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Sucrose taste but not Polycose taste conditions flavor preferences in rats

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Abstract

Rats have an inborn preference for sweet taste and learn to prefer flavors associated with sweetness. They are also strongly attracted to the taste of glucose polymers (e.g., Polycose). This “poly” taste differs in quality from the sweet taste of sugar. To determine if poly taste, like sweet taste, conditions flavor preferences rats were trained with a distinctive flavor (CS+) added to 2% Polycose solution and a different flavor (CS–) added to plain water. In a subsequent two-bottle test the rats did not prefer the CS+ to CS– when both flavors were presented in water. In contrast, other rats significantly preferred a CS+ flavor that had been paired with 2% sucrose. Adding saccharin to a flavored Polycose solution did not improve CS+ flavor learning; rather, Polycose appeared to overshadow saccharin-induced conditioning. Flavor conditioning by a 16% Polycose solution was assessed using a sham-feeding procedure to prevent post-oral reinforcement. Although the rats sham-fed substantial amounts of the CS+ flavored Polycose solution, they failed to prefer the CS+ to the CS– flavor. This contrasts with the preference other rats displayed for a CS+ paired with sham-fed sucrose. Why attractive sweet and poly tastes differ in their ability to condition flavor preferences is not certain, although some findings suggest they differentially activate dopamine and/or serotonin circuits involved in flavor learning.

Keywords

Flavor-taste conditioning; Sweet taste; Poly taste; Sham-feeding

1. Introduction

Twenty years ago, our laboratory published a series of papers (see [41]) demonstrating that, unlike humans, rats and other rodents (mice, hamsters, gerbils) are strongly attracted to the taste of starch-derived polysaccharides. We studied Polycose®, a soluble maltodextrin preparation containing primarily short-chain glucose polymers (also known as maltooligosaccharides) with small amounts of free glucose and maltose [39]. In two-bottle tests (3 or 30 min) rats preferred Polycose to sugar solutions (sucrose, glucose, maltose, fructose) at low isomolar concentrations [46,51]. Rats also preferred a maltooligosaccharide solution containing no sugar to isomolar glucose and maltose solutions in two-bottle tests [50]. Together these and other findings [52] indicate that the attractiveness of Polycose is not

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due to its low sugar content. Conceivably, the rat sweet taste receptor may respond to maltooligosaccharides as well as mono- and disaccharide sugars, which could account for Polycose palatability. However, conditioned taste aversion studies indicate that Polycose does not have a sweet-like taste. Conditioned aversions to sucrose and Polycose cross-generalize only weakly [34,37,40]. Electrophysiological findings also indicate that Polycose and sucrose activate different taste channels. In particular, taste inhibitors and enhancers differentially influence the chorda tympani nerve response to sucrose and Polycose [40] and the two carbohydrates produce different neural response profiles in brainstem gustatory nuclei [18, 33,60]. More recent studies with genetically modified mice indicate that sucrose and Polycose share intracellular taste signaling pathways (alpha gustducin, Trpm5 ion channel) but apparently not the T1R3 sweet taste receptor [25,55].

Thus, Polycose and sucrose have attractive but qualitatively different tastes to rats. There is no established name for the taste of glucose polymers and the term “poly” will be used in the present paper. [Footnote 1]. The palatability of poly and sweet tastes appears to be innate because rats show ingestive responses to intraoral infusions of sucrose and Polycose at an early age (6–9 days old) [61]. Adult rats consume substantial amounts of Polycose and sucrose in sham-feeding tests, in which the ingested solution drains out an open gastric fistula [35,52]. This indicates that with post-oral effects minimized, taste alone is sufficient to drive substantial intakes of both carbohydrates. Other studies suggest that the post-oral actions of Polycose and sucrose enhance their palatability. This can be inferred from experiments in which complex flavors (tastes and odors) were paired with the post-oral actions of the carbohydrates [43]. Rats developed strong and persistent preferences for a flavored noncaloric solution (e.g., cherry-saccharin) paired with intragastric (IG) infusions of Polycose or sucrose compared to a different flavored solution (e.g., grape-saccharin) paired with IG water infusions [4,53]. Other experiments demonstrated that pairing a CS+ flavor (e.g., cherry-saccharin) with IG infusions of glucose, which is the reinforcing component of Polycose and sucrose [4, 46,48], enhanced the palatability of the CS+ flavor as indicated by increased hedonic taste reactivity responses and increased lick cluster sizes [31,32]. Flavor preferences conditioned by the post-oral actions of nutrients is referred to as flavor-nutrient learning.

Flavor preferences are conditioned not only by the post-oral consequences of sucrose but also by its palatable sweet taste, a process called flavor-taste conditioning. Thus, rats trained to sham-drink a distinctively flavored sucrose solution and a less preferred flavored saccharin solution subsequently prefer the sucrose-paired flavor in a two-bottle test with each flavor presented in mixed sucrose + saccharin solutions [63,64]. (The rats were sham-fed in these studies to eliminate the post-oral conditioning effect of sucrose.) The flavor conditioning potency of sweet taste was originally demonstrated in an early study by Holman [24] in which rats were trained with flavored 0.32% saccharin and 0.065% saccharin solutions. In a subsequent two-bottle test with the two flavors presented in otherwise identical saccharin solutions the rats preferred the flavor that had been paired with the sweeter (0.32%) saccharin solution. Other investigators have reported saccharin-conditioned flavor preferences in animals given one-bottle training trials with flavored saccharin and flavored water followed by a two-bottle test with both flavors presented in water [19,38]. Another form of preference conditioning by sweet taste is provided by studies in which rats drank distinctively flavored fructose and saccharin solutions [5,6]. In choice tests with both flavors presented in saccharin solutions, rats consumed significantly more of the fructose-paired flavor. This is considered to be a form of flavor-taste conditioning rather than flavor-nutrient conditioning because fructose,

Footnote ¹Early papers used the term “starchy” to refer to the taste of starch-derived glucose polymers but this is inappropriate because rodents discriminate between the taste (or flavor) of Polycose and pure starch [37,54]. Ramirez [37] argued against naming the taste after a commercial product (i.e., Polycose) and instead recommended the term “maltooligosaccharide taste” but we prefer the shorter term “poly.”

unlike sucrose and glucose, does not condition flavor preferences in IG experiments that limit exposure to short (30 min) daily sessions [45,48].

