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Unconditioned Stimulus Devaluation Effects in Nutrient-Conditioned Flavor Preferences

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Abstract

Experiments with different temporal relations between the conditioned stimulus (CS) and the unconditioned stimulus (US) in conditioning assessed whether US devaluation effects can be obtained after nutrient-conditioned flavor preference learning. One flavor (CScarb) was paired with a carbohydrate, Polycose; a 2nd flavor (CSprot) was paired with a protein, casein; and a 3rd flavor (CS–) was presented by itself. Following conditioning, one of the nutrients was devalued through pairings with lithium chloride in the absence of the CS flavors. In a subsequent 2-bottle test, rats preferred CScarb over CSprot; however, this preference was smaller when the carbohydrate was devalued than when the protein was devalued. Results suggest that CS flavors are able to form associations with the sensory features of nutrient USs under a wide variety of circumstances.

Keywords

flavor preference; S-O associations; reinforcer devaluation; long delay learning; backward conditioning

It has been known for some time that presenting two flavors together will result in associations developing between them (e.g., Fanselow & Birk, 1982; Rescorla & Cunningham, 1978). One procedure that has been used to demonstrate such associations is sensory preconditioning, in which two affectively neutral flavors are paired, then an aversion is conditioned to one flavor, and finally a test of generalization is conducted with the other flavor. Early investigations have established that these associations occur in a wide variety of circumstances and that a number of different variables play an important role in their development. The importance of the interstimulus interval, for instance, has been demonstrated by the findings that simultaneous pairings of two flavors promote stronger flavor–flavor associations than do sequential pairings (Rescorla, 1980) and that with sequential stimuli, associations fail to occur when the stimuli are separated by as little as 27 s (e.g., Lavin, 1976; Lyn & Capaldi, 1994).

Associations between flavors also have been studied in experiments on conditioned flavor preference learning. In one approach, a relatively neutral flavor conditioned stimulus (CS+) is paired with a hedonically positive but nonnutritive flavor unconditioned stimulus (US), for example, saccharin. As a result of these pairings, the usual finding is that the animal acquires a learned preference for the CS+ flavor over a different flavor (the CS-) that has not been paired with the US (e.g., Forestell & LoLordo, 2003; Holman, 1975). Related research has shown that learned preferences can also be established under sham feeding conditions, where the CS- flavor is mixed into a nutrient solution that is consumed by rats with an open gastric fistula that allows the ingested solution to drain out of the stomach, thereby minimizing any postingestive nutrient actions (e.g., Yu, Silva, Sclafani, Delamater, & Bodnar, 2000). In these experiments the conditioned flavor preference is assumed to reflect an association between the cue flavor and the flavor of the US.

In most demonstrations of flavor preference conditioning, though, the flavor CS has been paired with a nutritionally rich US. One noteworthy finding is that associations based on pairing a flavor with a nutrient (e.g., dextrose mixed with quinine) survive a longer CS-US interval than associations based on pairing a flavor with a more palatable but nonnutritive solution (e.g., saccharin; Holman, 1975). This result implies that preferences can be established through an association between the flavor CS and the positive postingestive consequences of the nutrient US. Direct evidence to support this view comes from intragastric (IG) conditioning studies in which the CS+ flavor is presented in a nonnutritive solution that is paired with IG infusions of the nutrient US (Sclafani & Nissenbaum, 1988). Because the nutrient bypasses the oral cavity with this technique, any preference conditioned for the CS+ flavor can be attributed to the postoral actions of the nutrient.

In sensory preconditioning experiments, associations are believed to occur between two relatively neutral flavors or, to put it differently, between the sensory representations of those flavors. In conditioned flavor preference paradigms, on the other hand, the cue flavor is paired with a US that has unique sensory and general hedonic and/or nutritional effects. We may ask, therefore, whether the presence of strong hedonic and/or nutritional effects of the US might influence learning about the sensory characteristics of the US. It seems plausible that learning about the different features of a multifaceted US might proceed differently than learning about the features of a US that contains relatively few features (Miller & Matute, 1998; Rescorla, 1980). In particular, it might be the case that a potent postingestive effect of a nutrient would overshadow learning about the sensory qualities of the nutrient. Alternatively, there is reason to believe that associations with sensory features of a US under some circumstances may be facilitated by the concurrent presence of a potent motivational component (Gewirtz, Brandon, & Wagner, 1998).

Surprisingly, there is very little evidence in the literature to suggest that associations between the cue flavor and the sensory qualities of the US are actually learned in nutrient conditioning preparations. This question needs to be addressed before other issues regarding interactions between sensory, hedonic, and nutritional components of the US can be evaluated. One approach to this question has been to pair different flavor CSs with different nutrients having the same caloric density but different sensory characteristics and consequent hedonic effects. If one flavor becomes preferred as a result of this operation, then the flavors must have become associated with the different sensory qualities and/or the different hedonic reactions to the two nutrients.

Mehiel and Bolles (1984) failed to find evidence that this was the case. They found that different isocaloric nutrients with unequal palatabilities supported equivalent flavor preferences. On the other hand, more recent findings have provided evidence to suggest that isocaloric nutrients with different palatabilities can differentially support preference learning

(Ackroff, Rozental, & Sclafani, 2004; Warwick & Weingarten, 1994). Warwick and Weingarten (1994), for example, first determined that rats strongly preferred 20% glucose to a mixture of 20% glucose and 0.4% citric acid, suggesting that the plain glucose is more palatable than the mixture (see also Yildiz et al., 1996). Then Warwick and Weingarten repeatedly paired (i.e., mixed) a different Kool-Aid flavor with each reinforcer. Finally, the rats received a two-bottle test in which each bottle contained one of the Kool-Aid flavors mixed with both reinforcers, that is, with 20% glucose plus 0.2% citric acid. The rats strongly preferred the bottle containing the flavor that had been mixed with the plain glucose, indicating that oral, and not just postingestive, factors governed the preference. This effect was replicated by Yildiz et al. (1996), whose final two-bottle test simply pitted the flavors against each other. Taken together, these four studies provide reasonably good evidence that flavor associations are learned when a flavor is paired with a nutrient US. However, they cannot readily distinguish between the possibilities that such flavor-flavor preferences arise from associations between the CS flavor and the sensory properties of the nutrient US, or between the CS flavor and the hedonic properties of the nutrient US.

The present research takes a different approach to the question of whether associations between the cue flavor and the sensory properties of the nutrient can occur in a nutrient-conditioned flavor preference paradigm. To provide reasonably clear evidence for associations with the sensory properties of the nutrient, we used a US devaluation procedure (e.g., Holland & Rescorla, 1975). Following Perez, Lucas, and Sclafani (1995), we initially paired one flavor CS with a carbohydrate, paired a second flavor CS with a protein, and presented a third flavor CS with no nutrient. After this conditioning phase, an aversion was established to one of the two nutrients, and then the rats were allowed to choose between the two nutrient-paired cue flavors. A reduction in the preference for the nutrient-paired flavor whose nutrient was devalued would imply that subjects had learned to associate the cue flavor with the sensory qualities of the nutrient.

