AUTOSHAPING OF ETHANOL DRINKING: AN ANIMAL MODEL OF BINGE DRINKING

ARTHUR TOMIE*, JASON DI POCE, CHRISTOPHER C. DERENZO and LARISSA A. POHORECKY

Department of Psychology and Center of Alcohol Studies, Rutgers University, New Brunswick, NJ 08903, USA

(Received 25 April 2001; in revised form 30 July 2001; accepted 1 September 2001)

Abstract — To examine the hypothesis that Pavlovian autoshaping provides an animal learning model of drug abuse, two studies evaluated the induction of ethanol drinking by autoshaping procedures. In Experiment 1, the sipper tube conditioned stimulus (CS) contained saccharin/ethanol solution and was repeatedly paired with food as an unconditioned stimulus (US). The CS–US paired group consumed more of the 0.1% saccharin–6% ethanol solution than did the CS–US random group, revealing that autoshaping conditioned responses (CR) induce ethanol drinking not attributable to pseudo-conditioning. Experiment 2 employed saccharin-fading procedures and showed that the paired vs random group differences in ethanol drinking were maintained, even as the saccharin was eliminated from the solution. The results show that Pavlovian autoshaping procedures induce high volumes of ethanol drinking when the presentation of a sipper tube containing an ethanol solution precedes the response-independent delivery of food. The high volume of ethanol consumed in a brief period of time suggests that Pavlovian autoshaping may be a model of binge drinking.

INTRODUCTION

Tomie (1995, 1996, 2001) has proposed that Pavlovian conditioning of autoshaping conditioned responses (CR) may contribute to the development of prominent features of the drug abuse syndrome. The present experiments evaluated the autoshaping model of drug abuse by asking if Pavlovian autoshaping procedures enhance ethanol drinking in rats. In Pavlovian autoshaping procedures, the presentation of a localized visual stimulus, conditioned stimulus (CS), is followed by the response-independent presentation of a rewarding substance, unconditioned stimulus (US). Repeated CS–US pairings lead to the acquisition of the Pavlovian autoshaping CR, which is a complex sequence of motor responses directed at the CS (Brown and Jenkins, 1968; Tomie et al., 1989).

A widely employed Pavlovian autoshaping procedure in rats provides for the brief insertion of a stainless steel retractable lever CS, which is followed immediately by the response-independent delivery of the food US. As a result of repeated lever CS–food US pairings, many rats acquire the autoshaping CR, the topography of which consists of complex sequences of skeletal–motor consummatory-like responses directed at the lever CS. Thus, the rat will approach the lever CS, contact the lever CS, grasp the lever CS in its forepaws, culminating in the expression of mouthing, licking, and chewing of the lever CS (for review see Tomie et al., 1989). Important to the understanding of autoshaping, the lever CS-directed responding (i.e., autoshaping CR performance) is not required in order to procure the food US; rather, the food US is delivered regardless of whether or not the rat contacts or presses the lever. Thus, lever CS-directed autoshaping CR develop merely as a consequence of repeated Pavlovian lever CS–food US pairings.

The present study investigates whether ethanol drinking may be induced in rats by Pavlovian autoshaping procedures. The traditional rat autoshaping apparatus was modified by replacing the retractable lever CS with a retractable sipper tube CS that was attached to a Plexiglas bottle filled with solution. The sipper tube/solution CS was inserted briefly and immediately prior to the response-independent delivery of the food US. After repeated sipper tube/solution CS–food US pairings, the rats may acquire the Pavlovian autoshaping CR, as revealed by sipper tube/solution CS-directed consummatory-like responding. Thus, repeated pairings of the availability of the solution via the sipper tube CS with the rewarding food US may induce Pavlovian autoshaping of oral consumption of the solution. In the present study, the solution CS consisted of 6% ethanol in 0.1% saccharin (Experiment 1) or 6% (v/v) ethanol in saccharin concentrations that were systematically reduced (saccharin-fading) during the study (Experiment 2).

The present studies were designed to allow for the assessment of the effects of pseudo-conditioning. Sipper tube CS-directed consummatory responding may be due to the Pavlovian pairings of the sipper tube CS and the food US, or, alternatively, such responding may be due to factors other than the pairing of CS and US. For example, periodic presentations of food may induce motor activity and psychomotor activation (Wise and Rompre, 1989), or intermittent insertions of the sipper tube may incite target-biting behaviour (Tomie et al., 1993), or rats may drink the saccharin/ethanol solution for the intrinsic positively reinforcing effects of the saccharin (Dufour and Arnold, 1966; Domjan et al., 1976; Stefrak and Van der Kooy, 1992), or for the calorie component of the ethanol solution (Heyman, 1997). While none of these effects derives from the pairing of CS and US, all serve to increase sipper tube CS-directed consummatory-like responding. A pseudo-conditioning control group, receiving presentations of food US randomly with respect to the insertion of the sipper tube CS, estimates sipper tube-directed consummatory-like responding due to factors other than Pavlovian CS–US pairings, and will clarify the degree to which Pavlovian autoshaping procedures induce sipper tube-directed drinking behaviour beyond that due to pseudo-conditioning effects.

