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CONDITIONED CARBOHYDRATE APPETITE IN RATS

by

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Abstract

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by

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The present study examined the appetite conditioning effect of carbohydrates using a conditioned flavor preference paradigm. Adult female rats were fitted with two intragastric (IG) catheters and were trained to drink flavored water (CS+; e.g., cherry-water) paired with IG infusions of 32% Polycose. On alternate days a different flavor (CS–; grape-water) was paired with IG water infusions. The CSs and chow were available 23 hours/day. In two-choice tests the rats displayed strong preferences for the CS+ over the CS- as well as over plain water. The latter finding contrasts with the mild aversion naive rats displayed to the CS flavors. The CS+ preference persisted for several weeks during extinction tests when both the CS+ and CS– were paired with IG water or no infusions. The acquired preference for the CS+ flavor was not as strong, however, as the rats' innate preferences for the taste of saccharin or Polycose. Also, unlike their response to saccharin and Polycose, the rats' acceptance (absolute intake) of the CS+ was not elevated. Increased acceptance was also not obtained in a second experiment in which the initial palatability of the CS flavors was enhance by the addition of Polycose. However, in a third

experiment, which used a different set of flavors (bitter vs. sour), a conditioned increase in CS+ intake was observed. The third experiment also examined the rats' orofacial reactions to intraoral infusions of the CS+ and CS- flavors. Despite their strong conditioned preference for the CS+ over the CS-, the rats did not differ in their ingestive or aversive orofacial reactions to the CSs. This suggests that the palatability of the CS+ was not enhanced by the conditioning process.

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INTRODUCTION

The aim of this dissertation was to investigate the role of orosensory and postingestive factors in the carbohydrate appetite of rats. In order to dissociate and independently manipulate orosensory and postingestive factors, a conditioned flavor preference paradigm was used. An arbitrary flavor was paired with intragastric starch infusions, while a different flavor was paired with water infusions. Flavor preference was assessed in two-choice tests. Flavor acceptance (i.e., total intake) and caloric intake were also examined. Experiments were conducted to determine if flavor palatability influences appetite conditioning and, in turn, if conditioning affects flavor palatability.

Food selection is critical to the survival of omnivores, who, faced with a variety of potential food sources - many beneficial and some that are dangerous - must determine which to consume and which to avoid. Such determination is both innate and learned. It is well established that omnivores have an inborn attraction for sweet tastes as demonstrated in infant animals' readiness to consume sugar solutions (Hall & Bryan, 1981; Jacobs, Smutz, & DuBose, 1977; Sclafani, 1987a). Also, animals have an innate aversion for bitter tastes, which are generally associated with the presence of toxins (Jacobs, et al., 1977; Rozin & Kalat, 1971). In addition to innate preferences and aversions, animals can select foods based on their postingestive consequences. That is, omnivores have the ability to learn to avoid foods that have toxic or negative effects (Braveman & Bornstein, 1985) and to prefer foods that have positive postingestive consequences (Booth, 1985). The findings

have revealed that postingestive actions of carbohydrates can condition a strong appetite, as measured by flavor preferences, but they have not clearly identified the impact of the conditioning process on flavor palatability.

Most of the research examining the role of learning on food selection has focused on food aversions conditioned by negative postingestive consequences, including poisoning and X-irradiation and their sequelae of gastrointestinal disturbances and nausea. In this research paradigm, a rat is given access to a novel food which is then followed by an experimental manipulation that produces gastrointestinal malaise in the rat. After recovery from the illness, the rat is again permitted access to the test food. A comparison of the rat's post-illness intake with a control rat's intake of the same food (which was not paired with illness) provides an assessment of the emergence of an aversion to the novel food. Garcia and colleagues have demonstrated that rats readily form aversions to novel foods paired with X-irradiation, apomorphine injection, or lithium-chloride injection (Garcia, Kimoldorf, & Hunt, 1961; Garcia, Ervin, & Koelling, 1966; Garcia & Koelling, 1966). In addition, rats fed a thiamine-deficient diet will develop an aversion to the diet (Rozin, 1967). These investigators have argued that the capacity of the rat to make associations between flavors, i.e., conditioned stimuli (CS), and aversive gastrointestinal effects is highly adaptive to survival in the wild.

In contrast to the extensive literature showing that many species can learn to avoid foods that have been paired with illness, there is relatively little research on food preferences based on associations between food flavors and <u>positive</u>

postingestive consequences of foods. Conditioned flavor preferences have been difficult to show, although several studies report that hungry rats acquire preferences for flavors added to calorically-rich solutions (Bolles, Hayward, & Crandall, 1981; Capaldi, Campbell, Sheffer, & Bradford, 1987; Mehiel & Bolles, 1984). In one paradigm, for example, rats were fed a flavored starch solution and a differently flavored saccharin solution on alternate days. When subsequently offered the choice between the two flavors, the rats displayed a preference for the starch-paired flavor (Booth, 1985; Elizalde & Sclafani, 1988; Mehiel & Bolles, 1984, 1986, 1987). Similar results were obtained using glucose, sucrose, and ethanol solutions, and pure oil as well as oil emulsions (Elizalde & Sclafani, 1990; Fedorchak & Bolles, 1987; Mehiel & Bolles, 1984, 1987; Simbayi, Boakes, & Burton, 1986). These conditioned flavor preferences (CFPs) have been attributed to the postingestive caloric effects of the nutrients.

The conditioned preference paradigm described above involves a CS flavor mixed directly into the calorically-rich solution. However, nutrients such as sugar and starch are very palatable to rats (Jacobs, et al., 1977; Sclafani, 1987a). Several studies have reported that rats can be conditioned to prefer flavors associated with the sweet taste of saccharin (Fanselow & Birk, 1982; Holman, 1975). In order to dissociate the orosensory properties (e.g., palatable taste) of nutrients from their postingestive properties (e.g., calories), a CS flavor can be paired with nutrient infusions; thus, the nutrient bypasses the oral cavity. Attempts to condition flavor preferences by pairing a flavor with intragastric, intraduodenal, or intravenous

nutrient infusions have not always been successful. While some studies have produced conditioned flavor preferences with nutrient infusions (Holman, 1969; Mather, Nicolaidis, & Booth, 1978; Sherman, Hickis, Rice, Rusiniak, & Garcia, 1983; Tordoff & Friedman, 1986), others have failed to obtain positive results, or in some cases, have produced nutrient-induced flavor aversions (Deutsch, Molina, & Puerto, 1976; Koopmans & Maggio, 1978; Puerto, Deutsch, Molina, & Roll, 1976; Revusky, Smith, & Chalmers, 1971). Furthermore, the preferences that have been obtained are, in most cases, rather modest (60-75%), and some findings suggest that preferences extinguish very rapidly (Holman, 1969).

These and other findings have led to the view that conditioned preferences are more difficult to establish and less robust than are conditioned aversions (Rozin & Zellner, 1985). However, a recent study from Sclafani's laboratory demonstrated that very strong flavor preferences can be produced by intragastric (IG) infusions of hydrolyzed starch (Polycose) using an "electronic esophagus" preparation (Sclafani & Nissenbaum, 1988). With this preparation, rats were fitted with chronic intragastric catheters and were automatically infused with Polycose as they drank flavored water during 24-hour/day tests. The rats displayed a 96-98% preference for the flavor (CS+) paired with IG infusions of Polycose over the flavor (CS–) paired with water infusions. In addition, the rats continued to prefer the CS+ flavor during a four-day extinction test when both the CS+ and CS– flavors were paired with IG water infusions.

The present study sought to further investigate this important observation that

rats learned to prefer flavors associated with IG Polycose infusions. The persistence of the flavor preference was assessed by administering extinction tests. Whereas in the prior experiment (Sclafani & Nissenbaum, 1988) extinction testing lasted for four days, extinction was extended for several weeks in Experiment 1. In addition, the acquired preference for the CS+ flavor was compared to the rats' innate preference for sweet as well as polysaccharide tastes. The rats were given two-bottle tests between the CS+ and saccharin-sweetened water and between the CS+ and a Polycose solution. The impact of conditioning on total kilocalorie intake was also examined. Other experiments determined the effects of reversal training on the rats' preferences for the CS+ and CS– flavors as well as the ability of the rats to detect IG Polycose infusions in the absence of unique flavor cues.

The effect of flavor preference conditioning on flavor acceptance (i.e., absolute intake) was also examined. There is a distinction made between flavor "preference" (as measured in two-bottle choice tests) and flavor "acceptance" (as measured in one-bottle tests), but both preference and increased acceptance are taken as measures of palatability. However, preference and increased acceptance do not always go hand in hand: in one-bottle short- or long-term tests, rats consumed more of a dilute sugar solution (e.g., 4% sucrose) than of a concentrated solution (e.g., 32% sucrose); but when given the choice between the two solutions, rats preferred the concentrated to the dilute solution (Cagan & Maller, 1974; Collier & Bolles, 1968; Sclafani & Nissenbaum, 1987; Young & Greene, 1953). Therefore, the most accepted solution in terms of amount consumed may not be the most preferred

solution. When given the choice between a carbohydrate solution (e.g., 4% sucrose) and plain water, rats typically prefer and overconsume the carbohydrate solution, depending on its concentration (Sclafani & Nissenbaum, 1987). In Sclafani and Nissenbaum's (1988) study, the rats strongly preferred the CS+ over the CS– as well as over plain water, yet the rats' CS+ intake did not exceed their water baseline intake (unpublished data). Thus, the rats displayed a robust preference for the CS+, but no increased acceptance was exhibited. To assess whether the rats would overconsume the CS+ if it were made more palatable, saccharin was added to the CS+ solution.

The results of Experiment 1 suggested that the initial palatability of the CS flavors may influence the conditioning of increased acceptance as well as flavor preference. Experiment 2 investigated this possibility by comparing the conditioned preference and acceptance in two groups of rats. One group was trained with unpreferred (relative to water) CS flavors, while the second group was trained with preferred flavors. Any differences in conditioned acceptance observed between the two groups could then be attributed to the initial palatability of the CS solutions.

Experiment 3 investigated whether IG conditioning alters the palatability response to the CS+ flavor. According to Rozin and Zellner (1985), conditioned flavor preferences may involve an increased liking for the CS+ flavor or, alternatively, the CS+ flavor may act as a cue for the postingestive consequences. This issue was investigated using the taste reactivity test (Grill & Norgren, 1978), which is thought to be a measure of palatability. The taste reactivity test analyzes

the pattern of orofacial consummatory responses (fixed action patterns, FAPs) that are elicited by the infusion of taste solutions directly into the mouths of rats. The FAPs can be divided into two response domains, ingestive and aversive. Ingestive FAPs are accompanied by periods of uninterrupted ingestion and consist of mouth movements, tongue protrusions, lateral tongue movements, and lip flaring. Aversive responses result in the removal of the stimulus from the oral cavity and consist of gaping, chin rubbing, head shaking and forelimb flailing. Certain flavors, such as the sweet taste of sugar, elicit ingestive FAPs, and other flavors, such as the bitter taste of quinine, elicit aversive FAPs. A bitter-sweet solution may elicit both sets of responses (Grill & Berridge, 1985).

Several studies have used the taste reactivity test to assess the acquisition of dislikes in the conditioned taste aversion paradigm (Grill, 1975; Pelchat, Grill, Rozin, & Jacobs, 1983; Spector, Breslin, & Grill, 1988). In one such experiment (Spector et al.,1988), rats were intraorally infused with a sucrose solution after they had been injected with lithium chloride. Control rats were infused with a sucrose solution after being injected with sodium chloride. In addition to developing a conditioned taste aversion to the sucrose solution, the lithium-injected rats decreased the number of ingestive FAPs and increased the number of aversive FAPs to the sucrose solution; that is, the lithium-injected rats exhibited quinine-like responses to the taste of sucrose. The control rats maintained high levels of ingestive FAPs to the sucrose solution (Spector et al., 1988). Pelchat and colleagues (1983) found that not all conditioned flavor aversions involve the acquisition of dislikes. These investigators

found that an aversion to sucrose paired with LiCl poisoning (upper gut discomfort) was associated with an increase in aversive FAPs and a decrease in ingestive FAPs, whereas an aversion for sucrose paired with lactose intolerance (lower gut discomfort) was not associated with a change in orofacial responding (Pelchat et al., 1983)

Grill and colleagues also utilized the taste reactivity test to examine the conditioned preference for the taste of morphine (Zellner, Berridge, Grill, & Ternes, 1985). Rats prefer to drink water rather than a bitter morphine solution. However, this preference is reversed when they are forced to drink a morphine solution for an extended period of time (Stolerman & Kumar, 1970). Zellner et al. (1985) investigated whether this acquired preference for morphine was associated with changes in the orofacial responses to morphine taste. They found that morphine-raised rats exhibited a greater number of ingestive FAPs to an intraoral infusion of a morphine solution than water-raised rats. Zellner et al. concluded, therefore, that the rats came to like the taste of the morphine solution. The acquired preference for morphine involves an association between the drug's taste and its postingestive effects. Another study used the taste reactivity test to examine conditioned preferences for flavors associated with sweet taste (Breslin, Davidson, & Grill, 1990). When a normally avoided cue flavor (e.g., quinine) signalled a preferred taste (e.g., sucrose), the aversive responses elicited by the cue flavor decreased over time, while the ingestive responses increased.

Experiment 3 of the present study examined whether the robust preference rats

display for flavors associated with IG infusions of Polycose is accompanied by changes in the orofacial reactions to the flavors. The taste reactivity behaviors of rats conditioned to prefer the CS+ over the CS– were compared to that of control rats who had been given the same flavors paired with IG water infusions. The degree to which the conditioned rats displayed more ingestive FAPs and/or less aversive FAPs to the Polycose-paired flavor over the control rats provided an assessment of the acquired liking to the flavor.

In addition to the taste reactivity test, short-term (30 minutes/day) consumption tests were conducted in Experiment 3. The willingness of nondeprived rats to consume solutions in short-term tests has been taken as evidence that the rats find the solutions to be palatable (Sclafani, 1987a). Thus, if the conditioned rats consumed more of the CS+ solution during short-term tests than did the control rats, this would suggest that IG conditioning increased the palatability of the CS+ flavor.

GENERAL METHODS

Surgery

The rats were fitted with a gastric cannula using a procedure adapted from Kraly, Carty, and Smith (1978). The cannula consisted of a stainless steel cylinder (O.D., 8 mm, length, 14 mm) threaded on the inside to accept a stainless steel screw. One end of the cylinder was flanged (O.D. 16 mm); a slot (1 x 2 mm) in the flange was used to thread the cannula into the stomach.

The rats were anesthetized with Chloropent (3 ml/kg). A 3-cm incision was

made just lateral to the midline below the xiphisternum. The stomach was located and then lifted outside of the body cavity and was kept moist with isotonic saline. A small incision was made into the rumen of the stomach along the greater curvature. The cannula was inserted through the incision and fixed in place with a single purse-string suture which was tightened against the shaft of the cannula. A piece of Marlex mesh (Bard Inc., Billerica, MA) was slipped over the shaft of the cannula, pressed against the stomach, and fixed against the shaft with cranioplastic cement (Plastic Products, Inc., Roanoke, VA). The stomach was returned back to the body cavity, and the shaft of the cannula was passed through a stab wound in the abdominal wall and skin. The animal was then sutured and the wounds were treated with Bacitracin ointment (Henry Schein, Inc., Port Washington, NY). A stainless steel washer was placed over the shaft, pressed firmly against the skin, and was fixed in place by a small plastic tie. The plastic tie and washer were removed 48 hours after surgery. When not in use, the cannula was kept closed with a stainless steel screw.

Apparatus

The electronic esophagus infusion system, illustrated in Figure 1, consisted of 12 stainless steel cages similar to the rats' home cages (24.5 cm long x 18 cm wide x 18 cm high); the test cages were kept in an isolated room maintained on a 12:12 hour light-dark cycle. The cages were modified in that affixed to the front wall was a plexiglas plate with two 1.9-cm holes (5 cm apart) through which the rats could lick at stainless steel sipper tubes; the tubes were attached to 100-ml graduated

cylinders mounted on the outside of the cage. A slot (1 cm wide x 22.5 cm long) in the center of the cage floor allowed passage of the infusion tubes.

The rat was attached to the infusion system by replacing the screw in its gastric cannula with a stainless steel connector containing two 20-gauge stainless steel tubes. Attached to the tubes were two lengths (28 cm) of PE-90 tubing that were protected by a flexible stainless steel spring (3.7 mm OD x 20 cm long). The spring-covered tubing passed through slots in the cage floor and bedding pan located below the cage and was connected to the output ports of a dual-channel infusion swivel (Model No. 375/D/20, Instech Laboratories, Horsham, PA). The swivel was suspended below the bedding pan at one end of a counterbalanced lever mechanism. This system allowed the rats relatively unrestrained movement within their test cages.

The input ports of the swivel were connected via PE-90 tubing to larger Tygon tubing (ID=1.6 mm; Cole-Parmer Instrument Company, Chicago, IL). The Tygon tubing passed though peristaltic infusion pumps (pump drive: Model No. 7543-06; pump head: Model No.7021-20; Cole-Parmer) to draw fluid from 100-ml graduated cylinders. The infusion pumps were automatically operated by drinkometers whenever the rat drank from one of the two sipper tubes at the front of the cage; the pumps stayed on as long as the rat continued to lick faster than one lick/three seconds. Approximately 1.3 ml of fluid were infused intragastrically (IG) for each 1 ml of fluid orally consumed; the amount infused varied from 1 to 2 ml per 1 ml consumed as a function of the rat's licking pattern. With this system the rats were free to eat and drink normally. As they drank fluid from the sipper tube

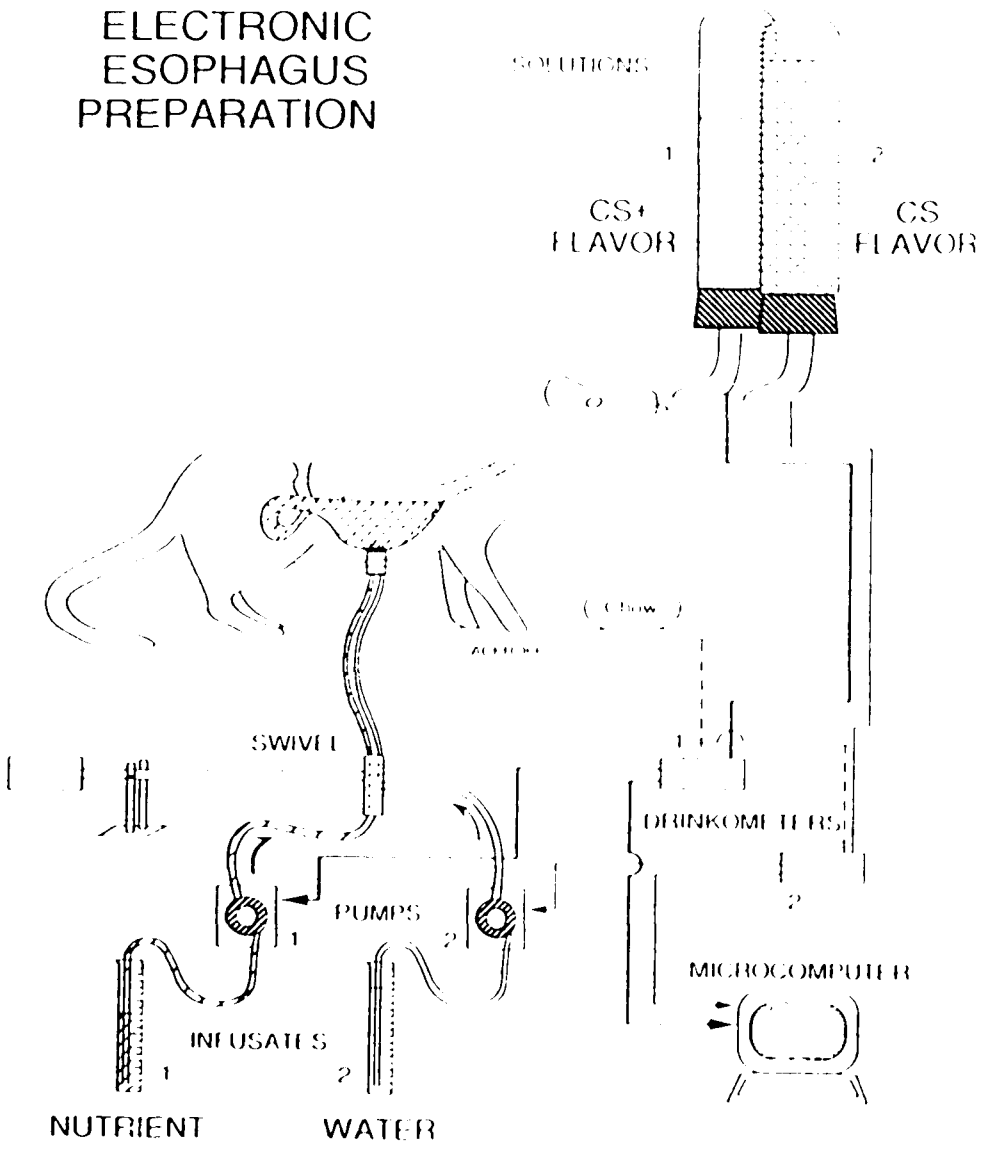

Figure 1. A schematic representation of the Electronic Esophagus Preparation. As the rat drinks flavored water from drinking bottles 1 or 2, a drinkometer activates pumps 1 or 2 which infuse a nutrient or water through a dual-channel swivel into the rat's stomach. A microcomputer records the number of licks emitted each minute. In the present study, consumption of the CS+ flavor was paired with infusions of a 32% Polycose solution and consumption of the CS– flavor was paired with water infusions. The rats had ad libitum access to Purina chow (except for Experiment 1E).

they received concurrent IG infusions which commenced with their first lick and ended three seconds after their last lick of a bout. When two sipper tubes were available, the rats could be infused IG with two different infusates (e.g., Polycose and water).

EXPERIMENT 1A: INITIAL CONDITIONING

In the first experiment the rats were adapted to the infusion system and were trained to associate the CS+ and CS– flavors with the IG Polycose and water infusions, respectively. The procedures used were similar to those employed in the study by Sclafani and Nissenbaum (1988) except that the rats were infused with a 32% Polycose rather than a 16% Polycose solution.

Subjects

The subjects were 12 adult female rats (CD strain, Charles River Breeding Lab, Wilmington, MA) weighing 245-302 g; an additional nine rats were used in a supplementary experiment (Experiment 1F). In order to facilitate the presentation and discussion of the results, Experiment 1 was divided into nine phases. There was no delay between each succeeding phase, except where noted (Experiment 1J). During the course of the nine phases of Experiment 1, which spanned 133 days, several of the initial 12 rats were discarded because of problems with their gastric cannulas. The number of rats included in each phase of Experiment 1 is specified in the individual Procedure sections.

The rats were individually housed in stainless steel cages kept in a vivarium maintained at 21°C and under a 12:12 hour light-dark cycle. Except where noted, the rats were given ad libitum access to Purina Lab Chow (#5001) and plain or flavored water.

CS Solutions

The conditioned stimuli (CS) consisted of water flavored with either 0.05% cherry or grape Kool-Aid® (unsweetened mix; General Foods Corp., White Plains, NY). These drink mixes contain citric acid and artificial flavors and colors that give them a flavor. For half of the rats cherry was the CS+ flavor and was paired with IG Polycose infusions, and grape was the CS– flavor and was paired with IG water infusions; the CS flavors were reversed for the remaining animals. A preliminary study with naive rats (n=8) revealed that the cherry and grape solutions were isohedonic as measured by two-bottle choice tests (cherry vs. grape: 24.2 vs. 25.0 ml/24 hours). Furthermore, the two solutions were equally unpreferred to plain water (cherry vs. water: 15.8 vs. 37.0 ml/24 hours, $t(7)=8.1$, $p<.001$, percent cherry intake = 29.9%; grape vs. water: 13.9 vs. 37.5 ml/24 hours, $t(7)=4.7$, $p<.01$, percent grape intake = 27.0%).

In Experiments 1C and 1D the rats were additionally tested with a saccharin solution (0.2% sodium saccharin; Sigma Chemical Company, St. Louis, MO) and a saccharin-sweetened CS+ solution.

Intragastric Infusions

The rats were infused with a 32% Polycose solution or water maintained at room temperature. Polycose® (Ross Laboratories, Columbus, OH) is a hydrolysate of corn starch that contains soluble polysaccharides and has a low sugar content (2% glucose, 5% maltose). The infused Polycose was diluted by the flavored water that the rats orally consumed such that the Polycose concentration in the stomach was approximately 18%.

Data Analysis

The primary data include the amount of fluid orally consumed each day during one-bottle training and two-bottle preference tests. The amount of a particular fluid consumed in the preference tests was also expressed as a percent of total intake. Fluid intakes were also subjected to a drinking pattern analysis in which daily bout number and bout size were calculated from the intake data and the licks/minute computer records. The minimum lick criterion for a bout was 10 licks, and the bout-termination criterion was 10 minutes without licking. Additional analyses were performed on the caloric intake data which included calories consumed as Purina Chow (3.3 kcal/g) and calories consumed as Polycose solution (1.2 kcal/ml).

In most cases the data were averaged over two-day periods and were analyzed using repeated-measures analysis of variance followed by simple main effects tests and Newman-Keuls tests, where appropriate. Data not involving multiple comparisons were analyzed with Student's t-tests.

Procedure

The rats were allowed several weeks to recover from surgery. The criterion used for recovery was the return of postoperative body weight and chow intake to preoperative levels. At the start of the experiment, they were placed in the test cages, connected to the infusion system, and given ad libitum access to chow and water. On the first day no infusions were given. On the following seven days the rats were infused IG with water as they drank water from the sipper tube; the last two days were taken as the water baseline period. The rats were then trained and tested over six-day cycles. On days 1 and 3 they were given one-bottle access to the CS+ solution paired with IG Polycose infusions, and on days 2 and 4 to the CS– solution paired with IG water infusions. The rats were then given a two-bottle preference test with the CS+ and CS– on days 5 and 6; intakes of the CS solutions remained paired with the appropriate infusions. The six-day cycle was then repeated. In these and subsequent tests, the right-left positions of the CS+ and CS– solutions were counterbalanced. The CS solutions and Purina chow were available ad libitum 23 hours/day; the animals and equipment were serviced during the remaining one hour/day. Oral fluid intakes and IG infusions were recorded to the nearest 0.5 ml; chow intakes were recorded to the nearest 0.1 g.

Results

CS intakes. The results of the one-bottle training and two-bottle preference tests are summarized in Figure 2. Overall, the rats did not differ in their one-bottle intakes of the CS+ and CS– solutions during training, but there was a CS x cycle

interaction [$F(1,11) = 24.7$, $p<.01$]. That is, the rats consumed marginally less CS+ than CS– in cycle 1 ($p<.06$) and reliably more ($p<.05$) CS+ than CS– in cycle 2. Their CS+ intake in cycle 2 was comparable to their water intake baseline (22.4 vs. 22.9 ml/day), whereas their CS– intake was less than their water baseline (16.3 vs. 22.9 ml/day, $p<.05$).