The main assumption underlying this logic is that when an aversion is established to the nutrient, the aversion is established to the sensory-specific properties of the nutrient and not to the more general hedonic or postingestive reinforcing properties that the nutrient in question shares with other reinforcing nutrients. Orosensory stimuli are prominent among stimuli that are specific to a nutrient. Different nutrients may also produce different sensory signals postingestively (e.g., Tracy, Phillips, Chi, Powley, & Davidson, 2004). If orosensory or viscerosensory aspects of the devalued nutrient became associated with the aversion-inducing agent and the cue flavor had earlier become associated with the relevant sensory properties of the nutrient, then the aversion should transfer to the cue flavor because the cue flavor should be capable of evoking a representation of the sensory properties of the nutrient rendered aversive by nutrient aversion conditioning. On the other hand, if the cue flavor failed to enter into an association with the sensory-specific properties of the nutrient but instead became associated with some general hedonic response or postingestive reinforcing effect (e.g., effects of calories) common to the two nutrients, then there would be no basis for the aversion established to the sensory properties of the nutrient to transfer back to the flavor of the cue that had been paired with the devalued nutrient any more than to the other CS+ flavor. Thus, the main point of the present studies was to use the US devaluation technique to determine whether flavor CSs associate with sensory-specific properties of nutrient USs in a nutrient-conditioned flavor preference paradigm.

Further, we began the investigation of the similarity of the associations in this situation to those reported in sensory preconditioning procedures by varying the interstimulus interval in an effort to determine the sensitivity of learning these associations to this variable. In our first study, the cue flavors and their paired nutrients were mixed together in solution during the training phase, but in the subsequent studies the cue flavors and their paired nutrients

were presented sequentially in either a forward (Experiment 2) or a backward manner (Experiment 3).

Experiment 1

The purpose of the first experiment was to extend the results reported by Perez et al. (1995). These investigators demonstrated that carbohydrate and protein nutrient USs were equally effective at supporting learned preferences for flavor cues that preceded the nutrients by a 10-min trace interval. In Experiment 1a, we determined the relative abilities of protein and carbohydrate nutrients to establish flavor preferences in hungry rats drinking different cue flavors mixed in solution with the nutrients. To this aim, one cue flavor, CScarb, was mixed in solution with a carbohydrate (16% Polycose); a second cue flavor, CSprot, was mixed with a calorically matched protein (16% casein hydrolysate); and a third cue flavor, CS–, was presented alone. Following a series of conditioning trials with each of these distinctively flavored solutions, preference for the two nutrient-paired cue flavors was assessed relative to CS–. Having established that these two nutrients can support flavor preference learning, in Experiment 1b we assessed the effects of establishing a flavor aversion to one of the nutrients following the conditioning phase on preference for the two nutrient-paired cue flavors.

Experiment 1a

Method

Subjects: Twelve experimentally naive female Sprague–Dawley rats participated in the experiment. These rats were bred at Brooklyn College and derived from Charles River labs (Raleigh, NC). Their weights at the beginning of the experiment ranged from 247 g to 294 g. The rats were housed individually in a colony room that was on a 16:8-hr light–dark cycle, and they were maintained at 85% of their ad lib body weights by daily supplemental feedings (given 2 hr after the experimental session).

Apparatus and solutions: The experiment was performed in the rats' home cages in the colony room starting approximately 4 hr after the lights were turned on. These cages were of standard dimensions (24 cm × 18 cm × 17.5 cm) and made of wire mesh with solid stainless steel side walls. The various solutions were presented in 50-ml centrifuge tubes attached to the outside of the cage, with their metal spouts protruding approximately 5 cm into the front of the cage. Unsweetened grape, cherry, and orange Kool-Aid solutions (0.05%) containing 0.2% sodium saccharin were used as CSs in these experiments. During training, one flavored solution (the CScarb) contained 16% Polycose (Ross Laboratories, Cincinnati, OH), a second flavor solution (the CSprot) contained 16% casein hydrolysate (Research Diets, New Brunswick, NJ) supplemented with 0.24% DL-methionine (Sigma-Aldrich), and the third flavor solution (the CS–) contained only water. The specific flavors paired with the nutrients were counterbalanced across subjects. During two-bottle testing, the CS flavors were presented without any nutrient present. All solutions were prepared daily with tap water.

Procedure: Initially the rats were given several days to accommodate to a drinking schedule. They were permitted to drink the 0.2% saccharin solution for a 24-hr period prior to introduction of their restricted feeding schedule. When subjects achieved their 85% free-feeding target weights, they were given limited access to the saccharin solution over a 5-day period (30 min for 2 days, 20 min for 2 days, and 10 min for 1 day).

The conditioning phase consisted of five 3-day cycles. Water bottles were removed just prior to the session, and they were returned 2 hr after the daily session. On the 1st day of

each cycle subjects were presented with the CScarb solution (containing Polycose). On the 2nd day, the CSprot solution (containing casein) was presented, and on the 3rd day the CS– solution was presented. On each day, subjects were first weighed, then their water bottles were removed, and then a bottle containing 20 ml of the appropriate CS solution was placed on their cages. Two hours later subjects were given their daily ration of chow, and their water bottles were placed back on their cages. By this time most of the rats had consumed their entire 20 ml of the CS solution. However, if a rat did not consume the entire amount, the bottle was left on its cage overnight.

The test phase was conducted over the next 4 days. On each day, subjects were given a 2-hr, two-bottle choice between one of the nutrient-paired flavors and CS–. Half of the subjects were tested with CScarb versus CS– on Days 1 and 2 and with CSprot versus CS– on Days 3 and 4. The remaining subjects were tested in the reverse order. The left–right position of the two solutions was counterbalanced across subjects on any given test day, and the positions of the solutions on Days 2 and 4 were the reverse of their positions on Days 1 and 3. Intakes were measured after 30 min as well as after the 2-hr period on each test day.

Statistical analysis: Here and throughout, standard analysis of variance (ANOVA) techniques were used to analyze the intake data. Although similar patterns were seen with the 30-min and 2-hr measures, the 30-min intake data are reported below. A Type I error rate of .05 was adopted.

