ELSEVIER

Pharmacology, Biochemistry and Behavior 74 (2002) 41–51

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Ethanol flavor preference conditioned by intragastric carbohydrate in rats

Karen Ackroff *, Anthony Sclafani

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

Received 7 March 2002; received in revised form 1 July 2002; accepted 10 July 2002

Abstract

The unpalatable flavor of ethanol solutions greater than ~6% may limit their consumption by rats. We determined if ethanol flavor avoidance, like bitter or sour taste avoidance, can be reversed by intragastric (IG) carbohydrate conditioning. Ad lib fed rats drank 5% ethanol and a matched flavor (0.05% citric acid + 0.5% maltodextrin, CM) on alternate days. For control rats, postingestive effects were equated: when they drank one solution they were infused IG with the other. Conditioned rats were also infused with 5% ethanol when they drank CM, but when they drank 5% ethanol they were infused with CM + 16% maltodextrin, a potent reward in flavor preference learning. In choice tests, only the conditioned rats preferred ethanol to CM; both groups preferred 5% ethanol to water. Conditioned rats but not controls preferred ethanol to water when the concentration was raised to 10%, and sustained their preference when the infusate carbohydrate was gradually removed. When ethanol concentration was gradually raised to 25%, ethanol preference declined from 48% to 30% in the control rats and from 84% to 50% in the conditioned rats. Thus, ethanol flavor avoidance can be reversed or reduced by postingestive nutritive conditioning, which may combine with the pharmacological effects of ethanol to produce the acquired appetite for the flavor of alcoholic beverages.

Keywords: Flavor conditioning; Ethanol; Gastric infusions; Maltodextrin

1. Introduction

There has been considerable discussion in the literature concerning the "unpalatable" flavor of ethanol and how this may be a limiting factor in ethanol consumption by animals. Detailed studies in rats indicate that the flavor of ethanol flavor is complex, and includes both sweet and bitter components as well as a strong olfactory element (Kiefer et al., 1986, 1988, 1990; Kiefer and Mahadevan, 1993; Kiefer and Lawrence, 1988). To naive, outbred rats, ethanol flavor appears to be hedonically neutral, relative to water, at low to moderate concentrations (0.5–6%) and aversive at concentrations above 6% (Kiefer et al., 1987; Richter and Campbell, 1940). At acceptable concentrations, intakes are usually too low to elevate blood ethanol to pharmacologically relevant levels. Because the goal of animal models of alcoholism is self-administration of significant doses of ethanol, many studies have sought to induce rats to drink ethanol at concentrations of 10% or higher.

One common procedure to promote the intake of higher concentrations involves starting with a sweet sucrose solution, adding a low concentration of ethanol, and then gradually increasing the ethanol concentration and reducing the sucrose concentration until the rats are drinking unsweetened ethanol (Samson, 1986; Tolliver et al., 1988). Several factors may contribute to the increased ethanol appetite produced by the sucrose fading procedure. The sweet taste of sucrose may stimulate rats to drink sufficient ethanol so that they experience its pharmacological reward properties, which they associate with ethanol flavor as the sucrose is faded out of the solution. In addition, the postingestive nutritive reward actions of ethanol as well as the sucrose may condition an increased preference for the flavor of ethanol through a flavor–nutrient conditioning process (Ackroff and Sclafani, 2001; Sclafani, 1999). Finally, sweet taste may enhance the "hedonic value" of the ethanol flavor via a flavor–flavor conditioning process. Studies with nonnutritive solutions demonstrate that mixing a sweet taste with a neutral or aversive flavor conditions an increased preference for the target flavor (Capaldi, 1996). The relative contributions of pharmacological reward, nutritive reward and sweet taste reward to the increased ethanol appetite

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

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00948-6

produced by the sucrose fading procedure are uncertain. The present study investigated the ability of postingestive nutritive reward to condition a preference for the flavor of ethanol.

Learned flavor preferences can be produced by pairing the intake of a cue flavor (the conditioned stimulus or CS+) with intragastric (IG) infusions with various carbohydrates, proteins or fats (Sclafani, 1999). We recently reported that IG ethanol infusions can also condition flavor preferences in nondeprived rats (Ackroff and Sclafani, 2001). The animals were given ad libitum access to food, water and a flavored noncaloric solution (the CS+) that was paired with a concurrent IG infusion of 6% ethanol. On other days, a different flavored solution (the CS−) was paired with IG water infusions. In subsequent two-bottle tests, the rats significantly preferred the CS+ to the CS− , and this preference was sustained as the ethanol concentration of the infusate was gradually increased to 24%. Several other reports of ethanol-conditioned flavor preferences have used food and/or water deprived rats (Cunningham and Niehus, 1997; Deems et al., 1986; Fedorchak and Bolles, 1987; Mehiel and Bolles, 1984; Sherman et al., 1983; Waller et al., 1984). Since our rats were not deprived during training, their learned preference for the ethanol-paired flavor was not based on restoration of energy or hydration deficits. This does not necessarily mean, however, that the flavor preference was reinforced by the pharmacological rather than the nutritional effects of ethanol. For example, IG carbohydrate infusions, even at what would appear to be calorically insignificant concentrations, can condition flavor preferences in nondeprived animals (Ackroff and Sclafani, 1994).

Carbohydrate conditioning is also notable because it can produce strong preferences for flavors that rats normally avoid, including unsweetened Kool-Aid flavors, sour citric acid, bitter sucrose octaacetate and pungent peppermint odor (Sclafani, 1999). Once conditioned, these flavor preferences are quite persistent even when no longer paired with carbohydrate infusions (Sclafani, 1999). In view of these results, we investigated if carbohydrate infusions can condition a preference for the flavor of ethanol, if this preference persists when the ethanol is no longer paired with the carbohydrate infusions and if this conditioned preference would promote the intake of ethanol concentrations that rats normally avoid.

In order to determine if the flavor of ethanol can be enhanced by associating it with postingestive carbohydrate reward, it is necessarily to control for the postingestive actions of the ethanol. This was accomplished by training rats with an ethanol solution and a second flavored solution that was paired with IG ethanol infusions. In pilot work, we developed a mixture of citric acid and maltodextrin (referred to as CM) that was matched in initial acceptability to 5% ethanol. The maltodextrin concentration was 0.5%, which has a negligible caloric value and does not condition a flavor preference when given IG (Ackroff and Sclafani, 1994). Although the taste of maltodextrin, even at 0.5%, is attractive to rats, it appears to be qualitatively different from the sweet taste of sucrose (Sclafani, 1987) and the inclusion of 0.05% citric acid reduced intake of the CM mixture to levels similar to that of the 5% ethanol. While the ethanol and the CM solutions had distinctive tastes (“bitter–sweet” vs. “sour–malty”), their postingestive consequences were equated by counterinfusions. That is, as control rats drank 5% ethanol they were infused with a matched volume of CM and, as they drank CM, they were infused with a matched volume of 5% ethanol. Experimental rats were treated similarly except that when they drank 5% ethanol they were infused with a CM mixture supplemented with 16% maltodextrin, a concentration that is very effective in conditioning flavor preferences (Sclafani, 1999). Following one-bottle training with these solutions and matched infusions, preferences for the 5% ethanol and CM solutions were assessed in two-bottle choice tests. The oral ethanol concentration was then increased to 10%, the 16% maltodextrin was faded out of the experimental rats’ CM infusions and the preference for ethanol vs. plain water was determined. Additional tests compared ethanol preference in the experimental and control rats as oral ethanol concentration increased to 25%. We predicted that the experimental rats trained with 16% maltodextrin infusions would show stronger and more persistent preferences for oral ethanol than would the control rats. This would demonstrate that postingestive nutrient reward can have lasting effects on the rats’ response to the flavor of ethanol.