While there are numerous instances of flavor preferences reinforced by the sweet taste of sugar or saccharin, the ability of poly taste to condition flavor preferences is not well established. Given its highly attractive taste, rats would be expected to develop preferences for conditioned stimulus (CS) flavors associated with the taste of Polycoze. Elizalde and Sclafani [15] investigated this question by training hungry rats to drink a distinctly flavored saccharin solution (the CS+) that contained 16% Polycoze and a different flavored saccharin solution (the CS-) without Polycoze. In a subsequent two-bottle test with both flavors presented in saccharin solutions the rats displayed a significant preference (90%) for the CS+ flavor. To determine if this preference was reinforced by the taste or post-oral nutrient properties of the Polycoze solution, other rats were identically trained except that the Polycoze solution contained the drug acarbose, which delays carbohydrate digestion and blocks IG Polycoze conditioning [42]. These animals failed to prefer (52%) the CS+ flavor in the subsequent choice test with the CS- flavor (see also [11]). Because acarbose does not appear to alter the taste of Polycoze [52] the results suggested that poly taste by itself does not support flavor conditioning. It is possible, though, that delaying carbohydrate digestion has an aversive consequence that interferes with flavor-taste conditioning [13].

A recent study which compared flavor conditioning by sucrose and a maltodextrin product similar to Polycoze provided some evidence that poly taste can condition flavor preferences [14]. To minimize post-oral influences, nondeprived rats were trained in separate sessions (30 min/day) with calorically dilute 2% sucrose and 2% maltodextrin solutions containing CS+ flavors and a CS- flavor added to a noncaloric saccharin solution; saccharin was added to the sucrose and maltodextrin solutions as well. In subsequent two-bottle tests with all flavors presented in saccharin-only solutions the rats display preferences for the sucrose-paired CS+ (64%) and the maltodextrin-paired CS+ (62%) over the CS- flavor, although the maltodextrin-based preference only approached statistical significance.

Given the weak evidence that poly taste supports flavor-taste conditioning, the present study investigated this question in greater detail using different training procedures. In several experiments nondeprived animals were trained with 2% Polycoze and 2% sucrose solutions to minimize post-oral influences. Both male and female rats were compared to reveal possible sex differences in flavor-taste conditioning. In a final experiment, flavor conditioning was studied using a 16% Polycoze solution and a sham-feeding procedure to minimize post-oral conditioning effects.

2. Experiment 1A

This experiment compared flavor-taste conditioning in two groups of female rats trained with 2% Polycoze or 2% sucrose solution. The experimental procedure was based on previous studies demonstrating flavor preferences conditioned by saccharin and dilute sucrose solutions in freely fed animals trained 24 h/day [19,38].

2.1. Methods

2.1.1. Animals—Female Sprague-Dawley rats (n=18, 5 months old) bred in our laboratory of parents purchased from Charles River Laboratories (Wilmington, MA) were used. They were individually housed in hanging wire-mesh cages in a colony maintained on a 12:12 h light:dark cycle (lights on at 0800 h) at 21°C. The rats had ad libitum access to chow pellets (5001, PMI Nutrition International, Brentwood, MO) and tap water or test solutions.

2.1.2. Solutions—All test solutions were prepared on a weight/weight basis with tap water. The conditioned flavor stimuli (CS) were 0.05% unsweetened cherry and grape Kool-Aid drink mixes (Kraft Foods, White Plains, NY). The CS+ flavor was mixed with a carbohydrate (carb) and the CS− flavor was not mixed with a carbohydrate during training. The flavors serving as CS+ and CS− were counterbalanced across animals. The carbohydrates were 2% Polycose (Ross Laboratories, Columbus, OH) and 2% sucrose (Domino Foods, Yonkers, NY). The CS +/carb notation refers generically to the solutions containing the CS+ flavor and the carbohydrate while CS+P/Polycose and CS+S/Sucrose refer specifically to the two different training solutions. CS+P and CS+S refer specifically to the flavors that were mixed with Polycose and sucrose during training while CS+ refers generically to the two flavors. The solutions were presented in 250-ml glass bottles with rubber stoppers and stainless steel sipper tubes 23 h/day. During the remaining 1 h/day solution intakes were measured to the nearest 0.1 g and the bottles were cleaned and refilled.

2.1.3. Procedure—Training and testing were conducted in the animals' home cages. The rats were divided into two groups of 9 each matched for body weight and water intake. The Sucrose group was trained with a CS+S/Sucrose solution and the Polycose group with a CS +P/Polycose solution. During the 5 days of one-bottle training, the CS+/carb and CS− solutions were presented on alternate days starting with the CS−. Following previous experiments [19, 38], the animals were given 3 days of CS− and 2 days of CS+/carb training because they were expected to consume more of the CS+/carb than CS−. At the end of training, the animals were given plain water for 1 day followed by a 2-day, two-bottle preference test with the CS+ and CS− flavors in plain water. The left-right position of the bottles varied during training using an LRRLR sequence and alternated during testing to reduce side biases.

Training intakes were expressed as the sum of 2- or 3-day intakes across sessions. Two-bottle intakes were averaged across the 2 test days. Statistical analyses of training and test intakes were conducted with analysis of variance, using simple main effects to evaluate interactions. Percent CS+ preferences were calculated as the proportion of CS+ intake relative to total two-bottle test intake. Differences between groups in percent CS preferences were examined with independent t-tests.

2.2. Results

During one-bottle training, the rats consumed more CS+/carb than CS− ($F(1, 16) = 27.07$, $P < 0.01$). The groups did not significantly differ in their training intakes (Table 1). During two-bottle testing (Fig. 1A) the Sucrose group displayed a significant preference for the CS+S flavor over the CS− flavor, whereas the Polycose group did not consume significantly more CS+P than CS− (Group $\times$ CS interaction $F(1, 17) = 4.94$, $P < 0.05$). The percent preference for the CS+S exceeded that for the CS+P (74% vs. 54%, $t(16) = 2.54$, $P < 0.05$).

3. Experiment 1B

The lack of a CS+P preference (54%) in the first experiment contrasts with the marginally significant CS+maltodextrin preference (62%) reported by Dwyer [14]. Experiment 1A and the Dwyer study differed in many respects, as discussed further below. One major difference is that Dwyer studied male rats whereas female rats were used in the first experiment. This may be important because in an early study male rats preferred 2% Polycose to 2% sucrose whereas females displayed the opposite pattern [49]. Two subsequent studies, however, observed sucrose preferences in male and female rats [36,44]. To determine if the differential flavor conditioning effects of Polycose and sucrose observed in Experiment 1A are unique to females, male rats were tested using the same procedures as the first experiment.

3.1. Methods

Male rats ($n = 20$; 4 months old) were studied using the same solutions and procedure as in Experiment 1A.

