ORIGINAL INVESTIGATION

Influence of caffeine on the liking of novel-flavored soda in adolescents

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Abstract

Background Soda manufacturers claim that caffeine is added to soda as a flavor enhancer, but many researchers have speculated that caffeine is added to increase the hedonic and reinforcing properties of the soda. Studies in adults have demonstrated that caffeine can condition flavor preferences when added to novel-flavored beverages.

Objectives The purpose of this study was to test the hypothesis that caffeine added to novel-flavored drinks would increase liking and preference in adolescents.

Methods Adolescents ($n=99$) between the ages of 12 and 17 rated and ranked seven novel soda drinks. They were then randomly assigned to consume one of these beverages paired with either caffeine (1 or 2 mg/kg) or placebo over four consecutive days and rate liking. On the final visit, participants retasted the seven beverages and provided hedonic ratings and rankings.

Results Participants in the 2-mg/kg caffeine group increased the liking of the beverage over the exposure period after an initial decrease, but there was no change in liking for those in the placebo group or in the 1-mg/kg group. The increase in liking in the 2-mg/kg group was accompanied by a decrease in perceived bitterness, but no change in beverage ranking or consumption during the post-test.

Conclusions Caffeine added to novel beverages results in a decrease in liking followed by an increase in liking with repeated exposures that may result from habituation to the bitterness of caffeine. Change in bitter perception may be the mechanism by which adolescents establish regular caffeine use.

Keywords Caffeine · Flavor preference · Adolescents · Dose–response · Reinforcement · Hedonics

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Introduction

Caffeine use is on the rise, particularly among children and adolescents (Frary et al. 2005; Kaminer 2010; Pennington et al. 2010). This increase in caffeine consumption is partially attributable to an increase in availability of caffeine-containing beverages that are marketed to youth (reviewed in Pennington et al. 2010). Unlike coffee and tea, which are the primary sources of caffeine in adults, soda and energy drinks do not contain caffeine naturally. Instead, caffeine is added to these beverages to, according to beverage manufacturers, enhance their flavor (Beverage 1999). This seems unlikely because most people cannot distinguish between caffeinated and non-caffeinated beverages at typical caffeine concentrations on the basis of taste, and at higher doses, caffeine is extremely bitter (Griffiths et al. 1990). Many researchers believe that caffeine is added to beverages in order to increase their hedonic and reinforcing properties (Griffiths and Vernotica 2000).

Studies in adults have demonstrated that caffeine can condition flavor preferences for novel beverages (Yeomans et al. 1998, 2000a, b, 2005). For example, when caffeine was paired with novel-flavored juices and teas over five to ten trials, liking ratings were increased (Richardson et al. 1996; Yeomans et al. 1998). Conversely, the liking of a non-caffeinated, novel-flavored beverage decreased with repeated presentations (Yeomans et al. 2002). The positive impact

of caffeine on flavor preference in adults appears to be related to removal of caffeine withdrawal symptoms, as it is only observed in regular caffeine consumers that have been deprived of caffeine use (Yeomans et al. 1998, 2002; Tinley et al. 2004). In non-caffeine consumers, caffeine may have the opposite effect on pleasantness (Tinley et al. 2004).

Our previous work in adolescents has demonstrated that caffeine increases the reinforcing properties of soda (Temple et al. 2009) and increases ratings of subjective responses (Temple et al. 2010; Temple and Ziegler 2011). The purpose of this study was to assess the ability of caffeine to condition flavor preferences in adolescents. We hypothesized that caffeine added to novel-flavored carbonated beverages would increase liking and preferences for these beverages. We also hypothesized that these effects would be independent of usual caffeine consumption.

Subjects and methods

Participants and recruitment

Participants were 12- to 17-year-old boys and girls (*n*=99), recruited through direct mailings, e-mails, referrals, as well as flyers posted around the University at Buffalo campuses. Eligibility criteria included: previous ingestion of caffeine without adverse reaction, not using hormone-based contraceptives, not taking medication known to have interactions with caffeine (such as stimulant medications for ADHD), and not smoking. A further requirement was that both the participant and one parent or guardian were willing to visit the laboratory on six occasions for 60 min each.

Screening

Potential participants contacted our lab and underwent screening via phone or through Survey Monkey™ software online. Individuals were excluded if they did not meet the eligibility criteria above. Only one participant was accepted per household. We obtained information about participants' usual caffeine consumption and stratified the sample by regular caffeine use into groups consuming <25 mg/day (*n*=29), 25–50 mg/day (*n*=26), 50–75 mg/day (*n*=8), or >75 mg/day (*n*=36). The sample consuming 50–75 mg/day may have been small because this amount of caffeine falls between the amount contained in one and two cans of soda.

