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Flavor preferences conditioned by intragastric infusion of ethanol in rats

Karen Ackroff*, Anthony Sclafani

Brooklyn College and The Graduate School, City University of New York, Brooklyn, NY 11210, USA

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Abstract

Sprague–Dawley rats were trained 22 h/day to associate a flavored solution [conditioned stimulus (CS+)] with intragastric infusions of 6% ethanol and another flavored solution (CS−) with water infusions. The infusions were matched to the CS intakes so that the animals determined their timing and size. In Phase 1, chow and water were available ad libitum, and both CS flavors were initially sweetened with saccharin that was then faded out. The rats displayed a preference for the CS+ over the CS− under both reinforced and extinction conditions. When food-restricted in Phase 2, the rats displayed an increased preference for the CS+. In Phase 3, the rats were fed ad libitum chow and given preference tests with the CS+ paired with ethanol infusions of increasing concentration (6%, 12%, 18%, and 24%). Their preference for the CS+ over the CS− persisted, and self-administered ethanol dose increased with concentration to 5 g/kg/day. The ethanol-based conditioned flavor preference resembled those conditioned by carbohydrate and fat infusions, suggesting that at least some of reinforcing ability of ethanol may be related to its postingestive nutritive effects.

Keywords: Flavor conditioning; Ethanol; Gastric infusions; Saccharin

Rats learn to associate the flavors of foods and fluids with their postingestive consequences. Most extensively documented are the conditioned aversions for flavors that are paired with drugs, which produce gastrointestinal malaise, such as lithium chloride (Nachman and Ashe, 1973; Riley and Tuck, 1985). Flavor aversions may also be produced by drugs of abuse, although animals will self-administer these drugs under some test conditions (Hunt and Amit, 1987). In contrast to these drug-induced aversions, strong preferences can be produced by pairing flavors with the postingestive actions of nutrients in a Pavlovian conditioning procedure. Rats will even learn to prefer flavors that they normally avoid (e.g., bitter or sour tastes) when the flavors are paired with intragastric carbohydrate infusions (Drucker et al., 1994; Pérez et al., 1998). Ethanol, which is both a drug of abuse and a nutrient, is reported in many animal studies to produce flavor aversions (Berman and Cannon, 1974; Cannon and Carrell, 1987; Crawford and Baker, 1982; Eckhardt et al., 1974; Marfaing-Jallat and Le Magnen, 1979; Miceli et al., 1980; Sinclair, 1984). However, under some conditions, ethanol has been shown to condition flavor preferences in rats (Cunningham and Niehus, 1997; Deems et al., 1986; Mehiel and Bolles, 1984; Sherman et al., 1983; Waller et al., 1984). Ethanol-conditioned preferences are of particular interest, since humans who drink alcoholic beverages acquire preferences for the flavors of these beverages.

Ethanol-conditioned flavor preferences have been obtained by training rats to drink an ethanol solution containing the cue flavor (oral method) or by having them drink a flavored solution that is paired with intragastric ethanol infusions (intragastric method). With the oral training method, the flavor of the ethanol may influence preference conditioning to the cue flavor through a flavor–flavor conditioning process. That is, to the extent that the animal is attracted or adverse to the flavor of ethanol, its preference for the cue flavor may be enhanced or reduced. With the intragastric method, however, only the postingestive actions of ethanol contribute to the flavor preference or aversion conditioning process. Using this method, Deems et al. (1986) and Sherman et al. (1983) trained food-restricted rats (Sprague–Dawley strain) to drink differently flavored sucrose solutions after being

* Corresponding author. Department of Psychology, Brooklyn College, City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA. Tel.: +1-718-951-5606; fax: +1-718-951-4824.

E-mail address: kackroff@gc.cuny.edu (K. Ackroff).

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given a fixed intragastric infusion of 5% ethanol or water during alternating one-bottle sessions. In subsequent two-bottle tests, the rats preferred the flavor that had been paired with intragastric ethanol at a dose of 0.5 g/kg relative to the water-paired flavor. Higher ethanol doses failed to produce a reliable preference (10% ethanol, 1.0 g/kg) or a flavor aversion (20% ethanol, 2.0 g/kg). Deems et al. (1986) further reported that the 0.5-g/kg ethanol infusion conditioned a flavor preference in food-deprived, but not in water-deprived, rats. These experimenters (Deems et al., 1986; Sherman et al., 1983) concluded that it was the "caloric restoration" provided by the ethanol infusions that reinforced the flavor preferences in the food-restricted rats, and that aversive effects of the higher ethanol doses counteracted preference conditioning. The interpretation of these findings is complicated, however, because the rats drank more flavored sucrose on ethanol training trials than on water training trials. Thus, the additional sucrose energy may have contributed to the ethanol-conditioned preferences.

In another intragastric conditioning study (Waller et al., 1984), ethanol-preferring (P) and -nonpreferring (NP) rats were trained to drink flavored water paired with concurrent infusions of 20% ethanol and a differently flavored water paired with intragastric water infusions. The rats were water-restricted during the initial short-term training sessions. In subsequent 24-h/day choice tests with food ad libitum, the P rats showed a strong preference for the flavored water paired with the concurrent intragastric ethanol infusions, whereas, the NP rats avoided that flavor. However, in extinction tests with both flavors paired with intragastric water, the P rats rapidly lost their preference for the ethanol-paired flavor and drank more of the water-paired flavor. This contrasts with the flavor preferences conditioned by intragastric carbohydrate infusions that are very resistant to extinction (Drucker et al., 1994; Elizalde and Sclafani, 1990).

In view of the limited evidence for flavor preference conditioning by intragastric ethanol, the present study further examined ethanol conditioning in rats using training procedures that are very effective in obtaining nutrient-conditioned preferences. In the first phase, the rats were given ad libitum access to flavored, noncaloric solutions paired with intragastric infusions of 6% ethanol or water. During training, the rats had plain water available, and thus, were not forced to self-administer ethanol while obtaining fluid. They were neither food- nor water-restricted during initial training, because restriction is not required to obtain robust-conditioned preferences by nutrients, such as carbohydrates (Drucker et al., 1993; Elizalde and Sclafani, 1990; Sclafani et al., 1993). Showing that nondeprived animals can acquire ethanol-based flavor preferences would support the idea that ethanol is treated like other nutrients in this learning process. It is also relevant to alcohol appetite in humans, which is not dependent upon food or fluid deprivation. In a second phase, the rats were given additional training and testing while food-restricted. Prior research with other nutrients indicates that this training schedule enhances preference conditioning and conditions increased one-bottle acceptance of the conditioned stimulus (CS)+ flavor (Pérez et al., 1998). In a third phase, we returned the rats to ad libitum food and determined how higher concentrations of ethanol affected total intake and preference for the CS+ relative to the CS − flavor paired with intragastric water.