2. Method

2.1. Subjects

Adult male Sprague–Dawley rats ($n = 23$) purchased from Charles River Laboratories (Wilmington, MA) were 12 weeks old and weighed 375–429 g at the start of the study. 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 water. The animal colony and experimental rooms were maintained on a 12:12 light–dark cycle (lights on 1000 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 (Elizalde and Sclafani, 1990). 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 infusion cages and circuitry used for IG infusion were similar to the "electronic esophagus" system previously described (Elizalde and Sclafani, 1990). In brief, the rats were housed in stainless steel hanging cages (24 × 18 × 18 cm) with powdered chow available from a food cup accessible through a hole in the back wall of the cage. Incursions into the cup were monitored by a photocell. Drinking fluids were available from stainless steel ball point 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 ~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 (1000–1200 h), chow and fluids were not available while the intakes were measured and the infusion system serviced.

2.4. Solutions

The oral training fluids (CSs) were a 5% ethanol solution and a mixture (CM) of 0.05% w/w citric acid (Fisher Scientific, Springfield, NJ) and 0.5% w/w maltodextrin (soluble hydrolyzed starch: Maltrin M580, Grain Processing, Muscatine, IA). Left/right positions of the bottles were counterbalanced across days. The infusates were water, ethanol solutions, CM and CM + additional maltodextrin (16%, 8% and 4% w/w). The ethanol solutions, which ranged from 5% to 25% during testing, were prepared v/v by mixing 95% ethanol and water. The energy density of the 5% ethanol training solution was 0.287 kcal/g. The CM solution contained 0.019 kcal/g; addition of 16% maltodextrin increased solution energy density to 0.627 kcal/g. The amounts of fluid consumed and infused were recorded to the nearest 0.1 g.

2.5. Procedure

After a postsurgery recovery period (5–9 days), the rats were given a two-bottle preference test with 5% ethanol vs. water in their home cages for 2 days. Then the rats were transferred to the infusion cages where they lived for the remainder of the experiment. They were adapted to the cages for 6 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 4 days. Two groups were formed, experimental (Exp, $n = 12$) and control (Con, $n = 11$), matched for pretraining ethanol intake and preference, food and fluid intake and body weight.

Flavor training involved alternate-day access for 10 days to 5% ethanol and CM. On even-numbered days, the rats were given ethanol to drink; the Exp group was coinfused with CM + 16% maltodextrin and the Con group with CM (without any added maltodextrin). On odd-numbered days, both groups drank CM and were infused with 5% ethanol. Following training, the rats were given a series of two-bottle preference tests. For 4 days, they were offered 5% ethanol vs. CM, for 2 days water vs. CM and for 2 days 5% ethanol vs. water. Throughout these tests, intakes of the training solutions were still paired with their respective IG infusions and intake of water was paired with IG water infusions. The left–right alternation of solution positions continued in all preference tests, using a minimum of 2 days to ensure that the preference is for the solution rather than its position on the cage.

Two-bottle testing of ethanol vs. water continued. First, the ethanol concentration was increased to 10% for 5 days, still paired with CM + 16% maltodextrin for the Exp group and CM only for the Con group. Then the concentration of the added maltodextrin in the Exp group's infusate was reduced to 8% (2 days), then 4% (2 days) and then 0% (4 days). The Exp and Con groups were next given a series of ethanol vs. water tests, with the oral ethanol concentration increasing by 2.5% every 2 days, to a maximum of 25%. Intake of ethanol remained paired with CM infusions and water with water infusions. The experimental conditions are summarized in Table 1. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Brooklyn College.

2.6. Data analysis

The intake data were averaged over the 5-day periods of training and over 2 or 4 days of preference tests. Unless specified, all data represent oral intakes of ethanol solution, CM solution or water; IG infusions closely matched oral intakes. Drinking patterns were analyzed with a bout defined as a period of drinking containing at least 30 licks and interlick intervals no longer than 5 min. In analyses of ethanol bout patterns on two-bottle days, animals that drank little or no ethanol were not included. Ethanol intakes per day and per bout were calculated as grams of pure ethanol contained in the oral solutions or infusates. To obtain an estimate of the average ethanol per bout of CM intake, the ratio of infused ethanol solution to oral intake was used as a correction factor to account for small variations from the targeted 1:1 ratio. Body weights were obtained periodically and used to calculate ethanol doses in grams per kilogram. The data were entered in repeated measures analyses of variance, except for single variable comparisons using *t*-tests. For significant main effects, tests of differences between specific means or groups of means were performed with Newman–Keuls tests. For descriptive purposes, an

Table 1
Summary of experimental conditions

Oral fluid	IG fluid	Number of days
One-bottle training (alternate days):		10
0.05% citric acid + 0.5% maltodextrin (CM)	5% ethanol (all rats)	
5% ethanol	Con: CM, Exp: CM + 16% maltodextrin (MD)	
Two-bottle test: 5% ethanol vs. CM	same pairing as training	2
Two-bottle tests vs. water (paired with IG water):		
CM	5% ethanol	2
5% ethanol	Con: CM, Exp: CM + 16% MD	2
10% ethanol	Con: CM, Exp: CM + 16% MD	5
10% ethanol	Con: CM, Exp: CM + 8% MD	2
10% ethanol	Con: CM, Exp: CM + 4% MD	2
10% ethanol	CM	4
12.5% ethanol	CM	2
15% ethanol	CM	2
17.5% ethanol	CM	2
20% ethanol	CM	2
22.5% ethanol	CM	2
25% ethanol	CM	2

individual animal was said to have a preference if its intake of a particular fluid was 60% or more of its total two-bottle intake. Statistical comparisons of two-bottle preference scores (CS+ intake/total intake × 100) were conducted on inverse sine transformed percentage scores (Kirk, 1995). A probability level of .05 was used in all tests.

3. Results

3.1. Overview

During training, the two groups drank similar amounts of 5% ethanol but the Exp rats drank less CM than did the Con rats. In the choice tests, the Exp group strongly preferred 5% ethanol to CM and to water, but preferred water to CM. The Exp rats continued to prefer ethanol to water when the concentration was raised to 10%, and this preference persisted as 16% maltodextrin was faded out of the paired IG infusion. The Con group, in contrast, drank 5% ethanol and CM equally and preferred both to water, but did not prefer 10% ethanol to water. As ethanol concentration increased to 25%, the Exp group gradually lost their ethanol preference while the Con rats displayed an ethanol avoidance.

