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Effects of removing food on maintenance of drinking initiated by pairings of sipper and food

Arthur Tomie *, Nicu R. Costea, Kashfia Vohra, Larissa A. Pohorecky

Department of Psychology, Rutgers University, 152 Freylinghuysen Road, Piscataway, NJ 08854–8020, USA
Center of Alcohol Studies, Rutgers University, 607 Allison Road, Piscataway, NJ 08854–8001, USA

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ABSTRACT

Two experiments evaluated the effects of removing food presentations on the maintenance of drinking induced by experience with sipper — food pairings. In Exp 1, ethanol drinking was induced in non-deprived Long-Evans rats by Pavlovian conditioning procedures employing an ethanol sipper as conditioned stimulus (CS) and food pellet as unconditioned stimulus (US). The Paired/Ethanol group received presentations of the ethanol sipper CS followed immediately by the response-independent presentation of the food pellet US. The Random/Ethanol group received the ethanol sipper CS and food US randomly with respect to one another. For both groups, the concentration of ethanol in the sipper CS [(3%, 4%, 6%, 8% (vol./vol.)] was increased across sessions, and, as in previous studies employing low concentrations of ethanol in non-deprived rats (i.e., maintained with free access to food in their home cages), the two procedures induced comparable levels of sipper CS-directed ethanol drinking. Removing food US presentations had no effect on sipper CS-directed ethanol drinking in either group. In Exp 2, groups of non-deprived Long-Evans rats were trained either with water or ethanol in the sipper CS paired with food US. Removing food US presentations had no effect on ethanol drinking in the Paired/Ethanol group, but water drinking in the Paired/Water group declined systematically across sessions. Results indicate that food US presentations contribute to the maintenance of water drinking but not to the maintenance of ethanol drinking. Implications for accounts of ethanol drinking based on Pavlovian sign-tracking, behavioral economics and intermittent sipper procedures are considered.

1. Introduction

There is abundant evidence that rats are readily induced to initiate and maintain ethanol drinking from sippers presented in the home cage (for reviews see Pohorecky, 1977, 1990; Wolffgramm and Heyne, 1995), but outside of their home cage (i.e., in drinking, testing, or operant chambers), the initiation of ethanol drinking is far more problematic. For example, investigators have employed food-deprivation (for review see Falk and Tang, 1988), use of sweeteners (Samson and Falk, 1974; Samson, 1986; Koob and Weiss, 1990; Heyman, 1993; Roberts et al., 1998) or ethanol exposure in the home cage (Samson, 1986; Glasner et al., 2005) to facilitate the initiation of ethanol drinking in rats outside of the home cage. While enabling investigators to study the effects of a broader range of environmental and experimental variables in the testing chamber, the use of these alcohol drinking facilitation techniques complicate the interpretation of ethanol drinking data (Holman and Myers, 1968; Kampov-Polevoy et al., 1995; Gahtan et al., 1996; Lau et al., 1996).

More recently, Tomie and his associates have reported that Pavlovian pairings of sipper conditioned stimulus (CS) with food unconditioned stimulus (US) reliably induces CS-directed sign-tracking conditioned response (CR) of robust ethanol drinking from the sipper CS (Tomie, 1995, 1996; Tomie et al., 2008). Most significantly, the Pavlovian sign-tracking procedures that are conducted in operant drinking chambers induce the reliable initiation and maintenance of ethanol drinking in Long-Evans rats without the use of food deprivation, sweeteners, or ethanol acclimation procedures (Tomie et al., 2004b, 2005, 2006). Under these conditions, pairings of sipper CS with food US did not induce more rapid initiation of drinking of low concentrations of ethanol (2% to 10%) than control procedures that provided presentations of sipper CS and food US randomly (Tomie et al., 2004b, 2005), indicating that the initiation of sipper CS-directed ethanol drinking was not due to Pavlovian sign-tracking CR performance.