This is an important consideration, as ethanol has been reported to induce psychomotor activation in rats (Carlsson et al., 1972; Imperato and Di Chiara, 1986; Waller et al., 1986; Moore et al., 1993), to increase motor responding in Pavlovian learning tasks in rats (Cappeliez and White, 1981; Silverman, 1990; Cunningham and Noble, 1992), and, most significantly,
to increase the performance of lever CS-directed autoshaping CR in rats when the lever CS is paired with food US (Tomie et al., 1998). The pseudo-conditioning control group will clarify whether ethanol’s effects on sipper tube CS-directed consummatory responding are mediated by ethanol’s effects on Pavlovian autoshaping CR or by ethanol’s effects of pseudo-conditioning.

EXPERIMENT 1

Materials and methods

Animals and treatment. Adult male Long–Evans (Blue Spruce strain) rats (n = 24) obtained from Harlan–Sprague–Dawley (Almont, NY, USA) initially weighing 260–280 g were used. Rats were housed individually in suspended steel cages in a colony room with a 12 h light:12 h dark (lights on at 04.00) cycle. Continuous access to water in their home cages was supplied while the rats were maintained at 80% of their free-feeding body weights by providing supplemental rat chow after each daily session. Principles of laboratory animal care (Institute of Laboratory Animal Resources, 1996) were followed.

Apparatus. Autoshaping chambers were four Plexiglas cubicles (24 × 24 × 26 cm) with a stainless steel grid floor all enclosed in sound-attenuating, ventilated outer casings. One house light (GE 1821) was mounted directly above the operant chamber on the ceiling of the outer hull. The front panel of each chamber was equipped with a retractable stainless steel sipper tube containing a stainless steel ball-bearing with an inserted rubber stopper holding the fluid in a 50 ml Plexiglas graduated tube (Kimble-Kontes, Vineland, NJ, USA; model 58320). The Plexiglas tube was mounted on a mechanical bottle insertion mechanism (BCS Machine, South Plainfield, NJ, USA) which inserted the stainless steel sipper tube 3.5 cm above the grid floor and 3 cm to the left of the centre-line. The bottle insertion mechanism moved the sipper tube a total of 2.75 cm from fully retracted to fully inserted. In the fully retracted position, the sipper tube was 2.0 cm removed from the chamber. A contact lickometer recorded licks (Med Associates, St Albans, VT, USA; model ENV-250). A metal food pellet receptacle was mounted 3 cm to the right of the centre-line, and 4 cm above the floor. The food pellet dispenser (BRS/LVE, model PDC/PPD) delivered 45 mg food pellets (BioServ F0165; Frenchtown, NJ, USA). Masking noise (88 dB, linear scale) was provided by the operation of ventilating exhaust fans mounted on the outer hull. IBM personal computers controlled session events.

Drugs. Bulk ethanol (95%) was obtained from Rutgers University Chemical Stores. Saccharin sodium was obtained from Sigma Chemical Co. (St Louis, MO, USA).

Autoshaping procedure. Rats were run 5 days per week on a double alternation schedule (a.m.–a.m.–p.m.–p.m.–p.m.). Morning sessions were between 09.00 and 12.00, whereas afternoon sessions were between 13.00 and 16.00. Prior to each autoshaping session, rats were weighed and then immediately placed in the autoshaping chamber. Twelve rats were randomly assigned to the paired group, whereas 12 rats were assigned to the random group. For the paired group, the sipper tube (CS) was inserted for 5 s followed immediately by the response-independent 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. For both the paired and random groups, the delivery of the food pellet US occurred regardless of whether the subject consumed the ethanol solution CS. Both the paired and random groups received a total of 25 trials per daily autoshaping session. The mean inter-trial interval was 60 s, with a minimum of 45 s and a maximum of 75 s. The session duration was ~30 min. Volume of fluid consumed (ml) during each autoshaping session was determined by recording the volume in the tube immediately before and after each session. Throughout the experiment, the fluid in the Plexiglas tube contained 6% ethanol (v/v) in 0.10% saccharin.

Statistics. Effects of groups (paired vs random) and blocks of autoshaping sessions on mean ml fluid consumed, mean g/kg of ethanol consumed, and mean sipper tube licks were assessed by two-way repeated-measures multivariate analysis of variance using MANOVA (Systat). Effects of groups and time of day (a.m. vs p.m.) on mean ml fluid consumed were assessed by two-way repeated measures multivariate analysis of variance using MANOVA (Systat).