3.2. Results and Discussion

During one-bottle training, the rats consumed more CS+/carb than CS- ($F(1, 18) = 28.22$, $P < 0.01$). The groups did not differ in their training intakes (Table 1). During subsequent two-bottle testing (Fig. 1B), the Sucrose group expressed a significant preference for CS+S over CS-, while the Polycose group consumed similar amounts of CS+P and CS- (Group $\times$ CS interaction $F(1, 18) = 6.34$, $P < 0.05$). The Sucrose group drank more CS+ and less CS- than did the Polycose group. The percent preference for the CS+S exceeded that for the CS+P (72% vs. 54%, $t(18) = 2.55$, $P < 0.05$).

Male rats, like the female rats of Experiment 1A, developed a preference for a CS+ flavor paired with sucrose but not for one that was paired with Polycose. Furthermore, the percent CS+S intakes displayed by male and female rats were nearly identical (72 vs. 74%) and their percent CS+P intakes were identical (54%). As in the case of the female rats, the lack of a conditioned preference for the CS+P flavor in the male rats was not due to a lower intake of the CS+P/Polycose solution compared to the CS+S/Sucrose solution. Rather, intake of the CS+P/Polycose was actually slightly higher than that of the CS+S/Sucrose (229.0 vs. 190.4 g).

To determine if the differential conditioning effects obtained with sucrose and Polycose were related to the particular CS flavors (grape and cherry Kool-Aid) used in this study, Experiment 1A was repeated with new female rats trained with different CS flavors (0.5% vanilla or strawberry extracts, McCormick). The results were similar to those obtained with the Kool-Aid flavors: the Sucrose rats displayed a significant preference for the CS+S (65%) whereas the Polycose rats did not prefer the CS+P (55%) to the CS- (Bonacchi, Ackroff and Sclafani, unpublished findings).

4. Experiment 2

The simplest explanation why sucrose-trained but not Polycose-trained rats developed a CS+ preference is that 2% sucrose has a more preferred taste than does 2% Polycose. In fact, two-bottle tests (30 min or 24 h) with these solutions revealed a sucrose preference in some but not all instances [36,44,49]. However, the rats in these earlier studies had one-bottle or two-bottle experience with the carbohydrates prior to the choice test with the 2% solutions, which may have influenced their choices. In general, rats prefer Polycose to sucrose at low isocaloric (1%) and isomolar (0.0125 – 0.05 M) concentrations, but sucrose to Polycose at higher concentrations. The Polycose to sucrose cross-over point varies from experiment to experiment depending upon test duration, and whether the solutions are presented at isocaloric or isomolar concentrations [2,35,46,49,51]. Experiment 2 evaluated the preference for 2% sucrose versus 2% Polycose in male and female rats with no prior experience with the carbohydrate solutions. The solutions were presented 23 h/day for 2 days, which replicates the 2-day exposure the animals received in Experiment 1.

4.1. Methods

Adult male and female rats (8 males and 8 females, 3 months old) with no prior experience except with chow and water were studied. They were given a 2-day, two-bottle test between unflavored 2% sucrose and 2% Polycose; chow remained available ad libitum. The left-right position of the carbohydrates alternated daily. Intakes of each carbohydrate were averaged over the 2 test days.

4.2. Results and Discussion

The female and male rats consumed somewhat more Polycose than sucrose but intakes were quite variable; there were no overall carbohydrate, group or carbohydrate $\times$ group differences (Fig. 2). The mean percent Polycose intakes for the female and male rats were 67% and 63%, respectively, but individual percent Polycose intakes ranged from 20% to 96%.

The lack of a significant difference in Polycose and sucrose intake contrasts with some previous reports of a 2% sucrose preference in rats that had prior one- or two-bottle experience with these carbohydrates [36,44,49]. Perhaps with additional experience the rats in the present experiment might have developed a preference for sucrose. Nevertheless, the rats consumed slightly more Polycose than sucrose in the two-bottle test, which is consistent with the slightly higher one-bottle intakes of Polycose observed during one-bottle training in the first experiment. Given that 2% Polycose has one third the molarity of 2% sucrose (20 vs. 58.4 mM) the fact that the rats consumed near equal amounts of the two solutions demonstrates the potency of dilute Polycose solutions to rats. Thus, the hypothesis that 2% Polycose fails to condition a CS+ preference because it is less palatable than 2% sucrose is not supported by the present findings. Rather, the data suggest that differences in taste quality may account for the differential flavor conditioning effects of sucrose and Polycose.

5. Experiment 3

As previously noted, Dwyer [14] reported similar, albeit weak preferences (62 – 64%) for CS + flavors paired with 2% maltodextrin and 2% sucrose solutions compared to a CS– flavor. Experiment 1 revealed, using a different training paradigm, that Polycose did not condition CS + preferences in female and male rats. Although Dwyer [14] used the same CS flavors (grape and cherry Kool-Aid) as those used in Experiment 1, he added saccharin to the CS+/carb and CS– training solutions. The addition of saccharin may have facilitated the development of a CS+maltodextrin preference because the combination of the sweet and poly tastes may be more effective than poly taste alone in conditioning a flavor-taste preference. In fact, a prior study observed that rats prefer a mixture of Polycose + sucrose to a Polycose (or sucrose) solution alone [1]. Experiment 3 tested the hypothesis that saccharin enhances flavor conditioning by comparing CS+ preferences in rats trained with a 2% Polycose solution or a 2% Polycose + 0.2% saccharin solution. For comparison, a third group was trained with a 2% sucrose + 0.2% saccharin solution. As in the Dwyer [14] study, the rats in the two saccharin groups had saccharin added to both the CS+ and CS– training and test solutions. Note that Dwyer [14] used a 0.1% saccharin concentration but we inadvertently used 0.2%, which is the standard saccharin concentration used in our laboratory.

5.1. Method

The female rats ($n = 28$, 3 months old) were divided into three groups matched for body weight and water intake. The unsweetened Polycose group (Polycose; $n=10$) was trained and tested with the same solutions used in Experiment 1. The saccharin-Polycose and saccharin-Sucrose groups (sacPolycose and sacSucrose, $n=9$ rats each) were trained with 2% Polycose or 2% sucrose containing 0.2% saccharin and 0.05% Kool-Aid flavor (sCS+P/Polycose and sCS+S/Sucrose solutions, respectively, where s denotes saccharin) and a 0.2% saccharin + 0.05% Kool-Aid solution (sCS– solution). All sessions were 23 h/day.