More recently, Tomie et al. (2006) has shown that food US deliveries are not necessary to induce initiation of sipper CS-directed ethanol drinking. In that study, a group of rats that received Pavlovian sign-tracking procedures (pairings of ethanol sipper CS with food US) exhibited elevated ethanol intake relative to a control group that received the same schedule of food US presentations but with continuous access (over six times longer than the former group) to

* Corresponding author. Tel.: +1 732 445 3593; fax: +1 732 445 3500.
E-mail address: tomie@rci.rutgers.edu (A. Tomie).

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the sipper CS during the entire duration of the drinking session. The elevated ethanol drinking induced by the intermittent sipper procedure was not dependent upon the deliveries of the food US. Repeating the experiment, but without food presentations, produced the same pattern of results. Tomie et al. (2006) concluded that the sipper CS-directed ethanol drinking induced by Pavlovian sign-tracking procedures was due to intermittent presentations of the ethanol sipper CS and that intermittent food US presentations did not contribute to sipper CS-directed ethanol drinking in non-deprived rats.

The present experiments further evaluated the role of food US presentations on sipper CS-directed drinking induced by Pavlovian sign-tracking procedures. While previous studies compared the initiation of ethanol drinking in groups with and without food US presentations, (i.e., between-groups comparisons) and found no evidence of group differences, the present studies employed within-subjects comparisons, to assess for each individual subject the change from baseline drinking levels induced by the removal of food. The effect of removing food on sipper CS-directed ethanol drinking was also assessed in random controls that exhibited comparable ethanol consumption levels to the food-paired group prior to the removal of food, to determine if food presentations contributed differentially to the maintenance of ethanol drinking under these two conditions. In addition, to determine the extent to which the effects of food presentations are specific to the drinking of ethanol, a fluid control group received water (i.e., the solution in which the ethanol was diluted) in the sipper CS that was paired with food US, prior to the removal of food US presentations. If the effects of removing food pertain to sipper CS-directed drinking per se, and are unrelated specifically to ethanol drinking, then removing food should have similar effects on the drinking of ethanol or water.

2. Materials and methods

2.1. Animals

The two experiments used ($n=48$) experimentally naïve adult male Long-Evans (Blue Spruce strain) rats purchased from Harlan–Sprague–Dawley (Almont, NY, USA). At the beginning of the experiments the subjects weighed approximately 350 grams. All rats were individually housed in suspended stainless steel cages in a colony room with 12-hours light/dark cycle (light on at 04:00 h) with free access to food and water. All experimental procedures were performed in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996) and approved by the IACUC at Rutgers University.

2.2. Drugs

Bulk ethanol (95%) was obtained from Rutgers University Chemical Stores. For both experiments, ethanol solutions were prepared volume to volume (vol./vol.) by diluting 95% ethyl ethanol with tap water. To convert ml fluid consumed of ethanol concentrations (vol./vol.) to g/kg ethanol intake, the specific gravity of ethanol was estimated at 0.80.

2.3. Apparatus

The experimental chambers used in this study were purchased from MED Associates (Lafayette, IN) and have been described in detail in previous publications (Tomie et al., 2003a,b, Exp 2; Tomie et al., 2004a,b,c,d, 2005). Briefly, the drinking chambers were four cubicles (32×25.5×23 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 45-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 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 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 3.8 cm from fully retracted to fully inserted position. In the fully retracted position, the sipper 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-750 C), cabled to a connection panel (Model SG-215D), and operating under locally developed software, controlled the session events and data collection.

