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Effects of Age on Pavlovian Autoshaping of Ethanol Drinking in Non-Deprived Rats

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Previous studies of autoshaping of drinking report a positive relationship between experience with autoshaping procedures and drinking, but this effect was confounded with age, as the rats were older when they drank more. The present experiment evaluated the effects of the age of male Long-Evans hooded rats [90-days old (Younger group) vs. 135 days old (Older group)], at the beginning of the study, on drinking induced by Pavlovian autoshaping procedures. Autoshaping procedures consisted of pairings of sipper conditioned stimulus (CS) with food unconditioned stimulus (US). Rats were deprived of neither food or fluid, and sweeteners were not employed at any time during the study. For all rats (n = 32), during sessions 1-10, the sipper CS contained water. Thereafter, for rats in the Ethanol groups (n = 20), the sipper CS contained ethanol, with the concentration (1, 2, 3, 4, 5, 6%, v/v) increasing across autoshaping sessions. For rats in the Water groups (n = 12), throughout the experiment the sipper CS contained tap water (0% ethanol). Rats in the Younger group drank more ethanol and more water from the sipper CS than rats in the Older group, and across age groups there was more ethanol drinking than water drinking, an effect unlikely due to foraging for calories. Data support the hypothesis that ethanol's pharmacological effect was to enhance autoshaping, resulting in a positive feedback loop inducing still more ethanol drinking. The younger rats were more vulnerable to autoshaping effects. Implications for models of addiction are discussed.

Pavlovian autoshaping procedures providing for pairings of sipper conditioned stimulus (CS) and food unconditioned stimulus (US) induce autoshaping of sipper CS-directed drinking, as revealed by systematic increases in drinking of fluid (water or ethanol) from the sipper CS as a function of experience with autoshaping procedures (Tomie et al., 2002a, 2002b, 2003, 2004). This effect of experience was confounded with age, as the rats were older when they drank more. For example, at the beginning of these studies, male rats were approximately 75-90 days old and initiated modest levels of sipper CS-directed drinking. Following approximately 30-35 daily sessions of training with autoshaping procedures, the rats were approximately 120-135 days old and drank higher daily volumes of fluid from the sipper CS. Thus, higher levels of drinking were confounded with the age of the rats at the time of drinking as well as with amount of training with autoshaping procedures. The higher levels of drinking observed in older rats, therefore, may be due to age-related differences in neophobia (Lorens et al., 2003; Misanin et al., 1985), ethanol acclimation (Amit et al., 1971; Veale & Myers, 1969; Wise, 1973), or ethanol's age-related effects on motor performance (Brasser & Spear, 2002; Silveri & Spear, 2001) and learning processes (Garcia-Moreno et al., 2002; Walker et al., 1981), rather than to experience with autoshaping procedures per se.

The present study evaluated effects of 28 daily sessions of autoshaping procedures on ethanol and water drinking in groups of rats that were 90 and 135

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days old at the beginning of the experiment. Previous studies that confounded age with fluid intake reported more autoshaping of ethanol drinking (Tomie et al., 2002a, 2002b, 2003, 2004) and water drinking (Tomie et al., 2002b, 2003, 2004) in older than in younger rats, but other investigators have reported that ethanol drinking in rats depends on the age of ethanol drinking onset (Yoshimoto et al., 2002), with more ethanol drinking observed in younger rats (Campbell, 2001; Dominquez et al., 1993; McKinzie et al., 1998; Rodd-Hendricks et al., 2002a, 2002b; Wahlstroem, 1994; Wood, 1976). It should be noted that the age ranges employed in the present study were selected to clarify age-related effects in previous autoshaping studies rather than to explore the effects of distinct developmental stages.

