

## Ethanol-conditioned flavor preferences compared with sugar- and fat-conditioned preferences in rats

Karen Ackroff\*, Dayna Rozental, Anthony Sclafani

*Department of Psychology, Brooklyn College and the Graduate School of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA*

Received 19 December 2003; received in revised form 13 March 2004; accepted 15 March 2004

### Abstract

Rats can learn to prefer flavors paired with ethanol and various nutrients. The present study examined the relative strengths of flavor preferences conditioned by 5% ethanol and isocaloric solutions of 7.18% sucrose, 7.18% fructose, or 3.26% corn oil. In three experiments, nondeprived rats were trained with different flavored solutions (conditioned stimuli, CS) paired with intragastric (IG) infusions: a CS+E flavor paired with ethanol infusion, a second CS+ paired with a nutrient infusion, and a CS– paired with water infusion. In two-bottle tests, rats strongly preferred a sucrose-paired CS+S over the CS– and over the CS+E. The preference for the CS+E over CS– was weaker. These effects occurred when the rats drank substantially more CS+S than CS+E in training and when training intakes were matched. Similar results were obtained when the nutrient infusion was fructose or corn oil, except that preferences for the CS+F or CS+O over the CS+E were less pronounced than with CS+S. Consistent with the IG results, rats trained to drink flavored sucrose and ethanol solutions preferred the CS+S to CS+E in a flavored water test. These results confirm prior reports of ethanol-conditioned preferences but show that ethanol is less effective than other nutrients at isocaloric concentrations. The marked individual differences in ethanol-conditioned preferences may be related to the impact of the sugar or fat infusions on the reward evaluation of the ethanol-paired flavor.

© 2004 Elsevier Inc. All rights reserved.

*Keywords:* Flavor conditioning; Ethanol; Gastric infusions; Saccharin; Sucrose; Fructose; Corn oil

### 1. Introduction

It is well documented that the postingestive effects of various nutrients and nutrient mixtures can condition strong flavor preferences in rats [1]. Ethanol, which is both a nutrient and a drug of abuse, can condition both flavor preferences and aversions depending upon dose and training procedure used. Some studies have directly compared the flavor conditioning effects of ethanol with those of other nutrients, with somewhat contradictory results. This is an important issue because the degree to which ethanol is similar to or different from other nutrients in its behavioral effects has been taken as evidence for and against a nutritional interpretation of ethanol reward and reinforcement. The present study extends this analysis by directly comparing the flavor conditioning effects of ethanol with those of three other nutrients: sucrose, fructose, and corn oil.

Ethanol-conditioned flavor preferences have been produced using two basic procedures. In the oral conditioning method, rats are trained on some days to drink an ethanol solution containing a distinctive cue flavor (positive conditioned stimulus, CS+), and on alternate days a noncaloric fluid (water or saccharin) containing a different cue flavor (CS–). Flavor preferences are then evaluated in a two-bottle test with both CS flavors presented in water [2–4]. In the gastric conditioning method, in contrast, rats drank a flavored solution (the CS+) which is paired with intragastric (IG) infusions of ethanol and a different flavored solution (the CS–) which is paired with IG water infusions [5–9]. Flavor preferences are evaluated in two-bottle tests with the CS+ and CS– solutions paired with their respective IG infusions (reinforced tests) or with no infusions (nonreinforced tests). The advantages of the oral method include its ease of use and its resemblance to the normal route of ethanol administration by humans. On the other hand, the oral method has the disadvantage that the flavor of ethanol can adversely contribute to the animals' net evaluation of the cue flavor through flavor–flavor learning [10,11]. That

\* Corresponding author. Tel.: +1-718-951-5606; fax +1-718-951-4824.  
E-mail address: kackroff@gc.cuny.edu (K. Ackroff).

is, if the animal evaluates the flavor of ethanol as aversive, this could be associated with the cue flavor, working against positive postingestive effects. The IG conditioning method, while more complicated, eliminates the flavor of ethanol as a conditioning factor and thus provides a more direct measure of ethanol's postingestive reinforcing effects.

To date, only a few studies have directly compared flavor preference conditioning by ethanol with that of other nutrients. Using a between-group design and an oral training procedure, Mehiel and Bolles [4] reported that isocaloric solutions of ethanol, sucrose, Polycose, and corn oil conditioned comparable flavor preferences over a flavor paired with a noncaloric saccharin solution. Mehiel and Bolles [3] also reported, using a within-group design, that rats equally preferred a flavor (CS+E) that had been added to an ethanol solution and a flavor (CS+S) that had been added to an isocaloric sucrose solution when both flavors were presented in water. Sherman et al. [8] observed, using a between-group design, that rats acquired comparable preferences for flavored solutions paired with isocaloric IG infusions of ethanol and glucose when the alternate flavor was paired with IG water. However, in a second experiment, rats trained with one flavor (CS+E) paired with IG ethanol and another flavor (CS+G) paired with IG glucose significantly preferred the glucose-paired flavor to the ethanol-paired flavor. This conflicts with the equal preferences for CS+E and CS+S reported by Mehiel and Bolles. These studies differed in several respects, including type of sugar used (sucrose vs. glucose) and route of administration (oral vs. IG) as well as session length (24 h/day in the Mehiel and Bolles study vs. 20 min/day in the Sherman et al. study), and it is not clear which factors are responsible for the conflicting results.

Of particular interest in the disparities among these findings is the use of different sugars, because recent work indicates that carbohydrates differ in their flavor reinforcing effects. In particular, glucose-based carbohydrates (glucose, maltose, Polycose) are more reinforcing than fructose-containing carbohydrates (fructose and sucrose) [12–17]. Thus, the equal sugar vs. ethanol preference reported by Mehiel and Bolles and the unequal preference reported by Sherman et al. may have resulted in part because these studies used sucrose and glucose, respectively. The present study re-examined the issue of the relative reinforcing effects of ethanol and sugar using the IG conditioning method. In this way, the preferred taste of sugar and unpreferred taste of ethanol were eliminated as conditioning factors. The initial plan was to use sucrose as the comparison sugar because sucrose is widely used in studies evaluating ethanol reinforcement in animals. If sucrose and ethanol were found to be equally effective, a subsequent experiment was to compare glucose and ethanol. A within-group design was used to compare sugar vs. ethanol conditioning. In particular, rats were trained with three flavored, noncaloric solutions: one (CS+E) was paired with IG ethanol, the second (CS+S) was paired with IG sucrose, and the third (CS–) was paired with IG water. This three-CS design allowed for comparisons for each

CS+ to the CS– as well as between the CS+S and CS+E. The rats were trained and tested 22 h/day under food ad libitum conditions which prior studies show support preference conditioning with both sucrose and ethanol [5,18].

Experiment 1 tested the hypothesis of Mehiel and Bolles that ethanol and sucrose are equally reinforcing when presented at isocaloric concentrations. When this prediction was not confirmed, we examined possible explanations based on procedural differences. Experiment 2 compared flavor conditioning by IG sucrose and ethanol when exposure to the CS+S, CS+E, and their paired infusions were matched. Experiment 3 used the oral conditioning and food restriction procedures of Mehiel and Bolles to determine if the route of administration and/or hunger state were critical determinants of sucrose and ethanol reinforcement. Finally, Experiment 4 expanded the comparison to other nutrients, fructose and corn oil, which should be less potent in reinforcing flavor preferences than isocaloric sucrose [13–17,19–21] and therefore might have reinforcing effects more similar to that of ethanol.

## 2. Experiment 1

In several prior studies, we have compared the reinforcing effects of IG nutrients using a within-subject design, in which each animal is exposed to two nutrient-paired flavors and a third water-paired flavor for comparison. In one study [18], which compared sucrose and maltose, rats were given unlimited access to each flavor and its paired infusion four times in a rotating sequence of one-bottle training days prior to a series of two-bottle tests to determine preferences between pairs of flavors. This design was adopted for comparison of sucrose and ethanol in Experiment 1. An important aspect of this experiment is that the rats were not food restricted during training and testing and thus energy need was presumably not a major factor influencing the reinforcing effects of the IG sucrose and ethanol infusions.

### 2.1. Subjects

Adult male Sprague–Dawley rats ( $n = 12$ ; Charles River Laboratories, Wilmington, MA) weighed 350–400 g (mean 375 g) at the start of training. They were housed in stainless steel hanging cages with ad lib access to powdered chow (No. 5001, PMI Nutrition International, Brentwood, MO; 3.3 kcal/g) and fluid in rooms maintained on a 12:12 light/dark cycle (lights on 0800 h) at 21 °C.

### 2.2. Surgery

The rats were anesthetized with a mixture of ketamine HCl (63 mg/kg) and xylazine (9.4 mg/kg), and implanted with a stainless steel gastric cannula used to attach the infusion catheters as described previously [22]. Briefly, the cannula was inserted into the fundus of the stomach and

secured with a purse-string suture, polypropylene mesh and dental cement. The shaft of the cannula was passed through a small incision in the abdominal wall and skin. When not in use, the cannula was kept closed with a stainless steel screw.

### 2.3. Apparatus

The test cages used for IG infusion were similar to the “electronic esophagus” system previously described [22]. In brief, the rats were housed in stainless steel hanging cages ( $24 \times 18 \times 18$  cm) with powdered chow available from a food cup accessible through a hole in the back wall of the cage. Drinking fluids were available from stainless steel sipper tubes located through two small holes (19 mm diameter) at the front of the cage. A slot in the cage floor permitted two catheters attached to the rat’s gastric cannula to be connected to a dual-channel infusion swivel located below the cage; the catheters were protected by a flexible stainless steel spring. Plastic tubing connected the swivel to two peristaltic infusion pumps. The pumps were operated automatically by drinkometer circuits and a microcomputer whenever the rat drank from the sipper tubes. The flow rate of the pumps was 1.6 ml/min and they were controlled by computer software to infuse  $\sim 1$  ml of fluid for each 1 ml of fluid orally consumed. The microcomputer stored on disk the number of licks emitted during 6-s bins for offline analysis of drinking patterns. The infusion system operated 22 h/day; during the remaining 2 h, chow and fluids were not available while the intakes were measured and the infusion system serviced.