2.4. Experimental procedures

For both experiments, the sipper CS — food US paired procedures employed were similar to those described in a previous report documenting initiation and maintenance of drinking of unsweetened ethanol by rats maintained in the colony room with free access to food and water (Tomie et al., 2004a,b,c,d, 2005). In Experiment 1, 32 rats were randomly assigned to the Paired/Ethanol ($n=16$) group or to the Random/Ethanol ($n=16$) group. In Experiment 2, 16 rats were randomly assigned to the Paired/Ethanol ($n=8$) group or to the Paired/Water ($n=8$) group. In both experiments, all rats were run 5–6 days per week and received one training session per day conducted between 0900 and 1600 h. Before each training session rats were weighed and then placed immediately in the drinking chamber. During the drinking procedure all rats received a total of 25 trials per session, and session duration was approximately 30 min. For rats in the Paired/Ethanol groups, the ethanol sipper tube CS was inserted into the drinking chamber for 10 s followed immediately by the response-independent operation of the pellet dispenser that delivered a sucrose-enriched 45 mg (Formula 0021, Bioserv, Frenchtown, NJ) food pellet US. The Random/Ethanol group received similar training, except that the delivery of the food pellet US was programmed to occur randomly with respect to the insertion of the ethanol sipper CS. The Paired/Water group received training similar to the Paired/Ethanol group except that the sipper tube CS contained tap water instead of ethanol. For all groups delivery of the food pellet US was response-independent, occurring regardless of whether or not the rat contacted the sipper CS. The mean inter-trial interval duration was 60 s. Volume of fluid consumed (ml) was determined by weighing the sipper bottle immediately before and after each session.

In Experiment 1, during the first 10 sessions of training with sipper CS — food US paired procedures, the sipper CS contained 3% ethanol (vol./vol.) for the Paired/Ethanol and Random/Ethanol groups. During the next 6 sessions (11 to 16) the sipper CS contained 4% ethanol (vol./vol.). During the next 5 sessions (17 to 21) the sipper CS contained 6% ethanol (vol./vol.). During the next 8 sessions (22 to 30) the sipper CS contained 8% ethanol (vol./vol.). The ethanol concentration in the sipper CS was increased when mean daily ml drinking for the Paired/Ethanol groups did not vary by more than 10% between sessions for 5 consecutive sessions. On session 31, both groups received the first of 15 daily sessions (31 to 45) of training with No Food US test procedures, during which the training conditions were identical to those of session 30 except that the food pellet US was not delivered. Thus, the Paired/Ethanol and Random/Ethanol groups received 8% ethanol in the sipper CS, which was presented on the

same schedule as previously, but, for both groups, the food US was not presented at any time during sessions 31–45.

In Experiment 2, all procedures for the Paired/Ethanol group were identical to those described for the Paired/Ethanol group of Experiment 1. The Paired/Water group of Exp 2 received the same procedures as the Paired/Ethanol group of Exp 2, except that the sipper CS contained 0% ethanol (tap water) during all sessions (1 through 45). During No Food US test procedures, the Paired/Ethanol group received 8% ethanol in the sipper CS, while the Paired/Water group received 0% ethanol (tap water) in the sipper CS. During the No Food US test procedures, the sipper CS was presented on the same schedule as during training, but for both groups the food US was not presented at any time during sessions 31–45.

2.5. Statistical analysis

For each subject, for each session, milliliters (ml) of fluid consumed and kg of body weight were recorded, then grams of fluid consumed per kilogram of body weight (g/kg fluid intake) and grams of ethanol consumed per kilogram of body weight (g/kg ethanol intake) were derived. The mean of the last 5 sessions of training with each ethanol concentration [(± the standard error of the mean (S.E.M.)] was derived to provide stable estimates of asymptotic drinking for each group. Effects of Groups (Paired/Ethanol vs. Random/Ethanol; Paired/Ethanol vs. Paired/Water) and ethanol Concentrations [3%, 4%, 6%, 8% (vol./vol.)] on mean ml fluid consumed, mean g/kg fluid intake, and mean g/kg ethanol intake were assessed by two-way, repeated-measures univariate analysis of variance using MANOVA (Systat Statistical Software, Richmond, CA, USA). Drinking data for the 15 sessions of training with No Food US test procedures is divided into 3 blocks of 5 sessions each (sessions 31 to 35, 36 to 40, and 41 to 45). To allow for comparisons of drinking during No Food US test procedures between groups that differed in their previous levels of drinking, each rat's drinking was expressed as a proportion of its baseline previous to the No Food US test procedure by calculating a suppression ratio for each daily session using the following formula: suppression ratio = {(mean drinking during the No Food US test procedure)/[(mean drinking during the No Food US test procedure) + (mean baseline of drinking previous to the No Food US test procedure)]}. Fisher's Least Significant Difference (LSD) provided pairwise comparisons at individual points (alpha = 0.05).