Method

Subjects

Adult male Long-Evans (Blue Spruce strain) rats (n = 32) were obtained from Harlan-Sprague-Dawley (New York, U.S.A.). The Younger group of rats (n = 16) were approximately 80-days old upon arrival in the colony and weighed approximately 275-300 g. The Younger group of rats were maintained in the colony room for approximately 10 days prior to the beginning of training with autoshaping procedures (September 2002). On the first day of autoshaping, the Younger rats were approximately 90-days old and weighed approximately 325 g. The Older rats (n = 16) were approximately 80-days old upon arrival in the colony and weighed approximately 275-300 g. The Older rats were maintained in the colony room for approximately 55 days prior to the beginning of training with autoshaping procedures (September 2001). On the first day of autoshaping, the Older rats were approximately 135-days old weighed approximately 425 g. Though the groups of rats were run one year apart, they were run during the same season providing for comparable laboratory conditions. Rats were housed individually in suspended steel cages in a colony room 12L: 12D (on 04:00 h) cycle. Rats were maintained at their free-feeding body weights by providing continuous access to supplemental rat food (Lab Diet, Formula 5012, PMI Nutrition International, Missouri, U.S.A.) and tap water in their home cages.

Apparatus

Autoshaping chambers were four Med Associates (Indiana, U.S.A.) cubicles (32 cm L x 23 cm W x 26 cm H) made of stainless steel front intelligence panel, stainless steel back wall, and a stainless steel grid floor (Model ENV-008). The ceiling, left wall, and right wall were clear Plexiglas, and the left Plexiglas wall was hinged to the back wall and opened with a side latch. One house light (ENV-215) mounted to the top-middle portion of the back wall of the cubicle was equipped with a GE 1821 lamp (28 Vdc), which provided ambient illumination to the chamber. On the front intelligence panel, the operation of a pellet dispenser (Model ENV-203M) delivered a single 45 mg food pellet containing approximately 50% sucrose (#F0021, BioServ; New Jersey, U.S.A.) to a metal pellet dispenser trough (Model ENV-200R2M) placed 1.0 cm from the back wall and 0.25 cm above the grid floor. A stainless steel sipper tube was inserted into the chamber through an oval hole in the intelligence panel located 3.0 cm from the right wall and 3.5 cm above the grid floor. The stainless steel sipper tube contained a stainless steel ball-bearing with an inserted rubber stopper holding the solution in a 400 ml Plexiglas bottle. The bottle insertion mechanism (ENV-252) moved the sipper tube a total of 3.8 cm from fully retracted to fully inserted position. In the fully retracted position, the sipper tube was 3.2 cm removed from the chamber. Each testing chamber was enclosed in sound-attenuating, ventilated outer casings (Model ENV-022) which provided masking noise by the operation of a ventilating exhaust fan (Model ENV-025F) mounted on the outer casing. An IBM PC, equipped with a relay interface card (Model DIG-750C) cabled to a connection panel (Model SG-215D) operating under locally developed software, controlled session events and data collection.

Ethanol (95% bulk) was obtained from Rutgers University Chemistry Department. Addition of tap water was used to create volume to volume dilutions of all ethanol concentrations.

Procedure

Rats in the Younger (n = 16) and Older (n = 16) groups received one autoshaping session per day, 5-6 days per week which was conducted between 09:00 and 16:00 h. All subjects were weighed immediately before being placed in the autoshaping chamber. Ten rats in each age group were randomly assigned to the Ethanol groups, while 6 rats in each age group were assigned to the Water groups. For all groups, the sipper tube (CS) was inserted for 5 s followed immediately by the response-independent operation of the pellet dispenser (US), which delivered a 45 mg food pellet to the feeder trough regardless of whether or not the subject contacted the sipper tube CS. All groups received 25 autoshaping trials per session. Throughout the experiment, the mean intertrial interval duration between successive autoshaping trials was 60 s, with a minimum 45 s and a maximum of 75 s. The session duration was approximately 30 min. Sipper tubes were tested for leakage prior to the start of the first session of each day. At the end of each daily session, the floors beneath the sippers were inspected for fluid. Volume of fluid consumed (ml) during each autoshaping session was determined by recording the weight of the Plexiglas bottle before and after each autoshaping session. Sipper tube licks were not recorded.