### 2.4. Solutions

The oral test fluids (conditioned stimuli, CS solutions) were water flavored with 0.2% saccharin (Sigma, St. Louis, MO) and 0.05% (w/v) unsweetened Kool-Aid drink mixes (Kraft Foods, White Plains, NY). Unflavored 0.2% saccharin and tap water were also available to drink during some phases of the experiment. Left/right positions of the test fluids were counterbalanced across days. The infusates were tap water, 5% ethanol (v/v; prepared by mixing 95% ethanol and tap water), and 7.18% sucrose (w/w; commercial grade). The ethanol and sucrose solutions were isocaloric at 0.287 kcal/g. Flavor–infusate pairs were counterbalanced across rats. The amounts of fluid consumed and infused were recorded to the nearest 0.1 g.

### 2.5. Procedure

After a postsurgery recovery period (7 days), the rats were transferred to the infusion cages where they lived for the remainder of the experiment. They were adapted to the cages for 4 days with chow and water available ad lib. Then their gastric catheters were attached and they were infused with water whenever they drank water during the next 2 days.

The rats were given 12 days of one-bottle training, in which oral intake of CS flavor solutions was paired with IG infusions. Half the rats received the flavors in the order CS+S, CS–, CS+E, paired with sucrose, water, and ethanol infusions, respectively; the other half received the order CS+E, CS–, and CS+S. These triplets were repeated so that each CS solution and its paired infusion were presented on 4 days. The flavors were cherry, grape, and orange, with flavor assignment counterbalanced across rats.

Then the rats were given a series of two-bottle tests, in which intake of the CS solutions was still accompanied by matched infusions (reinforced tests). First, each CS+ was compared to the CS–; half the rats received CS+E vs. CS– for 2 days, followed by CS+S vs. CS– for 2 days, and the other rats received the CS+S test first followed by the CS+E test. Finally, all rats received 2 days of CS+S vs. CS+E.

### 2.6. Data analysis

Intake data during one-bottle training and the two-bottle test sessions were averaged over days and analyzed using repeated-measures analyses of variance. Individual comparisons were evaluated using simple main effects or *t* tests as appropriate. A significant difference between the two-bottle intakes of the CS+ and CS– was taken as primary evidence for a preference. The two-bottle intakes of the individual rats were also expressed as percent CS+ intakes (CS+ intake/total intake  $\times 100$ ) and analyzed following an inverse sine transformation to normalize the distribution [23].

### 2.7. Results

The averaged one-bottle training intakes are shown in Table 1. In the training period, the rats consumed significantly more CS+S solution than CS+E and CS–; intakes of the latter two did not differ [ $F(2,22) = 18.05$ ,  $P < .01$  and simple main effects]. Because there were large increases in intake during training (Fig. 1), the individual days were also analyzed to characterize the changes. Intakes did not differ significantly on the first day of each solution, but thereafter CS+S intake increased while the intakes of CS+E and CS– solutions remained relatively constant [interaction  $F(6,66) = 8.73$ ,  $P < .01$  and simple main effects]. The average CS+E intake was 61.6% of CS+S intake. Self-administered ethanol doses averaged 5.1 g/kg/day on CS+E training days.

Average intakes in the preference tests are shown in Fig. 2. The rats drank somewhat more CS+E than CS– (62% CS+E) but the difference was not significant. However, 9 of the 12 rats preferred the CS+E to the CS– by at least 60%. The animals drank substantially more CS+S than CS– [90% CS+S,  $t(11) = 6.12$ ,  $P = .01$ ]. In the final test, the rats overwhelmingly preferred the CS+S to the CS+E [93%,  $t(11) = 6.56$ ,  $P < .01$ ]. All rats preferred the CS+S in both tests by 60% or more.

Table 1  
Mean (S.E.M.) intake (g) during training periods in Experiments 1, 2, and 4<sup>a</sup>

	CS+S	CS+E	CS-
<i>Experiment 1</i>			
All rats	85.3 (11.9)	43.1 (4.2)	57.5 (7.5)
S-first ( <i>n</i> = 6)	104.7 (11.6)	50.1 (5.7)	62.2 (9.8)
E-first ( <i>n</i> = 6)	65.9 (18.5)	36.2 (5.2)	52.8 (11.5)
<i>Experiment 2</i>			
2A: CS+S limited to CS+E			
all rats	47.6 (2.6)	51.3 (2.9)	58.8 (3.9)
cP ( <i>n</i> = 12)	45.6 (3.6)	49.8 (4.6)	51.8 (5.6)
cNP ( <i>n</i> = 11)	49.8 (3.8)	52.9 (3.6)	66.5 (4.7)
2B: unlimited CS+E			
	–	53.0 (2.8)	59.0 (3.2)
<i>Experiment 4</i>			
4A: fructose			
	CS+F	CS+E	CS-
all rats	58.8 (5.6)	47.3 (3.7)	55.1 (4.7)
cP ( <i>n</i> = 12)	43.0 (6.9)	40.8 (5.10)	41.9 (5.7)
cNP ( <i>n</i> = 12)	74.7 (6.1)	53.8 (4.9)	68.3 (5.3)
4B: oil emulsion			
	CS+O	CS+E	CS-
all rats	80.5 (4.0)	51.0 (2.2)	59.9 (3.2)
cP ( <i>n</i> = 13)	71.0 (8.1)	45.1 (3.1)	51.4 (5.0)
cNP ( <i>n</i> = 11)	91.8 (12.3)	57.8 (6.9)	69.9 (8.4)

<sup>a</sup> CS+S is the flavor paired with IG 7.18% sucrose, CS+E is paired with 5% ethanol, CS+F is paired with 7.18% fructose, CS+O is paired with 3.26% corn oil, and CS- is paired with water. "S-first" refers to rats given the CS+S first in the training sequence; "E-first" rats were given CS+E first. "cP" refers to the subset of rats that preferred the CS+E by 60% or more in two-bottle tests vs. CS-; "cNP" rats did not prefer the CS+E.

Additional analysis of the preference data revealed differences between the subgroups of animals that started training with the CS+E and with the CS+S. That is, rats that began

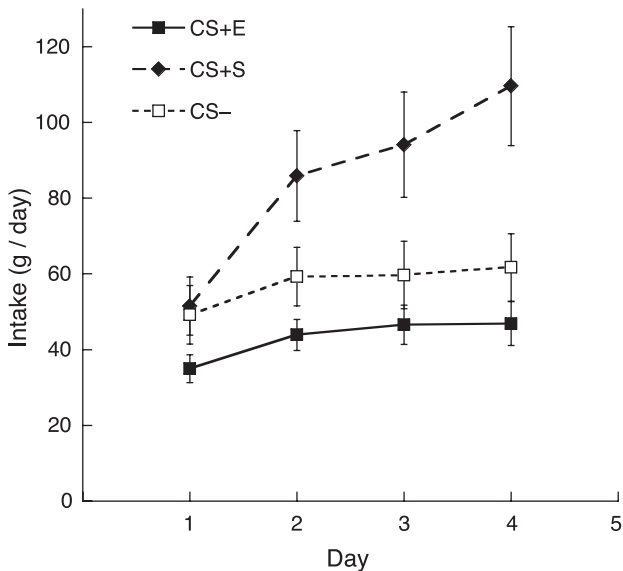


Fig. 1. Mean  $\pm$  S.E.M. daily intakes of CS solutions on the one-bottle training days of Experiment 1. Intakes of the solutions were paired with matched-volume intragastric infusions (CS+S, 7.18% sucrose; CS+E, 5% ethanol; CS-, water).

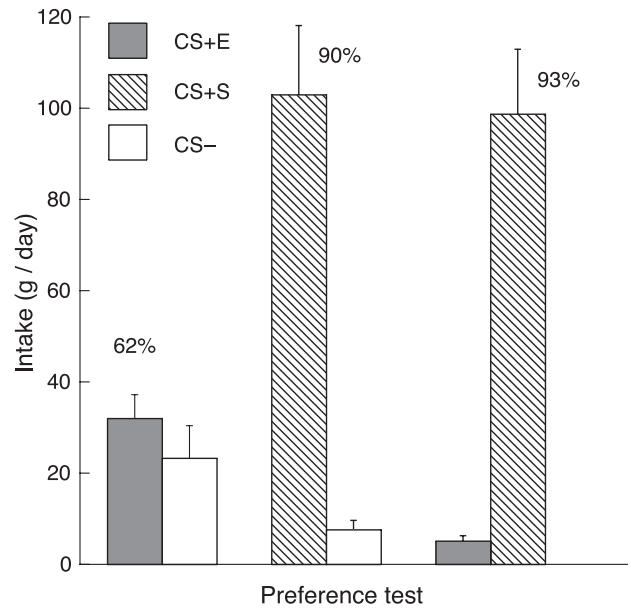


Fig. 2. Mean  $\pm$  S.E.M. daily intakes of CS solutions in the two-bottle tests of Experiment 1. Intakes of the solutions remained paired with the infusions given during training (CS+S, 7.18% sucrose; CS+E, 5% ethanol; CS-, water). Mean percentage of total intake consumed as CS+ is shown atop the bars.

training with the CS+S first ("S-first" subgroup) subsequently drank more CS+E than CS- in the two-bottle test (43.3 vs. 12.2 g, 75% CS+E), while the rats that received the CS+E first during training did not prefer the CS+E in the two-bottle test (20.7 g CS+E vs. 34.4 g CS-, 48% CS+E), interaction [ $F(1,10) = 5.76$ ,  $P < .05$ ]. The outcomes of the CS+S vs. CS- and CS+S vs. CS+E tests were not affected by training order. The S-first rats tended to drink more of all three CS solutions during one-bottle training than did the E-first rats (Table 1), but these differences were not significant.

