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Rapid Acquisition of Conditioned Flavor Preferences in Rats

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Abstract

Rats learn to prefer flavors paired with the post-oral effects of glucose. The present study examined how rapidly they acquire this preference. In Experiment 1, food-restricted rats were given repeated three-session training/testing cycles: one 30-min session with a CS+ flavor paired with intragastric (IG) infusion of 16% glucose, another session with a CS− flavor paired with IG water, and a third session with a choice between the flavors with their infusates. The rats preferred the CS+ (69%) in the first choice session, and preference increased across the six cycles to 86%. These data demonstrate that the post-oral reinforcing action of glucose is potent enough to support one-trial learning. In Experiment 2, two groups of rats were trained in the same way, with the CS+ flavor paired with IG infusion of 16% glucose or 7.1% corn oil emulsion, but tests were conducted under extinction conditions, with both CS+ and CS− flavors paired with IG water. Significant preference for the CS+ was acquired more rapidly with glucose (71% CS+ in test 1) than with oil (69% CS+ in test 4). Consistent with previous work, the post-oral stimulation by glucose was more potent than that of isocaloric oil emulsion in conditioning preferences. The last experiment examined the acquisition rate for a flavor-taste conditioned preference. Rats were trained with a CS+ flavor mixed into an 8% fructose + 0.2% saccharin solution and a CS− flavor in 0.2% saccharin. The same three-session training/testing cycles were used, and in the tests the flavors were presented in saccharin. A significant 74% preference for the CS+ flavor was apparent by the second test. Together these studies show that the acquisition of flavor preferences, whether based on flavor-taste or flavor-nutrient associations, can be quite rapid.

Keywords

Flavor conditioning; Intragastric infusion; Reinforcement; One-trial learning; Nutrient

1. Introduction

Animals readily learn to avoid the flavor (taste, odor, texture) of foods or fluids that are associated with gastrointestinal malaise. Extensive laboratory research on conditioned flavor avoidance has revealed several notable features including one-trial learning. For example, after a single experience with a novel conditioned stimulus (CS) flavor (e.g., saccharin solution) followed by the administration of a toxic unconditioned stimulus (US; e.g., lithium chloride), rats will consume less of the CS solution when it is next presented [21]. The magnitude of the CS avoidance is determined, in part, by the intensity of the toxic treatment. When treated with

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low doses of LiCl, rats will display only a weak CS avoidance, although the conditioned avoidance will increase with repeated flavor-LiCl pairings [9].

Animals also learn to prefer flavors that are associated with positive postingestive consequences. Conditioned flavor preferences have been extensively demonstrated in the laboratory by pairing the ingestion of a novel CS flavor with a nutritive US solution (e.g., glucose) using a variety of procedures. Strong preferences (often >90%) are conditioned by infusing animals intragastrically (IG) with a nutritive solution as they drink a novel flavored solution (CS+). In other training sessions a different flavor (CS-) is paired with IG infusions of a nonnutritive solution (e.g., water). Flavor preferences are then assessed in a two-bottle test with both flavors presented together [e.g., 11,13,15,19,22,26,27,38]. Typically, several one-bottle training sessions are conducted prior to the first two-bottle choice test, by which time a significant preference is displayed. Consequently, these studies provide little information on the acquisition rate of conditioned flavor preferences.

The ability of animals to learn a flavor-nutrient association in one exposure, as they can with flavor-toxin associations, would demonstrate the relevance of this learning process in their natural environment, where the opportunities for multiple learning experiences may be limited. Rapid identification of nutritious food would confer a selective advantage, perhaps equal in importance to rapid learning about toxins. The neural bases of flavor learning have received considerable attention. Some areas of the brain are differentially involved in mediating acquisition vs. expression of learned aversions [34]. Recent studies [5,6,8,14,18,33,37] have demonstrated differential sensitivity to pharmacological treatment during acquisition of flavor preferences and in expression of already-acquired preferences, which suggests shifts in the relative importance of central circuits as learning proceeds. However, these studies were conducted with multiple training trials prior to testing; there is a need for techniques that can assess the acquisition phase in greater detail.

The present study, therefore, determined if rats can learn a flavor preference after a single flavor-nutrient pairing, if this preference improves with further training, and if this rapid learning varies as a function of nutrient type. Following the initial demonstration in Experiment 1 of rapid learning with IG glucose infusions, Experiment 2 extended the analysis to compare the flavor conditioning effect of IG glucose with that of IG fat (corn oil) infusions. Experiment 3 investigated flavor conditioning by orally consumed fructose. In contrast to glucose, IG fructose infusions do not condition flavor preferences in rats trained during short (30-min) sessions [2,29]. However, rats learn to prefer a flavor mixed into an orally consumed fructose solution after several short training sessions [28]. This flavor preference is assumed to be reinforced by the sweet taste of fructose rather than its postingestive actions. Experiment 3 determined if this flavor-taste learning could occur in a single trial.

After the completion of the first glucose experiment Myers [20] published a study demonstrating that rats can learn a preference for a flavor after only one pairing with IG glucose infusion. As discussed below, his training procedures differed in several respects from those of the present study. The current report confirms and extends his finding.

2. Experiment 1

In prior work with multiple training exposures to CS+ and CS- flavors, glucose and glucose-containing saccharides (sucrose, maltose, Polycose) are potent reinforcers [4,13,29]. To evaluate how rapidly rats could learn to prefer a glucose-paired flavor over a water-paired flavor, Experiment 1 used a training-test cycle consisting of one exposure to the CS+, one exposure to the CS-, and then a choice test with both flavors offered simultaneously. Six cycles were conducted, with the order of one-bottle CS presentation counterbalanced and the paired infusions maintained during testing to avoid partial reinforcement of CS+ intakes.