3.2. Baseline intakes

In the two-bottle preference test prior to training, the Exp and Con groups drank similar amounts of 5% ethanol (18 and 20.9 g/day) and water (22.7 and 25.8 g/day). Ethanol intake averaged 44% of total intake for the Exp group and 45% for the Con group. Four rats in each group drank 60% or more of total intake as ethanol solution.

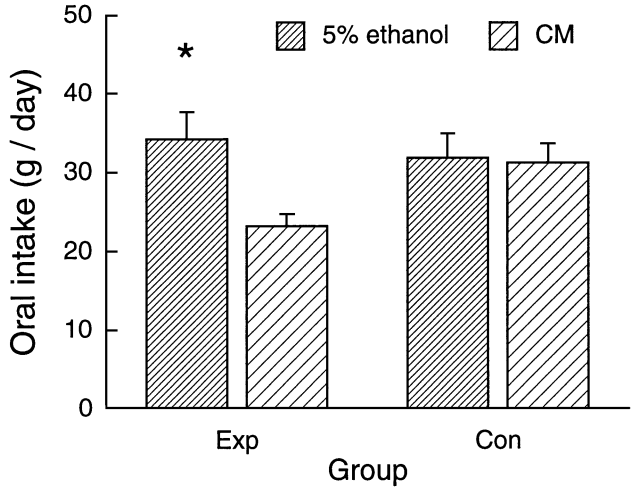

Fig. 1. Mean + S.E.M. daily oral intakes of 5% ethanol and CM solutions by Exp and Con groups during one-bottle training. Intake of 5% ethanol was paired with matched intragastric infusions of CM + 16% maltodextrin for the Exp group and CM only for the Con group. Intake of CM was paired with matched infusions of 5% ethanol for both groups. Asterisk denotes a significant difference between ethanol and CM intakes ($P < .001$).

3.3. Training intakes

The mean intakes during one-bottle training are shown in Fig. 1. There was an interaction of group and fluid type [$F(1,21) = 7.09$, $P < .02$]. Intakes of 5% ethanol were similar in the two groups and intake of CM and ethanol did not

Table 2
Mean (+S.E.M.) ethanol doses, energy intakes (kcal) and bout patterns on training days

	Exp group		Con group	
	Oral CM	Oral ethanol	Oral CM	Oral ethanol
Ethanol dose (g/kg/day)	$2.09^{a,*}$ (0.14)	3.17^{b} (0.30)	2.93^{b} (0.22)	2.96^{b} (0.29)
Energy from				
Ethanol	6.5^{a} (0.5)	9.8^{b} (1.0)	8.8^{b} (0.7)	9.1^{b} (0.9)
Maltodextrin	0.4^{a} (0.0)	20.2^{b} (0.3)	0.6^{a} (0.0)	0.6^{a} (0.1)
Chow	78.2^{a} (3.2)	74.5^{b} (3.1)	81.8^{a} (2.1)	83.8^{a} (1.8)
Total	85.1^{a} (3.2)	104.5^{b} (2.5)	91.2^{a} (2.5)	93.5^{a} (2.2)
Bout patterns				
Bout number	11.8^{a} (0.8)	13.3^{a} (1.2)	13.5^{a} (1.3)	13.5^{a} (1.7)
Bout size (g)	2.09^{a} (0.15)	2.59^{b} (0.19)	2.44^{a} (0.15)	2.64^{b} (0.19)
Bout dose (g/kg)	0.20^{a} (0.02)	0.24^{b} (0.02)	0.22^{a} (0.01)	0.24^{b} (0.02)
Bout energy (kcal)	0.64^{a} (0.05)	2.31^{b} (0.16)	0.73^{a} (0.03)	0.80^{a} (0.06)
Bout duration (min)	2.9^{a} (0.1)	2.8^{a} (0.2)	3.0^{a} (0.1)	3.1^{a} (0.2)

* Within rows, values with the same superscript do not differ significantly.

differ in the Con group. The Exp group, in contrast, drank more ethanol than CM [$F(1,21)=16.43$, $P<.001$] and less CM than the Con group [$F(1,33)=4.20$, $P<.05$]. To determine how the groups' intakes of CM diverged, separate analyses of the day by day training intakes were performed. The CM analysis revealed an interaction of group and day [$F(4,84)=2.55$, $P<.05$]; simple main effects showed that intake increased over days in the Con group only, and that the groups did not differ in CM intake on the first 3 days.

The average ethanol doses on oral CM and ethanol days (Table 2) paralleled the oral intakes, with an interaction of fluid and group [$F(1,21)=8.10$, $P<.01$]. The Con group self-administered similar ethanol doses on CM and ethanol days. The Exp group's ethanol dose was greater on oral ethanol than on CM days [$F(1,21)=17.94$, $P<.001$] and its dose on CM days was less than that of the Con group [$F(1,35)=5.93$, $P<.05$]. The groups did not differ on ethanol days, or in average daily dose across the training period (Exp: 2.63 g/kg/day and Con: 2.95 g/kg/day).

Energy intakes from oral and infused solutions and from chow are shown in Table 2. Ethanol energy paralleled the daily dose measure and maltrin contributions were minimal except on the Exp group's ethanol days. Analysis of chow intake showed differential effects of group and fluid [interaction $F(1,21)=7.85$, $P<.05$ and simple main effects], with the Exp group eating less chow than the Con group on oral ethanol days and eating less chow on oral ethanol days than CM days. Total energy intakes also showed an interaction of group and fluid type [$F(1,21)=29.36$, $P<.001$]. Total energy was very similar on oral ethanol and CM days for the Con group, but the Exp group's total energy was greater on oral ethanol days than CM days [$F(1,21)=78.57$, $P<.001$] and higher that of the Con group on oral ethanol days [$F(1,29)=8.60$, $P<.01$]. However, average group energy intakes did not differ (the group main effect was not significant).

Bout patterns were compared for oral CM and oral ethanol days (Table 2). Main effects of fluid type were apparent in several measures. The rats drank the ethanol and CM in similar numbers of bouts, but ethanol bouts were larger than CM bouts as measured by weight [2.6 vs. 2.3 g, $F(1,21)=11.80$, $P<.01$], dose [0.24 vs. 0.21 g/kg ethanol, $F(1,21)=15.19$, $P<.001$] and energy [1.59 vs. 0.68 kcal, $F(1,21)=143.10$, $P<.0001$]. Bout durations did not differ significantly and averaged 2–3 min. With the exception of energy/bout, there were no significant group differences in the bout patterns. A Group × Fluid interaction for energy per bout [$F(1,21)=118.30$, $P<.0001$ and simple main

