INTRODUCTION

Researchers have developed several procedures to study daily discrete episodes of voluntary, oral ethanol intake in drinking chambers in rats (Falk and Tang, 1988; for review see Samson et al., 2000). Three methods of dispensing ethanol in drinking chambers have traditionally been employed: dippers, fixed-position drinking tubes, and, more recently, retractable drinking tubes. Dipper procedures provide the rat with brief, repeated, and intermittent access to restricted and small volumes of the ethanol solution (Meisch and Beardsley, 1975; Samson, 1986; Samson et al., 1988; Weiss and Koob, 1991; Files et al., 1994; Heyman, 1997; for review see Samson et al., 2000; Samson and Chappell, 2001; Ciccocioppo et al., 2004). Fixed-position drinking tubes provide the rat with continuous access to the ethanol solution during the entire duration of the drinking session (Falk et al., 1972; Samson and Falk, 1975, 1977; Falk and Tang, 1988; Le et al., 2005). Retractable drinking tubes, on the other hand, allow the experimenter to control the duration of availability of the tube, while, at the same time, allowing the rat unrestricted access to ethanol while the tube is in the drinking chamber (Samson et al., 1998, 1999; Czachowski and Samson, 1999; Files et al., 2000; Tomie et al., 2002a, b, 2003, 2004b, 2005a; Czachowski et al., 2003; for review see Samson et al., 2000).

Recently, Tomie and his associates reported that inserting and retracting the drinking tube intermittently (intermittent sipper procedure) induced much longer duration access to the ethanol solution (Tomie et al., 2005a). This effect of the intermittent sipper procedure was observed when access to the intermittent ethanol sipper was co-mingled with the opportunity to interact socially with a conspecific male rat. The objective of this study was to evaluate the effects of the intermittent sipper procedure in situations providing for intermittent presentations of food, and, in addition, in situations that do not provide for intermittent presentations of another rewarding event.

Materials and Methods

Animals

Thirty-two Long-Evans (Blue Spruce strain) rats (~300 g at the beginning of the experiment) obtained from Harlan–Sprague–Dawley (Almont, NY) were housed individually in suspended steel cages in a colony room with a 12L:12 D (on 04:00 h) cycle. The rats had continuous access to food (PMI Rat Chow, Formula 5012) and water in their home cages. 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, 1996) and approved by the IACUC at Rutgers University.

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.

Apparatus

The drinking chambers were four cubicles (32 × 25.5 × 23 cm³ each), purchased from MED Associates (St Albans, VT),
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, ~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-750C), cabled to a connection panel (Model SG-215D), and operating under locally developed software, controlled the session events and data collection.

**Procedures**

The rats were run 5–6 days a week in daily drinking sessions conducted between 09:00 and 16:00 h. The rats were weighed before each drinking session and then placed immediately into the drinking chamber. Prior to the initiation of the study, the rats were randomly assigned to one of four groups. For rats in the Intermittent Sipper/Food group (n = 8), the ethanol sipper was inserted for 10 s, followed immediately by the response-independent operation of the pellet dispenser, which delivered a 45 mg food pellet. For rats in the Intermittent Sipper/No Food group (n = 8), the ethanol sipper was presented on the same schedule as for the Intermittent Sipper/Food group, but the food pellet was not presented at any time during the session. Rats in the Continuous Sipper/Food group (n = 8), were provided with continuous access to the ethanol sipper throughout the session while the pellet dispenser was operated on the same schedule as for the Intermittent Sipper/Food group. Rats in the Continuous Sipper/No Food group (n = 8), received training similar to the Continuous Sipper/Food group, except that the food pellet was not presented at any time during the session. For all groups, the delivery of the food pellet was response-independent, occurring regardless of whether the subject contacted the ethanol sipper or consumed the ethanol fluid in the sipper. All groups received a total of 25 trials per drinking 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 ~30 min. The volume of fluid consumed (ml) during each session was determined by recording the weight of the bottle immediately before and after each drinking session.