The rats were trained as in Experiment 1 with the CS+/carb (or sCS+/carb) on days 2 and 4, and the CS– (or sCS–) on days 1, 3, and 5. Because adding saccharin to the test solutions would be expected to increase solution intake [47], to equate exposure to CS flavors among groups, the rats in the sacPolycose and sacSucrose groups were limited to the average daily intakes of the Polycose group. Therefore, the two saccharin groups began their training one day after the

Polydose group. To avoid exposing the saccharin groups to fluid deprivation if they drank all of their allotted CS solution early in the training day, water was presented in a second bottle. Upon completion of training, the Polydose group was offered ad libitum water for 2 days, while the sweetened groups were offered ad libitum water for 1 day. This was done so that all groups would start preference testing on the same day. The rats then received a 2-day, two-bottle test between CS+ and CS- flavors for the Polydose group and between the sCS+ and sCS- flavors for the sweetened groups. An additional 2-day, two-bottle test was conducted between CS+/carb and CS- (Polydose group) or sCS+/carb and sCS- (sacPolydose and sacSucrose groups).

5.2. Results and Discussion

During one-bottle training, the rats consumed more CS+/carb than CS- ($F(1, 25) = 160.72$, $P < 0.01$). The sweetened groups were limited to the intakes of the Polydose group, and there were no significant group differences in training intakes (Table 1). Note that some of the rats in the saccharin groups failed to consume all of their allotted CS solutions during training, which explains why the intakes of the saccharin groups were somewhat lower than that of the Polydose group.

In the two-bottle test with the CS flavors only (Fig. 3A), the groups differed in their overall CS intakes due to the low intakes of the unsweetened CS solutions by the Polydose group compared to the intakes of the sweetened sCS solutions by the sacPolydose and sacSucrose groups ($F(2,25) = 9.04$, $P < 0.01$); the overall intakes of the two saccharin groups were similar. The groups also differed in their intakes of the two CS flavors (Group $\times$ CS interaction, $F(2,25) = 7.00$, $P < 0.01$). Only the sacSucrose group drank significantly ($P < 0.01$) more sCS+ than sCS-, whereas the sacPolydose group consumed more ($P < 0.05$) sCS- than sCS+. In contrast, the Polydose group consumed more CS+ than CS- but this difference was not significant. The percent CS+ (or sCS+) intakes of the sacSucrose, sacPolydose, and Polydose groups (69%, 40%, and 64%, respectively) did not differ significantly.

Because of the unexpected sCS+ avoidance displayed by the sacPolydose rats, the preferences for the CS+/carb (or sCS+/carb) training solution over the CS- (or sCS-) solution were evaluated in the three groups (Fig. 3B). All three groups consumed more CS+/carb than CS- ($F(2,25) = 90.7$, $P < 0.0001$). The groups had similar intakes of the CS+/carb solution but differed in their CS- intakes (CS $\times$ Group interaction $F(2,25) = 4.62$, $P < 0.02$). This was due to the higher CS- intake of the sacPolydose rats compared to the Polydose and sacSucrose rats. As a result, the CS+/carb preference of the sacPolydose group was weaker than that of the Polydose and sacSucrose groups (66% vs. 98% and 96%, $F(2,25) = 17.29$, $P < 0.0001$).

Contrary to the prediction that adding saccharin to a 2% Polydose solution would enhance its conditioning effect, it actually had the opposite effect. In the first test without carbohydrate in the CS+ solutions, the sacPolydose rats consumed less sCS+ than sCS- while the Polydose rats consumed somewhat more CS+ and the sacSucrose rats significantly more sCS+ than sCS-. Although the sacPolydose rats preferred the sCS- in the choice test, they drank more sCS+P/Polydose solution than sCS- solution during one-bottle and two-bottle sessions with the training solutions. Also, their sCS+/carb intakes in training and in the second two-bottle test did not significantly differ from those of the sacSucrose group. The groups differed, however, in their sCS- intakes in the sCS+/carb vs. sCS- test. Apparently, the sCS- solution was a more attractive alternative to the rats given the sCS+P/Polydose solution than to the rats given the sCS+S/Sucrose solution. This by itself, however, does not explain why the sacPolydose rats developed a preference for the sCS- flavor over the sCS+P flavor.

It appears that rather than saccharin enhancing flavor conditioning by Polydose, the presence of Polydose diminishes flavor conditioning by saccharin. In a prior study [19], rats trained with flavored saccharin and flavored water displayed a 65% preference for the saccharin-paired

flavor in a choice test with both flavors in water. This is not too dissimilar to the 60% preference displayed by the sacPolydose rats for the sCS⁻ flavor over the sCS+P flavor. It may be that poly taste overshadowed the sweet taste of saccharin in the sCS+P/Polydose solution so that the animals perceived the sCS⁻ as being sweeter and developed a preference for the CS⁻ flavor. Yet, in direct choice tests rats in the present experiment preferred the sCS+P/Polydose to the sCS⁻ solution, suggesting that Polydose was a more salient cue than saccharin.

The mild avoidance displayed by the sacPolydose rats for the sCS+P flavor contrasts with the findings of Dwyer [14]. The training and test procedures used in the Dwyer study differ in several respects from that of the present experiment. In a within-subject design with somewhat different experimental goals, his rats were trained and tested with three flavored solutions (sCS+M/Maltodextrin, sCS+S/Sucrose, and sCS⁻). The sCS+ vs. sCS⁻ choice tests were not conducted immediately after training but after the animals were tested for satiety-induced changes in their sCS+M vs. sCS+S preference. In the final tests with the sCS⁻ flavor, the rats preferred the sCS+S significantly (64%) and the sCS+M marginally (62%, $p = 0.065$). Given that each rat was tested with all three sCS solutions, it is possible that the animals learned to avoid the sCS⁻ rather than prefer the sCS+M. In contrast, the present experiment made direct comparisons between a single sCS+ and a single sCS⁻. Another difference between the Dwyer [14] and present study is that his rats were tested in short daily sessions (30 min/day) whereas our rats were tested in long sessions (23 h/day) but this does not readily explain the discrepant results.