1. Method

1.1. Subjects

Adult male Sprague–Dawley rats ($n = 14$) were purchased from Charles River Laboratories (Wilmington, MA). They were housed in stainless-steel hanging cages with ad libitum access to 3.3-kcal/g powdered chow (No. 5001, PMI Nutrition International; Brentwood, MO) and water. The animal colony and experimental rooms were maintained on a 12:12 light/dark cycle (lights on 08:00 h) at 21°C.

1.2. Surgery

The rats were 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.

1.3. Apparatus

The test cages and circuitry used for intragastric 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 ($24 \times 18 \times 18$ 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 gastric cannula of the rat 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 1.3 ml/min, and they were controlled by computer

Table 1
Summary of experimental conditions

Condition	Oral fluids	Saccharin in flavors	Ethanol
Phase 1: acquisition (chow and fluids available 22 h/day)			
Training (20 days)	two bottles, flavor vs. water: CS+ and CS − on alternate days	0.2% (2 days/flavor), 0.1% (2 days/flavor), 0.05% (3 days/flavor), 0% (3 days/flavor)	6%
Tests (10 days)	two bottles, CS+ vs. CS − flavors	none	6% (4 days), 0% (2 days), 6% (4 days)
Phase 2: food restriction (2 h chow and water, 2 h nothing, 20 h flavors)			
Training 1 (10 days)	one bottle, CS+ and CS − on alternate days	none	6%
Tests (5 days)	two bottles, CS+ vs. CS − flavors	none	6%
Training 2 (4 days)	one bottle, CS+ and CS − on alternate days	none	6%
Phase 3: concentration tests (chow and flavors available 22 h/day)			
Tests (24 days)	two bottles, CS+ vs. CS − flavors	none	6% (5 days), 12% (6 days), 18% (7 days), 24% (6 days)

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.

1.4. Solutions

CS solutions were water-flavored with 0.05% (w/v) unsweetened grape and cherry Kool-Aid drink mixes (General Foods; White Plains, NY). These flavors are equally unpreferred to plain water (Elizalde and Sclafani, 1990). The CS solutions were initially sweetened with sodium saccharin (Sigma; St. Louis, MO) added at 0.2%, 0.1%, and 0.05% (w/v) concentrations. (This was done because naive animals consume relatively little of the unsweetened Kool-Aid solutions when water is also available.) Unflavored 0.2% saccharin and tap water were also available to drink during some phases of the experiment. The infusates were tap water and 6% ethanol prepared by mixing 95% ethanol and tap water. The energy density of the ethanol solution was 0.345 kcal/g. For half the rats, grape was the CS+ flavor paired with intragastric ethanol, and cherry was the CS − flavor paired with intragastric water. The flavor–infusate pairs were reversed for the remaining rats. The amounts of fluid consumed and infused were recorded to the nearest 0.1 g.

1.5. Procedure

After a postsurgery recovery period (7–10 days), the rats were transferred to the test cages where they lived for the remainder of the experiment. They were adapted to the cages for 5 days with chow and two water bottles available ad libitum. Then their gastric catheters were attached, and they were infused with water whenever they drank water during the next 2 days. The rats were then familiarized with the 0.2% saccharin solution that was available along with water for 2 days. Water was infused intragastrically whenever they drank either fluid.

The phases of the experiment are summarized in Table 1.

1.5.1. Phase 1: acquisition

During the training phase, the rats had 22-h/day access to chow, a CS flavor solution, and water. Data were collected, and the infusion system was serviced in the remaining 2 h each day. On odd-numbered days, the CS − flavor solution was paired with water infusion, and on even-numbered days, the CS+ flavor solution was paired with 6% ethanol infusion. Intake of plain water was paired with water. The positions of the CS solution and water were counterbalanced across days, so each flavor appeared equally often on the left and on the right. This counterbalancing was in effect for all phases. Both the CS+ and CS − solutions were initially sweetened with saccharin at 0.2% concentration (2 days each) that was reduced to 0.1% (2 days each), 0.05% (2 days each), and 0% (3 days each).

Next, the rats were given two-bottle tests with the unsweetened flavor solutions to evaluate their preferences for the CS+ and the CS − . For the first 4 days, the rats were infused with ethanol and water whenever they drank the CS+ and CS − , respectively. During the next 2 days (extinction test), intake of both CS was paired with intragastric water. Finally, the rats were given 4 more days of two-bottle testing with the original infusates. Plain drinking water was not presented during these tests.

1.5.2. Phase 2: food restriction

The rats were placed on a "2-2-20" schedule: 2-h access to chow and water, 2 h with no food or fluid available, and 20 h with one or two fluids paired with intragastric infusion. The 20-h drinking period began at 14:00 h and included the 12-h night period. For the first 5 days, the animals were allowed to adjust to the schedule and had water to drink paired with intragastric water during the 20-h period. Thereafter, the fluids offered during the 20-h period were the unsweetened CS flavors. For 10 days, they were given alternate-day one-bottle access to the CS+ solution paired with intragastric 6% ethanol and the CS − paired with

intragastric water. This was followed by a two-bottle choice test between CS+ and CS−, still paired with their respective infusions. Because of an error on the second day of the preference test, 5 days were run. Only the last 2 days were analyzed. Finally, they were returned for 4 more days to one-bottle access to the CS+ and CS− paired with their respective infusions.

1.5.3. Phase 3: concentration tests

At the end of Phase 2, the rats were first returned to ad libitum food and water for 4 days. They were then given 23-h access to both the unsweetened CS+ and CS− flavor solutions paired with ethanol and water infusions, respectively. Initially, the concentration of the ethanol was 6%, as in the previous phases. Then, the concentration was increased to 12%, 18%, and 24%, with each concentration in effect for 5–7 days. Chow was available ad libitum throughout this period.

1.6. Data analysis

Two of the 14 rats developed problems with the gastric cannula during the study, and their data were excluded. The intake data were averaged over the 2- or 3-day periods in the initial training and over the 2 or 4 days of preference testing. In Phase 3, the data were averaged over 4- or 6-day periods. The first day of each new phase was not included. 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. Ethanol intakes per day and per bout were also calculated. To obtain an estimate of the average ethanol per bout of CS+ 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. In Phase 3, the bout analysis focused on the 6% and 24% periods. The data were entered in repeated-measures analyses of variance. For significant main effects, tests of differences between specific mean and groups of mean were performed using least-square mean contrasts. A probability level of .05 was used in all tests.

