the differential positive post-oral actions of the two sugars. IG infusion studies indicate that glucose conditions much stronger flavor preferences than does fructose in rats and mice (4; 46; 47)(Sclafani, unpublished observations). In Experiment 1 we investigated the time course over which glucose stimulates drinking, relative to sucralose. Food-restricted, sugar-naïve mice were adapted to drink a 0.8% sucralose solution during three daily 1-h sessions and were then switched to an 8% glucose solution for three sessions. A second group of mice was similarly tested but were switched from sucralose to an 8% fructose solution. The drinking response to the three sweeteners was monitored by recording licks every min. Based on prior findings obtained with the sugars (4; 46; 47), we predicted that glucose but not fructose would stimulate more licking than sucralose but whether this would occur in the first or subsequent test sessions was uncertain.

Methods

**Animals.** Adult male B6 mice (12 weeks old) born in the laboratory from Jackson Laboratories (Bar Harbor, ME) stock were singly housed in plastic tub cages kept in a test room maintained at 22°C
with a 12:12-h light-dark cycle. The mice were maintained on chow (5001, PMI Nutrition International, Brentwood, MO) prior to food restriction. During testing they were fed fixed-size chow pellets (0.5 or 1 g, F0171, F0173, Bio-Serv, Frenchtown, NJ) which allowed for precise adjustment of the daily food ration. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**Apparatus.** Drinking tests were conducted in plastic test cages where fluid was available from one or two stainless-steel sipper spouts through slots (5 x 20 mm, 32 mm apart) at the front of the cage. Motorized bottle holders positioned the sipper spouts 1 mm in front of the slots at the start of a trial and retracted them at the end of the trial as previously described (65). Licking behavior was monitored with electronic lickometers (ENV-250B, Med Associates) interfaced to a computer. In this and subsequent experiments, the assessment of licks per min began with each animal’s first non-zero 1-min bin to remove the effects of differences in latency to begin licking in the session.

**Procedure.** The mice (n=22) were adapted to drink in the test cages by housing them overnight with ad libitum access to water and food. They were then returned to their home cages and two days later were water restricted overnight. The next day they were given 1-h access to the 0.8% sucralose (Tate & Lyle, Dayton, OH) solution in the test cage. (Water restriction in this and subsequent experiments was used to stimulate drinking during the initial sessions.) After this session, the mice were given ad libitum access to water in their home cages but food access was restricted (2-3 g/day) to maintain their body weights at 85-90% of ad libitum level. While food restricted, the mice were given three test sessions (1 h/day) with the sucralose solution. They were then divided into two groups (n=11 each) equated for their sucralose licking response (mean of sessions 2 and 3) and body weight. The Glucose group was tested with 8% glucose for three daily 1-h sessions while the Fructose group was tested with 8% fructose. The sugars were food grade (Tate & Lyle, Dayton, OH) and obtained from Honeyville Food Products, Rancho Cucamonga, CA.
Data Analysis. Sucralose licks and intakes during the last two 1 h/day sessions were averaged. The mean data from these two sessions, referred to as Test 0, were compared to the licks and intakes recorded in the three sugar sessions (Tests 1-3). The 1-h total lick and intake data were analyzed separately using a mixed model analysis of variance (ANOVA) with a group factor (Glucose vs. Fructose) and repeated measure factor (Tests 0-3). Cumulative lick curves were generated for each test, and licking rates were also expressed as licks per 3-min bin for each test. The lick bin data were analyzed separately for each group with repeated measure ANOVA (Test x Bins) with each sugar test compared to sucralose test 0. If there was a Test x Bin interaction, simple main effects tests compared each sucralose vs. sugar bin.

Results

As illustrated in Figure 1, the Glucose mice increased their 1-h licks when switched from sucralose to glucose (Tests 0 to 1) and then further increased their licks in sugar Tests 2 and 3; the percent increase in licks over the sucralose Test 0 was 30%, 44%, and 74%, respectively, for glucose Tests 1-3. The Fructose mice, on the other hand, decreased their licks by 30% when switched from sucralose to fructose (Tests 0 to 1) and continued to lick less in Tests 2 and 3. Statistical analysis confirmed that there was a Group x Test interaction \[ F(3,60) = 30.9, P < 0.001 \]; the groups did not differ in Test 0 but the Glucose mice licked more than the Fructose group in Tests 1-3. In addition, Glucose mice increased their licks from Tests 0 to 1 and from Tests 1 to 3, whereas the Fructose mice decreased their licks from Test 0 to Tests 1-3. Analysis of the solution intake data revealed a similar pattern. The Glucose mice increased their intakes from Test 0 (2.1 g/h) to Tests 1-3 (3.0 to 3.7 g/h) whereas the Fructose mice decreased their intakes from Test 0 (1.9 g/h) to Tests 1-3 (1.4 to 1.5 g) \[ Group \times Test \text{ interaction, } F(3,60) = 47.4, P < 0.001 \].

The lick curves are presented in Figures 2 and 3. For the Glucose group, the cumulative lick curve in the first glucose test began to diverge from the sucralose curve at about min 9 whereas in Tests 2 and 3 the glucose lick curves diverged from the sucralose curve at min 1. Analysis of the 3-min bin data confirmed that the mice licked more for glucose than sucralose and that the differences varied over time.
[Test x Bin interactions, F(19,190) ≥ 3.8, P < 0.001]. In Tests 1 vs. 0, the mice licked slightly less glucose than sucralose in bin 1, but significantly more (P < 0.05) glucose in bins 2 to 7. Thus, the mice started licking at a faster rate for glucose than for sucralose 4-6 min into Test 1; this increased licking rate was not evident until min 9 of the cumulative lick curve because of the reduced glucose licking rate in bin 1. In Tests 2 and 3 the mice licked more (P < 0.05) glucose than sucralose (Test 0) in bins 1 to 7 and 1 to 8, respectively. Analysis of min 1 data revealed that mice licked more (P < 0.01) for glucose in Tests 2 and 3 than in Test 1 (120.0 and 124.7 vs. 37.7 licks) and more than they did for sucralose in Test 0 [66.1 licks, F(3,30) = 10.6, P < 0.001]. While the mice licked avidly for glucose at the beginning of Tests 2 and 3, their lick rate declined such that it was similar to their sucralose lick rates during the last 30 min of the sessions (Figure 2).

In contrast to the Glucose group, the Fructose mice licked less for fructose than sucralose throughout Tests 1 to 3 as indicated by the cumulative lick and 3-min bin curves (Figure 3). Analysis of the bin data indicated that overall sucralose licks exceeded fructose licks in the three sugar tests and only the Test 2 vs. 0 comparison revealed a Test x Bin interaction [F(19,190) = 2.2, P < 0.01]; in Test 2 the mice licked less for fructose than sucralose in bins 1-5, 8-9, 15 and 20.

