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Autoshaping induces ethanol drinking in nondeprived rats: evidence of long-term retention but no induction of ethanol preference

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Abstract

The effects of autoshaping procedures (paired vs. random) and sipper fluid (ethanol vs. water) on sipper-directed drinking were evaluated in male Long-Evans rats maintained with free access to food and water. For the paired/ethanol group ($n = 16$), autoshaping procedures consisted of presenting the ethanol sipper (containing 0% to 28% unsweetened ethanol) conditioned stimulus (CS) followed by the response-independent presentation of food unconditioned stimulus (US). The random/ethanol group ($n = 8$) received the sipper CS and food US randomly with respect to one another. The paired/water group ($n = 8$) received only water in the sipper CS. The paired/ethanol group showed higher grams per kilogram ethanol intake than the random/ethanol group did at ethanol concentrations of 8% to 28%. The paired/ethanol group showed higher sipper CS-directed milliliter fluid consumption than the paired/water group did at ethanol concentrations of 1% to 6%, and 15%, 16%, 18%, and 20%. Following a 42-day retention interval, the paired/ethanol group showed superior retention of CS-directed drinking of 18% ethanol, relative to the random/ethanol group, and superior retention of CS-directed milliliter fluid drinking relative to the paired/water group. When tested for home cage ethanol preference using limited access two-bottle (28% ethanol vs. water) procedures, the paired/ethanol and random/ethanol groups did not differ on any drinking measures.

Keywords: Pavlovian; Autoshaping; Ethanol; Retention; Preference; Pseudoconditioning; Rats

1. Introduction

Human beings drink ethanol from containers or glassware that may serve as conditioned stimuli (CSs) when paired repeatedly with unconditioned stimuli (USs), such as the eating of palatable foods. If ethanol glassware or sippers can serve as CSs for food reward USs, then, human beings may develop reflexive and involuntary drinking from the ethanol sipper CS through a process similar with Pavlovian autoshaping (Tomie, 1995, 1996, 2001). Recently, Tomie et al. (2002a,b) have provided evidence that autoshaping may contribute to the initiation and escalation of ethanol drinking in rats. Their autoshaping procedures closely resemble those employed by Pavlovian autoshaping investigators, who provide for the brief presentation of a localized visual stimulus, conditioned stimulus (CS, e.g., lever), followed closely by the response-independent presentation of a rewarding substance, unconditioned stimulus (US, e.g., food). Repeated CS–US pairings induce acquisition of the Pavlovian autoshaping conditioned response (CR), which is a complex sequence of directed motor responses targeted at the CS (Brown and Jenkins, 1968; Tomie et al., 1989). Important to the understanding of autoshaping, the performance of the autoshaping CR is not required to procure the US; rather, the rewarding US is delivered regardless of whether the rat contacts the CS.

Tomie et al. (2002a,b) modified the autoshaping apparatus by replacing the retractable lever with a retractable sipper tube CS. The insertion of the sipper tube CS into the chamber immediately before the response-independent delivery of the food US induced sipper CS-directed approach and contact responses, culminating in mouthing, chewing, licking, and swallowing responses, resulting in drinking of the 6% ethanol (vol./vol.) solution in the sipper CS. Several effects suggest that autoshaping contributes to ethanol drinking. For example, the autoshaping of sipper-CS-directed ethanol drinking increases as a function of experience with sipper CS–food pairings (Tomie et al., 2002a,b), and more ethanol drinking is observed in groups receiving pairings of CS and US than in controls receiving CS and US randomly with respect to one

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another (Tomie et al., 2002a, Experiments 1 and 2), suggesting that autoshaping induces ethanol drinking beyond that due to pseudoconditioning. In addition, asymptotic drinking rates are negatively related to sipper-CS duration (Tomie et al., 2002b), and drinking is retained across a 27-day retention interval (Tomie et al., 2002b). In addition, these effects are also consistent with autoshaping analysis of CS-directed drinking. Finally, autoshaping of ethanol drinking results in elevated postsession blood ethanol levels (Tomie et al., 2002b, 2003).