In the two-bottle preference tests, the rats consumed significantly more CS+ than CS– [$F(1,11) = 8.2$, $p<.05$], and this difference increased from cycle 1 to 2 [$F(1,11) = 20.5$, $p<.01$; see Figure 2]. The percent CS+ intakes were 77.6% in cycle 1 and 90.0% in cycle 2.

Drinking patterns. Analysis of the drinking patterns during the one-bottle training tests revealed that the rats did not differ in their CS+ and CS– bout number. Overall, the rats increased their CS bouts/day from cycle 1 to 2 [12.2 to 13.7 bouts/day, $F(1,11) = 19.2$, $p<.001$]. The CS+ and CS– bout number in cycle 2 was comparable to the bout number during the water baseline period (13.8, 13.7, 14.1 bouts/day, respectively). Mean bout size of the CS+ and CS– solutions did not differ in cycle 1 (1.5 and 1.4 ml/bout, respectively), but by cycle 2 CS+ bout size exceeded CS– bout size [1.7 vs. 1.2 ml/bout; CS x cycle interaction, $F(1,11) = 6.4$, $p<.05$]. The rats' CS+ bout size in cycle 2 was comparable to their bout size when drinking plain water (1.7 and 1.7 ml/bout, respectively), whereas their CS– bout size was smaller than their water bout size (1.2 vs. 1.7, $p<.05$).

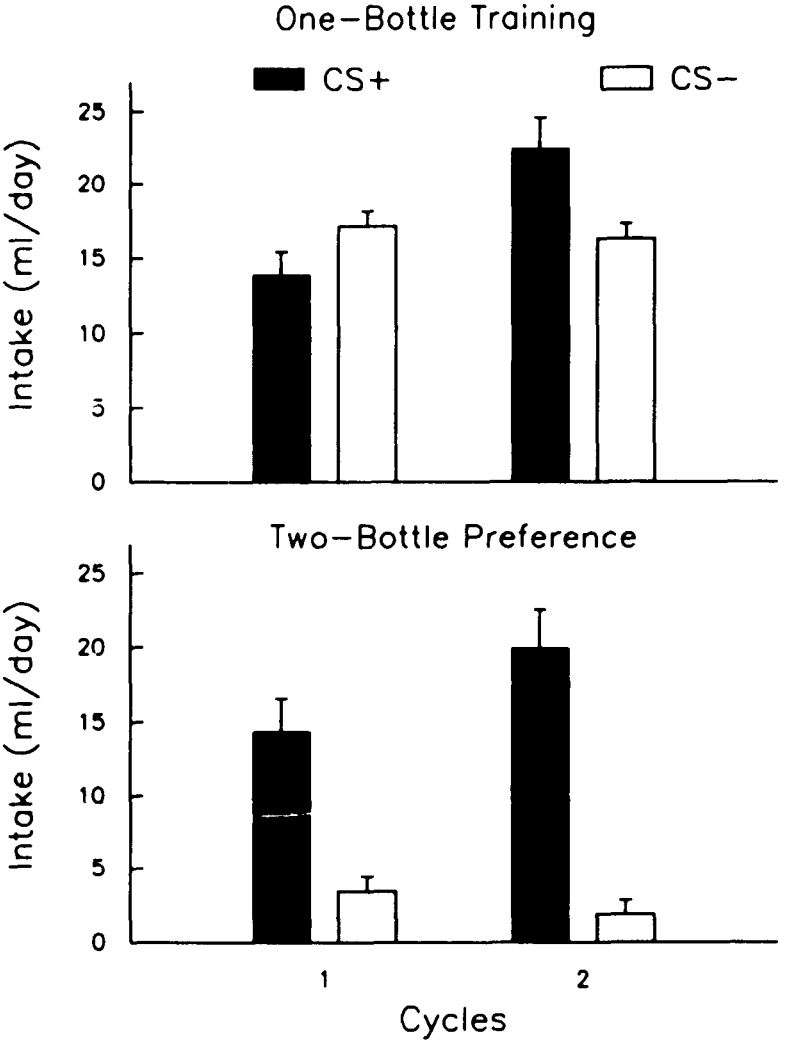

Figure 2. Experiment 1A: Mean (+SE) oral intake of CS+ and CS– during cycles 1 and 2 of the one-bottle training and two-bottle preference tests; each bar represents a mean of two days. The CS+ and CS– were cherry- or grape-flavored water and were paired with IG Polycose infusions and IG water infusions, respectively.

In the two-bottle preference test of cycle 2 the rats consumed more bouts of the CS+ than of the CS– [13.4 vs. 3.2 bouts/day, $t(11) = 5.6$, $p<.001$]. The mean bout size of the CS+ was also greater than that of the CS– [1.5 vs. 0.3 ml/bout, $t(11) = 4.9$, $p<.001$]. (The meal pattern data for the cycle 1 preference test were lost because of a computer malfunction.)

Caloric intakes. In the water baseline period the rats consumed 70.5 kcal/day in the form of chow. When infused with Polycose on CS+ training days the rats reduced their chow intake (cycle 1: 49.5 kcal/day; cycle 2: 44.4 kcal/day), but this decrease did not completely compensate for the calories provided by the IG Polycose (cycle 1: 26.4 kcal/day; cycle 2: 35.7 kcal/day). As a result, the rats' total caloric intakes on CS+ training days during cycles 1 and 2 exceeded their intake during the water baseline period (75.9 and 80.1 kcal/day vs. 70.5 kcal/day); these differences were marginally significant [$F(2,22) = 3.3$, $p<.06$]. The rats' caloric intakes on CS– training days (cycle 1: 62.6 kcal/day; cycle 2: 59.9 kcal/day) were less than their intakes on CS+ days [$F(1,11) = 34.8$, $p<.001$] as well as their intake during the water baseline [$F(2,22) = 5.1$, $p<.05$]. The reduced intake on CS– days compensated for the rats' elevated intake on the CS+ days. That is, the mean caloric intakes over the four CS+ and CS– days of cycles 1 and 2 (69.2 and 70.0 kcal/day) were similar to the rats baseline intake (70.5 kcal/day).

Discussion

These findings confirm and extend the recent report of Sclafani and Nissenbaum (1988) that IG Polycose infusions produce robust conditioned flavor

preferences in ad libitum fed rats. The conditioned flavor preference produced by the 32% Polycose infusions was acquired at a somewhat slower rate compared to that obtained in Sclafani and Nissenbaum's (1988) experiment with 16% Polycose infusions. That is, the rats reinforced with 16% Polycose displayed a 96% CS+ preference after one training cycle, whereas the rats reinforced with 32% Polycose displayed only 78% CS+ preferences after one training cycle and 90% preference after a second cycle; note that stronger preferences were obtained in subsequent experiments (see below). Concentrated Polycose solutions (32%) have also been found to be less effective than dilute solutions (8 or 16%) in conditioning flavor preferences using some, but not all, other conditioning paradigms (see Sclafani, 1990). It may be that concentrated Polycose solutions have both rewarding and mildly aversive postingestive effects and the aversive component may retard the acquisition of conditioned flavor preferences in some situations.

The increase in total caloric intake observed on CS+ training days also confirms previous results (Sclafani & Nissenbaum, 1988). On the CS- training days the rats decreased their caloric intakes to below that of their water baseline period. This reduction compensated for the caloric overconsumption on CS+ days. The rats' reduced food intake on CS– training days may explain why their one-bottle CS- intake was less than their one-bottle CS+ and water intakes.

EXPERIMENT 1B: CS VS. WATER PREFERENCE

Naive animals prefer plain water to the grape and cherry CS flavors used in this study (see CS Solutions above). In the present experiment, the rats were given preference tests with the CS solutions vs. water to determine whether their conditioning experience in Experiment 1A altered their preference for the CS flavors relative to water.

Procedure

The rats (n=12) were given a two-day, two-bottle preference test with the CS+ vs. water which was followed by a two-day, two-bottle test with the CS- vs. water. Intake of the CS+ was paired with IG Polycose infusions while intake of the CS- and water was paired with IG infusions of water.

Results

As illustrated in Figure 3, the rats consumed considerably more CS+ than water [$t(11) = 6.8$, $p<.01$]; their percent CS+ intake was 93.7%. In contrast, the rats consumed slightly, but not reliably more water than CS–; their percent CS- intake was 48.3%. The animals took more bouts of the CS+ than water [15.1 vs. 4.0 bouts/day, $t(11) = 7.5$, $p<.001$] and their bout size of the CS+ was greater than that of water [1.7 vs. 0.4 ml/bout; $t(11) = 6.0$, $p<.001$]. Neither bout number nor size reliably differed between CS– and water (8.5 vs. 11.2 bouts/day; 0.7 vs. 0.8 ml/bout).

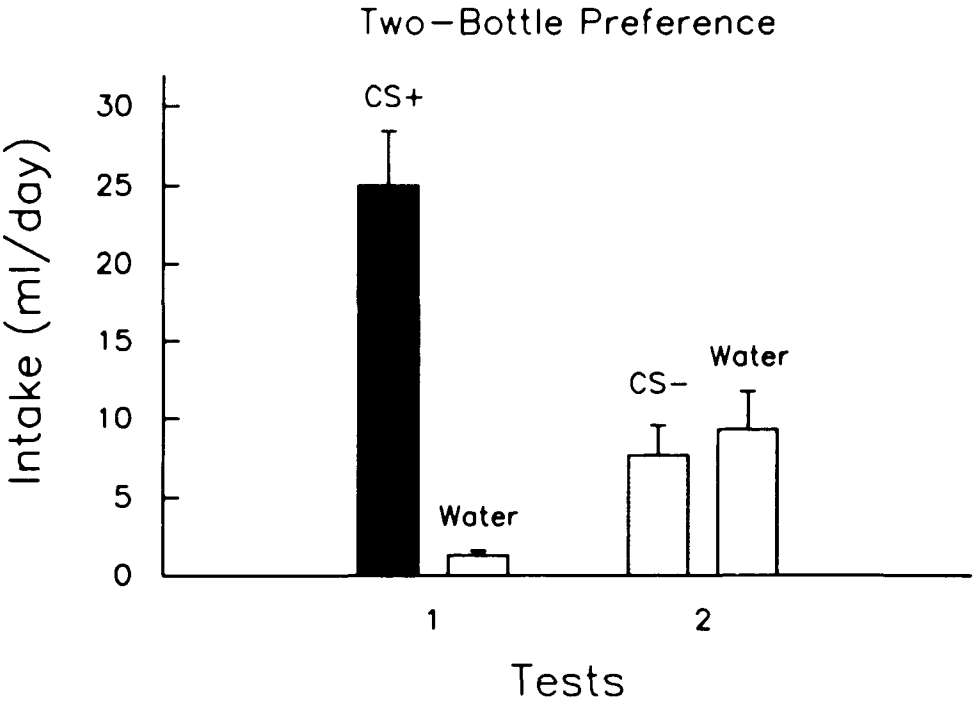

Figure 3. Experiment 1B: Mean (+SE) oral intake of CS+ and plain water, and of CS– and plain water during two-bottle preference tests; each bar represents a mean of two days. The CS+ was paired with IG Polycose infusions and the CS– and plain water were paired with IG water infusions.

Discussion

Taken together, the findings of Experiments 1A and 1B demonstrate that the rats not only preferred the CS+ to the CS– but also strongly preferred the CS+ to plain water. These results confirm previous findings (Sclafani & Nissenbaum, 1988) and suggest that the rats had acquired a true preference for the CS+ rather than an aversion for the CS– (see Rozin & Zellner, 1985). The 94% preference displayed by the rats for the CS+ over water is noteworthy given that naive rats prefer water to the grape and cherry flavors used as the CS+. Thus, IG infusions of Polycose converted a mild flavor aversion to a strong flavor preference.

The finding that the rats showed nearly equal preferences for the CS– solution and plain water was surprising in light of the mild aversion naive rats have for the flavors used in this study. This may have occurred because the exposure to the CS- reduced the animals' neophobia to the flavor or because the IG water infusions had a reinforcing effect. Alternatively, the rats' conditioned preference for the CS+ flavor may have partially generalized to the CS– flavor because the two flavors shared some common elements; e.g., both the grape and cherry flavor mixes contained citric acid. In support of this latter interpretation, preliminary findings indicate that when other flavor pairs are used as CSs (i.e., grape Kool-Aid and sucrose octaacetate), rats develop a strong preference for the CS+, but avoid the CS– in favor of water (Elizalde and Sclafani, unpublished observations; see also Experiment 3).

EXPERIMENT 1C: CS+ VS. SACCHARIN

In Experiment 1B the rats displayed a strong preference for the CS+ over water. The present experiment compared the rats' acquired preference for the CS+ with their innate preference for sweet taste. Therefore, the rats were given two-bottle tests between the CS+ and a saccharin solution rather than plain water.

Procedure

The rats (n=10) were first given a two-day, two-bottle test with the CS+ vs. plain water. They were then given a four-day, two-bottle test with the CS+ vs. a 0.2% saccharin solution; data analysis was based on the last two days of the test. The consumption of the CS+ was paired with IG Polycose infusions, whereas consumption of the saccharin solution was paired with IG water.

Results

CS intakes. As illustrated in Figure 4, when given the choice of the CS+ and water, the rats drank the CS+ and virtually no water [$t(9) = 10.1$, $p<.001$]; their percent CS+ intake was 96.9%. However, when given the choice of the CS+ and the saccharin solution the rats consumed more saccharin than CS+, [$t(9) = 4.8$, $p<.001$], and their CS+ intake was only 25.3% of their total intake. While the rats consumed a substantial amount of saccharin solution (73.8 ml/day plus 86.7 ml/day water infused IG), they still continued to drink the CS+. In particular, CS+ intakes were not reliably less when saccharin rather than water was the alternative fluid available (18.0 vs. 21.2 ml/day, ns).

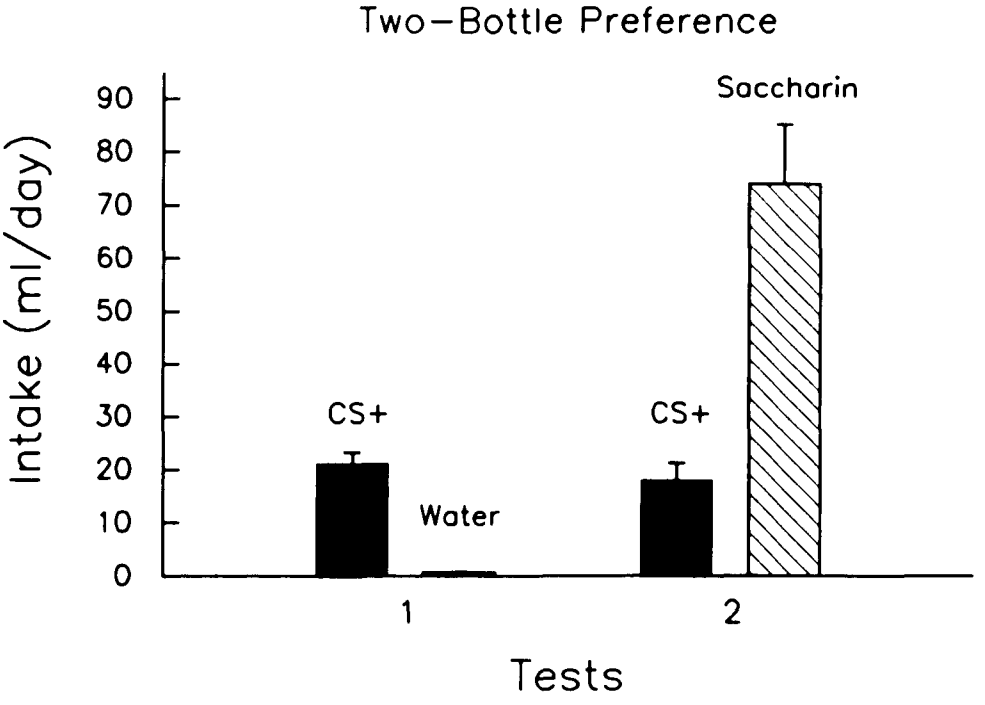

Figure 4. Experiment 1C: Mean (+SE) oral intake of CS+ and plain water, and of CS+ and a 0.2% saccharin solution during two-bottle preference tests; each bar represents a mean of two days. The CS+ was paired with IG Polycose infusions, and the plain water and the saccharin solution were paired with IG water infusions.

Drinking patterns. In the CS+ vs. water preference test, the rats took more and larger bouts of the CS+ solution than of plain water [15.2 vs. 1.6 bouts/day, $t(9) = 7.8$, $p<.001$; 1.5 vs. 0.1 ml/bout, $t(9) = 8.2$, $p<.001$]. In the subsequent CS+ vs. saccharin solution test, the animals consumed slightly more bouts and significantly larger bouts of the saccharin solution than of the CS+ solution [19.9 vs. 15.6 bouts/day, ns; 3.3 vs. 1.4 ml/bout, $t(9) = 4.29$, $p<.01$].

Discussion

Two new findings emerged from this experiment concerning the rats' conditioned preference for the CS+ flavor. First, although the rats strongly preferred the CS+ to the CS– and plain water, they drank considerably more of the saccharin solution than of the CS+ solution. Also, the rats consumed much larger bouts when drinking saccharin than when drinking the CS+. The interpretation of these results is complicated, however, by the fact that CS+ intake was paired with IG infusions of 32% Polycose whereas saccharin intake was paired with IG water infusions. It could be that caloric load provided by the Polycose infusions limited the CS+ bout size and total intake. The results of a subsequent experiment show that when CS+ intake was paired with IG infusions of water rather than Polycose, the rats did in fact increase their CS+ intake (see Experiment 1E). Nevertheless, the rats' CS+ intake under this condition was still considerably less than their saccharin intake in the present experiment (40.3 vs. 73.8 ml/day). Taken together, these findings suggest that the rats' acquired preference for the CS+ flavor was not as strong as their innate preference for the sweet taste of saccharin.

The other new finding is that, although saccharin was the preferred solution, the availability of the saccharin solution did not reliably suppress the rats' intake of the CS+. This suggests that the rats "recognized" that the CS+, unlike the saccharin solution, was a nutrient source. Note that rats fed chow and a 32% sucrose or Polycose solution will, when given access to a saccharin solution, consume substantial amounts of saccharin but do not reduce their sucrose or Polycose intake (Kenney & Collier, 1976; Sclafani, unpublished observations). Thus, rats do not treat saccharin as a carbohydrate substitute.

EXPERIMENT 1D: CS+ PLUS SACCHARIN

Experiment 1D further examined the influence of saccharin on CS+ solution intake. In this case, saccharin was added to the CS+ rather than being offered as an alternative solution. The question asked was would sweetening the CS+ with saccharin increase CS+ intake? This was of interest because, despite their strong conditioned preference for the CS+ solution, the rats did not overconsume the solution. That is, their CS+ intakes in the one-and two-bottle tests (14 - 25 ml/day) did not exceed their water intake baseline (23 ml/day). In contrast, when rats drink Polycose by mouth rather than "by stomach" their Polycose intake is substantially above their water baseline intake (Sclafani & Nissenbaum, 1987). It may be that direct infusions of Polycose into the stomach has an inhibitory effect that limits consumption; if so, the addition of saccharin to the CS+ solution would have little effect on CS+ intake. Alternatively, it may be that the initial palatability of the CS+

flavor was not sufficient to promote overconsumption. In this case, adding a sweet taste to the CS+ flavor might enhance CS+ intake.

Procedure

The rats (n=10) were first given a two-day, two-bottle test with the CS+ vs. water (Test 1). They were then given a four-day, two-bottle test with a CS+/0.2% saccharin solution vs. water (Test 2); data analysis was based on the last two days of this test. As part of the next experiment the rats were next given a two-day, two-bottle test with the CS+ vs. CS– (Test 3). The results of this latter test proved to be of interest to the present experiment and thus are presented here as well as in Experiment 1E. In all tests, the intake of the CS+ or CS+/saccharin solution was paired with IG Polycose infusions, while the intake of water or CS– was paired with IG water infusions.

Results

CS intakes. As illustrated in Figure 5, when saccharin was added to the CS+ solution (Test 2) the rats doubled their intake as compared to their CS+ intake in Test 1; the rats then decreased their CS+ intake when saccharin was removed from the solution (Test 3). Statistical analysis confirmed that the animals drank more CS+/saccharin in Test 2 than CS+ in Tests 1 and 3 [$F(2,18) = 34.2$, $p<.001$]. Water and CS– intakes were negligible (less than 0.7 ml/day). Percent CS+ and CS+/saccharin intakes in Tests 1 to 3 were 97.9%, 99.3%, and 97.9%, respectively.

Drinking patterns. The number of CS+ bouts consumed by the rats was not influenced by saccharin adulteration; the rats drank 18.9, 18.6 and 19.8 bouts/day,

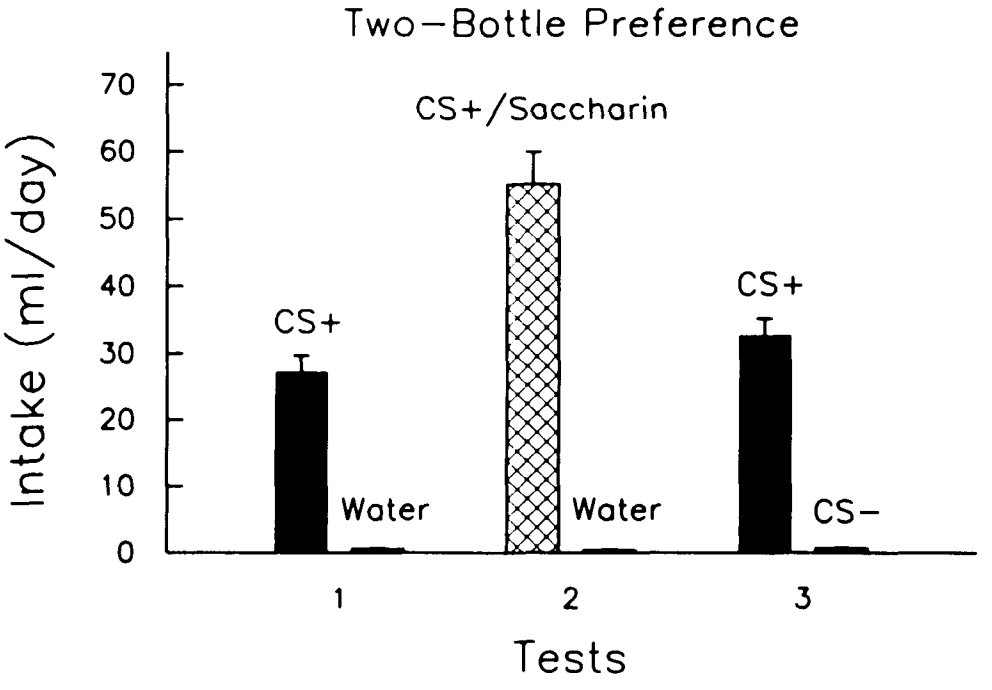

Figure 5. Experiment 1D: Mean (+SE) oral intake of CS+ and plain water, a CS+/Saccharin solution and plain water, and CS+ and CS- during two-bottle preference tests; each bar represents a mean of two days. The CS+ and the CS+/Saccharin solution were paired with IG Polycose infusions and the water and CS- were paired with IG water infusions.

respectively, in Tests 1 to 3. Bout size, however, was increased by adding saccharin to the CS+ solution [$F(2,18) = 38.1$, $p<.001$]. The rats' mean bout size with the CS+/saccharin solution was 3.2 ml/bout as compared to bout sizes of 1.5 ml/bout (Test 1) and 1.7 ml/bout (Test 3) with the CS+ solution.

Caloric intakes. The rats consumed more total calories when offered the saccharin-sweetened CS+ (96.9 kcal/day) than when given the plain CS+ in Tests 1 and 3 (81.6 and 85.6 kcal/day) [$F(2,18) = 8.7$, $p<.01$]. The enhanced caloric intake with the CS+/saccharin solution was due to an increase in IG Polycose intake which was not completely compensated for by a reduction in chow intake. In Test 2 the rats' intake of Polycose was 69.8 kcal/day compared to 45.4 and 51.2 kcal/day, respectively, in Tests 1 and 3 [$F(2,18) = 43.7$, $p<.001$]. The rats' chow intake in Test 2 was 27.0 kcal/day as compared to their intakes of 36.2 and 34.4 kcal/day, respectively, in Tests 1 and 3 [$F(2,18) = 5.8$, $p<.05$].

Discussion

These results demonstrate that the rats were capable of overdrinking the CS+ solution; sweetening the CS+ with saccharin produced a two-fold increase in CS+ intake. This increase was accomplished by a doubling of bout size; bout number remained unchanged. The addition of saccharin to the CS+ solution also significantly increased the rats' IG Polycose intake and total caloric intake. Note that the stimulatory effect of saccharin on CS+ intake was not dependent upon the rats' previous experience with the plain saccharin solution in Experiment 1C; similar

results have been obtained in rats that were not pre-exposed to a saccharin solution (Nissenbaum and Sclafani, unpublished observations; see also Experiment 2).

In view of these results, the fact that the rats did not overconsume the CS+, relative to their water baseline, in Experiments 1A to 1C cannot be attributed to a ceiling effect imposed by the IG Polycose infusions. Rather, it would appear that pairing the CS+ with IG Polycose infusions, while sufficient to produce a strong CS+ preference (compared to CS– or water), was not sufficient to increase the acceptance, i.e., absolute intake, of the CS+. This may be related to the fact that the particular flavors used (grape and cherry Kool-Aid) are moderately unpalatable to rats. Perhaps if neutral or slightly preferred flavors had been used, then pairing the CS+ with IG Polycose would have increased CS+ acceptance as well as preference. This possibility was addressed in Experiment 2.

The CS+ intakes in this experiment, while less than that of the CS+/saccharin solution, were greater than the CS+ intakes observed in the previous experiments. In particular, the rats consumed more CS+ in Test 1, which immediately followed the CS+ vs. saccharin test of Experiment 3, than they did in the CS+ vs. water test in the preceding experiment (27 vs. 21 ml/day). The rats further increased their CS+ intake to 33 ml/day following the CS+/saccharin test; this is 50% greater than their CS+ or water intakes in Experiment 1A. This increase in CS+ intake may have been due to the animals' repeated exposure to the CS+ solution and IG Polycose during the course of the first four experiments. Alternatively, the rats may have increased their CS+ intake as a result of the pairing of the CS+ flavor with the sweet taste of

saccharin. This latter interpretation is supported by results obtained with other rats that had been conditioned to prefer CS+ flavors (Elizalde & Sclafani, unpublished observations). One group of rats (n=6) showed a 47% increase in CS+ intake after the animals had been offered a CS+/saccharin solution for six days. A second group (n=6) was offered only the unsweetened CS+ during this period and showed no increase in CS+ intake. These findings suggest that associating the CS+ flavor with the sweet taste of saccharin enhanced its palatability. Previous studies have also observed "flavor-flavor" conditioning in animals offered a CS flavor in a saccharin solution (Fanselow & Birk, 1982; Holman, 1975; Rozin & Zellner, 1985). It may be that the combined effects of the flavor-nutrient conditioning with IG Polycose and the flavor-flavor conditioning with saccharin increased the palatability of the CS+ to a level that promoted increased acceptance of the CS+.