## 2.8. Discussion

In confirmation of prior research, the rats showed a robust preference (90%) for the CS+S over CS- [18]. They also tended to drink more CS+E than CS- (although this difference was not significant) which contrasts with our prior reports of significant CS+E preferences in nondeprived rats [5,6]. The weaker CS+E preference obtained here might be related in part to fewer CS+E training trials in the present experiment than in our prior work (4 vs. 10). However, in one recent experiment, rats showed an 80% CS+E preference after only five training sessions, which increased to 89% after another five sessions [6]. Perhaps more important is the fact that the present rats were trained concurrently with a CS+S paired with IG sucrose infusion. Their weak CS+E preference could reflect an effect of comparison with the alternate CS+S and paired sucrose infusion; perhaps the stronger reinforcing effect of sucrose reduced the animals' evaluation of the CS+E.



Some evidence that CS+E conditioning was influenced by experience with the CS+S is provided by the differential intakes and preferences of the subgroups given different training orders. Rats that received the CS+S first during training subsequently expressed a stronger preference for CS+E over CS–. The subgroups did not differ strongly in CS+E training intake, so differential ethanol exposure does not explain the outcome. Perhaps the evaluation of the first CS+ flavor generalized more readily to the second CS+ flavor, promoting a more positive response to the CS+E in rats experiencing sucrose first and reducing the response to the CS+S in rats given ethanol first. However, the small numbers of rats ( $n=6$ ) in these subgroups suggest caution in generalizing these results.

Irrespective of training order, all rats strongly preferred the CS+S to the CS+E. Additional evidence that IG sucrose was a more potent reinforcer than IG ethanol comes from the much larger daily intakes of CS+S than CS+E during training. The rats also drank more CS+S than CS– during training and this increased CS+S acceptance confirms prior findings that IG sucrose infusions can greatly stimulate the acceptance of flavored saccharin solutions [18,24]. Because the rats drank much more of the CS+S during training, and therefore self-infused more sucrose than ethanol, their strong preference for CS+S over CS+E could be secondary to their increased exposure to the CS+S. Note that in the oral conditioning study of Mehiel and Bolles [3], intake of the CS+S/sucrose solution during training was matched to that of the CS+E/ethanol solution. In Experiment 2, therefore, we trained new rats with their CS+S intakes matched to those of their CS+E intakes.

### 3. Experiment 2

This experiment controlled for the large difference in CS+S and CS+E training intakes, which could have influenced the preferences obtained in Experiment 1. In the prior study of Mehiel and Bolles [3], CS+ intakes were yoked by training all rats with the CS+E first and then limiting their intake of CS+S to the prior day's CS+E intake. Since Experiment 1 revealed a significant training order effect, the present experiment used a counterbalanced training order. After this initial training phase with matched exposures to the CS+E and CS+S, the rats were given additional one-bottle sessions with the CS+E and CS– only to see if preference for the CS+E relative to the CS+S would be enhanced after greater training with the CS+E paired with IG ethanol.

#### 3.1. Method

##### 3.1.1. Experiment 2A

Naive male Sprague–Dawley rats ( $n=23$ ) weighed 435–515 g (mean 477 g) at the start of training. Details of housing, surgery, and apparatus were the same as in

Experiment 1. After a postsurgery recovery (8–14 days), they were placed in the test cages and adapted to the infusion system as in Experiment 1 (9 days). For another 3 days they drank unflavored 0.2% saccharin paired with water infusions.

The training and testing in Experiment 2A resembled that of Experiment 1 except as noted. Prior to the start of one-bottle training, the subjects were familiarized with unflavored 0.2% saccharin paired with water infusions for 3 days. Training with the CS solutions then occurred over an 18-day period, with each rat's intake of the CS+S solution yoked to its intake of the CS+E solution. In the case of the rats in the S-first subgroup, their intake of CS+S on the first training day was arbitrarily limited to a fixed amount estimated to represent their average intake of CS+E. The subsequent CS+S limits were then individually adjusted for the S-first rats so that their total training intakes of CS+S and CS+E were equivalent. The flavors were 0.05% grape, cherry, and strawberry Kool-Aid sweetened with 0.2% saccharin. Flavor–infusate assignment was counterbalanced and flavors were rotated across training days with the CS+E, CS+S, and CS– each presented on 6 training days. After training, a series of two-bottle reinforced tests (2 days each) was conducted, with unlimited access to the solutions and paired infusions. The CS+S vs. CS+E test was conducted first, followed by the counterbalanced CS+E vs. CS– and CS+S vs. CS– tests.

##### 3.1.2. Experiment 2B

The animals of Experiment 2A were given additional one-bottle training with unlimited access to CS+E and CS– on alternate days for 12 days. At the end of training, a 4-day, two-bottle test was conducted with the CS+E vs. CS–. The rats were next given a 2-day CS+S vs. CS– test, then another 4-day CS+E vs. CS– test, and finally, a 2-day CS+E vs. CS+S test. With one exception, throughout training and testing, intake of the CS flavors remained paired with the appropriate infusions. This exception occurred midway during one-bottle training when, due to an error, the rats were given a single two-bottle test with CS+E vs. CS– and 14 of the 22 rats were given the wrong flavors paired with the ethanol and water infusions. This error did not affect the CS+E preferences observed in the CS+E vs. CS– tests conducted at the end of training.

#### 3.2. Results

##### 3.2.1. Experiment 2A

The yoking procedure produced similar training intakes of CS+S and CS+E; these data are shown in Table 1. In an analysis of mean CS solution intakes, CS+E and CS+S did not differ and were less than CS– intake [ $F(2,44)=9.84$ ,  $P<.01$ ]. Analysis of intakes over days showed a main effect of days [ $F(5,100)=13.88$ ,  $P<.01$ ; intakes increased over days] and an interaction of days and flavors [ $F(10,220)=2.38$ ,  $P<.05$ ] which was largely due to higher

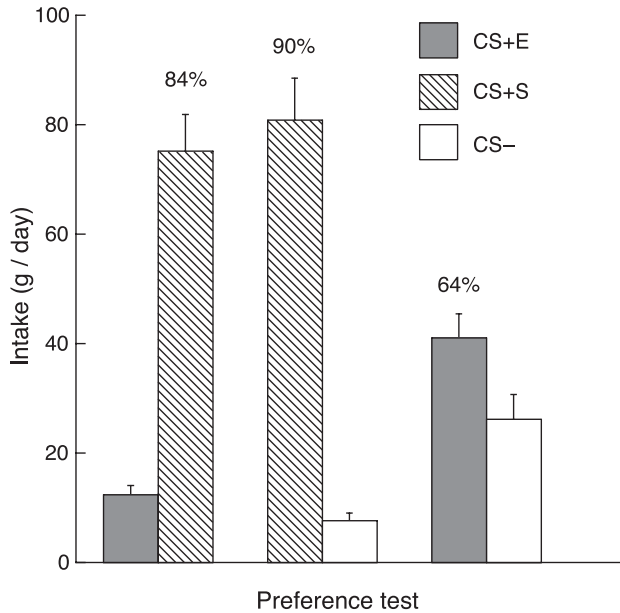


Fig. 3. Mean  $\pm$  S.E.M. daily intakes of CS solutions in the two-bottle tests of Experiment 2A. Intakes of the solutions remained paired with the infusions given during training (CS+S, 7.18% sucrose; CS+E, 5% ethanol; CS-, water). Mean percentage of total intake consumed as CS+ is shown atop the bars.

CS- than CS+ intakes. These and subsequent training data are not presented in graphic form. Self-administered ethanol doses averaged 4.0 g/kg on CS+E days.

The preference data for Experiment 2A are shown in Fig. 3. Preliminary analysis revealed no differences between the subgroups given CS+E or CS+S first in training. In the choice test with the two CS+ solutions, the rats consumed significantly more CS+S than CS+E [ $t(22)=8.74, P<.01$ ]. The overall CS+S preference was 84% and 21 of the 23 rats preferred the CS+S by at least 60%. In the choice tests with the CS-, the animals consumed significantly more CS+S than CS- [ $t(22)=9.32, P=.01$ ]; percent CS+ intake averaged 90% with all rats showing a CS+ preference. Mean CS+E intake exceeded CS- intake and this difference approached significance [ $t(22)=2.02, P=.055$ ]. Percent intake of CS+E averaged 64% and 12 of 23 rats consumed at least 60% of their intake as CS+E. Mean CS+S intakes per day during the two-bottle tests exceeded that of the one-bottle tests [ $t(22)=3.00, P<.01$  vs. CS+E,  $t(22)=3.47, P<.01$  vs. CS-], which indicates that the yoking procedure did in fact limit the intake of the CS+S during training.

To obtain a more detailed picture of rats' preferences, we analyzed two subgroups based on preference for the CS+E over the CS-. The "cP" (conditioned Preferring) set consisted of the 12 rats mentioned above, which had CS+E preferences ranging from 69–96% (mean 85%); the remaining 11 rats (the "cNP," conditioned Nonpreferring subgroup) had a mean percent CS+E intake of 41% (range 22–55%). Although they differed markedly in CS+E vs. CS- preference, we found that the two subgroups showed similar preferences for the CS+S over the CS- (91% cP and 89%

cNP) and for the CS+S over the CS+E (82% and 86%). Self-administered doses during training did not differ in the subgroups (cP 3.8 g/kg/day, cNP 4.1 g/kg/day,  $t<1$ ) and intakes of the CS+E and CS+S during training were similar (Table 1).

### 3.2.2. Experiment 2B

In the one-bottle training period, intakes were similar to those obtained during training in Experiment 2A, with average CS+E intake less than that of CS- [Table 1;  $F(1,22)=9.96, P<.01$ ]. Analysis of intakes over days showed a main effect of days [ $F(5,100)=7.90, P<0.01$ ; intakes decreased over days] and an interaction of days and flavors [ $F(5,110)=3.84, P<.01$ ]; CS+E was less than CS- only during the first half of the training period. The self-administered ethanol dose on CS+E training days averaged 3.8 g/kg/day.