For autoshaping sessions 1-10, the sipper CS contained tap water (0% ethanol) for all rats in the Ethanol groups (n = 20) and the Water groups (n = 12). During sessions 11-13, for all rats in the Ethanol groups, the sipper CS contained 1% ethanol (v/v). For all rats in the Ethanol groups, during sessions 14-16, 17-19, 20-22, 23-25, 26-28, the sipper CS contained 2%, 3%, 4%, 5%, and 6% ethanol (v/v), respectively. During the entire duration of the study, during sessions 1-10 and during sessions 11-28, for all rats in the Water groups, the sipper CS contained tap water (0% ethanol).

On the first autoshaping session, the Younger rats were approximately 90-days old and weighed approximately 325 g. During the last 3 autoshaping sessions in which the sipper CS contained tap water (0% ethanol), the Younger rats were approximately 98-100 days old, and weighed approximately 355 g. The Younger rats were approximately 101-103, 104-106, 107-109, 110-112, 113-115, and 116-118 days old, during the 3 autoshaping sessions in which the sipper CS contained 1%, 2%, 3%, 4%, 5%, and 6% ethanol (v/v), respectively. The Younger rats weighed approximately 363 g, 372 g, 379 g, 387 g, 396 g, and 403 g, during the 3 autoshaping sessions in which the sipper CS contained 1%, 2%, 3%, 4%, 5%, and 6% ethanol (v/v), respectively.

On the first autoshaping session, the Older rats were approximately 135-days old and weighed approximately 425 g. During the last 3 autoshaping sessions in which the sipper CS contained tap water (0% ethanol), the Older rats were approximately 143-145 days old, and weighed approximately 433 g. The Older rats were approximately 146-148, 149-151, 152-154, 155-157, 158-160, and 161-163, days old, during the 3 autoshaping sessions in which the sipper CS contained 1%, 2%, 3%, 4%, 5%, and 6% ethanol (v/v), respectively. The Older rats weighed approximately 434 g, 443 g, 450 g, 455 g, 464 g, and 468 g, during the 3 autoshaping sessions in which the sipper CS contained 1%, 2%, 3%, 4%, 5%, and 6% ethanol (v/v), respectively.

For each subject and for each autoshaping session, body weight in gms and gms of solution consumed were recorded. Due to large differences in body weights between the age groups, weight-adjusted measures of g/kg ethanol intake and g/kg fluid intake were derived. For each measure, the means of the last 3 days of autoshaping during which the Ethanol group received training with each ethanol concentration (0%, 1%, 2%, 3%, 4%, 5%, and 6%) in the sipper CS, were derived and analyzed statistically. Effects of age (Younger vs Older) and autoshaping sessions 1-10 on mean ml water drinking and g/kg water intake were assessed using repeated-measures, multivariate analysis of variance (MANOVA, SYSTAT). Effects of age (Younger vs Older), sipper fluid (Ethanol vs Water), and ethanol concentration (1%, 2%, 3%, 4%, 5%, and 6%) on mean ml drinking and mean g/kg fluid intake were assessed using repeated-measures, multivariate analysis of variance (MANOVA, SYSTAT). Effects of age (Younger vs Older) and ethanol concentration (1%, 2%, 3%, 4%, 5%, and 6%) on mean ml drinking and mean g/kg ethanol intake were assessed using repeated-measures, multivariate analysis of variance (MANOVA, SYSTAT). Fisher's Least Significant Difference (LSD) provided comparisons between individual pairs of means (alpha = 0.05).