EXPERIMENT 1E: EXTINCTION TEST

In the preceding experiments the rats consistently displayed strong preferences for the CS+ over the CS– or plain water. In these tests the consumption of the CS+ was paired with IG Polycose infusions. The present experiment determined if the rats would continue to prefer the CS+ when it was no longer reinforced with IG Polycose. That is, the rats were given an extinction test in which both the CS+ and CS- were paired with IG water infusions. The rats were tested under both ad libitum and limited access conditions; the rationale for the limited access condition is presented below.

Procedure

The rats (n=10) were first given a two-day, two-bottle preference test with the intakes of the CS+ and CS– paired with IG Polycose and water infusions, respectively. They were then given a 14-day extinction test during which the intakes of the CS+ and CS– solutions were both paired with IG water infusions. During the first eight days (phase 1) the rats had ad libitum access to the CS solutions and chow. They were then given limited access to the CS solutions and chow for four days (phase 2) followed by two more days of ad libitum access (phase 3). On the limited access days the rats were given chow and water (which was paired with IG water infusions) for two hours at midday, no food or fluid for two hours, and then two-bottle access to the CS+ and CS– solutions for 20 hours in the absence of chow.

Results

CS intakes. As illustrated in Figure 6, prior to the extinction test the rats consumed 32.7 ml/day of the CS+ and virtually none of the CS– (0.6 ml/day); their percent CS+ intake was 97.9%. The rats continued to consume almost all their fluid as CS+ during the first eight days of extinction (phase 1). In fact, the rats initially increased their CS+ intake to 41.7 ml/day on day 2 of extinction, which reliably exceeded their pre-extinction intake of 32.7 ml/day [$t(9) = 5.1$, $p<.01$]. They then reduced their CS+ intakes over the next six days to 32.4 ml/day, which was comparable to their pre-extinction intake. During this time CS– intakes remained unchanged and were less than 1 ml/day. Analysis of the eight-day extinction period confirmed that the rats consumed more CS+ than CS– [$F(1,63) = 152.6$, $p<.001$], and

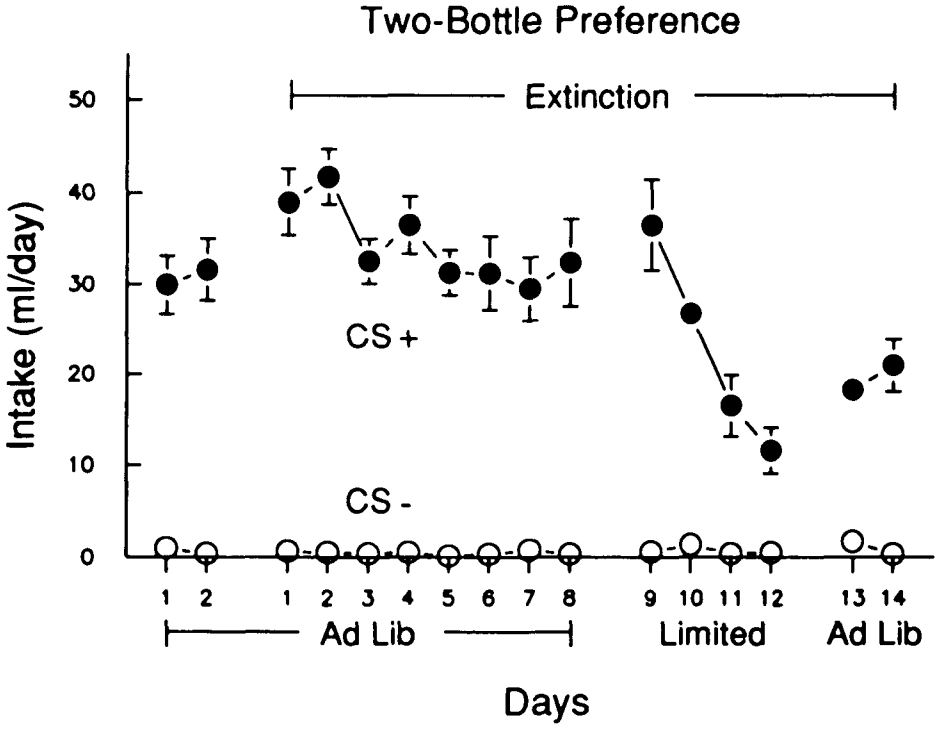

Figure 6. Experiment 1E: Mean (±SE) oral intake of CS+ and CS– during two-bottle reinforced tests (days 1 and 2) and extinction tests (days 1-14). During the reinforced tests the CS+ was paired with IG Polycose infusions and the CS– was paired with IG water infusions; during the extinction tests both the CS+ and CS– were paired with IG water infusions. During days 1-8 (phase 1) and days 13-14 (phase 3) of the extinction test the rats had ad libitum access to the CS solutions and chow. During days 9-12 (phase 2) the rats were given limited access to chow and water for two hours and, following a two-hour delay, to the CS solutions for 20 hours each day. Note that some error bars are hidden by the symbol.

that their CS+ but not their CS– intakes changed over days, CS x days interaction [$F(7,63) = 3.8$, $p<.01$]. The percent CS+ intakes during this phase ranged from 98.6% (day 1) to 98.9% (day 8).

In the second phase of the extinction test, when access to the CS solutions and chow was limited, the rats continued to drink more CS+ than CS– [$F(3,27) = 19.5$, $p<.001$; see Figure 6]. However, their CS+ intakes decreased ($p<.001$) over the four-day period, from 36.4 to 11.6 ml/day, while their CS– intakes showed small, but reliable ($p<.001$) fluctuations, from 0.5 to 1.3 ml/day, CS x days interaction [$F(3,27) = 18.2$, $p<.001$]. As a result of these changes, percent CS+ intake declined ($p<.01$) from 98.0% (day 9) to 91.0% (day 12). Note that although the rats consumed water during the two-hour/day feeding period, their oral water intake (5.8 ml/day) did not compensate for the 20.8 ml decline in CS+ intake on the fourth limited access day. (Phase 2 was terminated after four days because the animals were eating relatively little chow during the two-hour/day feeding period.)

When ad libitum access to the CS solutions and chow was restored in phase 3 the rats increased their CS+ intake to 19.6 ml/day, and their percent CS+ intake to 95.1%. The rats' percent CS+ intake did not reliably differ from their percent intake prior to the limited access phase (days 13-14 vs. days 7-8: 95.1% vs. 97.9%, ns) but their absolute CS+ intake in phase 3 remained below their intake in phase 1 [days 13-14 vs. days 7-8: 19.6 vs. 30.9, ml/day, $t(9) = 9.6$, $p<.001$].

Drinking patterns. Prior to extinction the rats took 19.2 bouts/day of the CS+ with a mean bout size of 1.7 ml/bout; their CS– bout number and size were

significantly smaller at 2.8 bouts/day and 0.2 ml/bout [$t(9) = 16.3, 10.8, p<.001$, respectively]. During the 14-day extinction test, the rats continued to drink only a few, small bouts of the CS– and therefore only changes in CS+ drinking patterns are described here. During the first eight days of extinction (phase 1) CS+ bouts/day increased from 22.7 (day 1) to 25.5 (day 2) and then decreased to 20.3 (day 8) [$F(7,63) = 3.6, p<.01$]. CS+ bout size did not change reliably during this period and ranged from 1.8 (day 1) to 1.6 ml/bout (day 8).

During the limited access phase, the rats reduced their CS+ bouts/day from 20.4 (day 9) to 14.1 (day 12) [$F(3,27) = 7.3, p<.001$]. CS+ bout size also decreased during this phase, from 1.9 ml (day 9) to 0.8 ml (day 12) [$F(3, 27) = 9.7, p<.001$]. When ad libitum access was restored in phase 3, the rats increased their CS+ bout size from 0.8 ml (day 12) to 1.5 ml (day 14) [$t(9) = 3.8, p<.01$]. They did not, however, increase their bout number, 14.1 (day 12) vs. 14.2 (day 14).

Caloric intakes. The rats consumed 85.6 kcal/day in the form of chow (34.4 kcal/day) and IG Polycose (51.2 kcal/day) in the two days preceding the extinction test. During the extinction test, calories were only available from chow since CS+ intake was paired with IG water. Over the first eight days of extinction (phase 1) the rats increased their chow intake from 46.6 kcal/day (day 1) to 70.1 kcal/day [$F(7,63) = 6.8, p<.001$]. During the limited-access phase, chow intake was reduced to 23.5 kcal/day on day 9 and increased only slightly to 28.2 kcal/day by day 12. When ad libitum access was restored in phase 3, chow intake rose to 77.6 kcal/day (day 14).

Discussion

These results demonstrate that the rats' preference for the CS+ persisted when the CS+ was paired with water rather than Polycose infusions. Over the two-week extinction test the rats displayed a near-total preference for the CS+ flavor over the CS– flavor. Although the animals reduced their absolute and percent CS+ intakes when access to the solutions and chow was limited in phase 2, these reductions do not appear to represent an extinction of the CS+ preference. That is, the rats continued to take most of their fluid from the CS+ solution; their CS– intake increased less than 1 ml/day during phase 2. Also, when ad libitum access was restored in phase 3, the rats' CS+ preference increased to 95.1% although CS+ intake was still paired with IG water infusions. Food restriction is known to reduce fluid intake in rats and thus the reduced CS+ intake observed in phase 2 may have been a secondary consequence of the rats' reduced food intake (secondary hypodipsia). This interpretation was evaluated in a supplementary experiment (see Experiment 1F).

The purpose of the limited-access phase was to determine if the failure of the rats to extinguish their CS+ preference was due to their associating the CS+ with the calories provided by the chow. Since rats are meal-associated drinkers, the rats presumably drank the CS+ solution along with their chow meals in phase 1. In phase 2, CS+ intake was disassociated from the chow meals, and yet the animals continued to display a strong preference (>90%) for the CS+. It is unlikely,

therefore, that the postingestive feedback provided by the chow was responsible for maintaining the CS+ preference during the extinction test.

At the onset of the extinction test, the rats did not immediately and fully compensate for the loss of Polycose calories by increasing their chow intake. This does not necessarily mean that the animals were insensitive to the change in the IG infusions. Rats that are returned to a chow-only diet after being fed chow and a 32% Polycose solution ad libitum will undereat the chow for several days as they recover their normal, i.e., pre-Polycose, body weight (Sclafani & Xenakis, 1984).

EXPERIMENT 1F: CS+ INTAKE AND FOOD RESTRICTION

The reduced CS+ intake observed during the limited-access phase of the preceding experiment may have been secondary to the rats' reduced food intake (secondary hypodipsia). Alternatively, the reduced CS+ intake may represent a partial extinction of the rats' conditioned response to the CS+. In an attempt to distinguish between these two alternatives, the present experiment measured the fluid intake of naive rats exposed to the same flavored solutions and feeding schedule used in Experiment 1E. The naive rats were not conditioned to prefer the flavored solutions. Thus, if the naive rats showed a decline in solution intake during food restriction that paralleled that displayed by the conditioned rats, this would indicate that the reduced CS+ intake of the conditioned rats was due to their limited food intake rather than to extinction.

Procedure

The naive rats (n=9) were similar in sex and strain to the conditioned rats but had no prior experience with the CS solutions, Polycose, or IG infusions; the rats were not implanted with gastric cannulas. At the start of the experiment they were given 23 hours/day, one-bottle access to cherry-flavored (n=4) or grape-flavored (n=5) water (CS solution) and chow. After four days of ad libitum access (phase 1), the rats were given limited access to the CS solution and chow for four days (phase 2), followed by ad libitum access for two additional days (phase 3). During each day of phase 2, the rats were given chow and water for two hours, no food or fluid for two hours, and the CS for 20 hours.

The fluid intake of the naive rats was compared to that of the conditioned rats from Experiment 1E. However, since the naive rats were not infused with IG water, their oral fluid intake exceeded that of the conditioned rats. Therefore, the CS intake of the naive rats was compared to the conditioned rats' total fluid intake derived from CS+ solution, i.e., oral CS+ intake plus IG water infusion (referred here as tCS+ intake).

Results

As illustrated in Figure 7, the naive rats showed an immediate and substantial (68%) decline in their CS intake on the first day of the limited access phase. They maintained their CS intake at this low level during the remaining three days of phase 2. The rats then increased their CS intake to a level slightly above their pre-restriction level when ad libitum access was restored in phase 3.

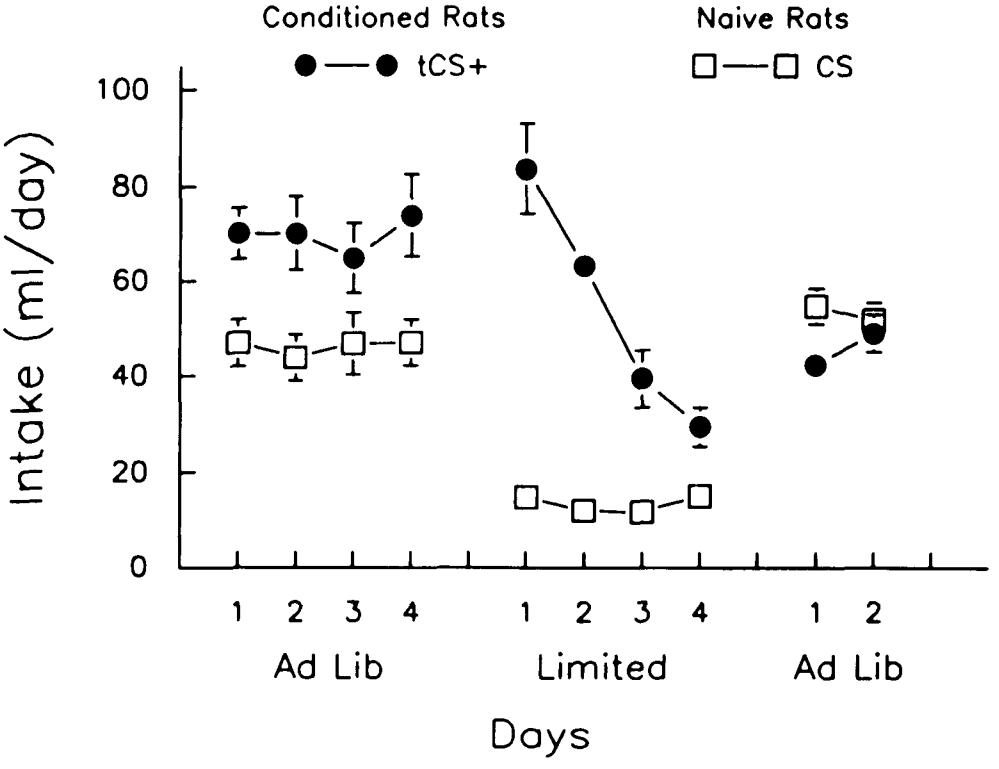

Figure 7. Experiment 1F: Mean (±SE) intake of tCS+ by the Conditioned Rats and CS by the Naive Rats during ad libitum and limited access conditions. tCS+ represents oral intake of CS+ (cherry- or grape-flavored water) plus IG water infusion. CS represents oral intake of cherry- or grape-flavored water; the naive rats were not infused IG. CS+ or CS and chow were freely available during ad libitum days. During limited access days chow and water were available two hours and, following a two-hour delay, the CS+ or CS were available 20 hr/day. Note that some error bars are hidden by the symbol.

In contrast to the naive rats, the conditioned rats showed a small increase (9 ml) in their tCS+ intake on the first day of limited access which was followed by a gradual decline in their fluid intake to a level comparable to that of the naive rats. The two groups also differed in that the conditioned rats did not fully recover their fluid intake following phase 2. As a consequence, whereas the conditioned rats consumed more fluid than the naive rats in phase 1, the intakes of the two groups were comparable in phase 3.

Statistical analysis indicated a significant group x days interaction [$F(9,153) = 15.2$, $p<.001$]. Individual comparisons revealed that the fluid intakes of the conditioned rats exceeded ($p<.05$) that of the naive rats during phase 1 and the first three days of phase 2; the intakes of the two groups did not differ on the last day of phase 2 and during phase 3. The conditioned and naive rats also did not reliably differ in their chow and water intakes during the two-hour/day feeding period of phase 2 (chow: 24.1 vs. 27.9 kcal/2 hours; water: 13.4 vs. 11.6 ml/2 hours, respectively).

Discussion

The finding that the naive rats reduced their fluid intake as much or more than the conditioned rats when access to food and CS solution was limited demonstrates that the hypodipsia response was not dependent upon prior flavor preference conditioning. The naive rats' suppressed CS intake can be attributed to their reduced food intake and lack of prandial drinking, and these factors presumably contributed to the hypodipsia displayed by the conditioned rats. On the other hand, the fact that

the naive rats displayed an immediate decline in fluid intake in phase 2 whereas the conditioned rats showed a gradual decline suggests that an extinction process contributed to the conditioned rats' hypodipsia response. The conditioned and naive rats also differed in that prior to the limited access phase the conditioned rats consumed more fluid and, unlike the naive rats, did not recover their pre-restriction intake following phase 2.

These latter findings suggest that the gradual reduction in CS+ intake displayed by the conditioned rats during phase 2 represents an extinction of their conditioned acceptance of the CS+. Recall that the rats increased their acceptance of the CS+ after it had been paired with saccharin in Experiment 1C and 1D. In the extinction test of Experiment 1E, the rats initially responded to the CS+ the way rats respond to saccharin solutions; that is, their intake was elevated compared to the naive rats, and they tended to increase rather than decrease their CS+ intake on the first day of food restriction. The rats' subsequent decrease in CS+ intake may represent an extinction of their conditioned acceptance response as they associated the CS+ flavor with an increasing state of deprivation. This would explain why, following the limited access phase, the rats' CS+ intake did not return to the elevated pre-restriction level, but rather to a level similar to that of Experiments 1A to 1C (and to that of the naive rats). According to this analysis, during the limited access phase the rats extinguished their conditioned *acceptance* but not their conditioned *preference* response to the CS+. It should be noted that the resistance to extinction displayed by the conditioned rats in Experiment 1E was not dependent upon their

previous experience with the CS+/saccharin solution; similar results have been obtained in rats that had no prior exposure to saccharin (Sclafani and Nissenbaum, 1988).

EXPERIMENT 1G: CS+ VS. POLYCOSE

The strong and persistent flavor preferences produced by the IG Polycose infusions indicate that the postingestive effects of Polycose are reinforcing to rats. Rats are also very attracted to the taste of Polycose, i.e., to the taste of starch-derived polysaccharides contained in Polycose (Sclafani, 1987). This polysaccharide taste preference, like the rat's sweet preference, appears to have an innate origin since Polycose elicits ingestive responses in neonatal rats with no prior experience with polysaccharides (Vigorito & Sclafani, 1988). The present experiment determined how the flavor preference conditioned by the postingestive effects of Polycose compares with the rats' unconditioned preference for the taste of Polycose. This was accomplished by giving the rats a two-bottle test with the CS+ solution vs. a Polycose solution.

Procedure

Following the extinction test of Experiment 1E, the rats (n=9) were given ad libitum access to the CS+ paired with IG Polycose infusions for four days (one-bottle test); chow was also available ad libitum. They were then given a two-day, two-bottle test with the CS+ solution and a 32% Polycose solution. Consumption of the CS+ remained paired with IG Polycose (32%) infusions while the consumption of

the 32% Polycose solution was paired with IG water infusions (this was done to equate for the infusions associated with the CS+).

Results

CS+ and Polycose Intakes. As illustrated in Figure 8, in the two-bottle test the rats drank a considerable amount of Polycose and very little CS+ [$t(8) = 28.2$, $p<.001$]; their Polycose intake represented 96.8% of their total intake. Furthermore, the rats consumed nearly twice as much Polycose in the two-bottle test as they drank of the CS+ in the preceding one-bottle test [$t(8) = 9.8.$, $p<.001$; see Figure 7].

Drinking patterns. During the two-bottle test the rats consumed more and larger bouts of Polycose than of the CS+ solution [11.2 vs. 1.3 bouts/day, $t(8) = 10.3$, $p<.001$; 3.8 vs. 0.5 ml/bout, $t(8) = 10.5$, $p<.001$]. Compared to their drinking pattern during the one-bottle CS+ test, the rats consumed fewer, but larger bouts of Polycose than of the CS+ [11.2 vs. 15.3 bouts/day, $t(8) = 4.40$, $p<.01$; 3.8 vs. 1.5 ml/bout, $t(8) = 8.54$, $p<.001$].

Caloric intakes. The rats consumed more total calories in the two-bottle Polycose vs. CS+ test than they did during the one-bottle CS+ test [93.8 vs. 82.5 kcal/day, $t(8) = 4.8$, $p<.01$]. This difference was due to their increased Polycose intake. In the two-bottle test the rats' oral Polycose intake was 53.3 kcal/day whereas in the one-bottle CS+ test their IG Polycose intake was 32.0 kcal/day [$t(8) = 7.9$, $p<.001$]. The rats consumed less chow in the two-bottle test than in the one-bottle test [40.7 vs. 50.5 kcal/day, $t(8) = 3.9$, $p<.01$] but this did not compensate for their increased Polycose intake.

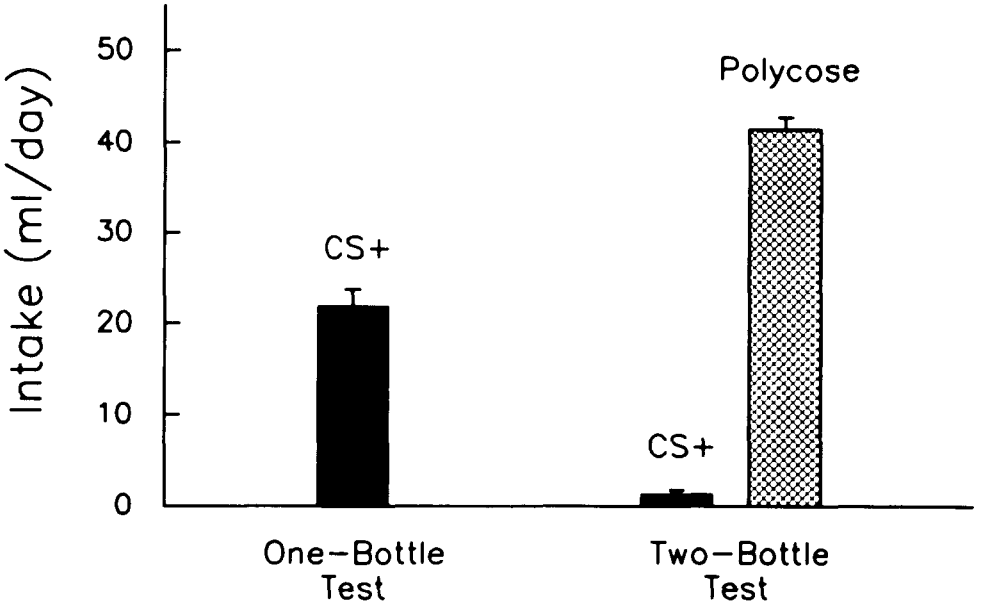

Figure 8. Experiment 1G: Mean (+SE) oral intakes of CS+ during one-bottle test and CS+ and 32% Polycose during two-bottle preference tests. The CS+ was paired with IG Polycose and the Polycose solution was paired with IG water infusions.

Discussion

The rats not only preferred the Polycose solution to the CS+, but they also consumed more of the Polycose than they did of the CS+ in the one-bottle test. These findings indicate that the rats' preference for the CS+ flavor was not nearly as strong as their preference for the taste of Polycose. Since the rats had not orally consumed Polycose prior to this experiment, their immediate and near-total preference for the Polycose solution can be attributed to their innate preference for the taste of starch-derived polysaccharides. This innate preference was presumably enhanced during the test as the rats associated the taste of Polycose with its postingestive effects. It is unlikely, however, that postingestive factors were an important determinant of the Polycose preference observed in the present experiment since the nutritional benefits of the Polycose solution and CS+ (paired with IG Polycose) were similar. It is possible, though, that the taste of Polycose triggered cephalic phase postingestive events which influenced the preference for the polysaccharide solution.

The rats not only preferred the Polycose solution to the CS+, but they also consumed more of the Polycose than they did of the CS+ in the one-bottle test. The rats also consumed more total calories when Polycose rather than the CS+ was available to drink. Similar effects were observed when the rats were offered the CS+/saccharin solution in Experiment 1D. With both the Polycose solution and the CS+/saccharin solution the rats' increased fluid intake was accomplished by an increase in bout size; bout number was not increased or, in the case of Polycose, was

actually decreased. These latter results are consistent with previous findings that changes in diet taste and palatability influence total intake primarily by altering meal size rather than meal number (LeMagnen, 1971; Sclafani & Berner, 1976).

EXPERIMENT 1H: CS+/+ VS. CS+/– TEST

The strength of the conditioned flavor preference observed in the preceding experiments suggests that the postingestive feedback produced by IG Polycose infusions is very salient. The present experiment investigated the ability of the rats to respond to this viscerosensory feedback in the absence of unique flavor cues. Specifically, the rats were given a two-bottle test with two identical CS+ solutions, except that one bottle was paired with IG Polycose infusions and the other with IG water infusions. The question asked was would the rats select the bottle that provided the IG Polycose infusions?

Procedure

The rats (n=7) were first given ad libitum access to a single bottle of the CS+ solution paired with IG Polycose for three days. They were then given a four-day, two-bottle test with each bottle containing the CS+ solution. Intake from one bottle (designated the CS+/+ bottle) was paired with IG Polycose, while intake from the other bottle (designated the CS+/– bottle) was paired with IG water.

To determine if the rats were actually tracking the IG Polycose infusions or if they had found some other orosensory cue with which to associate the IG infusions, the designation of the bottles was reversed during the next two days. That

is, the bottle that had been the CS+/+ became the rCS+/– and was now paired with IG water infusions; the bottle that had been the CS+/– became the rCS+/+ and was now paired with IG Polycose infusions. If the rat were able to track the postingestive feedback produced by the IG Polycose infusions, then the rat should switch to the "reinforced" bottle/sipper tube. Note that up to this point in the study each rat had received the CS+ in the same bottle with the same sipper tube; similarly, the CS– was always presented in the same bottle. At the start of the two-bottle test the CS+ bottle became the CS+/+ bottle, and the CS- bottle became the CS+/– bottle. The left-right position of two bottles was alternated daily.

Results

CS intakes. On day 1 when the rats were offered the two bottles containing the CS+ solution, they drank similar amounts from the CS+/+ and CS+/– bottles (Figure 9); their percent CS+/+ intake was 55.3% of their total intake. By the second day of the test, however, the rats displayed a strong preference for the CS+/+ bottle which persisted for the next two days; their percent CS+/+ intakes on days 2-4 were 88.7%, 91.7%, and 90.8%, respectively. Statistical analysis confirmed that the rats consumed more CS+/+ than CS+/– on days 2 to 4, but not on day 1 [$F(3,18) = 13.3$, $p<.001$].