For all four groups, during the first 10 sessions of training with drinking procedures, the ethanol sipper tube contained 3% ethanol (vol./vol.), followed by 10 sessions (11–20) of training with 4% ethanol (vol./vol.), 6 sessions (21–26) of training with 6% ethanol (vol./vol.), 6 sessions (27–32) of training with 8% ethanol (vol./vol.), and 10 sessions (33–42) of training with 10% ethanol (vol./vol.). Training was conducted with each ethanol concentration until mean session-to-session variability in g/kg ethanol intake for all four groups did not vary by more than 10% between three consecutive sessions.

**Statistical analysis**

For all subjects for each drinking session, fluid consumed (ml) and body weight (kg) were recorded, and grams of ethanol consumed per kilogram of body weight (g/kg ethanol intake) derived. Analyses of the effects of drinking sessions on initiation of ethanol intake were conducted on the 10 days of training with 3% ethanol. All analyses of the effects of ethanol concentrations were conducted on the mean of the last three sessions of training with each concentration. Effects on g/kg ethanol intake of Sipper Procedure (Intermittent vs Continuous), Food Procedure (Food vs No Food), and ethanol concentration [3, 4, 6, 8, and 10% (vol./vol.)] were assessed by 3-way mixed-design 2 × 2 × 5 repeated-measures multivariate analysis of variance using MANOVA (Systat Statistical Software, Richmond, CA, USA), then separate mixed-design 2-way repeated-measures MANOVA were conducted on each level of Food Procedures. Corrections for familywise error for all ANOVAs were based on an overall alpha level of 0.05. For the overall 2 × 2 × 5 ANOVA, seven effects were evaluated (alpha = 0.007). For each 2 × 5 ANOVA, three effects were evaluated (alpha = 0.017). Preexperimental predictions regarding differences between groups based on Sipper Procedure (Intermittent Sipper vs Continuous Sipper) were derived from previously published reports of more ethanol drinking and intake in the Intermittent Sipper than in the Continuous Sipper group (Tomie et al., 2005a); therefore, main effects of Sipper Procedure were evaluated statistically by using an adjusted family-wise alpha level = 0.033, one-tailed. Significant interaction effects between Sipper Procedure and ethanol concentration were evaluated using Fisher’s Least Significant Difference (LSD) subsequent test, which provided pairwise comparisons at individual points (alpha = 0.05).

**RESULTS**

All 32 rats initiated drinking of unsweetened 3% ethanol from the sipper during sessions 1–10, with group mean daily drinking during sessions 8–10 of at least 0.4 ml; however, there were no group differences in milliliters of drinking or grams per kilogram of ethanol intake during the first 10 sessions. Analysis of escalation of mean daily grams per kilogram ethanol intake across the five ethanol concentrations [(3, 4, 6, 8, and 10% (vol./vol.))] revealed a significant main effect of Sipper Procedure [F(1,28) = 15.07, P = 0.001], no significant main effect of Food Procedure [F(1,28) = 1.691, P = 0.204], and a significant main effect of ethanol concentration [F(4,112) = 63.35, P = 0.001]. Within the Food condition (Intermittent Sipper/Food vs Continuous Sipper/Food) analysis revealed (see Fig. 1) a significant main effect of Sipper Procedure [F(1,14) = 5.91, P = 0.029], a significant main effect of ethanol concentration [F(4,56) = 34.47, P = 0.001], and no significant interaction between Sipper Procedure and ethanol concentration [F(4,56) = 1.37, P = 0.267]. A similar
analysis conducted on the No Food condition (Intermittent Sipper/No Food vs Continuous Sipper/No Food) revealed (see Fig. 2) a significant main effect of Sipper Procedure \[F(1,14) = 10.11, P = 0.007\], a significant main effect of ethanol concentration \[F(4,56) = 30.94, P = 0.001\], and a significant interaction between Sipper Procedure and ethanol concentration \[F(4,56) = 5.46, P = 0.001\]. Fisher’s LSD revealed significantly higher mean daily g/kg ethanol intakes \((P < 0.05)\) in the Intermittent Sipper/No Food group than in the Continuous Sipper/No Food group when the concentration of ethanol in the sipper CS was 6, 8, and 10%. Thus, the Intermittent Sipper Procedure increased the rate of escalation of mean daily g/kg ethanol intake relative to the Continuous Sipper Procedure, and this effect was significant in both the Food and No Food conditions.

