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Conditioned Flavor Preferences in Children

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Children's ability to acquire conditioned flavor preferences was examined in two experiments. In Experiment 1, 57 children between the ages of 5 and 12 years consumed 4-ml samples of three flavors of herbal tea, one of which was mixed with sucrose (CS++), another with aspartame (CS+), and the other alone (CS-) over 42 trials during one conditioning session. A three-bottle consumption task, which was completed before and after conditioning, indicated that conditioned flavor preference (CFP) did not form as a result of flavor-taste conditioning. In Experiment 2, 49 children between the ages of 4 and 10 years consumed two flavors of herbal teas, one of which was mixed with either sucrose (a sweet, calorific reinforcer), aspartame (a sweet, non-calorific reinforcer), or Polycose (a mildly sweet, calorific reinforcer) (CS+), while the other was presented alone (CS-) over an eight-day conditioning phase at home. A 2-bottle consumption task completed pre- and post-conditioning indicated that those in the sucrose group showed a marginal increase in preference for the CS+, whereas those in the aspartame and Polycose groups did not. Moreover, more of the participants in the sucrose group increased their preference for the CS+ than those in the aspartame and Polycose groups. These findings suggest that sucrose might be a stronger reinforcer than aspartame or Polycose due to its ability to condition through both flavor-taste and flavor-calorie associations. This information could help us develop evidence-based strategies to encourage healthy eating habits in childhood, which in turn could impact healthy food choices throughout the lifespan.
Conditioned Flavor Preferences in Children

Introduction

Over the past thirty years, the incidence of obesity has risen dramatically in American youth. Today, more than 20% of children and adolescents are considered obese (Centers for Disease Control and Prevention [CDC], 2012). These statistics are alarming given that obesity places adults and children alike at increased risk for several serious health complications including diabetes, gallbladder disease, and high blood pressure (CDC, 2012). Although it cannot be denied that genetics have an important impact on weight differences, environment has also been shown to have a significant influence (Wadden, Brownell, & Foster, 2002). Along with inadequate physical activity, consuming a diet that is high in sugar and fat contributes to obesity (United States Department of Health and Human Services, 2000). The increase in obesity observed over the past several decades is thought to be caused, in part, by the abundance of high calorie, low nutrient foods that are cheaper and more convenient than healthier options, such as fruits and vegetables (McCrory, et al., 1999).

Despite the serious consequences associated with poor diet, only 30% of children between the ages of 6 and 11 years of age eat the recommended amount of vegetables in a day (United States Department of Health and Human Services, 2000). Poor food choices combined with an increasingly sedentary lifestyle (involving television, computers, and video games) have created what Wadden and colleagues (2002) have dubbed a “toxic” environment for children. Given that obese children are more likely to become overweight adults (CDC, 2012), it is a public health priority to promote healthy eating in children. However, in order to design effective interventions it is important to
understand the factors involved in the development of children’s flavor and food preferences.

Previous research has shown that liking for flavors can be enhanced through repeated exposure. Over the past 30 years, several research studies (Birch & Marlin, 1982; Forestell & Mennella, 2007; Gerrish & Mennella, 2001; Lakkakula, Geaghan, Zanovec, Pierce, & Turri, 2010) have demonstrated that when a child is repeatedly exposed to an initially disliked flavor or food they gradually learn to like and accept that flavor. Repeated exposure to flavors begins to occur prenatally through exposure to flavors in the mother’s amniotic fluid, which reflect her diet. In this manner, fetuses are exposed to a wide array of flavors within the mother’s culture (for a review see Forestell & Mennella, 2008). These exposures continue for newborns who are breastfeeding, as flavors in the mother’s diet are also passed to the infant through her breast milk (Mennella, Jagnow, & Beauchamp, 2001). Once children undergo the weaning process, these early exposures enhance the acceptance of the solid foods that the mother ate during pregnancy and lactation (Forestell & Mennella, 2007; Mennella, Jagnow & Beauchamp, 2001).

**Flavor Preference Conditioning in the Rat**

Animal research has given us insight into other ways flavor preferences can form. By using the rat as a model, research has demonstrated that flavor preferences can be acquired through Pavlovian conditioning (Sclafani, 1990). In these paradigms, a conditioned stimulus (CS+) is paired with a sweet-tasting or calorific reinforcer (which serves as an unconditioned stimulus or US). Another flavor (CS-) is paired with a non-calorific, neutral tasting source (for example water). After repeated trials, animals
demonstrate conditioned preferences by consuming more of the CS+ relative to the CS-
when presented with each solution without the reinforcer simultaneously in a 2-bottle test
(e.g., Elizalde & Sclafani, 1990; Fanselow & Birk, 1982; Mehiel, 1991). Because sweet-
tasting reinforcers are typically also calorific, animals may form two types of associations
during conditioning: a flavor-taste association, in which the sweet taste of the sugar
becomes associated with the flavor, and a flavor-calorie association, in which the calories
of the reinforcer become associated with the flavor. Therefore, in conditioning paradigms
that involve simultaneous ingestion of the flavor and the sugar, it is unclear to what
extent flavor-taste associations and flavor-calorie associations are responsible for the
resulting conditioned flavor preference (CFP).

In order to address this issue, studies have attempted to separate the sweet taste
from calories by using saccharin as a reinforcer, which has a sweet taste but is not
calorific. In one such experiment, Fanselow and Birk (1982) paired two neutral flavors
with saccharin and quinine (which has a bitter flavor) over several trials. After
conditioning, rats showed a preference for the flavor that had been paired with saccharin.
Similarly Holman (1980) demonstrated that a flavor paired with saccharin may become
preferred to one that has paired with water or to another that has been paired with a more
dilute saccharin solution (Holman, 1975).

