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Flavor preferences conditioned by sucrose depend upon training and testing methods: Two-bottle tests revisited

Anthony Sclafani*

Department of Psychology, Brooklyn College of the City University of New York, Brooklyn, NY 11210, USA

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Abstract

In confirmation of prior work, rats given one-bottle training with flavored 5% and 30% sucrose solutions (CS5 and CS30) strongly preferred the CS5 when both flavors were presented in intermediate 17.5% sucrose solutions. The CS5 preference has been attributed to a conditioned satiety response to the CS30 flavor, but equal intakes of CS5 and CS30 in one-bottle tests did not support this view. To determine if sweetness differences between training and test solutions contributed to the CS5 preference, new rats were trained and tested with flavored 10% sucrose solutions. One flavor (CS5) was paired with matched intragastric (ig) water infusions (= net 5% solution) and another flavor (CS30) was paired with matched infusions of 50% sucrose (= net 30% solution) during one-bottle training. In two-bottle tests with both flavors paired with an intermediate infusion (25% = net 17.5%), the rats initially showed no overall preference for the CS5 or CS30. Following additional training, the rats significantly preferred the CS30 to the CS5. The intragastric data suggested that a change in sweet taste context between training and testing might have accounted for the strong CS5 preference obtained in the first experiment. This was confirmed in a third experiment in which rats were trained with flavored 5% and 30% sucrose solutions and then given two-bottle tests with both flavors presented either in 5% sucrose or 30% sucrose. Rats tested with 30% sucrose strongly preferred the CS5 flavor, whereas rats tested with 5% sucrose significantly preferred the CS30 flavor. Thus, the outcome of two-bottle flavor preference tests and presumably other tests of conditioned flavor reward may be greatly influenced by the solutions used in the tests. The impact of this variable may be greatest when the training solutions do not substantially differ in their net postingestive reinforcing actions. This appears to be the case with 5% and 30% sucrose solutions because the satiating effect of the concentrated solution tends to counteract its nutrient reinforcing action.

Keywords: Conditioned preferences; Conditioned satiety; Intragastric infusions; Taste contrast; Context effects

1. Introduction

Learned associations between a food's flavor and its postingestive nutritive consequences can influence subsequent food intake and preference [6,19]. This is clearly demonstrated in rats by pairing the intake of an arbitrary flavored solution (positive conditioned stimulus or CS+) with an intragastric (ig) infusion of a nutritive solution and a different flavored solution (the CS −) with an intragastric infusion of water [19]. In subsequent choice tests with the CS+ and CS − flavors paired either with their respective infusions (reinforced test) or with both paired with water infusions (extinction test), rats typically prefer the CS+ flavor to the CS − flavor. Preferences can also be conditioned for different flavors that are paired with concentrated and dilute nutrient solutions, but it is not always the case that the more concentrated nutrient infusion will condition the stronger preference. For example, we trained rats with intragastric infusions of 8%, 16% or 32% maltodextrin (Polycose) solutions paired with the intake of different flavored saccharin solutions (referred here as CS8, CS16 and CS32) [14]. In subsequent choice tests, the rats preferred the CS16 to the CS8 but did not prefer the CS32 to the CS16. We proposed that postingestive reinforcement increases with nutrient concentration, but at high concentrations the nutrient's satiating action can limit its reinforcing action [14].

Warwick and Weingarten [25] also reported that learned flavor preferences vary as a function of nutrient concentra-

* Tel.: +1-718-951-5606; fax: +1-718-951-4824.
E-mail address: asclafani@gc.cuny.edu (A. Sclafani).

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tion using orally consumed rather than infused nutrients. In one experiment, rats that were trained to drink differently flavored 1% and 5% sucrose solutions on alternate days subsequently preferred the CS5 flavor in a choice test when both flavors were presented in intermediate sucrose solutions (3%). Other rats trained with flavored 5% and 30% sucrose solutions also preferred the CS5 flavor to the CS30 when both were presented in intermediate sucrose solutions (17.5%). Similar results were obtained with different sucrose concentrations (5% and 40%) and with different saccharide solutions (glucose, fructose and maltose) [23]. Warwick and Weingarten [25] proposed that the sugar solutions had opposing postingestive effects: a positive reinforcing action that promotes intake and preference and a satiating action that inhibits intake and preference. At high sugar concentrations, the satiating action predominates and the rats display a preference for the flavor paired with the less concentrated solution, i.e., CS5 is preferred to CS30. More specifically, Warwick and Weingarten [25] hypothesized that it was the anticipatory (or conditioned) satiety produced by the concentrated sucrose solution that reduced the preference for its associated flavor. According to their view, at the time of the two-bottle test, the rats may find the CS30 flavor more palatable or rewarding but nevertheless drink less of it because they anticipate its potent satiating effect. The authors concluded, therefore, that two-bottle tests do not provide an "unambiguous measure of a flavor's palatability."

Because of its important implication for the interpretation of two-bottle tests, the present study reexamined the Warwick and Weingarten [25] conditioning results obtained with 5% and 30% sucrose solutions. The first experiment repeated their basic conditioning procedure but added measures of conditioned satiety (drinking patterns and one-bottle tests). Experiment 2 compared the postingestive conditioning effects of dilute and concentrated sucrose solutions by delivering different concentrations by intragastric infusions rather than by mouth as in the Warwick and Weingarten [25] study. The intragastric method eliminates potential confounds produced by the differences in the sweet taste intensity of the training (5% and 30% sucrose) and test (17.5% sucrose) solutions. The third experiment further examined the impact of sweet taste differences in training and test solutions on sucrose-conditioned flavor preferences by testing rats with the flavors presented in either 5% or 30% sucrose solutions.

2. Experiment 1

As noted above, Warwick and Weingarten [25] hypothesized that rats consumed less of the CS30 flavor than of the CS5 flavor in the two-bottle tests because of a conditioned satiety response to the CS30 flavor. However, no direct evidence was presented in support of this hypothesis. In the original studies of conditioned satiety, rats were trained with differently flavored concentrated and dilute saccharide solutions and then given separate one-bottle test meals with the flavors presented in an intermediate concentration [2,4]. Conditioned satiety was evidenced by a smaller meal of the flavor previously paired with the concentrated saccharide than of the flavor previously paired with dilute saccharide. More recent evidence of conditioned satiety is provided by the results of one-bottle sham-feeding tests: rats sham-fed less of a flavored sucrose solution that they had previously real-fed compared to a different flavored solution that was not previously real-fed [26]. In contrast to these earlier studies, Warwick et al. [23] and Warwick and Weingarten [25] used two-bottle data to infer conditioned satiety, but factors other than learned satiety may influence the outcome of choice tests. Furthermore, Booth [3] reported that conditioned satiety does not have a simple effect on flavor preferences in two-bottle tests: rats preferred the flavor paired with concentrated saccharide at the beginning of the meal, were indifferent in the middle of the meal and avoided it at the end of the meal.

The present experiment investigated the conditioned satiety interpretation by training rats with flavored 5% and 30% sucrose solutions, as in the Warwick and Weingarten [25] study, and then administering two-bottle and one-bottle tests with both flavors presented in an intermediate solution (17.5% sucrose). If conditioned satiety was responsible for the low intakes of the CS30 in the choice test, then the rats should also drink less CS30 than CS5 in the one-bottle tests. In addition, mean bout size should be smaller when the rats drink the CS30 flavored solution than when drinking the CS5 flavored solution. Bout size and number were therefore measured during one-bottle and two-bottle testing.