2. Results

2.1. Phase 1: acquisition

2.1.1. Training data

2.1.1.1. CS intakes. Fig. 1 shows the CS solution and water intakes during the saccharin and no saccharin training periods. In separate analyses, intakes of CS+ and CS− solutions were compared with those of water offered on the same days. CS+ solution intake was greater than water intake [$F(1,11)=41.9$, $P<.0001$], and there was an interaction of saccharin concentration and fluid type [$F(3,33)=3.52$, $P<.05$]. Sweetened CS+ intakes at the three saccharin concentrations did not differ and were greater than unsweetened CS+ intake ($P<.001$), while water intake on CS+ days did not differ across the four conditions. CS+ solution intake exceeded water intake in all four conditions (P's $<.0001$). Overall, intake of the CS− solution also exceeded that of water [$F(1,11)=21.1$, $P<.001$], and saccharin concentration interacted with fluid type [$F(3,33)=25.3$, $P<.001$]. For both the CS− and water, intake during the three saccharin periods was similar and differed from intake in the unsweetened period (P's $<.005$). CS− solution intake exceeded water intake when the flavor was sweetened (P's $<.0001$), but intakes of CS− and water did not differ when the saccharin was removed from the CS− solution. Thus, when the rats had the choice of unsweetened CS solutions and water, they consumed 51% of their fluid intake as CS− but 79% as CS+.

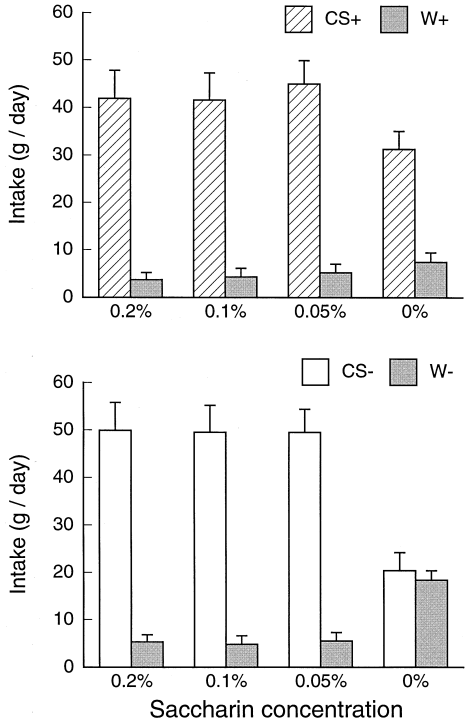

Fig. 1. Mean±S.E.M. daily intakes of training solutions in Phase 1. On CS+ days, intake of the CS+ flavor solution (upper panel) was paired with intragastric infusion of 6% ethanol, and intake of water (W+) was paired with intragastric water. On CS− days, intake of the CS− flavor solution (lower panel) and water (W−) were paired with intragastric water. The concentration of saccharin in the CS solutions was reduced over days (see text).

Analysis of the individual data revealed that 11 of the 12 animals consistently drank more CS+ solution than water at all saccharin levels (0.2–0%). The remaining animal (Rat 168) drank more CS+ and CS− than water at the 0.2% saccharin level, but then drank less CS+ and CS− than water at all remaining concentrations. This animal had a high-saccharin preference threshold or may have developed an ethanol-conditioned aversion to the CS+ that generalized to the CS− flavor. This cannot be attributed to the rat being

Table 2
Phase 1: mean ± S.E.M. bout patterns in 22-h training sessions

	0.2% Saccharin		0.1% Saccharin		0.05% Saccharin		0% Saccharin	
	CS+	CS−	CS+	CS−	CS+	CS−	CS+	CS−
Bout number	14.1 ± 1.7	15.1 ± 2.4	14.7 ± 2.2	17.0 ± 2.5	15.1 ± 1.6	17.7 ± 2.5	12.3 ± 1.6	7.9 ± 1.6
Bout size (g of CS)	3.04 ± 0.33	3.22 ± 0.35	2.99 ± 0.24	2.99 ± 0.31	3.00 ± 0.30	3.12 ± 0.40	2.69 ± 0.24	3.18 ± 0.53
Bout size (g/kg ethanol)	0.30 ± 0.03		0.29 ± 0.03		0.26 ± 0.02		0.28 ± 0.04	

infused with an unusually large amount of ethanol on the initial training days when the CS+ solution contained 0.2% saccharin, because its ethanol intake (1.4 g/kg/day) was less than the group average (2.1 g/kg/day).

A direct comparison between group mean intakes of CS+ and CS− solutions during training revealed differences as a function of saccharin concentration [interaction: $F(3,33) = 7.4$, $P < .001$]. In particular, the rats consumed more CS− than CS+ solution at the two higher saccharin levels (P's < .05) but more CS+ than CS− solution when the flavors contained no saccharin ($P < .005$). CS intakes did not differ at the 0.05% saccharin concentration.

2.1.1.2. Bout patterns. The differences in CS solution intakes across saccharin concentrations were the result of shifts in bout patterns. The bout data from the four periods are shown in Table 2. Mean numbers of CS bouts per day differed with saccharin concentration [$F(1,11) = 10.72$, $P < .0001$]. This concentration effect was due to the reduced numbers of bouts in the unsweetened period compared to the similar bout numbers in the three saccharin periods (P's < .001). The main effect of CS type was not significant. Sweetening and CS type interacted in their effects on bout number [$F(3,33) = 5.81$, $P < .005$]. Bout numbers were similar on CS+ days: only the 0.05% and 0% saccharin difference approached significance ($P = .06$). On CS− days, bout numbers during the saccharin periods were similar and greater than those of the unsweetened period (P's < .0001). Within all three saccharin concentrations, bout numbers were similar for CS+ and CS−, but when the flavors were unsweetened, there were more bouts on CS+ than on CS− days ($P < .005$). Bout sizes did not differ as a function of saccharin concentration, CS type, or their interaction.

2.1.1.3. Ethanol doses. The self-administered doses of ethanol were compared across saccharin periods. The self-administered ethanol dose per bout, like CS+ bout size, did not differ as a function of saccharin concentration, averaging about 0.28-g ethanol/kg/bout (Table 3). Daily ethanol self-administration differed with saccharin concentration [$F(3,33) = 5.70$, $P < .005$]. Like the total CS+ solution intake, total ethanol was similar in the three saccharin periods and exceeded that of the unsweetened period ($P < .0005$).