In order to demonstrate that calories alone can also produce CFP, an early study,
which paired flavors with low doses of ethanol intubated directly into the stomach,
demonstrated that rats acquired conditioned preferences for the flavor paired with ethanol
(Sherman, Hickis, Rice, Rusiniak, & Garcia, 1983). In this experiment, they concluded
that the calories provided by the ethanol served as the US. More recently, Sclafani and
colleagues have developed an infusion procedure in which rats are implanted with intagastric catheters that automatically deliver either a calorific reinforcer or water directly into stomach as the animal consumes flavored solutions in their home cages (e.g., Ackroff & Sclafani, 1994; Elizalde & Scalafani, 1988; Sclafani & Nissenbaum, 1988). After repeated trials, rats show a preference for the flavor that was paired with the calorific infusion relative to another flavor that is paired with an infusion of water. In a series of CFP studies with rats that use this procedure, Ackroff, Dym, Yiin, and Sclafani (2009) found that CFPs can be acquired quite rapidly with calorific reinforcers. Using glucose delivered intragastrically, rats acquired a preference for the glucose-paired flavor after only one 30-minute exposure to the CS+ paired with a glucose infusion and the CS- paired with a water infusion.

Recent work investigating the neurochemical basis of CFPs supports the idea that flavor-calorie and flavor-taste conditioning are a function of two distinct (although not mutually exclusive) mechanisms. Through localized administration of dopamine receptor antagonists prior to conditioning, researchers have examined the role of the dopamine systems in flavor preference conditioning. A study by Hsiao and Smith (1995) found that D2-like antagonism caused sweet tastes to become less rewarding to rats. Tousazani, Bodnar, and Sclafani (2010) expanded on this finding with a series of studies by investigating the roles of the dopamine systems in flavor-taste and flavor-calorie conditioning separately. The acquisition and expression of flavor-taste conditioning based on sweet taste was found to be reduced by both D1 and D2 antagonists, indicating that both of these systems play a role in flavor-taste learning. On the other hand, flavor-calorie learning was only influenced by D1 antagonists, suggesting that the D2 system is
not involved in this type of learning. Moreover, the D1 antagonist administration weakened only the acquisition and not the expression of flavor-calorie learning. These data suggest that although both flavor-taste and flavor-calorie learning involve the dopamine systems they do not appear to have identical neuropharmacological pathways.

**Flavor Preference Conditioning in Humans**

Early research that investigated flavor conditioning in humans demonstrated conditioned aversions. For example, when adult humans, like rats, received flavors paired with a bitter tasting reinforcer (i.e., Tween20), they acquired aversions to the cue flavor (Baeyens, Crombez, De Houwer, & Eelen, 1996). In other studies, CFPs were demonstrated by pairing flavors with sweet-tasting, calorific reinforcers (Mobini, Chambers, & Yeomans, 2007). However, many other studies have failed to find evidence of CFPs in humans (see Brunstrom, 2005 for a review).

The reported inconsistencies in the literature with respect to CFPs may be a result of individual differences in personal characteristics or prior experiences that are difficult to control in human studies, which may contribute to error variance. One such factor is the hunger level of the participants. Although it has been demonstrated in rodent models that hunger is important for the expression but not the acquisition of calorie-mediated conditioned preferences (Fedorachak & Bolles, 1987; Forestell, Schellnick & LoLordo, 2001), Brunstrom and Fletcher (2007) have demonstrated that hunger levels may also impact the acquisition of taste-mediated conditioned preferences in humans. In this study, only those who were hungry during conditioning acquired a conditioned preference for the flavor paired with saccharin relative to the flavors presented alone.
Additionally, independent of hunger levels, people vary in the extent to which they like sweet tastes (e.g., Pepino & Mennella, 2005). This natural variation in liking for sweet-tasting reinforcers appears to play a role in the effectiveness of flavor preference conditioning. Studies that have preselected only participants who like sweet tastes have found increased liking for flavors and odors paired with sweet taste (Yeomans & Mobini, 2006; Yeomans, Mobini, Elliman, Walker, & Stevenson, 2006) while many studies that have not measured sweet liking have failed to find evidence of CFPs (Bayens, Eelen, Van Den Burgh, & Crombez, 1990; Stevenson, Boakes, & Prescott, 1998; Stevenson, Boakes, & Wilson, 2000; Stevenson & Prescott, 1995). Participants also may differ in the extent to which they like the CS flavors. As a result of this variability, both study design and individual differences must be carefully considered when working with humans.

**Conditioned Flavor Preferences in Children**

Relative to adults, less research has been conducted to investigate CFPs in children. This is surprising considering that many flavor preferences are formed in childhood. The few studies that have been conducted have paired sweet tasting, calorific reinforcers with vegetable (Havermans & Jansen, 2007; Zeinstra, Koelen, Kok, & de Graaf, 2009) or fruit drinks (Capaldi & Privitera, 2008). As in some early rat studies, it is difficult to distinguish between the effects of the sweet taste and the postingestive consequences of the calories that are driving the flavor learning in these studies.

For example, in a study by Havermans and Jansen (2007) children consumed vegetable juices that were either sweetened with sucrose or unsweetened over six conditioning trials. After conditioning, when the children were asked to rate the
vegetable juices presented in their unsweetened form, they demonstrated CFPs by consuming more of the vegetable juice that had been previously sweetened relative to the vegetable juice that had not. This finding was further supported by a study that found that 2 to 5 year-old children increased their preference for unsweetened grapefruit juice after they had been repeatedly exposed to grapefruit juice sweetened with sugar (Capaldi & Privitera, 2008). When tested again several weeks later, the increased liking persisted. While the authors of these studies attributed these preferences to flavor-taste associations, it is possible that calorific reinforcement may have also contributed to these preferences.

Other studies have failed to demonstrate conditioning (Zeinstra, Koelen, Kok, & de Graaf, 2009) in children. As demonstrated by Zeinstra et al. (2009), one barrier to conditioning is that children typically find the taste of vegetables bitter and unpalatable (Drewnowski & Gomez-Cárneros, 2000; Sandell & Breslin, 2009; Pepino & Mennella, 1995), and as a result they often refuse to consume sufficient amounts of the solutions during conditioning, thereby precluding the development of conditioned preferences.

The lack of definitive research in conditioned preferences in children highlights the need for more carefully designed studies that will expand our knowledge of how CFP operates in young humans. The current experiments were designed with the following goals in mind: (1) To determine whether children can acquire flavor-taste associations in a single conditioning session consisting of several trials which precludes flavor-calorie conditioning (Experiment 1), and (2) To determine the relative contributions of flavor-taste and flavor-calorie conditioning to CFP using a more protracted conditioning phase (Experiment 2).