Results

The Younger and Older groups both systematically increased mean daily ml water drinking from the sipper CS during the first 10 daily sessions of training

with autoshaping procedures wherein the sipper CS contained tap water (0% ethanol) for both groups. Mean daily ml water drinking during autoshaping sessions 8-10 were 1.93 and 1.31 for the Younger and Older groups, respectively. Analysis revealed a significant main effect of age, $F(1, 30) = 4.26$, $p < 0.05$, a significant main effect of sessions, $F(9, 270) = 10.66$, $p < 0.01$, and a significant interaction effect between age and sessions, $F(9, 270) = 3.29$, $p < 0.01$. Fisher's LSD revealed that mean daily ml water drinking in the Younger group was significantly higher on autoshaping sessions 2, 4, 6, and 8-10. Analysis of mean daily g/kg fluid intake (see Figure 1), revealed a significant main effect of age, $F(1, 30) = 9.32$, $p < 0.01$, a significant main effect of sessions, $F(9, 270) = 8.77$, $p < 0.01$, and a significant interaction effect between age and sessions, $F(9, 270) = 3.55$, $p < 0.01$. Fisher's LSD revealed that mean daily g/kg fluid intake in the Younger group was significantly higher on autoshaping sessions 2, 4, and 6-10.

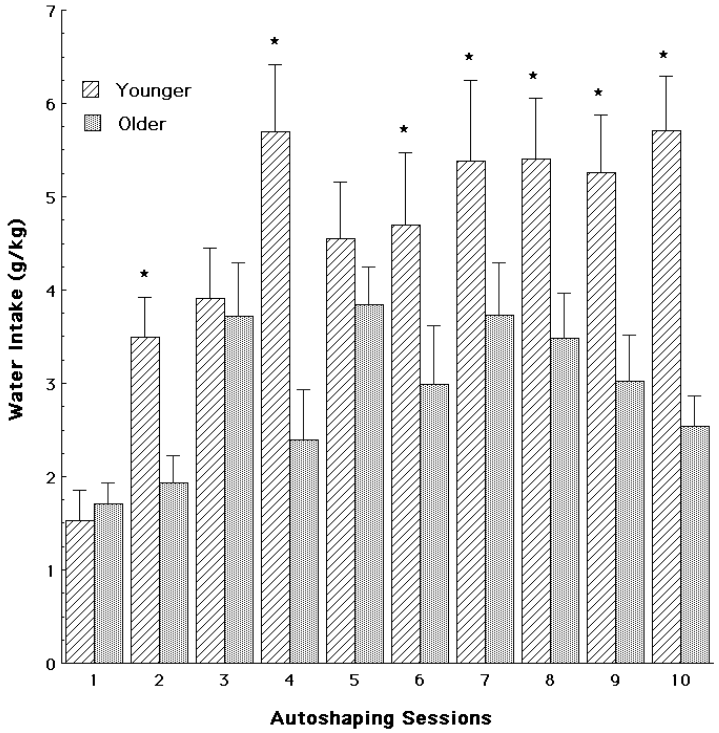

Figure 1. Mean daily g/kg water intake from the sipper CS during autoshaping sessions 1 through 10 for rats in the Younger group ($n = 16$; approximately 90-days old) and the Older group ($n = 16$; approximately 135-days old). The vertical bars represent the standard errors of the mean (SEM). The asterisk (*) indicates that the groups differ significantly ($p < 0.05$) on that session (Fisher's LSD).

For both the Younger/Ethanol and Older/Ethanol groups, mean daily ml drinking increased systematically as the concentration of ethanol in the sipper CS increased across sessions. Mean daily ml drinking of the 6% ethanol solution in the sipper CS were 2.80 and 1.87 for the Younger and Older groups, respectively. Analysis revealed a significant main effect of age, $F(1, 19) = 11.68$, $p < 0.01$, a significant main effect of concentrations, $F(5, 90) = 5.80$, $p < 0.01$, and no significant interaction effect between age and concentrations, $F(5, 90) < 1$. For both the

Younger/Ethanol and Older/Ethanol groups, mean daily g/kg fluid intake increased systematically as the concentration of ethanol in the sipper CS increased across sessions (see Figure 2). Analysis revealed a significant main effect of age, $F(1, 18) = 31.98$, $p < 0.01$, a significant main effect of concentrations, $F(5, 90) = 2.86$, $p < 0.05$, and no significant interaction effect between age and concentrations, $F(5, 90) < 1$.