6. Experiment 4

In Experiment 3, the sacPolydose rats trained with saccharin-sweetened CS solutions developed a preference for the sCS⁻ rather than the sCS+P. This unexpected result is not readily explained by the sweetener reducing the palatability of the Polydose solution given that the one- and two-bottle intakes of the sCS+P/Polydose exceeded that of the sCS⁻ solution. Rather it is more likely that the attractiveness of the saccharin-sweetened CS⁻ solution was competing with that of the sCS+P/Polydose solution. Experiment 4 investigated this possibility by training rats with a saccharin-sweetened sCS+/carb solution and an unsweetened CS⁻ solution. The presence of saccharin in the sCS+P/Polydose solution but not the CS⁻ solution should enhance the palatability difference between the training solutions and therefore facilitate the development of a sCS+P preference. As in prior experiments, flavor preference conditioning by Polydose was compared to that produced by sucrose. In addition, preferences were evaluated with the CS+ and CS⁻ flavors presented in plain water as well as in saccharin solutions.

6.1. Method

The female rats ($n = 20$, 2 months old) were purchased from Charles River. They were divided into two groups ($n = 10$ each) matched for body weight and water intake and trained with sCS+/carb solutions containing either 2% sucrose or 2% Polydose mixed with 0.2% saccharin and 0.05% Kool-Aid (cherry or grape). The CS⁻ solution contained 0.05% Kool-Aid in water.

The rats received one-bottle training with the sCS+/carb and CS⁻ solutions on alternate days for 5 days, starting with the CS⁻. The sCS+/carb solution was limited to about 132 g/day in order to equate exposure to the sCS+/carb intakes of Experiment 3. To avoid exposing the rats to fluid deprivation, water was presented in a separate bottle along with the sCS+/carb on days 2 and 4. The rats were then offered plain water for one day, followed by a 2-day, two-bottle preference test between CS+ and CS⁻ flavors presented in plain water. This was followed by a 2-day, two-bottle preference test between the flavors mixed with 0.2% saccharin (sCS+ vs. sCS⁻). Note that this was the first time the CS⁻ flavor was presented in a saccharin solution.

6.2. Results and Discussion

The one-bottle training intakes of sCS+/carb exceeded those of CS− ($F(1, 18) = 72.68$, $P < 0.01$). The training intakes of the sacSucrose and sacPolycose groups did not significantly differ (Table 1). During the two-bottle test with unsweetened solutions (Fig. 4A), the sacSucrose group drank more CS+ than CS−, whereas the CS+ and CS− intakes of the sacPolycose group did not differ significantly (Group $\times$ CS interaction $F(1, 18) = 7.08$, $P < 0.05$). In addition, the sacSucrose rats drank more CS+ and less CS− than the sacPolycose rats and their CS+ preference exceeded that of the sacPolycose rats (81% vs. 59%, $t(18) = 2.79$, $p = 0.01$). In the two-bottle preference test with the saccharin-sweetened solutions (Fig. 4B), the sacSucrose group drank more sCS+ than sCS−, whereas the sacPolycose group consumed similar amounts of the two solutions (Group $\times$ CS interaction $F(1, 18) = 12.24$, $P < 0.01$). The sacSucrose rats drank more sCS+ and less sCS− than the sacPolycose rats and their percent sCS+ intakes exceeded that of the sacPolycose rats (80% vs. 44%, $t(18) = 3.48$, $P < 0.01$). The CS+S and sCS+S preferences of the sacSucrose group were very similar at 81% and 80%, respectively. In contrast, the percent intakes of the sacPolycose group were less with the sCS+P solution (44%) than with the CS+P solution (59%) although this difference was not significant.

Altering flavor conditioning by adding saccharin to the CS+/carb but not the CS− did not change the differential effectiveness of the carbohydrates. Sucrose conditioned the strongest CS+ preference (81%) observed so far in this study whereas Polycose remained ineffective. The fact that the rats did not develop a preference for the CS+ flavor added to the Polycose + saccharin solution over the CS− flavor added to plain water is surprising given several prior reports of saccharin-conditioned preferences [16,19,24,59]. The present findings suggest that mixing Polycose and saccharin together in a solution overshadows flavor conditioning by the sweet taste of saccharin.

7. Experiment 5

The rats in the previous experiments consistently developed preferences for a CS+ flavor paired with 2% sucrose, but not for one paired with 2% Polycose. The ineffectiveness of Polycose to support flavor-taste preference learning may not be limited to a 2% solution. In earlier studies rats failed to learn a preference for a CS+ flavor mixed in a 16% Polycose solution containing acarbose, which suggests that even at this high concentration Polycose taste does not condition flavor preferences [11,15]. An alternate explanation, however, is that the acarbose-induced delay in Polycose digestion had an aversive action that blocked flavor learning [13]. The present experiment further investigated flavor-taste conditioning using a 16% Polycose solution. In this case, a gastric sham-feeding procedure was used to minimize the post-gastric actions of Polycose, which would otherwise condition a strong flavor preference [29,30]. Earlier studies show that rats trained to sham-feed a CS+ flavored 16% sucrose solution and a less preferred CS− flavored saccharin solution subsequently display a significant preference for the CS+ over the CS− flavor [63,65]. Experiment 5 determined if sham-fed 16% Polycose would also condition a CS+ flavor preference. The conditioning effect of sham-fed 16% Polycose was compared to that of 8% sucrose in separate groups of rats. An 8% sucrose solution was used because an earlier sham-feeding study showed that rats prefer 16% sucrose to 16% Polycose in a two-bottle test [35] whereas a pilot study indicated that 8% sucrose and 16% Polycose were equally preferred (Bonacchi and Sclafani, unpublished findings).

7.1. Method

7.1.1 Surgery and Pretraining—The female rats ($n = 24$, 3 months old) were fitted with a stainless steel gastric cannula according to a previously described procedure [35], and were given 3 weeks to recover from surgery. The rats were adapted to the sham-feeding procedure by first housing them in the test cages for 2 days. These cages were similar to their home cages

but had drinking spouts positioned just outside access holes (19 mm diameter) in the front of the cage; the cages were kept in an isolated room. During this time the rats were given ad libitum access to chow and water and 35 g/day of a solution containing 2% Polycose and 2% sucrose. The rats were then returned to their home cages and food-restricted to maintain them at 85% of ad libitum body weight.

The rats were exposed on two consecutive days to the gastric rinsing procedure used in the sham-feeding training sessions. Each rat's cannula was opened and the stomach was flushed twice (at 15-min intervals) with warm water until it appeared empty of food. The rats were next accustomed to 30-min two-bottle drinking sessions preceded by gastric washing. For the first two sessions the cannula was closed (real-feeding) and for the next two sessions the cannula was open (sham-feeding). In these sessions, one bottle contained unlimited amounts of the 2% sucrose + 2% Polycose mixture and the other contained water. At the end of the sham-feeding sessions the rats' stomachs were flushed again with warm tap water to ensure minimal nutrient absorption. The cannula was then closed and the rats were returned to their home cages. Chow rations were given 1 h later.