Results

Data on ml of 6% ethanol in 0.10% saccharin consumed during the first ten blocks of two sessions each (sessions 1–20) are presented in Fig. 1. The analyses of these data revealed a significant effect of blocks [F(9,198) = 4.573, P < 0.05], a significant effect of blocks [F(9,198) = 49.419, P < 0.01], but no significant groups × blocks interaction effect [F(9,198) = 1.073, P > 0.10]. The mean (± SEM) ml of ethanol consumed during the last four sessions (17–20) for the paired group was 6.8 ± 0.4 (range 4.8–8.0) and for the random group was 5.7 ± 0.4 (range 3.8–7.6).

Data on g/kg of 6% ethanol in 0.10% saccharin consumed during the first ten blocks of two sessions each (sessions 1–20)

![Graph](image-url)
are presented in Fig. 2. The analyses revealed a significant effect of groups \( F(1,22) = 4.092, P < 0.05 \), a significant effect of blocks \( F(9,198) = 47.386, P < 0.01 \), but no significant groups \( \times \) blocks interaction effect \( F(9,198) = 1.029, P > 0.10 \). The mean g/kg of ethanol consumed during the last four sessions (17–20) for the paired group was 1.7 ± 0.1 (range 1.1–2.0) and for the random group was 1.4 ± 0.1 (range 0.8–1.8). The paired group drank 21% more ethanol on a g/kg basis than the random group.

Analysis of licks of 6% ethanol in 0.10% saccharin during the first ten blocks of two sessions each (sessions 1–20) revealed a marginally significant effect of groups \( F(1,22) = 4.208, P < 0.06 \), a significant effect of blocks \( F(9,198) = 24.179, P < 0.01 \), but no significant groups \( \times \) blocks interaction effect \( F < 1.0 \).

To assess the effects of time of day (a.m. vs p.m.) on drinking, a three-way MANOVA of ml of solution consumed was performed with groups and time of day as factors. These data revealed a statistically significant main effect of group \( F(1,22) = 4.573, P < 0.05 \), a significant main effect of time of day \( F(1,22) = 49.051, P < 0.01 \), but no significant groups \( \times \) time of day interaction effect \( F < 1.0 \).

Discussion

These data reveal that the paired group consumed more saccharin/ethanol solution than the random group. These group differences in fluid consumption are consistent with the hypothesis that Pavlovian autoshaping procedures, consisting of repeated forward pairings of a sipper tube containing a saccharin/ethanol solution (CS) and response-independent presentation of food (US), induced sipper tube-directed consummatory conditioned responses (autoshaping CR). In addition, the paired group showed a systematic increase in ml of fluid consumed as a function of autoshaping sessions. This function resembles a Pavlovian autoshaping acquisition curve, and suggests that the sipper tube CS-directed consummatory responding induced by CS–US pairings are Pavlovian autoshaping CR.

The pseudo-conditioning control group, which received presentations of CS and US randomly with respect to one another (random group), provided less evidence of a systematic increase in fluid consumption across sessions of experience with the random CS–US procedure. Thus, the paired group consumed more fluid, and this difference developed as a function of experience with pairings of the sipper tube filled with saccharin/ethanol solution (CS) with food (US). This relationship between fluid consumption and experience is entirely consistent with the Pavlovian autoshaping hypothesis.

The paired group consumed more saccharin/ethanol fluid than did the random group, and, consequently, ingested larger volumes of ethanol during each autoshaping session. Analysis of g of ethanol consumed per kg of body weight during the last four sessions (17–20), revealed that the paired group consumed ~1.7 g, whereas the random group consumed 1.4 g of ethanol per kg body weight per session. In addition to revealing that higher levels of ethanol consumption are induced by the paired procedures, these data also confirm that the group differences in ethanol consumption are not due to confounding differences in mean body weight.

While the paired group yielded somewhat higher mean sipper tube licks than the random group, the differences did not achieve statistical significance. In view of the observed group differences in fluid consumption, the lack of a more pronounced effect on the sipper tube licking measure warrants further consideration. Some investigators employing operant drug self-administration procedures have reported highly positive relationships between fluid consumption and licking scores in rats (Stolerman and Kumar, 1972), and other investigators have actually inferred volume of fluid consumed based on licking scores (Marcucella et al., 1984; Marcucella and Munro, 1986). The absence of a stronger relationship between fluid consumption and licking in the present study may be due to the topography of autoshaping CR induced in the paired group. Unsystematic observations of the precise topography of the licking responses revealed that several of the rats in the paired group grasped the sipper tube with their forepaws or maintained contact with the sipper tube with their snout. These are classic features of autoshaping CR, and may in the paired group reduce the recorded number of licks, which are measured as makes and breaks of the contact circuitry. Notably, these response topographies were less often observed in rats in the random group. Thus, operant self-administration procedures yield high correlations between fluid consumption and licking, but these Pavlovian autoshaping procedures do not.