The preference data following the additional CS+E training of Experiment 2B are shown in Fig. 4. In the first 4-day test, the rats drank more CS+E than CS- (72% CS+E, 18 of 23 preferring),  $t(22)=5.61, P<.01$ , as they did in the second CS+E test,  $t(22)=5.26, P<.01$  (72% CS+E, 16 of 23 preferring). The cP and cNP subgroups from Experiment 2A still differed in the first CS+E vs. CS- test [81% vs. 63%,  $t(21)=2.51, P<.05$ ] but began to converge by the second test [79% vs. 65%,  $t(21)=1.79, P=.08$ ]. In the intervening CS+S test, the rats consumed more CS+S than CS- [ $t(22)=11.89, P<.01$  (91% CS+S, all prefer-

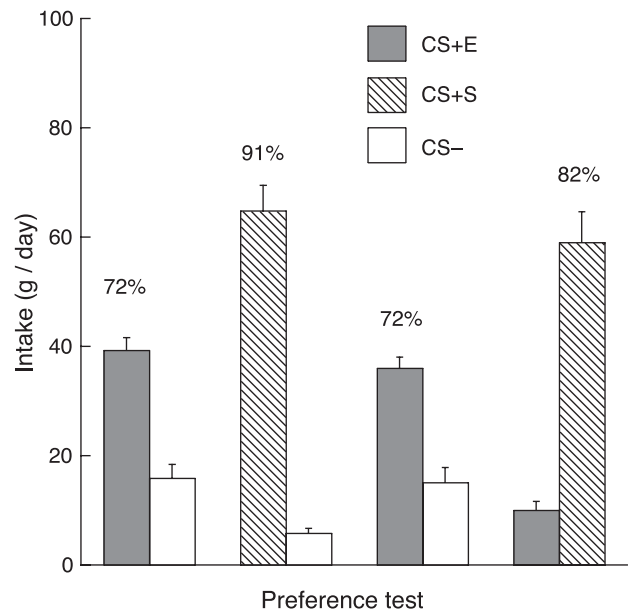


Fig. 4. Mean  $\pm$  S.E.M. daily intakes of CS solutions in the two-bottle tests of Experiment 2B. Intakes of the solutions remained paired with the infusions given during training (CS+S, 7.18% sucrose; CS+E, 5% ethanol; CS-, water). The tests are shown in the order they were conducted, from left to right; there were two CS+E vs. CS- tests in this series. Mean percentage of total intake consumed as CS+ is shown atop the bars.

ring)]. In the comparison of the two CS+ solutions, the CS+S was preferred (82%) over the CS+E [ $t(22)=7.35$ ,  $P<.01$ ; 20 of the 23 rats preferred the CS+S and the others were indifferent].

### 3.3. Discussion

Equating the training intakes of the ethanol- and sucrose-paired flavors did not alter the basic outcomes of preference tests in comparison to Experiment 1. The rats still showed a strong preference for the CS+S over CS–, a weaker preference for the CS+E over CS–, and a strong preference for the CS+S over the CS+E. Thus, the elevated training intakes of the CS+S and IG sucrose, relative to the CS+E and IG ethanol, were not responsible for the CS+S preference obtained in the first experiment.

Additional training with the CS+E and CS– enhanced the CS+E preference somewhat, as suggested by the increase from a marginal 64% in Experiment 2A to a significant 72% preference in Experiment 2B. In addition, the proportion of the rats expressing a CS+E preference increased after the extra training. However, the preference for the CS+S over the CS+E was unaltered by the additional experience; CS+S was preferred to CS+E by 84% in Experiment 2A and by 82% in Experiment 2B. These data suggest that while the relative reinforcing effects of sucrose and ethanol are relatively stable, the absolute reinforcing value of ethanol may be strengthened as experience with its postingestive effects increases.

In contrast to Experiment 1, order of presentation of CS+S and CS+E during training did not affect CS+E vs. CS– preference in the present experiment. This may have occurred because exposure to unsweetened saccharin prior to CS training and the limited intakes of CS+S throughout training prevented the rats' positive experience with the CS+S, when presented first, from generalizing to the CS+E flavor.

Although not related to training order, the rats could readily be grouped based on preference for the CS+E over the CS–. The existence of the cP and cNP subgroups, in contrast to the unanimous preference for the CS+S over CS–, suggests important individual differences in the post-ingestive reinforcing effect of ethanol, consistent with other results obtained with outbred rats [25–27]. The hint of convergence between these subgroups with accumulating experience in Experiment 2B suggests that one aspect of the difference involves the rate of acquisition of ethanol-based preferences, such that some animals require more exposure to learn to prefer ethanol-paired flavors.

## 4. Experiment 3

In Experiments 1 and 2, IG ethanol was a weaker reinforcer than IG sucrose, as demonstrated by the strong CS+S preference over the CS+E. This occurred despite the

lack of opportunity to taste the ethanol and sucrose, so that the basis for comparison was entirely postingestive. This result is similar to the finding that rats preferred a CS+G flavor paired with IG glucose over a CS+E flavor paired with IG ethanol [8], but inconsistent with the Mehiel and Bolles [3] finding of equal preference for flavors that had been mixed into orally consumed ethanol and isocaloric sucrose. Taken together, the results suggest that ethanol and sugar reinforcing effects are more similar when these nutrient sources are tasted (oral method) than untasted (IG method). This seems counterintuitive, however, because the sweet taste of sugar is normally preferred to the taste of ethanol. Experiment 3 sought to replicate the Mehiel and Bolles findings using orally consumed flavored ethanol and sucrose solutions. As in their study, the rats were food restricted during training with the flavored ethanol and sucrose solutions and during the 4-h preference test with the CS+S and CS+E flavors presented in water. The choice test was extended another 20 h with food available ad libitum to approximate the food ad libitum tests conducted in Experiments 1 and 2.

### 4.1. Method

Adult male Sprague–Dawley rats ( $n=16$ ) weighed 340–396 g (mean 364 g) at the start of the study. They were housed in standard stainless steel hanging cages. The CS solutions were prepared from tap water flavored with 0.25% (w/v) unsweetened Kool-Aid and contained either 5% ethanol (CS+E/E) or 7.18% sucrose (CS+S/S); the flavors paired with the ethanol and sucrose were counterbalanced across rats. The Kool-Aid concentration was based on that used by Mehiel and Bolles [3]. Solutions were presented in glass bottles with rubber stoppers and stainless steel spouts, which passed through the left and right sides of the front grid of the cage. The amounts of fluid consumed were recorded to the nearest 0.1 g.

After a 2-week acclimation to the lab with ad lib chow and water, the rats were adapted for 4 days to a 12-g/day chow ration; water remained available ad lib. This ration, along with the rats' intakes of flavored solutions, maintained them at 90% of ad lib body weight. During training, the rats were given alternating access to the flavored ethanol and 7.18% sucrose solutions, with the amount of sucrose yoked to the previous days' ethanol intake for each rat. The CS flavors were grape and cherry. This alternation continued for a total of 8 days, with left/right positions of the bottles counterbalanced so that the animals became accustomed to drinking from both sides and received the two solutions equally often on the left and right.

After the last day of training, the rats were given 3 days of ad lib access to chow and water, followed by 24 h chow deprivation. They were then given a two-bottle test with the Kool-Aid flavors mixed in water (now designated CS+S and CS+E). Intakes were measured at 1 and 4 h, and then ad lib chow and the CS+ solutions were returned for an additional

20 h. Positions of the bottles were counterbalanced across test periods.

#### 4.2. Results

During training, CS+E/E intakes increased over days, which approximately matched CS+S/S intakes due to the yoking procedure. However, the matching was not complete, and CS+S/S intakes were slightly higher than CS+E/E intakes [36.1 vs. 34.8 g/day,  $F(1,15)=8.38$ ,  $P<.05$ ]. The increase from the first to subsequent days accounted for the day effect [ $F(3,45)=14.06$ ,  $P<.05$ ], and there was a CS solution  $\times$  Day interaction [ $F(3,45)=89.95$ ,  $P<.01$ ]. Self-administered doses of ethanol averaged 4.3 g/kg/day.

Fig. 5 shows the intakes in the two-bottle tests. The rats consumed more CS+S than CS+E at 1 h [ $t(15)=4.50$ ,  $P<.01$ ] and this difference persisted at the 4-h measure [ $t(15)=2.90$ ,  $P<.05$ ]. During the 20-h period with ad lib chow, the animals continued to drink more of the CS+S flavor [ $t(15)=5.45$ ,  $P<.01$ ]. The CS+S preferences at 4 and 20 h were similar (67% and 70%), although somewhat less than the 80% preference obtained at the 1-h time point.

#### 4.3. Discussion

The present results failed to confirm the findings of Mehiel and Bolles that rats equally preferred CS+E and CS+S flavors after oral training sessions with flavored ethanol and sucrose solutions. Rather, consistent with Experiments 1 and 2, the rats in this experiment consumed more CS+S than CS+E. This preference was obtained at 1- and 4-h time points while the animals were food restricted

as well as during the 20-h period when they had ad libitum access to food. The rats consumed significantly more sucrose than ethanol during training, but this difference was small and unlikely to account for the CS+S preference. We also obtained a significant CS+S preference (78%) in another experiment in which rats ( $n=12$ ) were trained as in the present experiment except that the ethanol and sucrose solutions were flavored with 0.05% Kool-Aid, the concentration used in Experiments 1 and 2, rather than the 0.25% concentration used here and by Mehiel and Bolles (Ackroff and Sclafani, unpublished data).