7.1.2. Conditioning Procedure—The rats were divided into two groups ($n=12$ each) matched for body weight and 30-min intakes of sucrose+Polycose solution. The Sucrose group was trained with 8% sucrose and the Polycose group with 16% Polycose. The CS+/carb solutions contained the carbohydrate and 0.05% grape or cherry flavors. The alternate CS− flavor was mixed into a 0.2% saccharin solution (sCS−). The two-bottle preference tests were conducted with CS+ and CS− flavors presented in 0.2% saccharin solutions (sCS+ vs. sCS−). The addition of saccharin to the CS− during training and testing and to CS+P and CS+S solutions during testing was necessary to stimulate intakes during the 30-min sessions; hungry rats drink very little of unsweetened Kool-Aid solutions in brief daily tests.

During one-bottle training, the CS+/carb and sCS− solutions were presented on alternate days for a total of 9 days. On days 1, 4, 6, and 8, the rats were given the CS+/carb solution, and on days 2, 3, 5, 7 and 9, the rats were given the sCS−. Intakes were limited to 10 g/session in order to equate exposure to the CS solutions, and for this reason an additional sCS− session was run. Training was followed by a two-bottle test between the sCS+ and sCS− for two 30-min sessions. The rats were then given another cycle of training and testing. In the second training cycle, access the CS+/carb and sCS− solutions were unlimited during alternate sessions for a total of 6 sessions. The rats were then given another two-bottle test between sCS+ and sCS− for two 30-min sessions.

7.2. Results and Discussion

During one-bottle limited exposure training (Table 1), total intakes of the CS+/carb and sCS− solutions were similar, and the groups did not differ. During subsequent two-bottle testing (Fig. 5A), the Sucrose group drank more sCS+ than sCS−, whereas the Polycose group consumed comparable amounts of the two solutions (Group $\times$ CS interaction $F(1, 22) = 5.30$, $P < 0.05$). The Sucrose rats sham-fed more ($p < 0.05$) sCS+ than the Polycose rats and their percent sCS+ preference exceeded that of the Polycose rats although this difference was marginal (66% vs. 52%, $t(22) = 2.01$, $p = 0.0567$).

During the second training cycle with unlimited CS solutions (Table 1), both groups sham-fed considerably more CS+/carb than sCS− ($F(1, 22) = 264.32$, $P < 0.01$). The Polycose group sham-fed more CS+/carb and less sCS− than did the Sucrose group (Group $\times$ CS interaction $F(1, 22) = 5.86$, $p = 0.02$), although the simple main effects were not significant. In the subsequent two-bottle test (Fig. 5B) the Sucrose group drank more sCS+ than sCS−, whereas the Polycose group consumed comparable amounts of the two flavors (Group $\times$ CS interaction

$F(1,22) = 8.12, P < 0.01$). In addition, the Sucrose group sham-fed more sCS+ and had a higher sCS+ preference than did the Polycose group (64% and 46%, $t(22) = 3.20, P < 0.01$).

As in the preceding experiments in this series, the Sucrose group but not the Polycose group expressed a significant preference for the CS+ flavor over CS-, providing further evidence that sweet taste is more effective than poly taste in conditioning flavor-taste preferences. The flavor preference conditioning by sham-fed 8% sucrose extends prior conditioning results obtained with sham-fed 16% sucrose solutions. The results obtained with the sham-fed 16% Polycose solution, on the other hand, confirm the early suggestion that the taste of 16% Polycose does not support flavor-taste preference learning [11,15]. This is noteworthy because 16% Polycose has a very attractive taste to rats as evidenced by their robust sham-feeding response in the present and previous studies, and by their vigorous licking response in short-term tests [12,35,46].

There was one difference in the treatment of the sham-fed groups that should be acknowledged as a potential limitation of the experiment. The addition of saccharin to the CS+ solution during testing introduced a novel taste quality (sweet) to the flavor of the CS+ for the Polycose rats, but not the sucrose rats. It is possible that the addition of saccharin to the CS+P flavor during testing interfered with the expression of a CS+P preference. However, rats conditioned in real-feeding sessions (which support post-oral nutrient conditioning) with unsweetened CS+P/16% Polycose displayed a significant CS+P preference during testing with saccharin-sweetened CS+P and CS- solutions (Bonacchi, Ackroff, & Sclafani, unpublished findings). This extends the earlier findings obtained with real-feeding rats both trained and tested with a saccharin-sweetened CS+P solution [15]. Thus, a change in the taste quality of the CS+P flavor from training to testing need not prevent the expression of a robust preference.

8. General Discussion

The rats in the present study consistently acquired preferences for a CS+ flavor paired with the sweet taste of sucrose. This confirms prior results obtained with sucrose as well as with saccharin and fructose solutions [16,19,24]. In contrast, the rats failed to display significant preferences for a CS+ flavor paired with the taste of Polycose, which supports an early suggestion that poly taste may not be effective in flavor-taste conditioning [11,15]. Nevertheless, given that poly taste is quite attractive to rats, its failure to support flavor-taste preference conditioning is surprising.

In Experiment 1 male and female rats were similar in displaying a ~73% preference for a sucrose paired CS+ flavor but no preference (54%) for a Polycose paired CS+ flavor. Because the 2% carbohydrate solutions were calorically dilute (0.076 kcal/g) and the animals had free access to food, the sucrose-conditioned preferences are assumed to result primarily from an association between the sugar's sweet taste and the CS+ flavor. It is possible that the post-oral nutritive consequences of sucrose contributed to the flavor conditioning effect but it is unlikely that it was a major factor; otherwise, the Polycose rats should have acquired significant CS+ preferences. IG infusion studies demonstrate that Polycose and other glucose polymers are very effective in conditioning flavor preferences, even more so than IG sucrose infusions [4, 29,30]. Experiment 5 provides further evidence that sweet and poly tastes differ in their flavor conditioning potency. In this experiment, post-oral influences were minimized using a sham-feeding procedure, which allowed the use of more concentrated carbohydrate solutions. Whereas the rats trained with 8% sucrose acquired a significant CS+ flavor preference, the rats trained with 16% Polycose were indifferent to the CS+ and CS- flavors. This indicates that even at a highly attractive concentration poly taste is not an adequate stimulus for flavor preference conditioning. An early study of neonatal rats reported that intraoral infusions of a still higher Polycose concentration (32%) failed to condition an odor preference in contrast to

infusions of 7.5% sucrose [57]. The authors speculated that 6-day old rats may be insensitive to the taste of Polycose, although an alternative explanation suggested by the present findings is that poly taste simply does not reinforce flavor preferences. An investigation of odor preference conditioning in 12-day old neonates, which show behavioral responsiveness to both sucrose and Polycose [56,61], would be informative.