2.1. Subjects

Twelve adult female Sprague Dawley rats bred from stock obtained from Charles River Laboratories (Wilmington, MA) were used. The animals weighed 235–290 g and were housed in individual cages kept in a room maintained at 21°C with a 12:12 h light:dark cycle (lights on 0800 h). Tap water was always available in the home cages. Powdered chow (No. 5001, PMI Nutrition International, Brentwood, MO) was available as described below.

2.2. Surgery

The rats were anesthetized with a ketamine (63 mg/kg) and xylazine (9.4 mg/kg) mixture and surgically implanted with dual gastric catheters using a method modified from that of Davis and Campbell [10]. Two silastic tubes (0.76 mm i.d., 1.65 mm o.d.) were inserted into the fundus of the stomach and secured with sutures and polypropylene mesh. The catheters were routed subcutaneously to the head, where they connected to a Luer-lock assembly secured to the skull with stainless steel screws and dental cement.

2.3. Apparatus

Training and testing were conducted in plastic cages (23 × 24 × 31.5 cm) with stainless steel mesh flooring. Above the cage a counterbalanced lever held a dual-channel infusion swivel connected, by plastic tubing, to two syringe pumps (A-99, Razel Scientific, Stamford, CT) and at the other end to the rat's Luer-lock assembly. The rats drank from one or two stainless steel spouts accessible via two holes at the front of the cage; a motorized bottle holder (ENV-252M, Med Associates, Georgia, VT) automatically inserted and removed the spouts at the beginning and the end of a session. Fluid spillage, which was minimal, was collected in trays below the spouts; spillage amounts were recorded and used to correct the intake data. Licking was monitored by an electronic lickometer (ENV-250B, Med Associates) connected to a microcomputer that activated the pumps as the animal drank. The intragastric infusion rate was 1.3 ml/min, and was controlled by computer software to infuse ~ 1 ml of fluid for each 1 ml of fluid consumed orally.

2.4. Test Solutions

All solutions were prepared on a weight/weight basis using tap water. The CS solutions contained 0.05% unsweetened grape or cherry Kool-Aid mix (Kraft Foods, White Plains, NY). Naive rats are indifferent in a choice between these flavors, which are equally unpreferred to water [13]. To ensure that the animals would consume the flavors in 30-min tests, the solutions also contained 0.2% sodium saccharin (Sigma Chemical Co., St. Louis, MO). For half the rats, cherry-saccharin was the CS+ solution which was paired with IG infusions of 16% glucose solution (BioServ, Frenchtown, NJ) and grape saccharin was the CS– solution which was paired with IG infusions of water. The flavor-infusate pairs were reversed for the remaining animals.

2.5. Procedure

Prior to surgery, the rats were familiarized with the unflavored sweet solutions and the test cages. They were given 40 ml of 2% sucrose + 0.2% saccharin and ad libitum water in their home cages for 2 consecutive days, followed by one overnight session in the test cages with the same fluids. During this session fluids were available for 30 min every h to accustom the animals to the automated presentation and removal of the bottles. They were returned to their home cages and food restricted during additional daily 30-min training sessions in the test cages. They were given five sessions of one-bottle 2% sucrose + 0.2% saccharin, one session of 1% sucrose + 0.2% saccharin, and four sessions with 0.2% saccharin. This “sucrose-fading” procedure enhances subsequent intake of saccharin solutions [5]. During this and all subsequent

phases of the experiment, chow rations were delivered 1 h after the sessions in the animals' home cages. Chow was provided ad libitum at the end of pre-training, prior to surgery.

After surgery, the animals were allowed to recover for 1 week and then were returned to food restriction; thereafter body weights were recorded daily and chow rations were adjusted to maintain the animals at 85% of their free-feeding weights. After 4 days, daily 30-min sessions in the test cages resumed, with additional pre-training to adapt the animals to the infusion system with one- and two-bottle sessions. Four one-bottle 0.2% saccharin sessions without infusions were conducted; the rats were attached to the infusion system for the last two of these sessions. Then they were infused with water when they drank saccharin for two one-bottle sessions, and finally they were given two sessions of two-bottle training with saccharin and water, both paired with IG water. During this and all subsequent phases of the experiment, chow rations were provided in the home cage 1 h after the 30-min session.

On the first day of flavor training, all animals were given 30-min access to the grape-saccharin solution which, for half the rats was the CS+ paired with IG glucose and for the remaining rats was the CS- paired with IG water. On day 2, the cherry-saccharin solution was available paired with IG water (for rats that had IG glucose on day 1) or glucose (for rats that had IG water on day 1). On day 3, the rats were given a two-bottle choice test with the CS+ vs. CS-, with intake of each solution paired with its appropriate infusate. This 3-day training-test cycle was presented a total of six times. To counterbalance side of presentation, the 3-day sequence in cycles 1, 3 and 5 was grape left, cherry right, then test with CS+ left and CS- right, and for the other cycles was cherry left, grape right, then test with CS- left and CS+ right. After the sixth two-bottle test, another two-bottle test was conducted (test 7), which was followed by two additional tests (tests 8 and 9) during which intake of both the CS+ and CS- were paired with IG water infusions (non-reinforced test).

2.6. Data analysis

Averaged one-bottle intakes of CS+ and CS- solutions were compared with a paired t-test. Intakes of the CS+ and CS- solutions in two-bottle sessions were compared in repeated-measures ANOVA. Significant interaction effects were evaluated using simple main effects tests. For the comparison of reinforced and non-reinforced tests, intakes of the two sessions of each test were averaged. Percent preference for the CS+ was calculated as CS+ intake divided by total intake $\times 100$. Preferences in the two tests were compared using paired t-tests.