These procedures reveal induction of drinking by Pavlovian autoshaping procedures that employ food US. It is interesting to note that several 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 following the eating of large amounts of food (Meisch and Thompson, 1974; Neill et al., 1994; Cunningham and Niehus, 1997), and schedule-induced polydipsia (SIP) models of ethanol drinking provide for intermittent schedules of food presentations in a situation where ethanol is also available (Falk et al., 1972;
Colotla and Keehn, 1975; Riley et al., 1979). In the SIP procedure, rats also are observed to drink in the post-pellet interval after eating (Hymowitz and Freed, 1974; McMillan et al., 1976).

It is appropriate, therefore, to ask if the group differences in ethanol drinking observed here may be due to either post-ingestive prandial drinking or schedule induction effects. While it is possible that either or both of these factors contributed to drinking in this study, it is unlikely that they account for the group differences in drinking. This is because the paired group drank more than the random group, and drinking in the paired group occurred only prior to the ingestion of food, rather than after. Prandial drinking and SIP are far more likely to contribute to drinking in the random group, where by chance, the presentation of the food US may occur prior to the delivery of the fluid solution CS. Therefore, prandial drinking and SIP contribute only to drinking in the pseudo-conditioning (random) control, and, to the extent that such effects are present, they serve only to reduce differences in drinking between the paired and random groups. Thus, these group differences are likely to be very conservative estimates of the effects of the Pavlovian paired procedures on ethanol drinking, as they are obtained despite the operation of several factors that would serve to increase drinking in the random group but not in the paired group.

The time of day of the running of the autoshaping sessions (a.m. vs p.m.) was counterbalanced across groups in a double alternation sequence (a.m.–a.m.–p.m.–p.m.–a.m.–a.m.–p.m.–p.m.) to ensure that drinking was assessed equally often in both groups during both parts of the day. While the data revealed that rats in both the paired and random groups drank more ethanol solution in the p.m. sessions, there was no evidence of sequence effects related to time of day, i.e. consumption in an a.m. session following a p.m. session was comparable to when the a.m. session followed another a.m. session.

It is appropriate to justify the use of food-deprived rats in these studies. First, it should be noted that the use of food-deprived rats is typical of traditional autoshaping studies employing food as the US (Tomie et al., 1989). Presumably, this is to ensure that the rat is hungry enough to eat the food US and experience the CS–US pairing. For this reason, it seems appropriate to initiate the testing of the autoshaping model of ethanol drinking by using the food deprivation procedures typically employed in autoshaping studies. In addition, it should be noted that the sipper CS–food US autoshaping procedures are intended to provide a model of how, in humans, the pairing of the ethanol sipper with food induces autoshaping of ethanol drinking. Therefore, to the degree that humans consume ethanol prior to eating their meal, and to the degree that humans are at the table because they are somewhat hungry, it seems perfectly reasonable to evaluate the effects of pairing the ethanol sipper with food in the laboratory under conditions of food deprivation.

With regard to the issue of the appropriateness of employing food deprivation in studies of ethanol drinking, it is important to note that the autoshaping model of ethanol drinking is a Pavlovian conditioning model, rather than an operant or instrumental model of ethanol self-administration. The autoshaping model is intended to evaluate the effects of non-contingent 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 proposed studies are 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). Therefore, the data derived from these autoshaping procedures do not address important and fundamental issues related to ethanol-seeking behaviour, the distinction between ethanol seeking and ethanol consumption, or the relationship between ethanol seeking and ethanol consumption (Samson et al., 1998). While 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 these studies, which was to characterize the effects on ethanol drinking of experiencing the ethanol sipper just before eating.

EXPERIMENT 2

Experiment 1 showed that CS–US paired autoshaping subjects drank more saccharin/ethanol solution CS than CS–US random subjects. The purpose of Experiment 2 was to determine whether this relationship between autoshaping and drinking is also observed under conditions where the CS solution contains 6% ethanol but no saccharin. It is well established in the ethanol drinking literature that the presence of sweeteners, such as saccharin or sucrose, facilitates the initiation of ethanol drinking, and, moreover, substantial levels of ethanol drinking are maintained even as the concentration of sweetener is systematically reduced by fading procedures (Samson, 1986; Samson et al., 1988; Weiss et al., 1990; Weiss and Koob, 1991). Experiment 2 employed procedures similar to Experiment 1, but included saccharin-fading procedures, to determine if autoshaping-induced ethanol drinking is observed even when there is no saccharin in the drinking solution CS.

