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Moderate doses of ethanol partially reverse avoidance learning deficits in high-alcohol-drinking rats

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Abstract

We previously reported that ethanol-naive high-alcohol-drinking (HAD1 and HAD2) rats exhibited selective deficits in active avoidance learning, as compared to low-alcohol-drinking (LAD1 and LAD2) rats, in a signaled bar-pressing task [Alcohol. Clin. Exp. Res. 24 (2000) 1778]. In the current study, we used appetitive and aversive learning tasks to assess whether administration of ethanol influences approach and avoidance learning in HAD and LAD rats. Rats were administered 0.0, 0.5, 1.0, or 1.5 g ethanol/kg body weight during appetitive and aversive conditioning sessions. We found that ethanol impaired acquisition of the appetitive conditioned response in a dose-dependent manner in both HAD and LAD rats, with 1.5 g/kg ethanol producing the greatest deficits. Notably, moderate doses of ethanol (0.5 and 1.0 g/kg) partially reversed avoidance learning deficits in HAD rats, but only when appetitive conditioning preceded aversive conditioning. The highest dose (1.5 g/kg EtOH) abolished avoidance responding altogether in HAD rats. Avoidance responding in LAD rats was not affected by any dose of ethanol. These results are consistent with previous studies suggesting that alcohol preference may be associated with increased fear or anxiety, but the conditions under which ethanol produces a reduction of fear and anxiety in HAD rats appear to be relatively complex.

Keywords: Alcohol-preferring rats; Appetitive conditioning; Aversive conditioning; Fear; Alcohol-seeking behavior; Ethanol; Anxiolytic effects

1. Introduction

Numerous studies have demonstrated the involvement of genetic factors in the development and maintenance of alcohol-seeking behaviors in both humans (Cloninger et al., 1981; Viken et al., 1999) and rats (Li et al., 1981; Lumeng et al., 1995). Moreover, during the last two decades, animal models of alcoholism, such as the alcohol-preferring (P) and high-alcohol-drinking (HAD1 and HAD2) rats, have proven valuable for studying etiological factors, behavioral and neural correlates, and consequences of alcohol consumption in a controlled environment that are simply not possible with humans. As a result, much is known about the neuroanatomical, physiological, and neurochemical factors associated with alcohol preference (Waller et al., 1983; Hwang et al., 1990; McBride et al., 1990; Krimmer and Schechter, 1992; Slawecki et al., 2000).

Among the lines of rats that have been selectively bred for alcohol preference, the P rats have been most extensively characterized (Waller et al., 1986; Froehlich et al., 1988; Schechter, 1992; Stewart et al., 1993; Blankenship et al., 1998; McKinzie et al., 2000). One difficulty with the use of P rats, however, is that no replicate line exists: Any results obtained using these rats cannot be tested in a replicate line to assure that the association with alcohol preference is not spurious. The high-alcohol-drinking (HAD) rats may be more useful in that one can be more confident in results implicating an association between a particular behavioral factor and alcohol preference if the same result is obtained in both the HAD1 and HAD2 replicate lines.

Most of the studies regarding lines of rats bred for alcohol-preference involve assessment of performance under the influence of ethanol, rather than in an alcohol-naive state. Alcohol-naive behavioral differences between lines of rats may contribute to the effects that ethanol has on these lines. Therefore, it is important to fully characterize the behavioral tendencies of these rats in order to gain a better understanding of one or more neurobiological substrates of alcohol-seeking behavior. Previous studies have shown that alcohol preference in HAD rats is associated with a number of behavioral factors, including increased locomotor activity to novel stimuli, decreased ultrasonic vocalizations, and

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greater immobility in a modified forced swim test (Overstreet et al., 1997; Nowak et al., 2000). Only recently, though, has there been a systematic assessment of alcohol-naive behaviors using associative learning tasks. In an attempt to determine what behavioral differences may exist between HAD and LAD rats, Blankenship et al. (2000) conducted a within-subject assessment of appetitive and aversive learning in HAD and LAD rats in an alcohol-naive state. Although no differences were observed between HAD1 and LAD1 rats in an appetitive signaled bar-pressing task, HAD1 rats exhibited severe deficits in active avoidance learning, as compared to LAD1 rats, in an aversive version of the task. That is, HAD1 rats failed to learn to avoid a footshock when signaled by a tone, regardless of whether the aversive task was presented before or after the appetitive task. This result was replicated in HAD2 and LAD2 rats.