Discussion. Given that B6 mice prefer 0.8% sucralose to 8% glucose and 8% fructose in 1-min choice tests (Zukerman and Sclafani, unpublished), they would be expected to reduce their drinking rate when switched from sucralose to sugar, which is exactly what happened in the Fructose group. The mice switched to the glucose solution, however, reduced their drinking rate slightly in the first 3 min of Test 1 and then significantly increased their glucose licking rate such that their total 1-h licks exceeded that of their sucralose licks by 30%. In the subsequent tests the mice drank the glucose solution at a high rate from the very first min and their total 1-h licks and intakes were further elevated above sucralose Test 0 levels. These findings suggest that post-oral signals generated by the ingested glucose but not fructose stimulated drinking early in the first session and maintained the elevated drinking in subsequent sessions. In addition, the post-oral glucose signals generated in Test 1 appear to have conditioned an increase in the reward value of the sugar’s taste such that the animals drank the glucose solution at an elevated rate at the
start of Tests 2 and 3. Prior studies indicate that initial lick rates vary as a function of sugar concentration and therefore palatability (14; 15). The Glucose mice did not sustain this elevated drinking rate, however, but rather reduced their rate of licking in Tests 2 and 3 during the first 30 min of the session, which presumably represents the post-oral satiating actions of the ingested glucose (15).

The finding that the Fructose mice consumed less sugar than sucralose in Tests 1 to 3 is remarkable given that the animals were food restricted and obtained energy from the sugar solution but not from the sucralose solution. In 24-h sweetener vs. water tests, B6 mice consumed similar amounts of 8% fructose and 0.8% sucralose when chow was available ad libitum, but consumed more fructose than sucralose when food restricted (Zukerman and Sclafani, unpublished). Thus, B6 mice are not completely insensitive to the nutritive value of fructose but apparently do not detect (or respond to) this property of fructose during 1-h sessions.

**Experiment 2. Glucose stimulation of intake and flavor conditioning in normal and capsaicin-deafferented mice**

The mice in the first experiment increased their rate of drinking within 4-6 min of the first glucose test session, suggesting that glucose rapidly generates a post-oral positive feedback signal. The post-oral actions of the glucose in Test 1 also resulted in a conditioned increase in the initial licking rate in the subsequent glucose test sessions (see General Discussion). In this experiment we determined if glucose would also condition a flavor preference. Although the taste of 0.8% sucralose is strongly preferred to 8% glucose (Zukerman and Sclafani, unpublished), we predicted the mice would learn to prefer a glucose-paired flavor over a sucralose-paired flavor based on the sugar’s post-oral reward effect.

A second aim of this experiment was to determine if glucose-stimulated intake and conditioned acceptance are mediated by visceral afferent feedback. According to one study, visceral deafferentation induced by systemic capsaicin treatment prevents rapid post-oral nutrient conditioning in rats (64). We previously reported that capsaicin-sensitive visceral afferents are not required for flavor conditioning by IG infusions of a glucose polymer (Polycose) in rats trained over several one-bottle sessions (29). It is possible, however, that capsaicin-sensitive visceral afferents mediate the glucose-stimulated licking by
mice in the first sugar test session and the conditioned licking response displayed in subsequent sessions. We investigated this possibility by comparing the licking response of control and capsaicin-treated mice using the paradigm described in Experiment 1 with two modifications. First, prior to the 1-h drinking tests, the mice were given 1-min, two-bottle tests with 0.8% sucralose vs. 8% glucose to determine if the capsaicin treatment altered the sweetener preference of the mice. Second, in the 1-h tests the mice were given sucralose and glucose solutions containing different flavors to determine if they would learn a preference for the glucose-paired flavor and if capsaicin treatment altered preference conditioning.

Methods

Animals. Male B6 mice (10 weeks old) purchased from the Jackson Laboratories were housed as in Experiment 1. At 10 weeks of age they were treated with systemic capsaicin (n=14; 50 mg/kg, Sigma Chemical Co., St. Louis, MO) or vehicle (n=13) according to the procedure of (62) except that the mice were initially anesthetized with isoflurane (2%). The efficacy of the capsaicin treatment was evaluated at the end of the experiment using a corneal chemosensory test which involved monitoring the eye wiping reflex after ocular administration of a 7.5 µl drop of 0.2% NH₄OH solution; this concentration was used because with the small drop size the typical 0.1% concentration (62) elicited few eye wipes in intact mice. The capsaicin treated mice showed little or no eye wiping compared to vehicle-treated controls (1.2 vs. 21.7 wipes in 15 sec, P < 0.01).

Procedure. One week after capsaicin treatment the mice were adapted to the test cages overnight. They were then given restricted access to water (1 h/day) in their home cages and trained to drink water from two bottles in the test cages for 5 min (day 1) and then for 8% glucose vs. 0.8% sucralose in 1-min tests (day 2). The animals were then given unrestricted access to water but maintained on a restricted feeding schedule as in the first experiment. While food restricted they were given 1-min glucose vs. sucralose tests on two successive days. In these 1-min choice tests, the mice were first given 5-s access to one sipper tube and 5-s access to the other sipper tube to allow them to sample the contents of each tube before being presented with both tubes for 1 min. The timing of each session for each mouse began with the 10th lick by the mouse and the bottles were automatically retracted 5 s or 1 min later. The mice were
then returned to their home cages and 1 h later they were given a second 1-min choice test. The left-right position of the solutions was alternated from the first to second test each day.

The mice were then given a series of one-bottle, 1 h/day tests with 0.8% sucralose (3 sessions) and then 8% glucose (3 sessions). For 7 mice in each group, the sucralose was flavored with 0.01% ethyl acetate and the glucose was flavored with 0.01% propyl acetate (Sigma); the flavors were reversed for the remaining mice in each group. The mice were then given four additional “reminder” sessions (1 h/day) with the flavored sucralose presented on days 1 and 3, and the flavored glucose presented on days 2 and 4. This was followed by a choice test (1 h/day for 2 days) with two bottles of 0.8% sucralose, one containing the flavor previously paired with 8% glucose (the CS+) and the other with the flavor previously paired with the 0.8% sucralose (the CS-). The left-right position of the flavors was alternated from day 1 to 2.

Statistical tests were performed as in the first experiment.