The reason for the discrepancy between our preference results and those of Mehiel and Bolles is not certain, although strain differences may be a factor. They used Long–Evans and Wistar rats in contrast to the Sprague–Dawley rats used in the present study. These strains have been found to differ from each other in oral ethanol intake but the relative rankings differ across studies [28,29], with Sprague–Dawley rats generally intermediate. Another possible factor is ethanol dose. Based on the reported ethanol intake and body weight data, the rats in the Mehiel and Bolles study self-administered ethanol doses averaging 7.5 g/kg/day, which is greater than the average 4.3 g/kg/day dose obtained in the present experiment. Based on their rats' equal preference for the CS+S and CS+E flavors, Mehiel and Bolles concluded that 5% ethanol and isocaloric sucrose produced comparable postingestive reinforcing effects. However, even if this is the case for the rat strains they studied, the rats would still have been expected to prefer the CS+S to the CS+E based on the preferred taste of sucrose relative to ethanol. CS preferences can be conditioned by associations between the CS flavor and a preferred taste (e.g., sweet taste) as well as with positive postingestive nutritive effects. Of particular relevance here are the findings of Warwick and Weingarten [30] obtained with rats trained with CS flavors added to two isocaloric glucose solutions; the palatability of one solution was reduced by the addition of citric acid. After consuming similar amounts of the two solutions during training (because of intake limits), the rats displayed a significant preference for the CS flavor paired with the more palatable sugar solution when both flavors were presented in identical solutions. Thus, in the absence of differences in caloric density, flavor preferences can be conditioned by differences in orosensory palatability.

Mehiel and Bolles also reported that rats trained with a flavored 5% ethanol solution and a flavored hypocaloric 1% sucrose solution subsequently preferred the CS+E to the CS+S. We have confirmed this result, which indicates Sprague–Dawley rats are not insensitive to ethanol reinforcement when it is pitted against hypocaloric sucrose (Ackroff and Sclafani, unpublished data). It thus appears that the anomalous result in this set of flavor-conditioning studies is the Mehiel and Bolles [3] finding of equal CS+E and CS+S preferences in rats trained with isocaloric solutions.

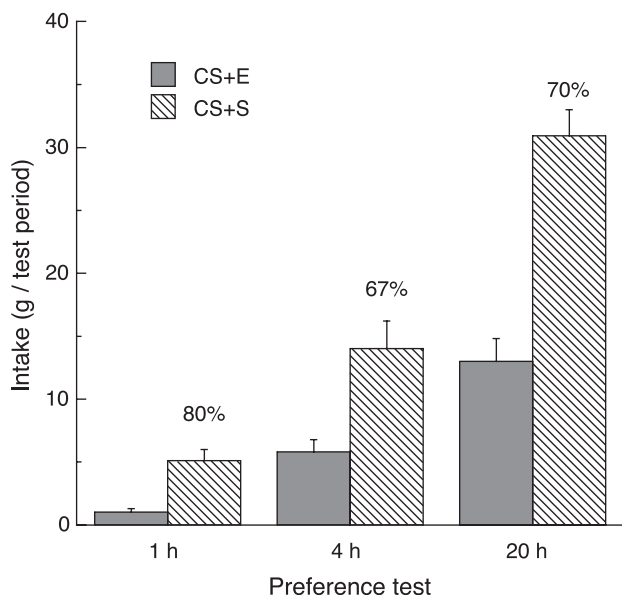


Fig. 5. Mean  $\pm$  S.E.M. intakes of CS solutions in the two-bottle tests of Experiment 3. The CS+E flavor was previously paired with 5% ethanol and the CS+S flavor was paired with isocaloric 7.18% sucrose. Mean percentage of total intake consumed as CS+S is shown atop the bars.



## 5. Experiment 4

Although early studies suggested that the postingestive actions of different isocaloric nutrient sources are comparable, subsequent work revealed significant differences among various types of carbohydrates, fats, and nutrient mixtures [1]. Thus, the findings of the first three experiments that sucrose and ethanol differed in their flavor conditioning effects were not surprising. Experiment 4 explored the possibility that ethanol reinforcement might be more closely matched to that of other nutrients. Fructose was selected as one nutrient because prior work indicates that fructose is less effective than glucose and sucrose in conditioning flavor preferences [14,16,21]. Corn oil was selected as another nutrient because of several reports that IG infusions of corn oil condition weaker flavor preference and acceptance than do infusions of isocaloric carbohydrate solutions [19,20,31]. Fructose and ethanol conditioning have not been previously compared and the only prior study to compare flavor conditioning by ethanol and corn oil used a between-group, oral training procedure and reported comparable flavor preferences [4].

As in Experiment 1, CS+ intakes were not limited during training so that the effect of the IG nutrient infusions on CS+F and CS+O intakes could be evaluated. Based on prior work, the IG corn oil and fructose infusions were not expected to stimulate CS+ intakes to the same degree as that observed in the first experiment [16,19,31,32]. An additional feature of Experiment 4 was that two sets of two-bottle tests were conducted following training. CS preferences were first evaluated, as in Experiment 1, with the various CS solutions paired with their respective IG infusions (reinforced tests). CS preferences were then compared with all the CS solutions paired with IG water (nonreinforced test) to determine if the CS+ flavor preferences remained stable when no longer associated with nutrient or ethanol infusions.

### 5.1. Method

#### 5.1.1. Experiment 4A

Male Sprague–Dawley rats ( $n=24$ ; Charles River) weighed 361–459 g (mean 415 g) at the start of training. Details of housing, surgery, and apparatus are the same as in Experiments 1 and 2. The CS solutions were 0.2% saccharin+0.05% Kool-Aid flavors (cherry, grape, and orange). The ethanol was prepared as 5% v/v as before, and the isocaloric nutrient was a 7.18% (w/w) fructose (Sigma) solution. After a postsurgery recovery (11–15 days), the rats were familiarized with saccharin by giving them ad libitum access to 0.2% saccharin and water for 2 days. The following day they were placed in the test cages, and adapted to the infusion system as in Experiment 2.

The animals were trained as in Experiment 1, with four triplets of CS days. The CS flavors paired with the infusates were counterbalanced. Half the rats got the CS+E (paired

with ethanol infusion) first, then the CS–, then the CS+F (paired with fructose infusion). The other rats received the order CS+F, CS–, CS+E. Then the standard set of two-bottle tests was conducted, with half the rats getting CS+F vs. CS– first and the others CS+E vs. CS– first. After all rats had received 2 days each of these comparisons, they were tested for 2 days with CS+F vs. CS+E. When these 6 reinforced test days were completed, they were repeated under nonreinforced conditions (intake of each CS was paired with water infusion).

#### 5.1.2. Experiment 4B

Male Sprague–Dawley rats ( $n=24$ ; Charles River) weighed 366–464 g (mean 416 g) at the start of training. Details of housing, surgery, and apparatus are the same as in Experiments 1 and 2. The training and testing procedures were similar to that of Experiment 4A except that the isocaloric nutrient was a 3.26% (w/w) corn oil emulsion. Because oil emulsions separate over time [33], we determined that an initial concentration of 3.53% accommodated the slow separation of the oil from the emulsion so that the average concentration infused during the 22-h sessions was approximately 3.26%. To prepare the emulsion, corn oil was added to hot water ( $\sim 80^\circ\text{C}$ ) along with 0.1% (w/w) sodium stearyl lactate (Emplex, American Ingredients, Kansas City, MO). This was mixed at high speed for 5 min in a rotor-stator homogenizer (Ultra-Turrax T25, IKA-Works, Cincinnati, OH), quickly cooled to  $21^\circ\text{C}$  in an ice bath, and then passed twice through a microfluidizer (HC 5000, Microfluidics, Newton, MA) to stabilize the emulsion further.

### 5.2. Results

#### 5.2.1. Experiment 4A

The mean training intakes of the three CS solutions differed [ $F(2,46)=8.30$ ,  $P<.01$ ]. One-bottle intakes of CS+F and CS–, which did not differ, exceeded that of CS+E ( $P<.01$ ; Table 1). Analysis of intakes over days showed an interaction of CS solutions and days [ $F(6,138)=2.41$ ,  $P<.05$ ]. Simple main effects tests showed that intakes of the CS solutions did not change significantly over days, with the interaction due to larger differences among solutions in the second and fourth triplets of training days. Self-administered ethanol doses averaged 3.3 g/kg/day on CS+E training days. The order of CS+ presentation did not affect training intakes or the resulting preferences.

The results of the reinforced and nonreinforced preference tests are shown in Fig. 6. Overall, the rats consumed more CS+E than CS– [ $F(1,23)=4.70$ ,  $P<.05$ ]. The CS+E preference was 61% and 65%, respectively, in the reinforced and the nonreinforced tests. There was a CS  $\times$  Test interaction [ $F(1,23)=4.09$ ,  $P=.05$ ]: the CS+ intake was significantly greater than that of CS– only in the nonreinforced test. The rats consumed more CS+F than CS– in both reinforced and nonreinforced tests [ $F(1,23)=44.50$ ,

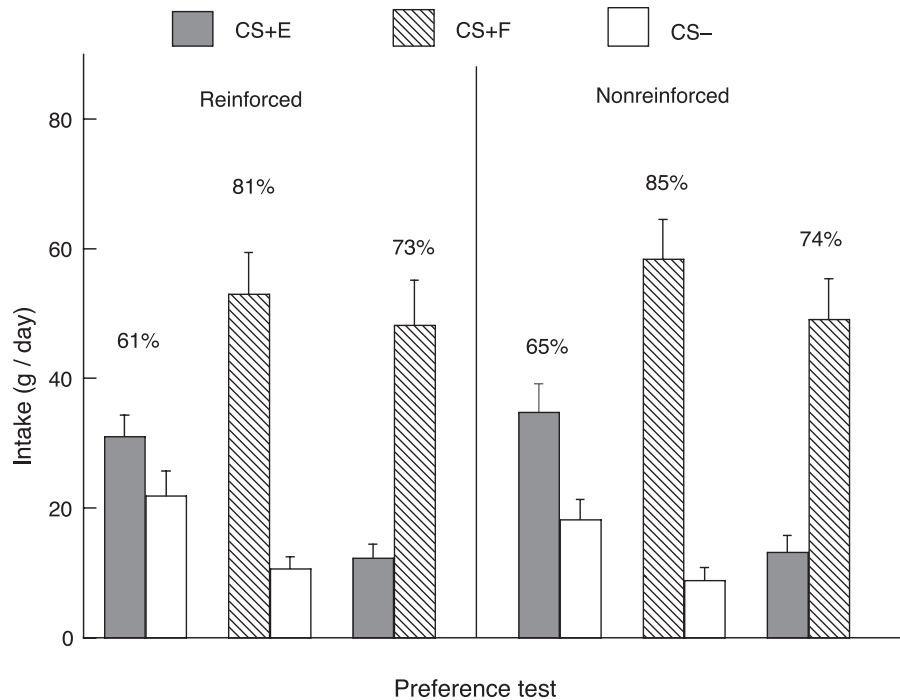


Fig. 6. Mean  $\pm$  S.E.M. daily intakes of CS solutions in the two-bottle tests of Experiment 4A. Left panel: reinforced test, in which intakes of the solutions remained paired with the infusions given during training (CS+F, 7.18% fructose; CS+E, 5% ethanol; CS-, water). Right panel: nonreinforced test, in which intakes of all CS flavors were paired with water infusion. Mean percentage of total intake consumed as CS+ is shown atop the bars.