Results and Discussion—Mean intakes averaged over the two tests with the CScarb versus CS– and CSprot versus CS– are presented in Figure 1. This figure shows that each nutrient was capable of supporting a conditioned preference for the flavor that was mixed in solution with it during training over CS–. The carbohydrate, however, was more potent at conditioning a preference compared with the protein. These data were analyzed using a Nutrient (carbohydrate vs. protein) $\times$ CS (CS+ vs. CS–) ANOVA, which revealed significant main effects of nutrient, $F(1, 11) = 6.34$, and CS, $F(1, 11) = 53.58$, as well as a significant Nutrient $\times$ CS interaction, $F(1, 11) = 12.74$. Separate tests of CS+ versus CS– using a pooled error term revealed significant preferences for CScarb over CS–, $F(1, 11) = 61.32$, and CSprot over CS–, $F(1, 11) = 9.36$.

An additional analysis was performed on the intake data. For this analysis, intakes of the nutrient-paired flavor were expressed as a percentage of total intake (nutrient-paired flavor plus CS– combined). CScarb was preferred to CS– (94%) more than CSprot was preferred to CS– (72%). This statistical comparison was highly reliable, $F(1, 11) = 15.42$.

The data from the present experiment agree with those reported by Perez et al. (1995) in showing that flavors paired with either 16% Polycose or 16% casein hydrolysate become preferred to CS–. Polycose was more effective than casein at conditioning a flavor preference in the present study, however, whereas these two nutrients were equally effective in the Perez et al. (1995) study. Regardless of the explanation for this difference, though, the present experiment demonstrates that the present procedures can be used to assess the effects of nutrient devaluation on nutrient-conditioned flavor preferences. Experiment 1b begins this examination.

Experiment 1b

Method

Subjects—Eleven experimentally naive female Sprague–Dawley rats participated in the experiment. These rats were bred at Brooklyn College and derived from Charles River labs.

Their weights at the beginning of the experiment ranged from 267 g to 405 g. Access to food was limited as described below. The rats were housed and maintained as in Experiment 1a.

Apparatus and solutions: The same apparatus and solutions were used as in Experiment 1a.

Procedure: Initially the rats were given several days to accommodate to a drinking schedule. When subjects achieved their 85% free-feeding target weights, they were given 24-hr access to a 0.2% saccharin solution for 2 days. This was followed over the next several days by limited access to the saccharin solution (30 min for 1 day, 20 min for 1 day, and 10 min for 1 day).

During the next 15 days, the conditioning phase was conducted exactly as in Experiment 1a. Over the next 6 days one of the nutrients was devalued. The Polycose solution (10 ml) was presented on odd-numbered days, and the casein solution (10 ml) on even-numbered days, during 30-min sessions. Six rats were injected intraperitoneally with lithium chloride (LiCl; 1.5% body weight, 0.3 M) at the end of the Polycose sessions and received no injection at the end of the casein session. The remaining 5 rats were treated with LiCl at the end of only the casein sessions. Daily food rations were given 2 hr following injections.

Testing began 2 days after the final nutrient devaluation session. The test procedures were, in general, the same as those used in Experiment 1a with the following exceptions. The rats were given a choice between the two nutrient-paired flavors, CScarb and CSprot, in each of the first two tests (which differed only in the right–left position of the tubes). Tests 3–6 pitted each of the nutrient-paired flavors against the CS– flavor. Six of the rats were tested with CScarb versus CS– during Tests 3 and 4 (which differed only in left–right bottle placements) and CSprot versus CS– during Tests 5 and 6. The remaining subjects received the opposite testing order. One week later, all of the rats were given a final test consisting of a choice between the Polycose and casein solutions (without flavorings), to confirm that the nutrients had been differentially valued.

Results—By the end of the nutrient devaluation phase, all subjects avoided the nutrient that had been paired with LiCl and consumed the nutrient not paired with LiCl. On the final devaluation day, rats that received the carbohydrate (Polycose) devalued consumed an average amount of 1.5 ml (out of a possible 10 ml) of Polycose and 9.5 ml of casein. Rats that received the protein (casein) devalued consumed 2.0 ml of casein and 9.0 ml of Polycose. Every rat displayed reduced intake of the nutrient that had been paired with LiCl.

The results from the CScarb versus CSprot choice tests are displayed in Figure 2. Similar patterns appeared in both the 30-min and 2-hr intake data, and only the 30-min data were statistically analyzed and appear graphically. Figure 2 illustrates that both groups preferred CScarb to CSprot but that the preference was reduced in subjects that had the carbohydrate devalued. A Stimulus (CScarb vs. CSprot) $\times$ Devaluation (carbohydrate vs. protein) ANOVA performed on these data revealed a significant main effect of stimulus, $F(1, 9) = 26.19$, as well as a significant Stimulus $\times$ Devaluation interaction, $F(1, 9) = 6.61$. An additional analysis revealed that the carbohydrate-devalued subjects displayed a weaker CScarb preference (64%) than the protein-devalued subjects (97%), $F(1, 9) = 12.32$.

Consistent with the results from Experiment 1a, the CS+/CS– tests (shown in Table 1) revealed that overall, the nutrient-paired flavors were preferred to CS–, $F(1, 9) = 13.85$, and that there was a greater preference for CScarb over CS– than for CSprot over CS–, $F(1, 9) = 9.97$. However, this did not interact with which nutrient had been devalued.

The final test between the carbohydrate and protein revealed that every rat showed a strong preference for the nutrient that had not been paired with LiCl. Rats that had the carbohydrate (Polycose) devalued consumed, on average, 0.4 ml of Polycose and 10.7 ml of casein, whereas rats that had the protein (casein) devalued consumed 0.1 ml of casein and 19.6 ml of Polycose.

Discussion—The present study indicates that a US devaluation effect can be obtained in a nutrient-based conditioned flavor preference learning procedure. Although the two nutrients used here, Polycose and casein, were calorically matched, they were shown to produce different magnitude preferences for flavors mixed in solution with them. More important, when preference between the two nutrient-paired flavors was assessed after one of the nutrients had been devalued by pairings with LiCl, the relative preference for the flavor that had been paired with the devalued nutrient was decreased. Recently Dwyer (2005), using rats with free access to food and water, observed a similar result with 2% sucrose and 2% maltodextrin as the reinforcers. Because of the low energy density of the 2% carbohydrate solutions, Dwyer assumed that the flavor preferences conditioned by sucrose and maltodextrin were based on the palatable tastes of the carbohydrate solutions rather than their postingestive nutritive effects. In the present study the nutrient solutions were more concentrated, and their postingestive actions presumably were importantly involved in the flavor conditioning process. In fact, some data indicate the flavor preferences conditioned by 16% Polycose are due primarily to an association between the CS flavor and the postingestive actions of the Polycose (Elizalde & Sclafani, 1988). Our devaluation results suggest, therefore, that the CS flavor preferences conditioned by the 16% Polycose and casein solutions involved an association between the CS flavor and some sensory-specific feature of the nutrient US solutions.