**Experiment 1**
As mentioned previously, CFP research in humans has been fraught with inconsistent findings (Brunstrom, 2005). The difficulty in consistently demonstrating CFPs in humans may be due to technical limitations that are not experienced when experimenting with rat models. For example, typically in human flavor studies, participants are asked to engage in a series of conditioning trials over several days. The participant must either take the solutions home to consume them daily or make repeated visits to a lab, which could lead to problems with compliance and attrition (Mobini, Chambers, & Yeomans, 2007). This may be especially true in studies with children, in which the quality of the data depends on the compliance of the both the mother and child.

Although many studies rely on home exposure trials, there is evidence in the literature that flavor-taste conditioning may occur when multiple trials are presented over a short period of time (Zellner, Rozin, Aron, & Kulish, 1983). In their study, college-age participants were asked to drink numerous small samples of different flavors of iced tea during one conditioning session. Some of the flavors of tea were paired with sucrose while others were presented alone. For the first experiment, each participant was presented with 64 4 ml-cups of tea. Of the first 48 cups presented, 24 contained one flavor paired with sucrose and the remaining 24 contained the flavor mixed in water. Throughout conditioning, participants were asked to rate the sweetness of each solution in relation to the first one that was tried (which always contained sucrose). Participants then completed a liking scale for the remaining 16 solutions, some of which were the same as those offered during the conditioning phase and some of which were different. The results showed that the addition of sucrose increased liking for all teas. This approach has some advantages over the traditional conditioning paradigm in that
everyone drinks the same amount of both teas and all of the conditioning takes place in the lab where it can be monitored, which allows for more experimental control.

If a single conditioning session that contains several trials is effective in producing CFPs in children, problems of compliance and attrition would be avoided. Thus, the goal of the present study was to replicate and extend the results of Zellner et al. (1983) using children. In the current study, flavored teas were paired with either sucrose (CS++), which has a sweet taste, an equally sweet non-calorific solution aspartame (CS+), or presented alone (CS-) during a single conditioning session. Participants were given a three-bottle test prior to and after conditioning to measure the strength of the CFPs. Sweet preference and hunger levels were additionally measured in order into account for these individual differences.

Because individuals were consuming both calorific and non-calorific solutions in close succession, we believe that any CFP that occurred would be a result of flavor-taste learning. It is unlikely that the postingestive consequences of the calories would provide differential reinforcement for the flavors. That is, even if participants did experience positive postingestive consequences from drinking the flavors mixed with sucrose, they would likely not be aware of which solutions contained calories. Therefore, it was hypothesized that children would show stronger CFPs for flavors paired with sucrose or aspartame as a function of their sweet taste than for a flavor presented alone.

Method

Participants

Thirty children between the ages of 5 and 12 years and their mothers were recruited for this study. Children were recruited through the use of advertisements,
mailings, and Craig’s List. Participants were healthy, with no allergies or medications that could interfere with their appetite. All procedures were approved by the College’s Protection of Human Subjects Committee, and written informed consent was obtained from each participant.

**Materials**

*Conditioning and Test Solutions.* Seven caffeine-free fruit-flavored herbal teas; Pleasantly Pear, Black Cherry Berry, Country Peach Passion, Raspberry Zinger, Tangerine Orange Zinger, True Blueberry all made by Celestial Seasonings (Boulder, Colorado) and Mango Passionfruit by Stash Premium (Portland, Oregon), were used for this study because they are calorie-free, unsweetened in their natural form, and their flavors are easily differentiated. Teas were prepared by steeping a tea bag in 600ml of boiled distilled water for 5 minutes. Red food dye was then added each tea to diminish any appearance differences that might influence preferences. All teas were served at room temperature. Each participant received three flavored teas during the conditioning phase, which were determined by the ranking task (described below). During the conditioning phase, two of the three teas selected were mixed with a reinforcer. Similar to Mobini, Chambers, and Yeomans (2007), sucrose (5% wt/vol), aspartame (0.01% wt/vol), and Acesulfame potassium (Ace K, 0.007% wt/vol) were added to the CS++ flavor. The CS+ was mixed with a sweet, non-calorific solution, which consisted of 0.02% wt/vol aspartame and 0.014% wt/vol Ace K. These concentrations were rated to be relatively equal to one another by a panel of adults. The CS- solution contained only the flavored tea made with distilled water. Four milliliters of each tea was presented in 5 ml solo cups. For the three-bottle test, 150 ml of each tea was presented in a plastic cup
with a sippy lid on top to prevent spills. All three cups used in the three-bottle test were always the same color.

**Testing Procedures**

*Three-Bottle Test.* Participants received 150 ml of the CS++, CS+, and CS- flavors in separate pre-weighed cups with covers. The participant was asked to try each tea and then told that they could drink as much of all the teas as they wanted. All teas were presented in their unsweetened form simultaneously for twenty minutes. At the end of the task, the cups were weighed to determine consumption.

*Ranking Task.* Participants were asked to rank the seven flavors of unsweetened tea using a procedure developed by Birch (1990). The participant was instructed to not swallow the teas but rather to take a sip of tea and swish it around in their mouth for five seconds. They were then asked to spit the tea out in a sink. Between each trial, the participant rinsed his or her mouth with water. For each tea the participant ranked it as “liked”, “just ok”, or “disliked” by placing it on a cartoon face that corresponded to the ranking. Once each of the teas was placed on a cartoon face, the participant was randomly presented with the flavors within each category in pairs. They tasted them each again without swallowing, and reported whether they preferred the first or second tea that they tried. Participants rinsed with water between pairs. This provided rankings from one (the most liked) to seven (the least liked).