Results

In the 1-min choice tests the Capsaicin-treated and Control mice licked more for sucralose than glucose [141.9 vs. 25.7 and 168.2 vs. 39.7 licks/min; F(1,25) = 155.2, P < 0.001] and the two groups were similar in their percent sucralose preference (84 vs. 80%). Total 1-min licks were marginally lower for the Capsaicin mice than for the Controls (167.7 vs. 204.9 licks/min, P < 0.06). In contrast, the Capsaicin mice licked slightly but not significantly more sucralose than the Control mice in Test 0 (Figure 4). When switched to the glucose solution the Capsaicin mice increased their 1-h licks to the same levels as those of the Control mice. The ANOVA confirmed that both groups increased (P < 0.001) their 1-h licks from Test 0 to 1, and then from Test 1 to Tests 2 and 3 [F(3,75) = 31.4, P < 0.001]; there were no significant group or group x test differences. Analysis of the solution intake data revealed a similar pattern. Overall, the two groups increased their solution intakes from Test 0 to 1, and then further increased their intakes in Tests 2 and 3 [Tests 0-3: 2.2, 3.2, 3.6, 3.6 g/1 h; F(3,75) = 84.1, P < 0.001]. The two groups also licked more glucose than sucralose in the one-bottle reminder trials that occurred after Test 3 [Capsaicin mice: 3915 vs. 3018 licks; Control mice: 3496 vs. 2570 licks, F(1,25) = 94.8, P < 0.001]. In the two-bottle choice test
conducted with the flavored sucralose solutions, the Capsaicin and Control mice licked considerably more for the CS+ than CS- solution \([F(1,25) = 145.7, P < 0.001]\) and displayed similar CS+ preferences (82% vs. 80%; Figure 4).

The lick curve data are presented in Figures 5 and 6. For the Control mice, the glucose Test 1 cumulative lick curve began to diverge from the sucralose curve at about min 13 in Test 1 but at min 1 in Tests 2 and 3. Analysis of the 3-min lick data confirmed that the mice licked more for glucose than sucralose and that the difference varied across bins \([\text{Test x Bin, } F(19,228) \geq 4.8, P < 0.001]\). In Test 1 the mice licked less \((P < 0.01)\) glucose than sucralose in bin 1 but more \((P < 0.05)\) glucose in bins 3-7 and bins 9, 12, 15-16, 18, and 20. In Tests 2 and 3 the mice licked more for glucose than sucralose in bins 1-8 and 1-9, respectively. For the Capsaicin mice, the cumulative lick curve in the first glucose test began to diverge from the sucralose curve at about min 19 in Test 1 but at min 1 in Tests 2 and 3. The 3-min bin data indicated that the Capsaicin mice licked more for glucose than sucralose and that the difference varied across bins \([\text{Test x Bin, } F(19,247) \geq 5.4, P < 0.001]\). In Test 1 the mice licked less \((P < 0.01)\) glucose than sucralose in bin 1, but more \((P < 0.01)\) glucose in bins 3-9 and 11. In Tests 2 and 3 the mice licked more glucose than sucralose in bins 1 to 5, 7 and 9 and in bins 1 to 4, and 6 to 7, respectively. A comparison of the 3-min bin data from the Capsaicin and Control groups revealed no significant group differences in Tests 0, 2 and 3. The groups differed only in Test 1, because the Capsaicin mice licked less \((P < 0.05)\) glucose than the Control mice in the first 3-min bin \([\text{Group x Bin interaction, } F(19,475) = 2.0, P < 0.01]\). Analysis of the first minute data indicated that both the Control and Capsaicin groups licked less in Test 1 but more in Tests 2 and 3 for glucose than for sucralose in Test 0 \([\text{Tests 0-3: Control group } 133.5, 50.6, 198.4 \text{ and } 211.5 \text{ licks}; \text{ Capsaicin group } 145.9, 34.1, 189.7, 198.7 \text{ licks}; F(3,75) = 54.5, P < 0.001]\).

**Discussion.** Overall, the data from the Control mice replicate the glucose stimulation of licking and intake observed in the first experiment. A new finding here is that the mice also learned to prefer the flavor paired with the glucose over the sucralose-paired flavor. This is notable given that in the 1-min choice test the mice strongly preferred sucralose to glucose, which confirms our prior results (Zukerman
and Sclafani, unpublished). Thus the glucose-stimulated intake and conditioned preference is attributable to the post-oral actions of the sugar rather than to its sweet taste per se.

Experiment 2 also revealed that glucose had very similar effects in the Control and Capsaicin groups. The two groups showed similar glucose-stimulated lick patterns in Test 1; both groups licked less for glucose in bin 1 but more starting in bin 3 compared to their sucralose lick pattern in Test 0. In addition, the Control and Capsaicin groups displayed comparable increases in their initial lick rates in glucose tests 2 and 3, and had similar preferences for the glucose-paired flavor over the sucralose-paired flavor in the two-bottle test. The present results confirm previous rat findings showing that capsaicin-sensitive visceral afferent fibers are not required for glucose-conditioned flavor preferences (29). The new finding here is that capsaicin-sensitive visceral afferent fibers are not required for glucose-stimulated ingestion in the first or subsequent sugar tests. Our data do not refute an earlier report of impaired rapid flavor conditioning in capsaicin-treated rats (64) because of the many differences between the two studies including species (rat vs. mouse), nutrients (milk vs. glucose), training protocols (two- vs. one-bottle training) and session lengths (10 vs. 60 min).

The present experiment is the first to report that rodents learn to prefer a sugar-paired flavor over a sucralose-paired flavor, but there are several reports of similar conditioning using saccharin as the non-nutritive sweetener (9; 18; 27; 34; 43; 61). However, in these earlier studies the sugar (sucrose, glucose, fructose) was initially preferred to the saccharin so that the conditioned preference could be due to the taste of the sugar, its post-oral actions, or both properties. In some studies quinine or citric acid was added to the sugar solution to reduce its palatability so that the sugar-conditioned preference could be attributed to post-oral rather than taste factors (9; 27; 43; 61). The natural preference of B6 mice for 0.8% sucralose over 8% glucose eliminates the need for adulteration of the sugar solution to reveal post-oral sugar conditioning.

**Experiment 3. Stimulation of intake and flavor conditioning by intragastric self-infusion of glucose**

The rapid stimulation of drinking displayed by the mice switched from sucralose to glucose in Experiments 1 and 2 is attributed to the post-oral rather than taste properties of the sugar. The present
experiment sought to provide direct evidence that post-oral glucose sensing stimulates drinking in B6 mice by having the mice self-infuse the sugar IG using an “electronic esophagus” system (20; 49). With this system, the mouse controls the IG infusion rate and volume by its licking response to a flavored CS solution. The test protocol was similar to that of Experiment 2 in that the mice were given three sessions with a flavored solution (the CS-) followed by three sessions with a different flavored solution (CS+). In this case, the CS- solution contained saccharin, as used in our prior conditioning studies, and was paired with an IG water self-infusion (49; 50). The CS+ solution was a flavored saccharin solution that was paired with an IG self-infusion of glucose. Previous reports demonstrate that IG glucose solutions condition strong flavor preferences in rats and mice (2; 5; 25; 31; 47). The question addressed here was whether IG glucose infusions would stimulate licking in the first test session and produce conditioned increase in licking rates in subsequent sessions.