Experimental procedures

Eligible participants were scheduled for six visits to take place within a 2-week period. Prior to their first visit, participants were randomized to a caffeine or a placebo condition using double-blind procedures. Initially, the caffeine condition was 2 mg/kg, based on findings from previous studies (Temple and Ziegler 2011), but after the data were analyzed, as we were nearing the end of the study, we noticed that participants in the 2-mg/kg group could perceive the bitterness of the caffeine in the drink, so we switched to a lower dose of 1 mg/kg for the remainder of the study. This resulted in unequal numbers of subjects across groups: we had a larger number in the placebo group (*n*=50) as we continued to recruit placebo participants to maintain the double-blind procedures, a small number of participants in the 1-mg/kg group (*n*=16), and the planned number of participants in the 2-mg/kg group (*n*=34). Participants were instructed to abstain from caffeine for 24 h and asked to fast for 2 h before all sessions. For the conditioning trials, this meant abstaining from caffeine throughout the duration of those trials.

Upon arrival to the laboratory for the first session, parents and participants read and signed consent and assent forms. Parents also completed a demographic questionnaire at that time. To remove expectations about the effects of caffeine, participants were told that the beverage they would be consuming "may have levels of one or more of the following substances manipulated: sugar, aspartame, Splenda®, caffeine, or artificial coloring." This minimal level of deception reduced the influence of expectancies on study outcomes. Participants' height and weight were measured. They also completed a dietary recall for the current day and the previous 24-h and provided a 2-mL saliva sample for verification of caffeine abstinence. Then, the participant completed the caffeine consumption questionnaire, to determine habitual caffeine use, and the behavioral checklist questionnaire to assess current psychological state and mood.

Once questionnaires were completed, the participant took part in a taste test to identify the target beverage to be used for flavor conditioning. Participants tasted seven novel-flavored soda beverages comprised of Seltzer®, Kool-Aid, and sugar (see details below). Participants sampled 300 mL of each beverage, labeled A–G, in clear, plastic cups labeled with the coded letter of the beverage. They rated each drink on a 100-mm visual analog scale (VAS) anchored with "Do not like at all" at 0 mm and "Like a lot" at 100 mm. While participants were required to taste each drink, they could drink as little or as much of each beverage as they desired. Participants also rated novelty, sweetness, sourness, bitterness, and acidity on the same 100-mm VAS. This taste-rating procedure has been previously described in detail (Tinley et al. 2004). Finally, participants ranked their preference for the drinks from 1 to 7. The beverage they ranked 4th was chosen as their target beverage.

Sessions 2–5 were conditioning sessions. These visits were scheduled on consecutive days, at least 3 days after the baseline visit. Upon arrival in the laboratory, subjects completed the dietary recalls, 2-mL saliva collection, and

behavioral checklist, and drank 300 mL of their target beverage with either placebo or caffeine (1 or 2 mg/kg) added, under double-blind procedures (described below). Participants then rated the beverage for all of the factors listed above using the 100-mm VAS. Thirty minutes later, while the caffeine was absorbed, participants completed the behavioral checklist again. They watched an age-appropriate TV show for the 30-min waiting period.

The sixth visit consisted of a post-exposure taste test involving all seven beverages, identical to that administered during the first session. The primary outcome measures were change in liking over time, beverage ranking before and after the conditioning trials, and beverage consumption pre- and post. Then, the participants and their parents were debriefed and compensated for participation in the form of a check for $120.00 made out to the participant. All study procedures were conducted in accordance with National Institutes of Health guidelines for the use of humans in research and approved by the University at Buffalo Social and Behavioral Sciences Institutional Review Board.

Caffeine preparation

The caffeine and placebo additives were prepared and coded by an individual who was not involved in data collection in order to ensure double-blind experimental design. A flattened Sprite™ stock solution was created by heating Sprite™ to 140°F and stirring it at 50 rpm for 25 min. This served as the placebo, and the caffeine solution was created by adding 10 mg caffeine/mL. The placebo and caffeine solutions were aliquoted into tubes, labeled with an A or a B, and frozen at −20°C until the day of the experiment. On the testing day, aliquots were removed from the freezer and thawed in warm water for several hours prior to the experimental session. Solutions were prepared for each subject, based on body weight.