For both the Younger/Ethanol and Older/Ethanol groups, mean daily g/kg ethanol intake increased systematically as the concentration of ethanol in the sipper CS increased across sessions (see Figure 3). Analysis revealed a significant main effect of age, $F(1, 18) = 29.23$, $p < 0.01$, a significant main effect of concentrations, $F(5, 90) = 84.09$, $p < 0.01$, and a significant interaction effect between age and concentrations, $F(5, 90) = 4.84$, $p < 0.01$. Fisher's LSD revealed that mean daily g/kg ethanol intake in the Younger group was significantly higher at all ethanol concentrations from 2% to 6%.

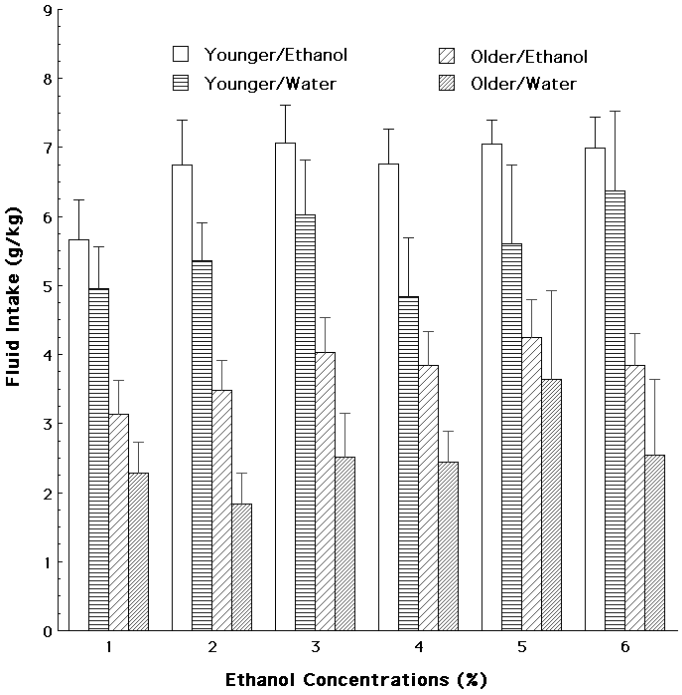

Figure 2. Mean daily g/kg fluid intake from the sipper CS during the 3 sessions of autoshaping when the sipper CS for the Ethanol groups contained each percent ethanol concentration [(1%, 2%, 3%, 4%, 5%, 6% (v/v)]. The sipper CS for the Younger/Ethanol group ($n = 10$) and the Older/Ethanol group ($n = 10$) contained the ascending sequence of ethanol concentrations. The sipper CS for the Younger/Water group ($n = 6$) and the Older/Water group ($n = 6$) contained water throughout the experiment. The vertical bars represent the standard errors of the mean (SEM).

For both the Younger/Water and Older/Water groups, mean daily ml drinking did not increase systematically during those sessions when the concentration of ethanol in the sipper CS for the Ethanol groups was increased across sessions. Mean daily ml water drinking when the Ethanol groups had 6% ethanol solution in the sipper CS were 2.62 and 1.18 for the Younger and Older groups, re-

spectively. Analysis revealed a significant main effect of age, $F(1, 10) = 5.26$, $p < 0.05$, no significant main effect of concentrations, $F(5, 50) = 2.12$, $p < 0.01$, and no significant interaction effect between age and concentrations, $F(5, 50) < 1$. For both the Younger/Water and Older/Water groups, mean daily g/kg fluid intake did not increase systematically as the concentration of ethanol in the sipper CS for the Ethanol groups increased across sessions (see Figure 2). Analysis revealed a significant main effect of age, $F(1, 10) = 9.50$, $p < 0.05$, no significant main effect of concentrations, $F(5, 50) = 1.48$, $p > 0.20$, and no significant interaction effect between age and concentrations, $F(5, 50) < 1$.