On day 5 of the test, when the designation of the bottles was reversed, the rats did not switch their preference to the rCS+/+ bottle but rather drank almost exclusively from the rCS+/– bottle, i.e., the original CS+/+ bottle. The rats' rCS+/– intakes on days 5 and 6 exceeded their rCS+/+ intakes [$F(1,6) = 50.0$, $p<.001$].

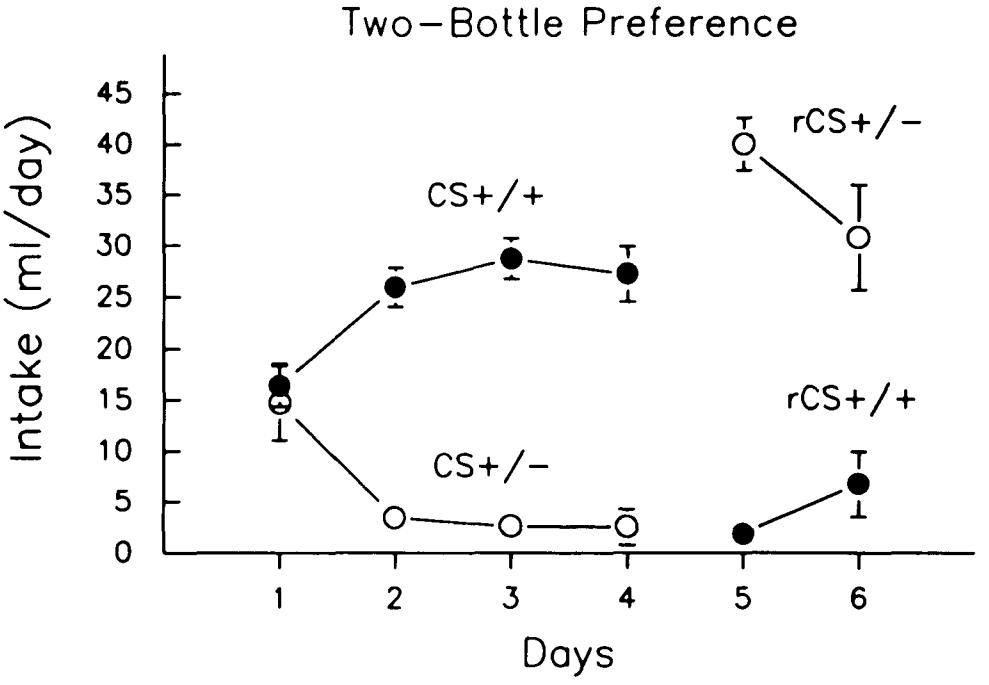

Figure 9: Experiment 1H: Mean (±SE) oral intake from the CS+/+ and CS+/– during two-bottle preference tests. The two bottles contained CS+ flavored water but intake from the CS+/+ bottle was paired with IG Polycose infusions whereas intake from the CS+/– bottle was paired with IG water infusions during days 1-4. During days 5-6 the reinforcement contingencies were reversed and intake from the rCS+/+ bottle (formerly the CS+/– bottle) was paired with IG Polycose, and intake from the rCS+/– (formerly the CS+/+) bottle was paired with IG water. Note that some error bars are hidden by the symbol.

Percent rCS+/– intakes were 93.6% and 79.6%, respectively, on days 5 and 6. The lower percent intake on day 6 was due to the fact that two of the seven rats drank slightly more rCS+/+ than rCS+/– on that day.

Drinking patterns. Analysis of the drinking pattern data revealed significant interactions between bout number x days [$F(4,24) = 29.2$, $p<.001$], and bout size x days [$F(4,24) = 17.4$, $p<.001$]; these analyses were based on days 1-3 and 5-6; the data from day 4 data were lost due to a computer malfunction. On the first day of the test the rats' CS+/+ and CS+/– bout numbers and sizes were similar (12.6 vs. 11.6 bouts/day, 1.3 vs. 1.3 ml/bout). On days 2 and 3 they took more frequent and larger bouts from the CS+/+ bottle than from the CS+/– bottle (2-day means: 18.8 vs. 4.1 bouts/day; 1.6 vs. 0.6 ml/bout). With the IG infusion conditions reversed on days 5 and 6, the rats consumed more and larger bouts from the rCS+/– bottle (i.e., the original CS+/+ bottle) than from the rCS+/+ bottle (2-day means: 20.6 vs. 4.8 bouts/day; 1.7 vs. 0.6 ml/bout).

Discussion

The present findings demonstrate that, in the absence of unique flavor cues, the rats developed a preference for the bottle that was paired with IG Polycose infusions over the bottle that was paired with IG water infusions. The lack of preference on day 1 of the test indicates that the rats did not immediately recognize the postingestive effects of the IG Polycose infusions. Conceivably, the rats learned to ignore the orosensory cues (e.g., taste, odor, or texture) provided by the CS bottles and to attend to viscerosensory cues provided by the IG Polycose infusions. The

results of the reversal test, however, refute this interpretation. That is, when the reinforcement contingencies were reversed, the rats "tracked" the drinking bottle rather than the IG Polycose infusions. It would seem that, without flavor cues to guide them, the rats learned to associate the IG Polycose infusions with other cues (e.g., tactile cues) provided by the drinking bottles. For example, the rats may have learned to distinguish the somatosensory "feel" of the sipper tubes on the CS+/+ and CS+/– bottles (see Nachman, Rauschenberger, & Ashe, 1977). Note though that there was, to the human eye, no obvious difference in the sipper tubes and they were all from the same supplier.

In light of the presence of a somatosensory cue, further research is needed to determine whether rats can directly monitor the viscerosensory feedback provided by the IG Polycose infusions. The present experiment failed to remove all somatosensory cues when the rats were tested with the CS++ and CS+– solutions; the rats found a cue -- the sipper tube -- for tracking the CS++. In order to correctly address this issue, sipper tubes and bottles must not be associated with a particular solution. Previous intragastric infusion studies (Borer, 1968; Holman, 1969) reported that rats have difficulty directly monitoring the viscerosensory feedback produced by the infusions. For example, in one study rats that had been trained to bar press for sucrose and water in a two-choice test displayed a strong sucrose preference when the sugar solution and water were delivered both to the mouth and to the stomach, but lost this preference when the fluids were delivered only to the stomach (Borer, 1968). Taken together, these findings indicate that the viscerosensory cues produced

by nutrients in the gut influence ingestive behavior primarily, if not exclusively, by modulating the animals' response to orosensory stimuli.

EXPERIMENT 1I: HOME CAGE EXTINCTION TEST

In Experiment 1E the rats continued to prefer the CS+ flavor to the CS– flavor during a two-week extinction test in which both flavors were paired with IG water infusions. The present experiment further investigated the duration of the rats' preference for the CS+ flavor in the absence of reinforcement. In this case the rats were tested without IG infusions and in a different environment, i.e., their home cages.

Procedure

The rats (n=7) were first "retrained" with the CS+ and CS– solutions in the test cages. The animals were given one-bottle access to the CS+ paired with IG Polycose infusions for one day, and one-bottle access to the CS– solution paired with IG water infusions for a second day. They were next given a four-day, two-bottle test with the CS+ and CS- solutions paired with the appropriate IG infusions; data analysis was based on the last two days of this test. The rats were then disconnected from the electronic esophagus apparatus and were returned to their home cages where chow and the CS solutions were available ad libitum. (The CS+ and CS– solutions were presented in different bottles with different sipper tubes than those used in the test cages.) The rats were given two-bottle access to the CS+ and CS–

for 28 days. They were then given only the CS– solution (plus chow) for two days, followed by another ten days of access to both the CS+ and CS– solutions.

Results

In the two-bottle test with IG infusions the rats consumed 24.3 ml/day of the CS+ compared to only 1.5 ml/day of the CS– [$t(6) = 9.4$, $p<.01$]; their percent CS+ intake was 93.7%. The rats continued to drink more CS+ than CS– in their home cages (without IG infusions), although their CS+ preference was reduced. That is, their percent CS+ intake during the first two days in their home cages was 78.3% as compared to their 93.7% preference in the test cages [$t(6) = 6.0$, $p<.001$]. Nevertheless, as illustrated in Figure 10, the rats persisted in drinking more CS+ than CS– over the 38 days of two-bottle testing in their home cages. Analysis of variance of the daily CS intakes confirmed that there was a significant CS effect [$F(1,6) = 27.3$, $p<.01$], as well as a days effect [$F(37,222) = 2.3$, $p<.001$], but that there was no solution x days interaction. Percent CS+ intakes did not reliably differ over days and averaged 74.8% for the 38-day, two-bottle test.

When the rats were given only the CS– solution to drink for two days they drank as much as they did of the CS+ solution in the two-bottle tests (Figure 10). Yet, when returned to the two-bottle choice condition, the rats again preferred the CS+ to the CS–. The rats' one-bottle test with the CS– tended to reduce their subsequent CS+ preference but this effect was not significant; that is, their percent CS+ intake in the 10-day period following the one-bottle CS– test was not reliably

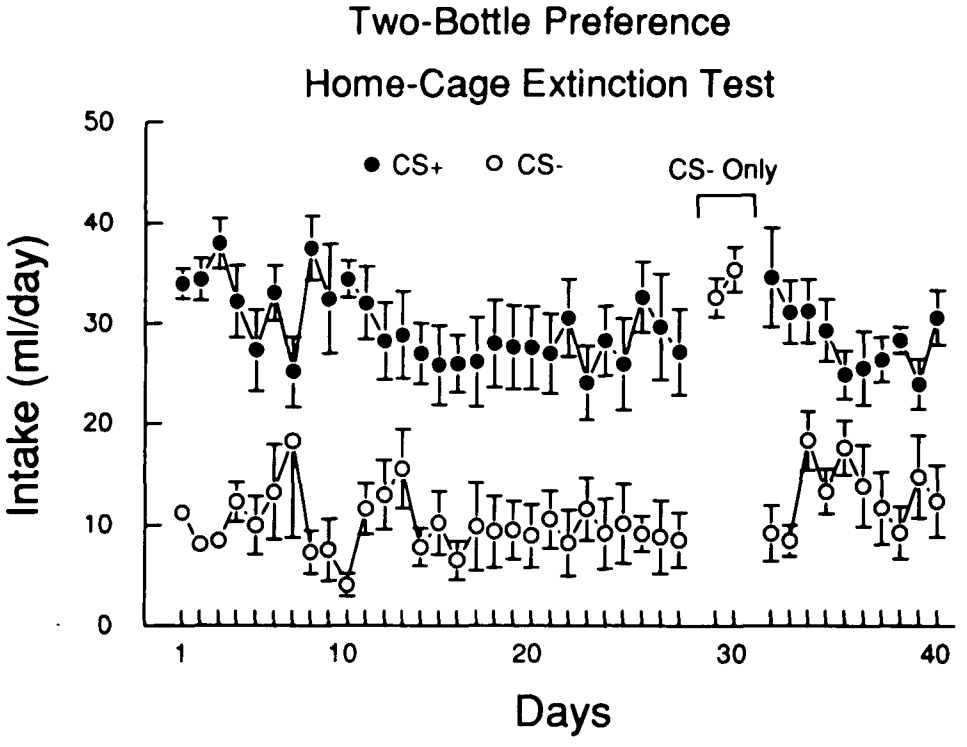

Figure 10: Experiment 1I: Mean (±SE) oral intake of CS+ and CS– during two-bottle preference tests in the home cages; the rats received no IG infusions during this experiment. On days 29 and 30 the rats were given one-bottle access to the CS– only. Note that some error bars are hidden by the symbol.

less than their percent intake in the 10-day period prior to the one-bottle test (70.6% vs. 77.8%, n.s.).

Discussion

These results confirm and extend the findings of Experiment 1E and show that the rats' conditioned preference for the CS+ was remarkably resistent to extinction. The animals did show an immediate decrease in their CS+ preference, from 94% to 78%, when they were switched from the test cages to the home cages. Since the rats did not reliably reduce their CS+ preference during the remainder of the test it is not clear that this initial decline was due to extinction per se. Rather, the preference decline may represent a generalization decrement related to change from the test cages to the home cages.

In particular, different drinking bottles were used in the test and home cages which, in view of the rats' "bottle training" experience in Experiment 1H, may have been responsible for the reduced CS+ preference displayed in the home cages. Also, in the home cages, unlike the test cages, the rats were not intragastrically infused with water as they drank the CS solutions. However, results obtained in another experiment suggest that the presence or absence of IG infusions does not influence CS+ preference during extinction (Elizalde & Sclafani, unpublished observations). The rats in this latter experiment displayed similar preferences for the CS+ during test-cage extinction tests during which they were infused with water or not infused (98% and 97%, respectively).

Since the rats drank relatively little of the CS– during the two-bottle extinction test, they had little opportunity to learn that this flavor was associated with the same postingestive consequences as the CS+ flavor. For this reason, the rats were given one-bottle access to the CS– for two days. Although the rats readily drank the CS–, this experience did not reliably reduce their subsequent CS+ preference. Thus, the rats' failure to extinguish their CS+ preference cannot be attributed to their minimal intake of the CS–. It is possible, though, that more prolonged one-bottle exposure to the CS– might have caused the rats to lose their preference for the CS+. However, results obtained in another experiment demonstrated that eight days of one-bottle exposure to the CS– during extinction did not weaken the rats' CS+ preference (Elizalde & Sclafani, unpublished observations).

EXPERIMENT 1J: CS REVERSAL TRAINING

The results of Experiment 1E and 1I indicate that the conditioned preference for the CS+ solution was rather immune to extinction. The present experiment determined whether the rats would lose their CS+ preference and come to prefer the CS– when the reinforcement contingencies were reversed, i.e., when the CS– flavor was paired with IG Polycose and the CS+ flavor was paired with IG water. Note that by this phase of the study (day 115) only four rats had viable gastric cannulas. Despite the small number of subjects, the results of this experiment were unambiguous.

Procedure

The present experiment began 32 days after the end of Experiment II; during the intervening period the rats had been given chow and water ad libitum in their home cages. At the start of this experiment the rats (n=4) were first retested for their CS+ vs. CS– preference in their home cages for two days before they were returned to the electronic esophagus apparatus. The animals were readapted to the infusion system for one day by allowing them to drink water from two sipper tubes; water intake was paired with IG water infusions; chow was available ad libitum. Then on training days 1 and 3, the rats were given one-bottle access to the "reversed" CS+, (rCS+, i.e., their old CS– flavor) which was paired with IG Polycose infusions. On days 2 and 4, the rats had one-bottle access to the "reversed" CS- (rCS–, i.e., their old CS+ flavor) which was paired with IG water infusions. On days 5 and 6 the rats were given a two-bottle test (test 1) with the rCS+ and rCS– each paired with the appropriate IG infusions. The rats were then given another four-day, one-bottle training cycle with the rCS– being presented on days 1 and 3, and the rCS+ being presented on days 2 and 4. On days 5 and 6 a two-bottle preference test was conducted (test 2A) followed by another two-day, two-bottle preference test (test 2B). The rats were then given a two-day, two-bottle extinction test in which the intakes of both the rCS+ and rCS– solutions were paired with IG water infusions.

Results

In the two-day, home-cage extinction test, the rats consumed more CS+ than CS- (38.8 vs. 15.1 ml/day); this difference was significant using a one-tailed t-test

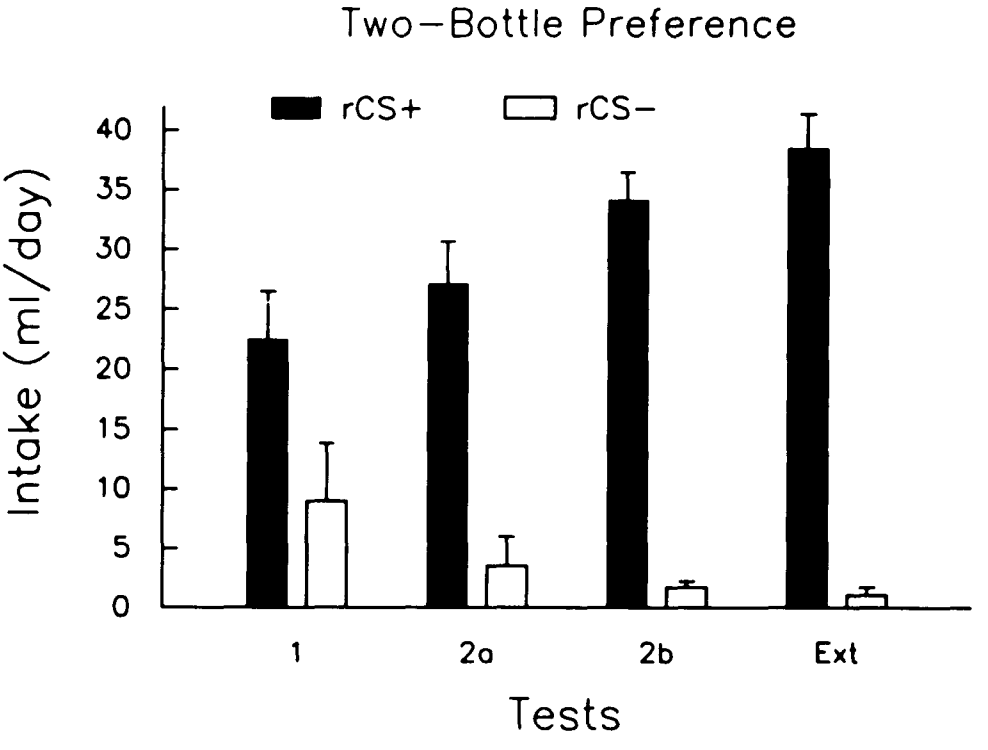

Figure 11: Experiment 1J: Mean (+SE) oral intake of rCS+ and rCS– during two-bottle reinforced tests (Tests 1, 2A, and 2B) and extinction tests; each bar represents a mean of two days. The rCS+ (formerly the CS–) was paired with IG Polycose and the rCS– (formerly the CS+) was paired with IG water during the reinforced tests; both were paired with IG water during the extinction test.

[t(3) = 2.4, p<.05]. The percent CS+ intake was 70.3% which was comparable to the rats' percent CS+ intake at the end of Experiment 1I.

During the one-bottle reversal training tests, the rats consumed similar amounts of the rCS+ and rCS– (28.0 vs. 30.7 ml/day, ns). However, as indicated in Figure 11, the rats consumed significantly more of the rCS+ than of the rCS– during preference tests 1 to 2B [F(1,3) = 38.1, p<.01]. The rats' percent rCS+ intakes were 72.9% in test 1, 87.8% in test 2A, and 95.0% in test 2B. In the subsequent two-day extinction test, the rats continued to drink much more rCS+ than rCS- [t(3) = 13.9, p<.001; see Figure 11]; their percent rCS+ intake was 97.6%.

Discussion

The rats readily learned to prefer the rCS+ flavor (i.e., their old CS- flavor) when it was paired with IG Polycose infusions. Furthermore, their rCS+ preference was as strong as their original CS+ preference and was undiminished during the two-day extinction test. Thus, while the rats did not respond to the lack of reinforcement in that they continued to prefer the CS+ during the extinction tests, they did respond to the reversal of reinforcement contingencies by acquiring a new flavor preference.

General Discussion: Experiment 1

Experiment 1 provides further evidence that the postingestive effects of nutrients can condition exceedingly strong flavor preferences in rats. In addition, this experiment provides new information concerning the persistence of acquired preferences, the relative strengths of learned and innate preferences, the differential conditioning of flavor preference and flavor acceptance, and the role of orosensory

and postingestive factors in caloric intake regulation. These effects will be discussed in turn.

Conditioned Preference. In agreement with the previous report of Sclafani & Nissenbaum (1988), the rats acquired a robust preference for the flavor that was paired with IG Polycose infusions. Although compared to the earlier results obtained with 16% Polycose infusions, the acquisition of the preference was somewhat retarded with the 32% Polycose infusions, ultimately the rats displayed near-total (98%) preferences for the CS+ flavor. Furthermore, the rats not only preferred the CS+ to the CS- but also to plain water; this contrasts with the mild aversions naive rats display toward the CS flavors. This latter finding is of significance since previous attempts to transform flavor aversions to preferences in rats have not been very successful (Rozin & Zellner, 1985).

The CS+ flavor preference displayed by the rats was remarkable not only for its potency but also for its resistance to extinction. Few studies have looked at the persistence of a conditioned flavor preference. One previous study reported that flavor preferences produced by IG infusions using a short-term testing paradigm extinguished rapidly (Holman, 1969). In the extinction test of Experiment 1E the rats continued to display a greater than 90% preference for the CS+ over a 14-day period. In the second extinction test (Experiment 1I), the CS+ preference was maintained for 40 days, although at a reduced level, and was still apparent when a subgroup of the rats was retested a month later (Experiment 1J). Attempts to block the preference during the extinction tests by giving the rats the CS+ in the absence

of chow or giving them the CS– only for two days were ineffective. In another experiment, Elizalde and Sclafani (unpublished observations) found that the rats' CS+ preference during extinction was not blocked when they were given the CS– only for eight days. While it is possible that other manipulations would have produced extinction, the present results demonstrate that the rats maintained their CS+ preference for at least several weeks in the absence of reinforcement with IG Polycose infusions. The rats were not insensitive to reinforcement contingencies, however. That is, they readily reversed their flavor preference when the original CS– flavor was paired with IG Polycose and became the rCS+.

Lack of extinction was also reported by Capaldi, Myers, Campbell, and Sheffer (1983). These investigators found that rats' preference for a flavor was greater if the flavor had previously been consumed under a low instead of a high level of deprivation. The magnitude of this preference was small but persisted for 28 days. Thus, not only are conditioned taste aversions resistant to extinction (Logue, 1979), but conditioned preferences can also persist under extinction conditions (Experiments 1E and 1I; Capaldi, et al., 1983).

The IG Polycose infusions not only produced a strong flavor preference, but, in the absence of unique flavor cues, produced a bottle or sipper tube preference (Experiment 1H). This finding indicates that food preference learning can involve somatosensory cues as well as taste and odor cues. The results of Experiment 1H, along with previous data (Borer, 1968; Holman, 1969), also suggest that the

viscerosensory stimuli produced by nutrients do not directly control ingestive behavior, but do so by altering the animal's evaluation of orosensory stimuli.

In contrast to the present findings (see also Sclafani & Nissenbaum, 1988), previous conditioning studies involving IG sugar infusions have obtained no or only weak flavor preferences. One study (Sherman et al., 1983) reported a small (≈64%) but reliable preference for a flavor that was paired with IG infusions of 9.2% glucose. Two other studies, however, failed to obtain preferences for flavors paired with IG infusions of an 18% glucose solution or an 18% glucose + 18% fructose mixture (Koopmans & Maggio, 1978; Puerto et al., 1976). In these studies, food-deprived rats were trained to associate CS flavors with IG infusions of sugar during brief daily training trials. Using similar training procedures, Nissenbaum and Sclafani (1987) have obtained reliable conditioned preferences (67-77%) with IG infusions of 8% to 32% Polycose; see Sclafani (1990). As discussed below, these latter findings suggest that Polycose is more effective than sugars in conditioning flavor preferences.

Thus, the strong conditioned flavor preferences obtained in the present study can be attributed to both the training procedure and the unconditioned stimulus (Polycose) used. With the present procedure the rats received multiple pairings (≈15) of the CS flavors and IG infusions each day. In addition, the animals directly controlled the size, duration, and frequency of the IG infusions by their voluntary drinking behavior. Thus, the rats were able to terminate an IG infusion before it became discomforting. The importance of this training procedure is evidenced by

the finding that food-deprived rats infused IG with 32% Polycose during once/day training sessions displayed a maximal flavor preference of 70% (Nissenbaum & Sclafani, 1987) compared to the 98% preference obtained in the present study.

With respect to the unconditioned stimulus, the available data suggest that hydrolyzed starch, such as Polycose, is more effective than sugars in conditioning flavor preferences. This is likely due to the fact that Polycose, while digested to and absorbed as glucose, is much lower in osmolarity than glucose at equicaloric concentrations. Hypertonic sugar solutions can have aversive postingestive consequences that would attenuate or even reverse the rewarding effect of the nutrient (Booth, 1985).

Innate vs. Acquired Preferences. The flavor preference conditioned by the IG Polycose infusions was not nearly as strong as the rats' innate preference for sweet and polysaccharide tastes. This is indicated by the results of the CS+ vs. saccharin and CS+ vs. Polycose preferences tests in Experiments 1C and 1G, respectively. Furthermore, not only did the rats prefer Polycose and saccharin to the CS+, but their absolute intake (acceptance) of these solutions was substantially greater than that of the CS+. It may be that with other CS flavors and/or other IG infusions (e.g., less concentrated Polycose), flavor preferences can be conditioned that are more comparable to the rats' innate preferences for carbohydrate tastes. Alternatively, it may not be possible to produce acquired flavor preferences that match the magnitude of innate preferences. This issue requires further investigation.

Much also remains to be learned about the behavioral and physiological mechanisms that mediate learned flavor preferences. In the case of conditioned flavor *aversions*, at least some acquired aversions appear to involve a reduction in the palatability or the "liking" of the flavor. In particular, behavioral and electrophysiological data suggest that pairing a sweet taste with lithium chloride causes the taste to evoke bitter-like responses (Chang & Scott, 1984; Pelchat et al., 1983). However, other types of flavor aversions, e.g., lactose aversion, do not appear to involve a "distaste" response (Pelchat et al., 1983). Animals may learn to avoid the taste of lactose, not because they come to dislike the taste, but because they anticipate the lower gut discomfort it produces (Rozin & Zellner, 1985).

Acquired flavor preferences may also involve different types of responses. Animals may come to prefer a flavor paired with a nutrient because they acquire an increased "liking" for the flavor or because they anticipate a beneficial consequence, e.g., caloric repletion, or both (Rozin & Zellner, 1985). These distinctions have received relatively little attention in the literature, and it has been assumed that conditioned preferences involve changes in the hedonic evaluation of the CS+ flavor (Booth, 1985). It appears likely that the strong flavor preference obtained in the present study was due at least in part to a conditioned increase in the palatability of the CS+; this issue will be addressed in Experiment 3.

Preference and Caloric Intake. The relative importance of orosensory and postingestive factors in diet-induced overeating has been the subject of considerable interest (Ramirez, Tordoff, & Friedman, 1989; Sclafani, 1987), and Experiment 1

provides new data relevant to this issue. When offered the initially unpreferred CS+ flavor paired with IG Polycose infusions, the rats increased their total caloric intake by about 14% (Experiment 1A). They displayed an even greater hyperphagia when saccharin was added to the CS+; the rats now increased their caloric intake by about 37% relative to their water baseline level (Experiment 1D). The rats also consumed more total calories when they drank the Polycose by mouth than when they drank the CS+ by mouth and received the Polycose by stomach infusions (Experiment 1F). The caloric intakes obtained with the CS+/saccharin and Polycose solutions (96.9 and 93.8 kcal/day, respectively) were comparable to that obtained in a study in which rats had ad libitum access to a 32% Polycose solution and chow during a 40-day period (90.6 kcal/day) (Sclafani, 1987b).