Beverage preparation

Seven novel-flavored soda beverages were created from a combination of flavored seltzer, Kool-Aid, sugar, and water, referred to as solutions A–G. In each case, 1.0 L of seltzer was mixed with 100 g of granulated, white sugar and 200 mL of cold water. The weight of powdered, unsweetened Kool-Aid mix varied from 1.0 to 6.0 g between flavors. Beverages were refrigerated until use, poured into 300-mL portions, and recarbonated with three pulses from a Soda-stream® carbonator.

Measurements

Anthropometrics A participant's weight was assessed by the use of a digital scale, and height was assessed using a SECA stadiometer. BMI was calculated according to the following formula: (BMI=kilograms/square meters). Weight was used to calculate the amount of caffeine or placebo to be administered.

Dietary recalls To ensure compliance with the protocol (no vigorous physical activity, no food or drink other than water for 2 h prior to testing, and no caffeine for 24 h prior to testing), the child recalled his/her dietary intake and physical activity for the previous 24 h and current day. Any participant who did not comply with the study protocol was rescheduled or excluded.

Questionnaires

Demographic questionnaire A general demographic questionnaire was used to assess parental education, annual household income, parental employment status, and parent and participant race and ethnicity.

Caffeine use questionnaire Average daily caffeine consumption was calculated based on the participants' self-report on a caffeine use questionnaire adapted from Miller (2008) that was designed to assess sources, amounts, and frequency of caffeinated food and beverage intake as well as reasons why adolescents use and/or do not use caffeine. Participants were asked "Do you drink _____" and "How often do you drink _____ (pop with caffeine, hot tea or iced tea, coffee, energy drinks)?" "How often do you eat chocolate?" and "How often do you take _____ (Excedrin or No-Doze)?" The possible answers for each of these questions were: once a month, two to three times a month, one time per week, two to three times per week, four to five times per week, and everyday. Then, participants were asked "When you are drinking _____, how many cups (or cans) do you drink?" The possible answers ranged from one to more than seven cups. Amounts of caffeine consumed were estimated based on information from the US Department of Nutritional Services and include the following: tea (40 mg/5 oz), soda (40 mg/12 oz), coffee (100 mg/5 oz), energy drinks (150 mg/12 oz), chocolate (10 mg/oz), and caffeine-containing pills (Excedrin or No-Doze—130–200 mg/pill).

Behavioral checklist A questionnaire containing 31 adjectives describing mood and physiological symptoms was presented to the subjects before and after each session. The subject was asked to rate how each adjective describes how they feel "right now" on a 9-point Likert-type scale anchored by "Not at all" (1) and "Extremely" (9). This questionnaire was adapted from the Profile of Mood States bipolar form and the Activation–Deactivation Adjective Checklist. This questionnaire has been used by multiple

investigators and is an indicator of both acute effects of caffeine intoxication and of caffeine withdrawal (Greden et al. 1981; Griffiths and Woodson 1988; Richardson et al. 1995, 1996; Yeomans et al. 2002; Temple et al. 2009, 2010).

Salivary caffeine analysis In order to verify 24-h caffeine abstinence, a 2-mL sample of saliva was collected at the beginning of each session. The participant expectorated a saliva sample into a sterile tube which will be stored at −20°C until analyzed. We selected one vial from each participant from a session during the conditioning trials to analyze for caffeine to verify caffeine abstinence. Salivary caffeine content was analyzed using the standard gas chromatography method with a structural analog of caffeine used as an internal standard (LabStat; Kitchner, Ontario). Participants were considered compliant if caffeine levels fell below 0.85 μg/mL, which is consistent with overnight caffeine abstinence. Data from one individual with salivary caffeine above this level were removed from analysis.

Analytic plan

Participant characteristics were compared among the three different dose groups (placebo, 1 mg/kg, or 2 mg/kg) using one-way analysis of variance for continuous data (BMI, age, caffeine use, caffeine provided in the laboratory) and chi-squared for categorical data (race, income, and parental education). Potential differences in liking and bitterness scores across the six sessions were analyzed using a mixed analysis of variance with gender and caffeine dose as between-subjects variables, trial number as the repeated measure, and regular caffeine use (in milligrams per day) as a covariate. Post hoc group comparisons were conducted using linear contrasts, with a Bonferroni correction for multiple comparisons ($p < 0.0167$). Changes in consumption of the target beverage from visit 2 to visit 6 were compared using a mixed analysis of variance with gender and caffeine dose as the between-subjects characteristics, pre/post as the within-subjects variable, and regular caffeine use (in milligrams per day) as a covariate. Ranking of the target beverage on the final visit was compared using one-way analysis of variance with gender and caffeine dose as the between-subjects variables and regular caffeine use (in milligrams/day) as a covariate. We also analyzed responses in the behavioral checklist using a mixed analysis of variance with caffeine dose (0, 1, or 2 mg/kg) and gender as between-subjects variables and pre/post as the repeated measure. In addition, usual caffeine use (in milligrams per day) and baseline responses (visit 1 prior to sampling beverages) for each adjective were covariates. For this analysis, we used data from the visit that we used for salivary caffeine analysis to assure that caffeine abstinence was verified empirically. All analyses were conducted using SYSTAT 11.0 (Chicago,