Experiments 3 and 4 investigated the possibility that adding saccharin to a 2% Polycose solution would facilitate the acquisition of a CS+ preference. This proved not to be the case. Instead, the addition of saccharin produced a mild avoidance of the CS+P flavor when the alternative was a sweetened CS- flavor and did not produce a significant CS+ preference when the alternative was an unsweetened CS-. The results from Experiments 3 and 4 suggest that poly flavor may overshadow the flavor conditioning effect of saccharin, although this requires further investigation.

The failure of Polycose to condition a flavor preference was not due to the specific CS flavors (grape and cherry Kool-Aid) used in this study because similar results were obtained with other flavors (vanilla and strawberry extracts; Bonacchi, Ackroff and Sclafani, unpublished findings). It cannot be attributed to a less preferred taste of Polycose than sucrose at the concentrations tested. The naïve rats in Experiment 2 consumed slightly more 2% Polycose than 2% sucrose in two-bottle tests and a pilot study indicated that the 16% Polycose and 8% sucrose solutions used in the sham-feeding experiment were isoprefered. In all the conditioning experiments the Polycose rats consumed as much or more of the CS+/carb during one-bottle training as did the sucrose rats. This indicates that underconsumption of the CS+P/Polycose solution, which would have resulted in less experience with the CS+P flavor and less associated carbohydrate compared to the CS+S flavor, cannot account for the weak CS+P preferences. The tonicity of 2% Polycose is less than that of 2% sucrose but this does not readily explain the differential conditioning effect given the low tonicity of both solutions. As noted above, post-oral factors also do not readily explain the differential flavor conditioning effects of sucrose and Polycose. Rather, it appears that the different taste qualities of Polycose and sucrose account for their differential flavor conditioning effects but why this should be is not known.

While poly taste does not serve as a reinforcer in flavor preference conditioning, it is an effective CS in other learning paradigms. In particular, rats learn to avoid both Polycose and sucrose when the carbohydrates are paired with an aversive treatment (e.g., LiCl) [34,37,40, 58]. Some findings, however, suggest differences in poly and sweet taste aversion learning. Barot and Bernstein [7] reported that rats display similar aversions to novel and familiar Polycose solutions, which differs from the reduced aversion rats develop for familiar sucrose and saccharin solutions [7,28]. One explanation offered for the lack of a novelty-potentiated aversion to Polycose is that the Polycose taste even on first exposure may not be particularly novel to rats [7]. This might occur because of generalization between the taste of Polycose and that of the starch-rich chow fed to the rats. However, other data indicate that rodents distinguish between the taste of Polycose and starch [37,54]. Furthermore, the absence of a novelty effect was documented at only one Polycose concentration (30%) and may not exist at lower concentrations. This is suggested by the finding of similar taste aversions in rats trained with dilute Polycose (2.5 – 3%) and sucrose (3 – 3.4%) solutions [34,58]. Relevant to the present study, we observed nearly identical aversions in rats conditioned with 2% Polycose or 2% sucrose solutions (Touzani and Sclafani, unpublished findings). In some of these studies, though, the Polycose aversion extinguished more rapidly than the sucrose aversion but this may be due to differences in the post-oral rather than the taste properties of the two carbohydrates ([58], Touzani and Sclafani, unpublished findings). Taste aversion learning and extinction studies conducted with rats sham-feeding different concentrations of Polycose and sucrose would clarify this issue.

Polycose and sucrose have also been studied using a combination of flavor preference and taste aversion paradigms. In the previously mentioned Dwyer study [14], after rats were trained to associate different CS+ flavors with 2% maltodextrin or 2% sucrose, they had either poly taste or sweet taste devalued by pairing intake of maltodextrin or sucrose, respectively, with LiCl injections. In subsequent CS+M vs. CS+S choice tests, the rats preferred the CS+ flavor associated with the non-devalued carbohydrate. Similar results were obtained in a second experiment in which the poly or sweet tastes were devalued by prefeeding the animals with maltodextrin or sucrose just prior to the CS+M vs. CS+S choice tests. Non-flavor cues can also be associated with poly and sweet tastes. Galarce et al. [17] trained hungry rats to associate different auditory cues with the presentation of 4% maltodextrin and 4% sucrose rewards and then to respond on different levers for these carbohydrates. In a subsequent Pavlovian-to-instrumental transfer test, presentation of the CS+M cue facilitated responding to the maltodextrin-paired lever, while the CS+S cue facilitated responding to the sucrose-paired lever. Additional experiments revealed that presentation of the CS+M and CS+S cues selectively enhanced maltodextrin and sucrose consumption, respectively, and devaluation (with LiCl injections) of maltodextrin or sucrose selectively altered the behavioral response to the CS+M and CS+S cues. These findings demonstrate that rats discriminate between poly and sweet tastes and can associate them with different flavor or non-flavor cues and instrumental responses.

The preference conditioning results obtained in the present study suggest that poly and sweet tastes differentially activate brain reward systems involved in flavor learning. Circumstantial support for this hypothesis is provided by some recent findings. In one study the obesity-prone OLETF rat, which lacks CCK-1 receptors, displayed increased licking responses to sucrose and other sweeteners, but near-normal responses to Polycose, compared to control (LETO) rats [21]. Altered dopamine D2 receptor signaling is implicated in the enhanced sucrose appetite displayed by OLETF rats [20,22]. There is considerable evidence linking sweet taste preference with brain dopamine activity, including reports that sweet taste stimulation promotes dopamine release in the nucleus accumbens and that chronic sugar intake alters dopamine receptor sensitivity [3,23]. Whether poly taste also stimulates brain dopamine release is not known. If sweet and poly tastes differ in their effects on brain dopamine activity, this could account for their different flavor conditioning effects. In several studies flavor conditioning by sweet taste was attenuated by systemic, and to a lesser degree, nucleus accumbens administration of dopamine receptor antagonists [5,10,64].

Serotonin (5-hydroxytryptamine, 5-HT) receptors are also implicated in the unconditioned and conditioned responses to sweet taste. In one study systemic injection of the 5-HT agonist dexfenfluramine decreased glucose solution intake and blocked glucose-conditioned flavor preferences [62]. In another study, inhibiting 5-HT receptors with methysergide increased sucrose intake by 30% at doses (0.5, 2.5 mg/kg) that had no effect on the intake of a maltodextrin solution [26]. Together these data suggest differential involvement of 5-HT receptors in the appetitive and perhaps conditioning responses to sweet and poly tastes.