2.1. Methods

2.1.1. Subjects

Adult female Sprague–Dawley rats ($n=8$) were purchased from Charles River Laboratories (Wilmington, MA) and were 19 weeks old at the start of the experiment. The rats were given ad libitum access to powdered chow (No. 5001, PMI Nutrition International, Brentwood, MO). Water was also available ad libitum except where noted. The experimental room was maintained on a 12:12 light/dark cycle at 21 °C. The rats were housed in modified stainless steel cages ($24 \times 18 \times 18$ cm) that provided access to the chow in a food cup accessible through a hole in the back wall of the cage. Drinking fluid was available from one or two stainless steel sipper tubes located through two small holes (19 mm diameter) at the front of the cage.

2.1.2. Test solutions

The training solutions were prepared with sucrose (generic supermarket brand) and tap water prepared at 5% and 30% (w/v) concentrations. They were flavored with 0.1% grape or cherry unsweetened Kool-Aid mix (General Foods, White Plains, NY). For half the rats, the flavor added to the 5% sucrose (referred to as the CS5) was grape and the flavor added to the 30% sucrose (the CS30) was cherry. The flavor–concentration pairs were reversed for the remaining rats. The training solutions were presented in 50-ml plastic bottles, each filled with 32.5 ml of fluid, which provided the rat access to about 30 ml of fluid. In two-bottle tests, the rats were offered the choice between grape-flavored and cherry-flavored sucrose solutions prepared at a 17.5% concentration, which is intermediate between the 5% and the 30% training concentrations. Test solutions were presented in 220-ml bottles and intakes were unlimited. The flavored 5% and 30% sucrose solutions used during training are referred to as CS5/5 and CS30/30, while the flavored 17.5% sucrose solutions used in testing are referred to as CS5/17.5 and CS30/17.5.

2.1.3. Procedure

During a 10-day training period, the rats were given access, on alternate days, to ~30 ml of the CS5/5 and CS30/30 solutions. For half the rats, the 5% solution was presented on odd-numbered days and the 30% solution was presented on even-numbered days. The order of presentation was reversed for the remaining rats. Following the last training day, a two-bottle preference test (1 day) was conducted with the rats given unlimited access to the CS5/17.5 and CS30/17.5, i.e., both flavors presented in 17.5% sucrose solutions. Next, the rats were given one-bottle solution tests with unlimited access to the CS5/17.5 and CS30/17.5 for 1 day each, with the order of presentation counterbalanced. This was followed by another two-bottle test (1 day) with the two solutions. All training and test sessions were 23 h in length. During the remaining 1 h, food and fluid were replaced and data were collected. Ad libitum water was available during one-bottle solution training and test days but not during two-bottle solution test days. Water intake was minimal and these data are not presented.

Daily solution intakes were recorded to the nearest 0.1 g and converted into milliliters. Drinking patterns were recorded using drinkometers and a microcomputer that stored lick data in 6-s bins for off-line analysis. A drinking bout was defined as a period of drinking containing at least 30 licks and interlick intervals no longer than 5 min. The intake and bout data were entered in repeated-measures analyses of variance (ANOVA), except for single variable comparisons using *t* tests. For significant main effects, tests of differences between specific means and groups of means were performed using least-squares means contrasts. The two-bottle intakes of the individual rats were also expressed as percent CS5/17.5 intakes (CS5/17.5 intake/total intake × 100) and analyzed by ANOVA following an inverse sine transformation [12].

2.2. Results

During the 10-day one-bottle training period, all the rats consumed the allotted 30 ml/day of CS5/5 solution. Five rats also consumed all of the available CS30/30 solution during training, while three rats did not always drink all 30 ml. Average intakes of CS5/5 and CS30/30 solutions did not reliably differ (30.2 vs. 28.8 ml/day). As illustrated in Fig. 1, in the two-bottle tests with the 17.5% sucrose solutions, the rats consumed substantially more of the CS5/17.5 solution than of the CS30/17.5 solution. ANOVA confirmed that this difference was significant [$F(1,7)=21.51$, $P<.01$] and indicated that intakes did not change from the first to the second two-bottle test. The percentage of total intake consumed as CS5/17.5 declined, however, from 87% in Test 1 to 73% in Test 2 [$t(7)=2.42$, $P<.05$]. All rats displayed a CS5 preference of 60% or greater in the first test and six of eight rats preferred the CS5 by this margin in the second test. In contrast to the two-bottle results, the rats consumed comparable amounts of the CS30/17.5 and CS5/17.5 solutions during the intervening one-bottle tests.

The drinking pattern data for the two-bottle and one-bottle tests are presented in Fig. 2. Training data were not analyzed because the rats emptied the drinking tubes during the sessions and subsequent licking would distort the results. In the two-bottle tests, the rats took more CS5/17.5 bouts than CS30/17.5 bouts [$F(1,7)=18.92$, $P<.01$]. Mean bout sizes were also greater with the CS5/17.5 than with the CS30/17.5, but this difference was significant ($P<.05$) only during the first two-bottle test [CS × Day interaction, $F(1,7)=17.46$, $P<.01$]. On one-bottle test days, the rats took slightly larger bouts of the CS30/17.5 than of the CS5/17.5, but this difference was not significant. The number of CS30/17.5 and CS5/17.5 bouts were similar during the one-bottle tests.

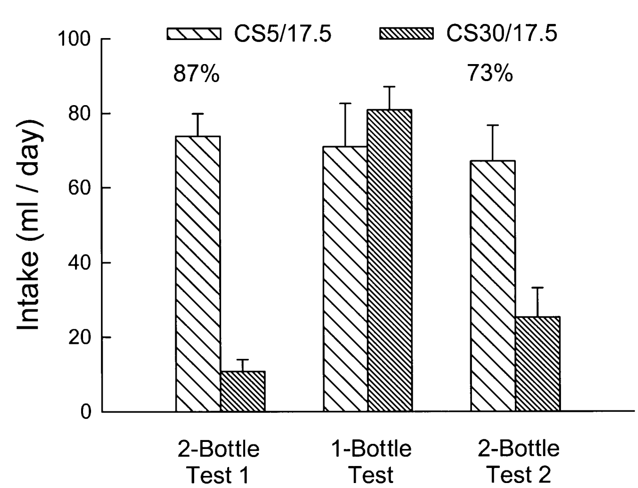

Fig. 1. Experiment 1. Mean + S.E.M. intakes of test solutions during two-bottle and one-bottle test days. The CS5/17.5 solution contained the flavor paired with 5% sucrose during training presented in a 17.5% sucrose solution during testing. The CS30/17.5 solution contained the flavor paired with 30% sucrose during training presented in a 17.5% sucrose solution during testing. The numbers atop the bars represent the means of the individual rats' percent intakes of CS5/17.5.