2.7. Results and discussion

In the six cycles of training and testing, the rats treated the CS+ and CS- similarly on one-bottle days, but not on two-bottle days (Fig. 1). During one-bottle training sessions, the rats consumed comparable amounts of the CS+ and CS-. When given the choice of the CS+ and CS- paired with IG glucose and water, respectively, the rats consumed more CS+ overall ($F(1,11) = 33.19$, $p < 0.001$). The CS+ preference was observed in the very first two-bottle test (11 of 12 rats consumed more CS+ than CS- in test 1) and did not differ as a function of whether the rats were exposed to the CS+ before or after the CS- in the preceding one-bottle sessions (71% vs. 67%). The absolute intakes of the CS+ and CS- did not differ over test cycles, i.e., cycle main effect and CS $\times$ cycle interaction were not significant. However the percent CS+ preference increased with testing, from 69% in test 1 to 86% by test 6 ($F(5,55) = 2.55$, $p < 0.05$).

The comparison of reinforced and non-reinforced tests showed greater intakes of CS+ than CS-, $F(1,11) = 52.21$, $p < 0.001$. Intakes of the CS+ but not CS- were higher in the non-reinforced test than in the reinforced test (15.0 vs. 10.1 g/30 min CS+, 2.5 vs. 1.8 g/30 min CS-; CS $\times$ test interaction, $F(1,11) = 20.19$, $p < 0.001$). The rats also displayed a stronger CS+ preference

in the non-reinforced test than in the reinforced test (89% vs. 80%, $t(11) = 3.28$, $p < 0.01$). The reduced CS+ intake and preference in the reinforced test may have been due to the satiating effect of the infused glucose.

The present finding that rats acquired a 69% preference for a glucose-paired flavor over a water-paired flavor after a single exposure to each is close to the 72% CS+ preference recently reported by Myers [20]. The similar results are noteworthy because of the different procedures used in the two studies. In particular, Myers trained food and water restricted male rats to drink coffee and vinegar flavored saccharin solutions during 10-min one-bottle sessions. Furthermore in the CS+ vs. CS- test conducted in the Myers study the rats were infused with water whereas in the present experiment intake of the CS+ was paired with IG glucose. The similar preferences obtained in the two experiments indicate that glucose infusion during the first two-bottle test is not essential for obtaining a significant preference. It may, however, contribute to the increased preference observed over training cycles in the present experiment. The rats in the Myers experiment were given one training cycle followed by an additional six non-reinforced two-bottle tests during which they maintained their CS+ preference at 72%. This latter finding confirms earlier reports that nutrient-conditioned preferences are very resistant to extinction [12,13].

3. Experiment 2A

Experiment 1 and the Myers study demonstrate that 16% glucose is effective in producing one-trial preference conditioning. Experiment 2A expanded this analysis to a different nutrient, corn oil. Prior research indicates that corn oil emulsions generally condition weaker preferences than isocaloric glucose or glucose polymer solutions [16,17]. Two groups of rats were trained with the CS+ paired with IG infusions of 16% glucose or isocaloric 7.1% corn oil emulsion. The rats were trained and tested as in the first experiment, except that they were infused with water during the two-bottle tests following the procedure of Myers [20].

3.1. Procedure

Adult female rats (259–301 g) of similar provenance were given single gastric catheters and maintained as in Experiment 1. They were trained with the same procedures as Experiment 1, with the following exceptions. Two-bottle test days were always non-reinforced: the rats were co-infused with water when they drank from either CS solution. One group ($n=12$) was infused with 16% glucose on CS+ training days, and the other group ($n=12$) was infused with 7.1% corn oil emulsion. The emulsion was prepared with commercial corn oil (Mazola, ACH Food, Memphis TN) using the emulsifier sodium stearoyl lactylate (Emplex, American Ingredients, Grandview MO; 0.2%). The oil and emulsifier were added to hot water and mixed at high speed for 5 min in a rotor-stator homogenizer (Ultra-Turrax T-25, IKA Works, Cincinnati OH), rapidly cooled, and passed twice through a microfluidizer (HC 5000, Microfluidics, Newton, MA) to stabilize the emulsion further. Six cycles of training and testing were conducted.

3.2. Results and Discussion

The rats treated the CS+ and CS- solutions similarly on one-bottle days, but not on two-bottle days (Fig. 2). Average one-bottle intakes were analyzed with 2 (group) by 2 (CS) ANOVA, which showed that the rats consumed comparable amounts of the CS+ and CS- solution during training. Although the oil group drank somewhat more of the solutions than the glucose group, and intake of the CS- was slightly greater than that of the CS+, these differences were only marginally significant (group $F(1,22) = 3.68$, $p = 0.07$; CS $F(1,22) = 4.133$, $p = 0.054$). The preference data were analyzed with a 2 (group) by 2 (CS) by 6 (cycle) ANOVA. When tested with the choice of the CS+ and CS- both paired with IG water, the rats consumed more CS+ overall ($F(1,22) = 80.72$, $p < 0.001$). Total CS intakes increased over sessions ($F(5,110) =$

10.39, $p < 0.001$), with a $CS \times \text{cycle}$ interaction ($F(5,110) = 11.57$, $p < 0.001$) due to similar intakes of the two solutions in the second test but not the others. Within-group planned comparisons showed that intake of the $CS+$, but not the $CS-$ increased over sessions for both groups. The glucose group's $CS+$ intake exceeded ($p < 0.05$) $CS-$ intake on all but the second test ($CS \times \text{cycle}$, $F(5,55) = 6.00$, $p < 0.001$), whereas the oil group's preference was not significant ($p < 0.05$) until test 4 ($CS \times \text{cycle}$, $F(5,55) = 6.33$, $p < 0.001$). Percent $CS+$ intakes also increased over sessions. The glucose group's preference ranged from 71% in test 1 to a peak of 88% in test 5 and then 81% in test 6 ($F(5,55) = 3.99$, $p < 0.01$). The percent $CS+$ intakes of the Oil group ranged from 56% in test 1 to a peak of 86% in test 6.