In previous studies reporting the autoshaping of ethanol drinking, rats were food deprived, and the autoshaping of sipper-CS-directed drinking was initiated by employing a sweetened ethanol solution [6% ethanol (vol./vol.) in 0.1% saccharin]. Across sessions, the concentration of saccharin was gradually reduced (saccharin-fading procedure) until the rat was drinking unsweetened 6% ethanol from the sipper CS. In the present study, the rats are maintained with free access to food and water and were evaluated for autoshaping of CS-directed drinking without using saccharin-fading procedures. The autoshaping of CS-directed drinking is initiated using water (0% ethanol) in the sipper CS, and thereafter, the concentration of ethanol in the sipper CS is systematically increased across the autoshaping sessions. The durability of sipper-CS-directed ethanol drinking is evaluated by testing for drinking following a 42-day retention interval, and the issue of whether the autoshaping procedures establish a preference for ethanol drinking outside of the autoshaping situation is addressed by evaluating drinking in the home cage using two-bottle (28% ethanol vs. water) limited access procedures. Thus, the procedures employed in the present experiment will allow, for the first time, an evaluation of the effects of autoshaping procedures on the initiation of sipper-CS-directed drinking of unsweetened ethanol in rats deprived of neither food nor water, as well as the escalation of ethanol intake using higher concentrations (up to 28%) than had previously been evaluated (up to 16%). In addition, the present experiment will evaluate the retention of autoshaping of ethanol drinking across a longer retention interval (42 vs. 27 days) by comparing drinking before and after the retention interval in the paired/ethanol and random/ethanol groups. Finally, this study will evaluate postautoshaping two-bottle ethanol preference, assessed outside of the autoshaping situation (in the home cage).

2. Materials and methods

2.1. Subjects

Thirty-two male Long-Evans (Blue Spruce strain) rats (310–330 g at the beginning of testing) from Harlan–Sprague–Dawley (Almont, NY) were house individually in suspended steel cages in a colony room with a 12-L:12-D (on 0400 h) cycle. The rats had continuous access to food (PMI Rat Chow, Formula 5012) and water in their home cages. All animal handling procedures were approved by the Institutional Animal Care Review Board of Rutgers University and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) (Publication No. 85-23, revised 1985).

2.2. Apparatus

The autoshaping chambers were four cubicles ($32 \times 25.5 \times 23$ cm each), purchased from MED Associates (Lafayette, IN), made of stainless steel walls, a stainless steel grid floor (Model ENV-008), clear Plexiglas back wall and ceiling, and a Plexiglas front panel that opened with a side latch. A house light (GE 1821) was mounted to the top–middle portion of the right wall of the cubicle. On the opposite wall, a pellet dispenser delivered 4-mg food pellets (Formula 0021, approximately 50% sucrose, BioServ, Frenchtown, NJ) to a metal pellet dispenser trough (Model ENV-200R2M) placed 2.0 cm from the back wall and 3.5 cm above the grid floor. A retractable stainless steel sipper tube (Model 58320, Kimble-Kontes, Vineland, NJ) delivered the solution into the chamber 3 cm from the front Plexiglas panel and 3.5 cm above the grid floor. This stainless steel sipper tube contained a stainless steel ball bearing with an inserted rubber stopper that held the solution in a 400-ml Plexiglas bottle. The bottle insertion mechanism moved the sipper tube 3.8 cm from fully retracted to fully inserted position. In the fully retracted position, the sipper tube was 3.2 cm removed from the chamber. Each testing chamber was enclosed in sound-attenuating, ventilated outer casings (Model ENV-022). An IBM PC, equipped with a relay interface card (Model DIG-750C), cabled to a connection panel (Model SG-215D), and operating under locally developed software, controlled the session events and data collection.

2.3. Drugs

Ethanol solutions were made volume to volume (vol./vol.) by diluting 95% ethyl alcohol (Rutgers University, Chemical Stores, New Brunswick, NJ, USA) with tap water.

2.4. Autoshaping procedures

The rats were run 5–6 days a week in daily autoshaping sessions conducted between 0900 and 1600 h. The rats were weighed immediately prior to each autoshaping session and then immediately placed in the autoshaping chamber. Prior to the initiation of the study, the rats were randomly assigned to one of three groups. Sixteen rats were assigned to the paired/ethanol group, eight rats were assigned to the random/ethanol group, and eight rats were assigned to the paired/water group. More rats were assigned to the paired/ethanol group than to either of the two control groups because the paired/ethanol group was the experimental

group that would provide the basis for all comparisons with the two orthogonal control conditions. For the *paired* groups, the sipper tube CS was inserted for 5 s, followed immediately by operation of the pellet dispenser US. Subjects in the *random* group received similar training, except that the sipper tube CS and the food pellet US were operated randomly with respect to one another. Thus, for the random/ethanol group, the probability of delivery of the food US during each 5-s period, during which the sipper CS was inserted, was 0.083, and the probability of delivery of the food US during each 5-s period, during which the sipper CS was not inserted, was also 0.083. For the random/ethanol group, therefore, approximately 2.08 times per autoshaping session, the food US, by chance, was presented during the 5-s period that the sipper CS was inserted. For all groups, the delivery of the food pellet US occurred regardless of whether the subject contacted the sipper CS or consumed the liquid in the sipper CS. All groups received a total of 25 trials per autoshaping session. The mean intertrial interval (ITI) duration was 60 s, with a minimum of 45 s and a maximum of 75 s. The session duration was approximately 30 min. The volume of fluid consumed (ml) during each autoshaping session was determined by recording the volume in the tube immediately before and after each session.