Methods

Subjects. Male B6 mice (n=11, 10 weeks old) born in the laboratory were housed as in prior experiments. The mice were anesthetized with isoflurane (2%) inhalation and fitted with a gastric catheter, as described previously (48). Two weeks after surgery the mice were briefly (5 min) anesthetized with isoflurane, and tubing was attached to the gastric catheter and then passed through an infusion harness with a spring tether (CIH62; Instech Laboratories, Plymouth Meeting, PA). The tubing was then attached to an infusion swivel mounted on a counterbalanced lever (Instech Laboratories). The body weight of each mouse was measured before and after it was fitted with the infusion tether/swivel system; daily body weights were monitored by weighing the mouse with the attached infusion tether/swivel system. Each animal was then returned to its home cage and the swivel counterbalanced lever was attached above the cage.

Apparatus. IG infusion tests were conducted in plastic test cages (48). The sipper spouts were interfaced via electronic lickometers (Med Electronics, St. Albans, VT) to a computer, which operated a syringe pump (A-99; Razel Scientific, Stamford, CT) that infused liquid into the gastric catheters as the animals licked. The pump rate was nominally 0.5 ml/min, but the animal controlled the overall infusion
rate and volume by its licking response. In particular, the computer accumulated licks during 3-sec bins and activated the pump for 3 sec when a criterion number of licks was recorded. The oral-to-infusion intake ratio was maintained at ~1:1 by adjusting the lick criterion for each mouse. Daily oral fluid intakes were measured to the nearest 0.1 g, and IG infusions were recorded to the nearest 0.5 ml.

Test solutions. The CS solutions contained 0.025% sodium saccharin (Sigma) flavored with 0.01% ethyl acetate or propyl acetate. The saccharin concentration was based on pilot work indicating that at this concentration the fluid volume (oral + IG) consumed during the CS- tests would approximate the sucralose intakes observed in the first two experiments. The CS- solution was paired with IG infusion of water while the CS+ solution was paired with IG infusion of 16% glucose. The 16% concentration was used because the sugar infusion was diluted to 8% in the stomach by the orally consumed CS+ solution, matching the 8% concentration used in the first two experiments.

Procedure. The mice were trained in the infusion test cages following a procedure similar to that of the first experiment with the following exceptions. They were first given a 1-h drinking session with an unflavored 0.025% saccharin solution while water deprived and then another four 1-h sessions while food-restricted; saccharin intakes were paired with matched volume infusions of water. The mice were then given three 1-h test sessions with a CS- saccharin solution paired with IG water infusions followed by three sessions with the CS+ saccharin solution paired with IG infusions of 16% glucose. A two-bottle preference test was then conducted with the CS+ vs. CS- solutions no longer paired with IG infusions in two 1-h sessions.

Statistical tests were performed as in the first experiment.

Results

Figure 7 shows the 1-h lick data averaged during the last two CS- (Test 0) and the three CS+ 1-h sessions (Tests 1-3). The mice significantly (P < 0.001) increased their 1-h licks when switched from the CS- solution paired with IG water self-infusion (Test 0) to the CS+ solution paired with IG glucose self-infusion (Test 1) and then further increased (P < 0.01) their licks over the following two CS+ tests [F(3,30) = 19.4, P < 0.001]. Total 1-h licks increased from Test 0 to Tests 1-3 by 44%, 94% and 90%,
respectively. The total intake (oral + IG infusion) data revealed a similar pattern: intakes increased (P < 0.05) from Test 0 to 1 (1.8 to 2.4 g) and then further increased from Test 1 to Tests 2 and 3 [2.4 g to 3.1, 3.1 g, F(3,30) = 13.6, P < 0.001]. In the subsequent two-bottle test with the CS solutions no longer paired with IG infusions the mice licked significantly more for the CS+ than CS- [t(10) = 5.0, P < 0.001, Figure 7] and consumed more CS+ than CS- (1.4 vs. 0.5, t(10) = 5.8, P < 0.001]. The CS+ preferences were 72% and 74%, respectively, when expressed as licks and intakes.

As illustrated in Figure 8, the cumulative lick curve during the first CS+ test began to diverge from the Test 0 CS- curve at about min 15 whereas the CS+ curves in Tests 2 and 3 diverged from the CS- curves at min 1. Analysis of the 3-min lick data confirmed that the mice licked more for the CS+ than CS- and that the difference varied across bins [Test x Bin, F(19,190) ≥ 3.1, P < 0.001]. In Test 1, the mice licked less (P < 0.05) in bin 1 for the CS+ than they did for the CS- in Test 0, but then significantly more for the CS+ in bins 5-10. In Tests 2 and 3, the mice licked more for the CS+ than CS- in bins 1 to 5 and 1 to 4, respectively. Further analysis indicated during the first min of testing the mice licked slightly less in CS+ Test 1 but significantly more (P < 0.001) in Tests 2 and 3 than for the CS- in Test 0 [Tests 0-4: 42.6, 28.6, 127.5 and 150.3 licks/min; F(3,30) = 30.2, P < 0.001].

Discussion. These findings demonstrate that IG glucose self-infusion stimulated licking within the first 1-h test, produced conditioned increases in licking early in subsequent test sessions, and increased total 1-h CS+ intakes. The glucose infusions also conditioned a preference for the CS+ flavor, consistent with prior reports (2; 5; 25; 31; 47). Overall, the pattern of results obtained with the IG glucose self-infusions was similar to that obtained with the orally consumed glucose in the first two experiments. However, the glucose-stimulated drinking produced by the IG infusions and by oral ingestion differed in one respect. Whereas the orally consumed glucose stimulated licking in the second or third 3-min bin in Test 1 of the oral experiments, the IG glucose self-infusions did not stimulate licking until the fifth 3-min bin of Test 1. The more rapid stimulation of ingestion observed in the first two experiments suggests that oral glucose sensing may contribute to the sugar’s stimulation of drinking, although there is another explanation. In particular, the flavored 0.025% saccharin solutions used as CS solutions in the IG
experiment were less sweet and probably less distinctive than the 0.8% sucralose and 8% glucose solutions used in Experiments 1 and 2. Prior rat studies indicate that the intensity and/or quality of CS solutions influence flavor conditioning by IG nutrient infusions (3; 4; 36; 50). The difference in the flavor cues may also account for the weaker CS+ preference observed in this experiment compared to the second experiment (72% vs. 80%). However, another important factor which may have enhanced the CS+ preference is that the mice in the second but not the third experiment were given “reminder” trials with the CS+ and CS- solutions prior to the two-bottle CS flavor test.