The glucose group's preference of 71% on the first test is similar to the 69% preference observed in the first test of Experiment 1 and the 72% preference reported by Myers [20]. The present findings provide further evidence that reinforced testing is not critical for the expression of significant $CS+$ preference after a single training session with the $CS+$ and $CS-$. However, unlike the glucose-trained rats in Experiment 1, the present glucose group did not express a $CS+$ preference in the second two-bottle test. Possible explanations for this discrepancy are discussed in the next experiment.

As a further exploration of the effect of procedural difference (reinforced vs non-reinforced testing), the $CS+$ preference scores of the glucose groups in Experiments 1 and 2A were compared. The main effect of group was not significant, and the group $\times$ cycle interaction was marginal ($F(5,110) = 2.15$, $p = 0.065$). (This was due to a difference in preference only in test 5). The similar strength and course of the preference tests suggests that infusion during test does not have much bearing on the outcome.

A significant $CS+$ preference appeared later (test 4) in the oil group than in glucose group (test 1). This is consistent with a number of previous studies showing that post-oral reinforcement by corn oil is not as potent as carbohydrate reinforcement [17,35]. The present findings suggest that part of the difference in potency is slower acquisition of oil-based preferences. Nevertheless, the percent $CS+$ preferences of the oil and glucose groups were similar by the last two-bottle test (86% vs. 81%).

4. Experiment 2B

While the glucose groups in Experiments 1 and 2A displayed similar $CS+$ preferences in the first test session (69% vs. 71%), they diverged in the second test. Unlike the rats in the first experiment, the glucose rats in Experiment 2A did not express a $CS+$ preference in the second two-bottle test. This may be related to the fact that in the second experiment $CS+$ intake was paired with IG water infusions during testing, which may have weakened the association between the $CS+$ and post-oral nutrient reinforcement. It is also possible, however, that a minor procedural difference between the experiments contributed to the discrepant results. Training and test sessions were run 6 days/week, as is common practice in our studies. The off days occurred between cycles in Experiment 1. However, in Experiment 2A the gap between sessions fell between the second one-bottle training day and the two-bottle preference day in cycles 2, 4, and 6. Thus the second test occurred after a 1-day gap, and the interruption may have disrupted behavior. Experiment 2B determined if non-reinforced testing disrupted $CS+$ preference conditioning when there were no gaps between the training and test sessions within the cycles.

4.1. Procedure

Adult female rats ($n=11$, 264–320 g) of similar provenance were given single gastric cannulas and maintained as in Experiment 2A. They were trained with the same procedures as the glucose group of Experiment 2A, except that the days off fell between cycles 2 and 3, and

cycles 4 and 5, which ensured that each two-bottle test occurred the day after the second training day of each cycle.

4.2. Results and Discussion

The rats treated the CS+ and CS− similarly on one-bottle days, but not on two-bottle days (Fig. 3). During one-bottle training sessions, the rats consumed comparable amounts of the CS+ and CS−. When given the choice of the CS+ and CS− paired with IG water, the rats consumed more CS+ overall ($F(1,10) = 37.6$, $p < 0.001$). The CS+ preference was apparent in the first two-bottle test (70%) and ranged from 73 to 82% in the remaining tests, although this variation was not significant.

The rats showed a clear preference for the CS+ on the first test, and this preference was sustained in succeeding tests. This outcome resembles that of Experiment 1, and suggests that testing under extinction conditions does not differ from reinforced testing. The reduced preference in the second test for the glucose group in Experiment 2A may therefore reflect the timing of session gaps within cycles or perhaps was due to random variation. Note that in the Myers [20] experiment the rats were given a non-reinforced session with unflavored saccharin between the last training session and the first two-bottle test session, which indicates that testing immediately after training is not critical.

5. Experiment 3

The findings so far indicate that one-trial flavor preference conditioning is produced by IG glucose but not IG corn oil infusions. The third experiment examined flavor conditioning by another nutrient, fructose. This sugar is of interest because it conditions flavor preferences when mixed with a CS+ flavor and orally consumed but not when infused IG in short daily sessions [1,2,6,7,29]. Preferences conditioned by orally consumed fructose therefore are considered to be a form of flavor-taste conditioning, in which the sugar's sweet taste is the unconditioned stimulus. In our prior fructose conditioning studies, animals were given eight to ten training sessions before preference testing and therefore it is not known how rapidly fructose-based preferences were acquired [1,6,7,28,31,32]. Recently, Golden and Houpt [14] investigated the acquisition of fructose-conditioned preferences by conducting flavor preference tests after successive blocks of four training trials. A significant CS+ flavor preference did not emerge until eight to twelve training trials, which suggests that oral fructose conditioning proceeds more slowly than does IG glucose conditioning. Experiment 3 investigated this question further by using the training and testing sequence of the prior experiments of this series. The animals were subsequently given eight training trials followed by a test to determine whether they would improve their preference following more conventional training.