IL), and significance was set at $p < 0.05$ for primary analyses and $p < 0.0167$ for post hoc analyses with multiple comparisons.

Results

Participant characteristics One hundred participants completed the study, but one female participant was removed from the analyses for having salivary caffeine levels above our cutoff. This left 54 males and 45 females with an average age of 14.1 ± 0.15 years. The N, mean$\pm$SEM, and ranges of caffeine use for the four consumption groups were: <25 mg/day ($n=29$, 14.5$\pm$1.5, 0–24.6 mg/day), 25–50 mg/day ($n=26$, 36.5$\pm$1.4, 26.1–49.3), 50–75 mg/day ($n=8$, 64.2$\pm$2.8, 53.2–74.6 mg/day), >75 mg/day ($n=36$, 106.4$\pm$4.8, 75.7–198.6 mg/day). For the purposes of data presentation (not data analysis), we have grouped the participants into "low" vs. "high" caffeine consumption groups based on a median split of the usual caffeine consumption data. This yielded the following number of individuals in each group, mean$\pm$SEM consumption, and range of consumption (in milligrams per day): low ($n=57$, 25.9$\pm$1.9, 0–54.3 mg/day) and high ($n=42$, 100.8$\pm$4.7, 62.1–198.6). The sample was predominantly Caucasian (86 %) from middle class families where at least one parent had completed college or graduate school (80 %). The consumption groups did not differ in gender or BMI, age, parental education, household income, or race (all $p > 0.07$; Table 1).

Caffeine increases the liking of beverages Ratings of liking increased across trials in the caffeine condition compared to the placebo condition (significant interaction between caffeine dose and trials ($F (8, 364)=4.6$; $p < 0.001$). Linear contrasts revealed that liking significantly increased from trial 2 to 6 in the 2-mg/kg group ($F (4, 132)=8.0$; $p < 0.001$), but did not change in the placebo ($F (4, 120)=0.9$; $p=0.4$) or the 1-mg/kg group ($F (4, 120)=1.4$; $p=0.3$), and the latter groups were not different from each other ($F (5, 375)=1.2$; $p=0.29$; Fig. 1). Neither gender nor regular caffeine use (in milligrams per day) influenced liking ratings or any other measures (all $p > 0.05$).

Caffeine increases ratings of bitterness in a dose-dependent manner Subjects rated their liking of the 2-mg/kg beverage on visit 2 lower than they rated liking the same beverage without caffeine on visit 1, suggesting that the addition of caffeine made the beverage bitter. This was confirmed in the analysis of bitterness ratings ($F (2, 85)=6.2$; $p=0.003$). However, this effect declined across the six sessions' interactions between dose and trials ($F (8, 364)=2.7$; $p=0.006$); linear contrasts revealed that the bitterness ratings of 2 mg/kg decreased from trials 2 to 6 ($F (4, 132)=6.1$;

Table 1 Participant characteristics

Category	Placebo Mean±SEM	1 mg/kg caffeine Mean±SEM	2 mg/kg caffeine Mean±SEM	p value
Age	14.18±0.210	14.27±0.431	14.06±0.263	NS
BMI	22.63±0.652	22.70±1.085	22.09±0.71	NS
Average daily caffeine consumption (mg/day)	52.91±5.534	55.83±11.528	66.87±8.51	NS
mg caffeine added	0	63.3±4.01[a]	119.7±5.66[b]	<0.0001
Race:	n (%)	n (%)	n (%)	
Caucasian	41 (84%)	13 (93%)	29 (88%)	NS
African American	6 (12%)	1 (7%)	2 (6%)	NS
Other	2 (4%)	0 (0%)	2 (6%)	NS
Parental education:	n (%)	n (%)	n (%)	
Some high school	1 (2%)	0 (0%)	0 (0%)	NS
High school	1 (2%)	0 (0%)	2 (6%)	NS
Some college or vocational	10 (20%)	1 (7%)	4 (12%)	NS
Completed college	29 (58%)	8 (57%)	18 (53%)	NS
Grad school	9 (18%)	5 (36%)	10 (29%)	NS
Household income:	n (%)	n (%)	n (%)	
<$30,000	3 (7%)	1 (7%)	2 (7%)	NS
$30,000–$50,000	6 (13%)	2 (14%)	7 (25%)	NS
$50,000–$70,000	7 (15%)	1 (7%)	8 (29%)	NS
$70,000–$110,000	17 (37%)	6 (43%)	6 (21%)	NS
>$110,000	13 (28%)	4 (29%)	5 (18%)	NS