3. Results

3.1. Experiment 1: initiation of ethanol drinking from the sipper CS

Comparable levels of mean daily g/kg ethanol intake from the sipper CS were observed for the Paired/Ethanol and Random/Ethanol groups (Fig. 1). Analysis of mean g/kg ethanol intake during the last 5 sessions of training with each of the 4 ethanol concentrations revealed no significant main effect of Groups [$F(1,30) < 1$], a significant main effect of Concentrations [$F(3,90) = 123.767$, $P < 0.01$], and no significant interaction effect between Groups and Concentrations [$F(3,90) = 1.004$, $P > 0.35$]. Thus, at the conclusion of training with Paired/Ethanol and Random/Ethanol procedures, comparable mean daily levels of g/kg ethanol intake were observed.

3.2. Experiment 1: effects of no food US test procedures

Analysis of the effects of Groups (Paired/Ethanol vs. Random/Ethanol) and Blocks of No Food US test sessions on mean daily ml fluid consumed, revealed no significant main effect of Groups [$F(1,30) = 1.001$, $P > 0.30$], no significant main effect of Blocks of No Food US test sessions [$F(2,60) = 2.416$, $P > 0.09$], and no significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,60) < 1$]. A similar analysis on mean daily g/kg ethanol intake (Fig. 2), revealed no

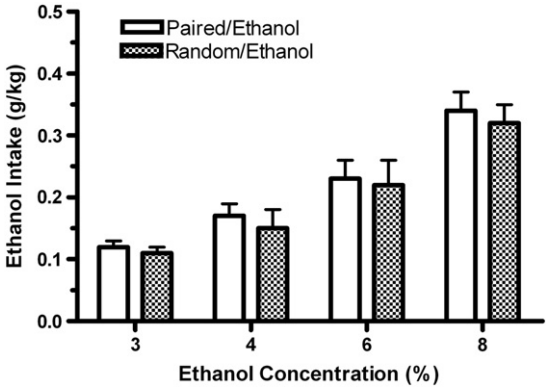

Fig. 1. Mean daily grams of ethanol consumed per kilogram of body weight (g/kg ethanol intake) as a function of the ethanol concentration [3%, 4%, 6% and 8% (vol./vol.)] in the sipper CS for rats in the Paired/Ethanol (n = 16) and Random/Ethanol (n = 16) groups. Group means were derived from the last 5 days of training at each of the 4 ethanol concentrations. The vertical bars represent the standard errors of the means (S.E.M.).

significant main effect of Groups [$F(1,30) < 1$], no significant main effect of Blocks of No Food US test sessions [$F(2,60) < 1$], and no significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,60) < 1$].

The suppression ratio provided a within-subject measure of a subject's change in drinking during No Food US test sessions relative to that subject's baseline drinking prior to the No Food US test sessions. Mean suppression ratios (± S.E.M.) for the Paired/Ethanol group during the 3 Blocks of No Food US test sessions were 0.50 ± 0.02, 0.53 ± 0.02, and 0.53 ± 0.03, respectively. For the Random/Ethanol group, mean suppression ratios for the Random/Ethanol group during the 3 Blocks of No Food US test sessions were 0.49 ± 0.01, 0.52 ± 0.01 and 0.50 ± 0.01, respectively. Analysis of the effects of Groups (Paired/Ethanol vs. Random/Ethanol) and Blocks of No Food US test sessions on suppression ratios of mean daily ml fluid consumed revealed no significant main effect of Groups [$F(1,30) < 1$], no significant main effect of Blocks of No Food US test sessions [$F(2,60) < 1$], and no significant interaction effect between groups and Blocks of No Food US test sessions [$F(2,60) < 1$]. A similar analysis on suppression ratios of mean daily g/kg ethanol intake revealed that mean suppression ratios did not differ [all Group F's < 1]. Thus, neither the Paired/Ethanol group nor the Random/Ethanol group decreased their mean daily ml drinking or mean g/kg ethanol intake. These data were consistent with the suppression ratio data, based on each individual subject's change from baseline levels of ethanol drinking