5.1 Procedure

Twelve adult female rats (247–300 g) of similar provenance were maintained on restricted rations to keep them at 85% of ad libitum weight. After pretraining to drink in test cages in 30-min sessions, they were given six 3-day cycles of training and testing in a parallel to the infusion experiments. Instead of receiving the nutrient intragastrically, the animals consumed it mixed into the CS+ solution (CS+/F). The fructose concentration was 8%, chosen to match the net concentration of glucose in the gut in the infusion experiments (16% glucose infusion plus an equal volume of ingested CS+ solution). Other aspects of the procedure were similar; all rats received grape on day 1 and cherry on day 2 of the cycle, with fructose in grape for half the rats and in cherry for the others. Both CS solutions contained 0.2% saccharin in addition to 0.05% Kool-Aid flavor. On two-bottle test days the rats were given the choice between the CS+ vs. CS− solutions without fructose. Like the tests in the non-reinforced infusion experiments,

this test procedure constituted an extinction condition. Days off were scheduled between rather than within cycles.

The rats were next retrained with the same flavors using a different training protocol, alternating the CS+/F and CS− flavors across 8 training days. Then they were given eight preference sessions with CS+ vs. CS−. There were no days off during this phase.

5.2 Results and discussion

The rats consumed more of the CS+/F solution than the CS− solution during one-bottle training ($t(11) = 4.47$, $p < 0.001$) and consumed more of the CS+ solution (without fructose) than the CS− solution during the two-bottle tests (Fig. 4; $F(1,11) = 21.9$, $p < 0.001$). Total CS intakes differed across cycles ($F(5,55) = 8.36$, $p < 0.001$), but the CS $\times$ cycle interaction was not significant. Nevertheless planned comparisons revealed that CS intakes did not reliably differ in test 1, in which only 6 of the 12 rats consumed more CS+ than CS−. CS+ and CS− intakes significantly differed ($p < 0.05$) in tests 2, 4 and 5 and nearly so ($p = 0.051$) in test 6. Percent CS+ intakes did not significantly differ over cycles, although they ranged from 62% in test 1, to a peak of 79% in test 4, and then a decline to 61% in the last test.

In one-bottle retraining without interspersed test sessions, the rats drank more CS+/F than CS− (Fig. 5; $t(11) = 3.85$, $p < 0.01$). In the 8-day extinction test that followed, the rats continued to prefer the CS+ over the CS− ($F(1,11) = 27.5$, $p < 0.001$). A CS $\times$ day interaction ($F(5,55) = 2.23$, $p < 0.05$) reflected vacillation in CS+ but not CS− intake across days (Fig. 5). CS+ intakes significantly ($p < 0.05$) exceeded CS− intakes in tests 2, 4, 6, 7, and 8 and nearly so ($p < 0.063$) in tests 1 and 3. Percent CS+ intakes did not significantly vary over testing and were 72% in test 1 and 76% in test 8. The decline in CS+ intake in test 5 was largely due to one rat which drank virtually no CS+, possibly due to a clogged sipper tube. With this test excluded, the rats averaged a 77% CS+ preference over the 8 extinction tests, which was greater than their average 66% preference during the first 6 preference tests that were interspersed among the one-bottle training sessions, $t(11) = 2.26$, $p < 0.05$.

The present finding of a significant CS+ preference after four training trials (test 2) contrasts with an earlier report that eight to twelve training trials were required to produce a significant preference for a CS+ flavor paired with fructose [14]. The two experiments differed in a number of respects, which may account for the discrepant results. In particular, whereas the present study used female rats given blocks of two 30-min training sessions, the Golden study used male rats given blocks of four 2-h training sessions. Also, the CS+ training solution used in the Golden study included 8% fructose and 0.05% Kool-Aid flavor, whereas the CS+ training solution used in the present experiment included 0.2% saccharin as well. This is a potentially important difference because mixtures of sugar and saccharin are, in general, more attractive to rats than a sugar-only solution [25,30].

6. General Discussion

The present findings show that rats rapidly learned a glucose-based flavor preference, demonstrating a preference for a flavor paired only once with an IG glucose infusion, relative to a flavor paired once with a water infusion. In contrast to glucose, IG corn oil infusions did not produce one-trial flavor conditioning but rather required four CS+ training sessions to produce a significant preference. The flavor preference conditioned by the sweet taste of fructose also required more than one training trial and was also not very stable when training and test sessions were interspersed. Thus, flavor preference conditioning, like avoidance conditioning, can be quite rapid, although acquisition rate varies as a function of the nutrient unconditioned stimulus.

Flavor-nutrient learning

The ability of IG glucose infusions to produce one-trial learning was quite consistent. Following the first IG glucose training trial, the rats in the first three experiments displayed CS+ preferences of 69, 70, and 71%. These preferences are comparable to the 72% CS+ preference reported in male rats given a single IG glucose training trial using a different pair of CS flavors [20]. With repeated training trials, the rats in the present study displayed maximum preferences of 82 – 88%. This approaches the 91% CS+ preference observed in an earlier study with female rats given three CS+ and CS– training trials prior to testing [29]. The one-trial CS+ preferences were obtained in food-restricted (present study) and food- and water-restricted rats [20] tested under reinforced or non-reinforced conditions. Whether one-trial conditioning is possible with non-deprived animals remains to be determined; we have reported CS+ flavor conditioning in non-deprived animals after multiple training trials (four CS+ and four CS– exposures) [36].