For the Younger groups, mean daily ml drinking of ethanol and water when the sipper CS for the Ethanol groups contained 4%-6% ethanol were 2.74 and 2.26, respectively. For the Older group, mean daily ml drinking of ethanol and water when the sipper CS for the Ethanol groups contained 4%-6% ethanol were 1.90 and 1.28, respectively. Three-way MANOVA on mean daily ml drinking revealed a significant main effect of age, $F(1, 28) = 16.02$, $p < 0.01$, a significant main effect of sipper fluid, $F(1, 28) = 5.41$, $p < 0.05$, and a significant main effect of concentrations, $F(5, 140) = 6.55$, $p < 0.01$, but no significant interaction effects between factors, all Fs < 1.

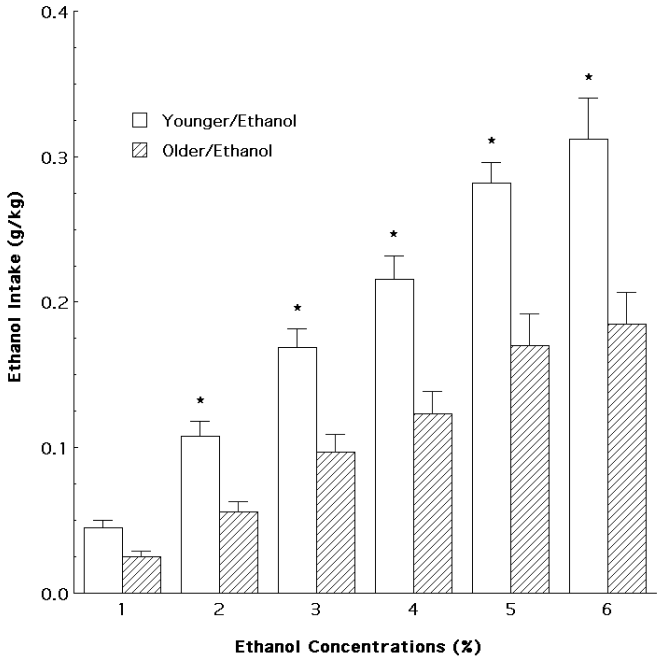

Figure 3. Mean g/kg of ethanol intake from the sipper CS during the 3 daily autoshaping sessions with each percent ethanol concentration [(1%, 2%, 3%, 4%, 5%, 6% (v/v)] for rats in the Younger/Ethanol and Older/Ethanol groups. The vertical bars represent the standard errors of the mean (SEM). The asterisk (*) indicates that the groups differ significantly ($p < 0.05$) at that ethanol concentration (Fisher's LSD).

For the Younger groups, mean daily g/kg fluid intake when the sipper CS for the Ethanol groups contained 4%-6% ethanol were 6.94 and 5.60 for the Younger/Ethanol and Younger/Water groups, respectively. For the Older group,

mean daily g/kg fluid intake when the sipper CS for the Ethanol groups contained 4%-6% ethanol were 3.98 and 2.87, respectively. Three-way MANOVA on mean daily g/kg fluid intake revealed a significant main effect of age, $F(1, 28) = 35.14$, $p < 0.01$, a significant main effect of sipper fluid, $F(1, 28) = 5.82$, $p < 0.05$, and a significant main effect of concentrations, $F(5, 140) = 3.54$, $p < 0.01$, but no significant interaction effects between factors, all ps > 0.35.

Discussion

Experience with autoshaping procedures that provided for pairings of the water sipper CS with food US, induced rats in both age groups to initiate water drinking from the sipper CS. Rate of acquisition and asymptotic levels of autoshaping of water drinking were significantly greater in the Younger group. Increasing the concentration of ethanol in the sipper CS across autoshaping sessions increased g/kg fluid intake in the Ethanol groups, but on those same sessions g/kg fluid intake in the Water groups did not exhibit an ascending trend. On autoshaping sessions when the sipper CS for the Ethanol groups contained ethanol concentrations of 2% to 6%, mean daily g/kg fluid intake was significantly higher in the Younger/Ethanol and Younger/Water groups, as compared to the Older/Ethanol and Older/Water groups, respectively. Thus, younger rats exhibited more evidence of autoshaping than older rats, and this effect of autoshaping was observed using mean session fluid intake measures of autoshaping CR performance, rather than the trial-by-trial CR measures typically reported by autoshaping investigators. Increasing the concentration of ethanol in the sipper CS induced reliable increases in g/kg ethanol intake. Asymptotic ethanol intake for both age groups was observed when the sipper CS contained 6% ethanol. Ethanol intake increased more rapidly as a function of ethanol concentrations in the Younger group, which showed approximately 70% greater ethanol intake than the Older group, at each of the ethanol concentrations.