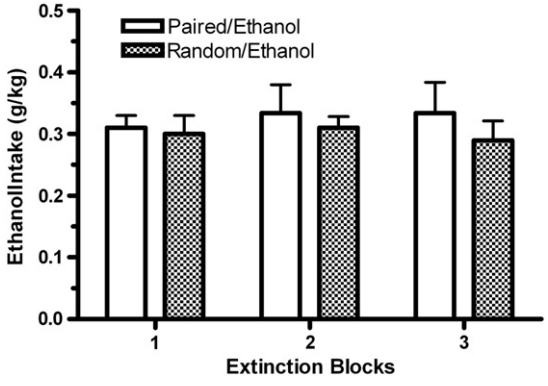

Fig. 2. Mean daily grams of ethanol consumed per kilogram of body weight (g/kg ethanol intake) when the sipper CS contained 8% ethanol (vol./vol.) for rats in the Paired/Ethanol ($n = 16$) and Random/Ethanol ($n = 16$) groups, as a function of the 3 blocks of 5 extinction sessions of the No Food US test procedures. Group means for each block were derived from the 5 days of training at each of the 3 blocks of extinction sessions. The vertical bars represent the standard errors of the means (S.E.M.).

during the No Food US test sessions, which also revealed that ethanol drinking was maintained at baseline levels during the test procedure.

3.3. Experiment 2: initiation of ethanol and water drinking from the sipper CS

Mean daily g/kg fluid intake from the sipper CS was comparable for the Paired/Ethanol and Paired/Water groups (Fig. 3). Analysis of mean daily g/kg fluid intake during the last 5 sessions of training when the Paired/Ethanol group received each of the 4 ethanol concentrations revealed no significant main effect of Groups [$F(1,14) = 1.969$, $P > 0.15$], a significant main effect of Concentrations [$F(3,42) = 5.164$, $P < 0.01$], and no significant interaction effect between Groups and Concentrations [$F(3,42) < 1$]. Thus, at the conclusion of training with Paired/Ethanol and Paired/Water procedures, comparable mean daily levels of g/kg fluid intake were observed.

3.3.1. Experiment 2: effects of no food US test procedures

Analysis of the effects of Groups (Paired/Ethanol vs. Paired/Water) and Blocks of No Food US test sessions on mean daily ml fluid consumed revealed a significant main effect of Groups [$F(1,14) = 15.330$, $P < 0.01$], no significant main effect of Blocks of No US test sessions [$F(2,28) = 1.159$, $P > 0.30$], and a significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,28) = 9.662$, $P < 0.01$]. Fisher's LSD revealed significantly higher mean daily ml fluid consumed ($P < 0.05$) in the Paired/Ethanol group than in the Paired/Water group on the second No Food US test block (No Food US test sessions 6–10), and the third No Food US test block (No Food US test sessions 11–15). A similar analysis on mean daily g/kg fluid intake (Fig. 4), revealed a significant main effect of Groups [$F(1,14) = 16.053$, $P = 0.01$], no significant main effect of Blocks of No Food US test sessions [$F(2,28) = 2.506$, $P > 0.10$], and a significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,28) = 8.801$, $P < 0.01$]. Fisher's LSD test revealed significantly higher mean daily g/kg fluid intake ($P < 0.05$) in the Paired/Ethanol group on the second No Food US test block (No Food US test sessions 6–10), and the third No Food US test block (No Food US test sessions 11–15).