NS not significant

[a] Significantly different from the placebo and 2-mg/kg group

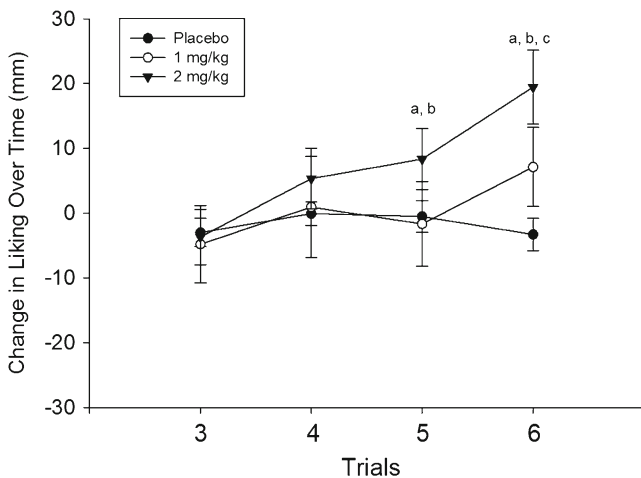[b] Significantly different from the placebo and 1-mg/kg group

Fig. 1 Mean±SEM change in liking ratings on a 100-mm visual analog scale anchored by "do not like at all" at 0 mm and "like a lot" at 100 mm for the target beverage from baseline conditioning ratings to visits 3–6. There was a main effect of trials ($F_{(5, 430)}$=5.3; $p<0.0001$) and a significant interaction between caffeine dose and trials ($F_{(10, 430)}$=6.9; $p<0.0001$). Linear contrasts revealed that liking significantly increased from trial 2–6 in the 2-mg/kg group ($F_{(4, 120)}$=9.2; $p<0.0001$), but did not change in the placebo ($F_{(4, 120)}$=0.9; $p=0.4$) or the 1-mg/kg group ($F_{(4, 120)}$=1.4; $p=0.3$), and the latter groups were not different from each other ($F_{(5, 375)}$=1.2; $p=0.29$). *a* 2-mg/kg group significantly different from trial 3, *b* 2-mg/kg group significantly different from the placebo and 1-mg/kg group, *c* 1-mg/kg group significantly different from the placebo

$p<0.001$), but neither the placebo ($F_{(4, 180)}$=0.3; $p=0.900$) nor the 1-mg/kg group ($F_{(4, 44)}$=1.5; $p=0.200$) showed this change in bitterness ratings (Fig. 2) (Table 2).

Changes in liking and bitterness over time In order to examine the relationship between changes in liking and changes in bitterness, we correlated the change in each of these measures from trial 2 to trial 6. There was a significant negative correlation between liking and bitterness ratings in the 2-mg/kg group (R^2=−0.44, $p=0.008$), but no significant correlations in the placebo or in the 1-mg/kg group (both $p>0.100$).

Changes in consumption or ranking On average, subjects increased their liking ratings of the beverages they had sampled during conditioning. The beverage they ranked fourth at baseline was ranked higher after conditioning. However, this effect was related to the dose condition ($F_{(2, 97)}$=4.6; $p=0.013$) and most apparent in the placebo and 1-mg/kg groups, and not the 2-mg/kg group (Fig. 3). Consumption of the tasted beverages did not change (all $p>0.200$) (data not shown).