The finding that, unlike glucose, corn oil infusions required multiple training trials to produce a significant CS+ flavor confirms prior reports that corn oil, in general, conditions weaker flavor preferences than do glucose or glucose polymers (e.g., Polycose, maltodextrin) at isocaloric concentrations [16,17,35,36]. A recent study also found a slower acquisition of flavor learning with fat than carbohydrate in a conditioned satiety paradigm using two different concentrations of sucrose solution or corn oil emulsion [24]. The greater strength of glucose-based than fat-based conditioning is most clearly demonstrated by the preference rats display for a CS+ flavor paired with IG Polycose infusions over a different CS+ flavor paired with IG corn oil infusions [17,35]. Note that maintaining rats on a high-fat rather than standard low-fat chow enhances the flavor conditioning actions of IG corn oil infusions, although the animals still prefer a CS+Polycose flavor to a CS+fat flavor [17]. This high-fat maintenance diet effect appears to be related, in part, to enhanced fat digestion and/or absorption. It is possible, therefore, that IG corn oil infusions would condition flavor preferences more rapidly in rats fed a high-fat rather than a low-fat chow. That direct comparison has not yet been conducted. The identity of the reinforcing stimuli generated by IG infusions of fat and glucose remains unknown. The differential potency of isocaloric fat and glucose infusions to condition flavor preferences rapidly suggests that these nutrients generate different reinforcing stimuli or that they generate a common stimulus but at different rates. By titrating the concentration or volume of infusion, it may be possible to match IG reinforcers that initially differ in potency so that rate of acquisition of flavor preferences is similar. This could help to narrow the focus on their shared reinforcing properties.

An alternative procedure used to study rapid nutrient conditioning involves training animals in short sessions with concurrent access to both CS+ and CS– flavors and their paired infusions. In one series of studies, food and water restricted rats were given brief daily access to two flavors, one of which was paired with matched milk infusions and the other with matched saline infusions (or no infusions). In some studies [23,38], rats preferred the CS+ within a single 7- or 10-min session, though in others significant preferences were not evident until the fifth session [39]. Other rats infused with 1 M (18%) glucose when they drank the CS+ did not acquire a preference during a series of nine sessions [23]. Why infusions of a complex nutrient mixture (milk) but not a simple nutrient (glucose) supports conditioning with two-bottle training is unclear. However, it may be related to the hydration state of the animals used in these studies; their only access to fluid was during the daily 7-min training sessions. Perhaps the IG milk infusions were more reinforcing to the thirsty rats than were the presumably more hypertonic glucose infusions. In studies using our IG infusion system, food-restricted rats readily learn to prefer a glucose-paired CS+ over a concurrently available water-paired CS– within one 20-h session [2], but did not acquire a preference in the course of six 30-min concurrent sessions (unpublished data). Comparisons of the effectiveness of IG milk and

glucose infusions to condition flavor preferences in rats that are food but not water restricted would be informative.

Flavor-taste learning

Fructose is markedly less effective than glucose and corn oil in post-oral conditioning. When the training sessions are relatively brief (e.g., 30 min), rats trained with IG fructose are indifferent to the CS+ in preference tests [29]. With 20–24 h training sessions, however, IG fructose infusions can condition flavor preferences in some cases [2,3]. We have speculated that the reduced potency of fructose may reflect a delayed post-oral reinforcing effect relative to glucose [2]. Despite its shortcomings as a post-oral reinforcer, fructose is an effective US in flavor-taste conditioning with multiple short training sessions, with CS+ preferences of 74–82% [6–8,14,18,28]. It thus serves as an oral reinforcer in brief sessions with minimal, if any post-oral reinforcement. In the present study, pairing a CS+ flavor with orally consumed fructose did not yield a preference in the first test, requiring a second training cycle before a significant CS+ preference was evident. The flavor preference conferred by association with the sweet taste of fructose was less stable than that of IG glucose when tested after each pair of training days. Following massed training, there was a stable fructose-based preference in the prolonged extinction test, which replicates an earlier finding [28]. The instability during the interspersed training and test days may reflect the change in the CS+ flavor across sessions: the training CS+ solution containing fructose and saccharin was very sweet, in contrast to the mildly sweet test CS+ solution with saccharin only. This degradation of CS+ stimulus quality did not occur in the IG infusion experiments, in which the animals were always given saccharin-sweetened solutions. Perhaps the contrast between training and test solutions is less important when fructose-trained rats get more CS+ training trials before testing.

Mediation of flavor preference acquisition vs. expression

The neural systems serving acquisition and expression of conditioned flavor preferences are not identical. For example, antagonism of dopamine D1 receptors, but not D2 receptors, blocks the acquisition of flavor preferences based on post-oral sugar but has lesser effects on the expression of already-acquired preferences [5,33]. Systemic CB1 receptor antagonism and central (nucleus accumbens) dopamine antagonism have no effect on acquisition of fructose-based preference but attenuate expression [8,18]. Systemic dopamine antagonism has different effects on acquisition and expression of flavor preference based on sweet taste [6,37]. Systemic antagonism of the NMDA glutamate-glycine receptor also selectively affects the acquisition and expression of fructose-based flavor-taste preferences [14]. These disparities are strong evidence that the neural substrates involved in acquiring a flavor preference and in expressing that preference can be dissociated. The multiple session training procedures that have been used extensively to generate flavor preferences are well-suited for the study of expression, and the present method is suggested as an ideal way to observe the process of flavor preference acquisition in more detail.