Given results from earlier sensory-preconditioning studies, this result is perhaps not very surprising. Nevertheless, there has not been much examination of sensory-specific learning when a flavor cue is associated with a nutrient US, and we might not expect learning of this sort to be comparable in all respects to sensory-specific learning among neutral flavor cues. The presence of sensory-specific learning in a nutrient-conditioned preference procedure established here allows us to examine additional questions related to the sensitivity of this learning to other variables shown to be important in sensory preconditioning. The next experiment examined the generality of the devaluation effect found here by determining the effect of the CS–US interval on sensory-specific learning in this situation.

Experiment 2

Prior research has established that nutrient-conditioned preferences can be established when the cue flavor and the nutrient are separated by relatively short temporal gaps (e.g., Capaldi, Campbell, Sheffer, & Bradford, 1987; Holman, 1975). Perez et al. (1995) reported evidence for preference learning with the same cue flavors and nutrients as those used here under conditions in which the consumption of the cue flavors preceded consumption of the nutrients with a 10-min delay. It is of interest to determine whether any of the preference learning that occurs under these conditions is based on an association between the cue flavors and the sensory characteristics of the nutrients. As noted above, studies of sensory preconditioning have shown that associations between two relatively neutral cue flavors, in the absence of any significant nutritional consequences, do not survive a relatively short interval of time separating the two flavors (e.g., Lavin, 1976; Lyn & Capaldi, 1994). This interval, 27 s, is much shorter than the 10-min interval across which nutrient-conditioned preferences can be established.

To examine this issue, in Experiment 2 we used the same general procedures used in Experiment 1b with the exception that subjects were trained in the present study with sequential cue–nutrient compounds (rather than simultaneous compounds). In two different groups of rats, each of two different cue flavors was paired with a different nutrient (while a third cue flavor was presented alone) during the training phase, and relative preference for the nutrient-paired flavors was assessed after devaluation of one of those nutrients. During the training phase the cue flavors were followed by the relevant nutrients either immediately (Group Immediate) or after a 10-min trace interval (Group 10-min Trace). If the strength of the associations formed between the cue flavors and the sensory properties of the nutrients is reduced by the 10-min trace interval, then we would expect nutrient devaluation to have a reduced effect in Group 10-min Trace compared with Group Immediate.

Method

Subjects—Twenty-four experimentally naive female Sprague–Dawley rats participated in the experiment. These rats were supplied by Charles River labs. Their weights at the beginning of the experiment ranged from 256 g to 295 g, but access to food was limited as described below. The rats were housed and maintained as in Experiment 1.

Apparatus and solutions—The same apparatus was used as in Experiment 1. The Kool-Aid and nutrient solutions were as in the previous studies. However, the Kool-Aid and nutrient solutions were prepared and presented separately (not mixed together).

Procedure—Initially the rats were given several days to accommodate to a drinking schedule. When subjects achieved their 85% free-feeding target weights, they were given 24-hr access to a 0.2% saccharin solution for 3 days. This was followed over the next several days by limited access to the saccharin solution (30 min for 3 days, 20 min for 2 days, and 10 min for 4 days).

Over the next 15 days, the conditioning phase was conducted as in Experiment 1 but with the following exceptions. For 12 of the subjects (Group Immediate), 50 ml of the Kool-Aid saccharin solutions were presented for 10 min and then removed. This was followed immediately by presentation of a bottle containing 20 ml of the relevant nutrient (or by no bottle on CS– trials). The remaining 12 subjects (Group 10-min Trace) received the bottle containing the nutrient starting 10 min after the CS solutions had been removed. The bottle containing the nutrient remained on the cage until the 20 ml had been consumed (which almost always occurred within 30 min). After 2 hr, the subjects were given supplemental feedings of chow to maintain their 85% body weights, and their water bottles were returned at this time.

The nutrients were devalued as in Experiment 1, but over five 2-day cycles (rather than three). Subjects were given two extra nutrient–LiCl pairings in this experiment because of the extensive exposures given to the nutrients alone during the conditioning phase. On each of these days, 20 ml of the relevant nutrient (rather than 10 ml in the previous experiment) was presented for 30 min, and, depending on group assignment, this was followed immediately by an intraperitoneal injection of LiCl (1.5% body weight, 0.3 M) or by no injection. Half of the rats received LiCl injections on days when Polycose was presented, and the remaining half received LiCl injections on days when casein was presented. This created four groups in the experiment: Group Immediate with Polycose devalued, Group Immediate with casein devalued, Group 10-min Trace with Polycose devalued, and Group 10-min Trace with casein devalued. Daily food rations were given 2 hr after injections, and the water bottles were returned at this time as well.

Testing was begun on the day following the final nutrient devaluation session. The test procedures were the same as those used in Experiment 1b except that all tests lasted 30 min (instead of 2 hr).

Results

Intakes of the CSs steadily increased over the conditioning phase. By the end of training, the rats consumed more of the CScarb than either the CSprot or CS−, which did not differ ($M_s = 12.8, 11.0, \text{ and } 11.5 \text{ ml}$, respectively, in Group Immediate and $11.6, 9.5, \text{ and } 9.0 \text{ ml}$, respectively, in Group 10-min Trace). A Group $\times$ Flavor ANOVA performed on these data revealed a significant main effect of flavor, $F(2, 44) = 10.66$, but no other significant effects.

Intakes of the nutrients during the devaluation phase proceeded as expected. By the end of this phase, every rat had consumed nearly all of the nutrient that was not paired with LiCl and nearly none of the nutrient that was paired with LiCl. In Group Immediate, the mean intakes of the carbohydrate (Polycose) and the protein (casein), respectively, were 1.4 ml and 14.9 ml in subjects receiving Polycose devaluation, and they were 19.3 ml and 0.7 ml in subjects receiving casein devaluation. In Group 10-min Trace, these values were 2.4 ml and 18.8 ml in subjects receiving Polycose devaluation, and they were 17.7 ml and 1.5 ml in subjects receiving casein devaluation.

The results of primary importance are displayed in Figure 3, which shows intakes of CScarb versus CSprot solutions during the two-bottle tests (see upper panel). The data are shown separately for subjects in Group Immediate and Group 10-min Trace, and they are broken down further by whether the carbohydrate (Polycose) or protein (casein) was devalued. A similar pattern of results was seen in Groups Immediate and 10-min Trace. There was a preference for CScarb over CSprot in both of these groups, but the size of this preference was greater in subjects that received the protein devalued than in subjects that received the carbohydrate devalued. These impressions were confirmed by a Group $\times$ CS $\times$ Devaluation ANOVA performed on these data. This analysis revealed a significant CS main effect, $F(1, 20) = 22.21$, and a significant CS $\times$ Devaluation interaction, $F(1, 20) = 7.55$, which indicates that the preference for CScarb over CSprot was less in subjects that had the carbohydrate (Polycose) devalued. This CS $\times$ Devaluation interaction itself did not interact with the group variable, $F(1, 20) = 0.21$.