For the Paired/Ethanol group, mean suppression ratios (± S.E.M.) during the 3 blocks of No Food US test sessions were 0.50 ± 0.03, 0.51 ± 0.02, and 0.55 ± 0.04, respectively. For the Paired/Water group, mean suppression ratios during the 3 Blocks of No Food US test sessions were 0.48 ± 0.01, 0.41 ± 0.02, and 0.36 ± 0.02, respectively. Analysis of the effects of Groups (Paired/Ethanol vs. Paired/Water) and Blocks of No Food US test sessions on suppression ratios of mean ml fluid consumed revealed a significant main effect of Groups [$F(1,14) = 14.252$, $P < 0.01$], no significant main effect of Blocks of No Food US test sessions [$F(2,28) = 3.170$, $P > 0.05$], and a significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,28) = 14.917$, $P < 0.01$]. Fisher's LSD revealed significantly higher suppression ratios of mean daily fluid consumed in the Paired/Ethanol group than in the Paired/Water group ($P < 0.05$) on the second Block of No Food US test sessions (No Food US test sessions 6–10) and third Block of No Food US test sessions (No Food US test sessions 11–15). A similar analysis on suppression ratios of mean daily g/kg fluid intake revealed a significant main effect of Groups [$F(1,14) = 12.773$, $P < 0.01$], a significant main effect of Blocks of No Food US test sessions [$F(2,28) = 5.433$, $P < 0.01$], and a significant interaction effect between Groups and Blocks of No Food US test sessions [$F(2,28) = 14.383$, $P < 0.01$]. Fisher's LSD test revealed significantly higher suppression ratios of mean daily g/kg fluid intake in the Paired/Ethanol group on the second and third Blocks of No Food US test sessions ($P < 0.05$).

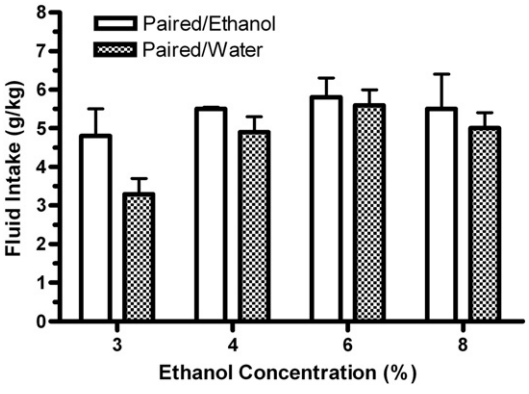

Fig. 3. Mean daily grams of fluid consumed per kilogram of body weight (g/kg fluid intake) as a function of the ethanol concentration [3%, 4%, 6% and 8% (vol./vol.)] in the sipper CS for rats in the Paired/Ethanol (n = 8) and Paired/Water (n = 8) groups. Group means were derived from the last 5 days of training when the sipper CS for the Paired/Ethanol group contained each of the 4 ethanol concentrations. Throughout the experiment, the sipper CS for the paired/water group contained water (0% ethanol). The vertical bars represent the standard errors of the means (S.E.M.).

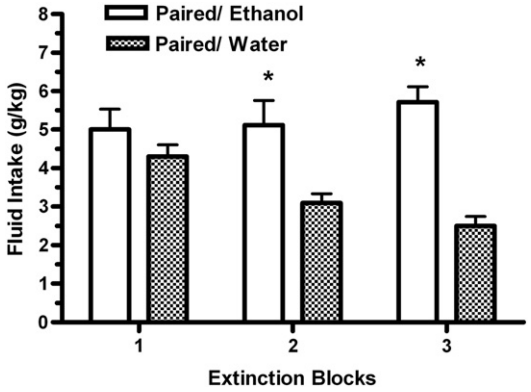

Fig. 4. Mean daily grams of fluid consumed per kilogram of body weight (g/kg fluid intake) when the sipper CS contained 8% ethanol (vol./vol.) for rats in the paired/ethanol ($n = 8$) and the sipper CS contained tap water (0% ethanol) for rats in the paired/water ($n = 8$) group, as a function of the 3 blocks of 5 extinction sessions of No Food US test procedures. Group means for each block were derived from the 5 days of training at each of the 3 blocks of extinction sessions. The vertical bars represent the standard errors of the means (S.E.M.). The asterisk (*) indicates that the observed differences between the paired/ethanol and paired/water groups were significant (Fisher's LSD, $P < 0.05$).