During the first 10 days of autoshaping, the sipper CS contained water (0% ethanol) for all three groups. For the remainder of the experiment, the sipper CS contained water for the paired/water group. For the *ethanol* groups, the concentration of ethanol in the sipper CS was systematically increased by 1% (vol./vol.) after every three autoshaping sessions, up to a concentration of 17%. For the *ethanol* groups, the sipper CS contained 18% ethanol for four daily sessions (Days 62–65), after which all groups received no autoshaping training for the duration of a 42-day retention interval (Days 66–107). During this time, all the rats were housed in their home cages in the colony room with free access to food and water. On Day 108, all rats received the first of four autoshaping sessions using procedures identical to those given prior to the 42-day retention interval. Thereafter, for the *ethanol* groups, the sipper CS contained 18% ethanol for six additional sessions (Days 112–117), then 20% ethanol (vol./vol.) for four autoshaping sessions (Days 118–121), and then the concentration of ethanol in the sipper CS was increased by 2% after every four autoshaping sessions. After the completion of the fourth autoshaping session with 28% ethanol, all rats were given the first of five daily two-bottle drinking tests in the home cage using limited access procedures. The two-bottle drinking test was conducted for 1 h/day between 1200 and 1300 h. During the test, each rat was given access in the home cage to two stainless steel sipper tubes attached to Plexiglas bottles (200-ml capacity). The position of the sipper tubes was randomized across days. One of the tubes contained 28% ethanol, while the remaining tube contained water. Volume drinking from each tube was determined by weighing each tube before and after each session.

2.5. Data analysis

For each subject, for each autoshaping session, the following data were obtained: milliliter fluid consumed, body weight, and grams per kilogram of ethanol intake. For each subject in each group, we derived the mean of the last 3 days for each ethanol concentration. The effects of the 42-day retention interval were evaluated by deriving for each subject in each group the mean of the 4 days of training with 18% ethanol before (Days 62–65) and the first 4 days of training with 18% ethanol after (Days 108–111) the retention interval. The effects of ethanol concentration and of retention interval on mean daily volume of fluid consumed and mean grams per kilogram ethanol intake were each assessed by repeated-measures, multivariate analysis of variance (MANOVA, SYSTAT). To assess the retention of drinking despite different baseline levels of drinking before the retention interval, each subject's drinking after the retention interval was expressed as a proportion of its preretention baseline by calculating a suppression ratio using the following formula: suppression ratio = (mean drinking before the retention interval)/[(mean drinking after the retention interval)+(mean drinking before the retention interval)]. The effects of the retention interval on the mean suppression ratios were assessed by univariate analysis of variance (ANOVA, SYSTAT). Fisher's LSD provided pairwise comparisons at individual points ($\alpha = .05$). To derive each rat's mean daily ethanol preference, that rat's daily milliliter ethanol drinking was divided by that rat's daily total drinking (ml ethanol drinking + ml water drinking).

3. Results

3.1. Acquisition of autoshaping of water drinking

During Autoshaping Days 1–10, all three groups showed systematic increases in milliliter of water drinking from the sipper CS. Mean milliliter drinking on Day 1 was between 0.25 and 0.75 ml for all groups, while on Day 10, mean milliliter drinking was between 1.70 and 2.17 ml for all groups. Analysis revealed a significant main effect of days [$F(9,261) = 13.52$, $P < .01$] but no effects of groups [$F(2,29) = 1.26$, $P > .05$].

3.2. Effects of ethanol concentration

The analysis of the effects of ethanol concentration [1% to 28% ethanol (vol./vol.)] on mean daily gram per kilogram ethanol intake for the paired/ethanol and random/ethanol groups revealed a significant main effect of groups [$F(1,22) = 6.17$, $P < .05$], a significant main effect of concentrations [$F(22,484) = 19.03$, $P < .01$], and a significant interaction effect between groups and concentrations [$F(22,484) = 2.66$, $P < .01$]. Fisher's LSD revealed that the paired/ethanol group had higher ethanol intake than the

random/ethanol group did when the sipper CS contained ethanol concentrations of 8% to 28% (Fig. 1).

The analysis of the effects of ethanol concentration [1% to 28% ethanol (vol./vol.)] on the mean daily milliliter drinking for the paired/ethanol and random/ethanol groups revealed a significant main effect of groups [$F(1,22)=4.59$, $P<.05$], a significant main effect of concentrations [$F(22,484)=9.73$, $P<.01$], and a significant interaction effect between groups and concentrations [$F(22,484)=2.34$, $P<.01$]. Fisher's LSD revealed that the paired/ethanol group had higher daily mean milliliter drinking than the random/ethanol group did when the sipper CS contained ethanol concentrations of 9% to 28% (Table 1). There was a 42-day retention interval during training with the 18% ethanol (vol./vol.) solution (indicated by the row marked Retention interval), and Table 1 depicts the mean of the last 3 days of autoshaping with the 18% ethanol (vol./vol.) after the retention interval (Days 114–117).