**Experiment 4. Stimulation of intake and flavor conditioning by intragastric self-infusion of fat**

This experiment determined if a nutrient other than glucose can stimulate intake during an ongoing ingestion bout and condition increased flavor acceptance in mice. Fat in the form of Intralipid (a soybean oil emulsion) was selected for study because we previously observed that IG Intralipid self-infusions increased CS+ solution intake and conditioned a strong CS+ preference in B6 mice (44; 50). Session length was 22 h/day in those studies and the ability of fat self-infusions to stimulate licking within a 1-h session has not been tested. IG fat self-infusions condition flavor preferences in rats trained with short sessions (30 min) but the preferences require more training trials than do glucose-conditioned preferences (2).

**Methods**

The male B6 mice (n=13, 11 weeks old) were tested as in Experiment 3 except that the CS+ was paired with IG infusions of 6.4% Intralipid, which is isocaloric to 16% glucose. The lipid infusion was prepared by diluting 20% Intralipid (Baxter, Deerfield, IL) with water.

**Results**

As illustrated in Figure 9, the mice significantly (P < 0.001) increased their 1-h licks when switched from the CS- solution paired with IG water infusions to the CS+ solution paired with IG Intralipid (Test 0 to 1) and then further increased (P < 0.01) their licks from Test 1 to 2, and from Test 2 to 3 [F(3,36) = 50.1, P < 0.001]. Total 1-h licks increased from Test 0 to Tests 1-3 by 57%, 96% and 120%, respectively. The total intake (oral + IG infusion) data revealed a similar pattern: intakes increased
(P < 0.05) from Test 0 to 1 (1.8 to 2.8 g) and then further increased in Tests 2 and 3 [3.3, 3.8 g/1 h, F(3,36) = 49.0, P < 0.001]. In the subsequent two-bottle test with the flavored solutions no longer paired with IG infusions the mice licked significantly more for the CS+ than CS- [t(12) = 8.3, P < 0.001, Figure 9] and they also consumed more CS+ than CS- (1.4 vs. 0.6, t(12) = 9.4, P < 0.001]. The CS+ preferences were 70% and 72% when expressed as licks and intakes, respectively.

As shown in Figure 10, the cumulative lick curve during the first CS+ test began to diverge from the CS- curve at about min 9 whereas the CS+ curves in Tests 2 and 3 diverged from the CS- curve at min 1. Analysis of the 3-min lick data confirmed that the mice licked more for the CS+ than CS- and that the difference varied across bins [CS x Test interaction, F(19,228) ≥ 2.0, P ≤ 0.01]. In Test 1 the CS+ licks did not differ from CS- licks (Test 0) in bins 1 to 3, but then exceeded CS- licks in bins 4, 6, 7, 11-17 and 19. In Tests 2 and 3, the mice licked more (P < 0.05) for the CS+ than CS- in bins 1 to 9, and 14, and in bins 1-12, and 14, respectively. Further analysis indicated that during the first min of testing the mice licked (P < 0.01) more for the CS+ in Tests 2 and 3 than for the CS- in Test 0 [Tests 0-3: 42.9, 47.1, 81.5 and 132.6 licks; F(3,36) = 10.9, P < 0.001].

Discussion. The present findings revealed that, like IG glucose, IG fat self-infusions stimulated licking in the first 1-h session, greatly stimulated initial lick rates and 1-h intakes in subsequent sessions, and conditioned a CS+ flavor preference. There are both similarities and differences between the IG fat and glucose effects observed in Experiments 3 and 4. In particular, the fat and glucose self-infusions first stimulated licking in 3-min bin 4 and 5, increased total Test 1 licking by 57% and 44%, and produced CS+ preferences of 70% and 72%, respectively. On the other hand, the fat infusions produced a more gradual but sustained stimulation of licking than did glucose in Test 1 (compare Figures 8 vs. 10), but stimulated total licking more than did glucose in Test 3 (120 vs. 90%).

The similar effects of IG glucose and fat self-infusions observed in the present study contrast with our earlier finding that IG glucose was more effective that IG fat infusions in conditioning flavor preferences in rats (2). In addition to the species differences, the present experiment differed from the earlier rat experiment in several respects including the training sequence, CS flavors, session duration,
and type of fat emulsion used, i.e., a lab-prepared corn oil emulsion in the rat study and the commercially prepared soybean oil emulsion (Intralipid) in the current experiment. Additional tests conducted with the mice of the present experiment after they completed the test series described above indicated that IG corn oil and Intralipid did not differ in their ability to stimulate 1-h licking. However, further study is needed with naïve mice to determine the effects of different types of fats on post-oral stimulation of intake in mice.

**GENERAL DISCUSSION**

This study reports for the first time that an ongoing ingestion bout of glucose or fat is stimulated by the post-oral actions of these nutrients in mice. This stimulation of intake contrasts with the well-characterized satiating actions these nutrients that terminate feeding. The first two experiments assessed glucose-induced intake stimulation by recording the licking response of mice during their first 1-h session with an 8% glucose solution after being adapted to drink a non-nutritive, but more preferred 0.8% sucralose solution. Experiments 3 and 4 investigated glucose- and fat-induced feeding stimulation by having the mice self-infuse the nutrients directly into the stomach as they drank a non-nutritive saccharin solution. The results revealed that glucose ingestion or self-infusion and fat self-infusion stimulated licking 4-15 min into the first test session and increased total 1-h intake, and produced a conditioned increase in licking rates at the start of tests 2 and 3. In addition, the results replicated prior studies showing that the post-oral actions of glucose and fat condition flavor preferences in mice (25; 44; 48-50).

**Glucose Stimulated Intake**

There are many reports that pairing the intake of a flavored solution with IG infusions of glucose or glucose-containing carbohydrate (sucrose, Polycose) conditions a strong flavor preference (2; 4; 31; 36; 47; 49; 50; 52; 54). In addition, some studies also observed increases in the intake of the flavored solutions paired with IG carbohydrate self-infusions during one-bottle testing. In rats given short daily tests (e.g., 30 min), this nutrient-induced increase in acceptance did not occur until after multiple test sessions (11; 32; 54). With long daily sessions (20-24 h), nutrient infusions can stimulate intake in the very first session but, as previously noted, animals consumed the CS+ in multiple bouts in these long
sessions and it is not clear when the post-oral stimulation of intake occurred within the session (33; 36; 37; 50). Taken together, these earlier IG conditioning studies provided no evidence that nutrients can stimulate ingestion within an initial feeding session in rodents. It is understandable, therefore, why the post-oral nutrient signals generated during an ingestive bout have been viewed as having primarily inhibitory effects that suppress ongoing intake (8; 10; 24; 56). The present findings demonstrate that this is not always the case and that some nutrients can rapidly stimulate ingestion with the appropriate test procedures.