Taken together, these results indicate that (1) the postingestive effects of Polycose are sufficient to produce a mild hyperphagia in the absence of an innately preferred taste; and (2) highly palatable tastes can exaggerate the overeating response. This latter point is worth emphasizing since there are few, if any, published data that clearly document a hyperphagia-promoting effect of taste. In most previous studies, dietary manipulations that enhanced caloric intake involved changes in both the orosensory and nutritional qualities of the diet. A limitation of the present data, however, is that they are based on short time periods (two or four days), and further research is required to establish the contribution of orosensory and postingestive factors to long-term food intake and body fat regulation. The electronic

esophagus preparation described in the present study, in addition to its use in conditioning experiments, is well suited to the study of long-term energy balance.

Conditioned Acceptance. Although the IG Polycose infusions produced a robust preference for the CS+ flavor, the infusions did not increase the acceptance of the flavor. That is, the rats did not drink more of the CS+ than of plain water during Experiments 1A and 1B. This was not due to an intake-limiting effect of the IG Polycose infusions since the rats doubled their CS+ intake when it was sweetened with saccharin in Experiment 1D. The findings indicate, therefore, that conditioned flavor preferences are not necessarily associated with increases in flavor acceptance. A similar situation exists with unconditioned flavor preferences as illustrated by results obtained in 24 hour/day Polycose vs. water preference tests (Sclafani & Nissenbaum, 1987). At low Polycose concentrations (0.01 to 0.3%) rats displayed up to a 92% preference for the Polycose solution but their Polycose intake (acceptance) did not exceed their baseline water intake. At higher concentrations (1 to 32%), however, not only did the rats strongly prefer the Polycose solution, but they increased their Polycose intake to as much as 250% of their water baseline.

The failure of the IG Polycose infusions to increase the acceptance of the CS+ may be related to the fact that the CS flavors were relatively unpalatable to begin with, i.e., were less preferred than water by naive animals. Thus, to the extent that the IG Polycose conditioned an increase in the palatability of the CS+ flavor (see above), it may be that the flavor-infusion pairings improved the palatability of the CS+ flavor to a level such that the CS+ was preferred to water (i.e., like a 0.3%

Polycose solution), but not to a level that it was overconsumed (i.e., like a 3% Polycose solution). Figure 12 depicts the hypothesis proposed to explain why rats who acquire robust flavor preferences may not display increased acceptance of those flavors. This hypothesis involves a continuum of palatability, ranging from something that is unpalatable to something that is quite palatable. Water, which has been traditionally thought to be a neutral substance, lies in the middle of the continuum as the zero point. To the right of water along the continuum would lie substances that are more palatable than water. The further to the right that substances lie, the more palatable they are. To the left of water would lie substances that are less palatable, or, in other words, more unpalatable.

Plain Kool-Aid, which is initially unpreferred to water, served as the CS in Experiment 1. After the conditioning process, the rats displayed a near-total preference for the CS+ over water. Thus, according to this hypothesis, the palatability of plain Kool-Aid was increased; however, this increase was not sufficient to drive intake above baseline water intake. It is proposed that there is a palatability threshold needed to be reached for there to be increased acceptance. The conditioning process in Experiment 1 presumably did not increase the CS+'s palatability to threshold. According to this analysis, if more palatable CS flavors are used, then conditioning with IG Polycose would increase flavor acceptance. This possibility will be addressed in Experiment 2.

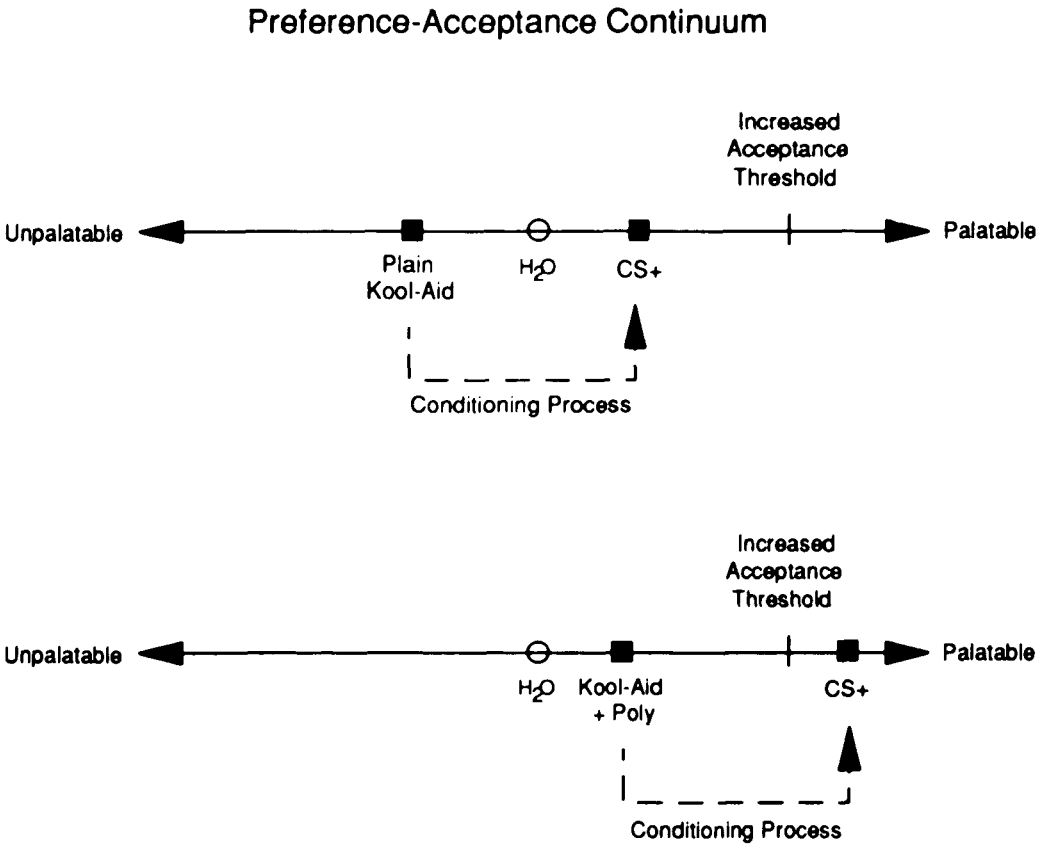

Figure 12. Diagram of a palatability hypothesis underlying conditioned flavor preferences and conditioned acceptance.

EXPERIMENT 2: PREFERENCE-ACCEPTANCE DISTINCTION

The findings indicate that the rats acquired a robust preference for the flavored solution (CS+) associated with IG Polycose infusions over the flavored solution (CS–) associated with IG water. The rats also displayed a strong preference for the CS+ over plain water. Yet despite their strong preference for the CS+ solution, the rats did not overdrink the CS+ solution relative to their baseline water intake. However, the rats overconsumed the CS+ solution when saccharin was added. The failure of the IG Polycose infusions to increase the intake of the CS+ may be due to the low initial palatability of the CS+ flavors.

The present experiment, therefore, investigated the influence of CS palatability on the conditioning of flavor preferences and acceptance. The influence of CS palatability on IG conditioning was manipulated by adding a small amount of Polycose to the CS solutions. Rats were conditioned in the same manner as in Experiment 1A using CS solutions (cherry- or grape-flavored water) containing 0.6% of Polycose. A second group was trained and tested using plain cherry- and grape-flavored water (no Polycose was added to the CS solutions). If the group tested with the more palatable CS solutions displayed increased acceptance of the CS+ while the control group did not, then the lack of increased acceptance of the CS+ in Experiment 1 could be attributed to the low initial palatability of the plain CS solutions used.

Subjects

Twelve adult female rats weighing 228-250 g of the same source and strain from the previous experiments were used. The rats were housed and maintained as in the previous experiments.

CS Solutions

For the Plain Kool-Aid group, the CS solutions consisted of water flavored with either 0.05% cherry or grape Kool-Aid (General Foods), and for the Kool-Aid/Polycose group, the CS solutions had 0.6% Polycose added to the Kool-Aid solutions. A preliminary study with naive rats (n=6) revealed that flavored Polycose solutions were preferred over water (Kool-Aid/Polycose vs. water: 42.8 vs. 15.0 ml/24 hours, $t(5) = 2.8$, $p<.05$; percent Kool-Aid/Polycose intake = 72.4%); this contrasts the mild aversions naive rats display to plain Kool-Aid solutions over plain water (see Methods section, Experiment 1). While naive rats prefer Kool-Aid/Polycose solutions over plain water in two-bottle tests, they drink as much Kool-Aid/Polycose solution as plain water in one-bottle tests (Kool-Aid/Polycose: 43.1 ml/24 hours; water: 42.0 ml/24 hours, ns). Naive rats (n=10) prefer Kool-Aid/Polycose solutions over plain Kool-Aid solutions (Kool-Aid/Polycose vs. plain Kool-Aid: 43.6 vs. 12.5 ml/24 hours, $t(9) = 4.8$, $p<.001$; percent Kool-Aid/Polycose intake = 75.8%). The Kool-Aid/Polycose flavor was specifically created so that it would be preferred over water, yet equally accepted to water. If the flavor were to be overconsumed from the start, then a ceiling effect may have occurred.

Intragastric Infusions

The infusates were a 32% Polycose solution and plain water.

Data Analysis

The data were analyzed as in the previous experiments.

EXPERIMENT 2A: INITIAL CONDITIONING

In this experiment, rats were trained to associate the CS+ with IG Polycose infusions and the CS– with IG water infusions. One group of rats was trained and tested with CS solutions that consisted of Polycose added to Kool-Aid, and a second group of rats was trained and tested using plain Kool-Aid solutions.

Procedure

The rats were implanted with a gastric cannula as in Experiment 1 and allowed several weeks to recover from surgery. They were then placed in the electronic esophagus apparatus and given water to drink for four days; days 3 and 4 were taken as the water baseline period. As they drank water from the sipper tube, the rats were infused IG with water. The test apparatus was changed so that the IG infusions were now controlled by computer software; in the present experiment, the infusion pumps would stay on for three seconds after the rat licked 15 times. Previously, the rate of infusion was controlled by mechanical relays and started with the rat's first lick and ended three seconds after the rat's last lick of a bout.

The rats were randomly divided into two groups equated for chow and water intake. Both groups of rats were subsequently trained and tested over two six-day

cycles as in Experiment 1A. One group, the Plain Kool-Aid group (KA group, N=6) was given plain Kool-Aid (cherry- or grape-flavored water) as the CS solutions, and the other group, the Kool-Aid/Polycose group (KA/P group, N=6) had 0.6% Polycose mixed into the CS solutions. Half of the rats in each group had cherry as the CS+ and grape as the CS–; the flavors were reversed for the remaining animals.

After the end of the second preference test cycle, the rats were given a two-bottle test between the CS– and plain water for two days; both the CS– and plain water were paired with IG water infusions. The rats were then given a two-day two-bottle test between the CS+, paired with IG Polycose infusions, and plain water, paired with IG water.

Results

CS intakes. The results of the one-bottle training and two-bottle preference tests between the CS+ and CS– are summarized in Figure 13. During training, the KA/P group consumed more CS– than CS+ in the first cycle but slightly more CS+ than CS– in the second cycle. Analysis of variance revealed that CS+ intake marginally increased across cycles ($p<.051$), whereas CS– intake did not reliably change. In addition, CS+ intake exceeded water baseline intake by the end of the second cycle, but this difference was not significant. The KA group drank comparable amounts of the CS+ and CS– in the first cycle, but in the second cycle CS+ intake exceeded CS– intake as well as water baseline intake. However, these differences were not significant. Absolute intakes of the two groups during training did not reliably differ.

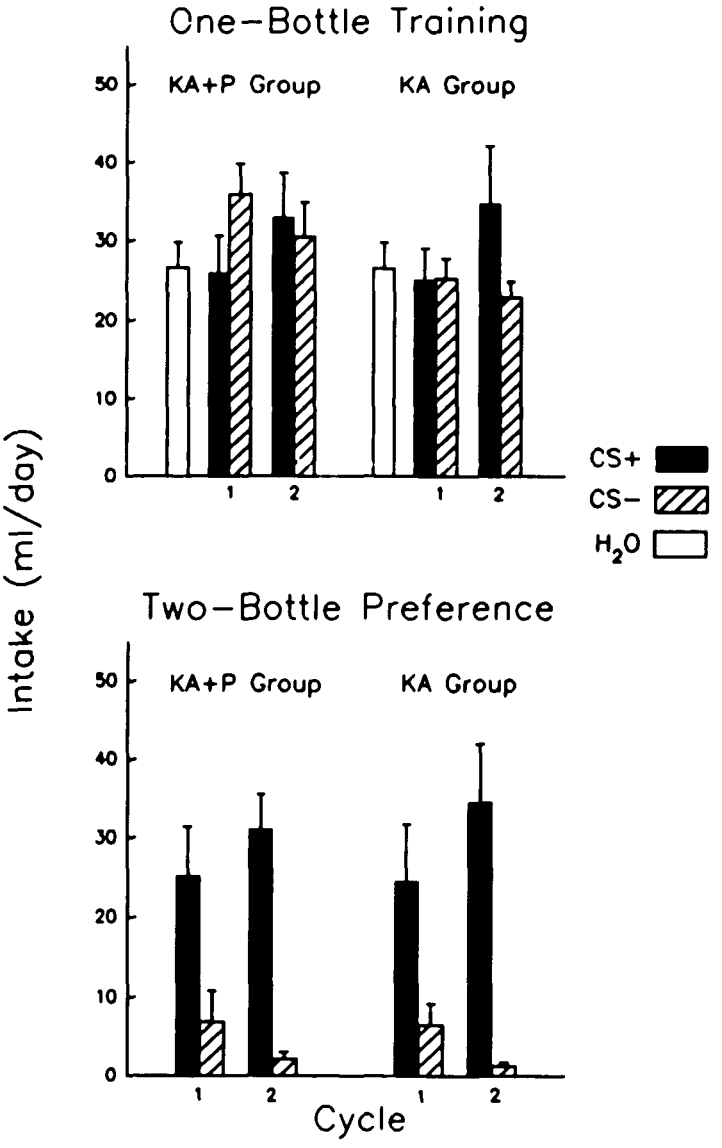

Figure 13. Experiment 2A: Mean (+SE) oral intake of CS+ and CS– during cycles 1 and 2 of the one-bottle training and two-bottle preference tests; each bar represents a mean of two days. For the KA/P group the CS+ and CS– were cherry- or grape-flavored water with Polycose added to it, and for the KA group the CS+ and CS– were plain cherry- or grape-flavored water; the CS+ and CS– were paired with IG Polycose infusions and IG water infusions, respectively.

In the two-bottle preference tests, the KA/P group consumed more CS+ solution than CS– solution [$F(1,5) = 11.2$, $p<.05$]; the strength of the CS+ preference increased from cycle 1 to 2, but, not significantly. The percent CS+ intakes were for the KA/P group 74.3% in cycle 1 and 92.2% in cycle 2. The KA group also consumed more CS+ than CS– [$F(1,5) = 9.8$, $p<.05$]; and this difference reliably increased from cycle 1 to 2 [$F(1,5) = 11.6$, $p<.05$]. Percent CS+ intake was 73.5% in cycle 1 and 95.2% in cycle 2. Absolute intakes of the two groups did not reliably differ.

The results of the CS vs. water tests are illustrated in Figure 14. The KA/P group consumed more CS+ than water [$F(1,10) = 25.8$, $p<.001$] and more CS– than water [$F(1,10) = 7.9$, $p<.05$]. In addition, the KA/P group's CS intakes as well as water intakes during the CS+ vs. water test and the CS– vs. water test did not reliably differ. The KA/P group's percent CS+ intake was 96.3% and percent CS– intake was 77.2%. The KA group consumed more CS+ than water [$F(1,10) = 20.6$, $p<.001$], but more water than CS– [$F(1,10) = 9.7$, $p<.05$]. The KA group's CS+ intake during the CS+ vs. water test exceeded their CS– intake during the CS– vs. water test [$F(1,10) = 16.5$, $p<.01$]. In addition, the KA group consumed less water during the CS+ vs. water test than during the CS– vs. water test [$F(1,10) = 19.4$, $p<.001$]. The KA group's percent CS+ intake was 88.0% and their percent CS– intake was 22.8%. The two groups' absolute intakes during the CS+ vs. water test did not reliably differ. However, during the CS– vs. water test the KA/P group consumed more CS– but less water than did the KA group [$F(1,16) = 14.1$, $p<.01$,

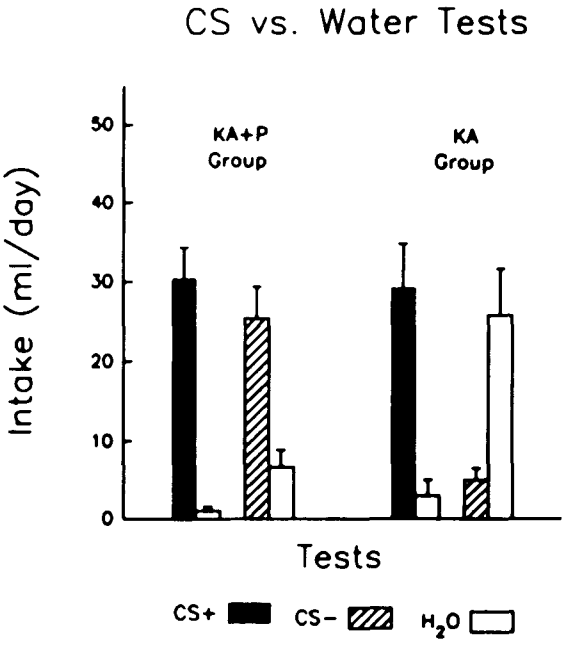

Figure 14. Experiment 2A: Mean (+SE) oral intake and plain water, and of CS– and plain water during two-bottle preference tests; each bar represents a mean of two days. The CS+ was paired with IG Polycose infusions, and the CS– and plain water were paired with IG water infusions.

and $F(1,16) = 12.4$, $p<.01$, respectively].

Caloric intake. Table 1 summarizes the kilocalorie intake during training. When infused with Polycose on the CS+ training days, the KA/P group reduced their chow intake compared to water baseline intake, but this decrease did not completely compensate for the calories provided by the IG Polycose. As a result, the KA/P group's total caloric intake on CS+ training days during cycles 1 and 2 exceeded their intake during the water baseline period. These differences were significant [$F(2,10) = 5.2$, $p<.05$]. Kilocalorie intake on CS+ training days during cycles 1 and 2 did not reliably differ. The KA/P group's kilocalorie intake on CS– training days was less than their total kilocalorie intake on CS+ days [$F(1,5) = 17.6$, $p<.01$], but comparable to the water baseline caloric intakes.

The KA group also decreased their chow intake during CS+ training days compared to water baseline days. Kilocalorie intake from chow together with the calories provided from the IG Polycose resulted in a reliable increase in total caloric intake on CS+ training days compared to water baseline days [$F(2,10) = 27.2$, $p<.001$]. In addition, Newman-Keuls test revealed that kilocalorie intake during CS+ days of cycle 2 was reliably greater than intake during CS+ days of cycle 1 ($p<.05$). The KA group's total kilocalorie intake on CS– training days was less than their intake on CS+ training days [$F(1,5) = 28.7$, $p<.01$], but comparable to their water baseline kilocalorie intake. There were no reliable group differences in intake.

Table 1. Experiment 2A: Mean (±SE) kilocalorie intake of the KA/P and KA groups during one-bottle training trials.

		Water Baseline	Cycle 1		Cycle 2	
			CS+	CS–	CS+	CS–
KA/P Group	Chow	68.3 ±3.4	58.0 ±2.8	67.8 ±3.8	53.2 ±4.5	63.8 ±4.0
	Polycose		30.8 ±5.6		39.3 ±5.2	
	Total	68.3 ±3.4	88.8 ±7.6	67.8 ±3.8	92.5 ±4.7	63.8 ±4.0
KA Group	Chow	72.2 ±4.4	64.2 ±5.6	69.1 ±7.6	64.2 ±3.0	66.3 ±4.1
	Polycose		25.4 ±2.5		38.4 ±4.4	
	Total	72.2 ±4.4	89.6 ±5.7	69.1 ±7.6	102.6±3.8	66.3 ±4.1

Discussion

The results of Experiment 2A, as well as of Experiment 1, demonstrate that rats strongly prefer to consume the CS+ over the CS– as well as over water. However, training rats with more palatable CS solutions failed to increase the acceptance of these solutions. Both the KA/P and KA groups strongly preferred the CS+ over the CS–, and neither group overconsumed the CS+ relative to water. Hence, even though the KA/P group was trained with an initially preferred solution (relative to water and the plain Kool-Aid solution), this group's intake did not differ from the KA group's intake.

Perhaps, the KA/P solution was not palatable enough to promote increased acceptance. However, a pilot study (Elizalde & Sclafani, unpublished observations) found that when more Polycose was added to the KA/P solution, naive rats immediately overconsumed the solution relative to water in one-bottle tests (presumably, because the level of palatability was past the increased acceptance palatability threshold; see Figure 12). Such immediate overconsumption may have a ceiling effect; therefore, any increased acceptance that could be produce by the conditioning process would not occur.

Alternatively, while flavor-IG infusion pairings produce strong conditioned preferences, perhaps they cannot induce conditioned acceptance by themselves. In Experiment 1D, conditioned acceptance was achieved only after the CS+ had been associated with the sweet taste of saccharin. When saccharin was added to the CS+, intake more than doubled; when saccharin was removed, intake dropped but to a

level that was still reliably greater than before the addition of saccharin (see Experiment 1D). Thus, conditioned acceptance was exhibited when the CS+ was paired with the sweet taste of saccharin. In other words, the flavor-calorie (CS+ paired with IG Polycose) association results in robust CFPs, while the juxtaposition of the flavor-calorie association with the flavor-taste (CS+ paired with the sweet taste of saccharin) results in increased acceptance of the CS+.

It may be that the conditioning process does not increase the palatability of the CS+. The rats may not come to "like" the CS+ after conditioning; they may have preferred the CS+ over the CS– because they were merely anticipating the postingestive effects associated with consumption of the CS+ (see Rozin & Zellner, 1985). This issue is further addressed in Experiment 3.

The results of the CS– vs. water test confirm the previous finding that naive rats prefer to consume KA/P solutions over plain water but do not overconsume them relative to water (Elizalde & Sclafani, unpublished observations). The KA/P group preferred the CS– to water but did not show increased acceptance of the CS–. In contrast, the KA group avoided the CS–. The water preference displayed by the KA group, however, differs from what was found in Experiment 1B. In Experiment 1B, the rats came to equally prefer the CS– and water. That finding was interpreted in terms of the rats' conditioned preference to the CS+ flavor partially generalizing to the CS– flavor, since both flavors share the key ingredient citric acid. In the present experiment, the conditioned preference for the CS+ did not generalize to the CS– flavor. One difference between the present experiment and Experiment 1B is that

whereas in Experiment 1B the CS+ vs. water test preceded the CS– vs. water test, the CS+ vs. water test followed the CS– vs. water test in the present experiment. An order effect may have been responsible for these results. More research is needed to determine the cause of the discrepant results (see Experiment 3).

The results of the present experiment as well as those of Experiment 1, demonstrate that rats will increase their total caloric intake when they have access to the CS+ and the IG Polycose. The rats' total caloric intake on CS+ training days exceeded their intake on CS– training days and water baseline days (percent increase on second cycle's CS+ training days compared to water baseline: KA/P group, 35%; KA group, 42%). The rats' increase in total caloric intake on CS+ training days was substantially greater than the rats in Experiment 1A who exhibited a 14% increase in total caloric intake. In addition, Experiment 1D showed that the rats further increased their caloric intake when the CS+ was made more palatable through the addition of saccharin. The following experiment determined whether the KA/P group would increase their intake of the CS+ (and, consequently, increase their caloric intake) if it were sweetened with saccharin.

EXPERIMENT 2B: CS+ PLUS SACCHARIN

The results of Experiments 1A and 2A demonstrate that despite their strong preference for the CS+ over the CS–, the rats did not reliably overconsume the CS+ compared to their water baseline intake; that is, there was no increased acceptance of the CS+. In order to determine if the rats in the KA/P group were capable of

overdrinking the CS+, they were tested with a saccharin-sweetened CS+ solution, while the KA group continued to receive an unsweetened CS+ solution.

Procedure

Both groups of rats were first given a two-day, two-bottle test with the CS+ vs. water (Test 1). The rats were then given a four-day, two-bottle test (Test 2); the KA/P group received CS+/0.2% saccharin solution vs. water, while the KA group continued to receive the CS+ vs. water (data analysis was based on the last two days of this test). Both groups of rats were then given a two-bottle test for two days (Test 3) between the CS+ (without any saccharin added) and plain water. In the three tests, intake of the CS+ or CS+/saccharin solution was paired with IG Polycose infusions, while the intake of water was paired with IG water infusions.

Results

CS intakes. Figure 15 illustrates the results of Tests 1 to 3. Since both groups of rats consumed less than two ml of water in each of the three tests, only the CS+ and CS+/saccharin solution intakes are shown in the figure. The KA/P group displayed near-total preferences for the CS+ and CS+/saccharin solutions over water [$F(1,5) = 51.6$, $p<.001$]. When saccharin was added to the CS+ solution (Test 2), the rats increased their intake by over 50%. However, this increase failed to be statistically significant. The lack of significance was due to one rat's decrease in intake when saccharin was added to the CS+, while the other five rats increased their intake. A rat's avoidance of saccharin is extremely rare (Mook, 1974); therefore, the KA/P group's data were also analyzed without the saccharin-avoiding rat's data

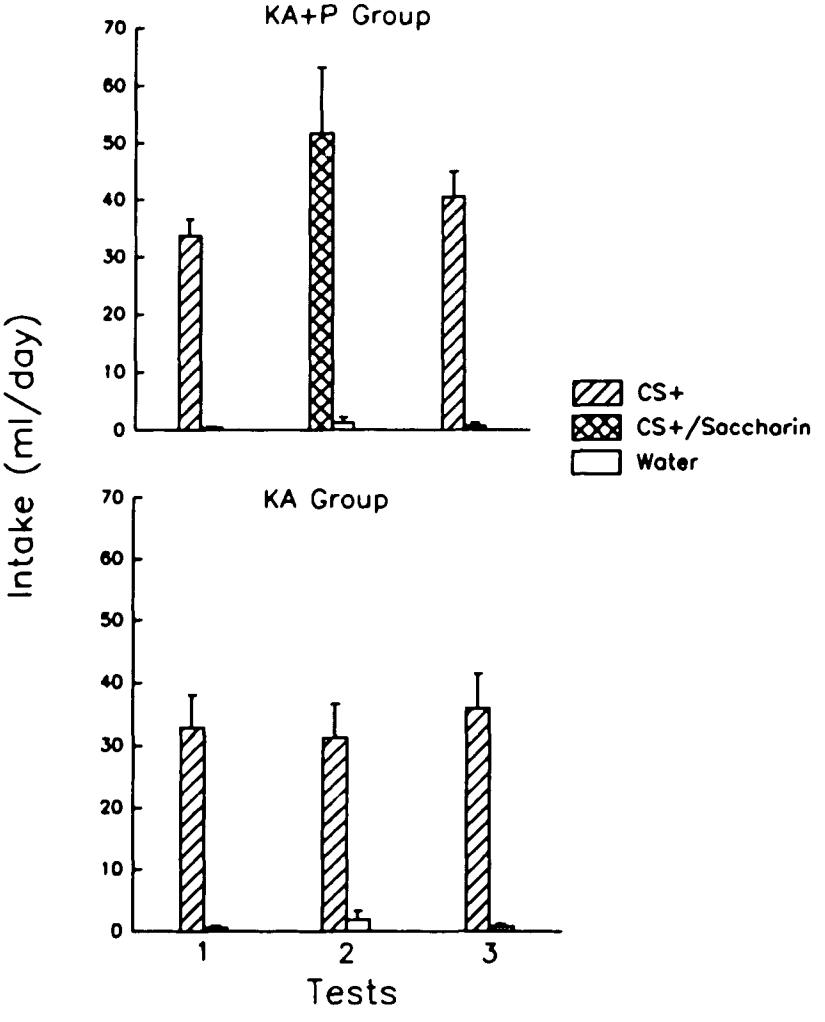

Figure 15. Experiment 2B: For the KA/P group, mean (+SE) oral intake of CS+ (Test 1), CS+/Saccharin solution (Test 2), and CS+ solution (Test 3); for the KA group, mean (+SE) oral intake of CS+ during Tests 1 to 3. Each bar represents a mean of two days. The CS+ and the CS+/Saccharin solution were paired with IG Polycose infusions.