Materials and methods

Animals and treatment, apparatus. These were the same as Experiment 1.

Drugs. Ethanol and saccharin were obtained as described above.

Autoshaping procedure. Twelve rats were randomly assigned to the paired group while 12 rats were randomly assigned to the random group. Procedures during the 0.10% saccharin–6% ethanol phase (first 28 sessions) were identical to Experiment 1. For the next 12 sessions (29–40), the 6% ethanol solution was diluted in 0.07% saccharin, then for the next 12 sessions (41–53), the 6% ethanol solution was diluted in 0.035% saccharin, and for the final 12 sessions (54–65), the 6% ethanol solution was diluted in 0.00% saccharin.

Statistics. Effects of groups (paired vs random) and blocks of autoshaping sessions on mean ml of fluid consumed, mean g/kg of ethanol consumed, and mean sipper tube licks were assessed by two-way repeated-measures multivariate analysis of variance using MANOVA (Systat). Effects of groups and saccharin concentration on mean ml of fluid consumed and mean g/kg of ethanol consumed were assessed by two-way repeated-measures multivariate analysis of variance using MANOVA (Systat). Effects of groups, saccharin concentration,
Groups and time of day on ml of fluid consumed were assessed by three-way repeated-measures multivariate analysis of variance using MANOVA (Systat).

Results

Millilitres of fluid consumed. Analysis of ml of fluid (6% ethanol in 0.10% saccharin) consumed during sessions 1–28 (Fig. 3) revealed a marginally significant effect of groups \([F(1,22) = 3.536, P < 0.10]\), a significant effect of sessions \([F(27,594) = 46.657, P < 0.01]\), and a significant groups \(\times\) sessions interaction effect \([F(27,594) = 3.671, P < 0.01]\). Fisher’s LSD revealed that the paired group differed from the random group on sessions 7–8, 11–12, 14–16, 19–21, 23–24, 26–28.

Analysis of ml of fluid (6% ethanol in 0.07% saccharin) consumed during sessions 29–40 revealed a marginally significant effect of groups \([F(1,22) = 4.052, P < 0.10]\), a significant effect of sessions \([F(11,242) = 4.463, P < 0.01]\), and a significant groups \(\times\) sessions interaction effect \([F(11,242) = 2.962, P < 0.01]\). Fisher’s LSD revealed that the paired group differed from the random group on sessions 30, 32–37, 39–40.

Analysis of ml of fluid (6% ethanol in 0.035% saccharin) consumed during sessions 41–53 revealed a marginally significant effect of groups \([F(1,22) = 3.570, P < 0.10]\), a significant effect of sessions \([F(12,264) = 3.609, P < 0.01]\), and a significant groups \(\times\) sessions interaction effect \([F(12,264) = 2.725, P < 0.01]\). Fisher’s LSD revealed that the paired group differed from the random group on sessions 41–44, 46, 50–51, 53.

The mean ml of ethanol consumed during sessions 54–65 (6% ethanol in 0.0% saccharin) for the paired group was 9.0 ± 0.4 (range 7.3–11.4) and for the random group was 7.1 ± 0.4 (range 5.6–9.8). Analysis of ml of fluid (6% ethanol in 0.0% saccharin) consumed during these sessions revealed a significant effect of groups, \(F(1,22) = 10.198, P < 0.01\), a significant effect of sessions \([F(11,242) = 4.585, P < 0.01]\), and a significant groups \(\times\) sessions interaction effect \([F(11,242) = 4.119, P < 0.01]\). Fisher’s LSD revealed that the paired group differed from the random group on sessions 54–56, 59–65.

Analysis of mean ml of fluid consumed during each of the last four sessions of each percentage of saccharin for the paired and random groups (Fig. 4) revealed a significant main effect of groups \([F(1,22) = 8.923, P < 0.01]\) and a marginally significant effect of percentage of saccharin \([F(3,66) = 1.199, P < 0.10]\). Analysis of 6% ethanol in 0.10% saccharin revealed that the paired group differed significantly from the random group \([F(1,22) = 5.781, P < 0.05]\). Analysis of 6% ethanol in 0.07% saccharin revealed that the paired group differed significantly from the random group \([F(1,22) = 8.336, P < 0.01]\). Analysis of 6% ethanol in 0.035% saccharin revealed that the paired group differed marginally from the random group \([F(1,22) = 3.263, P < 0.10]\). Analysis of 6% ethanol in 0.0% saccharin revealed that the paired group differed significantly from the random group \([F(1,22) = 17.381, P < 0.01]\).