*Sweet preference task.* In order to determine how much the participant liked sweet tastes, they were presented with sucrose mixed with water at five different concentrations (3%, 6%, 12%, 24%, 36%) using a procedure developed for children by Mennella, Pepino, Lehmann-Castor, and Yourshaw (2010). Samples were presented in pairs, starting with
$6\%$ and $24\%$. The child was asked to taste each sample, swish it in their mouth for five seconds, and then spit it out without swallowing it. The sample that the participant indicated that they preferred became one of the samples in the next pairs. Each subsequent sample in the pair was selected based on their choice (for example if they picked $6\%$ when given $6\%$ and $24\%$, the $6\%$ was then paired with $3\%$, if they picked $24\%$, this sample was then paired with $12\%$). Between pairs they were asked to rinse with water, which they also spit out, before tasting the next pair. This was done until the participant picked the same concentration in two consecutive trials where it was compared with both concentrations adjacent to it. Once that had been accomplished, the procedure was completed in the reverse order (for example if $6\%$ was chosen on the first trial in which it was paired with $24\%$, it would then be paired with the more concentrated $12\%$ instead of less concentrated $3\%$, similarly if $24\%$ was chosen, it would be paired with $36\%$). Average scores were calculated for the two sets of trials.

**Test Day Procedures**

Participants came into the lab on two separate days (no more than two days apart). During all testing, the experimenter kept all stimuli out of view of the participant until it was presented to them. During recruitment mothers were instructed to not feed their child for at least two hours before the appointments in order to ensure that the child was hungry on testing days.

*Test Day 1:* On Test Day 1, after informed consent and assent were completed children were asked whether they were currently hungry, and the last time that they ate was noted along with what it was that they last had to eat. The weight and height of all the children were recorded. Children then completed the ranking task to determine which of the seven
teas they rated as three, four, and five. These teas were randomly assigned to be the CS++, CS+, or CS- for the study. The three-bottle pre-test followed. This provided a baseline from which to compare any preference changes that might occur as a result of the conditioning phase.

*Test Day 2:* On the second test day conditioning and rating of the CS solutions was conducted. During the conditioning phase the CS solutions were presented in 5 ml solo cups separated by a one-minute break. Participants were asked to drink all 4 ml of the tea solutions in each cup and then rate how much they liked it on a five-point scale ranging from 1 (Bad) to 5 (Good). In total, each participant received 14 presentations of each CS solution for a total of 42 trials. The three-bottle consumption task was subsequently repeated after a short break in order to determine the influence of the conditioning phase on preference for the flavors.

**Results**

**Participant Characteristics**

Thirty children (14 females) between the ages of 5 and 12 years ($M= 9.1$, $SD = 2.5$ years) participated in the experiment. Twenty six of the children were Caucasian and 4 were African American. Average sweet preference scores, BMIs, and previous exposure to tea are shown in Table 1.

**Ratings of CS Solutions during the Conditioning Phase**

As seen in Figure 1, the average ratings for the teas during conditioning were between 1.87 and 4.17, however ratings for the teas differed according to whether or not they were sweetened. Across trials, children rated the sweetened teas (the CS++ and the CS+) higher on the 5-point liking scale than the unsweetened tea (the CS-). This was
supported by a 3 (CS) x 14 (Trials) repeated measures ANOVA that revealed a main effect of CS \( F(2, 56) = 32.43, p < 0.05 \) and a main effect of Trial \( F(13, 364) = 2.04, p = 0.043 \), but no significant interactions. Bonferroni pairwise comparisons revealed that the CS- (\( M = 2.17, 95\% \text{ CI} [1.73, 2.62] \)) was rated significantly lower than the CS+ (\( M = 3.57, 95\% \text{ CI} [3.21, 3.93], p < 0.01 \)) and CS++ (\( M = 3.64, 95\% \text{ CI} [3.31, 4.18], p < 0.01 \)), and the two sweetened teas were not rated differently from each other. Pairwise comparisons of the mean ratings from the first four and the last four trials indicated that although there was a main effect of trials, ratings did not increase as conditioning progressed.

**Three-bottle Test Consumption**

Consumption scores for the 3-bottle tests before and after conditioning were compared with a 3 (CS) x 2 (Time) repeated measures ANOVA. As shown in Figure 2, this analysis failed to reveal any significant main effects for CS \( F(2, 58) = 0.76, p > 0.05 \), Time \( F(1, 29) = 0.01, p > 0.05 \), or a CS x Time interaction \( F(2, 58) = 1.73, p > 0.05 \). Overall there was no evidence that the CS++ or CS+ became more preferred after conditioning relative to the CS- in children.

Further analyses were conducted in which children with a low sucrose preference were excluded as sweet preference has been shown to influence flavor-taste learning. Children were divided into groups according to their sweet preference using the cut-off score of 12 g/100ml, which was the middle sucrose concentration offered in the sucrose preference task. Participants with an average sucrose score of greater than or equal to 13 g/100ml were defined as having a high sucrose preference (\( n = 24 \)) and were included in the analysis. A 3 (CS) x 2 (Time) repeated measure ANOVA was then conducted. The ANOVA revealed no significant differences in the consumption tasks for the different CS
solutions ($F(2, 46) = 0.89, p > .05$) and there was no significant CS x Time interaction ($F(2, 46) = 1.87, p > .05$).

Given that previous research has shown that hunger may affect acquisition (Brunstrom & Fletcher, 2008) or expression of preferences (Fedorchak & Bolles, 1987), further analyses were then conducted which excluded participants who had eaten during the two hours preceding the visits to the lab. Twenty-eight children had refrained from eating and were included in the $3 \times 2 \times 2$ repeated measures ANOVA. However, this analysis failed to reveal significant differences in consumption of the CS solutions as a function of time and sucrose preference ($F(2, 52) = 1.21, p > .05$).

**Discussion**

The goal of the present study was to determine whether flavor-taste associations would occur in children using a procedure that controlled for the amount of solution that was ingested during conditioning, involved one conditioning session that consisted of several trials, and had previously been shown to be affective in producing CFPs in adults (Zellner et al., 1983). Based on Zellner et al.’s findings, we hypothesized that CFP would form in children after receiving flavors paired with either sucrose or aspartame. In the current study, later rankings of the CS solutions during the conditioning session did not differ from the early ratings, and consumption of the solutions did not change. These findings indicate that CFP did not occur in children using this paradigm.