(KA/P group's CS+ intake during Tests 1 to 3 with rat excluded: 33.6, 58.9, and 42.3 ml/day, respectively). The resulting analysis revealed that the KA/P group reliably increased their intake with the addition of saccharin [$F(2,8) = 4.9$, $p<.05$]. When saccharin was removed from the CS+ (Test 3), the KA/P group (with and without the saccharin avoider) decreased their intake of the CS+ but to a level that was greater than their intake in Test 1. However, CS+ intake in Test 3 did not reliably differ from intake during the first two tests (excluding the saccharin avoider). Analysis including the saccharin avoider's data revealed that CS+ intake in Test 3 was significantly greater than CS+ intake in Test 1. Percent CS+ and CS+/saccharin intakes of the KA/P group (including the deviant rat) in Tests 1, 2, and 3 were 98.7%, 95.0%, and 98.0%, respectively; excluding the saccharin-avoiding rat, percent intake was 99.2%, 99.5%, and 99.6%, respectively.

The KA group consumed more CS+ than water during Tests 1 to 3 [$F(1,5) = 35.3$, $p<.01$], and intake across the three tests did not reliably differ. Percent CS+ intake in Tests 1, 2, and 3 was 97.8%, 93.6%, and 98.0%, respectively. Although the KA/P group consumed more CS+ (with and without saccharin added to it) during Tests 2 and 3 than did the KA group, these differences were not significant ($p<.09$). However, when group comparisons were made excluding the saccharin-avoiding rat from the KA/P group, analysis revealed that the KA/P group consumed significantly more of the CS+/saccharin solution than did the KA group of the CS+ solution during Test 2 [$F(1,15) = 9.7$, $p<.01$]. In addition, there were no reliable group differences in absolute intake during Tests 1 and 3.

Caloric intake. Kilocalorie intake during Tests 1 to 3 is summarized in Table 2. The KA/P group increased their total kilocalorie intake when offered the saccharin-sweetened CS+ in Test 2 compared to their intakes in Test 1 or 3, but these differences were not statistically significant. However, the KA/P group's caloric intake derived from the infused Polycose differed across tests [$F(2,10) = 8.6$, $p<.01$]. Newman-Keuls test found that the KA/P group's caloric intake from infused Polycose increased when saccharin was added to the CS+ (Test 1: 42.4 kcal/day; Test 2: 58.6 kcal/day, $p<.01$), and decreased when saccharin was removed from the CS+ solution in Test 3 (47.9 kcal/day, $p<.05$). Caloric intake derived from Polycose in Tests 1 and 3 did not reliably differ. The increased caloric intake from Polycose intake in Test 2 [$F(2,10) = 8.6$, $p<.01$], was compensated for by a drop in chow intake when the CS+ was sweetened with saccharin [$F(2,10) = 7.4$, $p<.05$].

When the saccharin-avoider's data were excluded, analysis revealed that the KA/P consumed reliably more total kilocalories when they were given the saccharin-sweetened CS+ in Test 2 than when they were given the plain CS+ in Tests 1 and 3 [$F(2,8) = 4.5$, $p<.05$]. As the rats reliably increased their caloric intake derived from IG Polycose, $F(2,8) = 32.1$, $p<.001$, they decreased their caloric intake from chow [$F(2,8) = 9.2$, $p<.01$].

Total kilocalorie intake for the KA group when given the plain CS+ solution during Tests 1 to 3 did not reliably differ, although it marginally increased in the third test ($p<.054$). The KA group's caloric intake derived from Polycose and chow did not significantly differ across tests.

Table 2. Experiment 2B: Mean (±SE) kilocalorie intake of the KA/P and KA groups during Tests 1, 2, and 3. Kilocalorie intake derived from chow and from IG Polycose, as well as total kilocalorie intake, are indicated. In addition, the KA/P group's data with the saccharin-avoiding rat's data excluded is shown.

		Test 1	Test 2	Test 3
KA/P Group (n=6)	Chow	42.2 ±2.5	31.9 ±1.5	35.4 ±2.2
	Polycose	42.4 ±2.9	58.6 ±6.6	47.9 ±3.7
	Total	84.6 ±3.8	90.5 ±6.2	83.3 ±3.7
KA Group (n=6)	Chow	46.2 ±4.4	43.4 ±5.1	51.0 ±5.1
	Polycose	41.8 ±3.4	42.7 ±5.0	43.1 ±3.3
	Total	88.0 ±4.0	86.1 ±3.4	94.1 ±3.1
KA/P Group (n=5)	Chow	42.5 ±3.0	30.9 ±1.2	33.8 ±1.9
	Polycose	42.9 ±3.5	63.3 ±5.6	48.8 ±4.4
	Total	85.4 ±4.5	94.2 ±6.0	82.6 ±4.4

Comparisons of the two groups found that, overall, the KA/P group consumed fewer calories from chow than did the KA group [$F(1,10) = 5.5$, $p<.05$]. In contrast, the KA/P group consumed more calories derived from IG Polycose than did the KA group in Test 2 [$F(2,20) = 5.5$, $p<.05$]. When the two groups' total kilocalorie intakes were analyzed, a group x test interaction was found [$F(2,20) = 3.9$, $p<.05$]. However, simple main effects revealed that the KA group only slightly increased their total kilocalorie intake in Test 3 ($p<.08$; two out of the five rats increased their CS+ intake by 8-14 ml) . When the saccharin-avoiding rat's data were removed, group comparisons yielded the same effects.

Discussion

The effect of adding saccharin to the CS+ was to increase the KA/P group's intake of the CS+, though not significantly. The lack of significance was due to one (out of six) rat's avoidance of saccharin. When the saccharin-avoiding rat's data were excluded, statistical analysis revealed a reliable increase in intake when saccharin was added to the CS+. In addition, the rats consumed more total calories when saccharin was added to the CS+. These results confirm the findings of Experiment 1 which found enhancement of intake with the sweetening of the CS+. The KA/P group's percent increase (38%) in total kilocalorie intake when saccharin was added to the CS+ compared to water baseline days was comparable to the percent increase displayed by the rats in Experiment 1 (37%). The rats in Experiment 1 exhibited a 14% increase in total caloric intake on CS+ training days compared to water baseline intake, and they further increased their intake to about 37% above their water baseline

intake when the CS+ was sweetened with saccharin. The KA/P group, on the other hand, had already increased their caloric intake by 35% in the second training cycle. Therefore, a ceiling effect may have inhibited a greater increase in caloric intake, since these rats only increased their intake to 38% above water baseline when saccharin was mixed into the CS+ solution. Nevertheless, the rats' total kilocalorie intake when given the CS+ or the saccharin-sweetened CS+ exceeded their caloric intake on water baseline days (Experiment 1 and 2).

In addition, the results add to the accumulating evidence that rats acquire preferences for flavors associated with IG Polycose infusions. However, increasing the initial palatability of the CS solutions (by adding Polycose to the solutions) did not increase the acceptance of the CS+ solution; that is, while the CS+ was preferred over plain water, CS+ intake (nor the intake of the CS–) did not exceed water baseline intake. CS+ intake of the KA/P group exceeded water baseline intake only after the CS+ had been associated with the sweet taste of saccharin.

It may be that the palatability of the CS+ may not have been increased through the conditioning process. The rats may have preferred the CS+ over the CS– as well as over plain water because they were anticipating the postingestive consequences from consumption of the CS+ (see Rozin & Zellner, 1985). In other words, the rats may not come to "like" the CS+ due to an increase in its palatability produced by the conditioning process, but merely consume it for the calories associated with consumption. This issue of whether or not the preference for the CS+ involves an increase in its palatability is addressed in Experiment 3.

EXPERIMENT 3: THE TASTE REACTIVITY TEST

The findings demonstrate that the postingestive nutritive effects of Polycose can condition strong flavor preferences which are resistant to extinction. However, the rats did not reliably overconsume the CS+ compared to water baseline intake. Perhaps, the rats preferred the CS+, not because the palatability of the solution was increased, but because they were anticipating positive postingestive consequences.

The taste reactivity test, thought to be a measure of palatability, has been used in previous studies to assess the acquisition of likes (Breslin et al., 1990; Zellner et al., 1985). Intraoral (IO) infusions of taste stimuli elicit qualitatively different behaviors (fixed action patterns, FAPs) that involve stereotyped movement patterns, particularly involving the muscles of the tongue, jaw and face. The FAPs can be divided into two response domains, ingestive (or positive) and aversive (or negative). Breslin and colleagues (1990) found that when a normally avoided taste (quinine) was associated with a preferred taste (sucrose), the aversive responses produced by the avoided taste decreased over time, while the ingestive responses increased. Another study used the taste reactivity test to examine the conditioned preference for the bitter taste of morphine (Zellner et al., 1985). Morphine-raised rats exhibited a greater number of ingestive FAPs to an IO infusion of a morphine solution than water-raised rats (Zellner et al., 1985). An increase in the number of ingestive FAPs over time has been taken to mean that an acquisition of a "liking" for the flavor of the solution has occurred (Breslin et al., 1990; Zellner et al., 1985).

The taste reactivity test was used in the present experiment to address the issue of whether or not the preference conditioning process involves an increase in the liking of the CS+ flavor. New rats were implanted with two intraoral (IO) catheters, in addition to the IG catheters. After being conditioned to prefer the CS+ by pairing it with IG Polycose infusions, the rats' orofacial reactions to the CS+ and CS– were measured using the taste reactivity test. The orofacial responses of the conditioned rats were compared to behaviors displayed by rats that had the CS+ and CS– flavors paired with IG water infusions. The degree to which the conditioned rats displayed more ingestive reactions to the CS+ than the control rats provided an assessment of the acquired liking to the CS+.

In addition to the taste reactivity test, the rats were given short-term (30 minutes/day) tests. The willingness of nondeprived rats to consume solutions in short-term tests has been taken as evidence that the solutions are found to be palatable by the rats (Sclafani, 1987a). Therefore, if the conditioned rats consumed more of the CS+ solution during short-term tests than did the control rats, this would suggest that IG conditioning increased the palatability of the CS+ flavor.

Subjects

Twenty-four female rats of the same strain and source as in the previous experiments were used. They were housed and maintained as in the previous experiments. During the course of the different phases of this experiment, several of the rats were discarded because of problems with their gastric cannulas or because of

various health problems. One rat from the Experimental group and two rats from the Control group were discarded during the home-cage testing phases.

Surgery

All the rats were first implanted with an IG cannula, as in the previous experiments, and then with two IO cannulas. Subsequent to being implanted with a gastric cannula, the anesthetized rat was placed in ear bars and a midline incision was made from the ears to the back of the eyes. Four holes were drilled in the skull (one in each quadrant surrounding the bregma) and small screws were inserted into the holes. Dental acrylic was applied to form a block of cement containing the screws. The rat was then removed from the ear bars and placed on its back. A teflon washer was then threaded onto flared PE-90 tubing; this was repeated so as to yield two cannulas for each rat. A 9-mm piece of 20-gauge metal tubing was partially inserted into each piece of PE-90 tubing. A 20-gauge sharpened metal tube (a needle) was then attached to the metal tip of each cannula using a piece of PE-90 tubing. The cannula was inserted (using the sharpened tip) just anterior-lateral to the first maxillary molar (see Figure 16). The needle was then advanced subcutaneously until the washer was flush with the roof of the mouth between the cheek and gum, thereby having the distal end of the tubing protrude above the skull's surface. The procedure was repeated for the opposite side of the mouth. The rat was then placed back into the ear bars. More dental acrylic was applied over the acrylic block foundation and to the PE-90/20 G tubing juncture of the two cannulas. Following surgery, the IO cannulas were cleaned out every other day by infusing water into the cannulas.

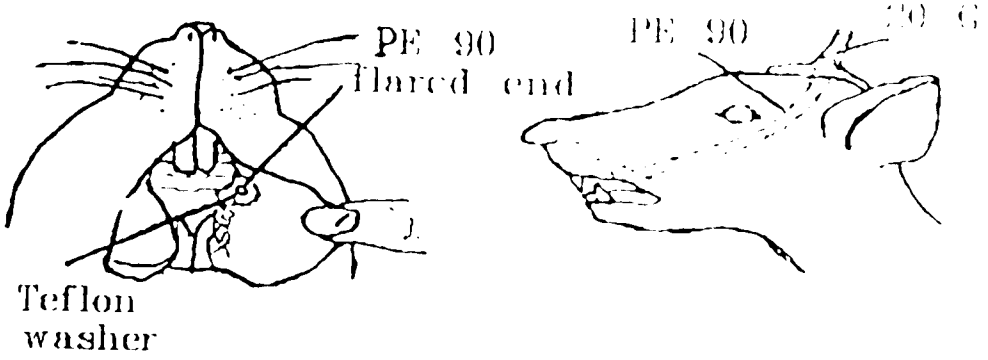

Figure 16. Diagram of placement of intraoral cannula. The intraoral end of the cannula is placed just rostral to the first maxillary molar. The tubing is led out subcutaneously to the skull and secured to a short piece of 20-gauge stainless-steel tubing and anchored to the skull with dental acrylic. (A) ventral view; (B) lateral view. (Adapted from Grill & Berridge, 1985.)

Apparatus

The taste reactivity test chamber, illustrated in Figure 17, was made up of the base and mirror, a clear plastic cylinder, and a plastic lid. The floor of the cylinder rested on the base. The lid was placed on top of the cylinder, and PE-90 tubing containing the CS solutions passed through a slot in the lid. One end of the PE-90 tubing attached to the rat's IO cannula, and the other end attached to a syringe needle. A mirror was mounted under the base of the chamber. A video camera was positioned approximately 90 cm from the surface of the mirror and was connected to a video cassette recorder (VCR). A monitor was connected to the VCR and was used as a guide to position the camera during testing.

CS Solutions

The CS solutions consisted of either 0.03% sucrose octaacetate (SOA; ICN Pharmaceuticals, Inc., Cleveland, OH) or 0.05% citric acid monohydrate (Fisher Scientific Company, Fairlawn, NJ) added to water. These two flavors were used instead of the Kool-Aid flavors for the taste reactivity test for two reasons. First, the Kool-Aid flavors have both taste and olfactory properties, whereas SOA and citric acid are primarily taste cues. With the Kool-Aid flavors, rats may use olfactory as well as taste cues. The use of an olfactory cue would be inappropriate for the taste reactivity test. With this intraoral procedure, a solution is directly infused into the mouth, thereby limiting any olfactory cues. Thus, flavors were chosen that were primarily taste cues. The second reason for not using cherry and grape Kool-Aid was that these two flavors share one key taste ingredient -- citric acid. Ideally, two unique taste cues

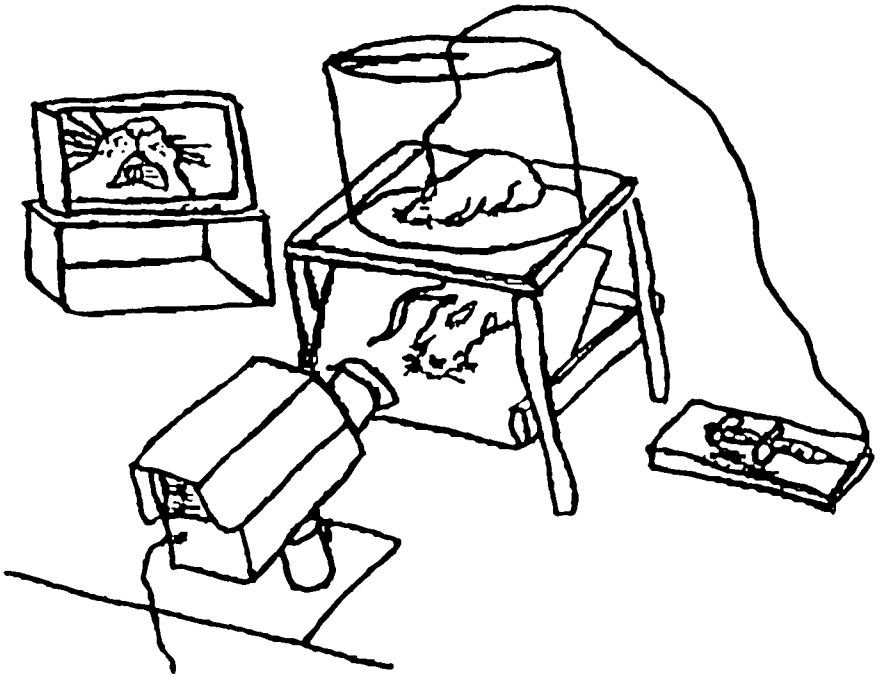

Figure 17. A schematic representation of the intraoral preparation for videotaping taste reactivity responses to taste stimuli infused into the mouth via chronic intraoral catheters. Videotaping is done via a mirror located beneath the Plexiglas floor of the cylinder test chamber. (Adapted from Grill & Berridge, 1985.)

should be used with the taste reactivity test. Citric acid was chosen as one flavor since it is the primary ingredient of the Kool-Aid flavors. SOA was also a salient cue flavor as evidenced by a pilot study using SOA-flavored water and grape-flavored water as the CS solutions. Rats preferred SOA over grape Kool-Aid when it was paired with IG Polycose infusions; the opposite preference was observed in different rats when the reversed pairings were presented (Elizalde & Sclafani, unpublished observations). Naive rats' intakes of SOA and citric acid solutions at these concentrations do not reliably differ (Elizalde & Sclafani, unpublished observations). In addition, naive rats consume more plain water than SOA or citric acid (Elizalde & Sclafani, unpublished observations).

Intragastric Infusions

The infusates were a 32% Polycose solution and plain water.

Procedure

The experiment was divided into five phases: Habituation, Conditioning and testing, Taste reactivity test, Home-cage short-term testing, Home-cage long-term testing.

Habituation phase. The rats were allowed several weeks to recover from surgery. Habituation training, which began during the second week of this recovery period, served to familiarize the rat with the taste reactivity test chamber and with receiving fluid intraorally; it also served to decrease agitation and allowed for easier videotaping. On days 1 and 2 of habituation training, the rats were individually placed in the test chamber and left undisturbed for approximately 10-20 minutes. On days

3 and 4, the rats were left undisturbed in the chamber for five minutes. On Days 5, 6, and 7 the rats were infused IO with tap water for one minute.

Conditioning and testing phase. After the end of the recovery/habituation phase, the rats were placed in the test cages and connected to the electronic esophagus infusion system. The rats were given water to drink on the first four days; they were infused IG as they drank water from the sipper tube. Days 3 and 4 were taken as the water baseline period. The rats were then divided into two groups [Experimental group (n=16) and Control group (n=8)] equated for water and chow intake. The two groups of rats were trained and tested over two six-day cycles as in Experiment 1A. For half of the rats in the Experimental group, the CS+ consisted of an SOA solution, paired with IG Polycose infusions, and the CS– consisted of a citric acid solution, paired with IG water infusions; for the rest of the rats in the Experimental group, the CS+ was a citric acid solution, paired with IG Polycose infusions, and the CS– was an SOA solution, paired with IG water infusions. The Control group received identical exposure to the SOA and citric acid solutions as did the Experimental group, except that the solutions were each paired with IG water infusions. Half of the rats in the Control group were given the SOA solution on Day 1 of training and the citric acid solution on Day 2 of training; the order of presentation was reversed for the remaining rats in the Control group. The solutions that the Control group received on the Experimental group's CS+ training days were designated as "CS+" solutions even though the Control group was always infused IG with water; likewise, the solutions

that were given to the Control group on the Experimental group's CS– training days were designated as "CS–" solutions.

After the second preference test, both groups of rats were given a two-bottle test between one CS solution and plain water for two days, and then a two-bottle test between the other CS solution and plain water for two days. Specifically, half of the rats from each group were given a CS+ vs. water test followed by a CS– vs. water test; the rest of the rats were tested in the reverse order. Following these last four days of testing, the animals were disconnected from the infusion system and placed back in their home cages. They were maintained on ad libitum water (not paired with any infusions) and chow.

Taste reactivity test phase. Subsequent to the end of the CS vs. water tests, both groups of rats were rehabituated to the taste reactivity test chamber. The rats were individually placed in the test chamber and left alone for five minutes; they were then each infused IO with tap water for one minute. On the following day, this procedure was repeated. On the third day, the taste reactivity test was administered. Each rat was placed in the chamber for five minutes and then given a one-minute IO infusion (at a rate of 1 ml/minute) of a particular CS solution (CS+ or CS–). On the following day, the taste reactivity test was repeated except that the rat received a one-minute IO infusion of the other CS solution (CS– or CS+). Half of the rats were infused with the CS+ on day 1 and then with the CS– on day 2; the order was reversed for the remaining rats.

Home-cage short-term intake test phase.

One-bottle tests with CS+ and CS–. Following the taste reactivity test phase, the Experimental group and the Control group were given daily one-bottle tests with the CS+ and CS– solutions in their home cages. On days 1, 3, 5, 7, 9, 11, and 13, half of the rats were given a 30-minute one-bottle test with the CS+; the rest received the CS– for 30 minutes. On days 2, 4, 6, 8, 10, 12, and 14, 30-minute access was given to the other CS solution. Consumption of the CS solutions was not paired with IG infusions of Polycose or water. All the rats had ad libitum access to chow; however, chow was not available during the 30-minute tests.

On the following 10 days, the rats were given restricted chow rations in order to examine whether the rats would increase their consumption of the CS solutions in the short-term tests. Each animal received half of the average amount of chow the same rat consumed on ad libitum days. During the food-restricted days, the rats were given 30-minute access to the CS+ and CS– solutions on alternate days. Hence, on days 15, 17, 19, 21, and 23, half of the rats received the CS+ solution for 30 minutes; the rest received the CS–; and on days 16, 18, 20, 22, and 24, 30-minute access was given to the other CS solution.

This same schedule of testing was continued on days 25 through 28, except that the rats were returned to ad libitum schedule of chow feeding.

One-bottle testing with the CS+ and plain water. Following the one-bottle testing period with the CS+ and CS–, the animals received one-bottle tests with the CS+ and plain water in order to determine if the rats would exhibit increased

acceptance of the CS+ relative to water. On days 29, 31, and 33, all the rats were given a 30-minute test with plain water, and on days 30, 32, and 34, the rats were given 30-minute access to the CS+ solution. The rats continued to have ad lib access to chow, except during the 30-minute tests.

Home-cage long-term intake test phase.

One-bottle testing. Subsequent to the short-term testing period, the rats received 24-hour one-bottle access to the CS+ solution and plain water on alternate days in order to assess long-term increased acceptance of the CS+. On days 35, 37, and 39, the rats were given the CS+; on days 36, 38, and 40, they were given access to plain water. On the following six days, the rats were given 24-hour one-bottle access to the CS– and water on alternate days.

Two-bottle testing. The rats then received 24-hour two-bottle preference tests with the CS+ and plain water for eight days and then the CS+ and CS– for the following eight days.

Data Analysis

The data collected during the conditioning and testing phase were analyzed as in the previous experiments.

All video taped records were scored in slow motion and frame by frame. Each test session was scored without knowledge of which CS solution was being presented. Table 3 describes four ingestive behaviors and four aversive behaviors. There are six additional categories that involve noningestive behavior: paw licking and face washing (each considered to be a grooming response); locomotion; passive drip (a neutral-to-

Table 3: **Taste Reactivity Behavior** (Adapted and modified from Spector, Breslin, & Grill, 1988)

BEHAVIOR	DESCRIPTION
Ingestive	
Tongue protrusion (TP)	Rhythmic protrusions of the tongue.
Lateral tongue protrusion (LTP)	Asymmetric parting of the lips; nonrhythmic protrusions of the tongue.
Mouth movement (MM)	Low amplitude, rhythmic openings of the mandible; looks like chewing.
Lip flair (LF)	Flaring of the portion of the lips overlying the upper incisors; the head moves up.
Aversive	
Gape (G)	Corner of mouth retracts; the mouth has a triangular shape.
Chin rub (CR)	The mouth is brought into direct contact with the substrate, and the body is projected forward by flexion of the dorsal neck and forelimb musculature. Fluid is often deposited onto the substrate during this action.
Forelimb flail (FF)	A burst of high frequency movements of one or both forelimbs.
Head shake (HS)	A burst of high frequency side-to-side movements of the head.
Other	
Paw licking (PL)	Rhythmic extensions of the tongue along the midline toward the forepaws, which were held either in front of the face or on the floor while being licked; this response was generally considered a grooming response; scored in terms of duration.
Face washing (FW)	Front paws are wiped over the top of head while rearing on hindquarters; this response was generally considered a grooming response.
Locomotion (L)	Quadrupedal movement in test chamber, given that mouth is still in view of video camera; scored in terms of duration.
Passive drip (PD)	Accumulation of fluid on the tip of the lower mandible; fluid drips on the substrate in the absence of any other overt behavior; scored in terms of duration.
No response (NR)	Absence of oral behavior for at least 1 sec.
No data (ND)	No data (≥1 sec) due to technical problems or rat movement out of view of video camera.

weak aversive response); no response; and no data. All the categories were scored but only the ingestive and aversive behaviors were included in the analysis.

The occurrence of each taste reactivity response was summed for each rat. The mean number of each type of response for each group of rats was then calculated. The analysis of these behaviors was divided into two scores, an ingestive score and an aversive score. The ingestive score represented the combined total of mouth movements, tongue protrusions, lateral tongue protrusions, and lip flares. The aversive score represented the combined total of gapes, chin rubs, head shakes, and forelimb flails. Means of these scores were computed for each group. The data were analyzed using multivariate analysis of variance and repeated measures analysis of variance followed by simple main effects tests and Newman-Keuls tests, where appropriate.

The data collected during the home-cage testing phases were analyzed in the same manner as were the data from the conditioning and testing phase.