Behavioral checklist Caffeine altered some of the ratings on the behavioral checklist. There were significant interactions between pre/post and caffeine dose for headache ($F_{(2, 89)}$=4.3; $p=0.017$) and mood swings ($F_{(2, 89)}$=13.5; $p<0.0001$), with decreases in both headache and mood swings with caffeine. There were also interactions among
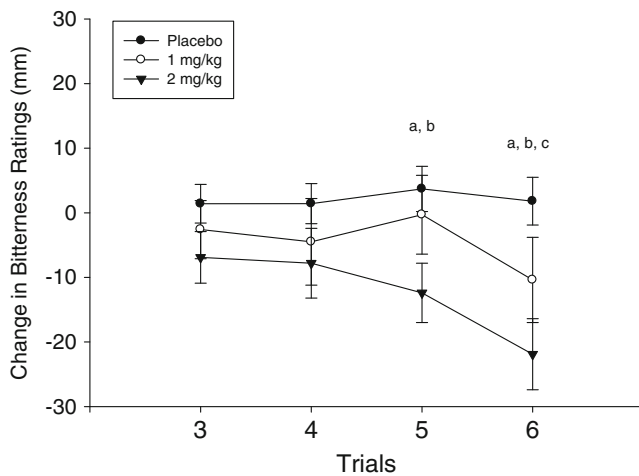
Fig. 2 Mean±SEM change in bitterness ratings on a 100-mm visual analog scale anchored by "not at all bitter" at 0 mm and "extremely bitter" at 100 mm for the target beverage from baseline conditioning ratings to visits 3–6. There was an interaction between dose and trials ($F_{(10, 430)}=4.8$; $p<0.0001$). Linear contrasts revealed that the 2-mg/kg group showed significant changes in bitterness ratings from trials 2 to 6 ($F_{(4, 120)}=5.5$; $p<0.0001$), but neither the placebo ($F_{(4, 180)}=0.3$; $p=0.9$) nor the 1-mg/kg group ($F_{(4, 44)}=1.5$; $p=0.2$) showed this change in bitterness ratings. *a* 2-mg/kg group significantly different from trial 3, *b* 2-mg/kg group significantly different from the placebo and 1-mg/kg group, *c* 1-mg/kg group significantly different from the placebo

Fig. 3 Mean±SEM ranking of the target beverage in each dose group. The *line* represents the ranking of the target beverage at baseline and a decrease in ranking is a higher rank (closer to 1). On average, all beverage rankings decreased. There was a main effect of dose on this decrease in ranking ($F_{(2, 97)}=4.6$; $p=0.013$), with the placebo and 1-mg/kg groups showing significant change in ranking, but not the 2-mg/kg group

pre/post, caffeine dose, and gender for alert ($F_{(2, 89)}=4.3$; $p=0.015$), mood swings ($F_{(2, 89)}=15.0$; $p<0.0001$), and stomach ache ($F_{(2, 89)}=4.3$; $p=0.017$), with decreases in mood swings and stomach ache after caffeine in males only and increased ratings of alertness in males and decreased ratings of alertness in females in the 2-mg/kg group (Table 3). There were no interactions or main effects of usual caffeine consumption on behavioral checklist ratings.

Discussion

Participants consuming novel-flavored beverages with 2 mg/kg of caffeine showed an initial decrease in liking followed by a gradual increase in liking over the subsequent
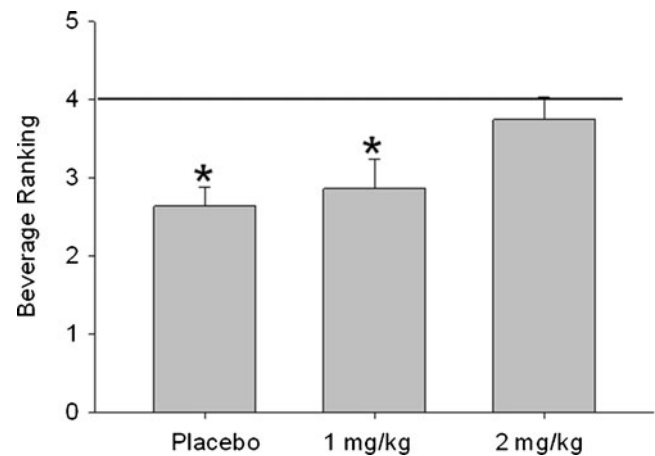
four trials. This pattern was different from that observed in the placebo or 1-mg/kg group. In addition, the 2-mg/kg group had a decrease in the ratings of bitterness over time that was inversely related to the increases in liking. Finally, despite the increase in liking from visit 2 to 6 in the 2-mg/kg group, no change in beverage ranking was observed. Conversely, the placebo and 1-mg/kg group both ranked the target beverage higher after the exposure period. When taken together, these findings suggest that caffeine added to novel-flavored beverages leads to a pattern of decrease in liking followed by an increase in liking of those beverages over time, but the corresponding changes in bitterness with repeated exposure suggest that the mechanism for increase in liking may be increased palatability of those beverages. In addition, our findings do not support a role for caffeine in enhancing the flavor of carbonated beverages.