In Experiments 1 and 2 glucose-stimulated drinking was assessed by recording the licking response of mice switched from a more preferred 0.8% sucralose solution to a less preferred 8% glucose solution. Consistent with this preference difference, in Test 1 the mice initially licked less for glucose than they did for sucralose in Test 0. However, at 4 to 9 min they began to increase their glucose licking rate which resulted in an increase in their 1-h solution intake. Mice switched to fructose, on the other hand, did not show this reversal in their licking rate, indicating that the glucose-stimulated intake was not due to some non-specific change in the taste or post-oral stimulation provided by the glucose solution.

A related case of sugar-stimulated drinking was reported by de Araujo et al. (16) in a study of the sweet taste ageusic TRPM5 knockout (KO) mice. In their study TRPM5 KO mice switched from a 1.2% sucralose solution to a 27% sucrose solution substantially increased their 1-h licks, with the increase occurring about 8 min after the start of the sucrose session. The authors interpreted this delayed stimulation of licking as a response to the post-oral actions of the sucrose. We concur with this interpretation but propose that the sucrose-stimulated drinking was due specifically to the glucose component of the disaccharide, given the failure of fructose to stimulate licking (Experiment 1) or condition flavor preferences (4; 6; 47). In contrast to the TRPM5 KO mice, de Araujo et al. (16) reported that B6 control mice reduced their 1-h licks when switched from sucralose to sucrose. This finding is likely due to the satiating actions of the concentrated (27%) sucrose solution and thus does not conflict with the present findings obtained with an 8% glucose solution. Sucrose satiation may have also limited the sugar intake of the TRPM5 KO mice but it did not prevent their increased licking response because
their sucralose lick rate was much lower than that of the B6 mice. In fact, because the TRPM5 KO mice were not attracted to the sweet taste of sucralose or sucrose, the mice were water and food restricted in order to induce them to drink the sweeteners.

Direct evidence for post-oral intake stimulation was provided by the findings of the third experiment that IG glucose self-infusions stimulated licking beginning 15 min into the very first CS+ session. This is a robust finding that we have obtained in a pilot experiment as well as in ongoing follow-up studies. The rapid first session stimulation of CS+ licking by IG glucose or fat self-infusions in the B6 mice contrasts with the more slowly developing across-session increase in CS+ intake observed in prior rat studies (11; 32; 54). The discrepant results may be due to species differences, i.e., mice may be more sensitive than rats to the post-oral positive feedback effects of glucose or fat, but many procedural variations between the present study and prior rat experiments may also be important and require investigation.

While the present and prior (16) mouse findings provide converging evidence for a potent post-oral sugar stimulatory effect, the site and mechanism of action remain uncertain. Previous studies of glucose-conditioned flavor preferences indicate a critical role for intestinal glucose sensing, although post-absorptive sites are also implicated. In particular, we observed that preferences for flavored saccharin solutions in rats are conditioned by intestinal but not hepatic portal glucose infusions (5). Tordoff and Friedman (58), on the other hand, reported that hepatic-portal glucose infusions conditioned preferences for a flavored chow. It may be that post-absorptive glucose actions can reinforce flavor preferences when there is also pre-absorptive nutrient stimulation as provided by the flavored chow used in the Tordoff and Friedman study. Currently, there is considerable interest in the role of intestinal T1R2+T1R3 sweet receptors in carbohydrate absorption and hormone release (19; 55), but these gut receptors are not implicated in sugar-stimulated intake or flavor conditioning. In particular, the failure of fructose to stimulate drinking in Experiment 1 and the relatively weak flavor conditioning effect of IG fructose observed in other studies (4; 46; 47) argues against the involvement of intestinal sweet receptors, because fructose is a potent sweet receptor ligand. In addition, we recently reported that IG sucralose self-
infusions condition a mild flavor avoidance rather than a preference in B6 mice and that knockout mice missing the T1R3 component of the sweet receptor show a normal flavor conditioning response to IG sucrose infusions (48). Other putative intestinal sugar sensors have been proposed, including SGLT-1 and SGLT-3 (39), which are of particular interest because they bind to glucose but not fructose. We are currently investigating the role of these sugar sensors in glucose-stimulated licking and flavor conditioning.

Another critical question concerns how glucose post-oral stimulatory signals reach central neural sites controlling ingestion. Nutrient signals generated in the intestinal tract rapidly reach the brain via visceral afferent pathways (7), but the findings of Experiment 2 indicate that capsaicin-sensitive fibers are not critical for glucose-stimulated drinking in the mouse. Rat studies also demonstrate that visceral afferents destroyed by systemic capsaicin treatment, subdiaphragmatic vagotomy, subdiaphragmatic vagal deafferentation, or celiac-superior mesenteric ganglionectomy are not essential for glucose-conditioned flavor preferences, although these studies did not specifically investigate glucose-stimulated drinking (29; 45; 51). Direct gut-brain hormone signaling is an alternative pathway, although many gut hormones act via the vagus (7). Also, as discussed elsewhere (5), most gut hormones released by glucose have inhibitory actions on feeding and thus would appear unlikely candidates to mediate glucose stimulation of intake. While the gut hormone ghrelin stimulates feeding and is implicated in reward processing, ghrelin release is suppressed rather than stimulated by glucose, which argues against its involvement in glucose-induced intake and preference conditioning (5). Obviously, further research is required to elucidate gut-mediated glucose stimulation of intake, and the oral and IG testing procedures described in the present study should be useful in this endeavor.

In addition to stimulating the intake of sweet solutions, the post-oral actions of glucose can stimulate the intake of a chow diet under certain conditions. Geiselman and Novin (23) reported that non-deprived rabbits given a fixed duodenal infusion of glucose (~10 ml of 5.4% glucose) reduced their latency to eat and increased their meal size compared to a saline control infusion. This feeding stimulatory effect was observed only when the glucose was infused at a high rate (3 ml/min) whereas
food intake was suppressed when the same amount of glucose was infused at a slower rate (1 ml/min). The authors hypothesized that the rapid intestinal absorption of glucose induced metabolic changes that increased hunger in the rabbits. Subsequent findings suggest that this chow intake stimulatory effect may not be directly related to the glucose-specific stimulation of sweet solution intake observed in the present study. In particular, rapid ID infusions of fructose were as effective as glucose infusions in stimulating chow intake whereas IG infusions of either sugar did not increase chow intake relative to the saline infusions (21; 22). Nevertheless, the rabbit findings provide another example in which post-oral sugar actions can promote rather than suppress ingestion.