Results

Conditioning and testing

CS intakes. Figure 18 summarizes the results of the one-bottle training and two-bottle preference tests between the CS+ and CS–. During the first training cycle, the Experimental group consumed slightly more CS– than CS+ solution; however, as CS– intake during training decreased from cycle 1 to cycle 2 [$F(1,15) = 15.2$, $p<.001$], CS+ increased substantially [$F(1,15) = 39.2$, $p<.001$]. As a result, CS+ intake exceeded CS– intake in the second training cycle [$F(1,15) = 38.9$,

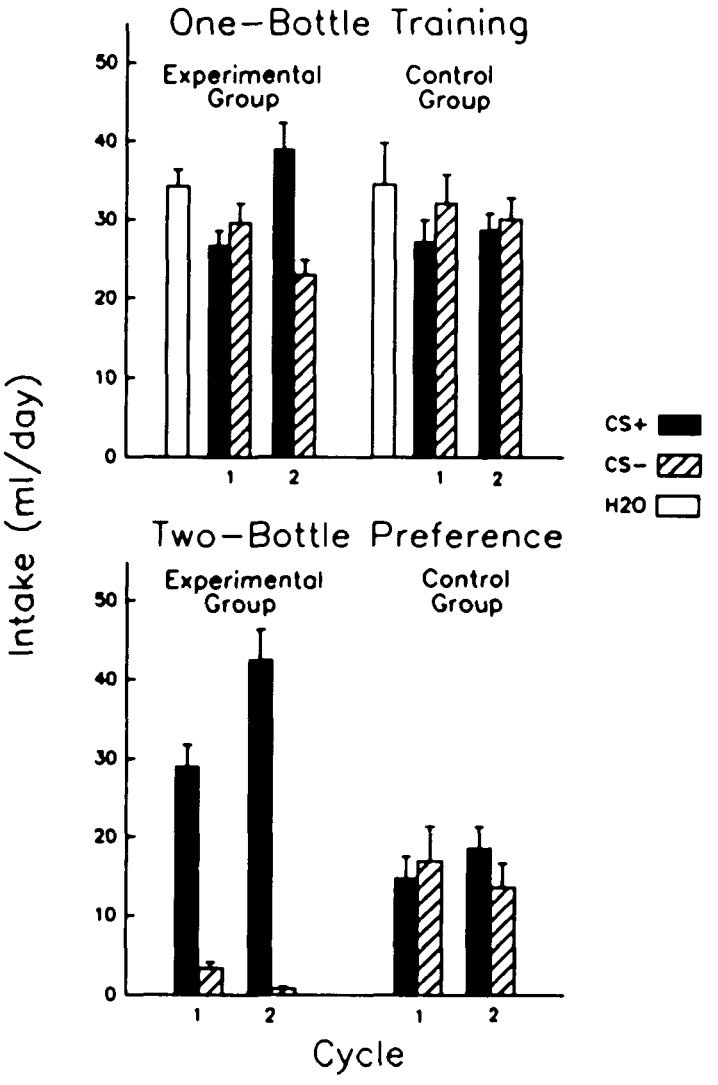

Figure 18. Experiment 3: Conditioning and testing phase. Mean (+SE) oral intake of CS+ and CS– during cycles 1 and 2 of the one-bottle training and two-bottle preference tests; each bar represents a mean of two days. For the Experimental group the CS+ and CS– were SOA- or citric acid-flavored water; the CS+ and CS– were paired with IG Polycose infusions and IG water infusions, respectively. The Control group received SOA- or citric acid-flavored water, each paired with IG water infusions.

$p<.001$]. The Experimental group's CS+ intake during the first training cycle was less than their water baseline intake, but by the second training cycle CS+ intake was greater than their water baseline intake [$F(2,30) = 15.5$, $p<.001$]. In contrast, their CS– intake during both training cycles was reliably less than their water baseline intake [$F(2,30) = 25.0$, $p<.001$].

The Control group consumed slightly, but not reliably, more CS– than CS+ during the first training cycle and consumed about the same amounts of both solutions during the second cycle. In addition, the Control group's intakes of the CS solutions during training was less than their water baseline intake, although these differences were not significant.

Absolute intakes of the two groups during the first training cycle did not reliably differ. However, by the second training cycle, the Experimental group consumed more CS+ solution than did the Control group [$F(1,35) = 6.1$, $p<.05$]. In addition, the Control group consumed more CS– than did the Experimental group during the second training cycle but this difference was not significant.

During the two-bottle preference tests, the Experimental group consumed more CS+ than CS– [$F(1,15) = 117.0$, $p<.001$], and this difference increased from cycle 1 to cycle 2 [$F(1,15) = 31.5$, $p<.001$]. The Experimental group's percent CS+ intake during the two preference tests was 88.0% in cycle 1 and 97.4% in cycle 2.

In contrast, the Control group's intakes of the CS+ and CS– during both preference tests were not reliably different. Note that both the CS+ and CS– solutions were paired with IG water infusions. The Control group's percent CS+

intake was 51.8% during the first preference test and 58.8% during the second preference test. In addition, analysis of the Control group's intake in terms of SOA and citric acid solution intake during the preference tests revealed no reliable differences in intake; percent SOA intake was 42.4% in the first preference test and 50.2% in the second preference test.

Between-group comparisons revealed that the Experimental group consumed more CS+ [$F(1,41) = 29.3$, $p<.001$], but less CS– [$F(1,41) = 13.8$, $p<.001$], than did the Control group.

The results of the CS vs. water tests are shown in Figure 19. During the CS+ vs. water test, the Experimental group consumed more CS+ than plain water [$F(1,15) = 142.4$, $p<.001$]; in contrast, they consumed more water than CS– in the CS– vs. water test [$F(1,15) = 30.4$, $p<.001$]. In addition, Newman-Keuls test revealed that the Experimental group's CS+ intake exceeded both CS– and water intake in the CS– vs. water test, $p<.01$. The Experimental group's percent CS+ and CS– intakes were 96.0% and 4.6%, respectively.

The Control group displayed a water preference during the CS+ vs. water test as well as during the CS– vs. water test [$F(1,7) = 16.7$, $p<.01$]. Absolute intake did not differ across both tests. The Control group's percent CS+ intake was 35.1% and their percent CS– intake was 22.2%.

Between-group comparisons found that the Experimental group consumed more CS+, but less water, during the CS+ vs. water test than did the Control group [$F(1,42) = 60.3$, $p<.001$, and $F(1,42) = 25.6$, $p<.001$, respectively]. During the CS–

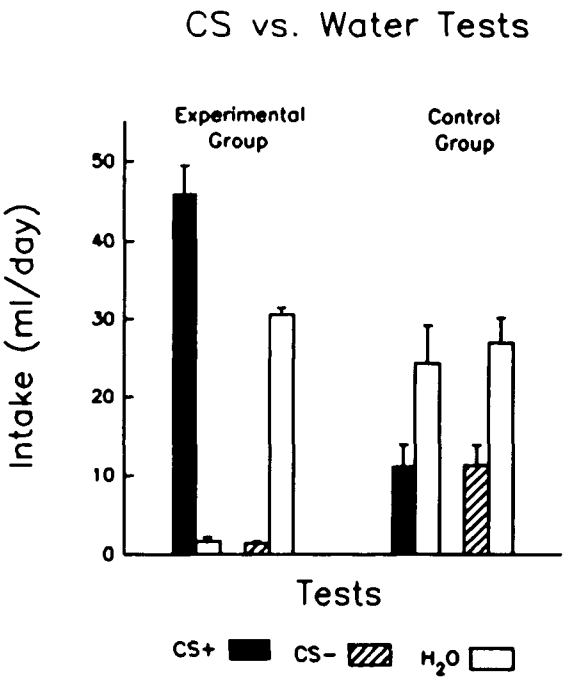

Figure 19. Experiment 3: Mean (+SE) oral intake of the CS+ and plain water, and of the CS– and plain water during two-bottle preference tests; each bar represents a mean of two days. For the Experimental group, the CS+ and CS– were SOA- or citric acid-flavored water; the CS+ was paired with IG Polycose infusions, and the CS– and plain water were paired with IG water infusions. The Control group received SOA-flavored water vs. plain water and citric acid-flavored water vs. plain water; all fluid intake for this group was paired with IG water infusions.

vs. water test, the Experimental group consumed less CS– than did the Control group [$F(1,44) = 6.9$, $p<.05$]; however, there were no group differences in water intake during this test.

Caloric intake. Table 4 summarizes the kilocalorie intake during training. When infused with Polycose on CS+ training days, the rats in the Experimental group reduced their chow intake but not to a level that compensated for the kilocalorie intake from the IG Polycose. As a result, the Experimental group's total kilocalorie intake on CS+ training days during cycles 1 and 2 exceeded their intake during the water baseline period [$F(2,30) = 69.5$, $p<.001$]. In addition, total kilocalorie intake on CS+ training days increased from cycle 1 to 2 [$F(1,15) = 18.7$, $p<.001$]. The Experimental group's caloric intake on CS– training days was less than their caloric intake on CS+ training days, [$F(1,15) = 239.6$, $p<.001$]. Also, kilocalorie intake on CS– training days decreased from cycle 1 to 2 [$F(1,15) = 10.4$, $p<.01$]. The Experimental group's caloric intake on CS– training days in the first cycle did not reliably differ from their water baseline level of intake, but by the second cycle caloric intake on CS– training days was less than water baseline intake [$F(2,30) = 9.9$, $p<.001$].

The Control group's kilocalorie intake on CS+ and CS– training days did not reliably differ; note that the CS+ and CS– were paired with IG water infusions. In addition, kilocalorie intake during training did not significantly differ from water baseline intake.

Between-group comparisons revealed that the Experimental group consumed

Table 4. Experiment 3: Mean (±SE) kilocalorie intake of the Experimental and Control groups during one-bottle training trials.

		Water Baseline	Cycle 1		Cycle 2	
			CS+	CS–	CS+	CS–
Exp'l Group	Chow	58.5 ±2.9	51.8 ±2.9	54.9 ±2.4	38.6 ±2.5	48.4 ±1.8
	Polycose		33.5 ±1.8		57.4 ±3.4	
	Total	58.5 ±2.9	85.3 ±2.2	54.9 ±2.4	96.0 ±2.8	48.4 ±1.8
Control Group	Chow	60.2 ±5.5	59.7 ±3.1	59.0 ±5.1	56.1 ±2.5	60.1 ±3.6
	Polycose					
	Total	60.2 ±5.5	59.7 ±3.1	59.0 ±5.1	56.1 ±2.5	60.1 ±3.6

more kilocalories on CS+ training days than did the Control group in cycle 1 as well as in cycle 2 [$F(1,52) = 29.8$, $p<.001$, and $F(1,52) = 72.2$, $p<.001$, respectively]. In contrast, while the two groups' caloric intakes on CS– training days in cycle 1 did not reliably differ, the Experimental group took in fewer calories in cycle 2 than did the Control group [$F(1,44) = 8.5$, $p<.01$].

Taste Reactivity Test

The results from the taste reactivity test in terms of an ingestive score and an aversive score are illustrated in Figure 20; Table 5 summarizes the mean number of occurrences of each type of FAP elicited. The data of one rat from the Control group were discarded because the number of ingestive FAPs she made to the SOA solution (234 ingestive FAPs) was vastly greater than the rest of the rats in the group, thereby greatly inflating the group mean.

The Experimental group displayed more ingestive than aversive FAPs to both CS solutions [$F(1,15) = 31.4$, $p<.001$]. In addition, the Experimental group made more ingestive and fewer aversive FAPs to the CS+ than CS–, but these differences were not significant. Like the Experimental group, the Control group exhibited more ingestive than aversive FAPs to the CS solutions [$F(1,6) = 16.3$, $p<.01$], but the number of FAPs made to the CS+ and CS– did not reliably differ. Between-group comparisons revealed that the Experimental group displayed a greater number of FAPs, overall, than did the Control group [$F(1,21) = 6.2$, $p<.05$].

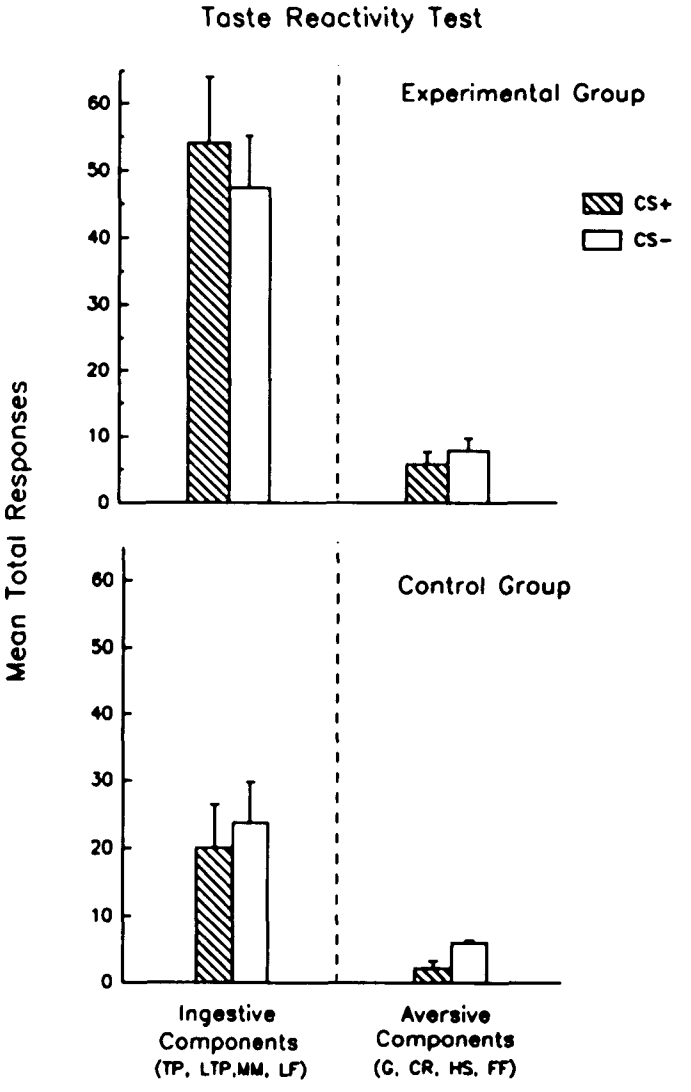

Figure 20. Experiment 3: Taste reactivity profiles. Taste-elicited responses to oral infusions of the CS+ and CS–; the CS+ and CS– were SOA- or citric acid-flavored water. Response categories: TP, tongue protrusions; LTP, lateral tongue protrusions; MM, mouth movements; LF, lip flaring; G, gapes; CR, chin rubs; FF, forelimb flailing; HS, head shakes.

Table 5. Experiment 3: Mean (±SE) number of FAPs displayed by the Experimental and Control groups to the CS+ and CS– solutions during the taste reactivity test.

CS+ Solution	Ingestive FAPs				Aversive FAPs				Other FAPs			
	TP	LTP	MM	LF	G	CR	FF	HS	PL	FW	L	PD
Experimental Group	23.4 ±4.6	13.2 ±3.2	16.6 ±4.4	0.8 ±0.4	3.4 ±1.8	0.0 ±0.0	1.8 ±0.7	0.5 ±0.2	6.6 ±2.1	5.5 ±1.4	16.5 ±2.2	0.0 ±0.0
Control Group	12.3 ±4.4	3.3 ±1.5	3.7 ±1.6	0.7 ±0.4	0.6 ±0.6	0.0 ±0.0	1.0 ±0.7	0.6 ±0.4	8.0 ±5.0	6.9 ±3.2	9.0 ±2.9	4.3 ±4.0

CS– Solution	Ingestive FAPs				Aversive FAPs				Other FAPs			
	TP	LTP	MM	LF	G	CR	FF	HS	PL	FW	L	PD
Experimental Group	25.2 ±4.1	10.4 ±2.4	11.3 ±3.6	0.4 ±0.2	2.6 ±0.9	0.0 ±0.0	4.3 ±1.1	0.9 ±0.3	13.4 ±3.3	9.3 ±2.5	12.5 ±1.7	0.3 ±0.3
Control Group	12.0 ±3.3	4.7 ±2.1	5.9 ±2.4	1.1 ±0.6	0.1 ±0.1	0.0 ±0.0	0.7 ±0.4	0.1 ±0.1	10.3 ±5.0	10.0 ±5.3	14.0 ±3.0	1.3 ±1.3

Home-cage short-term intake tests

The results of the short-term tests are illustrated in Figure 21. The Experimental group consumed more CS+ than CS– during the initial one-bottle tests [$F(1,14) = 41.1$, $p<.001$]. During the deprivation phase that followed, the Experimental group continued to consume more CS+ than CS– in the one-bottle tests [$F(1,14) = 22.4$, $p<.001$]. However, their CS+ intake decreased during the deprivation phase and failed to return to its predeprivation level of intake once ad libitum chow was restored [$F(2,28) = 8.1$, $p<.01$]. Nevertheless, the Experimental group's CS+ intake exceeded their CS– intake during all three deprivation conditions [$F(1,14) = 35.5$, $p<.001$]. The Experimental group's CS– intake did not change with deprivation.

The Control group consumed comparable amounts of the CS+ and CS– during the three deprivation conditions. However, when the Control group was food-restricted, they slightly decreased their intake of the two solutions, and, when ad libitum chow was restored, their overall solution intake was slightly greater than their predeprivation level of intake. Statistical analysis revealed that intake during the deprivation phase was not reliably less than predeprivation intake, but it was significantly lower than the rats' postdeprivation intake level [$F(2,12) = 5.2$, $p<.05$; see Figure 21].

Between-group comparisons found that the Experimental group consumed more CS+ than did the Control group [$F(1,39) = 16.0$, $p<.001$]; but both groups consumed comparable amounts of the CS–. While the Experimental group consumed

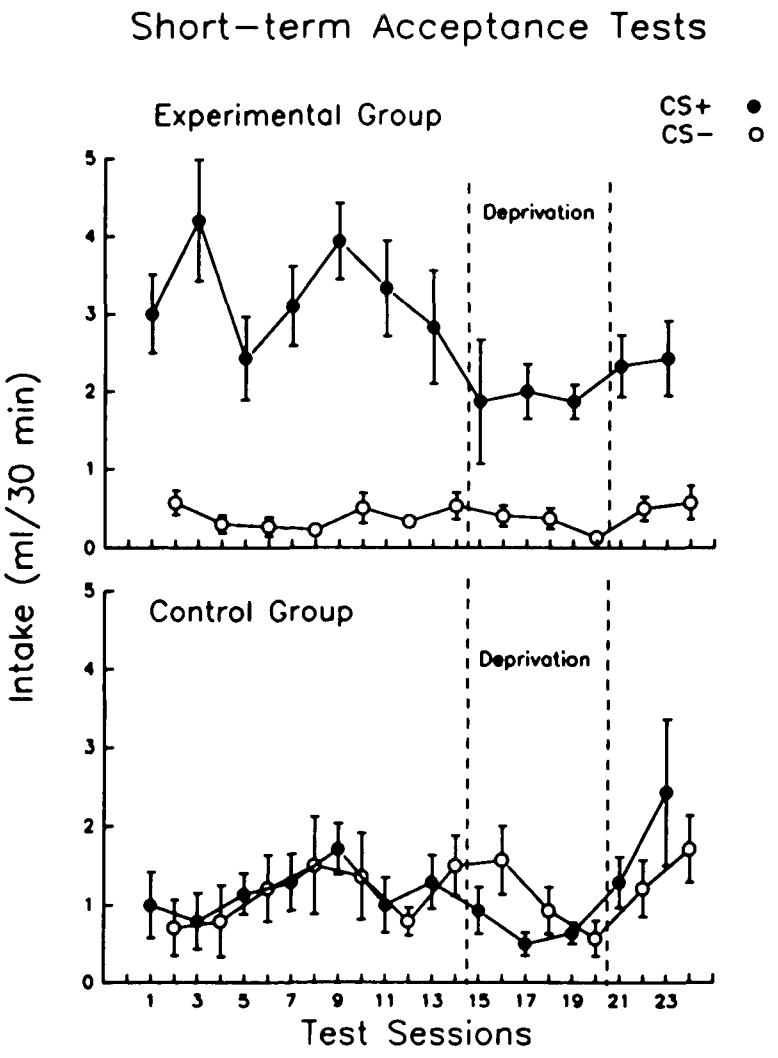

Figure 21. Experiment 3: Mean (±SE) intake of CS+ and CS– during 30-minute one-bottle tests. Fluid intakes were not paired with IG infusions.

more CS+ than CS– during the one-bottle tests [$F(1,20) = 7.8$, $p<.05$], the Control group's intake of the CS+ and CS– did not differ.

The results of the one-bottle tests between the CS+ and plain water are shown in Figure 22. The Experimental group consumed more CS+ than water during the one-bottle tests [$F(1,19) = 18.6$, $p<.001$]. In addition, while the Experimental group's CS+ intake did not reliably differ across the tests, their water intake decreased significantly from tests 1 to 3 [$F(2,28) = 5.5$, $p<.01$]. In contrast, the Control group's intake of the CS+ and water did not reliably differ. Between-group comparisons revealed that the Experimental group drank more CS+ than did the Control group[$F(1,38) = 19.8$, $p<.001$], while the two groups' intakes of plain water did not reliably differ.

Home-cage long-term tests

One-bottle tests. The results of the one-bottle tests with the CS+ solution and plain water, as well as with the CS– solution and plain water, are illustrated in Figure 23. During the one-bottle tests, the Experimental group consumed more CS+ than plain water [$F(1,19) = 30.6$, $p<.001$], but comparable amounts of the CS– and plain water. Comparisons of the CS+ and CS– intakes revealed that the Experimental group consumed more CS+ than CS– [$F(1,19) = 24.8$, $p<.001$]. The Experimental group's water intake did not reliably change across tests.

The Control group consumed more CS+ than water, but this difference failed to be significant ($p<.06$). The Control group also consumed slightly, but not reliably, more CS– than water during the one-bottle tests.

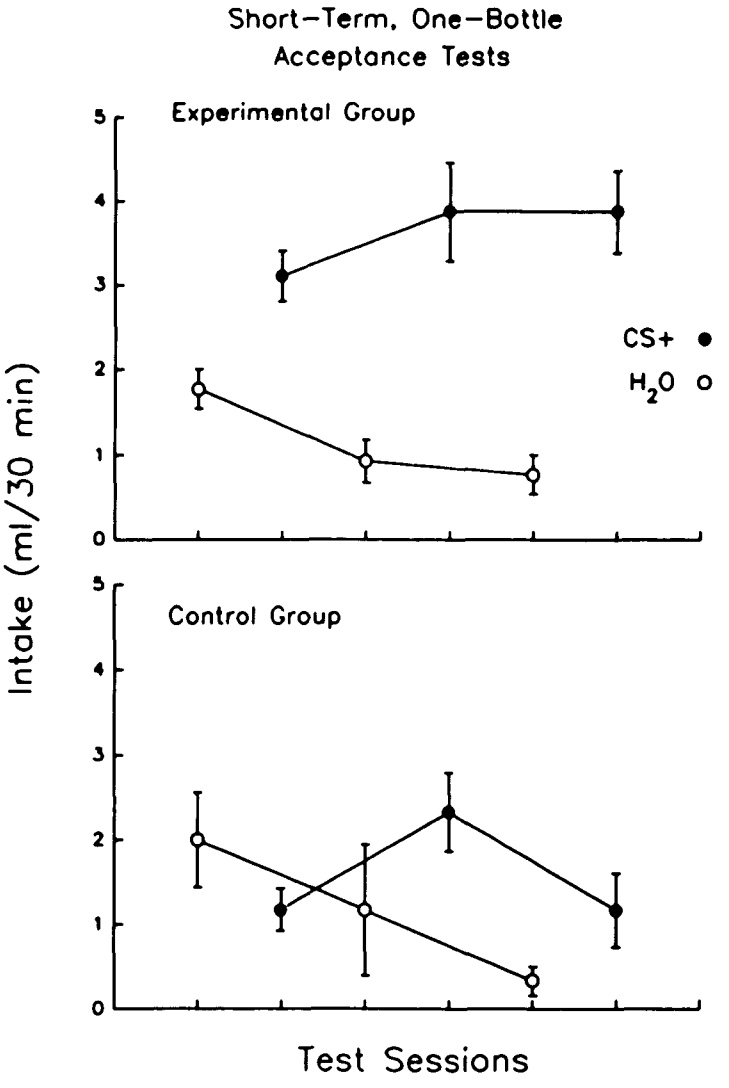

Figure 22. Experiment 3. Mean (±SE) intake of CS+ and plain water during 30-minute one-bottle tests in home cages. Note that fluid intake was not associated with IG infusions.

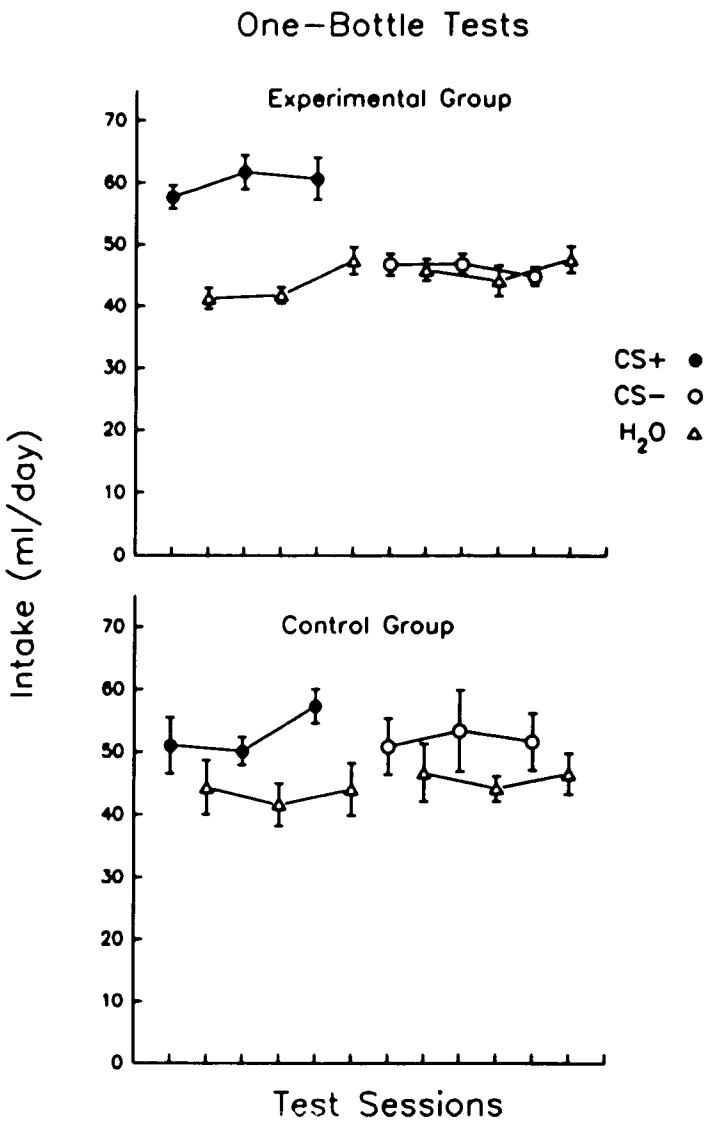

Figure 23. Experiment 3. Mean (±SE) intake of CS+ and water and of the CS– and water during 24-hour one-bottle tests in home cages. Note that fluid intake was not paired with IG infusions.