As mentioned previously, flavor preferences have been difficult to reliably condition in humans, with some studies finding evidence of conditioning (Brunstrom and Fletcher, 2007; Mobini, Chambers, & Yeomans, 2007; Zellner, et al., 1983) and others
failing to do so (Bayens, Eelen, Van Den Burgh, & Crombez, 1990; Stevenson, Boakes, & Prescott, 1998; Stevenson, Boakes, & Wilson, 2000; Stevenson & Prescott, 1995; Zeinstra, Koelen, Kok, & de Graaf, 2009).

The current study accounted for many of the individual difference variables, such as hunger and sucrose preferences, which have been thought to contribute to the variability in CFP studies. However, after controlling for these influences there was no evidence of CFPs in children in the present study. In contrast, Zellner et al. (1983) demonstrated CFPs with adults without controlling for these factors. However, there are several methodological differences between our study and that of Zellner et al. For example, Zellner et al. asked participants to rate the CS flavors as well as novel flavors that were presented both in their sweetened and unsweetened form after conditioning. Unlike Zellner et al., our study relied on consumption of the CS solutions in a three-bottle test to determine if CFP occurred. Although participants completed liking ratings during the conditioning phase of our study, they did not complete ratings during the three-bottle test and it is possible that if given the opportunity to complete post-conditioning ratings, the CS++ and CS+ solutions may have been rated more positively than the CS- solution (although probably not rated as highly as the solutions in their sweetened form).

It is important to note that during the three-bottle test all flavors were presented in their unsweetened form. Since unsweetened solutions were not favorably rated during our study, it is possible that participants' consumption of the solutions might not accurately reflect a CFP even if one did occur. That is, even if a participant liked an unsweetened form of a previously sweetened flavor more than they liked an unsweetened solution that had never been paired with a sweet tasting reinforcer, they still might not
have liked it enough to consume more of it. Thus, one possible explanation for the failure of this study to find CFP could be in the participants’ reluctance to consume unsweetened teas in the 3-bottle tests. On average, participants consumed only about 8.29 ml during the post test (out of an available 450 ml). Similarly, in a study by Zeinstra, Koelen, Kok, and de Graaf (2009) children were unwilling to consume sufficient amounts of the vegetable drinks during the conditioning phase. They concluded that children found the tastes of the drinks unpleasant and that in order to examine conditioning, the vegetable drinks would have to be made more palatable.

Although children in the present study consumed all of the 4 ml samples in the conditioning phase regardless of whether they were sweetened (as per experimental protocol), when they were given free choice to consume the unsweetened solutions during the three-bottle test, they were generally only willing to try the solutions, and were unwilling to drink any of them in great quantities. If participants had instead been asked to rate the solutions, we might have more readily observed conditioned preferences.

Another possible explanation for the lack of CFP could be due to the timeline of our study. While it has been established that rats (Ackroff Dym, Yiin, & Sclafani, 2009) and perhaps adult humans (Zellner et al., 1983), can acquire CFP very quickly it is not clear whether children acquire CFPs as quickly. In addition, children might have been overwhelmed with the procedures of the study (which were based of those used in Zeller et al., 1983) or have been unable to keep track of which reinforcers were paired with which flavors. Because a separate group of adults were not included in the present study, it is not clear whether the children’s failure to acquire CPP was a result of the paradigm itself, their inability to acquire preferences within one conditioning session that consisted
of several trials, or both. It is also worth noting that the CFP studies that have been successful in children and adults have used sweet, calorific reinforcers, (Birch, 1990; Capalidi & Privitera 2008; Havermans and Jansen, 2007), suggesting that the effects of flavor-taste and flavor-calorie associations may be additive. The goal of the next experiment was to test this hypothesis.

**Experiment 2**

As Experiment 1 failed to find evidence of CFPs, a second study was designed to overcome the potential limitations identified above. In the second study we utilized a more traditional timeline for human CFP studies by sending the conditioning solutions home with the mother and child to be consumed over a period of eight days. Although this design increased the possibility of noncompliance and resulted in the loss of some experimental control, it provided an extended conditioning phase within a natural environment for the children to acquire conditioned preferences.

This procedural change also provided the ability to access the relative contributions of both flavor-taste and flavor-calorie learning. Thus, the current study sought to clarify how CFP operates in children by using a between groups design in which all children received two flavors of iced tea (CS+ and CS-) on separate days. Depending on the group to which they were randomly assigned, the CS+ flavor was paired with either a sweet tasting calorific reinforcer; i.e., sucrose, a sweet tasting non-calorific reinforcer; i.e., aspartame/Ace K, or Polycose; a starch-based minimally sweet tasting calorific reinforcer. Polycose is starch based, and contains calories while having a minimally sweet taste to humans, although rats find the taste palatable (Bonacchi,
Ackroff, & Sclafani, 2008; Zuckerman, Glendinning, Margolskee, & Sclafani, 2009). Interestingly, Bonnachhi and colleagues (2008) found that although rats would consume more of a Polycose-paired flavor (CS+) than a water-paired flavor (CS-) when presented with both simultaneously, Polycose did not produce a flavor-taste CFP, whereas sucrose did. Thus, although Polycose produces strong CFPs through flavor-calorie learning, it is not effective as a flavor-taste reinforce in rats. As a result, it is potentially an effective solution for looking at the effects of flavor-calorie learning without the influence of flavor-taste learning. For all three groups the CS+ alternated with the presentation of the CS- flavor in water over the eight day home exposure phase. Based on the principles of flavor-taste and flavor-calorie learning it is hypothesized that: (1) Children would develop greater CFPs for flavors that were paired with a reinforcer compared to flavors that were presented alone (2) Sucrose would produce stronger CFP than aspartame and Polycose because it provides reinforcement through sweet taste and calories.

**Method**

**Participants**

A total of 35 mothers and their 59 children between the ages of four and ten years were recruited for this study. Children were healthy, with no allergies or medications that could interfere with the study. Participants were recruited through online ads, telephone calls, and mass mailings. The College’s Protection of Human Subjects Committee approved all procedures, and written informed consent was obtained from each participant.