Analysis of the effect of time of day on mean ml of fluid consumed revealed a significant effect of groups \([F(1,22) = 6.34, P < 0.05]\), a significant effect of time of day \([F(1,22) = 12.20, P < 0.01]\), and a significant effect of saccharin concentration \([F(3,66) = 11.22, P < 0.01]\).

Grams per kilogram of ethanol consumed. Analysis of mean g/kg of ethanol consumed during sessions 1–28 (6% ethanol in 0.10% saccharin) revealed a significant effect of groups \([F(1,22) = 4.296, P < 0.05]\), a significant effect of sessions \([F(27,594) = 44.024, P < 0.01]\), and a significant groups \(\times\) sessions interaction effect \([F(27,594) = 3.698, P < 0.01]\). Fisher’s LSD revealed that the paired group differed from the random group on sessions 4, 7–8, 11–12, 14–16, 19–21, 23–24, 26–28.

Analysis of mean g/kg of ethanol consumed during sessions 29–40 (6% ethanol in 0.07% saccharin) revealed a significant
effect of groups \( F(1,22) = 4.789, P < 0.05 \), a significant effect of sessions \( F(11,242) = 5.726, P < 0.01 \), and a significant groups \( \times \) sessions interaction effect \( F(11,242) = 3.274, P < 0.01 \). Fisher’s LSD revealed that the paired group differed from the random group on sessions 30–37, 39–40.

Analysis of mean g/kg of ethanol consumed during sessions 41–53 (6% ethanol in 0.035% saccharin) revealed a significant effect of groups \( F(1,22) = 4.471, P < 0.05 \), a significant effect of sessions \( F(12,264) = 5.255, P < 0.01 \), and a significant groups \( \times \) sessions interaction effect \( F(12,264) = 3.244, P < 0.01 \). Fisher’s LSD revealed that the paired group differed from the random group on sessions 30–37, 39–40.

The mean g/kg of ethanol consumed during sessions 54–65 (6% ethanol in 0.0% saccharin) for the paired group was 1.9 ± 0.1 (range 1.4–2.5) and for the random group was 1.5 ± 0.1 (range 1.0–2.1). The paired group drank 27% more ethanol on a g/kg basis than the random group. Analysis of mean g/kg of ethanol consumed during these sessions revealed a significant effect of groups \( F(1,22) = 10.837, P < 0.01 \), a significant effect of sessions \( F(1,22) = 7.090, P < 0.05 \), and a significant groups \( \times \) sessions interaction effect \( F(1,22) = 4.073, P < 0.05 \). Fisher’s LSD revealed that the paired group differed from the random group on sessions 54–56, 59–65.

Analysis of mean g/kg of ethanol consumed during each of the last four sessions of each percentage of saccharin for the paired and random groups (Fig. 5) revealed a significant main effect of groups \( F(1,22) = 10.531, P < 0.01 \). Analysis of 6% ethanol in 0.10% saccharin revealed that the paired group differed significantly from the random group \( F(1,22) = 7.090, P < 0.05 \). Analysis of 6% ethanol in 0.07% saccharin revealed that the paired group differed significantly from the random group \( F(1,22) = 4.073, P < 0.05 \). Fisher’s LSD revealed that the paired group differed significantly from the random group \( F(1,22) = 19.620, P < 0.01 \).

Sipper tube licks. Analysis of the number of licks on the sipper tube during the last four sessions of training with each saccharin concentration revealed that the paired and random groups did not differ in mean lick frequency during any of these periods (\( F < 1 \) in all cases).

Discussion

The results of Experiment 2 replicate and extend the major observations reported in Experiment 1. In Experiment 2, the paired group consumed more saccharin/ethanol solution than did the random group, and this effect was observed, even as the concentration of saccharin was systematically reduced across autoshaping sessions. Notably, group differences in ethanol drinking were most evident after the saccharin had been eliminated, indicating that, following the initiation of drinking, the maintenance of group differences in ethanol consumption is not dependent on the presence of saccharin. While it remains unclear if saccharin is required to initiate acceptance of the ethanol solution CS when these autoshaping procedures are employed, it is encouraging that these autoshaping procedures are effective, even without saccharin, in maintaining substantial levels of ethanol drinking in rats.

The results of Experiment 2 also confirm that the group differences in ethanol drinking are not due to confounding differences in body weight, and both the paired and random groups consumed more ethanol solution during the p.m. sessions than during the a.m. sessions. Finally, Experiment 2 shows that the groups differed reliably in ml of fluid consumed but not in the number of sipper tube licks, and unsystematic observations revealed, as noted in the previous study, that the rats in the paired groups were more likely to grasp the sipper tube CS and maintain contact with the sipper tube CS with the snout, thereby reducing the frequency of discrete contacts recorded as licks.