**DISCUSSION**

In all four groups, mean daily g/kg ethanol intake was a positive function of the concentration of ethanol in the sipper tube, replicating previous reports of this effect in non-deprived rats in autoshaping studies employing either ethanol sipper CS and food US (Tomie et al., 2004b, 2005b) or ethanol sipper CS and social interaction opportunity US (Tomie et al., 2004a, 2004c, 2005a). The Intermittent Sipper procedure induced more rapid escalation of daily ethanol intake than the Continuous Sipper procedure, and this effect was observed in both the Food and No Food conditions. In the Food condition, with 10% ethanol in the sipper, even though the Intermittent Sipper/No Food group was provided with less opportunity to drink from the ethanol sipper during each session, their ethanol intake was \(\sim 65\%\) greater than the Continuous Sipper/No Food group. A similar comparison in the No Food condition, revealed that the ethanol intake of the Intermittent Sipper/No Food group was 94% greater than the Continuous Sipper/No Food group. The Intermittent Sipper groups had access to the ethanol sipper for a total of only 4 min and 10 s during each session, while the Continuous Sipper groups had access to the ethanol sipper for \(\sim 30\) min, or over seven times longer, during each session. Mean daily ethanol intake was inversely related to the total duration of access to the ethanol sipper during each daily drinking session, replicating our previous report of the intermittent sipper effect (Tomie et al., 2005a). The present study adds the important observation that the intermittent sipper effect is not dependent on intermittent presentations of another rewarding event, such as social interaction opportunity or food.

The procedures of the Continuous Sipper/Food group are similar to schedule-induced polydipsia (SIP) procedures employed to induce ethanol drinking in rats (Falk et al., 1972; Falk, 1977). It is unlikely, however, that SIP contributed substantially to ethanol drinking in the Continuous Sipper/Food group, as similar levels of ethanol drinking were observed in the Continuous Sipper/No Food group that did not experience an intermittent schedule of food deliveries. It should be noted that the levels of ethanol drinking in the Continuous Sipper/Food group were much lower than in many studies of SIP (Falk and Tang, 1988), though this may be due to differences in food-deprivation procedures. In the
present study, rats were maintained with free access to food in the colony room, while in SIP studies rats are typically food-restricted in the colony room, so as to be maintained at 80–85% of their free-feeding weights (Falk and Tang, 1988). Several investigators employing SIP procedures have reported that removal of food deprivation substantially reduces ethanol drinking (Meisch and Thompson, 1973, 1974; Lynch et al., 1982; Stiglick and Woodworth, 1984; Hepner and Kemble, 1987).