$P < .01$ ], and their percent CS+F intakes were 81% and 85%, respectively, in the two tests. The CS  $\times$  Test interaction was significant [ $F(1,23) = 5.93$ ,  $P = .05$ ], which was due to similar intakes of CS- but not CS+F in the two tests. In the comparison of the two CS+ flavors, CS+F intake exceeded CS+E intake in both reinforced and nonreinforced tests [ $F(1,23) = 19.91$ ,  $P < .01$ ]. The percent CS+F intake was 73% and 74% in the two tests, with 16 of 24 rats preferring the CS+F by 60% or more.

To obtain a more detailed picture of rats' preferences, we analyzed two subgroups based on preference for the CS+E over the CS- in the reinforced test. The "cP" set consisted of the 12 rats which had CS+E preferences ranging from 63 to 96% (mean 83%); the remaining 12 rats (the "cNP" subgroup) had a mean percent CS+E intake of 40% (range 16–56%). The preference for the CS+F vs. CS- was the same for the cP and cNP groups (81%; 22 of 24 rats preferred CS+F by at least 60%). However, the cP subgroup's preference for CS+F over CS+E was lower than that of the cNP subgroup [61% vs. 85%,  $t(22) = 2.63$ ,  $P < .05$ ]. This pattern of results persisted in the nonreinforced tests. As noted above, overall the rats consumed more CS+F than CS+E in the two-bottle test and while this difference was greater in the cNP subgroup than in the cP subgroup, the CS  $\times$  Subgroup interaction failed to reach significance ( $P = .06$ ). During one-bottle training, the cNP rats drank more CS solution than did the cP rats [ $F(1,22) = 10.11$ ,  $P < .01$ ]. The CS  $\times$  Group interaction [ $F(2,44) = 7.02$ ,  $P < .01$ ] reflected the contrast between the similar intakes of all three CS solutions by the cP subgroup and the differential

intakes of the cNP subgroup; their intakes of the CS+E did not differ (Table 1).

#### 5.2.2. Experiment 4B

The mean training intakes of the three CS solutions differed [ $F(2,44) = 24.25$ ,  $P < .01$ ; Table 1]. One-bottle intake of CS+O exceeded that of CS+E and CS- ( $P < .05$ ) and CS- intake exceeded CS+E intake ( $P < .05$ ). Analysis of intakes over days showed an interaction of CS solution and days [ $F(6,130) = 3.76$ ,  $P < .01$ ]. Simple main effects tests showed that CS+E intake did not change over days, while CS+O increased and CS- decreased. The order of CS+ presentation did not affect training intakes or the resulting preferences. Self-administered ethanol doses averaged 3.8 g/kg/day.

The preference tests are shown in Fig. 7. Overall, the rats consumed more CS+E than CS- [ $F(1,22) = 34.15$ ,  $P < .01$ ]. There was an interaction of CS solution  $\times$  Test [ $F(1,22) = 20.36$ ,  $P < .01$ ], which was due to greater intake of CS+E when it was paired with water (nonreinforced test) than when it was paired with IG ethanol. The percent CS+E intakes were 65% and 74% in the reinforced test and nonreinforced test, respectively. The rats drank more CS+O than CS- overall [ $F(1,22) = 60.49$ ,  $P < .01$ ], and intakes in the reinforced and nonreinforced tests did not differ; percent CS+O intakes were 88% and 86%, respectively. In the choice tests with the two CS+ solutions, the rats drank more CS+O than CS+E [ $F(1,22) = 15.38$ ,  $P < .01$ ]. Intakes differed as a function of test [reinforced intakes greater than nonreinforced,  $F(1,22) = 10.96$ ,  $P < .01$ ]

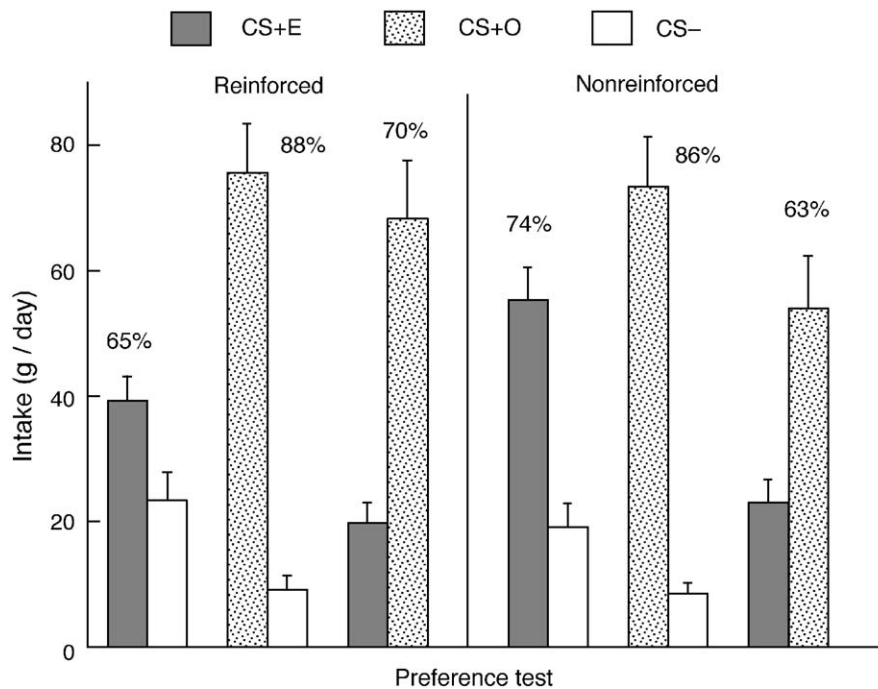


Fig. 7. Mean  $\pm$  S.E.M. daily intakes of CS solutions in the two-bottle tests of Experiment 4B. Left panel: reinforced test, in which intakes of the solutions remained paired with the infusions given during training (CS+O, 3.26% oil emulsion; CS+E, 5% ethanol; CS-, water). Right panel: nonreinforced test, in which intakes of all CS flavors were paired with water infusion. Mean percentage of total intake consumed as CS+ is shown atop the bars.

and there was a CS solution  $\times$  Test interaction [ $F(1,22) = 13.20, P < .01$ ], due to reduced CS+O but not CS+E intake in the nonreinforced test ( $P < .01$ ). The percent intake of CS+O in the reinforced test was 70% (with 16 of 24 rats preferring by 60% or more) and 63% (12 rats preferring) in the nonreinforced tests.

To obtain a more detailed picture of rats' preferences, we analyzed two subgroups based on preference for the CS+E over the CS- in the reinforced test. The "cP" set consisted of the 14 rats which had CS+E preferences ranging from 60% to 97% (mean 84%); the remaining 10 rats (the "cNP" subgroup) had a mean percent CS+E intake of 39% (range 19–54%). The two subgroups' preference for the CS+O over the CS- did not differ (91% cP vs. 82% cNP, with 22 of 24 rats preferring CS+O by at least 60%). The cP subgroup's preference for CS+O over CS+E was lower than that of the cNP subgroup, but not significantly (63% vs. 81%). This pattern of results persisted in the nonreinforced tests. As noted above, overall, the rats consumed more CS+O than CS+E in the two-bottle test and there was no interaction between CS+ intake and subgroup. The cNP rats tended to drink more of all CS solutions during training than the cP rats but these differences were not significant (Table 1).

### 5.3. Discussion

Significant flavor preferences were produced by IG fructose, corn oil, and ethanol relative to the IG water infusions. However, like sucrose, the isocaloric fructose

and corn oil infusions were more effective in conditioning flavor preferences than was ethanol. Nevertheless, the overall preferences for CS+F (73%) and CS+O (70%), relative to CS+E, were not as extreme as the preferences observed in Experiments 1 and 2 for CS+S over CS+E (93% and 84%). In particular, whereas all or nearly all of the rats preferred the CS+S to the CS+E in the first two experiments, only 16 of 24 rats preferred the CS+F or CS+O to the CS+E in the reinforced tests of the present experiment. This variability in relative preference for the CS+ solutions was related to the strength of the rats' CS+E preference: the ethanol preferring rats (cP subgroups) tended to show weaker preferences for the CS+F or CS+O over CS+E than did the ethanol nonpreferring rats (cNP subgroups). In Experiments 1 and 2, in contrast, the cP and cNP subgroups did not differ in their preference for CS+S over CS+E.

The 81% CS+F preference relative to CS- obtained in this experiment was unexpected: in prior studies with 16% fructose infusions, rats expressed no preference or only a weak preference unless they had been trained 20 h/day with food restriction [15,16]. A subsequent study revealed that the strong CS+F preference obtained here was due to the use of a dilute fructose concentration (7.18% vs. 16%) and saccharin-sweetened rather than unsweetened CS solutions [32]. Nevertheless, the available data indicate that fructose is less effective in conditioning flavor preferences compared to sucrose or glucose. This includes the present finding that the preference for CS+F over CS+E was not as strong as that obtained with CS+S.