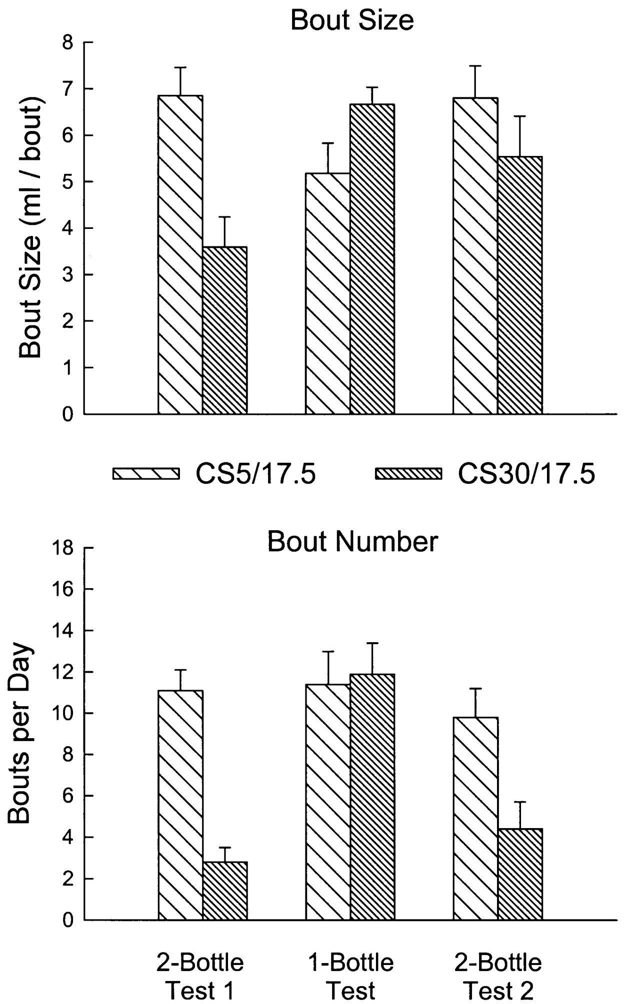

Fig. 2. Experiment 1. Mean + S.E.M. bout size and bout number during two-bottle and one-bottle test days. The CS5/17.5 solution contained the flavor paired with 5% sucrose during training presented in a 17.5% sucrose solution during testing. The CS30/17.5 solution contained the flavor paired with 30% sucrose during training presented in a 17.5% sucrose solution during testing.

2.3. Discussion

The results of the first two-bottle test closely replicate the findings of Warwick and Weingarten [25]. The rats displayed an 87% preference for the CS5/17.5 over the CS30/17.5, which is similar to the 86% preference reported in the earlier study. These near-identical results are noteworthy given that the present data were obtained with female Sprague–Dawley rats, whereas the prior data were obtained with male Long–Evans rats.

The finding that the rats consumed smaller bouts of the CS30/17.5 than of the CS5/17.5 during the initial two-bottle test is consistent with the conditioned satiety hypothesis proposed by Warwick and Weingarten [25]. However, arguing against this interpretation are the similar intakes and drinking patterns obtained with the CS5/17.5 and CS30/17.5 in the one-bottle tests. If the rats consumed less CS30/17.5 in the two-bottle test because of anticipated satiety, then they should also have consumed less CS30/17.5 in the one-bottle test. Conceivably, the similar one-bottle intakes and drinking patterns may have occurred because the conditioned satiety response to the CS30 flavor rapidly extinguished during the 23-h test periods. Note that prior reports of conditioned satiety all involved short test meals and there are no data showing that the response will persist over a 23-h period. An extinction explanation, however, does not explain why the rats continued to prefer the CS5/17.5 in the second two-bottle test. The most remarkable difference in the two-bottle intake patterns was that rats took far fewer CS30 bouts than CS5 bouts. This would appear to be inconsistent with Booth's [3] report that conditioned satiety does not influence flavor preference at the start of a meal.

3. Experiment 2

While the two-bottle preference results of the first experiment precisely replicate the findings of Warwick and Weingarten [25], the one-bottle results and bout pattern data do not fully support their conditioned satiety interpretation of the CS5 flavor preference. An alternative explanation of the results is that during one-bottle training the unconditioned satiating effect of the 30% sucrose solution counteracted the sugar's postingestive reinforcing action so that the net "reward" value of the 30% sugar solution was less than that of the 5% solution.

It is possible, however, that the rats' strong preference for the CS5/17.5 solution over the CS30/17.5 solution has more to do with the taste properties of the training and test solutions than with the postingestive consequences of the 5% and 30% sucrose solutions. In particular, a potential confound of the two-bottle test is that the sweet taste intensity of the training and test solutions differed. The rats may have consumed more of the CS5 flavor and less of the CS30 flavor in the choice test because of a combination of positive and negative taste contrast effects [10]. That is, the "hedonic" value of the CS5 flavor may have been enhanced because it was presented in a sweeter solution during the test than during training, whereas the value of the CS30 flavor may have been reduced because the test solution was less sweet than the 30% sucrose solution used in training.

The present experiment focused on the role of postingestive factors alone in the conditioning of preferences for CS5 and CS30 flavors by eliminating differences in the sweet taste intensity of the training and test solutions. This was accomplished by training and testing rats with "isosweet" flavored solutions and manipulating postingestive consequences using intragastric infusions. That is, the "CS5" and "CS30" flavors (grape or cherry) were both always presented in 10% sucrose solutions. As the rats drank the CS5 flavored 10% sucrose solution, they were simultaneously infused with a matched volume of water so that the net concentration of the sugar solution in the stomach was 5%. As they drank the CS30 flavored 10% sucrose solution, they were infused with a matched volume of 50% sucrose so that the net concentration of the sugar solution in the stomach was 30%. The two-bottle preference tests were then conducted with both the CS5 and the CS30 flavored 10% sucrose solutions paired with intragastric infusions of 25% sucrose to produce a net sucrose concentration of 17.5% in the stomach. Additional two-bottle tests were conducted with the CS5 and CS30 solutions paired with 0% and 50% sucrose infusions, respectively.

3.1. Methods

3.1.1. Subjects

Adult female Sprague–Dawley rats ($n=12$) were purchased from Charles River Laboratories and were 18 weeks old.

3.1.2. Surgery

The rats were anesthetized with a mixture of Ketamine HCl (63 mg/kg) and Xylazine (9.4 mg/kg) and implanted with a stainless steel gastric cannula used to attach the infusion catheters as described previously [9]. Briefly, the cannula was inserted into the fundus of the stomach and secured with a purse-string suture, polypropylene mesh and dental cement. The shaft of the cannula was passed through a small incision in the abdominal wall and skin. When not in use, the cannula was kept closed with a stainless steel screw. The rats were allowed 2 weeks to recover from surgery before beginning the experiment.

3.1.3. Apparatus

The infusion test system were similar to the ‘‘electronic esophagus’’ system previously described [9]. In brief, the rats were housed in the cages described in Experiment 1. A slot in the cage floor permitted two catheters attached to the rat’s gastric cannula to be connected to a dual-channel infusion swivel located below the cage. The catheters were protected by a flexible stainless steel spring. Plastic tubing connected the swivel to two peristaltic infusion pumps. The pumps were operated automatically by drinkometer circuits and a microcomputer whenever the rat drank from the sipper tubes. The flow rate of the pumps was ~1.6 ml/min and they were controlled by computer software to infuse ~1 ml of fluid for each 1 ml of fluid orally consumed. The microcomputer stored on disk the number of licks emitted during 6-s bins for off-line analysis of drinking patterns. The infusion system operated 23 h/day. During the remaining 1 h, fluid intakes were recorded and the infusion system was serviced.