**Materials**
Conditioning and Test solutions. The CSs consisted of six flavors of caffeine-free fruit-flavored herbal teas: Country Peach Passion, Lemon Zinger, Raspberry Zinger, Perfectly Pear, and Tangerine Orange Zinger all made by Celestial Seasonings (Boulder, Colorado) and Mango Passionfruit by Stash Premium (Portland, Oregon). For the last 38 participants, True Blueberry by Celestial Seasonings was substituted for Perfectly Pear, which became unavailable part way through the study. Teas were prepared as described in Experiment 1. Additionally 9 ml of aspartame solution (0.01% wt/vol of aspartame and 0.07% wt/vol Ace K) was also added to each 600ml of tea to provide a baseline sweetening. Given that children in Experiment 1 were reluctant to consume the unsweetened teas, the baseline sweetening was used to increase the palatability of the teas.

For the CS+ solutions, one of three reinforcers were added depending on the group to which the child was randomly assigned; sucrose, which is sweet and contains calories, an aspartame solution, which is sweet and does not contain calories, or Polycose, which is minimally sweet and has the same number of calories as sucrose. For the sucrose group, 14% wt/vol sucrose was added to the baseline flavor solution. The aspartame group received 0.02% wt/vol aspartame and 0.014% wt/vol of Ace K added to the baseline flavor condition. These concentrations of sucrose and aspartame have previously been shown to be equally sweet (Mobini, Chambers, & Yeomans, 2007). Because Polycose and sucrose are equally calorific, 14% Polycose was added to the baseline flavor solution for the Polycose group. Teas were prepared approximately 24-48 hours before the test day on which they were used. All teas were refrigerated and served cold throughout the study.
**Conditioning and Testing Procedures**

*Two-Bottle Test.* After the flavor rankings were determined, the teas that were chosen for CS+ and CS- were simultaneously presented to the child in their baseline form (i.e., no reinforcers were added). Each cup was filled with 150 ml of tea and weighed prior to presentation. The child was asked to take a sip of each tea and was then given 20 minutes to color as other studies have found this to be an appropriate activity to engage in during conditioning (Havermans & Jansen, 2007). The children were told that during this time they could drink as much or as little of either of the teas as they wanted. At the end of the 20 minutes, the cups were weighed. This gave a baseline from which to compare any preference changes that might occur as a result of the at home conditioning phase.

*Ranking task.* Children completed the same baseline flavor ranking task (Birch, 1990) used in Experiment 1, although this version contained only six varieties of tea. The tea flavors which were ranked as three and four were randomly assigned to be either the CS+ or CS- for the study.

*Sweet preference task.* In order to determine how much the child liked sweet tastes, the same sweet preference task from Experiment 1 was used (Mennella, Pepino, Lehmann-Castor, & Yourshaw, 2010).

*Conditioning phase.* At the end of Test Day 1, eight cups of tea were prepared for the at home conditioning phase. Each child was randomly be assigned to one of the three CS+ groups (sucrose, aspartame, or Polycose). In addition to four cups of the CS+ solutions, they also received four cups of the CS- solution, which was unsweetened. For each day, 150 ml of the appropriate tea was measured out and poured into a lidded cup that was weighed and labeled for the day it is to be consumed (Day 1, Day 2, etc.). Over the
eight day home conditioning phase, the cups of the CS+ and the CS- solutions were to be consumed in alternation on separate days. Mothers were instructed to present the cup with the appropriate label to the child at the same time of day over the next eight days. They were instructed to ask the child to drink as much or as little as they like. Mothers were asked to not pressure the child to drink and to make sure the teas are kept cold. The mothers were instructed to not dump out the remaining tea, but place a lid on the cup, return it to the fridge, and bring it back to the lab for Test Day 2.

Test Day Procedures

Test Day 1. During recruitment mothers were instructed to not feed their child for at least two hours before the appointments in order to ensure that the child was hungry on testing days. After informed consent and assent were completed, children were asked whether they are currently hungry, when and what they last ate, and their weight and height were measured. Children were tested in a separate room from the mother while they completed the baseline flavor ranking task, the two-bottle free tea task, and the sweet preference task as described above. Meanwhile, mothers completed questionnaires about demographic information.

Test Day 2. On Test Day 2 (which was Day 10 of the experiment) the mother and child returned to the lab. The remaining tea was returned and the amount of tea consumed was calculated for each day. The child was again asked if they were hungry, and the last time they ate and what they ate was recorded. The child then completed the two-bottle free tea task again.

Results

Participants
Of the 59 children who were recruited for this experiment, one child started the experiment but was excluded for not returning to the lab to complete Test Day 2, one child was excluded for not consuming the conditioning solutions, and eight additional children were excluded because they were categorized as obese for age and gender based on their BMI percentile. Obese children were not included because obese individuals have been shown to find foods more rewarding (McGloin et al., 2002; Rissanen et al., 2002) and are hypothesized to have dopamine dysregulation (Davis et al., 2009; Mathes et al., 2010). The 49 remaining children (23 males) had a mean age of 7.51 years, SD ± 1.87. The majority of participants were White (n = 40), with the remaining participants of the following races: 2 Black, 1 Asian, 6 mixed or “unknown”. The average sweet preference score for this sample was 19.71%, SD ± 11.53.

Children were randomly assigned to either the sucrose (n = 15), aspartame (n = 20), or Polycose (n = 14) groups. As shown in Table 2, there were no significant differences between these groups in regards to gender, age, racial makeup, or sweet preference. A one-way ANOVA revealed that the age of the mothers differed significantly by group (F(2, 45) = 5.80, p = 0.006). Post-hoc Tukey tests showed that mothers in the sucrose group (M = 43.16 years, SD ± 3.88, p < .05) were significantly older than those in the aspartame (M = 38.45 years, SD ± 5.59) or Polycose group (M = 36.47 years, SD ± 6.67).

Consumption during Training

To determine whether the groups differed in their consumption of the solutions during the 8-day conditioning period in which the CS+ and CS- solutions were each presented on four alternating trials, a 3 (Group: sucrose, aspartame, Polycose) x 4 (Trial) x 2 (CS) repeated measures ANOVA was conducted. As shown in Figure 3, this analysis
revealed a main effect of Trial ($F(3, 138) = 5.51, p = .001$). Post-hoc tests indicated that consumption of the CS solutions in Trial 2 and Trial 4 were significantly lower than consumption during Trial 1. There was no main effect for CS ($F(1, 46) = 2.77, p > .05$) and no interactions for CS x Trial ($F(3, 138) = 0.45, p > .05$), Trial x Group ($F(6, 138) = 1.58, p > .05$), or Trial x Group x CS ($F(6, 138) = 0.34, p > .05$).