At the 0.2% saccharin concentration, 40% of ethanol bouts were larger than 0.3-g ethanol/kg body weight. At 0.05%, this value was 33%, and when saccharin was removed, only 19% of bouts were larger than 0.3 g/kg. At all saccharin concentrations, fewer than 5% of the bouts lasted more than 10 min. The interbout interval (time between the end of one bout and the beginning of the next) was generally less than 1 h; 33% or fewer intervals exceeded 60 min at all saccharin concentrations.

2.1.1.4. Energy intakes. The contributions of ethanol and chow calories to total energy intake were examined across the saccharin conditions (Table 3). In parallel with the reduction in CS+ solution intake when saccharin was removed, ethanol calories varied with saccharin content [$F(3,33) = 5.76$, $P < .005$]. On CS+ days, the rats obtained ~14-kcal ethanol (~13% of the daily total kilocalories) with the sweetened CS+ and a significantly lower 9.2 kcal (8.4% of total) with the unsweetened CS+ (P's < .005). Although the rats ate less chow on CS+ than on CS− days [93.8 vs. 102.3 kcal/day; $F(1,11) = 84.35$, $P < .0001$], they consumed more total energy on CS+ than CS− days [106.6 vs. 102.3 kcal/day; $F(1,11) = 23.72$, $P < .0005$]. Chow and total intakes did not vary significantly with saccharin concentration.

2.1.2. Flavor preference tests

In the first preference test, when the CS+ and CS− remained paired with 6% ethanol and water infusions, respectively, the rats preferred the CS+, taking 72% of their total intake as that flavor (Fig. 2). Under extinction conditions, when the intakes of both flavors were paired with

Table 3
Phase 1: mean ± S.E.M. daily energy intakes and ethanol doses in 22-h training sessions

	0.2% Saccharin		0.1% Saccharin		0.05% Saccharin		0% Saccharin	
	CS+	CS−	CS+	CS−	CS+	CS−	CS+	CS−
Ethanol (kcal)	14.5 ± 1.9		13.7 ± 1.6		13.6 ± 1.3		9.2 ± 1.1	
Chow (kcal)	93.4 ± 5.0	102.9 ± 4.4	90.0 ± 3.4	100.7 ± 2.7	92.4 ± 2.9	102.8 ± 3.6	99.5 ± 2.9	102.8 ± 3.4
Total (kcal)	107.9 ± 5.7	102.9 ± 4.4	103.6 ± 4.0	100.7 ± 2.7	106.0 ± 3.4	102.8 ± 3.6	108.6 ± 2.9	102.8 ± 3.4
Ethanol (g/kg/day)	4.12 ± 0.54		3.89 ± 0.46		3.86 ± 0.36		2.61 ± 0.31	

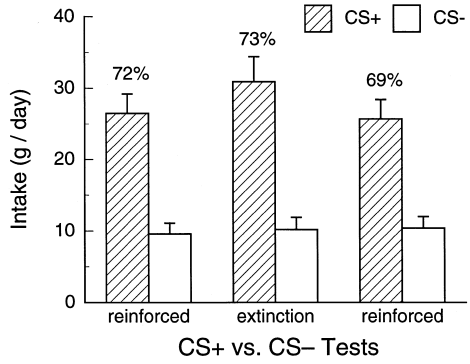

Fig. 2. Mean ± S.E.M. daily intakes of CS solutions in two-bottle tests of Phase 1. Shown are the initial reinforced preference test with the CS+ paired with intragastric infusion of 6% ethanol (left), the extinction test with both CS solutions paired with water (center), and the second reinforced preference test (right). Mean percentage of total intake consumed as CS+ is shown atop the bars.

intragastric water, the CS+ preference persisted at 73%. On return to testing with the original infusions, the rats continued to prefer the CS+ (69%) and to consume amounts similar to those in the first test. Analysis of intakes in the three tests indicated that CS+ solution intakes were significantly greater than CS − intakes [$F(1,11) = 23.07$, $P < .001$], and that extinction intakes were greater than original infusion intakes [$F(1,11) = 21.1$, $P < .001$]. CS and test types did not interact. Note that 11 of the 12 rats consumed more CS+ than CS − in these tests. Rat 168, which drank more water than unsweetened CS+ in training, preferred the CS − to the CS+. With the data of this rat excluded, the CS+ preferences during the three CS+ vs. CS − choice tests were 76%, 77%, and 74%, respectively.

2.2. Phase 2: food restriction

The 20-h CS solution intakes are shown in Fig. 3. During the initial one-bottle training period, the rats drank more

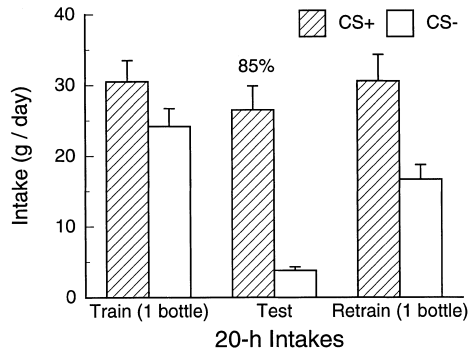

Fig. 3. Mean ± S.E.M. 20-h intakes of CS solutions in Phase 2. On CS+ training days, intake of the CS+ flavor solution was paired with intragastric infusion of 6% ethanol, and on CS − days, intake of CS − solution was paired with intragastric water. Shown are the one-bottle intakes during training (left), the two-bottle reinforced preference test (center), and the return to one-bottle training (right). Mean percentage of CS+ preference is shown atop the bar.

Table 4
Phase 2: mean ± S.E.M. one-bottle intakes and bout patterns

	20-h intake (g)	Bouts/20 h	Bout size (g)
Water	15.0 ± 1.4	9.3 ± 1.0	1.58 ± 0.13
Initial CS period			
CS +	30.5 ± 3.0	13.1 ± 0.6	2.38 ± 0.22
CS −	24.2 ± 2.5	11.5 ± 0.8	2.14 ± 0.18
Post-preference period			
CS +	30.6 ± 3.8	11.1 ± 1.1	3.08 ± 0.50
CS −	16.7 ± 2.0	8.3 ± 1.1	2.19 ± 0.27

CS+ than CS − [$t(11) = 4.01$, $P < .001$]. This difference was due to a greater number of CS+ than CS − bouts per day [$t(11) = 2.44$, $P < .05$]. The bout sizes did not differ significantly (Table 4). The average 2-h chow intake following CS − days was 53.3 kcal and was 50.7 kcal after CS+ days [$t(11) = 1.49$, $P = .08$]. The ethanol infusion averaged 9.3 kcal, so that the total daily energy intake was increased by about 18% during CS+ one-bottle days. The rats averaged 2.64 g/kg/day of ethanol on CS+ days, with a mean ethanol dose of 0.20 g/kg/bout.