To compare the daily mean milliliter drinking in the paired/ethanol and paired/water groups at ethanol concentrations comparable with those employed in previous autoshaping studies employing food-deprived rats (Tomie et al., 2002b, 2003, Experiment 2), an analysis was performed comparing the mean daily milliliter fluid consumption in the paired/ethanol and paired/water groups when the sipper CS for the paired/ethanol group contained 1% to 6% ethanol (Table 1). This analysis revealed a significant main effect of groups [$F(1,22)=4.37$, $P<.05$], no significant main effect of concentrations [$F(5,110)=2.02$, $P>.05$], and no significant interaction effect between groups and concentrations [$F(5,110)<1$, $P>.05$]. To compare the daily mean milliliter drinking in the paired/ethanol and paired/water groups when the paired/ethanol group received ethanol concentrations higher than 6% in the sipper CS, an analysis was performed on the daily mean milliliter drinking when the paired/ethanol group received training with the 17 concentrations from 7% through 28% ethanol in the sipper CS. This analysis revealed no significant main effect of groups [$F(1,22)>1$, $P>.05$], no significant main effect of concentrations [$F(16,352)<1$, $P>.05$], and a significant interaction effect between groups and concentrations [$F(16,352)=1.83$, $P<.05$]. Fisher's LSD revealed that the paired/ethanol group had higher mean milliliter fluid consumption than the paired/water group did at ethanol concentrations of 15%, 16%, 18%, and 20% (Table 1).

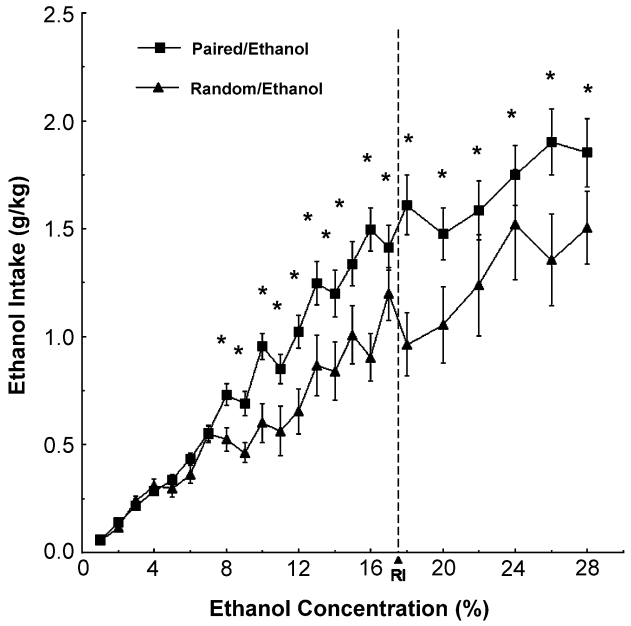

Fig. 1. Mean daily gram per kilogram ethanol intake during the last three sessions of autoshaping with each ethanol concentration [1% to 28% ethanol (vol./vol.)] in the sipper CS for the paired/ethanol ($n=16$) and random/ethanol ($n=8$) groups. The vertical bars represent the S.E.M., and the asterisk (*) indicates that the observed group differences are significant at the .05 confidence level (Fisher's LSD). There was a 42-day retention interval (RI in the figure) during training with the 18% ethanol (vol./vol.) solution (indicated by the dotted vertical line). The graph depicts the mean of the last 3 days of autoshaping with the 18% ethanol (vol./vol.) after the retention interval (Days 114–117).

Table 1
Mean daily fluid consumption (ml) across concentration

ET (%)	Groups		
	P/E	R/E	P/W
1	2.08 ± 0.17	1.98 ± 0.25	1.80 ± 0.23#
2	2.55 ± 0.16	2.20 ± 0.23	1.89 ± 0.23#
3	2.68 ± 0.19	3.06 ± 0.27	2.19 ± 0.27#
4	2.73 ± 0.18	3.03 ± 0.27	1.88 ± 0.26#
5	2.66 ± 0.23	2.34 ± 0.32	2.24 ± 0.32#
6	2.86 ± 0.22	2.45 ± 0.31	2.62 ± 0.32#
7	3.17 ± 0.22	3.16 ± 0.31	3.55 ± 0.31
8	3.72 ± 0.25	2.74 ± 0.36 *	3.21 ± 0.36
9	3.18 ± 0.23	2.15 ± 0.32 *	3.54 ± 0.32
10	4.03 ± 0.26	2.54 ± 0.37 *	3.54 ± 0.37
11	3.31 ± 0.28	2.24 ± 0.39 *	3.34 ± 0.39
12	3.70 ± 0.26	2.38 ± 0.37 *	3.61 ± 0.47
13	4.23 ± 0.33	3.03 ± 0.47 *	3.61 ± 047
14	3.83 ± 0.33	2.76 ± 0.46 *	3.53 ± 0.46
15	4.08 ± 0.31	3.15 ± 0.43 *	3.40 ± 0.43†
16	4.36 ± 0.29	2.66 ± 0.41 *	3.33 ± 0.41†
17	3.99 ± 0.27	3.34 ± 0.38 *	3.41 ± 0.38
Retention ± interval			
18	4.53 ± 0.35	2.75 ± 0.49 *	3.50 ± 0.49†
20	3.79 ± 0.32	2.70 ± 0.45 *	2.91 ± 0.45†
22	3.69 ± 0.35	2.88 ± 0.49 *	3.49 ± 0.49
24	3.77 ± 0.32	3.28 ± 0.46 *	3.39 ± 0.46
26	3.83 ± 0.32	2.70 ± 0.45 *	3.83 ± 0.45
28	3.49 ± 0.28	2.84 ± 0.39 *	3.00 ± 0.39