3.1.4. Test solutions

The CS test solutions presented for oral consumption were 10% sucrose solutions flavored with 0.1% grape or cherry unsweetened Kool-Aid mix. For half the rats, grape was the CS5 flavor that was paired with intragastric infusions of water and cherry was the CS30 flavor that was paired with intragastric infusions of 50% sucrose. The flavor–infusion pairs were reversed for the remaining rats. In the initial two-bottle tests, the CS5 and CS30 flavored 10% sucrose solutions were both paired with intragastric infusions of 25% sucrose. The oral solution and intragastric infusion combinations used in training are referred to as CS5/(5) and CS30/(30), while the combinations used in the initial two-bottle tests are referred to as CS5/(17.5) and CS30/(17.5). Additional two-bottle tests were conducted with the training solutions and their paired infusions, i.e., CS5/(5) vs. CS30/(30). The (5), (17.5) and (30) nomenclature refers to the net sucrose concentration in the stomach resulting from the mixture of orally consumed 10% solution and the infused 0%, 25% and 50% solutions, respectively.

3.1.5. Procedure

The rats were adapted to the infusion test cages for 6 days with food and water available ad libitum. During the last four of these days, intake of water was paired with intragastric infusions of water. Next, during a 10-day training period, the rats were given alternating one-bottle access to the CS5 and CS30 flavored 10% sucrose solutions paired with intragastric infusions of water and 50% sucrose, respectively. Daily intakes of the CS solutions was limited to 20 ml, which was combined with a 20-ml intragastric infusion. (To match the 30-ml/day CS limit used during training in Experiment 1, the oral CS intakes of the intragastric infused rats would have been limited to 15 ml/day, but this seemed too constraining.) Food and water (not paired with intragastric infusions) remained available ad libitum during the 23-h/day sessions. For half the rats, the CS5/(5) was presented on odd-numbered days and the CS30/(30) was presented on even-numbered days. The order of presentation was reversed for the remaining rats. Following the last training day, the rats were given unlimited two-bottle access to the CS5/(17.5) vs. CS30/(17.5) for 4 consecutive days. Water was not available during this or subsequent two-bottle solution tests.

The rats were then given 6 days of one-bottle training with the CS5/(5) and CS30/(30) oral + infusion combinations with intakes no longer limited. Water was also available. This was followed by a 4-day, two-bottle preference test with the CS5/(5) vs. CS30/(30). A final 4-day choice test was then conducted with CS5/(17.5) vs. CS30/(17.5) as in the original test.

Except where noted, one-bottle and two-bottle solution intakes are expressed as orally consumed solutions. Total intakes (oral + intragastric infusions) were approximately twice that of the oral intakes.

3.2. Results

During one-bottle training days with the CS5/(5), the rats consumed all of the allotted 20 ml/day and therefore were also infused with 20 ml/day. Nine of the 12 rats also consumed all of the CS30/(30) solution, while the remaining rats did not always drink all of the available solution. Consequently, mean oral intake of the CS5/(5) was slightly greater than that of CS30/(30) (20.7 vs. 19.8 ml/day, ns).

Fig. 3 summarizes the results of the first 4 two-bottle test days, during which both CS flavors were paired with intragastric infusions of 25% sucrose. The rats consumed more CS5/(17.5) than CS30/(17.5) on Day 1. Eight of 12 rats displayed a preference of 60% or more. The rats then reversed their preference and consumed somewhat more CS30/(17.5) than CS5/(17.5) on Days 3 and 4. Eight of 12 rats had a CS30 preference of 60% or more on Day 4. Overall, CS5 and CS30 intakes did not significantly differ, but total CS intakes increased over days [$F(3,33)=3.41$, $P<.05$]. There was a significant CS × Day interaction [$F(3,33)=4.10$, $P<.05$]. Simple main effect tests revealed that CS30/(17.5) intake increased over days ($P<.01$).

During the first two-bottle test day, the rats tended to take larger and more frequent bouts of the CS5/(17.5) compared to the CS30/(17.5), but these differences were not significant (3.63 vs. 3.14 ml/bout and 8.6 vs. 4.5 bouts/day, respectively). Mean CS5 and CS30 bout size and number also did not differ when averaged over the 4 days of two-bottle testing (2.86 vs. 3.02 ml/bout, 7.5 vs. 8.3 bouts/day).

In the second one-bottle training period, intakes were unlimited and the rats consumed somewhat more CS5/(5) than CS30/(30) (56.5 vs. 42.6 ml/day, ns). Total sucrose caloric intake (oral + intragastric), however, was much greater on CS30/(30) training days than on CS5/(5) days [92.3 vs. 23.6 kcal/day, $t(11)=10.13$, $P<.001$]. Mean bout size and number tended to be greater on CS5 days than

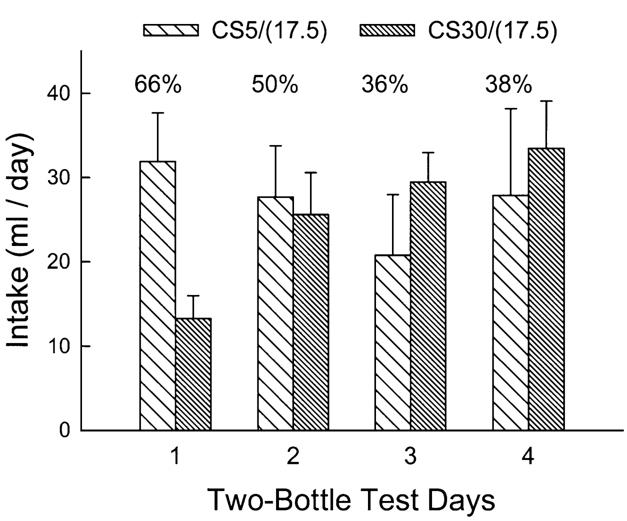

Fig. 3. Experiment 2. Mean + S.E.M. intakes of test solutions during two-bottle test days. All solutions contained 10% sucrose paired with matched intragastric infusions of 25% sucrose, which mimicked the postingestive effects of 17.5% sucrose. The CS5/(17.5) solution contained the flavor paired with the postingestive effects of 5% sucrose during training. The CS30/(17.5) solution contained the flavor paired with the postingestive effects of 30% sucrose during training. The numbers atop the bars represent the means of the individual rats' percent intakes of CS5/(17.5).