Schedule-induction may still play a role, nevertheless, as the ethanol sipper was also presented on an intermittent schedule and this may have contributed to the development of SIP, resulting in elevated levels of ethanol intake in the Intermittent Sipper groups relative to the Continuous Sipper groups. Consistent with this analysis is evidence that ethanol is rewarding or positively reinforcing (Samson et al., 1998). In addition the lower levels of ethanol intake in the Continuous Sipper groups is not unlike the effects of removing the intermittent reward schedule in studies of SIP. For example, Tang et al. (1982) exposed rats to intermittent food deliveries, which resulted in higher ethanol intake relative to controls given daily single-ration sessions of food; moreover, when intermittent foods were discontinued, drinking levels decreased (Tang et al., 1982). This is similar to the results of a study by Reynolds et al. (1977) who reported less SIP when 105 pellets where presented at the beginning of the session than when a single pellet was presented every 70 s. Moreover, increasing the amount of time between reward deliveries up to the 60 s interval employed in the present study increased the levels of drinking in rats (Falk, 1967; Freed and Hymowitz, 1972; Falk and Tang 1989; Hodgson and Bond, 1994), and larger food reward magnitudes induced lower levels of SIP (Freed and Hymowitz, 1972; Keenh and Stoyanov, 1986). Thus, in studies of SIP, less drinking is induced by procedures providing for mass ration feedings or shorter intervals between successive rewards, and these aspects of SIP procedures resemble the properties of the Continuous Sipper procedure of the present study. These data, therefore, suggest that a circumstance conducive to ethanol intake is an intermittent schedule of presentations of the sipper containing ethanol itself. Consistent with this analysis, there is evidence suggesting that intermittent exposure to ethanol increases ethanol intake. For example, intermittent exposure to ethanol vapour increases oral ethanol self-administration in rats more than continuous exposure to ethanol vapour (O’Dell et al., 2004), and interruptions in ethanol availability increased ethanol drinking in monkeys (Kornet et al., 1990). Moreover, intermittent ethanol exposures may influence the development of and loss of behavioural tolerance to ethanol’s unconditioned effects (Maier and Pohorecky, 1987; Pohorecky and Roberts, 1991), though levels of ethanol intake achieved in the present study were lower than those associated with the development of behavioural tolerance.

A theoretical account of the intermittent sipper effect is offered by the CAM/autoshaping model (Tomie, 1995, 1996), which proposes that the positive correlation between the ethanol sipper CS and ethanol US induces Pavlovian autoshaping CRs expressed as sipper tube CS—directed drinking of ethanol. The positive correlation between the sipper tube CS and ethanol US is maintained at a higher level by the Intermittent Sipper Procedure because neither are available during the majority of the session, improving their co-variation (Rescorla, 1967; Gibbon et al., 1974). Further support for the CAM/autoshaping model is derived from data showing that ethanol intake is a positive function of the duration of sipper tube retraction (Tomie et al., 2003, Experiment 2). These effects of trial spacing on autoshaping CR performance have been well documented by autoshaping investigators using a wide range of autoshaping techniques (Gibbon and Balsam, 1981; Killeen, 1984; Gallistel and Gibbon, 2000) and provides evidence of the involvement of Pavlovian autoshaping processes in the induction of ethanol drinking by the intermittent sipper procedure.

These data may have implications for models of drug-taking based on principles derived from behavioural economics (Hrusch and Bauman, 1987; Bickel et al., 1990; Carroll et al., 1991; Carroll, 1993, 1996; DeGrandpre and Bickel, 1996). For example, Carroll (1996) reviews evidence that the availability of non-drug alternative reinforcers reliably reduces drug self-administration in animals and humans. For example, availability of food decreases self-administration of cocaine and other drugs (Carroll et al., 1981, 1991; de la Garza et al., 1981; Carroll and Meisch, 1984), and, in addition, the availability of preferred dietary sweeteners also suppresses intake of cocaine and other drugs (Carroll, 1985; Kanarek and Marks-Kaufman, 1988a; b; Carroll et al., 1989, 1991; Carroll and Lac, 1992). In the present study, the groups receiving presentations of food during ethanol drinking sessions do not differ in ethanol intake from the groups that do not receive food during ethanol drinking sessions, suggesting that food does not substitute for ethanol. Moreover, presentations of food did not increase ethanol drinking, suggesting that food is not an economic complement of ethanol. There is evidence from studies of SIP that food and ethanol are economic complements, as removing intermittent presentations of food reduced ethanol drinking (Freed and Lester, 1970; Freed et al., 1970; Tang et al., 1982). In the present study, unlike in studies of SIP, rats were maintained with free access to food in their home cages, and this may have reduced the effectiveness of food as an alternative reinforcer. This explanation is consistent with reports that food deprivation increased drug-seeking or drug-taking behaviour reinforced by ethanol (Meisch and Thompson, 1973).

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REFERENCES