In the 20-h/day two-bottle test, the rats consumed substantially more CS+ than CS − solution [$t(11) = 7.08$, $P < .00001$]. Their CS+ solution intake of 85% was higher than the 69% preference obtained in the last reinforced test of Experiment 1 [$t(11) = 2.35$, $P < .05$]. Furthermore, all 12 rats now consumed more CS+ than CS − solution. In particular, Rat 168 had a CS+ preference of 85%, which is a reversal of its 18% CS+ preference during the last test of Phase 1.

When returned to one-bottle access following the choice test, the rats consumed nearly twice as much CS+ than CS − solution. Compared to the pretest one-bottle intakes, CS+ intake remained the same, while CS − intake was reduced [$t(11) = 6.21$, $P < .0001$]. Overall, CS+ solution intake was greater than CS − intake. The rats took more CS+ than CS − bouts [$t(11) = 1.82$, $P < .05$]. CS+ bout size was greater than that of the CS −, but this difference was not significant. The rats averaged 2.46 g/kg/day of ethanol on CS+ days, with a mean ethanol dose of 0.22 g/kg/bout.

One-bottle CS solution intakes were compared to those of plain water during the baseline period of adaptation to the 2-2-20 schedule (Table 4). One-bottle intakes of water and the CS solutions differed [$F(4,44) = 12.54$, $P < .0001$]. Intakes of water and CS − after the preference test did not differ, but CS − intake in the first one-bottle series and CS+ intake in both periods exceeded that of water (P's $< .005$). These differences reflect shifts in bout number [$F(4,44) = 6.78$, $P < .0002$] and size [$F(4,44) = 4.66$, $P < .005$] across conditions. CS+ bout number and size exceeded those of water in initial training, and CS+ bout size was also greater than water in the second one-bottle period (P's $< .05$). CS − bout numbers and sizes did not differ significantly from those of water.

2.3. Phase 3: concentration tests

As shown in Fig. 4, the rats consumed more CS+ than CS − solution at all ethanol concentrations [$F(1,11) = 38.0$, $P < .0001$]. However, CS+ intake declined, whereas, CS − intake remained unchanged as concentration increased [$F(3,33) = 5.3$, $P < .005$]. Percentage of CS+ intake also declined from 80% (6% ethanol) to 64% (24% ethanol). Nevertheless, total daily ethanol intake (g/kg/day) increased with concentration [$F(3,33) = 15.3$, $P < .0001$; Fig. 4, lower panel]. In particular, the rats self-infused 2.5 times more ethanol per day at the 24% concentration than they did at the 6% concentration. Rat 168 had intakes similar to the other rats, maintaining a CS+ preference as concentration increased.

Bout pattern comparison of the 6% and 24% conditions indicated that the declining intake of CS+ solution was due to a reduction in bout size [$t(11) = 3.83$, $P < .005$]. The reduction in bout number was not significant. The rats drank the CS+ in 7.4 bouts of 3.7 g at 6% and 6.7 bouts of 3.0 g at 24%. The corresponding infusions converted to ethanol doses were 0.25 and 0.81 g/kg/bout at 6% and 24%, respectively.

The distribution of energy from ethanol and chow changed across infusion concentrations (Table 5). Ethanol in kcal/day increased with ethanol concentration [$F(3,33) = 15.2$, $P < .0001$]. The 18% and 24% intakes did not differ, but all other differences were significant (P's < .05). Comparison of

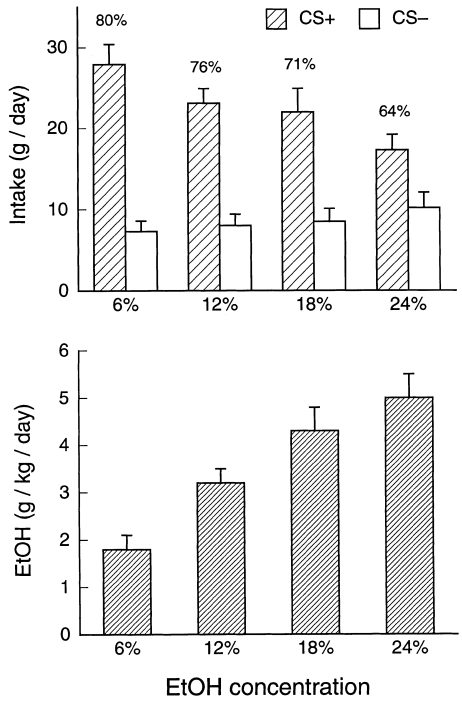

Fig. 4. Mean ± S.E.M. daily intakes of CS solutions in two-bottle tests (upper panel) and daily self-administered ethanol doses (lower panel) in Phase 3. The concentration of the ethanol infused during CS+ intake was increased over days (see text). Mean percentages of total intake consumed as CS+ is shown atop the bars in the upper panel.

Table 5
Phase 3: mean ± S.E.M. daily energy intakes in 22-h sessions

	6%	12%	18%	24%
Ethanol (kcal)	7.5 ± 1.0	13.0 ± 1.1	17.4 ± 1.9	20.2 ± 1.8
Chow (kcal)	106.8 ± 3.8	96.6 ± 3.4	87.0 ± 3.4	82.1 ± 2.6
Total energy (kcal)	114.3 ± 3.5	109.6 ± 3.1	104.4 ± 4.2	102.3 ± 2.8
% Energy as ethanol	6.7 ± 1.0	12.0 ± 1.1	16.5 ± 1.3	19.7 ± 1.6

chow in kcal/day at the four concentrations showed a significant decline [$F(3,33) = 44.6$, $P < .0001$], with decreases associated with each increase in ethanol concentration (P's < .05). Total energy per day also fell significantly as concentration increased [$F(3,33) = 5.78$, $P < .01$]. Intake at 6% was greater than at 18% and 24%, and intake at 12% exceeded 24% (P's < .05).