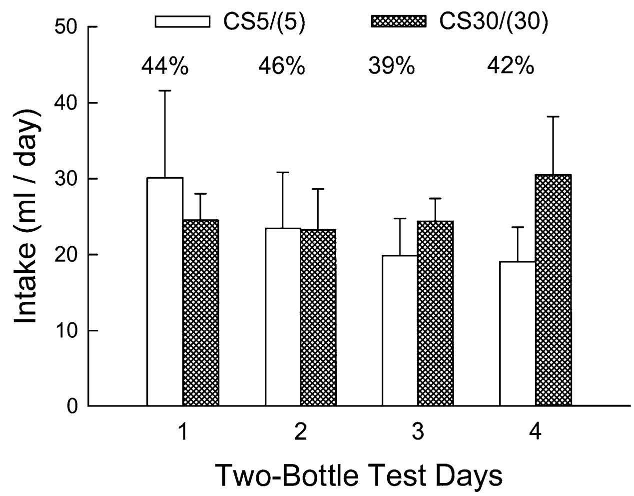

Fig. 4. Experiment 2. Mean + S.E.M. intakes of test solutions during two-bottle test days. All solutions contained 10% sucrose paired with matched intragastric infusions of water or 50% sucrose, which mimicked the postingestive effects of 5% sucrose (5) and 30% sucrose (30), respectively. The CS5/(5) solution contained the flavor paired with the postingestive effects of 5% sucrose during training. The CS30/(30) solution contained the flavor paired with the postingestive effects of 30% sucrose during training. The numbers atop the bars represent the means of the individual rats' percent intakes of CS5/(5).

CS30 days, but these differences were not significant (13.5 vs. 12.0 bouts/day, 4.25 vs. 3.64 ml/bout).

Intakes of the CS5/(5) and CS30/(30) solutions also did not significantly differ during the 4-day, two-bottle test (Fig. 4). Overall, the rats consumed 66% of their total intake as CS30/(30). In terms of energy intake, the rats consumed substantially more sucrose calories from the CS30/(30) solution than from the CS5/(5) solution during the two-bottle test [61.5 vs. 9.2 kcal/day, $t(11)=7.27$, $P<.001$]. Mean 4-day bout sizes and numbers of the CS30/(30) and CS5/(5) did not significantly differ (3.42 vs. 2.97 ml/bout and 8.6 vs. 7.8 bouts/day, respectively).

In the final 4 days of two-bottle testing, the rats now consumed significantly more CS30/(17.5) than CS5/(17.5) [$F(1,11)=11.22$, $P<.01$] (Fig. 5). Percent CS30 intakes ranged from 66% to 79% of total intake. Averaged over the 4-day test, the rats took more CS30/(17.5) bouts than CS5/(17.5) bouts [12.1 vs. 5.1 bouts/day, $t(11)=4.34$, $P<.01$]. CS30/(17.5) bout size was slightly but not significantly greater than CS5/(17.5) bout size (3.27 vs. 2.86 ml/bout).

3.3. Discussion

In this experiment, rats were trained and tested with flavored 10% sucrose solutions that had the postingestive consequences of 5% and 30% solutions or a 17.5% solution by intragastric coinfusions of 0%, 50% and 25% sucrose, respectively. These training and test conditions did not produce a robust CS5 flavor preference as was obtained in Experiment 1. Rather, the rats showed a weak 66% preference for the CS5 flavor on the first two-bottle test day and

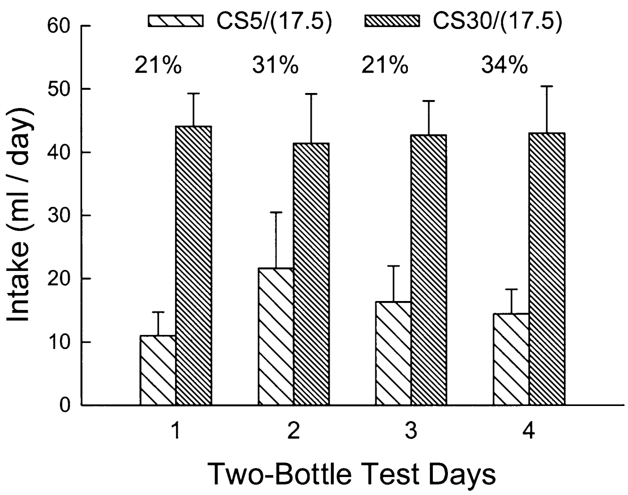

Fig. 5. Experiment 2. Mean + S.E.M. intakes of test solutions during two-bottle test days. All solutions contained 10% sucrose paired with matched intragastric infusions of 25% sucrose, which mimicked the postingestive effects of 17.5% sucrose. The CS5/(17.5) solution contained the flavor paired with the postingestive effects of 5% sucrose during training. The CS30/(17.5) solution contained the flavor paired with the postingestive effects of 30% sucrose during training. The numbers atop the bars presented the means of the individual rats' percent intakes of CS5/(17.5).

then tended to drink more CS30 on the last 2 days of the test. Subsequently, after additional one-bottle training and two-bottle testing with the CS5/(5) and CS30/(30) solutions, the rats significantly preferred the CS30 flavor over the CS5 flavor when both were again paired with the postingestive consequences of 17.5% sucrose solution.

Two factors may be responsible for the conflicting flavor preference results obtained in Experiments 1 and 2. First, intragastric infusions were used in the present experiment to manipulate the postingestive consequences of the CS flavors and it could be argued that this route of administration altered normal digestive processes. Prior studies have, in fact, reported differences in the postingestive disposition of orally consumed and infused nutrients [8,18]. In these studies, however, unlike the present experiment, the nutrients were not coinfused as the animals orally consumed flavored sweet solutions nor were the infusions under the animals' control. Concurrent oral stimulation appears to normalize the processing of intragastric nutrient infusions [18]. Furthermore, if the intragastric sugar infusions interfered with normal sucrose processing, then the rats should have displayed a decreased preference for the CS30 flavor, which was paired with intragastric infusions of 50% sugar relative to the CS5 flavor paired with intragastric water infusions during training. In contrast, the rats showed an enhanced preference for the CS30 flavor in this experiment compared to the noninfused rats of Experiment 1.

The second factor that may account for the different results obtained in Experiments 1 and 2 is that in the present experiment the CS flavors were always presented in iso-sweet solutions (10% sucrose), whereas in the first experiment the CS flavors were associated with different levels of sweetness during training (5% vs. 30% sucrose), which then changed to a common intermediate level (17.5% sucrose) during preference testing. The next experiment investigated the influence of sweet taste on the rats' preference for the CS5 and CS30 flavors.

4. Experiment 3

The strong preference rats displayed in Experiment 1 and in a prior study [25] for the CS5 flavor over the CS30 flavor when both were presented in a 17.5% sucrose solution may be secondary to the change in sweetness level from training to test solutions. This could occur because of a combination of positive and negative contrast effects as discussed in Experiment 2. Alternatively, Warwick et al. [24] hypothesized that differential "familiarity" of the CS5 and CS30 flavors when presented in the intermediate 17.5% sucrose solution may have influenced the rats' flavor preference. That is, the flavor of the CS5/17.5 solution may be more familiar to the rats than the flavor of the CS30/17.5 solution because the sweet taste intensity of the 17.5% solution may be closer to that of 5% sucrose than to that of 30% sucrose. Warwick et al. [24] examined this possibility by training rats with flavored 5% and 40% sucrose solutions (instead of 30% as used in their original study [25]) and then testing them with both flavors presented in 40% sucrose solutions rather than an intermediate concentration. The rats showed an 83% preference for the CS5 flavor, which is similar to the preferences obtained in prior studies (and Experiment 1) in which the CS5 and CS30 (or CS40) flavors were tested using intermediate sucrose solutions. This finding clearly indicates that the lack of preference for the CS40 flavor did not result because it was the less familiar choice in the two-bottle test. However, the data do not rule out a positive flavor contrast explanation of the preference data. That is, the rats may have been attracted to the CS5 flavor during the preference test because it was presented in a much sweeter solution than they had experienced during training.