The finding in the present study, that Younger rats more rapidly initiated and escalated intake of ethanol and water suggests that the higher levels of fluid intake reported in previous studies of autoshaping of drinking (Tomie et al., 2002a, 2002b, 2003, 2004) were likely due to experience with autoshaping procedures rather than to effects of age per se. For example, autoshaping of ethanol and water drinking was initiated in 90-day old rats, and on the first day of training, fluid drinking from the sipper CS was approximately 5 and 7 ml when the sipper CS contained ethanol or water, respectively. In contrast, on the 20th day of training, when the rats were approximately 110 days old, fluid drinking from the sipper CS was approximately 16 and 12 ml for the ethanol and water groups, respectively (Tomie et al., 2002b). While the increase in fluid intake was attributed to 20 days of experience with autoshaping procedures, the effects of experience with autoshaping procedures were confounded with the age of the rats at the time of drinking, and, therefore, changes in drinking within each group may be due to higher levels of drinking in older rats. The present data suggest that the Older rats did not drink more from the sipper CS than the Younger rats, and drinking increased systematically as a function of amount of experience with autoshaping procedures in both groups. These results are inconsistent with the hypothesis that increases in drinking across autoshaping sessions are due to increases in age rather than to

amount of experience with autoshaping procedures.

The present finding that autoshaping of ethanol drinking was negatively related to age at the onset of ethanol drinking is consistent with reports of investigators using a variety of alternative techniques to induce voluntary ethanol drinking in rats. For example, investigators that employed lever pressing for ethanol reinforcement in operant self-administration studies (Rodd-Hendricks, 2002a, 2002b) have reported more ethanol drinking in younger rats (33-60 days old) than in older rats (greater than 135 days old). A similar effect of age on ethanol drinking has been reported by investigators employing limited-access procedures. For example, 1- and 4-month old rats showed more ethanol drinking and ethanol preference than 10-month old rats (Yoshimoto et al., 2002; see also Wahlstroem, 1994). Thus, the present study adds to a growing body of literature indicating that younger rats initiated and escalated ethanol intake more rapidly than older rats, and these data provide, for the first time, evidence of this effect of age on the induction of ethanol intake by autoshaping procedures.

The effects of age were also evident in the acquisition of autoshaping of sipper CS-directed water drinking, as during the first 10 days, the Younger rats drank more water from the sipper CS on days 2, 4, 6-10. Thus, autoshaping of sipper CS-directed drinking was unlikely due to age-related effects of ethanol, but rather may be due to a more general effect of age on autoshaping CR performance. Though the specific ages investigated differed from those of the present study, investigators have reported superior performance in autoshaping tasks by younger as compared to older subjects. This age-related effect on autoshaping has been reported in rats (Meneses et al., 1996), chicks (Cunningham et al., 1989; Zolman et al., 1990), and humans (Anderson et al., 1981). For example, in autoshaping studies employing lever CS-food US pairings, investigators reported more lever-press autoshaping CR performance in 3-month old rats than in older rats (6, 9, 12, and 18-month old), while the oldest rats (24 months) acquired most slowly and achieved the lowest asymptotic levels of responding (Meneses et al., 1996). Age-related effects have also been reported by investigators employing other Pavlovian conditioning procedures. For example, 12-month old rats acquired cardiac conditioning more rapidly than 26-28-month old rats (Buchanan & Ginn, 1988), 4-week old kittens acquired cardiac conditioning more rapidly than adult cats (Soltysik et al., 1982), and 3-month old rabbits acquired nictitating membrane conditioning more rapidly than 21-55-month old rabbits (Woodruff-Pak et al., 1994).