As noted in the introduction, Mehiel and Bolles [4] previously reported comparable flavor preferences in a between-group, oral conditioning study using flavored ethanol and corn oil compared with flavored saccharin solutions. The present experiment differs in many respects from this earlier study (within- vs. between-group design, IG vs. oral procedure, nondeprived vs. deprived rats, rat strain), which may account for the conflicting results. Nevertheless, the IG data obtained here do not support the view that corn oil and ethanol are equally effective in conditioning of flavor preferences.

Overall, the preference results obtained in the reinforced and nonreinforced two-bottle tests were similar. Thus, the presence or absence of the IG nutrient/ethanol infusions did not influence the rats' relative intakes of the CS solutions. However, in the CS+E vs. CS- tests, the rats consumed more CS+E when it was paired with water infusions rather than ethanol infusions, indicating that concurrent ethanol infusions limited the intake of the CS+E solution. A similar pattern was observed in the reinforced and nonreinforced tests conducted with the CS+F vs. CS-, but not with the CS+O vs. CS-. Thus, the fructose infusions, but not the corn oil infusions, limited CS+ intakes during testing. Consistent with the two-bottle results, intakes of the CS+F and CS- did not differ during one-bottle training, whereas intakes of the CS+O exceeded CS- intakes during training. Therefore, corn oil infusion stimulated greater acceptance of its paired CS+ flavor, whereas fructose infusion did not. The one-bottle intakes of the CS+F and even more so of the CS+O exceeded those of the CS+E during training and it is possible that this contributed to the rats' preferences for the CS+F and CS+O over the CS+E. Arguing against this interpretation, the preferences for the CS+F (73%) and CS+O (70%), relative to the CS+E, were similar although the rats consumed 58% more CS+O, but only 24% more CS+F than CS+E in training.

## 6. General discussion

When CS+ flavors were paired with intragastric infusions of ethanol and sucrose, rats expressed preferences for each CS+ flavor over the water-paired CS- flavor, and consistently preferred the sucrose-paired flavor over the ethanol-paired flavor. This shows that both ethanol and sucrose are capable of reinforcing flavor preferences under the present conditions, and that sucrose is a more powerful reinforcer than ethanol at the isocaloric concentrations tested. This occurred even when the rats' tendency to self-administer more sucrose than ethanol during training was prevented in Experiment 2, ruling out an explanation based on differential exposure. Expanding the comparison to fructose and corn oil in Experiment 4 revealed similar, although less extreme, reinforcement disparities relative to ethanol. The basis for the reinforcement comparison is thus not energy concentration, per se, but some other feature(s) that differ

between ethanol and sucrose, fructose, or oil. This is consistent with other studies reporting differences in the flavor conditioning effects of different types of carbohydrates and fats [1].

In the first experiment, the rats were allowed to drink unlimited amounts of the CS solutions, and they rapidly increased their intakes of the CS+S, but not the CS+E or the CS-. This is compelling evidence that the animals distinguished among the postingestive consequences of various IG infusions. However, the selective increase provided an alternative explanation of the preference results, based on greater exposure to the CS+S than to the CS+E. The second experiment revealed that a simple exposure disparity was not a sufficient explanation: despite the elimination of differential intake of CS+ solutions during training, the magnitude of the CS+S preference over the CS+E was only slightly less than that observed in the first experiment (84% vs. 93%). Experiment 2 also revealed that further training experience with the CS+E only, while it improved the CS+E preference relative to the CS-, did not enhance the value of the CS+E compared to the CS+S: the rats still strongly preferred (82%) the CS+S to the CS-. These data indicate that the IG flavor conditioning effect of sucrose is more potent than that of isocaloric ethanol. Together, the data suggest a flavor conditioning ranking of sucrose > corn oil = fructose > ethanol. Note that this ranking is relative to ethanol only; whether rats trained with a CS+O and CS+F would show equal preferences for these two CS+ flavors in direct tests remains to be established.

The present data, combined with the earlier IG conditioning results of Sherman et al. [8], indicate that IG ethanol infusions can condition flavor preferences in rats, but the preference is weaker than that produced by isocaloric sucrose, fructose, glucose, or corn oil. This list is not exhaustive and other nutrient sources (e.g., protein) have yet to be compared with ethanol. Another approach is to compare ethanol reinforcement with that produced by hypocaloric nutrient sources. The finding that 1% sucrose conditioned a weaker flavor preference than did 5% ethanol in an oral conditioning study [3] suggests that an intermediate sucrose concentration would have a similar reinforcement effect to that of 5% ethanol when both are infused intragastrically. Consistent with this idea, we have previously reported that carbohydrate-conditioned flavor preferences increased in magnitude as the concentration of the infused nutrient (Polycose) increased from 1% to 4% [34]. The operant literature contains some direct comparisons of ethanol and sucrose reinforcement as assessed by lever-pressing. In an early study, Samson et al. [35] found that rats lever-pressed more for sucrose than 5% ethanol on a concurrent schedule when the sucrose concentration was as low as 1.25%. More recently, 3% sucrose responses did not differ from those for 10% ethanol [36], and these concentrations had equal reinforcer value in between-group comparisons of responding for sipper-tube access [37,38]. In a multiple schedule analysis with alternating access to ethanol and sucrose within



sessions, responding was equal for 2% sucrose and 10% ethanol. However, all these studies used ethanol-experienced rats that had been extensively trained to consume ethanol, so they may offer only initial guidance in choosing concentrations to test in ethanol-naive rats.

The CS+E preferences vs. CS– obtained in the present study (62–74%) were weaker than those obtained in a previous study in which rats were trained and tested with saccharin-sweetened CS+E and CS– solutions only. After five training sessions each with the CS+E and CS–, the rats in the earlier study showed an 80% CS+E preference, which increased to 89% after another 5 CS+E training sessions and to 92% during a nonreinforced two-bottle test with the CS+E vs. CS– [6]. The CS+E training intake (48.3 g/day) and infused ethanol dose (3.9 g/kg/day) in the earlier experiment were similar to those of the present study and thus these variables do not account for the different CS+E preferences obtained in the two studies. A difference in the schedule of ethanol presentation, every second day in the earlier experiment and every third day in present experiments, might contribute to the dissimilar CS+E preference results.

However, a difference between studies that is probably more important is the fact that the rats in the present study were concurrently trained with a second CS+ flavor paired with a more potent nutrient reinforcer, sucrose, fructose, or corn oil. These infusates generated preferences (over the water-paired CS– flavor) in most or all rats, consistent with many prior studies. The rats' experience with this second CS+ nutrient pair may have reduced their evaluation of the CS+E and infused ethanol. In particular, the presence of the second CS+ nutrient combination appears to have exposed individual differences in the susceptibility to ethanol's postingestive reinforcing effect. In our earlier study of CS+E vs. CS– conditioning, all the rats displayed a CS+E preference, whereas only half of the rats in the present study showed a CS+E preference exceeding 60%. The CS+E preferences of the cP subgroups (~85%) in Experiments 2 and 4 were comparable to the preference observed in our prior study [6]. Importantly, the CS+S, CS+F, and CS+O preferences of the cP and cNP subgroups were similar, so the dissimilar CS+E preferences displayed by these subgroups do not reflect a global difference in flavor-nutrient learning ability. Rather, it would appear that the postingestive reinforcing effect of ethanol is degraded more in some rats than others by experience with more potent nutrient reinforcers. This is consistent with marked differences in ethanol reinforcement and self-administration observed in outbred rat strains [25–27] and in rats selectively bred for ethanol preference and nonpreference (e.g., Refs. [9,29,39,40]). Additional measures of ethanol's reinforcing effect, such as oral intake and operant responding to obtain ethanol, would be needed to determine whether the subgroups observed here reflect a more general difference in responses to ethanol.

Pharmacological reinforcement may have contributed to the ethanol-conditioned preferences obtained here, although this seems unlikely because of the relatively low ethanol concentration used; note that in IG experiments the infused ethanol was diluted to 2.5% by the ingested CS+E solution. At higher ethanol concentrations, drug effects may contribute to or override nutritive effects so that different results may be obtained in choice tests with a CS+E flavor vs. a CS+nutrient flavor. Training outbred rats from the start with a CS+E paired with IG infusions of more concentrated ethanol infusions is likely to produce flavor aversions rather than preferences [8,41]. Nevertheless, outbred rats conditioned to prefer a CS+E by infusions of 5% ethanol continue to prefer the CS+E when it is subsequently paired with much higher ethanol concentrations [5,6]. Conceivably, after being accustomed to relatively high ethanol infusions, rats might shift their preference for the CS+E flavor relative to CS+ flavors paired with other nutrients. This would be most likely with nutrients like fructose, which have a reduced reinforcing effect at higher concentrations [32].

Another interesting line of research would be to compare flavor preference conditioning by IG infusions of ethanol and other nutrients in rat strains selected to prefer ethanol over water. Offering a palatable nutrient solution along with ethanol and water markedly reduces oral ethanol intake in some alcohol-drinking strains [42,43], although not in the genetically selected alcohol-preferring P strain [44]. The ethanol intakes of the corresponding nonpreferring (NP) strains were low and unchanged by the addition of the alternative, and both strains in each pair consumed large quantities of palatable chocolate drink. The preferring and nonpreferring strains might behave differently when offered the choice between identically sweetened CS solutions paired with IG infusions of ethanol vs. another nutrient. The previous work with IG presentation of ethanol in selected strains has only compared it to water infusions; in these studies, the expected strain difference was observed, with P but not NP rats acquiring a preference for an ethanol-paired flavor [9,45]. Because these experiments used procedures that may have been acceptable for P rats but quite unfavorable for acquisition by the NP rats (water deprivation and infusions of 20% ethanol), it would be useful to revisit the study of IG ethanol presentation using procedures that work well in outbred rats. It is possible, given some of the studies that have reduced or eliminated differences in P and NP ethanol intake [9,45,46], that the NP rats will not avoid an ethanol-paired flavor, and will thus resemble outbred animals that show a range of susceptibility to postingestive ethanol reinforcement.