*Fat Stimulated Intake*

There are several reports of fat-conditioned flavor preferences in rats but, in general, IG fat self-infusions produce weaker preferences than do isocaloric IG carbohydrate infusions (2; 30; 59). In addition, IG fat self-infusions stimulated the intake of a saccharin solution less than did IG carbohydrate self-infusions in a 24-h conditioning study (38). To date, only one study has compared fat and sugar conditioned flavor preferences in B6 mice, but it is notable because IG self-infusions of isocaloric fat (6.4% Intralipid) and carbohydrate (16% sucrose) increased the consumption of a CS+ flavored saccharin solution, relative to the CS- solution, by similar amounts (80-100%), and conditioned comparable CS+ preferences (96-98%) (50). Thus, the B6 mouse is rather sensitive to the post-oral stimulatory actions of fat and this was confirmed in Experiment 4. That is, IG fat self-infusion stimulated CS+ licking and intake in the first test session and even more so in subsequent sessions. Overall, the fat self-infusions increased 1-h licks as much or more as did the IG glucose self-infusions, and produced similar CS+ preferences. However, as previously noted, the licking stimulation produced by the fat infusions was more gradual but sustained compared to that observed with the glucose infusions. This may be because the infused fat must be first digested to fatty acids before it is detected by intestinal sensors. It will be of interest in future experiments to compare the intake stimulatory actions of IG fatty acid infusions with those of IG glucose infusions.
Little is known about the site and mechanism of action for fat-induced intake stimulation and flavor conditioning. However, the finding that IG infusion of Intralipid increased CS+ licking beginning 12 min into the first test session suggests a pre-absorptive site of action. Measures of fat absorption in rats indicate that little fat is absorbed within the first 30 min after a duodenal fat infusion (26; 63). If fat is absorbed at a similar rate in mice (see (13)) then the enhanced CS+ intake observed during the first 30 min of Test 1 in Experiment 4 must be due to stimulation of pre-absorptive fat sensors. Several different putative fatty acid sensors have been identified in the intestine, including three -- CD36, GPR120, and GPR40 -- which are also implicated as fatty acid taste receptors in the mouth (12; 28; 41; 44; 57). Which of these or others are involved in the fat stimulatory effect revealed in the present study is not known. CD36, however, would appear not to be involved since CD36 knockout mice, like control mice, developed a strong preference for a flavor paired with IG Intralipid infusions (44). How post-oral positive feedback signals generated by fat reaches the brain is also not clear. The findings that capsaicin-induced visceral deafferentation did not block flavor conditioning in rats by IG fat infusions (29) or glucose stimulated intake in mice (Experiment 2) suggest that capsaicin-sensitive visceral afferents are not involved in fat-induced stimulation of intake in Experiment 3, but this remains to be determined. It is possible that glucose and fat release a common, as yet to be identified, orexigenic hormone that stimulates ingestion and conditions flavor preferences.

**Conditioned vs. Unconditioned Post-oral Stimulation of Intake**

In all four experiments lick rates were not stimulated in the first nutrient test until 4 or more min into the session whereas lick rates were elevated from the very first min in Tests 2 and 3. It is unlikely that the elevated initial lick rates in these later tests were a direct response to post-oral stimulation by glucose or fat. Rather, they presumably represent a conditioned enhancement in the reward value of the CS+ flavor (Experiments 2-4) or glucose taste (Experiments 1-2) produced by the post-oral nutrient stimulation in the first test session. This is indicated by prior findings that increases in flavored solution intakes observed after several sessions with IG sugar self-infusions remain unchanged during a subsequent extinction test in which rats self-infused water rather than sugar (27; 31; 35). The substantially
elevated lick rates at the start of tests 2 and 3 presumably prevented the expression of any further post-oral nutrient stimulation of licking in these test sessions. Yet, prior work indicates that post-oral nutrient stimulation is required to maintain conditioned increases in intake, because intakes decline over repeated extinction sessions without nutrient infusions (33; 37). It will be of interest in future experiments to compare the decline in glucose- and fat-conditioned licking in B6 mice in extinction tests after Test 3.

The role of flavor conditioning in the stimulation of licking observed in Test 1 of the four experiments is not certain. The glucose- and fat-stimulated licking in the initial 1-h session may represent an unconditioned response to pre-absorptive (and perhaps post-absorptive) signals generated by these nutrients acting on central reward circuits. De Araujo et al. (16; 40) reported that the post-oral actions of sucrose or glucose in mice produce elevated dopamine release in the nucleus accumbens (NAc) as early as 10-30 min into a test session. Tsurugizawa et al. (60) reported fMRI data indicating that IG glucose infusion activated brain areas, including the NAc, in rats within 15 min and that, relevant to the present capsaicin results, subdiaphragmatic vagotomy had little effect on the brain response to the glucose infusion. Alternatively, the early activation of brain reward circuits may initiate the first stages of the flavor-nutrient associative process so that licking stimulation in Test 1 may reflect conditioned changes in the evaluation of the glucose (or CS+) flavor. The importance of brain dopamine signaling in flavor-nutrient preference learning is indicated by the finding that flavor conditioning by IG glucose infusions is blocked by microinjections of a D1 receptor antagonist (SCH23390) in the NAc as well as the amygdala and medial prefrontal cortex (53). Further studies are needed to determine if the learning processes responsible for nutrient-conditioned flavor preferences also mediate the early nutrient stimulation of ingestion revealed in the present study. It is clear, however, that early post-oral nutrient action within a test session is not required for flavor-nutrient learning. Rats acquire flavor preferences even when glucose is infused 30 min after the end of a CS+ training session (1).

Perspectives and Significance

The satiating actions of nutrients in the intestinal tract have been extensively studied and reviewed. Prior studies demonstrated that nutrients can have post-oral actions that promote ingestion and
condition strong flavor preferences, but the time course for these positive post-oral actions was not clear. The present findings demonstrate that glucose and fat rapidly stimulate ingestion and condition flavor acceptance and preference in B6 mice and the time course implicates an intestinal site of action. These results suggest that gut nutrient sensors are not only involved in generating negative feedback signals that terminate ingestion, but also generate positive feedback signals that stimulate ingestion and flavor conditioning. Much remains to be learned about the positive post-oral feedback process and how the brain integrates stimulatory and satiating signals from the gut to control feeding. The test paradigms used in the present study provide a window into the post-oral stimulatory actions of sugar and fat which should facilitate the further study of nutrient-induced appetite.

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES


FIGURE CAPTIONS

Figure 1. Experiment 1. Glucose stimulation and fructose inhibition of licking. All mice drank an 0.8% sucralose solution (1 h/day) in Test 0 before being switched to an 8% glucose solution or an 8% fructose solution in Tests 1 – 3. 1-h licks (mean ± sem) are plotted for each test. Significant differences (P < 0.05) between Test 0 vs. Tests 1 – 3 licks are indicated by an asterisk and between the licks of the Glucose and Fructose groups by a plus sign.