* Paired/ethanol>random/ethanol. $P<.05$ (Fisher's LSD).
† Paired/ethanol>paired/water. $P<.05$ (Fisher's LSD).
# Paired/ethanol>paired/water. $P<.05$ based on main effect of groups.

3.3. Effects of the retention interval

The analysis of the effects on group mean gram per kilogram ethanol intake during the 4 days immediately preceding and following the 42-day retention interval for the paired/ethanol and random/ethanol groups revealed a significant main effect of groups [$F(1,22)=6.82$, $P<.05$], a

significant main effect of retention interval [$F(1,22) = 5.18$, $P < .05$], and a significant interaction effect between groups and retention interval [$F(1,22) = 5.46$, $P < .05$]. Fisher's LSD revealed that the paired/ethanol group had higher mean gram per kilogram ethanol intake relative to the random/ethanol group during the last 4 days before the retention period and during the first 4 days after the retention period (Fig. 2). The mean suppression ratios ($\pm$ S.E.M.) for gram per kilogram ethanol intake for the paired/ethanol and random/ethanol groups were 0.50 ± 0.02 and 0.44 ± 0.01, respectively. Analysis revealed that this difference was significant [$F(1,22) = 8.16$, $P < .01$].

Group mean milliliter drinking ($\pm$ S.E.M.) during the 4 days immediately before and after the 42-day retention interval for the random/ethanol group were 3.18 ($\pm$ 0.42) and 2.67 ($\pm$ 0.45), respectively. Group mean milliliter drinking ($\pm$ S.E.M.) during the 4 days immediately before and after the 42-day retention interval for the paired/ethanol and paired/water groups are presented in Fig. 3. The analysis of the effects on group mean milliliter drinking during the 4 days immediately before and after the 42-day retention interval for the paired/ethanol and paired/water groups revealed no significant main effect of groups [$F(1,22) = 4.12$, $P > .05$], no significant main effect of retention interval [$F(1,22) = 1.65$, $P > .05$], and a significant interaction effect between groups and retention interval [$F(1,22) = 6.49$, $P < .05$]. Fisher's LSD revealed that the paired/ethanol group had higher mean milliliter drinking after the retention period (Fig. 3).

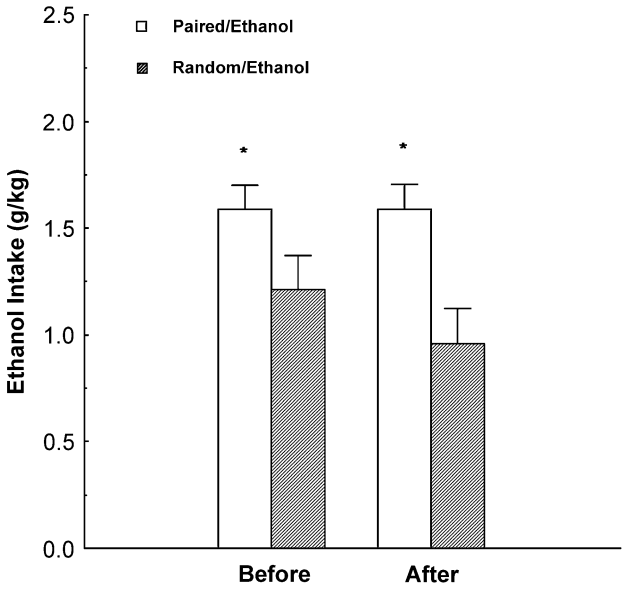

Fig. 2. Mean daily gram per kilogram ethanol intake of 18% ethanol (vol./vol.) for the paired/ethanol ($n = 16$) and the random/ethanol ($n = 8$) groups during the 4 days before (Days 62–65) and the 4 days after (Days 108–111) the 42-day retention interval. The vertical bars represent the S.E.M., and the asterisk (*) indicates that the observed group differences are significant at the .05 confidence level (Fisher's LSD).