It is unlikely that the age-related effects of ethanol observed in the present study were due to the differential effects of ethanol on cognitive processes related to autoshaping, such as spatial memory, in younger and older rats. Studies have demonstrated that ethanol inhibits memory processes (Garcia-Moreno et al., 2002) and memory-related synaptic activity and plasticity more so in younger rats than in older ones (for a review, see White et al., 2002). For example, Markwiese et al. (1998) reported that pretreatment with ethanol impaired acquisition of spatial memory in adolescent rats (30 days old) more so than in adult rats (65 days old). Thus, the memory-impairing effects of ethanol would be expected to reduce autoshaping more in the Younger than the Older rats, and this is opposite to the effects observed in the present study.

Ethanol's effects on motor impairment are age-related, with lower doses producing more impairment in younger than older rats (Silveri & Spear, 2001; see

also Silveri, 2001) but higher doses having the opposite effect (Little et al., 1996). Younger rats exhibited less tolerance to the motor-impairing effects of ethanol (Silveri & Spear, 2001; White et al., 2002; see also Silveri, 2001) and were more sensitive to the motor-impairing effects of lower doses of ethanol, showing more dramatic impairments in balance and motor coordination than adult rats; however, this effect was dose dependent, as older rats were more impaired following administration of higher doses of ethanol (2.0 g/kg and 3.0 g/kg ethanol) than were younger rats (White et al., 2002). There is also evidence that younger rats were less sensitive to the sedative effects of ethanol, as older rats (60 days old) showed a greater decrease in locomotor activity than younger rats (20 and 30 days old) when administered 2.5 g/kg ethanol (Little et al., 1996). Age-related differences in ethanol drinking and in sensitivity to ethanol's effects may also have been due to pharmacokinetic differences in ethanol metabolism between adolescent and adult rats (Little et al., 1996). In the present study, only lower doses of ethanol were achieved, which would be expected to induce more motor impairment and less autoshaping in younger rats, an effect opposite to that observed here.

In the present study, more fluid intake was observed when the sipper CS contained ethanol than water, and this effect was observed in both age groups. This effect has been previously reported by investigators employing sipper CS-food US autoshaping procedures (Tomie et al., 2002b, 2003, 2004), though typically in studies employing food-deprived rats. The rats in the present study were not food deprived, and this is significant, because higher levels of ethanol drinking in food-deprived rats are likely due, at least in part, to foraging for the caloric value of ethanol. An intriguing alternative interpretation is based on the premise that ethanol's pharmacological effect was to facilitate autoshaping CR performance. Evidence supporting this hypothesis was derived from data showing that presession injections of ethanol enhance autoshaping CR performance. For example, it has been reported that presession injections of ethanol (0.25–1.0 g/kg) facilitated lever-press autoshaping CR performance in rats receiving pairings of lever CS with food US (Tomie et al., 1998). If the pharmacological effect of ethanol was to enhance autoshaping, then autoshaping of sipper CS-directed ethanol drinking would result in ethanol intake, resulting in still further exaggeration of autoshaping, resulting in still further autoshaping of even more ethanol drinking. Thus, the pharmacological effect of ethanol on autoshaping may have provided the basis of a positive feedback loop, inducing exaggerated bouts of poorly controlled binge-like episodes of ethanol drinking (Tomie, 1995, 1996, 2001; Tomie et al., 2002a, 2002b, 2003, 2004). On the other hand, there would be no pharmacological effect of the sipper fluid that would have enhanced autoshaping CR performance when the sipper CS contained water.

The present data suggested that Younger rats were especially vulnerable to autoshaping of sipper CS-directed drinking and the pharmacological effect of ethanol was at least as evident in Younger rats as compared to Older rats. These data also provided evidence that autoshaping of sipper CS-directed drinking of unsweetened ethanol was readily obtained in all rats, even though rats were deprived of neither food nor fluid at any time during the study.

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