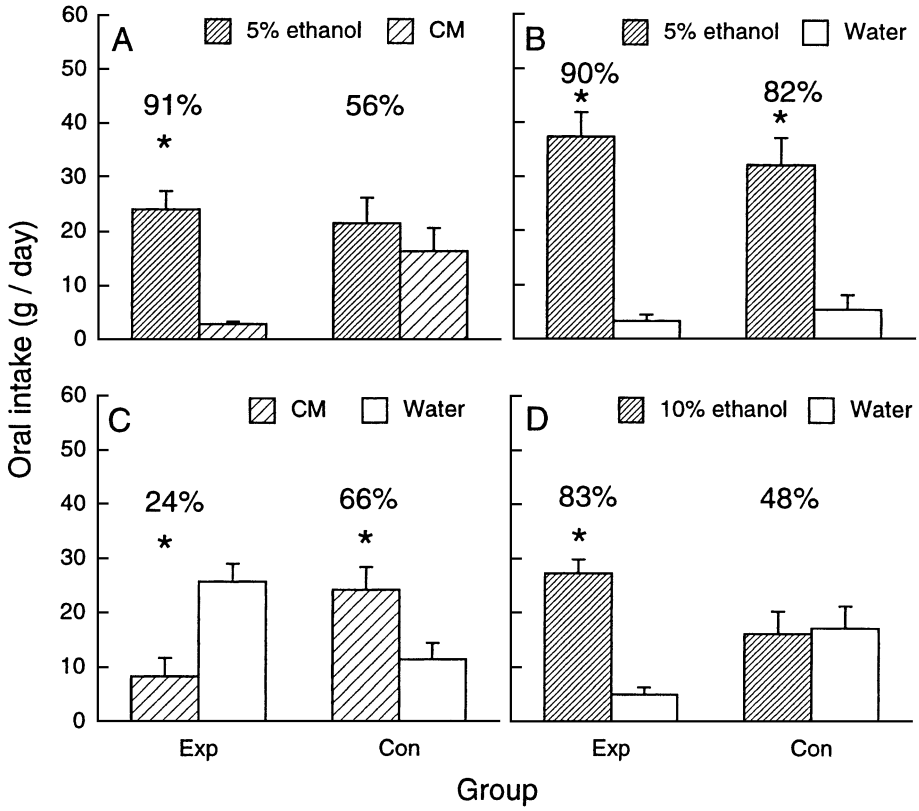

Fig. 2. Mean + S.E.M. daily intakes of ethanol and CM solutions and water by Exp and Con groups during two-bottle preference tests. (A) 5% ethanol vs. CM; (B) 5% ethanol vs. water; (C) CM vs. water; (D) 10% ethanol vs. water. Intake of 5% ethanol was paired with matched intragastric infusions of CM + 16% maltodextrin for the Exp group and CM only for the Con group. For both groups, intake of CM and water were paired with matched infusions 5% ethanol and water, respectively. The numbers atop the bars represent the mean of the individual rats' percentage of total intake consumed as ethanol (A, B, D) or CM (C). Asterisks denote a significant within-group difference between intakes of pairs of fluids ($P<.001$).

effects] reflected the higher energy content of the Exp group's ethanol-paired maltodextrin.

3.4. Initial preference tests

The initial two-bottle preference tests are shown in Fig. 2. In the ethanol vs. CM test (Fig. 2A), the Exp group's 91% preference for ethanol over CM was greater than the 56% ethanol intake of the Con group, $t(21)=3.68$, $P<.01$. This was apparent in the analysis of intakes as an interaction of group and fluid type [$F(1,21)=8.67$, $P<.01$]. The Exp group drank more ethanol than CM [$F(1,21)=25.96$, $P<.001$], whereas the Con group drank similar amounts of the two fluids. The groups differed in both ethanol [$F(1,30)=6.45$, $P<.02$] and CM intake [$F(1,30)=7.52$, $P=.01$]. All 12 Exp rats preferred ethanol by at least 60%, compared to 5 of 11 Con rats.

Analyses of each group's fluid vs. water intakes showed that the Exp group preferred 5% ethanol to water and water to CM, whereas the Con group preferred both fluids to water (Fig. 2B and C). For the Exp group, an interaction of test and fluid type [$F(1,11=80.92$, $P<.0001$ and simple main effects] confirmed that they drank more ethanol than water and less CM than water, and that ethanol intake exceeded CM intake in these tests. For the Con group, intake of the 5% ethanol and CM did not differ and exceeded that of water [$F(1,10)=17.44$, $P<.01$]. Eleven of 12 Exp rats and 9 of 11 Con rats preferred 5% ethanol to water; the groups did not differ in percent ethanol preference (90% vs. 82%). Percent preference for CM differed in the Exp and Con groups (24 vs. 66%, $t(21)=3.75$, $P<.01$); only 1 Exp rat preferred CM, compared to 7 of 11 Con rats.

The groups differed in ethanol preference when its concentration was increased to 10% (Fig. 2D): the Exp group continued to prefer ethanol to water, whereas the Con group drank similar amounts of ethanol and water [interaction $F(1,21)=7.79$, $P<.02$ and simple main effects]. Compared to the Con group, the Exp group drank more 10% ethanol [$F(1,26)=6.56$, $P<.02$] and less water [$F(1,26)=7.62$, $P<.02$]. Eleven of 12 Exp rats and 4 of 11 Con rats preferred 10% ethanol. Bout pattern analysis showed that the changes in intake from 5% to 10% were due to a reduction in bout numbers [from 15.6 to 10.9 bouts/day, $t(11)=7.06$, $P<.0001$] with only a small and nonsignificant reduction in sizes (from 2.52 to 2.38 g/bout). The smaller bouts did not compensate for the higher ethanol concentration, so that g/kg/bout rose from 0.22 to 0.42, $t(11)=14.24$, $P<.0001$).

Analysis of the self-administered daily ethanol doses revealed effects of concentration [5% less than 10%, $F(1,21)=9.72$, $P<.01$] and interaction of group and concentration [$F(1,21)=8.69$, $P<.01$]. The Exp group's dose increased from 3.17 g/kg/day at 5% ethanol to 4.67 g/kg/day at 10% ethanol [$F(1,21)=19.23$, $P<.01$], while the Con group's dose remained constant (2.64 vs. 2.68 g/kg/day). The groups' doses did not differ significantly in the 5% vs. water tests, but the Exp dose was greater than the Con dose at 10% [$F(1,27)=8.93$, $P<.01$]. Body weights of the groups during this period did not differ significantly (Exp: 483.9 g and Con: 494.1 g).

As the 16% maltodextrin was gradually removed from the Exp group's ethanol-paired infusion, they maintained their intake of 10% ethanol; the similar intakes of ethanol and water by the Con group were sustained through this period. The Exp group's ethanol intakes exceeded those of the Con group [27.6 vs. 15.2 g/day, $F(1,21)=6.50$, $P<.02$]; intakes did not change as a function of maltodextrin concentration. The differential ethanol dose thus was maintained, with the Exp group self-administering 4.60 g/kg/day and the Con group 2.45 g/kg/day at 0% added maltodextrin [$t(21)=2.70$, $P<.02$]. Total energy intakes did not differ as a function of maltodextrin concentration or group during this period, averaging 95 kcal/day. There were no major