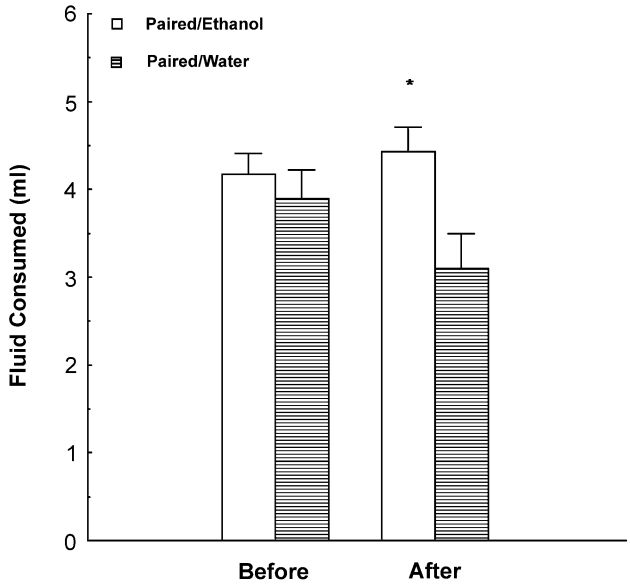

Fig 3. Mean daily milliliter fluid consumed from the sipper CS for the paired/ethanol ($n = 16$; sipper CS contained 18% ethanol) and the paired/water ($n = 8$; sipper CS contained water) groups during the 4 days before (Days 62–65) and the 4 days after (Days 108–111) the 42-day retention interval. The vertical bars represent the S.E.M., and the asterisk (*) indicates that the observed group differences are significant at the .05 confidence level (Fisher's LSD).

The mean suppression ratios for milliliter fluid consumed for the paired/ethanol, paired/water, and random/Ethanol groups were 0.51 ($\pm$ 0.01), 0.45 ($\pm$ 0.02), and 0.45 ($\pm$ 0.02), respectively. The analysis comparing the suppression ratios for the paired/ethanol and paired/water groups revealed that this difference was significant [$F(1,22) = 5.65$, $P < .05$]. Analysis comparing the suppression ratios for the paired/ethanol and random/ethanol groups revealed that this difference was significant [$F(1,22) = 10.33$, $P < .01$].

3.4. Ethanol preference

The mean daily milliliter drinking ($\pm$ S.E.M.) of 28% ethanol for the paired/ethanol, random/ethanol, and paired/water groups during the last 3 of the 5 days of the two-bottle drinking test were 2.39 ($\pm$ 0.15), 1.93 ($\pm$ 0.22), and 1.98 ($\pm$ 0.25) respectively. The difference between the paired/ethanol and random/ethanol groups was not significant [$F(1,22) = 3.06$, $P > .05$]. The difference between the paired/ethanol and paired/water groups was not significant [$F(1,22) = 1.85$, $P > .05$]. During this period, mean daily gram per kilogram ethanol intakes ($\pm$ S.E.M.) for the paired/ethanol, random/ethanol, and paired/water groups were 1.26 ($\pm$ 0.09), 1.03 ($\pm$ 0.12), and 0.99 ($\pm$ 0.11) respectively. The difference between the paired/ethanol and random/ethanol groups was not significant [$F(1,22) = 2.44$, $P > .05$]. The difference between the paired/ethanol and paired/water groups was not significant [$F(1,22) = 3.54$, $P > .05$]. During this period, the mean daily milliliter water

drinking (± S.E.M.) for the paired/ethanol, random/ethanol, and paired/water groups were 1.24 (± 0.25), 1.19 (± 0.35), and 2.48 (± 0.32), respectively. The difference between the paired/ethanol and random/ethanol groups was not significant [$F(1,22) < 1$, $P > .05$]. The difference between the paired/ethanol and paired/water groups was significant [$F(1,22) = 10.25$, $P < .05$]. During this period, the mean total daily milliliter fluid drinking [ml 28% ethanol + ml water (± S.E.M.)] for the paired/ethanol, random/ethanol, and paired/water groups were 3.63 (± 0.27), 3.12 (± 0.38), and 4.46 (± 0.49), respectively. The difference between the paired/ethanol and random/ethanol groups was not significant [$F(1,22) < 1$, $P > .05$]. The difference between the paired/ethanol and paired/water groups was not significant [$F(1,22) = 1.88$, $P > .05$]. The mean daily ethanol preferences [ml ethanol drinking/ml total drinking (± S.E.M.)] for the paired/ethanol, random/ethanol, and paired/water groups were 0.68 (± 0.05), 0.68 (± 0.07), and 0.44 (± 0.04), respectively. The difference between the paired/ethanol and random/ethanol groups was not significant [$F(1,22) < 1$, $P > .05$]. The difference between the paired/ethanol and paired/water groups was significant [$F(1,22) = 23.37$, $P < .01$].