As noted earlier, the random group provided an estimate of the effects of pseudo-conditioning, and in Experiment 2, the paired group consumed more ethanol than the random group, and this effect was largest when the saccharin had been eliminated. As noted earlier, the random group provides an estimate of the effects of pseudo-conditioning, and in Experiment 2, the paired group consumed more ethanol than the random group and this effect was largest when the saccharin had been eliminated. These group differences are likely to be very conservative estimates of the effects of the Pavlovian paired procedures on ethanol drinking, as they were obtained despite the operation of several factors (prandial drinking and schedule-induced drinking) that would serve to increase drinking in the random group but not in the paired group.

The induction of ethanol drinking by these Pavlovian autoshaping procedures would be expected to further facilitate the ingestion of still additional quantities of ethanol. As noted by Tomie and his associates, pre-session intraperitoneal injections of ethanol have been reported to enhance the rate of acquisition and asymptotic levels of autoshaping CR performance in rats trained with lever CS–food US autoshaping procedures (Tomie et al., 1998). Thus, for the rats in the paired group of the present study, autoshaping CR performance would be expected to increase ethanol drinking, which, in turn, would be expected to increase autoshaping CR performance.
resulting in the drinking of still additional quantities of ethanol. This synergistic interaction between autoshaping and ethanol drinking provides the basis for a positive feedback loop yielding a binge episode of excessive ethanol intake.

It is appropriate to compare the levels of ethanol consumption induced by these Pavlovian autoshaping procedures relative to alternative procedures developed to induce ethanol drinking in rats. The following procedures, selected for purposes of comparison, are not intended to cover the entire literature on animal models of alcoholism (for complete review, see Lester and Freed, 1973; Mello, 1973, 1976; Meisch, 1982; Samson, 1987). Comparisons are only intended to highlight the amounts of ethanol consumed by male rats, of comparable weight, run in daily sessions, providing for discrete episodes of availability of ethanol solutions. In the current study, the mean g/kg of ethanol consumed during sessions 54–65 (6% ethanol in 0.0% saccharin) for the paired group was 1.9 ± 0.1 (range 1.4–2.5) and for the random group was 1.5 ± 0.1 (range 1.0–2.1). Converting to g/kg per hour of ethanol drinking sessions, the paired group consumed 3.8 g/kg per hour and the random group consumed 3.0 g/kg per hour.

Several other procedures have been developed to facilitate ethanol consumption in rats run in test chambers in daily drinking sessions, though it is important to acknowledge that the procedures employed in the present study differed in many ways from these alternative procedures, and therefore the differences reported may be due to any of a number of these factors.

The dipper-presentation paradigm is a widely employed operant procedure used to induce ethanol self-administration in rats (Samson, 1986). To initiate ethanol consumption in male Long–Evans rats, sucrose-fading procedures were employed providing the rats with varying concentrations of sucrose and ethanol. The 0% sucrose/5% ethanol solution produced a mean intake of 0.28 g/kg per half hour session, or 0.56 g/kg per hour. Meisch and Thompson (1973) provided albino Sprague–Dawley rats with lever-press contingent access to a dipper containing 8% ethanol on fixed-ratio schedules and obtained a rate of 0.3 g/kg of ethanol consumption per hour. When the rats were food-deprived to 80% of their free-feeding weights, they consumed ~2.0 g/kg of ethanol per hour. Using the dipper procedures, Heyman (1993) provided Wistar rats, which were food-deprived to 85% of their free feeding weights, with access to a range of ethanol concentrations (5–20%) in 10% sucrose, on a variable interval 5-s schedule (estimated total ethanol access time = 8.57 min/session). Heyman reported that these food-deprived rats consumed 2.53–4.68 g/kg of ethanol per half hour session.

The sipper-tube presentation paradigm is another operant procedure used to induce ethanol self-administration in rats (Samson et al., 1999). To initiate ethanol consumption in male Long–Evans rats, sucrose-fading procedures were employed providing the rats with varying concentrations of sucrose and ethanol. The 0% sucrose/10% ethanol solution produced a mean intake of ~0.7 g/kg per half hour session, or 1.4 g/kg per hour.

The two-lever, free choice operant self-administration procedure developed by Koob and his associates employs saccharin-fading procedures to initiate and maintain ethanol consumption in rats (Weiss et al., 1990, 1996). Although several rats failed to develop reliable daily intakes of ethanol solutions and were subsequently excluded from experiments, most of the rats did reliably drink the ethanol solutions consuming on average 0.72 ± 0.10 g/kg per half hour session, or 1.44 g/kg per hour. Another study employed these procedures to compare ethanol drinking in alcohol-prefering (P) and heterogeneous populations of Wistar male rats. Mean daily fluid consumption was ~4.43 ± 0.4 ml per 30 min session for the P group and 3.31 ± 0.42 ml per 30 min session for the heterogeneous group, or approximately 1.6 g/kg per hour and 1.3 g/kg per hour for the P and heterogeneous groups, respectively.