3. Discussion

The results of this study demonstrate that flavor preferences are conditioned by intragastric infusions of ethanol in outbred rats given ad libitum access to the ethanol-paired flavor solution as well as to food and water. The preference was expressed in two-bottle choice tests with the CS+ and CS − when intake of the CS+ solution was paired with intragastric ethanol as well as with intragastric water. In addition, the rats preferred the unsweetened CS+ to plain water, whereas, untrained rats avoid the unsweetened flavors when water is available (unpublished findings). The ethanol-conditioned preference was enhanced by training the rats while food-restricted, and this training increased the one-bottle acceptance of the CS+ flavor solution relative to the CS − flavor and plain water. After extensive experience with 6% ethanol infusions, the rats continued to prefer the CS+ to the CS − when infused with ethanol at 12%, 18%, and 24% concentrations. At the highest concentration, the rats infused themselves with 5 g/kg/day, which is comparable to the daily dose obtained in alcohol-preferring P rats tested under similar infusion conditions (Waller et al., 1984).

The CS+ preferences observed in the CS+ vs. water and CS+ vs. CS − tests demonstrate for the first time that intragastric ethanol can condition flavor preferences in nondeprived rats. Previously published reports of flavor conditioning with intragastric ethanol used training procedures in which rats were food- (Deems et al., 1986; Sherman et al., 1983) or water-restricted (Waller et al., 1984). The strength of the preference is comparable to the 72–79% preferences reported for food-restricted rats trained in 30-min sessions with intragastrically administered ethanol (Deems et al., 1986; Sherman et al., 1983). This shows that the development of ethanol-conditioned flavor preferences is not dependent on energy need.

The strength of the preference for the CS+ over the CS − did not diminish when it was paired with intragastric water (extinction), demonstrating that the rats acquired a

true preference for the CS+ flavor that was not dependent upon concurrent ethanol infusions. The extinction test lasted 2 days, and thus, it is uncertain if the CS+ preference would persist over a longer test period. Flavor preferences conditioned by intragastric carbohydrate with this procedure are remarkably resistant to extinction and persist for several weeks or more in the absence of intragastric nutrient (Drucker et al., 1994; Elizalde and Sclafani, 1990). In contrast, the earlier study of Waller et al. (1984) reported that preference of P rats extinguished in 3 days when the CS+ flavor was paired with intragastric water rather than intragastric ethanol. This rapid extinction may represent a response characteristic of P rats and/or may have been related to the high ethanol concentration (20%) used in that experiment.

When further trained and tested with restricted access to food, the rats displayed a stronger CS+ preference (85%) than they did in the ad libitum food tests of the first phase (69–73%). Also, on one-bottle training days, the rats consumed more CS+ than CS− solution or plain water, showing that intragastric ethanol infusions increased the acceptance of, as well as the preference for, the CS+ flavor. This is consistent with prior results showing that food restriction during training enhances flavor conditioning by intragastric carbohydrate and fat infusions (Lucas and Sclafani, 1989; Pérez et al., 1998). This effect may occur, because the food restriction enhances the rewarding value of the infusate and/or provides the animal with unambiguous experience with the postingestive consequences of the training flavors. That is, with ad libitum food, rats typically drink just before and after their meals (a prandial drinking pattern; Kissileff, 1969), and the rewarding impact of the CS+/intragastric infusion may be reduced, because the infusate mixes with the nutrients provided by the meal.

In Phase 3, the rats were challenged with increasing ethanol concentrations in the infusion paired with the CS+, while the CS− paired with water infusion was concurrently available. This method of increasing the ethanol concentration had the advantage of sensitivity to potential aversive effects, because the animals could consume the benign CS− to satisfy their fluid requirements. Because it was conducted with ad libitum chow access, the rats did not need the ethanol calories and could easily shift intake away from ethanol if it was aversive. The rats reduced their absolute and % intake of the CS+ solution but did not increase their CS− intake. The reduced CS+ intake did not fully compensate for the increasing ethanol concentration. A consequence of this was a steady increase in daily ethanol intake as the concentration increased. Chow intake was reduced in parallel with the increasing ethanol calories, so that total energy intake did not increase. Taken together, these results indicate that the reduction in CS+ intake with increasing concentration was not due to a developing aversion but rather resulted from the satiating effects of the concentrated ethanol infusions.

In Phase 1, the bout pattern data provide a more detailed view of the responses of rats during acquisition. When saccharin was present, there were minimal differences between CS+ and CS−. When saccharin was removed from both flavors, the differential intake was due entirely to a sustained number of CS+ bouts. The amount consumed per bout remained the same as in the sweetened conditions for both the ethanol- and water-paired flavors. The sustained bout size in the unsweetened condition could reflect flavor–flavor conditioning, such that the Kool-Aid flavors retain some attractiveness due to the lengthy pairing with saccharin (Fanselow and Birk, 1982; Holman, 1975). Nevertheless, the unsweetened CS+ was more attractive than the unsweetened CS−, because the rats took more frequent CS+ bouts. This is evidence for a postingestive rewarding effect of ethanol under these conditions.

By the end of acquisition, the rats were self-administering, on average, 0.28-g/kg ethanol per bout, which is less than the 0.5-g/kg dose of the conditioned flavor preferences in the studies of Deems et al. (1986) and Sherman et al. (1983). The effectiveness of the low dose self-administered in the present experiment is presumably due to the multiple CS+/intragastric ethanol pairings that the rats experienced during the 22-h/day training sessions. Also, the fact that the rats controlled the size of the individual ethanol infusions by their CS+ drinking response may have contributed to the flavor preference learning. If bout sizes were limited by the satiating effects of the ethanol, for example, the animals may have limited themselves to infused amounts that were rewarding rather than larger amounts that were potentially aversive. This is consistent with the finding that distributed oral intake of ethanol does not condition a taste aversion, but massed intake of the same total amount does yield aversion (Eckhardt, 1975). Perhaps, procedures that do not produce ethanol aversion act in part by maintaining ethanol intake at a rate that does not exceed the metabolic capacity of 0.3-g ethanol/kg/h of rats. We suspect that the procedural differences between our study and those that find taste aversion (deprivation state and its influence on bout size, one vs. many bouts per day) are largely responsible for the different outcomes.

The bout patterns also detail the compensation that occurred in response to the increasing ethanol concentration. The average bout size for CS+ solution intake, which gradually rose through the course of experience of these rats with 6% ethanol in the first two phases, fell somewhat in volume as ethanol concentration increased. The decrease was not compensatory, which would have required a reduction to one-quarter of the original volume as ethanol concentration quadrupled. Instead, the minor reduction in volume intake resulted in a large increase in the amount of ethanol infused per bout. The number of bouts declined only slightly. These results resemble aspects of the altered bout patterns in outbreed Long–Evans rats (Samson et al., 1992) when oral ethanol concentration was increased from 10% to 20%: they reduced bout size without changing bout

number. In contrast, alcohol-preferring P rats (Files et al., 1993) did not alter bout size but took fewer bouts per day. The net result in both studies was an increase in daily ethanol dose with an increase in concentration, consistent with the present findings.