4. Discussion

A higher mean daily gram per kilogram ethanol intake was observed in the paired/ethanol group than in the random/ethanol group at ethanol concentrations of 8% to 28%, extending the range of ethanol concentrations at which this difference has been observed (Tomie et al., 2002a). Sipper CS–food US pairings induced ethanol drinking beyond that due to nonassociative factors related to pseudoconditioning, and this effect of autoshaping CR performance of sipper-CS-directed ethanol drinking was observed across a broad range of higher ethanol concentrations. In addition, higher mean daily milliliter drinking was observed in the paired/ethanol group than in the random/ethanol group at ethanol concentrations of 9% to 28%, and this extends the range of ethanol concentrations at which this difference has been observed.

Our previous reports showing more drinking when the sipper CS contained up to 6% ethanol than water employed food-deprived rats (Tomie et al., 2002b, 2003, Experiment 2), allowing for the possibility that this effect was due to the caloric value of ethanol (Heyman, 1993, 1997; Samson et al., 2000). The present study employed rats that are not food deprived, and the paired/ethanol group again showed more sipper-CS-directed drinking when the sipper CS contained up to 6% ethanol than did the paired/water controls, an effect unlikely due to foraging for calories. In addition, the nondeprived rats in the present study showed more sipper-CS-directed drinking when the sipper CS contained ethanol concentrations of 1% to 6% and, in addition, even higher concentrations of ethanol (15%, 16%, 18% and 20%), extending the range of ethanol concentrations at which this effect has been observed. The comparisons of drinking in the paired/ethanol and paired/water groups are confounded by the continuous availability of water in the home cage for all subjects. Thus, lower levels of drinking in the paired/water group relative to the paired/ethanol group may be due to the differences in novelty of the fluid in the sipper CS. To evaluate the effects of ethanol in the sipper CS on the autoshaping of ethanol drinking, independent of the novelty of ethanol, the *paired* and *random* groups, using a novel substance in the sipper CS rather than water, must be compared with the *paired* and *random* groups using ethanol in the sipper CS.

An intriguing interpretation of the enhanced drinking observed when the sipper CS contains ethanol, as compared with water, is based on the possibility that ethanol's pharmacological effect is to facilitate the autoshaping CR performance. For example, Tomie et al. (1998) observed that presession injections of ethanol increased, in a dose-dependent fashion, the lever-CS-directed autoshaping CR performance when lever CS was paired with food US. In studies of autoshaping employing ethanol sipper CS (Tomie et al., 2002a,b, 2003), the augmenting of sipper CS-directed autoshaping by ethanol would serve to further increase ethanol intake. Thus, autoshaping-induced ethanol drinking may provide the basis for a positive feedback loop, conducive to episodes of poorly controlled and exaggerated ethanol intake (Tomie, 1995, 1996).

It is important to note that autoshaping provides a Pavlovian conditioning, rather than operant or instrumental, model of ethanol drinking. The autoshaping model is intended to evaluate the effects of noncontingent pairings of ethanol sipper and food on ethanol drinking. The drinking induced by the autoshaping technique, therefore, is due solely to the experience of ethanol sipper then food, and does not necessarily reflect on the positively reinforcing effects of ethanol. The present studies were not designed to effectively isolate ethanol's positively reinforcing effect, or to provide information as to the environmental conditions most conducive to the expression of the positively reinforcing effects of ethanol (Samson et al., 2000). Although the assessment and analysis of the positively reinforcing effects of ethanol is an extremely important and complex issue, it remains orthogonal to the purpose of this study, which is to characterize ethanol drinking induced by autoshaping procedures.

Alternative models of ethanol drinking in rats also arrange for the drinking of ethanol to be accompanied by the presence of food. For example, prandial drinking models of ethanol drinking provide for ethanol availability after the eating of large amounts of food (Cunningham and Niehus, 1997; Meisch and Thompson, 1974; Neill et al., 1994), and schedule-induced polydipsia (SIP) models of ethanol drinking provide for intermittent schedules of food presentations in a situation where ethanol is also available (Colotla and Keehn, 1975; Falk et al., 1972; Hymowitz and Freed, 1974;

McMillan et al., 1976; Riley et al., 1979). Therefore, postingestive prandial drinking or schedule induction effects may contribute to drinking observed in autoshaping procedures. Although these factors may contribute to the pseudoconditioning of drinking observed in the random controls, it is unlikely that they account for the additional drinking observed in the paired condition. This is because drinking in the paired procedures occurs only in the brief intervals of time just before the ingestion of food, whereas the vast majority of drinking induced by prandial drinking or SIP procedures occurs during the postingestive intervals after the food has been consumed.