Brain opioid receptors constitute a third neurochemical system closely linked to sweet taste hedonics. However, opioid receptors are also implicated in poly taste hedonics. The general opioid receptor antagonist naltrexone produced comparable suppressions in the intake of sucrose and maltodextrin solutions [26], although some data suggest that different receptor subtypes mediate sucrose (κ , μ_2) and maltodextrin (μ_2) consumption [8,9]. In addition, long-term exposure to sucrose or Polycose enhances the feeding suppressive effects of naltrexone suggesting that both carbohydrates alter the activity of the endogenous opioid system [27]. Despite its involvement in sweet taste hedonics, the opioid system does not appear to have a central role in sweet taste conditioned flavor preferences. In two studies naltrexone treatment had little effect on flavor conditioning by sucrose or fructose solutions [5,64].

In conclusion, the present findings indicate that the taste of Polycose, unlike sweet taste, does not readily support flavor preference conditioning. Yet, Polycose has a very attractive taste to rats and post-oral actions that condition strong flavor preferences. Why preferred sucrose and poly tastes differ in their flavor preference conditioning actions is not certain, although some data suggest that they may differentially activate brain dopamine and/or serotonin receptor systems involved in flavor preference learning.

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Figure 1.

Mean (+sem) CS+ and CS- intakes in females (A) and males (B) during the 2-day, two-bottle choice tests in Experiment 1. The asterisk denotes a significant ($P < 0.05$) difference between CS+ and CS- intakes. Percentages above bars indicate percent preference for the CS+ solution. The standard error of the means (sem) for each percent value are A: 74% (4.9), 54% (5.9); B: 72% (4.6), 54% (4.8).

Figure 2.

Mean (+sem) 2% Polycose and 2% sucrose intakes during the 2-day, two-bottle choice test in Experiment 2. Percentages on above bars indicate percent preference for 2% Polycose. The standard error of the means (sem) for each percent value are: 67% (7.6) and 63% (8.4).

Figure 3.

Experiment 3. A. Mean (+sem) CS+ and CS- intakes during 2-day, two-bottle choice test. B. Mean (+sem) CS+/carb and CS- solutions during 2-day, two-bottle choice test. The Polycose group was trained and tested with unsweetened CS solutions while the sacPolycose and sacSucrose groups were tested and trained with saccharin-sweetened CS solutions. The asterisk denotes a significant ($P < 0.05$) difference between CS+ and CS- intakes or CS+/carb and CS- intakes. Percentages above bars indicate percent preference for the CS+ solution or CS+/carb solution. The standard error of the means (sem) for each percent value are A: 64% (5.2), 40% (10.8), 69% (8.3); B: 98% (0.5), 66% (7.7), 96% (0.8).

Figure 4.

Mean (+sem) CS+ and CS- intakes during 2-day, two-bottle choice tests with unsweetened (A) and saccharin-sweetened (B) flavors in Experiment 4. The Sucrose and Polycose groups were given saccharin-sweetened CS+/carb and unsweetened CS- solutions during one-bottle training. The asterisk denotes a significant ($P < 0.05$) difference between CS+ and CS- intakes. Percentages above bars indicate percent preference for the CS+ solution. The standard error of the means (sem) for each percent value are A: 81% (4.7), 59% (6.4) B: 80% (6.2), 44% (8.3).

Figure 5.

Mean (+sem) sCS+ and sCS- intakes during two-bottle sham-feeding choice tests (30 min/day) after one-bottle training with limited (A) and unlimited (B) amounts of CS solutions in Experiment 5. The Sucrose group was trained with CS+/8% sucrose solution and the Polycose group with CS+/16% Polycose solutions. Both groups were trained with a saccharin-sweetened sCS- and were tested with saccharin-sweetened sCS+ and sCS- solutions. The asterisk denotes a significant ($P < 0.05$) difference between sCS+ and sCS- intakes. Percentages above bars indicate percent preference for the sCS+ solution. The standard error of the means (sem) for each percent value are A: 66% (5.8), 52% (4.3); B: 64% (3.2), 46% (4.4).

Table 1
Mean (sem) One-Bottle Total Training Intakes of CS Solutions

Group	CS+/carb Solution	CS+/carb Intake g	CS- Solution	CS- Intake g
Experiment 1A: Females				
Polycose	CS+P/2% Polycose	200.8 (33.6)	CS-	95.5 (5.5)
Sucrose	CS+S/2% Sucrose	188.5 (29.5)	CS-	93.8 (6.3)
Experiment 1B: Males				
Polycose	CS+P/2% Polycose	229.0 (28.3)	CS-	116.8 (6.3)
Sucrose	CS+S/2% Sucrose	190.4 (22.9)	CS-	117.7 (6.3)
Experiment 3				
Polycose	CS+P/2% Polycose	261.9 (30.6)	CS-	99.1 (7.2)
sacPolycose	sCS+P/2% Polycose	229.5 (13.3)	sCS-	97.8 (6.4)
sacSucrose	sCS+S/2% Sucrose	226.3 (22.1)	sCS-	90.4 (6.6)
Experiment 4				
sacPolycose	sCS+P/2% Polycose	226.5 (18.0)	CS-	92.2 (4.4)
sacSucrose	sCS+S/2% Sucrose	178.0 (23.0)	CS-	87.4 (5.2)
Experiment 5				
<i>Cycle 1: Limited</i>				
Polycose	CS+P/16% Polycose	34.3 (1.0)	sCS-	26.9 (2.7)
Sucrose	CS+S/8% Sucrose	31.0 (1.2)	sCS-	33.8 (3.2)
<i>Cycle 2: Unlimited</i>				
Polycose	CS+P/16% Polycose	130.5 (6.7)	sCS-	53.4 (4.5)
Sucrose	CS+S/8% Sucrose	116.0 (8.8)	sCS-	58.9 (7.5)

CS = 0.05% Kool-Aid flavored solution; sCS = 0.05% Kool-Aid + 0.2% saccharin flavored solution

Experiments 1 – 4: CS+ intakes summed over two 23-h sessions; CS- intakes summed over three 23-h sessions.

Experiment 5, Cycle 1: CS+ intakes summed over four 30-min sham-feeding sessions; CS- intakes summed over five 30-min sessions. Cycle 2: CS+ and CS- intakes summed over three 30-min sham-feeding sessions with each CS solution.