**Two-Bottle Test Consumption**

To investigate whether preferences for the CS+ changed relative to the CS-, preference ratios were calculated for Test Day 1 and 2 by dividing the grams of CS+ consumed by the total grams consumed for each day (See Table 3 for average grams consumed for each group). These preference ratios were then subjected to a 2 (Time) x 3 (Group) repeated measures ANOVA. Although there was no main effect for Time ($F(1, 46) = 0.22, p > .05$), there was a significant main effect for Group ($F(2, 46) = 4.10, p = .023$). Post hoc test revealed that the Polycose ratios ($M = .41, SE = .04$) were significantly lower than sucrose ratios ($M = .58, SE = .04, p = .008$) and aspartame ratios ($M = .53, SE = .04, p = .042$) across both days. There was also a significant Time x Group interaction ($F(2, 46) = 3.28, p = .047$). Although simple main effects analyses revealed that there was no significant change between the pre and post tests for aspartame ($F(1, 19) = 2.84, p > .10$) or Polycose ($F(1, 13) = 0.35, p > .10$), there was a marginal increase for sucrose: ($F(1, 14) = 4.14, p = .061$).

Because visual inspection of the preference ratios (see Figure 4) suggested that initial preference ratios from Test Day 1 were not equivalent between groups, a univariate ANOVA was performed on the preference ratio from Test Day 1, where Group (sucrose, aspartame, Polycose) was the independent variable. A main effect for Group was
revealed \( F(2, 46) = 3.81, p = .029 \), post hoc tests showed that the initial preference ratio for children in the aspartame group \((M = .58, SE \pm .05)\) was significantly higher than those in the Polycose group \((p = .009, M = .39, SE \pm .05)\), whereas preference ratios for children in the sucrose group \((p > .05, M = .53, SE \pm .05)\) did not differ significantly from those in the aspartame or Polycose group.

In order to control for the difference between baseline ratios, consumption ratios for the 2-bottle post-test were then compared with a one-way Analyses of Covariance (ANCOVA), where Group (sucrose, aspartame, Polycose) was the independent variable and the preference ratio from Test Day 1 was the covariate. This analysis revealed a significant main effect of Group \( F(2, 45) = 3.49, p = .039 \). Post hoc tests revealed that the post conditioning preference ratio for children in the sucrose group \((M = .63, SE \pm .05)\) was significantly higher than those in the aspartame \((p = .019, M = .46, SE \pm .05)\) and Polycose \((p = .044, M = .47, SE \pm .06)\) groups. The preference ratios for the aspartame and Polycose groups did not significantly differ \((p > .05)\).

A further analysis was conducted to determine whether there were group differences in the proportion of participants whose CS+ preference increased as a function of conditioning. A chi-square analysis revealed that the proportion of participants who increased their preference for the CS+ after conditioning did indeed significantly differ between groups \( \chi^2(2, N = 49) = 7.45, p = .024 \). As seen in Figure 5, more children in the sucrose group \((73\%, \chi^2(1, N = 35) = 6.44, p = .011)\) and Polycose group \((64\%, \chi^2(1, N = 34) = 3.93, p = .048)\) increased their preference for the CS+ when compared to those in the aspartame group \((30\%)\). The children in the Polycose group did not differ significantly from those in the sucrose group \( \chi^2(1, N = 29) = 0.28, p > .05 \).
Discussion

The design of Experiment 2 addressed some of the limitations of Experiment 1. The conditioning timeline was increased to eight days and Polycose was added as a reinforcer to separate the effects of flavor-taste and flavor-calorie learning. One of our hypotheses was that sucrose would produce stronger CFPs than either aspartame or Polycose, because it has the ability to condition through both flavor-taste and flavor-calorie associations. Although none of the groups demonstrated a significant increase in their preference for the CS+, there was some suggestion that children in the sucrose group may have acquired a conditioned preference. Although they only marginally increased their CS+ preference after conditioning, their preferences for the CS+ were stronger than those of the aspartame and Polycose groups after conditioning, and significantly more children in the sucrose group increased their preference for the CS+.

In combination, these findings suggest that while these increases in CS+ preferences may have been small, there was some evidence that sweet taste and calorific reinforcement may have combined to begin to condition a preference for the CS+.

In addition to the fact that aspartame and Polycose only provide either sweet taste or calorific reinforcement, and not both, it is possible that the sensory properties of these reinforcers may have interfered with conditioning. It is possible that aspartame is not a very effective reinforcer for children as its bitter aftertaste may be a deterrent (Shinoda & Okai, 1985). Because children do not like bitter tastes (Mennella, Pepino, & Reed, 2005), the bitter aftertaste of aspartame may have reduced the liking of (and produced a reluctance to consume) the CS+ (Simons et al., 2008). Although Polycose is effective in producing CFP in rats (Ackroff, Drucker, & Sclafani; Elizade & Sclafani, 1988; Elizalde
& Sclafani, 1990), to our knowledge there are not studies demonstrating its effectiveness as a reinforcer in children. Zeinstra et al. (2009) used Polycose in their failed flavor conditioning study. However, children did not consume enough of the solutions for conditioning to occur, which supports the idea that children do not find the taste of Polycose palatable. The present study did not provide evidence that Polycose produced CFP in children. It is possible that children were deterred by the texture of Polycose, which produces a more viscous solution than either sucrose or aspartame, or it could be that sweet taste is a necessary component for CFP in children.

Based on the null findings from Experiment 1, the conditioning period for Experiment 2 was extended to eight days. This required solutions to be sent home with the children to be consumed, which resulted in a loss of experimental control. Parents were supplied with detailed instructions for how to conduct the at-home conditioning phase of the experiment, however we have no way of confirming how closely these guidelines were followed. It is conceivable that at least some parents did not complete the study exactly as instructed. Flavor-calorie conditioning in particular could have been interrupted if parents did not have their children refrain from eating for an hour before and after consuming the conditioning solutions.