The primary finding of this study was that as the liking of the beverage increased for the highest caffeine dose group, the perceived bitterness of the beverage decreased. One

Table 2 Liking and bitterness scores across different dose groups		Liking					
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Placebo	51.3±3.4	70.0±2.4	67.0±2.9	69.6±2.5	69.5±2.9	66.7±2.9
	1 mg/kg	47.9±6.7	63.5±6.5	58.7±6.1	64.5±7.0	61.9±7.4	70.7±5.6
	2 mg/kg	54.8±3.9	41.6±4.6	37.9±4.8	46.9±5.1	49.9±4.9*	61.1±5.4*
		Bitterness					
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Placebo	42.6±3.7	30.2±3.5	31.6±3.2	31.4±3.4	33.8±3.4	32.0±3.6
	1 mg/kg	36.4±8.3	41.5±8.6	38.9±8.3	37.0±7.1	41.2±8.9	31.1±7.1
*p<0.05 (significant difference from trial 2)	2 mg/kg	35.2±3.7	59.9±4.3	53.0±4.5	52.1±4.3	47.6±4.2*	38.1±4.6*

Table 3 Responses on the behavioral checklist

| | Male | | | | | | Female | | | | | | p value |
| | 0 mg | | 1 mg/kg | | 2 mg/kg | | 0 mg | | 1 mg/kg | | 2 mg/kg | | |
	Pre Mean (SEM)	Post Mean (SEM)	Pre Mean (SEM)	Post Mean (SEM)	Pre Mean (SEM)	Post Mean (SEM)	Pre Mean (SEM)	Post Mean (SEM)	Pre Mean (SEM)	Post Mean (SEM)	Pre Mean (SEM)	Post Mean (SEM)	
Alert	3.6 (0.5)	3.3 (0.4)	3.3 (1.4)	3.8 (1.0)	3.6 (0.5)	4.3 (0.5)	2.9 (0.4)	2.9 (0.4)	5.0 (0.5)	4.9 (0.6)	5.1 (0.6)	4.1 (0.6)	b
Headache	1.4 (0.2)	1.4 (0.2)	3.0 (2.0)	1.3 (0.3)	1.9 (0.3)	1.7 (0.2)	1.4 (0.2)	1.3 (0.1)	1.8 (0.6)	1.5 (0.2)	2.1 (0.4)	1.8 (0.4)	a
Mood swings	1.3 (0.1)	1.2 (0.1)	3.5 (1.9)	1.3 (0.3)	1.2 (0.1)	1.3 (0.2)	1.2 (0.1)	1.2 (0.1)	1.2 (0.2)	1.3 (0.3)	1.1 (0.1)	1.1 (0.1)	a, b
Stomachache	1.1 (0.1)	1.0 (0.04)	2.5 (1.5)	1.0 (0.0)	1.5 (0.2)	1.6 (0.2)	1.6 (0.2)	1.6 (0.2)	1.2 (0.1)	1.5 (0.2)	1.6 (0.3)	1.6 (0.4)	b

a significant interaction of pre/post and caffeine dose ($p<0.017$), *b* significant interaction among pre/post, caffeine dose, and gender ($p<0.017$)

mechanism for this is a habituation of the sensory receptors to the bitter taste over time. There are studies that have shown a short-term habituation of responses to tastants, such as salt (O'Mahony 1979; McCutcheon and Tennissen 1989). Conversely, studies that have looked at short-term and long-term adaptations to bitter taste have not found significant changes in response to exposure (Mela 1989; Mela et al. 1992). In addition, given the pattern of our data, it appears that a reduction in bitter perception may be the primary mechanism by which palatability of the beverage with the 2-mg/kg concentration of caffeine was increased. Although most adolescents begin consuming caffeine in soda, which lacks bitterness, transitioning to beverages such as coffee or espresso may necessitate a reduction in the perception or the unpleasantness of bitterness in order to consume enough to achieve the desired effect.