The amounts of ethanol taken by our rats at the higher concentrations are remarkably similar to those intragastrically self-infused by alcohol-preferring P rats given increasing ethanol concentrations (Waller et al., 1984). Infused ethanol bouts of our rats were even larger than the oral bouts recorded for an alcohol-preferring strain. P rats housed in operant cages with an FR1 requirement for 0.1-ml dippers of ethanol had average bouts of 0.2 and 0.4 g/kg with 10% and 20% ethanol, respectively (Files et al., 1993). In contrast, the intragastric infusion of 24% ethanol during CS+ intake yielded bouts of 0.86 g/kg in our outbred rats. This may reflect the gradual increases in the concentration, so that the animals effectively had multiple trials to adjust to each increase as they consumed successive bouts. The 24% ethanol might not have conditioned a preference if introduced at the beginning of training (Sherman et al., 1983), but once the preference conditioned with 6% was well established, the higher concentrations were accepted. If we had continued to increase the concentration, the animals might have reduced their intake still further, but under these benign conditions, they could still take ethanol and even dilute it with CS−. While this untasted ethanol infusion technique is not a direct analog of typical acquisition of ethanol intake, the sustained CS+ preference is another instance of acceptability of higher concentrations predicated on a learned acceptance of lower concentrations. This mechanism for accepting more concentrated ethanol could underlie human acquisition of preferences for more concentrated alcoholic beverages.

In several respects, the flavor preference conditioned by intragastric ethanol was similar to those obtained with other nutrients. That is, intragastric carbohydrate and fat infusions produce flavor preferences in rats trained under ad libitum conditions, the flavor preferences are resistant to extinction, and food restriction during 20-h training sessions strengthens the preferences and produces increased flavor acceptance as well (Pérez et al., 1998). Nutrients differ, however, in their ability to support flavor conditioning. In particular, isocaloric infusions of glucose were found to be much more effective than fructose (Sclafani et al., 1993), and maltodextrin infusions were more effective than corn oil (Lucas and Sclafani, 1999). Intragastric infusions of some nutrients (galactose) may even condition flavor avoidance in adult rats (Sclafani et al., 1999). The differential effectiveness of nutrients in preference conditioning may be related to differences in their postabsorptive rate of utilization and/or activation of pre- or postabsorptive nutrient-specific receptors. In the only intragastric study to compare ethanol directly with another nutrient, Sherman et al. (1983) reported that isocaloric glucose conditioned a stronger flavor preference than did ethanol (0.5-g/kg dose). The results are consistent with the finding that the ~70% CS+ preference observed with the nondeprived rats during acquisition is less than that typically produced by glucose or glucose polymer (maltodextrin) infusions (Elizalde and Sclafani, 1990). How ethanol compares with other nutrients that are less effective than glucose (e.g., corn oil and fructose) remains to be determined.

It is difficult to specify the rewarding quality of ethanol in the present study. The finding that intragastric ethanol, like other nutrients, can condition flavor preferences in rats is consistent with the hypothesis that energy-related signals mediate flavor conditioning by ethanol (Mehiel and Bolles, 1984; Sherman et al., 1983). The present data do not specifically support a ''caloric restoration'' hypothesis, however, because rats do not need to be energy-restricted in order to acquire flavor preferences with intragastric ethanol or other nutrients. Furthermore, nutrient-conditioned preferences can be obtained with calorically diluted infusions, which contribute very little to the daily energy intake of rats (Ackroff and Sclafani, 1994). Food restriction enhanced the ethanol-based preference, but food restriction is also reported to increase the reinforcing value of psychoactive drugs that are not nutritive (Carroll and Meisch, 1984). Deems et al. (1986) reported that water restriction blocks ethanol-conditioned flavor preferences. If water restriction does not block the reinforcing action of other drugs of abuse, this would provide more compelling support for a nutritional reward interpretation of ethanol-conditioned flavor preferences. The present data also do not exclude the possibility that central pharmacological actions may have mediated the preference conditioning by the intragastric ethanol infusions. The relatively low ethanol doses that the rats self-infused per bout and per day in acquisition suggest, however, that pharmacological actions may not have been the major factor responsible for the conditioned preference. Differentiation of the nutritive and drug effects of ethanol is a difficult issue that remains to be solved. However, it is not safe to assume that enhancement of ethanol acceptance is due only to an association between ethanol taste and its neuropharmacological activity (e.g., Slawecki and Samson, 1998).

The reduction in total energy intake by rats observed with the concentrated ethanol infusions does not mimic the effects of infusing other caloric solutions. Increased energy intake is typically observed with infusions of concentrated carbohydrate and fat solutions. The rats usually fail to reduce their chow intake sufficiently to compensate for the calories added by the infusion. Here, there was some overcompensation for ethanol calories, which has been shown previously (Luz et al., 1996). However, the acute effects of ethanol on feeding, such as the satiating (meal-terminating) effectiveness of premeal intragastric loads, are similar to those of carbohydrate (Seeley et al., 1997).

In contrast to the rat studies, the caloric contribution of ethanol appears to be poorly detected by humans. For example, consumption of both ethanol- and carbohydrate-

containing drinks did not reduce subsequent meal intake (Poppitt et al., 1996), and required daily drinks produced equivalent increases in total intake due to noncompensatory reductions in food intake (Foltin et al., 1993). Total energy intake also increases when ethanol is freely included in the diet (de Castro, 1993; de Castro and Orozco, 1990; Orozco and de Castro, 1991). It has been asserted that ethanol intake is a promotor of overeating and obesity in humans (see Suter et al., 1997).

In both humans and rats, overeating has been linked to the palatability of the ingested material. Rats offered carbohydrate solutions to drink do not compensate completely by reductions in chow intake, and therefore, overeat and gain weight (Sclafani, 1987). We have also observed greater self-infusion of carbohydrate solutions in rats that drank a palatable solution than those that drank an unpalatable solution (Sclafani et al., 1996). Although during acquisition, the rats in the present study drank more and were therefore infused with more ethanol when the CS+ was sweetened, in the concentration phase, they were tested with unsweetened CS+. It is possible that if a sweet CS+ was offered while ethanol concentration increased, greater ethanol intakes might have been obtained. In this context, it is interesting that a connection has been suggested between primate frugivory and ethanol intake (Dudley, 2000), implying that human alcoholism may be related in part to this historical nutritional strategy of consuming sweet, fermentable foods. This confluence may also be reflected in the strong association of attraction to sweetness with alcoholism (Kampov-Polevoy et al., 1999).