In a further analysis of these data, CScarb intake was expressed as a percentage of total intake during the test (lower panel of Figure 3). The percentage of CScarb consumed was reduced in subjects that had the carbohydrate (Polycose) devalued, and this was equally true in Groups Immediate and 10-min Trace. A Group $\times$ Devaluation ANOVA performed on these data revealed a significant main effect of devaluation, $F(1, 20) = 6.13$, but no interaction with group.

The data from the CS+ versus CS− test sessions (shown in Table 1) were also analyzed. These data revealed, once again (see Experiment 1a), that there was a greater preference for CScarb over CS− than for CSprot over CS−, $F(1, 20) = 4.60$; however, the critical interaction between these factors and which nutrient was devalued failed to reach significance, $F(1, 20) = 2.67, p = .12$. Apparently, the direct test pitting CScarb against CSprot is more sensitive at detecting these devaluation effects.

In the final test pitting the carbohydrate against the protein, no subject consumed more than 1 ml of the nutrient that had undergone devaluation training. Further, all subjects drank substantial amounts of the nondevalued nutrient, though the carbohydrate (Polycose) was consumed more than the protein (casein) when they were not devalued. Mean intakes of

nondevalued Polycose and nondevalued casein, respectively, were 19.8 ml and 12.8 ml in Group Immediate and 16.3 ml and 11.1 ml in Group 10-min Trace.

Discussion

The results from the present study demonstrate that a US devaluation effect similar to that found in Experiment 1b can occur under conditions in which nutrient-conditioned preferences are established through sequential cue flavor–nutrient pairings. Indeed, the outcomes were the same whether the nutrient occurred during training either immediately following the cue flavor or after a 10-min trace interval. In both cases, subsequent devaluation of one of the nutrients reduced the relative preference for the flavor that had been paired with that nutrient. The usual interpretation of a US devaluation effect like this is that the cue flavors became associated with some sensory characteristic of the nutrient, whose subsequent devaluation mediated the altered preference for the cue flavor. If we accept this interpretation, then it suggests that such associations can be formed when as much as a 10-min temporal gap separates the CS+ flavor and the nutrient US.

This conclusion is in stark contrast with earlier results from sensory preconditioning studies (Lavin, 1976; Lyn & Capaldi, 1994) that failed to find evidence of learning among neutral flavors separated by more than 27 s. These differences may point to the importance of the postingestive reinforcing effect of the nutrient in permitting such relatively long delay sensory-specific associations. Presently, we do not have the sorts of comparisons that would permit a detailed examination of this possibility, but at the very least, the results suggest that caution should be used in interpreting conditioned flavor preferences as reflecting processes other than associations between the flavor CS and sensory aspects of the nutrient under conditions in which the two flavors are separated by more than some minimal amount of time (e.g., Holman, 1975; Sclafani & Ackroff, 1994).

Experiment 3

Nutrient-conditioned preferences have also been established using a *reverse procedure*, in which a flavor cue and a nutrient are paired, but with the nutrient being consumed prior to the cue flavor. Under these conditions, Boakes and Lubart (1988) and Forestell and LoLordo (2000, 2003, 2004) demonstrated that preferences were established for the flavor CS that followed a glucose solution relative to a second flavor presented without a nutrient. Although this result has a natural interpretation in terms of the flavor CS entering into a forward excitatory association with the postingestive properties of the nutrient, it is possible that backward excitatory associations between the CS flavor and the sensory features of the nutrient might also have occurred and contributed to the observed preference. This interpretation is usually dismissed on the grounds that several minutes elapsed between presentations of the two flavors. However, as the results of Experiment 2 make clear, associations between the cue flavors and some sensory features of the nutrients can be formed with relatively long intervals separating the two.

In the present experiment we examined the possibility of backward excitatory associations between the cue flavor and sensory features of the nutrient by using the same experimental design as that used in Experiment 2, with the exception that the nutrients preceded the flavor CSs in one group and followed the CSs in a second group. A shorter trace interval was used in this experiment (5 min) compared with Experiment 2 (10 min), because shorter intervals have revealed preference conditioning using the reverse procedure (Boakes & Lubart, 1988; Forestell & LoLordo, 2000, 2003, 2004). If backward excitatory associations can be established between the sensory features of the nutrients and the CS flavor in the reverse order procedure, then devaluing one of the nutrients following the conditioning phase should weaken a preference for the flavor CS associated with the devalued nutrient. The forward-

conditioned group was run to compare the magnitude of the devaluation effect with forward and backward training procedures, as well as to replicate the effect found in Experiment 2.

Method

Subjects—Forty-eight experimentally naive male Sprague–Dawley rats participated in the experiment. These rats were supplied by Charles River labs. Their weights at the beginning of the experiment ranged from 393 g to 639 g. Access to food was limited as described below. The rats were housed and maintained as in Experiment 1. The experiment was run in three replications.

Apparatus and solutions—The same apparatus was used as in the previous experiments. The Kool-Aid and nutrient solutions were used as in Experiment 2.

Procedure—Initially the rats were given several days to accommodate to a drinking schedule. When subjects achieved their 85% free-feeding target weights, they were given 24-hr access to a 0.2% saccharin solution for 3 days. This was followed over the next several days by limited access to the saccharin solution (30 min for 2 days, 20 min for 2 days, and 10 min for 4 days).

The conditioning phase was conducted as in Experiment 2, but with the following exceptions. The conditioning phase lasted for ten 3-day cycles. Half of the subjects (Group Forward) were trained with the flavor CSs preceding the nutrient USs. The other half (Group Reverse) were trained with the nutrient USs preceding the flavor CSs. In each of the 10 conditioning cycles, the grape-flavored CS was presented on the 1st day, cherry on the 2nd, and orange on the 3rd. The appropriate nutrient (or nothing) was paired with the CS solution on each day, such that the identities of the CS flavors were counterbalanced in their roles as CScarb, CSprot, and CS–.

On a given conditioning session, the subjects were offered a limited amount (8 ml or less, as described below) of the CS solution to drink over a 10-min interval. On trials in which a nutrient was scheduled, they were also offered 20 ml of the nutrient to drink over a 15-min interval. For subjects in Group Forward, the appropriate nutrient was presented 5 min after the CS was removed. For subjects in Group Reverse, the nutrient was presented first, and then the CS was presented 5 min after the nutrient was removed. In the first three cycles, subjects were presented with 8 ml of the CS on any given conditioning trial. Thereafter, CS intakes were limited in such a manner as to roughly equate the relative intakes of CScarb, CSprot, and CS– across the two groups. Because Group Reverse subjects consumed more CS– than CScarb and more CScarb than CSprot, an effort was made to match intakes of these three solutions in Group Forward during Cycles 4–10.