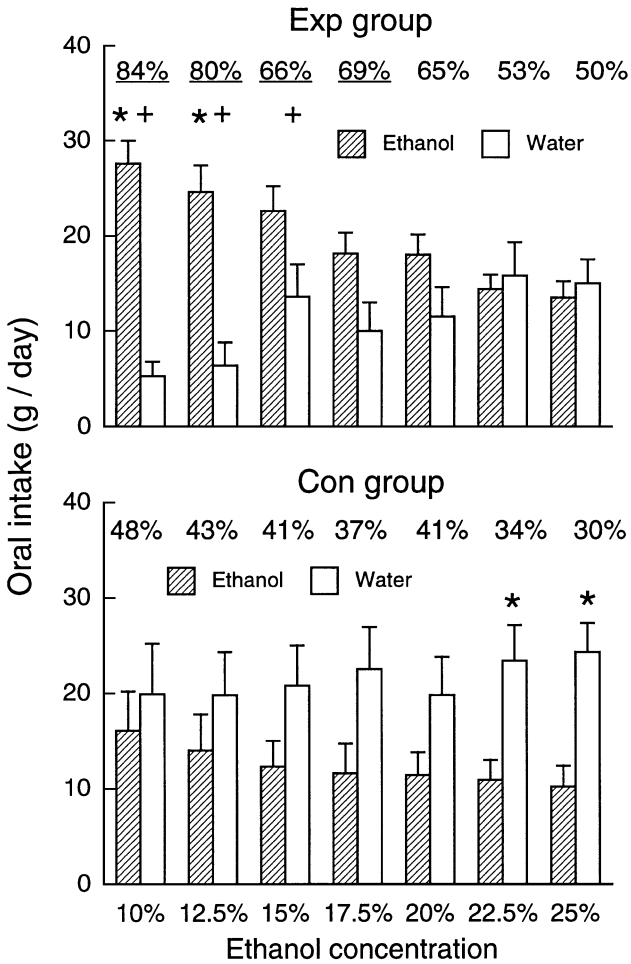

Fig. 3. Mean + S.E.M. daily intakes by Exp and Con groups during two-bottle preference tests as ethanol concentration increased from 10% to 25%. The numbers atop the bars represent the mean of the individual rats' percentage of total intakes consumed as ethanol. Asterisks denote a significant within-group difference between intakes of pairs of fluids ($P<.01$). Underlined percentages denote significant difference in preference between groups. Plus signs denote greater ethanol intake by the Exp group than the Con group.

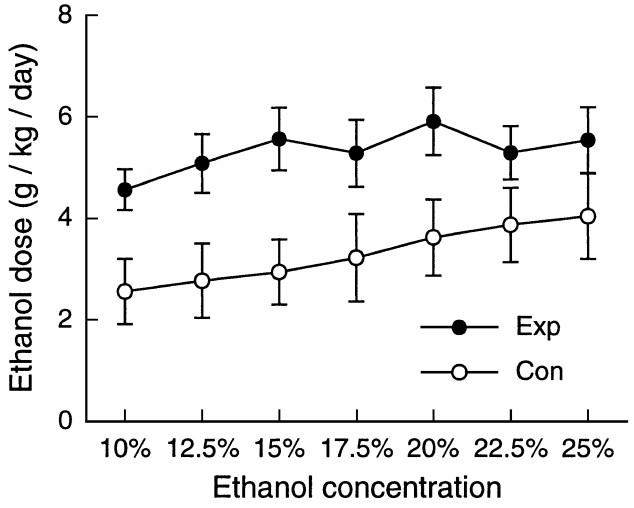

Fig. 4. Mean ± S.E.M. daily ethanol doses (g/kg) of Exp and Con groups as ethanol concentration increased from 10% to 25%.

shifts in bout sizes or numbers during this period, so that the Exp group's energy/bout gradually dropped; it no longer significantly exceeded that of the Con group by the end of maltodextrin fading.

3.5. Concentration series

Intakes of the ethanol solutions declined in both groups as ethanol concentration increased from 10% to 25% (Fig. 3). An interaction of group and concentration [$F(6,126) = 3.40$, $P < .01$] and simple main effects showed that the Exp group's ethanol solution intake was greater ($P < .01$) than that of the Con group at 10%, 12.5% and 15%, and that ethanol solution intake fell significantly for both groups [Exp $F(6,126) = 17.91$, $P < .001$; Con $F(6,126) = 2.61$, $P < .05$]. The Exp group's preference (percentage of fluid consumed as ethanol solution) decreased gradually as the concentration increased, remaining greater than that of the Con group ($P < .05$) from 10% to 17.5% ethanol concentration. Within group comparisons revealed that the Exp group drank more ($P < .05$) ethanol than water at the 10% and 12.5% concentrations and intakes did not differ at higher concentrations. The Con group, in contrast, drank similar amounts of ethanol and water at the 10% concentration and consumed significantly less ($P < .05$) ethanol than water at the 22.5% and 25% concentrations. The numbers of rats preferring ethanol over water by at least 60% decreased as ethanol concentration increased. Citing the low, intermediate and high concentrations, 11 of 12 Exp rats preferred ethanol at 10%, 8 at 17.5% and 3 at 25%. The comparable numbers for the 11 Con rats were 3, 2 and 1. Despite reductions in solution intake, the rats' absolute intake of ethanol increased with concentration [$F(6,126) = 4.36$, $P < .001$; Fig. 4]. Pairwise comparisons showed that the dose at 10% differed from those at 20% and higher concentrations. The average daily ethanol dose of the Exp group exceeded that of the Con rats [5.3 vs. 3.3 g/kg/day, $F(1,21) = 5.61$, $P < .05$]. The interaction of group and concentration was not significant.

The bout patterns of the Exp group are shown in Fig. 5A; the data represent 11 rats, because one rat drank less than 5 g/day of ethanol at concentrations above 15%. (The patterns for the ethanol drinkers in the Con group look similar, but analysis is prevented by the reduced and variable number of rats that drank sufficient ethanol to reveal meaningful patterns.) The Exp group's reductions in solution intake as concentration increased were accomplished largely by reducing the number of bouts per day [$F(6,60) = 28.09$, $P < .0001$]. Although there was an overall effect on bout size [$F(6,60) = 3.05$, $P < .05$], there was no clear trend, with bouts ranging from 2.58 to 3.45 g. This relative constancy yielded increasing ethanol doses per bout [Fig. 5B; $F(6,60) = 17.95$, $P < .0001$]. Pairwise comparisons showed that both bout number and dose per bout clustered into three subsets of concentrations: 10% and 12.5% differed from the middle three concentrations, which in turn differed from 22.5% to 25% ethanol. Average bout durations remained at 2–3 min.