Sweetening may also have been important from a procedural perspective. The inclusion of saccharin in both flavors during training and its gradual removal ensured that the animals would consume enough fluid to detect the differential postingestive effects of the CS+ and CS−. The similar bout patterns suggest that the flavors were treated similarly as long as they contained saccharin. In an oral ethanol-conditioning study, Cunningham and Niehus (1997) found a preference for the CS− when both CS were sweetened with saccharin, but a switch to a CS+ preference when the saccharin was removed from the flavors. Because preference tests were not conducted with the saccharin-sweetened CS in the present study, it is not clear whether a similar effect would be obtained with intragastric conditioning. The rats consumed more sweetened CS− than sweetened CS+ during training, but this does not necessarily reflect a greater attraction to the sweetened CS− solution. Rather, the lower intakes of the sweetened CS+ solutions may have been due to the satiating action of the intragastric ethanol infusions. Had the ethanol infusions been aversive, the rats could have consumed less CS+ and more water, but the very low water intakes indicate that ethanol was not avoided.

The enhancement of CS− intake during the food restriction phase may have stemmed in part from relative similarity of the flavors, even after the common saccharin element had been removed. In the initial one-bottle sessions without concurrent food, the CS− solution intake of rats, while significantly less than CS+ intake, was greater than their water baseline intake. This contrasts with the equal intakes of CS− solution and water at the end of acquisition. One explanation for the elevated CS− intake may be generalization to the CS+. The two flavors have a common citric acid base and have been shown to support generalization (Pérez et al., 1998). The difference from acquisition may reflect the shift from ad libitum to food-restricted status, i.e., when energy intake was reduced to half by the restriction to 2-h chow access, generalization to the CS+, and a history of association with chow calories may have led to the elevated CS− intakes. Following the two-bottle preference test, however, the one-bottle CS− intake declined to water baseline levels. We have observed a similar effect in a study using these flavors paired with intragastric carbohydrate and water infusions (Pérez et al., 1998). Two-bottle experience with the CS+ and CS− flavors appears to increase their discriminability, and thereby, reduces the reward value of the CS−.

How do these data fit into the existing literature on conditioned ethanol effects? Assessment of place preferences conditioned by ethanol typically finds avoidance of the ethanol location in rats. Lower doses have sometimes yielded a null result, neither preference nor avoidance. With flavor associations, the few reports of preference already cited are outweighed by many reports of aversion to ethanol-paired flavors. While there are no outstanding procedural details, which clearly differentiate these studies, there are some factors that may be important.

A not surprisingly major factor is dose: the amount given per training trial in studies that yield aversions may be greater than what the animals would self-administer. Furthermore, in the typical aversion design, a drug dose is injected rapidly, unlike the more gradual effect of a self-administered bout in this study. These features are not defining, however, since orally self-administered ethanol can also yield aversions (Stewart and Grupp, 1986). However, the animals have typically been deprived of food or water to encourage ethanol intake during training trials, thus, pitting hunger or thirst against factors that might normally terminate a drinking bout sooner.

Other differences, which may be important for the preference/aversion outcome, are the relative timing of ethanol and cues and the nature of the test measure. In flavor aversion studies, a thirsty animal is trained to drink water in brief daily trials and injected with saline. Then, a flavored solution is presented, followed by ethanol injection (or saline for controls). The measure of conditioning is change in intake of the flavor over trials compared to controls. In studies that have demonstrated flavor preference, however, each animal is trained with two flavors, only one of which is paired with ethanol, and the measure is relative to the intake of the two flavors in a choice. The process of comparison may lead to a different outcome than the simpler choice of

how much to consume. In the place preference studies, the animal is injected with ethanol and placed in one side of a test chamber or injected with saline and placed on the other side. Here, the measure is more similar in a sense that the rats distribute their time between two locations, much as they distribute their drinking between two flavors. Yet, studies usually find no effect at lower doses and place aversion at doses of 0.8–1.0 g/kg and higher (Asin et al., 1985; Bormann and Cunningham, 1998; Van der Kooy et al., 1983). Previous experience with self-administration of ethanol (Bienkowski et al., 1996; Gauvin and Holloway, 1992; Reid et al., 1985) or provision of food in compartments during training (Stewart and Grupp, 1981, 1985) can facilitate subsequent place preferences. One way to interpret these exceptions is to postulate that factors, which minimize the responsiveness of animals to the aversive effect of ethanol, permit the expression of responses based on positive effects.

A recent hypothesis for the resolution of the apparent paradox that drugs of abuse often condition taste aversions (Hunt and Amit, 1987) suggests that animals reduce their intake of a flavor in anticipation of a more positive event (drug injection) that it predicts (Grigson, 1997). That is, the reward value of the flavor (usually a saccharin solution) is less attractive than the drug, and the animals reduce their intake, just as they do when saccharin predicts a preferred sucrose solution (e.g., Flaherty et al., 1994). It would appear at first that our results are contrary to this hypothesis, but an important difference is that, unlike the taste aversion paradigm, flavor intake is required rather than merely predictive for ethanol administration. If our animals gradually reduced their intake, they would receive less ethanol. Animals in taste aversion procedures receive a fixed dose after each saccharin trial, independent of the amount they consume. Ethanol remains to be tested in the anticipatory contrast procedure (Grigson, personal communication), but at sufficiently low doses, we would expect it to reduce saccharin intake in the same way as morphine and cocaine.

Flavor preferences produced by ethanol are fundamentally similar to those of carbohydrate and fat in this intragastric infusion procedure. This suggests that at least part of reinforcing capability of ethanol may reflect its energy content even in the absence of caloric need. These experiments were not designed to distinguish caloric and pharmacological contributions to ethanol reinforcement. It is possible that the responses of animals to the mixture of energy and drug effects will yield a picture of ethanol reinforcement that is distinct from that of other nutrients. This would not be surprising in view of the differences that have already been found between different macronutrients and even within nutrient classes (Sclafani, 1999). We are currently expanding the comparison of ethanol with other nutrients in a variety of flavor-preference paradigms, which should lead to a clearer view of ethanol reinforcement of flavor preferences.

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