Following the conditioning phase, the nutrients were devalued as in Experiment 2, but over three 2-day cycles (Replications 1 and 2) or four 2-day cycles (in Replication 3). On each of these days, 20 ml of the relevant nutrient was presented for 15 min, and, depending on group assignment, this was followed immediately by an intraperitoneal injection of LiCl (1.5% body weight, 0.3 M) or by no injection. Half of the rats received Polycose devalued, and the remaining received casein devalued to create four groups in the experiment: Group Forward with Polycose devalued, Group Forward with casein devalued, Group Reverse with Polycose devalued, and Group Reverse with casein devalued. Daily food rations were given 2 hr following injections, and the water bottles were returned at this time as well.

For the next 2 days following the final nutrient devaluation session, all subjects were given 15-min two-bottle choice tests between saccharin in one bottle and water in the other. The position of the solutions was switched across these days. This was done in order to

familiarize subjects with a two-bottle testing procedure. The flavor tests began on the following day, and the procedures were the same as those used in Experiment 2 with the exception that all tests were 15 min in duration (rather than 30 min). Following the flavor tests all rats were given a final 15-min test session between Polycose and casein solutions to verify that these solutions were differentially valued.

Results

Intakes of the various solutions from the conditioning phase are displayed separately for each group in Figure 4. The graphs in the top row show intakes of CScarb, CSprot, and CS– over the 10 cycles of conditioning, and those in the lower row show intakes of the nutrients (the carbohydrate, Polycose, and the protein, casein). Both groups consumed nearly all of the nutrients offered in each conditioning cycle, with the exception of the first. Throughout training, Group Reverse consumed more of CS– than CScarb and more of CScarb than CSprot. Apparently, consumption of the nutrients suppressed subsequent intake of the CS flavors in Group Reverse. The satiating effects of protein have been reported previously to be greater than the satiating effects of carbohydrate (Bensaid et al., 2002). Because of the differential consumption of the three CSs in Group Reverse, starting on the fourth cycle access to the CS flavors was limited in Group Forward to match the relative intakes of the three CS flavors to those of Group Reverse.

The CS intake data averaged over Conditioning Cycles 7–10 were analyzed with a one-way ANOVA, and this analysis revealed significant differences among the three solutions, $F(2, 46) = 32.77$. Post hoc tests following the methods of Rodger (Rodger, 1974) revealed differences in CScarb and CSprot intakes, $F(2, 46) = 8.92$, and showed that CScarb and CSprot were consumed less than CS–, $F(2, 46) = 23.84$.

The nutrient devaluation phase proceeded normally. On the final devaluation cycle, every rat consumed nearly all of the nondevalued nutrient and approximately 1 g or less of the nutrient that had been paired with LiCl. There were no differences between the groups in this respect.

The data of most interest are shown in Figure 5, which displays the mean intakes during the CScarb versus CSprot tests. The upper panel shows for Groups Forward and Reverse the mean intakes of each CS, separated by whether the carbohydrate or the protein was devalued. The lower panel expresses these data in terms of percentage of CScarb intake. For both Groups Forward and Reverse, there was a weaker preference for CScarb over CSprot when the carbohydrate was devalued than when the protein was devalued.

These impressions were supported by a Group (Forward vs. Reverse) $\times$ Devaluation (carbohydrate vs. protein) $\times$ CS (CScarb vs. CSprot) $\times$ Replication (1, 2, or 3) ANOVA performed on the intake data. This analysis revealed a significant main effect of devaluation, $F(1, 36) = 10.66$, as well as a significant Devaluation $\times$ Group interaction, $F(1, 36) = 4.41$. This merely indicates that there was less overall consumption in protein-devalued subjects and that this was somewhat more true in Group Reverse than in Group Forward. There was also a significant main effect of CS, $F(1, 36) = 42.16$, which indicates that CScarb was preferred to CSprot. However, the critical CS $\times$ Devaluation interaction was also significant, $F(1, 36) = 5.19$, indicating that the preference for CScarb over CSprot was weaker in carbohydrate-devalued subjects than in protein-devalued subjects. There were no interactions with replication, and the lack of a significant CS $\times$ Devaluation $\times$ Group interaction, $F(1, 36) = 0.11$, indicates that the devaluation effect was comparable in Groups Forward and Reverse.

These conclusions were also supported by a Group $\times$ Devaluation $\times$ Replication ANOVA performed on the percentage of CScarb intake data (shown in the lower panel of Figure 5). This analysis revealed only a significant main effect of devaluation, $F(1, 36) = 8.97$, which confirms a weaker preference for CScarb over CSprot when the carbohydrate was devalued compared with when the protein was devalued.

The results of the CScarb/CS– and CSprot/CS– tests (shown in Table 1) were inconclusive. A Group $\times$ Devaluation $\times$ Replication $\times$ CS (CS+ vs. CS–) $\times$ Test (CScarb vs. CS– or CSprot vs. CS–) ANOVA performed on the data revealed only a significant main effect of CS, $F(1, 36) = 57.08$, and a main effect of test, $F(1, 36) = 6.91$. The former result indicates that the nutrient-paired flavors were preferred to CS–, and the latter indicates that there was somewhat greater intake, overall, in the CScarb versus CS– test than in the CSprot versus CS– test. There were no reliable interactions in this analysis, though the critical Test $\times$ CS $\times$ Devaluation interaction approached significance levels, $F(1, 36) = 2.17$, $p = .15$.

The results of the final test between the carbohydrate and the protein confirmed that these two nutrients were differentially valued at the end of the experiment. No subject in either Group Forward or Group Reverse consumed more than 1 g of the devalued nutrient. Further, all subjects drank substantial amounts of the nondevalued nutrient, though the carbohydrate (Polycose) was consumed more than the protein (casein) when they were not devalued. Mean intakes of nondevalued Polycose and nondevalued casein, respectively, were 19.5 g and 13.4 g in Group Forward and 18.1 g and 12.5 g in Group Reverse.

Discussion

The results from the present study were largely in agreement with those found in Experiments 1b and 2. Nutrient devaluation altered a conditioned preference for a CS flavor that had been associated previously with that nutrient. The present study included a forward-conditioned group trained with a 5-min trace interval separating the cue flavors and the nutrients, and the results resembled those from a group trained with a 10-min trace interval in Experiment 2. In addition, the present study found that a reverse-conditioned group was also equally sensitive to the US devaluation manipulation in spite of the fact that a 5-min trace interval separated the US and CS solutions. Apparently, associations between orally presented cue flavors and the sensory features of the nutrients with which they are paired can be established when using either forward or backward trace procedures. Thus, it appears to be unsafe to conclude that just because an interval of several minutes separates the presentation of a nutrient and a cue flavor, the resulting preference for the cue flavor is based solely on a forward association between the cue flavor and the general reinforcing postingestive effects of the nutrient.