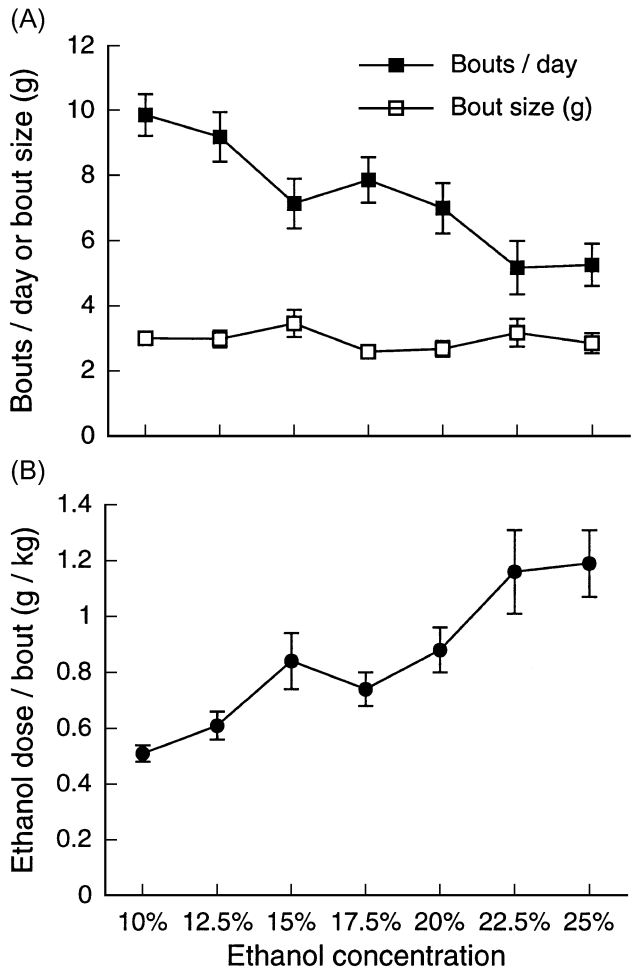

Fig. 5. Mean ± S.E.M. daily bout patterns (A: number and size; B: g/kg/bout) of Exp group ethanol intake as ethanol concentration increased from 10% to 25%.

4. Discussion

This experiment demonstrated in nondeprived rats that IG carbohydrate infusions conditioned a strong preference for a 5% ethanol solution over an alternate solution (CM) paired with IG infusions of 5% ethanol. Control rats, in which the 5% ethanol and CM had identical postingestive consequences, drank similar amounts of the two solutions in training and testing. While both groups preferred 5% ethanol to water, only the experimental rats preferred 10% ethanol to water. The experimental rats continued to prefer 10% ethanol as the 16% maltodextrin was faded out of the infusate, which demonstrates a true shift in preference, rather than ethanol intake as an instrumental response to obtain carbohydrate. The experimental rats also showed a stronger ethanol preference relative to control rats as concentration increased to 17.5% and, unlike controls, did not avoid ethanol at the highest concentration tested (25%). These findings confirm the prediction that nutritive reward can have lasting effects on the rat's response to ethanol.

The significant ethanol preference produced by the IG infusions of 16% maltodextrin is consistent with prior findings showing that such infusions condition preferences for a variety of other flavors, tastes and odors (Sclafani, 1999). In addition to have a direct reward effect, the 16% maltodextrin infusions may have also affected the postingestive reward value of the orally consumed 5% ethanol. That is, the presence of 16% maltodextrin in the Exp group's training infusions may have altered the disposition of the ingested ethanol, perhaps by altered gastric emptying. Thus, the postingestive reward actions of ethanol on ethanol training days and CM training days may have differed for the experimental rats. There is little published data that directly addresses this issue. Several studies have compared blood ethanol values following ethanol administration with and without accompanying sucrose, but results are not consistent: some find large effects of sucrose (Matthews et al., 2001; Roberts et al., 1999) and others find no effect (Czachowski et al., 1999; Gauvin, 1999). No studies have used concentrations of carbohydrate, which would permit comparisons with 16% maltodextrin and, in any case, information derived from sucrose would not necessarily apply to maltodextrin, which as a glucose polymer is digested and absorbed differently from sucrose, a glucose–fructose disaccharide. The impact of the added maltodextrin on ethanol disposition and reward remains an open question.

The control rats' lack of preference for ethanol vs. CM is consistent with preliminary data showing that the two solutions are matched in flavor palatability. If the control rats had not been counterinfused with ethanol as they drank CM, then they might have developed a preference for oral ethanol over CM because only ethanol flavor would be paired with ethanol's postingestive nutritive and pharmacological effects. Their preference for CM over water may, in fact, represent a conditioned preference produced by the ethanol infusions, although this requires verification, i.e., CM vs. water preference data are needed for rats infused with water rather than ethanol throughout training and testing. However, their increased intake of CM during the one-bottle training period is consistent with the idea of enhanced attraction to CM. The significant posttraining preference the control rats displayed for 5% ethanol over water may also represent a conditioned response, given that the rats did not prefer ethanol to water during the pretraining baseline period. Interpretation of the control rats' ethanol preference data is complicated, however, by the fact that in the posttraining test the rats were infused with CM as they consumed the 5% ethanol, so the net ethanol concentration in their stomach was 2.5%. The degree to which preference is controlled by the flavor vs. the postingestive actions of ethanol at a concentration range of 2.5–10% ethanol requires further investigation.

In marked contrast to the control group's CM preference, the experimental rats avoided the CM when water was available. Since CM was paired with IG ethanol for both groups, their opposite preference response to the CM, relative to water, can be attributed to the different postingestive effects of the alternate fluid. (An alternate explanation, that the groups differed in their initial acceptance of CM, is unlikely given the similar CM intakes of the groups at the beginning of training.) For the experimental animals, drinking ethanol provided both maltodextrin and ethanol postingestive sources of reward, but drinking CM provided only ethanol reward. Prior studies in which CS+ and CS− flavors were paired with IG carbohydrate and water, respectively, have reported both increases and decreases in CS− preference, relative to water (Elizalde and Sclafani, 1990; Pérez et al., 1998). The outcome appears to depend upon the distinctiveness of the flavor sets used; with more distinct flavors (citric acid and sucrose octaacetate), the CS− preference declined, but with the more similar Kool-Aid flavors, the CS− preference increased, perhaps due to generalization. The important point here is that the conditioning of a strong flavor CS+ preference does not depend upon concurrent conditioning of a CS− flavor avoidance (Myers and Sclafani, 2001a). The conditioned rats' evaluation of CM may have been reduced because of contrast with the stronger positive effects of ethanol.

The conditioned rats' energy intake was greater on oral ethanol than CM days during training. The infused maltodextrin provided about 20 kcal/day and, although the rats responded with some reduction of chow intake on oral ethanol days, their total intakes were greater than on CM days and greater than the intakes of the controls. While the additional energy may appear to be the source of the reward that enhanced subsequent ethanol intake, it should be noted that flavor preference conditioning can occur with lower concentrations of carbohydrate in the infusion, sometimes with very minor contributions to total energy intake (Ackroff and Sclafani, 1994). It is possible, therefore, that maltodextrin concentrations lower than 16% would also

condition an ethanol flavor preference; however, note that the 0.5% maltodextrin in CM is below the threshold concentration. Our working hypothesis is that nutrient detection is integrated with flavor information, and that it is not energy per se that is detected because isocaloric nutrients differ dramatically in their reward potency (e.g., Lucas and Sclafani, 1999; Sclafani et al., 1999).

Unlike the controls, the experimental rats drank more ethanol than water as the concentration increased from 5% to 10% and then to 12.5%, and did not avoid the higher concentrations tested (15–25%). This is consistent with prior results showing that IG carbohydrate infusions can condition preferences even for flavors that rats do not initially prefer (e.g., citric acid, sucrose octaacetate, unsweetened Kool-Aid flavors) and the preferences persist after the carbohydrate infusions have stopped (Sclafani, 1999). In the present study, the experimental rats showed no change in 10% ethanol preference, as the 16% maltodextrin was faded out of the IG infusions. The experimental rats' graded reduction in ethanol preference as concentration increased from 10% to 25% presumably represented a response to both the increasing flavor intensity of the ethanol and its increasing postingestive effects.