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References

1. Ackroff K, Sclafani A. Flavor preferences conditioned by sugars: Rats learn to prefer glucose over fructose. *Physiol Behav* 1991;50:815–824. [PubMed: 1775557]

2. Ackroff K, Touzani K, Peets TK, Sclafani A. Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency. *Physiol Behav* 2001;72:691–703. [PubMed: 11337001]
3. Ackroff K, Sclafani A. Fructose conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration. *Appetite* 2004;42:287–297. [PubMed: 15183920]
4. Azzara AV, Sclafani A. Flavor preferences conditioned by intragastric sugar infusions in rats: Maltose is more reinforcing than sucrose. *Physiol Behav* 1998;64:535–541. [PubMed: 9761229]
5. Azzara AV, Bodnar RJ, Delamater AR, Sclafani A. D₁ but not D₂ dopamine receptor antagonism blocks the acquisition of a flavor preference conditioned by intragastric carbohydrate infusions. *Pharmacol. Biochem. Behav* 2001;68:709–720.
6. Baker RM, Shah MJ, Sclafani A, Bodnar RJ. Dopamine D₁ and D₂ antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats. *Pharmacol. Biochem. Behav* 2003;75:55–65.
7. Baker RW, Li Y, Lee MG, Sclafani A, Bodnar RJ. Naltrexone does not prevent acquisition or expression of flavor preferences conditioned by fructose in rats. *Pharmacol Biochem Behav* 2004;78:239–246. [PubMed: 15219763]
8. Bernal SY, Dostova I, Kest A, Abayev Y, Kandova E, Touzani K, Sclafani A, Bodnar RJ. Role of dopamine D₁ and D₂ receptors in the nucleus accumbens shell on the acquisition and expression of fructose-conditioned flavor-flavor preferences in rats. *Behav Brain Res* 2008;190:59–66. [PubMed: 18339434]
9. Dacanay RJ, Mastropaolo JP, Olin DA, Riley AL. Sex differences in taste aversion learning: an analysis of the minimal effective dose. *Neurobehav Toxicol Teratol* 1984;6:9–11. [PubMed: 6325971]
10. Davis, JD.; Campbell, CS. Chronic intrajugular, intraportal, gastric, and duodenal cannulae for the rat. In: Singh, D.; Avery, DD., editors. *Physiological techniques in behavioral research*. Monterey: Brooks Cole; 1975. p. 163-177.
11. Drucker DB, Ackroff K, Sclafani A. Flavor preference produced by intragastric Polycose infusions in rats using a concurrent conditioning procedure. *Physiol Behav* 1993;54:351–355. [PubMed: 8372131]
12. Drucker DB, Ackroff K, Sclafani A. Nutrient-conditioned flavor preference and acceptance in rats: Effects of deprivation state and nonreinforcement. *Physiol Behav* 1994;55:701–707. [PubMed: 7800736]
13. Elizalde G, Sclafani A. Flavor preferences conditioned by intragastric Polycose: A detailed analysis using an electronic esophagus preparation. *Physiol Behav* 1990;47:63–77. [PubMed: 2109327]
14. Golden GJ, Houpt TA. NMDA receptor in conditioned flavor-taste preference learning: blockade by MK-801 and enhancement by D-cycloserine. *Pharmacol Biochem Behav* 2007;86:587–596. [PubMed: 17350084]
15. Horn CC, Mitchell JC. Does selective vagotomy affect conditioned flavor-nutrient preferences in rats? *Physiol. Behav* 1996;59:33–38.
16. Lucas F, Sclafani A. Flavor preferences conditioned by intragastric fat infusions in rats. *Physiol Behav* 1989;46:403–412. [PubMed: 2623061]
17. Lucas F, Sclafani A. Differential reinforcing and satiating effects of intragastric fat and carbohydrate infusions in rats. *Physiol Behav* 1999;66:381–388. [PubMed: 10357426]
18. Miner P, Abayev Y, Kandova E, Gerges M, Styler E, Wapniak R, Touzani K, Sclafani A, Bodnar RJ. Role of systemic endocannabinoid CB-1 receptor antagonism in the acquisition and expression of fructose-conditioned flavor-flavor preferences in rats. *Pharmacol Biochem Behav* 2008;90:318–324. [PubMed: 18407342]
19. Myers KP, Sclafani A. Conditioned acceptance and preference but not altered taste reactivity responses to bitter and sour flavors paired with intragastric glucose infusion. *Physiol Behav* 2003;78:173–183. [PubMed: 12576114]
20. Myers KP. Robust preference for a flavor paired with intragastric glucose acquired in a single trial. *Appetite* 2007;48:123–127. [PubMed: 16979259]
21. Nachman M, Ashe JH. Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiol Behav* 1973;10:73–78. [PubMed: 4697023]