The present study addressed the inconsistencies in prior reports comparing ethanol-conditioned flavor preferences with those produced by other nutrients [3,4,8]. The findings obtained here were quite consistent: 5% ethanol conditioned flavor preferences in male Sprague–Dawley rats that were weaker than those produced by isocaloric solutions of sucrose, fructose, and corn oil. The earlier conditioning

results, which were obtained in food-restricted rats, were taken as evidence that CS+E flavor preferences were reinforced by the postingestive nutritive properties of ethanol. The present findings are consistent with this interpretation even though nondeprived animals were tested and ethanol differed from the other nutrients in its preference conditioning potency. There is now abundant evidence that nutrients condition preferences in ad libitum fed animals and that flavor learning is not based on energy density per se [1,47]. However, the site(s) at which nutrients act to reinforce flavor preferences and the nature of the reinforcement signals they generate remain uncertain.

### Acknowledgements

This research was supported by a grant from the National Institute on Alcoholism and Alcohol Abuse (AA 11549) to K.A. and a National Institute of Mental Health Research Scientist Award (MH 00983) to A.S. Experiment 3 was an honors project submitted as part of a bachelor's degree (D.R.).

### References

- [1] Sclafani A. Macronutrient-conditioned flavor preferences. In: Berthoud H-R, Seeley RJ, editors. *Neural and metabolic control of macronutrient intake*. Boca Raton (FL): CRC Press; 1999. p. 93–106.
- [2] Cunningham CL, Niehus JS. Flavor preference conditioning by oral self-administration of ethanol. *Psychopharmacology* 1997;134:293–302.
- [3] Mehiel R, Bolles RC. Learned flavor preferences based on caloric outcome. *Anim Learn Behav* 1984;12:421–7.
- [4] Mehiel R, Bolles RC. Learned flavor preferences based on calories are independent of initial hedonic value. *Anim Learn Behav* 1988;16:383–7.
- [5] Ackroff K, Sclafani A. Flavor preferences conditioned by intragastric infusion of ethanol in rats. *Pharmacol Biochem Behav* 2001;68:327–38.
- [6] Ackroff K, Sclafani A. Flavor quality and ethanol concentration affect ethanol-conditioned flavor preferences. *Pharmacol Biochem Behav* 2002;74:229–40.
- [7] Deems DA, Oetting RL, Sherman JE, Garcia J. Hungry, but not thirsty, rats prefer flavors paired with ethanol. *Physiol Behav* 1986;36:141–4.
- [8] Sherman JE, Hickick CF, Rice AG, Rusiniak KW, Garcia J. Preferences and aversions for stimuli paired with ethanol in hungry rats. *Anim Learn Behav* 1983;11:101–6.
- [9] Waller MB, McBride WJ, Gatto GJ, Lumeng L, Li T-K. Intragastric self-infusion of ethanol by the ethanol-preferring and -nonpreferring lines of rats. *Science* 1984;225:78–80.
- [10] Fanselow MS, Birk J. Flavor-flavor associations induce hedonic shifts in taste preference. *Anim Learn Behav* 1982;10:223–8.
- [11] Holman EW. Immediate and delayed reinforcers for flavor preferences in rats. *Learn Motiv* 1975;6:91–100.
- [12] Ackroff K, Sclafani A. Sucrose to polycose preference shifts in rats: the role of taste, osmolality, and the fructose moiety. *Physiol Behav* 1991;49:1049–60.
- [13] Ackroff K, Sclafani A. Flavor preferences conditioned by sugars: rats learn to prefer glucose over fructose. *Physiol Behav* 1991;50:815–24.
- [14] Ackroff K, Sclafani A, Axen KV. Diabetic rats prefer glucose-paired flavors over fructose-paired flavors. *Appetite* 1997;28:73–83.
- [15] Ackroff K, Touzani K, Peets TK, Sclafani A. Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency. *Physiol Behav* 2001;72:691–703.
- [16] Sclafani A, Cardieri C, Tucker K, Blusk D, Ackroff K. Intragastric glucose, but not fructose conditions robust flavor preferences in rats. *Am J Physiol* 1993;265:R320–5.
- [17] Sclafani A, Ackroff K. Glucose- and fructose-conditioned flavor preferences in rats: taste versus postingestive conditioning. *Physiol Behav* 1994;56:399–405.
- [18] Azzara AV, Sclafani A. Flavor preferences conditioned by intragastric sugar infusions in rats: maltose is more reinforcing than sucrose. *Physiol Behav* 1998;64:535–41.
- [19] Lucas F, Sclafani A. Flavor preferences conditioned by intragastric fat infusions in rats. *Physiol Behav* 1989;46:403–12.
- [20] Lucas F, Sclafani A. Differential reinforcing and satiating effects of intragastric fat and carbohydrate infusions in rats. *Physiol Behav* 1999;66:381–8.
- [21] Sclafani A, Fanizza LJ, Azzara AV. Conditioned flavor avoidance, preference and indifference produced by intragastric infusion of galactose, glucose, and fructose in rats. *Physiol Behav* 1999;67:227–34.
- [22] Elizalde G, Sclafani A. Flavor preferences conditioned by intragastric polycose: a detailed analysis using an electronic esophagus preparation. *Physiol Behav* 1990;47:63–77.
- [23] Kirk RE. *Experimental Design: Procedures for the Behavioral Sciences*. Pacific Groves (CA): Brooks/Cole; 1995.
- [24] Ramirez I. Stimulation of fluid intake by carbohydrates: interaction between taste and calories. *Am J Physiol* 1994;266:R682–7.
- [25] Bell SM, Gosnell BA, Krahn DD, Meisch RA. Ethanol reinforcement and its relationship to saccharin preference in Wistar rats. *Alcohol* 1994;11:141–5.
- [26] Nadal R, Palleres M, Ferre N. Conditioned place preference for ethanol and individual differences in rats. *Pers Individ Differ* 1992;13:287–94.
- [27] Nadal R, Armario A, Janak PH. Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology* 2002;162:333–8.
- [28] Khanna JM, Kalant H, Shah G, Sharma H. Comparison of sensitivity and alcohol consumption in four outbred strains of rats. *Alcohol* 1990;7:429–34.
- [29] Gauvin DV, Moore KR, Holloway FA. Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or of the preparedness to learn? *Alcohol* 1993;10:37–43.
- [30] Warwick ZS, Weingarten HP. Dissociation of palatability and caloric effects in learned flavor preferences. *Physiol Behav* 1994;55:501–4.
- [31] Ramirez I. Stimulation of fluid intake by nutrients: oil is less effective than carbohydrate. *Am J Physiol* 1997;272:R289–93.
- [32] Ackroff K, Sclafani A. Fructose conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration. *Appetite*. In press. doi:10.1016/j.appet.2004.01.001
- [33] Smith BK, Volaufova J, West DB. Increased flavor preference and lick activity for sucrose and corn oil in SWR/J vs. AKR/J mice. *Am J Physiol* 2001;281:R596–606.
- [34] Ackroff K, Sclafani A. Flavor preferences conditioned by intragastric infusions of dilute polycose solutions. *Physiol Behav* 1994;55:957–62.
- [35] Samson HH, Roehrs TA, Tolliver GA. Ethanol reinforced responding in the rat: a concurrent analysis using sucrose as the alternate choice. *Pharmacol Biochem Behav* 1982;17:333–9.
- [36] Files FJ, Samson HH, Brice GT. Sucrose, ethanol, and sucrose/ethanol reinforced responding under variable-interval schedules of reinforcement. *Alcohol Clin Exp Res* 1995;19:1271–8.
- [37] Samson HH, Slawcecki CJ, Sharpe AL, Chappell A. Appetitive and consummatory behaviors in the control of ethanol consumption: a measure of ethanol seeking behavior. *Alcohol Clin Exp Res* 1998;22:1783–7.
- [38] Samson HH, Chappell A, Legg B. Effects of self-administered alcohol or sucrose preloads on subsequent consumption in the rat. *J Stud Alcohol* 2002;63:107–13.

- [39] Cannon DS, Leeka JK, Block AK. Ethanol self-administration patterns and taste aversion learning across inbred rat strains. *Pharmacol Biochem Behav* 1994;47:795–802.
- [40] Ritz MC, Garcia JM, Protz D, Rael A-M, George FR. Ethanol-reinforced behaviour in p, np, had and lad rats: Differential genetic regulation of reinforcement and motivation. *Behav Pharmacol* 1994; 5:521–31.
- [41] Ackroff K, Sclafani A. Ethanol flavor preference conditioned by intragastric carbohydrate in rats. *Pharmacol Biochem Behav* 2002; 74:41–51.
- [42] Lankford MF, Myers RD. Genetics of alcoholism: simultaneous presentation of a chocolate drink diminishes alcohol preference in high drinking HAD rats. *Pharmacol Biochem Behav* 1994;49:417–25.
- [43] Colombo G, Agabio R, Diaz G, Fa M, Lobina C, Reali R, et al. Sardinian alcohol-preferring rats prefer chocolate and sucrose over ethanol. *Alcohol* 1997;14:611–5.
- [44] Lankford MP, Roscoe AK, Pennington SN, Myers RD. Drinking of high concentrations of ethanol versus palatable fluids in alcohol-preferring (P) rats: valid animal model of alcoholism. *Alcohol* 1991; 8:293–9.
- [45] Murphy JM, Waller MB, Gatto GJ, McBride WJ, Lumeng L, Li T-K. Effects of fluoxetine on the intragastric self-administration of ethanol in the alcohol preferring P line of rats. *Alcohol* 1988;5:283–6.
- [46] Murphy JM, Gatto GJ, McBride WJ, Lumeng L, Li T-K. Operant responding for oral ethanol in the alcohol-preferring P and alcohol-nonpreferring NP lines of rats. *Alcohol* 1989;6:127–31.
- [47] Sclafani A. Oral and postoral determinants of food rewards. *Physiol Behav*. In press.