It is also possible that more than eight conditioning trials were required for CFP to form. This study was limited in how long the trials could extend as the teas were sent home and needed to stay fresh. Polycose solutions can mold if they are left too long, even if they are refrigerated. Even with the extended timeline for this experiment, children only had four conditioning trials for each CS solution, spread out over an eight-day period. There has not been enough research on flavor learning in children to
determine the optimal number of trials required for conditioning. Havermans and Jansen (2007) conditioned children over six trials that were presented over two days (three trials per day). In the Zeinstra et al. (2009) study children had seven trials for each CS solution, over a 14-day period. Birch, McPhee, Steinberg, and Sullivan (1990) used 8 conditioning trials for each CS, with a pair being presented each day. There is the possibility that with more trials, the CFP would have been conditioned in the sucrose group. Polycose and aspartame might require even more time than sucrose to condition preferences, as they each utilize only one conditioning mechanism.

**General Discussion**

The current study attempted to extend our limited knowledge of how flavor conditioning operates in children. Although Experiment 1 failed to replicate Zellner et al.'s findings in which preferences were conditioned after one session of conditioning that contained several trials, Experiment 2 suggested that CFP may form in children when sucrose was used as a reinforcer. Taken together, these experiments suggest that flavor conditioning in children might not occur rapidly, and that sucrose, which produces both flavor-taste and flavor-calorie associations, might be more effective than either aspartame or Polycose.

Consistent with previous research (Zeinstra et al., 2009) we found that children were reluctant to consume the CS solutions in Experiment 1, consuming an average of only 8.29 ml of a possible 450 ml during the post-conditioning three-bottle test. In order to overcome this problem in Experiment 2, we ensured that the flavored solutions contained more of the reinforcers. While this increased children’s willingness to consume the CS+ solutions throughout conditioning, when they received the CS solutions
without their corresponding reinforcer during the test the children may have experienced a contrast effect. Negative contrast effects occur when a palatable solution is replaced by a less preferred solution (Flaherty, 1982). When expectations about the solution are not matched, the less preferred solution tends to be rejected more than it would have been had it not been preceded by the palatable solution. Numerous rat studies have shown that consumption of a solution is reduced when it is presented after a more desirable solution, compared to when it is presented before the expectation for a more desirable solution forms (Becker & Flaherty, 1983; Capaldi, Sheffer, & Pulley, 1989; Lombardi & Flaherty, 1978). If a contrast effect occurred in this study when the CS solutions were presented unsweetened in the post conditioning bottle-tests any preferences that were conditioned during training would have been counteracted. Although children may like the CS+ flavor more after conditioning, they would consume less of this flavor during the post-conditioning bottle tests because it is no longer sweetened. The CS- flavor would not be impacted as participants had previously consumed it in its unsweetened form during conditioning. This may help explain some of the null effects in the current study. Zellner et al.'s use of liking ratings rather than a consumption test might have allowed them to bypass this issue to some extent in their study.

It is often difficult to show CFPs in humans (Bayens, Eelen, Van Den Burgh, & Crombez, 1990; Stevenson, Boakes, & Prescott, 1998; Stevenson, Boakes, & Wilson, 2000; Stevenson & Prescott, 1995), as there are many factors to consider when examining flavor conditioning in people. Although these experiments attempted to address the known issues (controlling for hunger, examining sweet preference, controlling for BMI), there could be additional factors that influence conditioning.
Future studies are needed to shed more light on the mechanisms of flavor conditioning in children. If we can improve our understanding of flavor conditioning in children then we might have the opportunity to determine the relative contributions of flavor-taste and flavor-calorie associations in flavor preference conditioning. This information could help us develop evidence-based strategies to encourage healthy eating habits in childhood, which in turn could impact healthy food choices throughout the lifespan.
References


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<th>Children (N=30)</th>
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<td>Sex (# Females)</td>
<td>14 (53%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.1 (2.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.7 (13.3)</td>
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<tr>
<td>Height (m)</td>
<td>139.8 (17.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>17.4 (3.6)</td>
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<tr>
<td>Sweet Preference (g/100ml)</td>
<td>20.8 (10.7)</td>
</tr>
<tr>
<td>How many have sucrose preference &gt;12 g/100ml?</td>
<td>24 (87%)</td>
</tr>
<tr>
<td>How many drink unsweetened tea?</td>
<td>8 (27%)</td>
</tr>
<tr>
<td>How many drink sweet tea?</td>
<td>11 (37%)</td>
</tr>
<tr>
<td></td>
<td>Sucrose (n= 15)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
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<tr>
<td>Sweet Preference (g/100ml)</td>
<td>14.6 (10.7)</td>
</tr>
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</table>
Table 3  

Experiment Two: Average of CS+ and CS- consumed for each group during test day 2 two-bottle test [grams (SE)]

<table>
<thead>
<tr>
<th></th>
<th>Sucrose</th>
<th>Aspartame</th>
<th>Polycose</th>
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<tr>
<td>CS+</td>
<td>53.78 (10.04)</td>
<td>45.35 (8.76)</td>
<td>49.77 (10.60)</td>
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<tr>
<td>CS-</td>
<td>39.09 (12.15)</td>
<td>61.30 (10.61)</td>
<td>57.10 (12.84)</td>
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</table>
Figure 1 *Experiment 1: Liking ratings of the CS solutions during conditioning*
Figure 2 Experiment 1: Children’s consumption of CS solutions during the 3-bottle pre and post tests
Figure 3 *Experiment 2: Consumption of solutions during at home conditioning phase*
Figure 4 *Experiment 2: Preference ratios for test day 1 and test day 2*

![Graph showing preference ratios for test day 1 and test day 2 for Sucrose, Aspartame, and Polycose. The graph includes error bars to represent the variability in the data. The x-axis represents the CS+ Group with Sucrose, Aspartame, and Polycose as categories. The y-axis represents the preference ratio ranging from 0 to 1.]
Figure 5 Experiment 2: Percentage of children increasing or decreasing relative consumption of CS+ post conditioning.