Figure 2. Experiment 1. Glucose-stimulated licking. Licks per 3-min bin are plotted for Test 0 with 0.8% sucralose and Tests 1 – 3 with 8% glucose. Inset graph plots cumulative lick curves for Tests 0 – 3. Analysis of the 3-min data indicated that, compared to Test 0 sucralose licks, the mice licked more (P < 0.05) for glucose in Test 1 (bins 2-7), Test 2 (bins 1-7) and Test 3 (bins 1-8).

Figure 3. Experiment 1. Fructose-suppressed licking. Licks per 3-min bin are plotted for Test 0 with 0.8% sucralose and Tests 1 – 3 with 8% fructose. Inset graph plots cumulative lick curves for Tests 0 – 3. Analysis of the 3-min data indicated that, compared to Test 0 sucralose licks, overall the mice licked less (P < 0.05) for fructose in Tests 1 and 3, and less in bins 1-5, 8-9, 15 and 20 of Test 2.

Figure 4. Experiment 2. Glucose stimulation of licking and flavor preference conditioning in capsaicin-treated and control B6 mice. The mice drank an 0.8% sucralose solution (1 h/day) in Test 0 before being switched to an 8% glucose solution in Tests 1 – 3. Left side: 1-h licks (mean ± sem) are plotted for one-bottle Tests 0 – 3. Right side: 1-h licks (mean + sem) are plotted for glucose-paired (CS+) and sucralose-paired (CS-) flavored sucralose solutions during the two-bottle preference test. Numbers atop bars represent mean percent preference for the CS+ solution. Significant differences (P < 0.05) between Tests 0 vs. Tests 1 – 3 licks and between CS+ vs. CS-licks are indicated by an asterisk. There were no significant differences between the Capsaicin and Control groups.

Figure 5. Experiment 2. Glucose-stimulated licking in control B6 mice. Licks per 3-min bin are plotted for Test 0 with 0.8% sucralose and Tests 1-3 with 8% glucose. Inset graph plots cumulative lick curves for Tests 0 – 3. Analysis of the 3-min data indicated that, compared to Test 0 sucralose
licks, in Test 1 the mice licked less (P < 0.01) glucose in bin 1 but more (P < 0.05) in bins 3-7 and bins 9, 12, 15-16, 18, and 20. In Tests 2 and 3 the mice licked more for glucose in bins 1-8 and 1-9, respectively.

Figure 6. Experiment 2. Glucose-stimulated licking in capsaicin-treated B6 mice. Licks per 3-min bin are plotted for Test 0 with 0.8% sucralose and Tests 1 – 3 with 8% glucose. Inset graph plots cumulative lick curves for Tests 0 – 3. Analysis of the 3-min data indicated that, compared to Test 0 sucralose licks, in Test 1 the mice licked less (P < 0.01) glucose in bin 1 but more (P < 0.05) in bins 3-9 and 11. In Tests 2 and 3 the mice licked more for glucose in bins 1-5, 7 and 9 and in bins 1-4, and 6-7, respectively.

Figure 7. Experiment 3. Intragastric glucose stimulation of licking and flavor preference conditioning. The mice drank (1 h/day) a CS- flavored saccharin solution paired with IG water infusions in Test 0 before being switched to a CS+ flavored saccharin solution paired with IG 16% glucose infusions in Tests 1 – 3. Left side: 1-h licks (mean ± sem) are plotted for one-bottle Tests 0 – 3. Right side: 1-h licks (mean + sem) are plotted for CS+ and CS- flavored saccharin solutions during the two-bottle preference test. CS+ and CS- intakes were not paired with IG infusions in the two-bottle test. Number atop bar represents mean percent preference for the CS+ solution. Significant differences (P < 0.05) between Tests 0 vs. Tests 1 - 3 licks and between CS+ vs. CS- licks are indicated by an asterisk.

Figure 8. Experiment 3. Intragastric glucose-stimulated licking. Licks per 3-min bin are plotted for Test 0 with CS- flavored saccharin solution paired with IG water infusions, and for Tests 1 – 3 with CS+ flavored saccharin solution paired with IG 16% glucose infusions. Analysis of the 3-min data indicated that, compared to CS- licks (Test 0), in Test 1 the mice licked less (P < 0.01) CS+ in bin 1 but more (P < 0.05) in bins 5-10. In Tests 2 and 3 the mice licked more for CS+ in bins 1-5 and 1-4, respectively.

Figure 9. Experiment 4. Intragastric fat stimulation of licking and flavor preference conditioning. The mice drank (1 h/day) a CS- flavored saccharin solution paired with IG water infusions in Test 0
before being switched to a CS+ flavored saccharin solution paired with IG 6.4% Intralipid infusions in Tests 1 – 3. Left side: 1-h licks (mean ± sem) are plotted for one-bottle Tests 0 – 3. Right side: 1-h licks (mean + sem) are plotted for CS+ and CS- flavored saccharin solutions during the two-bottle preference test. CS+ and CS- intakes were not paired with IG infusions in the two-bottle test. Number atop bar represents mean percent preference for the CS+ solution. Significant differences (P < 0.05) between Tests 0 vs. Tests 1-3 licks and between CS+ vs. CS- licks are indicated by an asterisk.

Figure 10. Experiment 4. Intragastric fat-stimulated licking. Licks per 3-min bin are plotted for Test 0 with CS- flavored saccharin solution paired with IG water infusions, and for Tests 1 – 3 with CS+ flavored saccharin solution paired with IG 6.4% Intralipid infusions. Analysis of the 3-min data indicated that, compared to CS- licks (Test 0), in Test 1 the mice licked more CS+ (P < 0.05) in bins 4, 6, 7, 11-17 and 19. In Tests 2 and 3 the mice licked more for CS+ in bins 1-9 and 14 and in bins 1-12 and 14 respectively.
Licks / 1 h

0 1000 2000 3000 4000

---------- Sugar ----------

One-Bottle Tests

Fructose
Sucralose

Glucose

* * *

* + +

* +

* +

0 1 2 3
One-Bottle Tests

0 1000 2000 3000 4000
Licks / 1 h

Sucralose
Sugar
Capsaicin and Control

Licks / 1 h

--- Glucose ---

One-Bottle Tests

Two-Bottle Tests

Sucralose

--- Glucose ---

CS+

CS−

82%        80%

* *

* *

* *

* *

80%

Capsaicin Group

Control Group

CS−

CS+
Licks / 1 h

<table>
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<th>CS-</th>
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<tr>
<td>IG H2O</td>
<td></td>
<td>72%</td>
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<td>IG Glucose</td>
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One-Bottle Tests

Two-Bottle Test

* Indicates significance at 72%