22. Pérez C, Lucas F, Sclafani A. Increased flavor acceptance and preference conditioned by the postingestive actions of glucose. *Physiol Behav* 1998;64:483–492. [PubMed: 9761222]
23. Puerto A, Deutsch JA, Molina F, Roll P. Rapid rewarding effects of intragastric injections. *Behav Biol* 1976;18:123–134. [PubMed: 985291]
24. Revelle, CH.; Warwick, ZS. Time course of acquisition of flavor-nutrient learning with fat vs carbohydrate calories. Steamboat Springs; CO: 2007.
25. Sclafani A, Einberg LT, Nissenbaum JW. Influence of saccharin on Polycose, sucrose, and glucose intake and preference in rats. *Neurosci Biobehav Rev* 1987;11:223–229. [PubMed: 3614790]
26. Sclafani A, Nissenbaum J. Robust conditioned flavor preference produced by intragastric starch infusion in rats. *Am J Physiol* 1988;255:R672–R675. [PubMed: 3140680]
27. Sclafani A, Cardieri C, Tucker K, Blusk D, Ackroff K. Intragastric glucose but not fructose conditions robust flavor preferences in rats. *Am J Physiol* 1993;265:R320–R325. [PubMed: 8368385]
28. Sclafani A, Ackroff K. Glucose- and fructose-conditioned flavor preferences in rats: Taste versus postingestive conditioning. *Physiol Behav* 1994;56:399–405. [PubMed: 7938256]
29. Sclafani A, Fanizza LJ, Azzara AV. Conditioned flavor avoidance, preference and indifference produced by intragastric infusion of galactose, glucose, and fructose in rats. *Physiol Behav* 1999;67:227–234. [PubMed: 10477054]
30. Smith, JC.; Foster, DF.; Bartoshuk, LM. The synergistic properties of pairs of sweeteners. In: Barker, LM., editor. *The psychobiology of human food selection*. Westport, CT: Avi Publishing; 1982. p. 123-138.
31. Touzani K, Sclafani A. Critical role of amygdala in flavor but not taste preference learning in rats. *Eur J Neurosci* 2005;22:1767–1774. [PubMed: 16197517]
32. Touzani K, Sclafani A. Insular cortex lesions fail to block flavor and taste preference learning in rats. *Eur J Neurosci* 2007;26:1692–1700. [PubMed: 17822433]
33. Touzani K, Bodnar R, Sclafani A. Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of nutrient-conditioned flavor preferences in rats. *Eur J Neurosci* 2008;27:1525–1533. [PubMed: 18336564]
34. Touzani, K.; Sclafani, A. Learned flavor aversions and preferences. In: Squire, LR., editor. *Encyclopedia of neuroscience*. Oxford: Academic Press; 2009. p. 395-399.
35. Tracy AL, Phillips RJ, Chi MM, Powley TL, Davidson TL. The gastrointestinal tract “tastes” nutrients: evidence from the intestinal taste aversion paradigm. *Am J Physiol* 2004;287:R1086–R1100.
36. Yiin YM, Ackroff K, Sclafani A. Flavor preferences conditioned by intragastric nutrient infusions in food restricted and free-feeding rats. *Physiol Behav* 2005;84:217–231. [PubMed: 15708774]
37. Yu WZ, Silva RM, Sclafani A, Delamater AR, Bodnar RJ. Role of D₁ and D₂ dopamine receptors in the acquisition and expression of flavor-preference conditioning in sham-feeding rats. *Pharmacol. Biochem. Behav* 2000;67:537–544.
38. Zafra MA, Simon MJ, Molina F, Puerto A. The role of the external lateral parabrachial subnucleus in flavor preferences induced by predigested food administered intragastrically. *Brain Res* 2002;950:155–164. [PubMed: 12231240]
39. Zafra MA, Molina F, Puerto A. Learned flavor preferences induced by intragastric administration of rewarding nutrients: role of capsaicin-sensitive vagal afferent fibers. *Am J Physiol* 2007;293:R635–R641.

Figure 1.

Experiment 1. Top: Mean ($\pm$ SEM) CS+ preferences during two-bottle tests. Bottom: Mean ($\pm$ SEM) intakes of CS+ and CS- solutions: means of six one-bottle sessions and individual means of two-bottle choice tests. The CS+ flavor was paired with IG infusion of 16% glucose, and the CS- flavor was paired with IG infusion of water, during both one- and two-bottle sessions. Asterisks indicate significantly greater CS+ than CS- intake.

Figure 2.

Experiment 2A. Top: Mean ($\pm$ SEM) CS+ preferences during two-bottle tests. Bottom: Mean ($\pm$ SEM) intakes of CS+ and CS- solutions: means of six one-bottle sessions and individual means of two-bottle choice tests. The left panels show the glucose group and the right panels show the oil group. The CS+ flavor was paired with IG infusion of 16% glucose or 7.1% corn oil emulsion during one--bottle sessions, and with IG water during two-bottle sessions. The CS- flavor was paired with IG infusion of water, during both one- and two-bottle sessions. Asterisks indicate significantly greater CS+ than CS- intake.

Figure 3.

Experiment 2B. A: Top: Mean ($\pm$ SEM) CS+ preferences during two-bottle tests. Bottom: Mean ($\pm$ SEM) intakes of CS+ and CS- solutions: means of six one-bottle sessions and individual means of two-bottle choice tests. The CS+ flavor was paired with IG infusion of 16% glucose during one--bottle sessions, and with IG water during two-bottle sessions. The CS- flavor was paired with IG infusion of water, during both one- and two-bottle sessions. Asterisks indicate significantly greater CS+ than CS- intake.

Figure 4.

Experiment 3. Top: Mean ($\pm$ SEM) CS+ preferences during two-bottle tests. Bottom: Mean ($\pm$ SEM) intakes of CS+ and CS- solutions: means of six one-bottle sessions and individual means of two-bottle choice tests. The CS+ flavor was mixed into 16% fructose + 0.2% saccharin during one-bottle sessions, and into 0.2% saccharin during two-bottle sessions. The CS- flavor was mixed into 0.2% saccharin during both one- and two-bottle sessions. Asterisks indicate significantly greater CS+ than CS- intake.

Figure 5.

Experiment 3. Top: Mean ($\pm$ SEM) CS+ preferences during two-bottle tests following massed training. Bottom: Mean ($\pm$ SEM) intakes of CS solutions: means of four one-bottle sessions of CS+/F and CS- solutions and individual means of two-bottle choice tests between CS+ and CS- solutions. The CS+ flavor was mixed into 16% fructose + 0.2% saccharin during one-bottle sessions, and into 0.2% saccharin during two-bottle sessions. The CS- flavor was mixed into 0.2% saccharin during both one- and two-bottle sessions. Asterisks indicate significantly greater CS+ than CS- intake.