Limited access paradigms assess ethanol drinking in the rats’ home cage during discrete periods of ethanol access. In our laboratory, male Long–Evans rats given a 1-h limited access to 6% ethanol solution in the home cage consumed 0.88 ± 0.02 g/kg per hour. Sinclair et al. (1992) described one technique in which male Wistar rats and male AA rats had continual access to 10% ethanol solution. During this period, the AA group consumed 7.99 ± 0.28 g/kg and the Wistar group consumed 2.29 ± 0.34 g/kg, or 0.30 g/kg per hour and 0.09 g/kg per hour, for the AA and Wistar groups respectively. After switching to a 1-h access period, the g/kg ethanol intakes of the AA and Wistar groups converged, so that they both consumed ~0.80 g/kg per hour. The levels of the amount of alcohol consumption reported by Sinclair et al. (1992) were considerably higher than those reported by Sinclair (1990) who used similar limited access procedures in male Long–Evans rats and observed only ~0.4 g/kg per hour level of ethanol consumption. In a free choice procedure with alternate days of ethanol available in the home cage and water continuously available, Boyle et al. (1997) found that male Long–Evans rats consumed between 0.75 and 1.0 g/kg of 6% ethanol per 23-h session, though at higher levels of alcohol concentration, increased g/kg intakes were observed.

Forced choice is a widely employed technique reported to rapidly initiate ethanol consumption in rats, and to maintain high daily volumes of ethanol intake. Variations of this procedure include presenting a nutritionally complete solution that includes ethanol, or an ethanol solution as the only source of liquid while providing additional food. Walker and Hunter (1974) used the former variation and reported that the mean daily ethanol consumption of male Long–Evans rats was 8.0 ± 0.8 g/kg/day. Employing the latter variation, Glavin and Rockman (1985) selected Wistar rats based on their ethanol consumption during 4 days of forced exposure to 6% ethanol solution. The low group consumed 1.5–2.5 g/kg per day, the medium group consumed 2.5–4.5 g/kg per day, and the high group consumed 4.5–6.0 g/kg per day. While both variations of the forced choice procedure result in greater daily ethanol intake than that reported in the present autoshaping studies, it should be noted that data on the ethanol consumed during these forced choice procedures are based on 23 h of daily access to the ethanol solution, rather than the 125 s (25 trials × 5 s/trial) of access to the ethanol solution provided by the autoshaping procedures.

Our work confirms that the fading procedures using sweetened ethanol solutions (Samson, 1986; Weiss et al., 1990; Samson et al., 1999) are effective in initiating and maintaining consistently higher levels of ethanol consumption, and the present studies extend the application of the fading procedure to the realm of Pavlovian autoshaping. While comparison of levels of ethanol drinking across different procedures is tenuous, at best, the levels of ethanol drinking induced by the Pavlovian autoshaping procedures appear to be at least as high
as those induced by alternative procedures and particularly when ethanol consumption is expressed as a function of ethanol availability time.

These Pavlovian autoshaping procedures induce relatively high volumes of ethanol consumption in very brief periods of time and, therefore, may provide an animal model of binge drinking. The rapid drinking of large volumes of ethanol that characterizes binge drinking in humans is characteristic of the drinking behaviour induced by the Pavlovian autoshaping procedures. In our studies, the paired rats consumed ethanol vigorously, drinking ~1.9 g/kg of ethanol per session even though the sipper tube was available for only a total of 125 s/session.

The finding that ethanol drinking is facilitated by the pairing of ethanol with food is not inconsistent with observations of the circumstances under which ethanol is often consumed by humans. It seems plausible that, for many people, ethanol consumption is differentially paired with the eating of food or with the presence of other highly valued and rewarding events, such as social interaction, entertainment, and other highly preferred activities. Griffiths et al. (1974, 1977, 1978) have reported that humans drink far less alcohol under conditions of isolation, as compared to when social interactions and other preferred activities are available, thus allowing for the pairing of alcohol with other rewards; these are precisely the conditions amenable to the acquisition and expression of autoshaping CR (Tomie, 1995, 1996, 2001).

Acknowledgements — This research was supported in part by NIAAA grant 12023-01A1 awarded to A.T. and NIAAA grant 10124-03 awarded to L.A.P.

REFERENCES


Marcucella, H. and Munro, I. (1986) Patterns of ethanol and water consumption as a function of restricted ethanol access and feeding condition. Psychopharmacologia (Berlin) 89, 145–149.