Experiment 3 further investigated the impact of sweet taste during testing on the rats' relative preference for CS5 and CS30 flavors. Rats were trained as in Experiment 1 with one flavor mixed in a 5% sucrose solution and a second flavor mixed in a 30% sucrose solution. The animals were then divided into two groups. One group had both flavors presented in 30% sucrose solutions during the two-bottle preference test, while the second group had both flavors presented in 5% sucrose solutions.

4.1. Methods

4.1.1. Subjects

Adult female Sprague–Dawley rats ($n = 20$) were purchased from Charles River Laboratories and were 13 weeks old at the start of training. They were housed as in Experiment 1.

4.1.2. Procedure

The rats were trained as in the first experiment with grape and cherry flavored 5% and 30% sucrose solutions and 30 ml of the CS5/5 and the CS30/30 were available on alternate days along with ad libitum water. The rats were then divided into two groups equated for body weight and training intakes. Group 5 was given two-bottle access to the CS5 and CS30 flavors both presented in 5% sucrose solutions for 2 consecutive days, i.e., CS5/5 vs. CS30/5. Group 30 was similarly treated, except that the two flavors were presented in 30% sucrose solutions, i.e., CS5/30 vs. CS30/30.

4.2. Results

The rats consumed all of the allotted 30 ml/day of the CS5/5 and CS30/30 solutions during training, except for one rat that consumed very little CS5/5 (~2 ml/day). The data for this rat were therefore excluded from the study. Fig. 6 presents the intakes during the 2 days of preference testing. Group 30 strongly preferred the CS5/30 to the CS30/30 on both days, with all 10 rats drinking more CS5 than CS30. In contrast, Group 5 preferred the CS30/5. Only one rat in this group drank more CS5/5 than CS30/5. This was confirmed by a significant Group × CS interaction [$F(1,17) = 36.85$, $P < .01$]. Individual within-group tests indicated that Group 30 rats consumed more CS5/30 than CS30/30 [$F(1,9) = 94.71$, $P < .001$], whereas Group 5 consumed more CS30/5 than CS5/5 [$F(1,8) = 5.38$, $P < .05$] . Furthermore, the rats tended to increase their intake of their preferred solution from Days 1 to 2 of testing, although this difference was significant only for Group 5 ($P < .01$). Averaged over the 2-day test, the percent CS5 intake was greater for Group 30 than for Group 5 [89% vs. 39%, $t(16) = 10.88$, $P < .01$].

Bout patterns averaged over the 2 days of testing are presented in Fig. 7. There was a significant Group × CS interaction for bout size [$F(1,17) = 8.81$, $P < .01$]. CS5 bout size exceeded CS30 bout size for Group 30 ($P < .01$) but not for Group 5. Also, Group 30 drank larger bouts of CS5 than did Group 5 ($P < .01$), but the groups did not differ in their CS30 bout sizes. The groups also differed in their bout number [Group × CS interaction, $F(1,17) = 40.34$, $P < .001$]. Group 5 rats had more CS30 bouts than CS5 bouts ($P < .01$), whereas the Group 30 showed the reversed pattern ($P < .01$). The two groups did not differ in their number of CS5 bouts, but CS30 bout number was much greater in Group 5 than in Group 30 ($P < .01$).

4.3. Discussion

The Day 1 CS5 preference displayed by the rats tested with both flavors presented in 30% sucrose (Group 30) was nearly identical to that obtained in Experiment 1 where an intermediate 17.5% sucrose concentration was used in the two-bottle test (86% vs. 87%). This replicates previous findings obtained with rats trained with flavored 5% and 40% sucrose solutions and then tested with the

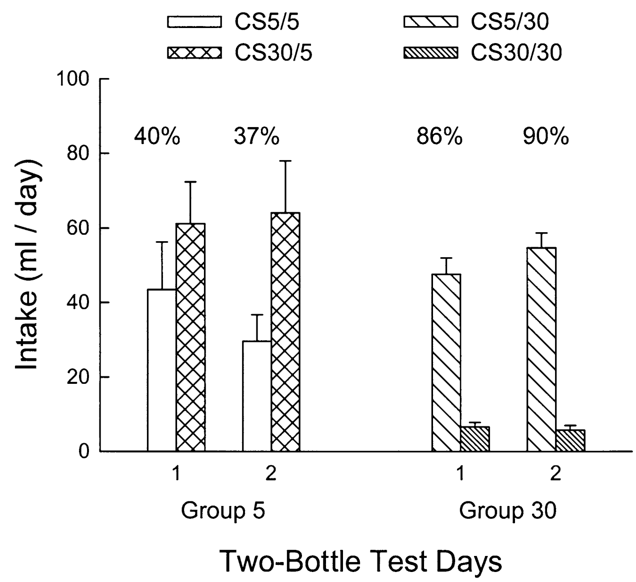

Fig. 6. Experiment 3. Mean + S.E.M. intakes of test solutions during two-bottle test days. Group 5 was tested with 5% sucrose solutions containing the flavors paired with 5% sucrose (CS5/5) and 30% sucrose (CS30/5) during training. Group 30 was tested with 30% sucrose solutions containing the flavors paired with 5% sucrose (CS5/30) and 30% sucrose (CS30/30) during training. The numbers atop the bars represent the means of the individual rats' percent intakes of CS5/5 or CS/30.

flavors presented either in 40% sucrose or in intermediate (22.5%) sucrose solutions [24,25]. Note that the CS5 preference in Group 30 increased from Days 1 to 2 of testing, whereas the CS5 preference decreased from the first to the second test in Experiment 1. This is most likely due to the interposed one-bottle tests in the first experiment, which presumably facilitated extinction of the CS5 preference, although it is possible that the CS5 preference is more persistent when the flavors are presented in 30% than in 17.5% sucrose solutions.

The new and important finding of the present experiment is the significant CS30 preference displayed by rats tested with both flavors presented in a 5% sucrose solution. This CS30/5 preference was replicated in a second group of nine rats trained and tested identically to Group 5 (Sclafani, unpublished findings). In addition to displaying opposite preference patterns, Groups 5 and 30 also differed in the magnitude of their preferences. That is, Group 30 rats showed a near total CS5 preference (89% averaged over 2 days) and drank very little CS30. They averaged only two CS30 bouts/day. In contrast, the CS30 preference of Group 5 was not as robust (61%) and they consumed almost as much CS5 as did Group 30. CS30 bout numbers were, in fact, nearly identical in the two groups (11.4 vs. 11.5 bouts/day). What most distinguished the two groups was that CS30 bout number was 10 times higher in Group 5 than in Group 30.