The present findings agree with existing associative theory (Wagner & Brandon, 1989) that discusses the possibility of backward excitatory associations forming between the CS and the sensory features of the US. However, to our knowledge the only existing evidence bearing on this issue documents sensory-specific inhibitory learning arising from backward pairings (e.g., Delamater, LoLordo, & Sosa, 2003; McNish, Betts, Brandon, & Wagner, 1997; Tait & Saladin, 1986). Whether one obtains sensory-specific excitatory or inhibitory associations from a backward procedure will undoubtedly depend on many different parametric considerations, including the type of US, the type of CS, and the US–CS interval.

In contrast, as alluded to in the general introduction, it may be possible to explain the results from the present study in terms of the organism forming an association between the flavor CS and nutrient-specific postingestive signals. Such signals could have occurred after the flavor CS was presented on each trial, and this could have resulted in a forward association forming between the flavor CS and the sensory-specific features of the nutrient. The present

experiment does not allow us to distinguish between associations involving the flavor CS with the orosensory versus viscerosensory aspects of the nutrient. We discuss these two possibilities later.

General Discussion

The present studies demonstrate that US devaluation effects can be obtained in a nutrient-conditioned preference paradigm. In three experiments, postconditioning devaluation of a nutrient was shown to reduce the rats' preference for a CS flavor that had been associated with that nutrient prior to its devaluation. This devaluation effect occurred reliably, and consistently, in two-bottle test procedures that pitted against one another two nutrient-paired flavors, only one of which had been associated with the devalued nutrient. Specifically, the preference for a carbohydrate-paired CS flavor over a protein-paired CS flavor was lower if the carbohydrate (Polycose) was devalued following conditioning than if the protein (casein hydrolysate) was devalued. The effect was shown to occur under several different training conditions, including (a) simultaneous pairings of the flavor CS and nutrient US; (b) forward pairings between CS and US with 0-s, 5-min, or 10-min trace intervals; and (c) backward pairings between CS and US with a 5-min backward trace interval. There are several issues regarding the data that deserve comment, as well as several implications for other research that are discussed in turn.

The locus of our US devaluation effects is unclear. We have shown that preference for CScarb over CSprot was greater when the protein (casein) was the devalued nutrient compared with when the carbohydrate (Polycose) was the devalued nutrient. It seems possible that there are either one or two US devaluation effects at work here. One way to examine this issue is by determining whether subjects differ in their preference for the nutrient-paired flavors against CS– as a function of which nutrient was devalued. Although we did not obtain significant devaluation effects in the present studies in the CS+ versus CS– tests in individual experiments, the relevant interactions sometimes approached significance. To examine this issue further, we conducted another analysis on the data from the CS+ versus CS– tests, collapsing across the results from the different experiments (see Table 1). Overall, there was a greater preference for CSprot over CS– when the protein had not been devalued (and carbohydrate had been devalued) than when the protein had been devalued. The analogous difference, though in the right direction, was not so apparent in the CScarb versus CS– tests.

The data were analyzed with a Devalued Nutrient (carbohydrate vs. protein) $\times$ Test (CScarb vs. CS– or CSprot vs. CS–) $\times$ CS (CS+ vs. CS–) ANOVA, and this analysis revealed a significant three-way interaction, $F(1, 81) = 5.67$. Subsequent Devalued Nutrient $\times$ CS ANOVAs on the data from each type of test revealed this interaction to be significant in the CSprot versus CS– tests, $F(1, 81) = 6.16$, but not in the CScarb versus CS– tests, $F(1, 81) = 0.46$. These results demonstrate clearly that the devaluation effects reported here involved the flavor cues associated with the protein. However, from this analysis it is unclear to what extent associations with the carbohydrate may have also contributed to the devaluation effects seen in the present studies as well. This is because the CS+ versus CS– tests always occurred following the CScarb versus CSprot tests, and because the CScarb was consistently preferred to the CSprot, there was a greater opportunity for these tests to have adversely affected the carbohydrate devaluation effect than the protein devaluation effect. Other research has shown that US devaluation effects are reduced in flavor stimuli that have undergone extinction (e.g., Harris, Shand, Carroll, & Westbrook, 2004). In the present studies there was a greater opportunity for extinction to have occurred to CScarb than to CSprot in the CScarb versus CSprot tests. Thus, the absence of an effect of carbohydrate devaluation in the CS+ versus CS– tests is not easily interpreted. In any case, this

uncertainty does not diminish the basic conclusion that rats form associations between the CS flavor and sensory aspects of some nutrients.

The nature of the association between the CS flavor and the sensory features of the nutrient is equally unclear from the present studies. The most obvious possibility is that the CS flavors were associated with orosensory components of the nutrients (i.e., with their gustatory and/or olfactory features). Another possibility, however, is that the CS flavors may have become associated with some postoral viscerosensory features of the nutrients (e.g., Baker, Booth, Duggan, & Gibson, 1987; Perez, Ackroff, & Sclafani, 1996). If, for example, our carbohydrate (Polycose) and protein (casein) generate different postoral sensations that can enter into associations both with the CS flavors and with LiCl injections, then the US devaluation effects reported here can be accounted for. Unpublished research in our laboratory has failed to find evidence that rats can associate IG infusions of the nutrients we used with LiCl injections, which would be revealed by a reduction in subsequent intake of the nutrients. However, some recent data do suggest that nutrient aversions can be formed in this way (Tracy et al., 2004). However, these same authors failed to find evidence that a CS flavor associated with an intragastrically presented nutrient was sensitive to nutrient devaluation achieved in this manner. Thus, although there exists evidence for the claim that different nutrients may elicit specific postingestive sensory signals, there is little evidence presently to suggest that flavor CSs can associate with these in a manner that would support US devaluation effects. The most parsimonious conclusion, therefore, is that subjects in our studies learned by associating the flavor CSs with the orosensory features of the nutrients with which they were paired. When these sensory features were later associated with LiCl, selective devaluation of the CS flavors occurred.

The present results are of additional interest with reference to research examining sensory preconditioning. As noted in the general introduction, sensory preconditioning and nutrient-conditioned flavor preference studies both bear on the idea that flavor–flavor associations develop when two flavors are paired. Though the conclusion that such associations actually consist of associations forming between the sensory properties of the two flavors in sensory preconditioning studies seems straightforward, the nature of this learning in nutrient conditioning has not been well understood. When a flavor CS is paired with an orally consumed palatable nutrient, the CS could become associated with the sensory, hedonic, or postingestive nutritive components of the US. The present results suggest that associations with either the orosensory or the viscerosensory features of the nutrient can occur. They do not permit, at this point, any strong conclusions about possible associations involving the CS and the hedonic or postingestive nutritive components of the US. However, a comparison between the US devaluation effects seen here and those seen in sensory preconditioning studies might suggest some important interactions between the various US features.