Previous studies in adults have reported a difference in the liking of beverages paired with caffeine compared to placebo (Yeomans et al. 1998; 2000a, b; 2002; 2005). Our data suggest that children and adolescents may have different responses to repeated exposure to caffeine. First of all, we observed an initial decrease in the liking of the caffeinated beverage in the 2-mg/kg group followed by a steady increase. The adult studies report a sustained increase in hedonic ratings over time (Yeomans et al. 2000a, b). It could be that adolescents are more sensitive to the bitter taste of caffeine than adults and may require habituation to the taste in order to find it pleasurable. Another difference is that the majority of the studies conducted in adults have reported differences between habitual caffeine users and low or non-users, with the consensus being that the conditioning of flavor preferences by caffeine is dependent on the reversal of withdrawal symptoms and is not observed in light to no caffeine users (Richardson et al. 1996). The average amount of daily caffeine intake in our study population was 58 mg/day, which is much lower than what is reported in adults (Frary et al. 2005). Because of this, our population may be considered light caffeine users, but we found an increase in liking following the initial decrease with 2 mg/kg of caffeine. When taken together, these data suggest that the mechanisms that relate to the liking of caffeinated beverages differ between adults and adolescents (Tinley et al. 2004).

Although we observed a greater increase in liking over the repeated exposures in the group with the highest dose of caffeine, this group did not rank the beverage higher than the initial exposure nor did they consume more of the beverage during the post-test than they did during the initial testing. In fact, the 1-mg/kg group and the placebo group both ranked the target beverage significantly higher after the exposure period than they did at baseline, despite the fact that liking ratings did not increase over time. This finding suggests that, in the placebo and 1-mg/kg group, mere exposure leads to a higher ranking of the beverage when it

was compared to six more novel beverages. This is consistent with the literature on the impact of repeated taste exposure on food liking (Anzman-Frasca et al. 2012). The fact that the 2-mg/kg group did not show this change in ranking suggests that the bitterness of the caffeine may have counteracted the impact of mere exposure on the ranking when that beverage was compared with six more novel beverages. The literature on using mere exposure to increase consumption of vegetables in children suggests that anywhere from 8 to 14 exposures may be necessary to significantly increase liking and/or consumption (Wardle et al. 2003; Lakkakula et al. 2010; Anzman-Frasca et al. 2012). Future studies will determine if a greater number of exposures to the beverage with the 2-mg/kg caffeine would have led to an increase in ranking or consumption.

This study had several strengths. First, we used a double-blind, placebo-controlled design, and we verified caffeine abstinence empirically. Second, we had a large sample size with a broad range of habitual caffeine use. Finally, we used adolescents, which are an understudied population. This study was not without limitations. First, our population was predominantly Caucasian, middle class, and well educated. Although we made attempts to recruit a sample that was more racially and economically diverse, the repeated travel to our laboratory was a barrier for some families. We are therefore limited in our ability to generalize these findings to the entire population. Second, the number of participants in the 1 mg/kg group is relatively low. This was due to the fact that this group was added toward the end after we began to suspect that the caffeine was perceptible by taste. The responses within this group were very consistent, and the variability was low, so we do not feel that the findings would be different if we had a larger number of participants. Third, despite the fact that we created the beverages in our laboratory, their flavors may have been similar to other types of beverages. We examined the relationship between self-reported novelty and liking scores and found them to be unrelated, but it is possible that our effects would have been stronger if the beverages were truly unlike anything that the participants had experienced in the past. Fourth, the doses of caffeine administered to the participants were, on average, higher than what is found typically in soda, which is the primary source of caffeine in this population. Although our caffeine levels were in the range of other caffeinated beverages that children and adolescents consume (coffee and energy drinks), the ecological validity of these findings is limited. Fifth, the taste of the caffeine was not appropriately masked in our 2-mg/kg condition. This reduced palatability and may have influenced our findings. Future studies will use test beverages in which the flavor of caffeine is properly masked or, if not possible, in which a bitter tastant is used in the placebo condition. Finally, we did not specifically assess withdrawal symptoms in this

population. Although we did not find any differences between the dose groups on responses to the behavioral checklist, which contains questions related to withdrawal (ex. headache, fatigue, alertness), this questionnaire does not explicitly ask about withdrawal.

In sum, we found that caffeine added to novel-flavored drinks leads to a decrease in liking following by an increase in beverage liking over time. This increase in liking was accompanied by a steady decrease in ratings of bitterness, suggesting that habituation to the bitterness of the caffeine may have been responsible for the increase in liking. Caffeinated beverages consumed most frequently by children and adolescents are sodas and energy drinks, to which caffeine has been added, unlike coffee and tea (preferred by most adults) in which caffeine is naturally occurring. The implication of this is that beverage manufacturers add caffeine to their products to increase hedonic value over time and reinforce beverage consumption and purchasing and not to enhance flavor, as they claim.

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