The function relating the mean daily milliliter ethanol drinking to ethanol concentration for the paired/ethanol group was biphasic in form (see Table 1), with the peak daily drinking volumes observed in the range of ethanol concentrations of 13% to 18%. Although the mean daily drinking volumes declined somewhat when higher concentrations of ethanol were employed, the function relating the mean daily gram per kilogram ethanol intake to ethanol concentration (see Fig. 1) was monotonic and ascending in form, with higher concentrations of ethanol yielding higher mean daily gram per kilogram ethanol intakes. The decline in mean daily drinking volumes at higher ethanol concentrations may be due to several factors including mild gastrointestinal distress, mild euphoria, changes in taste, or gross motor impairment (Agabio et al., 2000a).

These data reveal that the autoshaping of ethanol drinking is well retained across a 42-day retention interval. Our previous study evaluating the retention of autoshaping of ethanol drinking employed a 27-day retention interval and found superior retention in the paired/ethanol relative to the paired/water group (Tomie et al., 2002b). These data suggest that the autoshaping of ethanol drinking is better retained than is the autoshaping of water drinking. An alternative interpretation is based on the alcohol deprivation effect (ADE; Agabio et al., 2000b; Samson and Chappell, 2001; Sinclair and Senter, 1968; for review, see Li, 2000). The ADE is an increase in ethanol drinking following a period of abstinence and may account for the differences in retention of drinking between the paired/ethanol and paired/water groups observed here.

On the other hand, it should be noted that the ADE would serve to increase ethanol drinking after the retention interval in the random/ethanol group, reducing the deleterious effects of the retention interval on ethanol drinking in that group. The present study shows superior retention, as assessed by the suppression ratio, by the paired/ethanol relative to the random/ethanol group, indicating that the retention of the autoshaping of ethanol drinking is more durable than is the retention of sipper-CS-directed drinking induced by nonautoshaping procedures. This suggests that the retention of sipper-CS-directed autoshaping CR performance is not due merely to experience with ethanol (i.e., ADE) or to other factors related to pseudoconditioning, and this is consistent with reports of CR retention observed by other Pavlovian investigators (Mackintosh, 1974).

These data provide the first evaluation of the transfer of ethanol drinking induced by autoshaping procedures to situations outside of the autoshaping apparatus. In the home cage, the paired/ethanol and random/ethanol groups did not differ in ethanol intake or ethanol preference, suggesting that autoshaping per se induces neither additional ethanol drinking or ethanol preference outside of the autoshaping apparatus relative to the control group, which also drank ethanol in the autoshaping apparatus. On the other hand, relative to the paired/water control group, the paired/ethanol group showed lower daily mean milliliter drinking of water and higher preference for 28% ethanol over water. These comparisons, therefore, indicate that prior experience with ethanol drinking per se in the autoshaping apparatus induces a preference for ethanol outside of the autoshaping apparatus, while water controls exhibit a preference for water outside the autoshaping apparatus. This implicates acclimation to ethanol drinking during training in the autoshaping apparatus as the factor responsible for ethanol preference over water in the home cage.

It should be noted that during the daily two-bottle preference test in the home cage, the paired/ethanol and random/ethanol groups drank less 28% ethanol than during the daily autoshaping session, even though the total duration of access to the 28% ethanol solution was much briefer in the autoshaping chamber (125 s) than in the home cage (1 h). This suggests that although drinking of ethanol transfers somewhat to situations outside of the autoshaping apparatus, the total amount of ethanol drinking observed outside of the autoshaping apparatus is far lower than is observed in the autoshaping apparatus. The precise conditions responsible for the high levels of ethanol drinking in the autoshaping apparatus by both the paired/ethanol and random/ethanol groups may be due to the autoshaping of ethanol drinking induced by the pairings of the sipper CS with ethanol US in both groups (Tomie, 1995, 1996).

The data of the present study show initiation and escalation of ethanol intake in rats by Pavlovian autoshaping procedures. The rapid drinking of large volumes of ethanol in a brief period of time is characteristic of binge drinking in human beings; thus, these autoshaping procedures induce binge-like drinking and do so merely by providing for repeated pairings of the ethanol sipper with food. Ethanol-abuse researchers have extensively documented the Pavlovian conditioning of physiological responses and affective or emotional reactions to ethanol-related glassware CSs in human beings (Rohsenow et al., 1992). Thus far, however, ethanol-related glassware has not been evaluated experimentally as a CS for autoshaping in human beings. To the degree that, in human beings, the ethanol sipper is differentially paired with the eating of food or with other highly rewarding and preferred activities, it seems plausible that the Pavlovian conditioning of sipper CS-directed autoshaping

CRs may develop and serve to mediate the induction of reflexive and involuntary ethanol drinking, resulting in excessive and compulsive bouts of binge-like ethanol intake (Tomie, 1995, 1996, 2001).

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