4. Discussion

4.1. Acquisition and maintenance of sipper CS-directed ethanol drinking

In both experiments, Pavlovian pairings of ethanol sipper CS with food US induced consistent and reliable increases in sipper CS-directed ethanol drinking, and this is consistent with previous reports showing that Pavlovian paired procedures induced drinking of unsweetened ethanol in non-deprived rats and without the use of sweeteners or ethanol acclimation procedures (Tomie et al., 2004a,b,c,d, 2005, 2006). In Exp 1, the Paired/Ethanol and Random/Ethanol groups consumed comparable amounts of the ethanol concentrations (3%–8%) in the sipper CS. This is consistent with previous reports employing non-deprived rats (Tomie et al., 2004b, 2005; see also Tomie et al., 2004a,c) and contrasts to the results of studies employing food-deprived rats, where significantly more ethanol drinking was observed in Paired groups than Random controls, even when the sipper CS contained lower (2% to 8%) concentrations of ethanol (Tomie et al., 2002, 2003a,b; see also Tomie et al., 2004d), indicating that in food deprived rats, food US presentations are more effective in promoting sipper CS-directed ethanol drinking than in non-deprived rats.

4.2. Effects of removing food US on ethanol drinking

Removal of food US presentations did not reduce sipper CS-directed ethanol drinking in the Paired/Ethanol group (Exps 1 and 2)

or in the Random/Ethanol group (Exp 1). The Paired/Ethanol and Random/Ethanol groups maintained ethanol drinking at the levels observed prior to the removal of the food US, and did so for the entire 15 sessions of training with the No Food US test procedures. These data are consistent with the observations of Tomie et al. (2006), who reported that sipper CS-directed drinking of low concentrations of ethanol was acquired and maintained in non-deprived rats merely by providing, from the beginning of the study and for the entire duration of the study, intermittent presentations of the ethanol sipper CS without any food US presentations. In the present experiments, the effects of food US presentations on ethanol drinking in the Paired/Ethanol groups were assessed within-subjects, by comparing levels of ethanol drinking before and after the food US presentations were removed. In both experiments, removing food US presentations had no effect on several measures of sipper CS-ethanol drinking, including mean ml ethanol drinking, mean g/kg ethanol intake, and mean suppression ratio. The suppression ratio measure compares ethanol drinking before and after the removal of food US presentations, and, in addition, weighs equally the contribution of each subject, regardless of their absolute level of ethanol drinking. Thus, using within-subjects comparisons, these data provide additional evidence that food US presentations do not contribute to sipper CS-directed ethanol drinking when sipper CS and food US are paired, confirming results obtained earlier using between-groups comparisons (Tomie et al., 2006).

In Exp 1, in the Random/Ethanol group, removal of food US presentations had no effect on several measures of sipper CS-ethanol drinking, including mean ml ethanol drinking, mean g/kg ethanol intake, and mean suppression ratio, indicating that food US presentations do not contribute to sipper CS-directed ethanol drinking in random controls. This is the first study to evaluate the effects of food US presentations on sipper CS-directed ethanol drinking in Random controls, and the absence of a decrease in mean levels of ethanol drinking following the removal of food US presentations provides evidence indicating that sipper CS-directed ethanol drinking is not due to food US presentations. This is an important observation because it reveals that prandial drinking of ethanol (Kissileff, 1969; Meisch and Thompson, 1974; Samson et al., 1988; Neill et al., 1994; Cunningham and Niehus, 1997) or schedule-induced polydipsia (SIP) of ethanol drinking (Lester, 1961; Hymowitz et al., 1970; Falk, 1971; Falk et al., 1972; Colotla and Keehn, 1975; McMillan et al., 1976; Riley et al., 1979) do not account for the ethanol drinking observed in the present studies.