Between-group comparisons found that the Experimental group consumed marginally more CS+ than did the Control group ($p<.053$). The two groups did not reliably differ in their CS– and water intakes.

Two-bottle tests. The results of the CS+ vs. water tests are shown in Figure 24. The Experimental group consumed more CS+ than water during the two-bottle tests [$F(1,14) = 10.7$, $p<.01$]. The Experimental group's mean percent CS+ intake across the two-bottle tests was 65.0%. In contrast, the Control group consumed more water than CS+ across the two-bottle tests (except for the first one), but these differences were not significant ($p<.07$). Mean percent CS+ intake for the Control group was 39.7%. Between-group comparisons revealed that the Experimental group consumed more CS+, but less water, than did the Control group [$F(1,30) = 9.3$, $p<.01$, and $F(1,30) = 5.6$, $p<.05$, respectively].

Figure 25 summarizes the results of the CS+ vs. CS– preference tests. The Experimental group consumed reliably more CS+ than CS– during the preference tests [$F(1,14) = 11.9$, $p<.01$]. Mean percent CS+ intake was 66.3% for the Experimental group. The Control group, on the other hand, consumed comparable amounts of the CS+ and CS–; mean percent CS+ intake was 50.9%. There were no reliable between-group differences in absolute intake.

Discussion

The results of Experiment 3 have confirmed previous findings that rats acquire very strong preferences for flavors associated with IG Polycose infusions. The Experimental group displayed a strong (88%) preference for the CS+ after

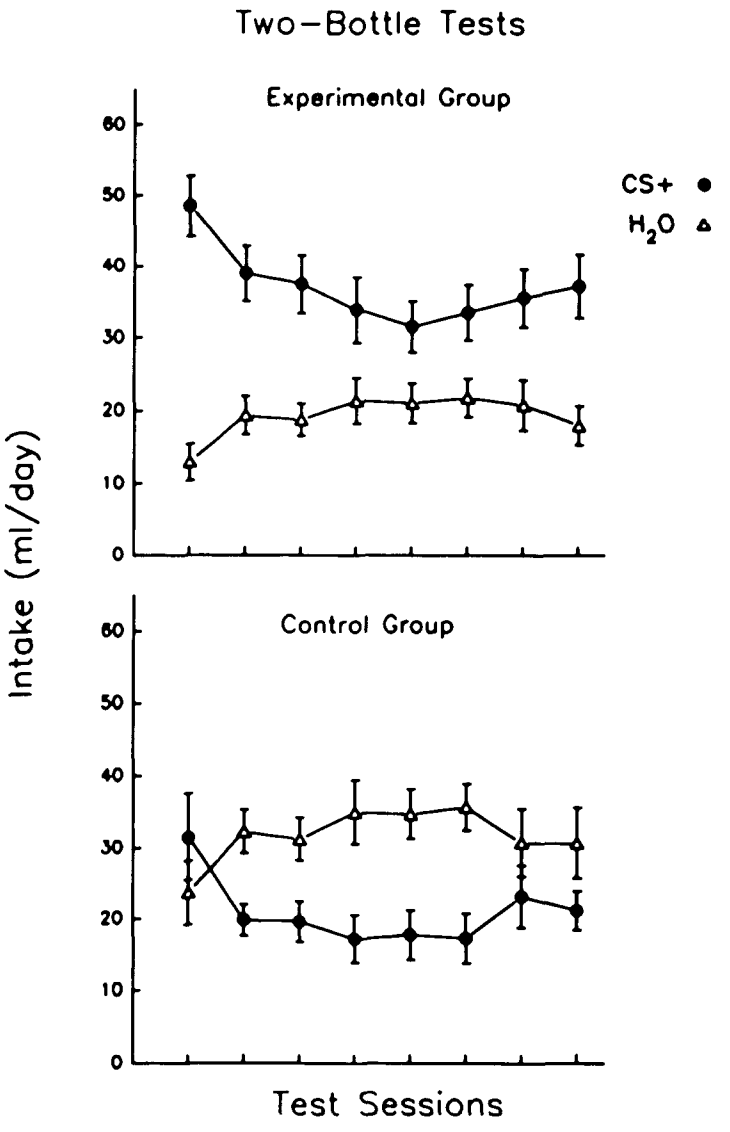

Figure 24. Experiment 3. Mean (±SE) intake of CS+ and plain water during 24-hour two-bottle tests in home cages. Note that fluid intake was not associated with IG infusions.

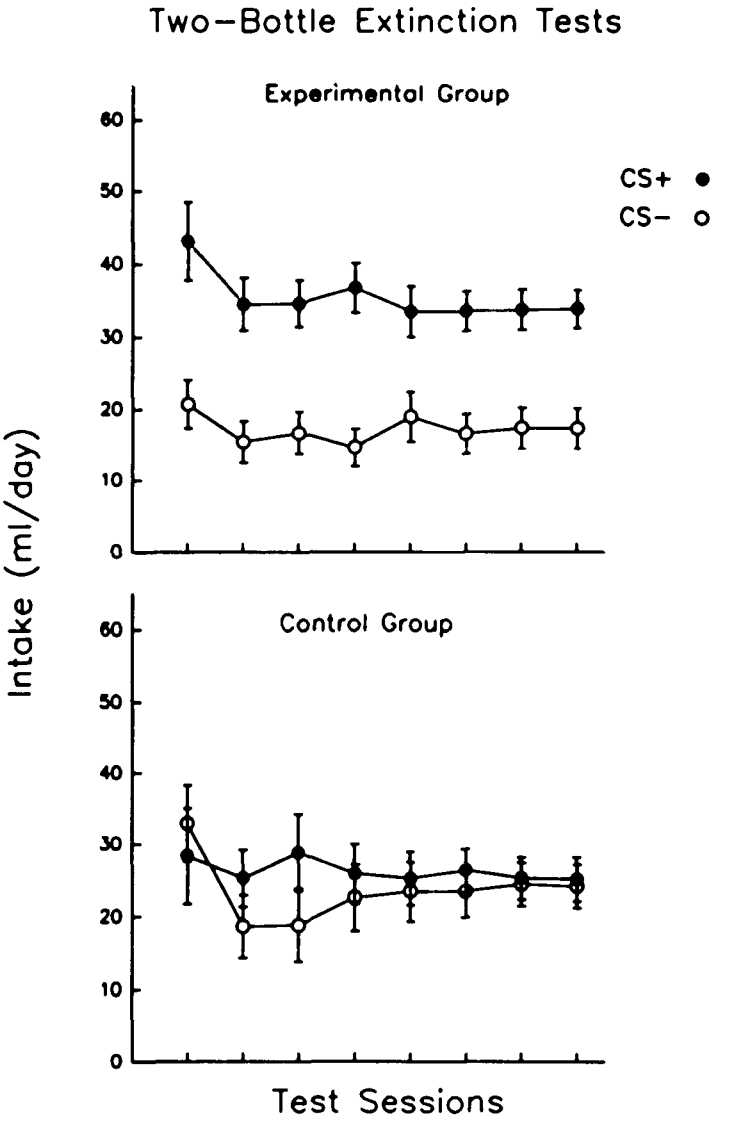

Figure 25. Experiment 3. Mean (±SE) intake of CS+ and CS– during 24-hour two-bottle tests in home cages. Note that fluid intake was not associated with IG infusions.

only four days of training, and this preference was strengthened by the end of the second training-test cycle (97%). The Control group, of course, exhibited no such preference since both the CS+ and CS– solutions were paired with IG water infusions. The rate of preference conditioning manifested by the Experimental group was much faster than that exhibited by the rats in the previous experiments. The rats in Experiment 1 displayed a 78% preference for the CS+ in cycle 1, and this preference increased to 90% in cycle 2. In Experiment 2, the KA/P group displayed a 74% CS+ preference in cycle 1 and a 92% preference in cycle 2, and the KA group displayed a 74% and a 95% CS+ preference, respectively, during cycles 1 and 2. The difference in rate of conditioning can be attributed to the CS flavors used. In Experiments 1 and 2, the grape and cherry Kool-Aid flavors, which share the key ingredient citric acid, were used. SOA and citric acid, which are quite distinct from each other, were the CS flavors used in the present experiment. The more discriminable that the CS+ and CS– are, the more that conditioning will be facilitated (see Mackintosh, 1974). It should be noted that Sclafani and Nissenbaum (1988) reported a 96% CS+ preference after one training cycle; however, in that study the rats were reinforced with 16% Polycose, whereas the rats in the present study were reinforced with 32% Polycose. Thus, it appears that more distinct CS cues facilitate the formation of a CS+ preference when more concentrated Polycose solutions are used.

Not only did the Experimental group strongly prefer the CS+ over the CS–, they also preferred the CS+ over water. In addition, the Experimental group avoided

the CS– (like the KA group did in Experiment 2), more so than did the Control group. The Control group preferred to drink plain water over both the CS+ and CS– solutions. Also, the Experimental group continued to prefer the CS+ over the CS– and over water during the home-cage long-term extinction tests. These preferences (65-66% CS+ preference) were substantially weaker than those exhibited in the test apparatus (97% CS+ preference), but these tests were conducted after the rats had had extensive one-bottle extinction experience. Nevertheless, the Experimental group continued to significantly prefer the CS+ over the CS– after 62 days of not receiving an IG infusion of Polycose.

One surprising result was that the Experimental group's CS+ intake during training exceeded baseline water intake. In Experiment 1, the rats exhibited a strong preference for the CS+ over the CS– as well as over plain water; however, those rats did not overconsume the CS+ solution. Experiment 2 sought to condition increased acceptance of the CS+ by training rats with a CS solution which was preferred over water. This contrasted the use of relatively unpalatable CS solutions in prior experiments (Experiment 1; Sclafani & Nissenbaum, 1988). When trained with the more palatable CS solutions (relative to water and to the CS solutions used in Experiment 1), the rats failed to exhibit increased acceptance of the CS+. Increased acceptance of the CS+ was only manifested when the CS+ had previously been associated with the sweet taste of saccharin (see Experiment 2B as well as 1D). The major difference between the first two experiments and the present experiment was the flavors used. The Kool-Aid flavors were quite satisfactory for the purposes of

conditioning robust preferences. However, these flavors were not used in the present experiment because they have both taste and olfactory properties. The taste reactivity test requires the use of taste cues since the solutions are infused directly into the oral cavity, thereby bypassing the olfactory system. Therefore, SOA and citric acid were chosen as the CS flavors. Apparently, SOA and citric acid are more salient stimuli for the conditioning of an increased acceptance. Irrespective of the underlying cause, the Experimental group did manifest increased acceptance of the CS+ compared to water baseline intake, whereas the Control group's CS+ and CS– intakes during training were both comparable to their baseline water intake. In addition, the Experimental group's CS+ intake exceeded the Control group's CS+ intake. The Experimental group also exhibited increased acceptance of the CS+ during the CS+ vs. water tests.

The results of the home-cage tests provide additional information concerning the increased acceptance of the CS+, specifically that the increased acceptance was still manifested in the short-term as well as the long-term tests. The purpose of the short-term tests was to see if the rats would, in fact, consume the CS solutions in a 30-minute period and if they would differentially consume the two solutions. The results of the short-term tests in the home cages paralleled the results of the one-bottle tests during training. During the 30-minute tests, the Experimental group exhibited increased acceptance of the CS+. The Experimental group decreased their intake of the CS+ when they were tested under food deprivation conditions. This contrasts with the finding that food-deprived rats increase their intake of sugar,

saccharin, Polycose, corn oil emulsions, and mineral oil emulsions during short-term tests (Ackroff, Vigorito, and Sclafani, in press; Sclafani, 1987a). The Experimental group's decrease in CS+ intake when deprived suggests that there is a difference between acquired vs. innate preference tests. Alternatively, the failure to obtain an increase in CS+ intake due to deprivation may have resulted from the rats' extinction experience. Ackroff and Sclafani (unpublished observations) found that rats given four-hour tests with the CS+ paired with IG Polycose infusions and the CS– paired with IG water infusions exhibited a 43% increase in intake when deprived of chow. Therefore, it appears that an extinction process contributed to the failure to obtain an increase in CS+ intake under deprivation.

The Experimental group's CS+ intake also exceeded water intake during the home-cage 24-hour one-bottle tests; this group's CS– and water intake did not differ. The Experimental group differed from the Control group in that the latter consumed comparable amounts of the CS+ and water and of the CS– and water during the one-bottle tests.

Even though the rats in the Experimental group exhibited a robust preference for the CS+ over the CS– and water and exhibited increased acceptance of the CS+, the results of the taste reactivity test do not support the hypothesis that the conditioning process involves an increase in the palatability of the CS+. The Experimental group, like the Control group, made comparable orofacial responses to the CS+ and CS–. One of the few significant effects was that the rats, overall, made more ingestive than aversive responses. In addition, the Experimental group made

more responses, overall, than did the Control group. The finding that the Experimental group exhibited more FAPs to the CS+ than did the Control group can be interpreted as being due to the conditioning experience. However, why the Experimental group also exhibited more FAPs to the CS– is quite puzzling.

To date, only two studies have reported reliable effects with the taste reactivity test in conditioned flavor preference paradigms. But the paradigms used differed greatly from that used in the present study. Zellner et al. (1985) examined preferences based on an association made between taste and drug effects. Specifically, morphine-raised rats exhibited more ingestive and fewer aversive FAPs to the taste of morphine than did water-raised rats. Another study looked at preferences based on associations made between a CS flavor and an innately preferred taste (Breslin, Davidson, & Grill, 1990). Breslin et al. (1990) used the taste reactivity method and successfully conditioned a reversal of orofacial responses to normally avoided tastes, specifically, quinine and hydrochloric acid (HCl). The investigators paired an IO infusion of a CS+ solution with an IO infusion of a sucrose solution; an infusion of the CS– was followed by nothing. Both quinine and HCl are avoided tastes and produce aversive orofacial responses in rats. When the normally avoided taste (CS+) signalled a preferred taste (sucrose), the aversive responses produced by the avoided taste decreased over time, while the ingestive responses increased. In contrast, the orofacial responses made to the CS– remained unchanged.

The present experiment involved taste-nutrient conditioning; that is, a CS+ flavor was paired with IG infusions of calorically-rich Polycose infusions. This paradigm contrasts the taste-drug and taste-taste paradigms just described. Therefore, the taste reactivity test results may have differed because the CS–US pairings differed. Another difference involved the number of CSs used. Some of the previous conditioning studies using the taste reactivity test have typically involved the presentation of only one CS (e.g., sucrose) (Berridge, Grill, & Norgren, 1981; Grill, 1975; Spector et al., 1988). Experiments 1 through 3 have involved the use of two stimuli which were experienced 24 hours a day for at least 16 days. If the present experiment only had the CS+ flavor and no CS– flavor (as was done in other studies), and a comparison were to be made between the number of ingestive FAPs made by the Experimental group and the Control group, one would see that the Experimental group exhibited more FAPs. One may arrive at a different conclusion than when there is a CS– as well. Therefore, more research has to be done before the hypothesis that the development of the CS+ preference reported in Experiments 1 to 3 involves an increase in the palatability of the CS+ can be rejected. For example, flavor-nutrient conditioning could be directly compared with flavor-flavor conditioning. Comparison criteria could include: number of ingestive FAPs relative to the number of aversive FAPs, in addition to strength of preference, short-term intake, and resistance to extinction.

The results of the taste reactivity test contrast with the results of the short-term one-bottle intake tests. The rats in the Experimental group voluntarily

consumed the CS+ during the 30-minute tests, and the amount they consumed exceeded water baseline levels. In addition, the Experimental group's CS– intake during the 30-minute tests was less than they "involuntarily" consumed during the one-minute taste reactivity test. In other words, the rats consumed more CS+ but less CS– than the 1 ml of solution that was intraorally infused. The willingness of non-hungry rats to drink solutions during short-term tests has been taken as evidence that the solutions are palatable to the rats (Sclafani, 1987a). Therefore, the results of the short-term tests suggest that the conditioning process increased the palatability of the CS+. More research is needed to clearly understand the mechanisms underlying the conditioning process. Nevertheless, the evidence accumulated so far unambiguously demonstrates that rats acquire strong preferences for flavors associated with IG Polycose infusions, and these preferences are resistant to extinction. In addition, as shown in the present experiment, increased acceptance of the CS+ can be conditioned, at least with the SOA and citric acid flavors.

GENERAL DISCUSSION

The results of this study have provided extensive evidence that the postingestive effects of nutrients can condition robust flavor preferences in rats, and that, once acquired, these preferences are not dependent on continued reinforcement. This study also provides new information about the differences between learned and innate preferences as well as the role of orosensory and postingestive factors in the control of caloric intake. In addition, the effect of palatability on flavor preference

as well as flavor acceptance was assessed. Finally, the issue of whether the flavor preference conditioning process involves an acquired liking to the CS+ flavor was also examined. Each of these issues will be discussed below.

Conditioned Flavor Preference. The results have confirmed previous findings (Sclafani & Nissenbaum, 1988) that rats acquire very strong preferences for flavors associated with IG infusions of Polycose. Compared to Sclafani and Nissenbaum's (1988) study which used 16% Polycose as the infusate, the rate of preference conditioning using 32% Polycose was slower. While Sclafani and Nissenbaum (1988) reported a 96% CS+ preference after one training cycle, the rats in Experiment 1 exhibited a 78% CS+ preference in cycle 1 and a 90% preference by the end of cycle 2. Likewise, the KA/P group of Experiment 2 displayed a 74% preference in the first cycle and a 92% preference in the second cycle, and the KA group displayed a 74% preference in cycle 1 and a 95% preference in cycle 2. However, the use of the SOA and citric acid flavors in Experiment 3, instead of the Kool-Aid flavors which were used in the previous experiments, together with 32% Polycose infusions, resulted in the development of a strong preference (88%) after one training cycle which increased to 97% after the second cycle. Whereas the Kool-Aid flavors share the common ingredient citric acid, the SOA and citric acid flavors are quite distinct from each other. The greater the difference there is between the CSs the quicker the preference would be acquired (see Mackintosh, 1974).

Although the rate of conditioning in Experiment 3 was faster than that exhibited by the rats of the first two experiments, it was slower than the rate of

acquisition reported by Sclafani and Nissenbaum (1988) in which 16% rather than 32% Polycose infusions were used. Other studies have also reported that dilute (8% or 16%) Polycose solutions are more effective than concentrated solutions (32%) in conditioning flavor preferences (see Sclafani, 1990). Concentrated Polycose solutions may have rewarding as well as mildly aversive postingestive effects, and this aversive component may be responsible for the slower rate of flavor preference conditioning in some situations.

Nevertheless, all the rats ultimately displayed near-total preferences for the CS+ flavor. The rats not only preferred the CS+ over the CS– but also over plain water. This finding contrasts the mild aversions naive rats exhibit toward the CS flavors. This is important since previous attempts to reverse flavor preferences have not been very successful (Rozin & Zellner, 1985). Experiment 3 showed that rats came to strongly prefer normally avoided tastes (bitter SOA and sour citric acid).

Not only were the CS+ preferences obtained in the present study robust, but they were also quite resistant to extinction. In Experiment 1E the rats continued to strongly prefer (>90%) the CS+ during 14 days of extinction. In addition, the CS+ preference was still apparent for 40 days during which the rats were given extinction tests in their home cages (Experiment 1I). After prolonged one-bottle nonreinforced exposure to the CS+ and CS– in Experiment 3, preference was substantially reduced but not eliminated. Several maneuvers were done to facilitate extinction of the CS+ preference but to no avail. Such attempts to block the CS+ preference included giving the rats the CS+ in the absence of chow (Experiment 1E) and giving the rats

the CS– only for two days (Experiment 1I). Elizalde and Sclafani (unpublished observations) found, in another experiment, that rats' continued preference for the CS+ during extinction was not blocked when the rats were given the CS– only for eight days. It is still possible that different attempts to facilitate extinction may have been successful, but the results unambiguously show that the CS+ preference is strong and not dependent on continued reinforcement with Polycose infusions. However, the rats were capable of having their CS+ preference reversed. When the CS+, which became the rCS–, was paired with IG water infusions and the CS–, which became the rCS+, was paired with IG Polycose infusions, the rats switched their preference to what became the rCS+ (Experiment 1H).

The strong flavor preferences displayed in the present study contrast with the results reported by previous studies involving IG infusions of glucose, fructose, or glucose + fructose mixtures. Such studies have reported no preferences or weak preferences or even aversions (Koopmans & Maggio, 1978; Mather et al., 1978; Puerto et al., 1976; Sherman et al., 1983). In addition, some results suggest that the preferences extinguish rapidly (Holman, 1969). These findings have led to the view that conditioned flavor preferences are difficult to establish (Rozin & Zellner, 1985). However, experiments using Polycose as the infusate have obtained reliable and replicable conditioned preferences (Experiments 1-3; Nissenbaum & Sclafani, 1987; Sclafani & Nissenbaum, 1988). Therefore, the findings suggest that Polycose is more effective than sugars. Polycose, while broken down to and absorbed as glucose, has a much lower osmolarity than glucose at equicaloric concentrations.

Hypertonic sugar solutions can have aversive postingestive effects that would weaken or even reverse the rewarding effect of the nutrient (Booth, 1985).

Not only does the effectiveness of Polycose contribute to the conditioning of such robust flavor preferences, but the training procedure as well is an important ingredient in the conditioning process. The conditioning procedure used in the three experiments of this study involved giving the rats multiple pairings of the CS+ and CS– and IG infusions. A critical aspect of this procedure is that the rats controlled the size, duration, and frequency of the IG infusions through their licking behavior; that is, the rats chose when to drink and when to stop drinking. Therefore, the rats were able to stop a meal before the IG infusion became uncomfortable.

The Polycose infusions were not only effective in conditioning strong flavor preferences, but when unique flavor cues were unavailable (Experiment 1H), the Polycose infusions produced a sipper tube preference. Thus, the findings of this study and others reported in the literature suggest that preference learning can involve somatosensory cues as well as taste and odor cues, and that viscerosensory stimuli produced by nutrients control ingestive behavior by altering the animal's evaluation of orosensory stimuli (Experiment 1H; Borer, 1968; Holman, 1969).

Experiment 3 assessed the effects of IG conditioning on the palatability response to the CS+ using the taste reactivity test. Although the Experimental group strongly preferred the CS+ over the CS–, the rats made comparable orofacial responses to the CS+ and CS–. Perhaps the taste reactivity test is not a valid measure of palatability, especially for use with taste-nutrient conditioning. Grill and

colleagues have demonstrated reliable orofacial response differences using taste-drug and taste-taste preference conditioning paradigms (Breslin et al., 1990; Zellner et al., 1985). The robust preference exhibited in the present study may involve an acquired liking for the CS+, but this still needs to be established.

Alternatively, the conditioning process may not involve an acquired liking for the CS+. The rats may have preferred the CS+ over the CS– and over plain water because they were anticipating the positive postingestive effects resulting from consumption of the CS+. But, it may also be that the conditioning process involves both the anticipation of calories and an increase in the liking of the CS+ flavor, but this remains to be demonstrated.

Conditioned vs. Innate Preferences. The conditioned preference for the CS+ exhibited by the rats was very strong, yet not nearly as strong as their innate preference for the taste of sweet and for the taste of polysaccharides. The rats preferred saccharin over the CS+ (Experiment 1C) as well as Polycose over the CS+ (Experiment 1G), even though the rats never experienced saccharin or Polycose before these tests. In addition, the rats' acceptance of these solutions was much greater than that of the CS+. Perhaps if other CSs and/or IG infusions are used, the acquired flavor preference may approximate the rats' innate preference for carbohydrate tastes. However, it may not be possible to condition flavor preferences that equal the strength of innate preferences. Further research is needed to develop greater understanding of this issue.

Conditioned Acceptance. The robust preference for the CS+ was not always associated with increased acceptance of the CS+. The CS+/IG Polycose pairings did not condition increased acceptance of the CS+ in Experiments 1 and 2. Only when the CS+ was previously associated with the sweet taste of saccharin did the rats overconsume the CS+ (Experiments 1D and 2B). Experiment 2 examined whether using more palatable CS flavors would produce increased acceptance of the CS+. However, the results of Experiment 2 suggest that more palatable CS flavors paired with IG Polycose infusions do not increase the acceptance of the CS+.

The Kool-Aid flavors were the CSs used in Experiments 1 and 2. Experiment 3 utilized SOA and citric acid as the CS flavors because they were better suited for the taste reactivity test. Surprisingly, when SOA or citric acid was paired with IG Polycose infusions, the rats exhibited conditioned acceptance. Increased acceptance was manifested during training as well as during the home-cage short-term and long-term tests. The only difference in the conditioning procedure between the first two experiments and the third experiment was the CS flavors used. Much needs to be learned about the mechanisms underlying conditioned acceptance and conditioned preferences; the mechanisms underlying these two processes may be quite different from each other.

Preference and Caloric Intake. The findings of this study provide data relevant to dietary hyperphagia and its causes. The relative importance of orosensory and postingestive factors in diet-induced overeating has been the focus of study for various researchers (Ramirez et al., 1989; Sclafani, 1987). Ramirez et al. (1989)

contend that postingestive factors play a major role in diet-induced overeating and that evidence for palatability as a possible factor in overeating has not been unambiguously shown.

The results of the present study demonstrate that when the rats were offered the initially unpreferred CS+ paired with IG infusions of Polycose, they increased their total caloric intake by 14% in Experiment 1A, 35-42% in Experiment 2A, and 64% in Experiment 3. The rats increased their caloric intake even further to 37-38% above their water baseline level when the CS+ was sweetened with saccharin (Experiments 1D and 2B). The rats also consumed more total calories when they were given the Polycose to drink than when they were given the CS+ to drink and Polycose was infused into their stomachs (Experiment 1F). The rats consumed as many calories when they had the CS+/saccharin and Polycose solutions (96.9 and 93.8 kcal/day, respectively; Experiment 1) to drink as rats in another study which had ad libitum access to a 32% Polycose solution and chow during a 40-day period (90.6 kcal/day; Sclafani, 1987b).

In summary, the results of this study demonstrate that the postingestive effects of IG Polycose, when paired with an unpreferred flavor, produced a mild hyperphagia. In addition, highly palatable tastes can reliably increase the overeating response. The findings of this study are quite relevant to this issue concerning the possible causes of diet-induced overeating since few, if any, published data unambiguously document a hyperphagia-promoting effect of a taste. In most previous studies, as pointed out by Ramirez et al. (1989), the dietary manipulations

that promoted hyperphagia involved changes in both the orosensory and nutritional characteristics of the diet. However, the hyperphagia-promoting effects reported in the present study are limited in that these effects are based on short-time periods (two or four days). Further research is needed to decisively establish the role of orosensory and postingestive factors in long-term energy balance. The electronic esophagus preparation is well-suited to study this issue.

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