A role for both flavor and postingestive factors in the declining ethanol preference is suggested by our prior findings obtained with rats tested with increasing IG rather than oral ethanol concentrations (Ackroff and Sclafani, 2001). The rats in the earlier study were first trained to prefer a non-nutritive flavored solution (CS+; grape or cherry) that was paired with IG infusions of 6% ethanol infusions; the alternate CS− flavor was paired with IG water infusions. They were then given CS+ vs. CS− choice tests with CS flavor intensity held constant as the IG infused ethanol was made more concentrated. As concentration increased from 6% to 12%, 18% and 24%, the magnitude of the CS+ preference declined from 80% to 76%, 71% and 64%. Although intake of the ethanol-paired flavor declined across these tests, it remained greater than that of the water-paired flavor even with the 24% ethanol infusion. This suggests that untasted ethanol may support continued flavor preference at higher concentrations than oral ethanol. However, there was little difference in total ethanol intake at high concentrations in the oral and IG experiments; self-administered doses of IG ethanol rose from less than 2 g/kg/day at 6% to 5 g/kg/day at the 24% concentration (Ackroff and Sclafani, 2001), which is quite similar to the experimental group's doses in the present study. Alcohol-preferring P rats trained with flavors paired with IG ethanol infusion also showed continued preference for the ethanol-paired flavor as the concentration of the infusion was raised from 10% to 20%, 30% and 40%, with a linear increase from 3 to 9 g/kg/day (Waller et al., 1984).

Intake bout patterns recorded in this experiment reflected total fluid intakes. Although the groups did not differ significantly during one-bottle training, the controls tended to drink CM and ethanol in similarly sized bouts, consistent with a lack of difference in palatability and postingestive effect. The experimental rats treated the fluids differently, drinking 25% larger bouts of ethanol than CM despite the fact that the ethanol + IG 16% maltodextrin had more than twice the caloric density of the CM + IG ethanol. The experimental animals appeared relatively resistant to changing their ethanol bout sizes. When oral ethanol concentration doubled from 5% to 10% they reduced their volume intake by reducing their ethanol bouts per day; bout size did not change. When ethanol concentration then increased from 10% to 25%, the experimental rats again reduced their volume intake largely by decreasing bouts per day. A consequence of the relative constancy in bout size is that the rats self-administered larger doses per bout at higher concentrations, and they continued to consume those doses over a short time period (2–3 min). At the highest ethanol concentrations (22.5–25%), the self-administered dose/bout (1.2 g/kg) was four times the rat's hourly rate of ethanol metabolism (Wallgren and Barry, 1970).

The tendency to maintain a constant bout size in spite of changes in the concentration of the orally consumed ethanol solution differs from the response to IG ethanol. The rats in our previous experiment, which drank a CS+ flavor paired with IG ethanol, responded to increasing ethanol concentration by decreasing bout sizes to a greater extent than bout numbers (Ackroff and Sclafani, 2001). Nevertheless, the smaller oral bouts did not prevent an increase in dose per bout, from 0.25 g/kg at the 6% concentration to 0.81 g/kg at 24%. In that study, the oral fluids were Kool-Aid solutions with unchanging concentration, so the animals had no oral feedback to indicate the increasing ethanol concentration. In contrast, the present rats had a flavor intensity cue to assist them. Reductions in bout size are usually regarded as reductions in palatability and/or increases in satiation in the food intake literature. With ethanol, reductions in bout size could also serve to avoid toxic effects. Thus, it is surprising that the experimental rats maintained a fairly constant bout size ($\sim$2.5–3 g/bout) as oral ethanol concentration increased from 10% to 25%. In contrast, when outbred rats drinking ethanol in an operant situation were shifted from 10% to 20% ethanol, bout sizes decreased by 33% while bout number did not change (Samson et al., 1992). Alcohol-preferring P rats tested the same way drank ethanol in constant bout sizes, so that their self-administered dose doubled when the concentration increases from 10% to 20% (Files et al., 1993). It is not clear why the behavior of experimental rats in the present study should resemble that of genetically selected animals more than outbred rats. Future work should extend the concentration series with more days at each concentration, to evaluate how bout patterns may change during prolonged access. Rats' adjustments to changes in the energy density of food provide an instructive parallel: the number of bouts per day changed immediately with the food's "concentration," whereas bout size and daily energy intake adjustments required several days (Johnson et al., 1986).

In one respect, the conditioning procedure used in the present experiment resembles the sucrose-fading method used to induce rats to drink plain ethanol (Samson, 1986; Tolliver et al., 1988). That is, with both techniques the animals experience the flavor of ethanol in association with postingestive carbohydrate actions. The present results demonstrate that this flavor–nutrient association is sufficient to condition a robust ethanol preference. The sucrose-fading procedure, unlike the conditioning method used here, also exposes the rats to the sweet taste of sucrose which, by stimulating total intake and bout size, increases the animals' exposure to the pharmacological and nutritive effects of ethanol. In addition, the rats have the opportunity to associate the flavor of ethanol with the sweet taste of sucrose, which may enhance their evaluation of ethanol flavor. The sucrose-fading procedure, therefore, might be expected to be even more effective than IG carbohydrate infusions in enhancing ethanol appetite. Alternatively, IG carbohydrate may be more effective than oral carbohydrate because with IG infusions the animal experiences only the ethanol flavor, which may facilitate the association of ethanol flavor and nutrient reward. Direct comparisons between oral and IG conditioning procedures are needed to resolve this issue. Based on the daily ethanol dose displayed by the experimental rats in the 10% ethanol vs. water test, the carbohydrate conditioning procedure appears to be at least as effective as the sucrose fading procedure in promoting high ethanol intakes (Tolliver et al., 1988).

While the present findings establish that postingestive nutritive reward can enhance the rat's preference for the flavor of ethanol, the nature of this conditioned flavor preference remains to be determined. One interpretation of nutrient-conditioned flavor preferences is that they represent an increase in the hedonic or palatability evaluation of the CS+ flavor (Mehiel, 1991). However, flavor preferences as measured in choice tests may be influenced by factors other then palatability, such as an expectation of some positive benefit (Capaldi, 1992; Rozin and Zellner, 1985). Berridge and Robinson (1998) also distinguish between the hedonic value and the incentive value of motivational stimuli, which they suggest is related to separate "liking" and "wanting" processes. Recent studies that used a variety of behavioral measures, including taste reactivity, indicate that IG carbohydrate conditioning does enhance flavor palatability, at least with saccharin-sweetened flavors that are already mildly preferred to water (Myers and Sclafani, 2001a,b). Whether ethanol palatability is also increased by carbohydrate conditioning requires further investigation.