Earlier sensory preconditioning research (Lavin, 1976; Lyn & Capaldi, 1994; Rescorla, 1980) demonstrated that flavor–flavor associations can be formed only when the flavors are either mixed together in solution or sequentially paired with very little time separating the two flavors (i.e., less than 27 s). However, the present study provides evidence that associations with the sensory characteristics of a nutrient can occur even when the CS and nutrient flavors are separated by up to 10 min (at least) in the forward direction and 5 min (at least) in the backward direction. What might account for these rather dramatic differences? One rather trivial explanation is that the sensory features of the nutritive solutions we used are simply more salient than the sensory features of the nonnutritive solutions studied in sensory preconditioning. Given the large difference in the effective flavor–flavor intervals used in these studies, we think that salience differences are unlikely to be the sole explanation. Another, somewhat related possibility is that postingestive nutritional consequences of nutrient intake stimulate motivational feedback (i.e., general

reinforcement signals) that serves to maintain the processing of the sensory features of the solution for a much longer time than could otherwise occur. In the terms of Wagner and Brandon's (1989) AESOP theory of associative learning, this assumption can be understood in terms of the sensory features of a stimulus decaying from their primary activation state more slowly if the stimulus in question is a potent motivational event than if it is not. Thus, if a CS is paired with a US whose sensory features are processed for a longer period of time, then perhaps associations can be established over a much longer trace interval. It may be of some interest to examine how well associations can be established with the very same sensory event when that event is accompanied by an independently controlled motivational effect or not. Such a scenario may be accomplished in the present paradigm by accompanying (or not accompanying) intake of a nonnutritive solution with intragastric infusion of a nutrient.

Another possible explanation is based on the idea that the CS might come to associate with an "integrated" US representation. One way for this to work is by assuming that the unique sensory and the general motivational features of a US become associated with one another. This association may be necessary for the motivational component of the nutrient to maintain processing of the sensory features beyond what would normally occur without this associative link with the motivational aspects of the nutrient. In other words, by virtue of this association, the rates at which the sensory and motivational components of the nutrient decay from their primary activation states may be much more comparable than if no such association existed. This additional processing would allow for the CS to be associated with the sensory features of a flavor US under conditions that might not normally be possible with nonnutritive flavors.

There is another way in which the "integrated" US representation idea can help explain the present results. Suppose that the flavor CS is associated with the postingestive reinforcing properties of the nutrient with which it is paired, even after a fairly long trace interval (e.g., Drucker, 1996). Further suppose that the orosensory and the postingestive reinforcing properties of the nutrient associate with one another. At the time of test, the flavor CS could associatively activate the postingestive reinforcing aspects of the nutrient with which it was paired, and this, in turn, could associatively activate the orosensory properties of that nutrient. If the orosensory aspects of the nutrient underwent devaluation, then there is a basis for decreased intake of the appropriate CS at test. Notice that for this mechanism to work, however, the "postingestive reinforcing" components of the two nutrients used here (Polycose and casein) must themselves be distinguishable. The results reported by Tracy et al. (2004) are consistent with this general claim.

Regardless of how one is to explain the present findings, it seems clear that the results reported here indicate that US devaluation effects can be found in nutrient-conditioned preference procedures. These effects imply, at the very least, that the CS flavors can enter into associations with the sensory features of a nutrient US, and that such associations can be formed with much longer forward or backward trace intervals separating the CS and US than would be expected in sensory preconditioning procedures. Precisely how the sensory and motivational features of a nutrient US might interact with one another in the conditioning of a flavor preference must await further research.

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Figure 1.

Mean intake in the CScarb versus CS- and CSprot versus CS- tests of Experiment 1a.

CScarb = flavor paired with carbohydrate; CS- = flavor presented by itself; CSprot = flavor paired with protein; CS+ = neutral flavored conditioned stimulus.

CScarb vs CSprot Devaluation Test

Figure 2.

Mean intake in CScarb versus CSprot tests for subjects receiving either the carbohydrate (Polycose) or the protein (casein) devalued in Experiment 1b. CScarb = flavor paired with carbohydrate; CSprot = flavor paired with protein.

Figure 3.

Mean intake (upper panel) for Group Immediate and Group 10-min Trace subjects in the CScarb versus CSprot tests of Experiment 2. Subjects received either the carbohydrate (Polycose) or the protein (casein) devalued. The lower panel expresses the data in terms of percentage CScarb intake. CScarb = flavor paired with carbohydrate (Carb); CSprot = flavor paired with protein (Prot); D = devaluation; Dev = devaluation; Gp = Group; Immed = Immediate.

Figure 4.

Mean conditioned stimulus (CS) and unconditioned stimulus (US) intakes for Group Forward and Group Reverse over the 10 conditioning cycles of Experiment 3. Gp = Group; CScarb = flavor paired with carbohydrate (Carb); CSprot = flavor paired with protein; CS- = flavor presented by itself.

Figure 5.

Mean intake (upper panel) for Group Forward and Group Reverse subjects in the CScarb versus CSprot tests of Experiment 3. Subjects received either the carbohydrate (Polycose) or the protein (casein) devalued. The lower panel expresses the data in terms of percentage of CScarb intake. CScarb = flavor paired with carbohydrate (Carb); CSprot = flavor paired with protein (Prot); Dev = devaluation; Gp = Group.

Table 1

Mean Intakes (ml) of Solutions in CS+ Versus CS– Tests

Devalued nutrient	<u>CScarb vs. CS– tests</u>		<u>CSprot vs. CS– tests</u>	
	CScarb	CS–	CSprot	CS–
Experiment 1b				
Carbohydrate	16.3	0.7	11.3	3.6
Protein	15.4	3.2	4.7	8.2
Experiment 2				
Group Immediate				
Carbohydrate	11.8	6.1	8.2	7.0
Protein	14.7	4.7	10.6	8.7
Group 10-min Trace				
Carbohydrate	8.6	7.1	10.1	6.2
Protein	12.7	5.7	8.0	8.0
Experiment 3				
Group Forward				
Carbohydrate	10.4	6.7	10.6	5.2
Protein	11.0	5.9	8.5	6.9
Group Reverse				
Carbohydrate	13.4	6.2	11.3	5.8
Protein	11.5	4.9	9.8	6.5
Overall means				
Carbohydrate	12.1	5.7	10.6	5.5
Protein	12.5	5.1	8.6	7.4

Note. CS = conditioned stimulus; CS+ = neutral flavored conditioned stimulus; CS– = flavor presented by itself; CScarb = flavor paired with carbohydrate; CSprot = flavor paired with protein.