4.3. Effects of removing food US on water drinking

In Exp 2, removal of food US presentations reduced sipper CS-directed water drinking by approximately 40%, relative to water drinking levels observed during Paired/Water procedures. During the 15 sessions of training with the No Food US test procedures, water drinking systematically declined, as measured by mean ml water drinking, mean g/kg water intake, and mean suppression ratio, indicating that food US presentations contributed to sipper CS-directed water drinking in the Paired/Water group. This substantial decline in water drinking was in contrast to the absence of any evidence of a decrease in ethanol drinking in the Paired/Ethanol group, indicating that the presence of ethanol in the sipper CS had an effect on the maintenance of drinking when food US presentations were discontinued. This is the first study that evaluated the effects of removing food US presentations on sipper CS-directed water drinking. The results suggest that the intermittent presentations of the food US during paired procedures contributed more to the drinking of water than to the drinking of ethanol.

It is appropriate to consider how further experimentation would serve to clarify these results. The present study did not include a pseudoconditioning control group that received the water sipper CS randomly with respect to the food US. Differences in water drinking between the Paired/Water and Random/Water groups would serve to clarify the extent to which the water sipper CS-directed drinking observed in the Paired/Water group was due to Pavlovian sign-tracking CR performance. Moreover, the reduction in water drinking induced by the removal of food US in the Paired/Water group may be due to Pavlovian extinction, and the performance of the Random/Water group under the No Food US condition might serve to confirm this interpretation. It should also be noted that the reduction in water drinking but not ethanol drinking may be due to the differential availability of these fluid outside of the test chambers. The ethanol groups were deprived of ethanol in their home cage and maintained ethanol drinking in the test chambers when the food US was removed. On the other hand, the water group was not deprived of water in the home cage and therefore, the decreased water drinking in the test chambers when the food US was removed may be due to this factor. In a future study, the effects of water deprivation in the home cage on water drinking in the test chamber will be evaluated.

4.4. Effects of intermittent sipper procedures

The present data contribute to a growing body of evidence suggesting that the ethanol drinking observed in studies employing Pavlovian sign-tracking procedures is not due to presentations of the food US. Additional evidence discounting the role of food presentations is provided by Tomie et al. (2006) who showed that in procedures providing for no presentations of food at any time during the experiment, repeated brief insertions and retractions of the ethanol sipper were sufficient to induce reliable initiation of ethanol drinking. The data indicate that intermittent insertions and retractions of the ethanol sipper are sufficient to maintain ethanol drinking in rats in operant testing chambers without the use of either food-deprivation, or saccharin-fading, or sucrose-fading, or alcohol acclimation procedures (see also, Tomie et al., 2004b, 2005, 2006). Thus, in between-groups and within-subjects assessment procedures, there is now evidence that intermittent sipper procedures per se are sufficient to induce initiation and maintenance of ethanol drinking, and that the ethanol drinking observed in intermittent sipper procedures is not dependent on food presentations.

It should be noted that in home cage studies of ethanol drinking employing limited access procedures, interruptions in the availability of the ethanol sipper have been reported to induce levels of daily ethanol intake that are comparable (Sinclair et al., 1992; Pinel and Huang, 1976; Wayner & Greenberg, 1972) or greater (Wise, 1973; Simms et al., 2008) than that observed when the ethanol sipper is continuously available. For example, Simms et al. (2008) provided Long-Evans rats with 24-h access to a sipper tube containing 20% ethanol but for only 3 days per week (Intermittent Procedure), while control groups were provided with continuous 24-h access to a sipper tube containing either 20% or 10% ethanol for 7 days per week (Continuous Procedures). The Intermittent Procedure induced significantly elevated daily ethanol intake in the home cage relative to the Continuous Procedures. While there is now much evidence indicating that intermittent sipper procedures elevate ethanol drinking above the levels observed in controls receiving continuous access to the ethanol sipper, the basis of this effect remains unclear. One intriguing possibility, suggested by Tomie et al. (2006) points to the improved covariation between the sipper and ethanol when the ethanol sipper is retracted from the drinking chamber for extended periods of time, during which neither the sipper nor ethanol are present. Further evaluation of the effects of intermittent sipper procedures on the initiation and maintenance of ethanol drinking is need to more fully characterize the basis of this important environmental determinant of ethanol drinking in rats.

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