In summary, the rat's attraction to the taste of ethanol, like their responses to bitter and sour tastes, is modifiable by nutrient conditioning. This suggests that procedures used to induce animals to take ethanol by mixing it with sugar and then fading out the sugar (e.g., Samson, 1986; Tolliver et al., 1988) may be effective in part because the sugar's postingestive effects make the ethanol flavor more acceptable and rewarding. In the case of the saccharin-fading procedure used in some studies (e.g., Rassnick et al., 1993), it may be the nutritive actions of the ethanol itself that contributes to the increased acceptability of the ethanol taste. By extension, human ethanol intake may be rewarded at least initially and partially by the nutritive actions of the carbohydrate and ethanol in mixed drinks. Of course, the addition of flavors, including sugars, to alcoholic drinks has the primary effect of increasing the palatability of the ethanol. This makes it difficult to specify the relative contributions of taste and postingestive factors in human learning. This analysis does not deny pharmacological effects on flavor preferences but suggests that oral and postingestive nutrient reward may supplement drug reward, perhaps bridging the period when drug reward effects are minimal. The contribution of conditioned attraction to ethanol's flavor, combined with its pharmacological effects, may represent a good model for the development of preferences for particular alcoholic beverages.

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.

Portions of this work were presented at the joint meeting of the Society for the Study of Ingestive Behavior and the International Conference on the Physiology of Food and Fluid Intake, Pécs, Hungary, July 1998 (Ackroff and Sclafani, 1998).

References

Ackroff K, Sclafani A. Flavor preferences conditioned by intragastric infusions of dilute polycose solutions. Physiol Behav 1994;55:957–62.

Ackroff K, Sclafani A. Flavor quality and ethanol concentration affect ethanol conditioned flavor preferences. Appetite 1998;31:290–1.

Ackroff K, Sclafani A. Flavor preferences conditioned by intragastric infusion of ethanol in rats. Pharmacol Biochem Behav 2001;68:327–38.

Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 1998;28:309–69.

Capaldi ED. Conditioned food preferences. In: Medin D, editor. The psychology of learning and motivation, vol. 28. New York: Academic Press, 1992. p. 1–33.

Capaldi ED. Conditioned food preferences. In: Capaldi ED, editor. Why we eat what we eat: the psychology of eating. Washington, DC: American Psychological Association; 1996. p. 53–80.

Cunningham CL, Niehus JS. Flavor preference conditioning by oral self-administration of ethanol. Psychopharmacology 1997;134:293–302.

Czachowski CL, Samson HH, Denning CE. Blood ethanol concentrations in rats drinking sucrose/ethanol solutions. Alcohol Clin Exp Res 1999; 23:1331–5.

Deems DA, Oetting RL, Sherman JE, Garcia J. Hungry, but not thirsty, rats prefer flavors paired with ethanol. Physiol Behav 1986;36:141–4.

Elizalde G, Sclafani A. Flavor preferences conditioned by intragastric polycose: a detailed analysis using an electronic esophagus preparation. Physiol Behav 1990;47:63–77.

Fedorchak PM, Bolles RC. Hunger enhances the expression of calorie- but not taste-mediated conditioned flavor preferences. J Exp Psychol Anim Behav Process 1987;13:73–9.

Files FJ, Andrews CM, Lewis RS, Samson HH. Effects of ethanol concentration and fixed-ratio requirement on ethanol self-administration by P rats in a continuous access situation. Alcohol Clin Exp Res 1993;17:61–8.

Gauvin DV. Blood alcohol concentrations in rats drinking or intubated with ethanol [letter]. Alcohol, Clin Exp Res 1999;23:1945–7.

Johnson DF, Ackroff K, Peters J, Collier GH. Changes in rats' meal patterns as a function of the caloric density of the diet. Physiol Behav 1986;36:929–36.

Kiefer SW, Mahadevan RS. The taste of alcohol for rats as revealed by aversion generalization tests. Chem Senses 1993;18:509–22.

Kiefer SW, Lawrence GJ, Metzler CW. Learned alcohol aversions in rats: gustatory and olfactory components. Alcohol 1986;3:27–31.

Kiefer SW, Lawrence GJ, Metzler CW. Alcohol preference in rats lacking gustatory neocortex. Alcohol 1987;4:37–43.

Kiefer SW, Lawrence GJ. The sweet–bitter taste of alcohol: aversion generalization to various sweet–quinine mixtures in the rat. Chem Senses 1988;13:633–41.

Kiefer SW, Morrow NS, Metzler CW. Alcohol aversion generalization in rats: specific disruption of taste and odor cues with gustatory neocortex or olfactory bulb ablations. Behav Neurosci 1988;102:733–9.

Kiefer SW, Bice PJ, Orr MR, Dopp JM. Similarity of taste reactivity responses to alcohol and sucrose mixtures in rats. Alcohol 1990;7:115–20.

Kirk RE. Experimental design: procedures for the behavioral sciences. Pacific Groves, CA: Brooks/Cole; 1995.

Lucas F, Sclafani A. Differential reinforcing and satiating effects of intragastric fat and carbohydrate infusions in rats. Physiol Behav 1999;66:381–8.

Matthews DB, Overstreet DH, Rezvani AH, Devaud LL, Morrow AL. Effects of sweetened ethanol solutions on ethanol self-administration and blood ethanol levels. Pharmacol Biochem Behav 2001;68:13–21.

Mehiel R. Hedonic-shift conditioning with calories. In: Bolles RC, editor. The hedonics of taste. Hillsdale, NJ: Lawrence Erlbaum Associates, 1991. p. 107–26.

Mehiel R, Bolles RC. Learned flavor preferences based on caloric outcome. Anim Learn Behav 1984;12:421–7.

Myers KP, Sclafani A. Conditioned enhancement of flavor evaluation reinforced by intragastric glucose: I. Intake acceptance and preference analysis. Physiol Behav 2001a;74:481–93.

Myers KP, Sclafani A. Conditioned enhancement of flavor evaluation reinforced by intragastric glucose: II. Taste reactivity analysis. Physiol Behav 2001b;74:495–505.

Pérez C, Lucas F, Sclafani A. Increased flavor acceptance and preference conditioned by the postingestive actions of glucose. Physiol Behav 1998;64:483–92.

Rassnick S, D'Amico E, Riley E, Koob GF. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. Alcohol Clin Exp Res 1993;17:124–30.

Richter CP, Campbell KH. Alcohol taste thresholds and concentrations of solutions preferred by rats. Science 1940;91:507–8.

Roberts AJ, Heyser CJ, Koob GF. Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. Alcohol Clin Exp Res 1999;23:1151–7.

Rozin P, Zellner D. The role of Pavlovian conditioning in the acquisition of food likes and dislikes. Ann NY Acad Sci 1985;443:189–202.

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food-and water-sated rats. Alcohol, Clin Exp Res 1986;10:436–42.

Samson HH, Schwarz-Stevens K, Tolliver GA, Andrews CM, Files FJ. Ethanol drinking patterns in a continuous-access operant situation: effects of ethanol concentration and response requirements. Alcohol 1992;9:409–14.

Sclafani A. Carbohydrate taste, appetite, and obesity: an overview. Neurosci Biobehav Rev 1987;11:131–53.

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.

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.

Sherman JE, Hickis 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.

Tolliver GA, Sadeghi KG, Samson HH. Ethanol preference following the sucrose-fading initiation procedure. Alcohol 1988;5:9–13.

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.

Wallgren H, Barry III H. Actions of alcohol. Biochemical, physiological and psychological aspects. Amsterdam: Elsevier; 1970.