The opposite flavor preferences displayed by Groups 5 and 30 demonstrate that the context in which the flavors are

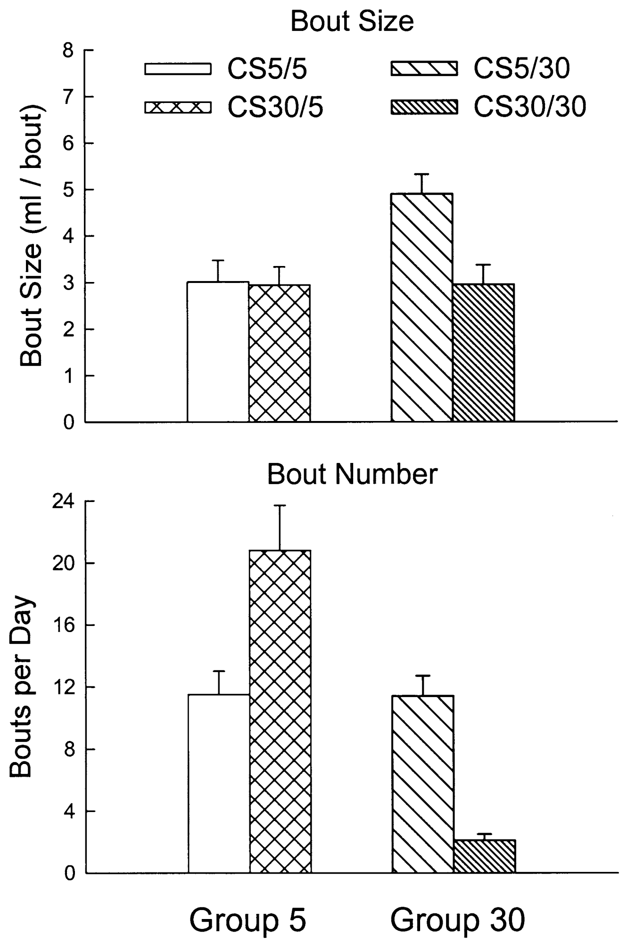

Fig. 7. Experiment 3. Mean + S.E.M. bout size and bout number averaged over the 2 two-bottle test days. Group 5 was tested with 5% sucrose solutions containing the flavors paired with 5% sucrose (CS5/5) and 30% sucrose (CS30/5) during training. Group 30 was tested with 30% sucrose solutions containing the flavors paired with 5% sucrose (CS5/30) and 30% sucrose (CS30/30) during training.

presented, i.e., sweet taste of the test solutions, greatly influences the outcome of the preference test. Possible explanations for this effect are discussed below.

5. General discussion

The present study compared the flavor preference conditioned by dilute (5%) and concentrated (30%) sucrose solutions in rats. Prior studies indicate that the postingestive reinforcing action of sugars increases with concentration, but at higher concentrations the sugar's satiating effect can counteract its reinforcing action. In particular, Warwick et al. proposed that the satiating action of a 30% or 40% sucrose solution opposed its reinforcing potency so much that rats preferred a flavor paired with a 5% sucrose solution over a flavor paired with the 30% (or 40%) sucrose solution [23–25]. The present findings confirmed the preference results reported by Warwick et al. but do not support their conditioned satiety interpretation of the results. Instead, the present study revealed that the outcome of the conditioned flavor preference tests is very much influenced by the training and test procedures, which has important implications for the interpretation of conditioned flavor preference findings.

In two-bottle preference tests with different sugar concentrations, rats typically prefer the more concentrated solution over a wide range of concentrations [7]. With glucose solutions, rats may reverse their preference with repeated testing and come to drink more dilute solution (5–10%) than concentrated sugar (30–50%) [5,17]. This switch in preference has been attributed to a learned change in taste preference due largely to the postingestive satiating effect of the concentrated glucose solution [5,17]. Similar preference reversals were obtained with fructose and glucose + fructose mixtures but not with sucrose, which suggested that the higher osmolarity of the monosaccharide sugars, relative to the disaccharide sucrose, was the critical determinant of the preference reversal [17]. In an important modification of this procedure, Warwick et al. [23] and Warwick and Weingarten [25] trained rats with dilute and concentrated sucrose solutions containing distinctive cue flavors and then measured their preference for the cue flavors presented in sucrose solutions at the same intermediate concentration. They obtained a robust preference for the flavor (CS5) paired with the dilute solution, which they attributed to a conditioned satiety response to the CS30 flavor. According to their hypothesis, the rats drank less CS30 in the two-bottle test, not because it was less palatable or less reinforcing but because it evoked a stronger conditioned satiety response than did the CS5 flavor. Note that the rats' strong preference for the CS5 flavor does not necessarily conflict with the failure to observe a preference reversal in rats tested with unflavored dilute and concentrated sucrose solutions. The sweeter taste of the concentrated solution may overcome the solutions' conditioned (or unconditioned) satiating effects so that rats continue to prefer the concentrated solution, although the magnitude of their preference may decline over test days [17].

Prior studies from my laboratory have also reported on the presumed interaction of postingestive satiety and nutrient reinforcement in the development of flavor preferences [13,14,20]. We reported that whereas rats learned to prefer a flavored saccharin solution paired with intragastric infusions of 16% Polycose over a different flavored solution paired with infusions of 8% Polycose, they did not learn to prefer a flavor paired with 32% Polycose over a flavor paired with 16% Polycose. The rats' preference for CS8 vs. CS32 was not examined [14]. The failure of the rats to acquire a preference for the CS32 over the CS16 was attributed to the unconditioned satiating action of the concentrated solution limiting its reinforcing action at the time of learning. This differs from Warwick et al.'s [23,24] and Warwick and Weingarten's [25] hypothesis that it is a conditioned satiety response to the cue flavor that reduces the rats' choice of the flavor at the time of testing.

In a test of the conditioned satiety hypothesis, Experiment 1 repeated the Warwick and Weingarten [25] sucrose conditioning study with the addition of one-bottle tests and bout pattern analysis. The initial preference results closely matched prior findings [25]: rats trained with flavored 5% and 30% sucrose solutions displayed a strong preference for the CS5 flavor when both flavors were presented in 17.5% sucrose solutions. However, the rats consumed no less of the CS30/17.5 solution than CS5/17.5 solution in one-bottle tests and their one-bottle drinking patterns did not differ. These results provide no support for a conditioned satiety explanation of the rats' CS5 preference.

While it may not be due to conditioned satiety, the strong CS5 preference obtained in Experiment 1 and prior studies [23,25] is a remarkable finding. The rats would have been expected to prefer the CS30 flavor, given that it was paired with more calories (flavor–nutrient learning) and with a sweeter taste (flavor–flavor learning) during training. Earlier studies indicate that rats learn to prefer flavors paired with the sweeter of two solutions, at least when postingestive consequences are minimal [11,27]. However, the interpretation of the CS5 preference is complicated because of the differences in sweet taste intensities of the test and training solutions. The results of Experiment 2 and 3 revealed that the CS5 preference observed in the first experiment was dependent upon the changes in sucrose concentration from the training to test solutions.

Experiment 2 eliminated the differences in the sweet taste by always presenting the CS flavors in 10% sucrose solutions and mimicking the postingestive effects of 5%, 17.5% and 30% sucrose solutions by coinfusions of different sucrose concentrations. With this test procedure, a strong CS5 preference did not emerge. Rather, the rats showed a weak (66%) CS5/(17.5) preference the first day of testing, which changed to a weak (63%) CS30/(17.5) preference by Days 3 and 4 of the initial test. Thus, when differences in sweet taste are eliminated, the postingestive flavor conditioning effects of 5% and 30% sucrose solutions appear to be relatively similar, at least after 10 days of training. However, after additional one-bottle and two-bottle experience with the CS5/(5) and CS30/(30) solutions, the rats displayed a significant preference for CS30/(17.5) over CS5/(17.5) in the final two-bottle test. Whether it was the additional experience with the training solutions alone, the change in the one-bottle training procedure (unlimited rather than limited access) or the rats' intervening two-bottle experience with the training and test solutions that resulted in the final CS30/(17.5) preference is not certain.

Similar to the delayed appearance of the CS30 preference in Experiment 2 are conditioning results reported by Sclafani et al. [20] with rats trained to consume differently flavored 32% Polycose solutions. In this case, the postingestive consequences of the solutions were manipulated during one-bottle training by having the gastric cannulated rats consume one flavored solution with the cannula open (sham-feeding) and the other flavor with the cannula closed (real-feeding). In initial two-bottle tests with both solutions, the rats tended to prefer the sham-fed flavor, but after repeated training and testing they switched their preference to the real-fed flavor. It may be that early in training, the postingestive satiating action of concentrated sucrose (Experiment 2) or Polycose [20] counteracts the carbohydrate's postingestive reinforcing action, but with further training the rat adapts to the satiety effect, which allows the reinforcing action to condition a flavor preference.

The different preference results obtained in Experiments 1 and 2 suggested that the sweet taste of the training and test solutions was an important factor influencing the expression of the CS preference. Experiment 3 investigated this possibility by training rats as in Experiment 1 but then testing them with the CS flavors presented either in 5% or in 30% sucrose solutions. This had a profound effect on the outcome of the two-bottle test. When tested with the 30% sucrose solutions, the rats displayed a CS5 preference as strong as that obtained with the 17.5% test solutions in Experiment 1 (89% vs. 87%). In contrast, rats tested with 5% sucrose solutions showed a significant preference for the CS30 flavor. Both groups were identically trained so that the opposite preference patterns were clearly due to the different sucrose concentrations used in the two-bottle test. For both groups, the preferred solution was the more novel of the two offered, i.e., the CS5 mixed in the 30% sucrose for Group 30 and the CS30 mixed in 5% sucrose for Group 5, but it seems unlikely that it was the novelty, per se, of the solution that attracted the rats. In Experiment 1, the test solutions (17.5% sucrose) were different from both training solutions. A conditioned satiety hypothesis could, in theory, account for the CS5 preferences displayed by Group 30, but it fails to explain the CS30 preference of Group 5.

A taste contrast analysis also does not readily explain both the CS5 and the CS30 preferences observed in Experiment 3. Conceivably, a positive taste contrast could account for the CS5 preference displayed by Group 30. That is, the rats may have preferred the CS5 because the hedonic value of the flavor was greatly increased when it was presented in the context of the 30% sucrose solution. A contrast analysis, however, would predict that Group 5 rats would reject the CS30 flavor (negative taste contrast) because the flavor, which had previously been paired with very sweet 30% sucrose, was now presented in the less sweet 5% solution. These considerations indicate that there is no single explanation for the CS5 and CS30 preferences observed in the groups. Perhaps flavor contrast was responsible for the CS5 preference in Group 30, whereas flavor–flavor conditioning was responsible for the CS30 preference in Group 5. In the case of Group 5 rats, their prior association of the CS30 flavor with 30% sucrose may have enhanced the flavor's hedonic value so that the CS30/5 solution was more palatable than the CS5/5 solution. With the Group 30 rats, on the other hand, the CS30 flavor may not have had much effect on the hedonic value of the 30% sucrose test solution, because it was quite sweet already. Rather, the rats may have been more attracted to the unexpected combination of the CS5 flavor and 30% solution. These explanations remain speculative and further research is needed to resolve this issue.

One reason why the sucrose concentration of test solutions may have had such large impact on the direction of the flavor preference in Experiment 3 is that, according to Experiment 2, the postingestive conditioning effects of the 5% and 30% sucrose solutions are not too dissimilar. At least early in testing, the strong satiety effect of the 30% sucrose may counteract its nutrient reinforcing action so that the net postingestive reinforcement produced by 30% sucrose is not much different from that produced by 5% sucrose.

An important implication of the present findings is that the outcome of flavor learning studies may depend upon the test solutions used to evaluate the conditioned preference. This is most critical in oral conditioning studies in which the CS flavor and US flavor or nutrient are mixed in the same solution during training and then a different combination is used in testing. In such studies, rats are typically trained with a CS+ flavor mixed in a nutrient solution and a CS– flavor mixed in a saccharin solution or plain water. Flavor preferences are then assessed with both flavors presented either in plain water or in saccharin solutions. The latter case is analogous to the treatment of Group 5 rats in the current study. Another common test procedure is to present both flavors in an intermediate solution, which is a combination of the two training solutions (e.g., a sugar–saccharin mixture), analogous to the procedure used in Experiment 1 and in Warwick's [23] and Warwick and Weingarten's [25] studies. Apparently, only one prior study evaluated conditioned flavor preferences with the flavors presented in both of the original US training solutions. That is, in the original flavor–flavor conditioning study of Holman [11], rats were given one-bottle training with a flavored 0.065% saccharin solution and a differently flavored 0.32% saccharin solution. Two-bottle tests were then conducted with the two flavors presented in 0.065% saccharin solutions and then with the two flavors in 0.32% saccharin solutions. In both tests, the rats preferred the flavor paired with 0.32% saccharin during training. The preference was weaker, however, when the flavors were mixed with 0.32% saccharin than with 0.065% (~63% vs. ~80%), but this is difficult to interpret because the order of testing was not counterbalanced. The generality of these data is also open to question because a 0.32% saccharin solution is not particularly palatable to rats, being "isohedonic" to about 2–4% sucrose [22]. It will be of interest to reexamine the impact of test solution sweetness on the expression of flavor–flavor conditioned preferences. This could be accomplished by giving rats one-bottle training with flavored 5% and 30% sucrose solutions under sham-feeding conditions to minimize postingestive consequences [27] and then testing them, in a counterbalanced order, with the flavors both presented in 5% sucrose and 30% sucrose (again under sham-feeding conditions).

A provocative aspect of the Warwick and Weingarten [25] study that stimulated the present research was the claim that two-bottle tests "do not provide an unambiguous measure of a flavor's palatability or the magnitude of the positive reinforcement." This view was based on their conditioned satiety interpretation of the two-bottle test data, which is not supported by the current study. Nevertheless, the present results raise a new concern about the interpretation of flavor conditioning tests. That is, when CS flavors are presented in different taste contexts in training and testing, this change in context may have a major impact on the expression of the conditioned response. Such taste context changes are almost always present in oral conditioning studies in which the conditioned and unconditioned stimuli are mixed in the same solution during training (for exceptions, see Refs. [1,21]) but are usually absent in intragastric conditioning studies. Thus, the interpretation of oral conditioning studies must consider the possibility of taste context effects. Importantly, this problem is not unique to the two-bottle test method. Other measures of conditioned flavor reward, such as one-bottle intake or lick rate measures, may also be influenced by taste context effects. The two-bottle test remains a valuable technique to assess learned and unlearned responses to flavors, but ultimately a complete description of flavor palatability and reward requires multiple experimental approaches (e.g., see Refs. [15,16]).

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