The current study was designed as a follow up to the study conducted by Blankenship et al. (2000). Given that alcohol-naive HAD rats exhibited a selective deficit in active avoidance learning, the current study was designed to systematically evaluate the performance of HAD and LAD rats on this learning task under the influence of ethanol.

2. Methods

2.1. Subjects

The subjects were 48 LAD1 (22 females, 26 males), 62 HAD1 (31 females, 31 males), and 31 HAD2 (21 females, 10 males) experimentally naive rats obtained from the Alcohol Research Center at the Indiana University School of Medicine, Indianapolis, IN. Because our primary motivation for these experiments was to measure the effects of ethanol on the avoidance learning deficits in HAD1 and HAD2 rats observed in an earlier study, and because no conditioning differences were observed between LAD1 and LAD2 rats when compared to each other in an earlier study (Blankenship et al., 2000), LAD2 rats were not used in the experiments reported here.

Rats weighed at least 180 g at the beginning of the study and were maintained at 85% of their free-feeding weight. All rats were housed in 12-h light/dark conditions (0700 light/1900 dark cycle) and were cared for by the Indiana University Animal Care Facility, which operates in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The experimental protocols used in this study were approved by the Indiana University Bloomington Animal Care and Use Committee.

2.2. Apparatus

All rats were trained in standard operant boxes consisting of two stainless steel and two Plexiglas walls placed in sound-attenuating chambers. Each operant box contained an operant lever (bar) located approximately 15 cm above the floor grid and a recessed food tray placed approximately 10 cm above the floor grid. The floor grid was composed of 0.5-cm stainless steel bars placed 1.5 cm apart. A speaker and a lamp were attached to the ceiling of the sound-attenuating chamber 5 to 10 cm above the operant box. A custom computer program (Chen and Steinmetz, 1998) generated stimulus presentations and recorded behavioral responses and onset latencies. The total number of bar-presses was recorded on a digital counter.

2.3. Procedure

2.3.1. Ethanol doses

In order to assess the dose–response relationship between ethanol and learning, HAD and LAD rats were assigned to groups in which they received one of three doses of alcohol or saline. Ethanol (EtOH) is known to produce biphasic effects, with low doses (e.g. <0.8 g/kg EtOH) producing excitatory effects, including locomotor stimulation, and higher doses (e.g. >1.0 g/kg EtOH) producing locomotor depression (reviewed in Pohorecky, 1977). Although some variation between experimental results has been noted, in general, administration of 1.0 g/kg ethanol tends to produce minimal or no motor effects (Pohorecky, 1977). Therefore, in order to assess the dose–response effects of ethanol, we used 0.5, 1.0, and 1.5 g/kg ethanol in this study.

Standard, within-subject conditioning procedures established by Steinmetz et al. (1993) were employed. Initially, HAD1 and LAD1 rats were randomly assigned to one of four dosage groups (saline, 0.5 g/kg EtOH, 1.0 g/kg EtOH, or 1.5 g/kg EtOH; in 20% v/v solution). As presented below, the 1.0- and 0.5-g/kg doses of ethanol produced a significant improvement in avoidance responding in HAD1 rats, while the other two doses did not. Based on this initial finding, we created a group of HAD2 rats that were given 1.0 g/kg ethanol to test the replicates for the effect noted in the HAD1 rats. Additional HAD1 and HAD2 rats were run to further explore the results we noted when 1.0 g/kg EtOH was given. Saline injections were given during all shaping procedures that preceded signaled bar-press training. All injections were given 12 to 15 min prior to each session. During conditioning, rats received intraperitoneal injections of the appropriate dose of ethanol (0.5, 1.0, or 1.5 g/kg EtOH) or physiological saline in equivalent total volume as the 1.0-g/kg EtOH group.

2.3.2. Appetitive training

Rats were first shaped to press the operant lever to obtain 45 mg sucrose pellets. They were trained on a continuous reinforcement schedule until they received 100 reinforcements in less than 30 min on two consecutive days. Following continuous reinforcement, rats were trained on a partial reinforcement schedule (one reward for every four

bar presses) until the criterion of 100 reinforcements in less than 30 min was reached. After rats reached criterion on partial reinforcement on two consecutive days, they were transferred to appetitive conditioning. The mean number of sessions required to meet this criterion was 12.8. No statistical differences between HAD and LAD rats were apparent during shaping, as HAD rats required a mean of 12.8 sessions and LAD rats required a mean of 12.7 sessions to meet criterion ($P>.05$).

Appetitive conditioning consisted of 15 sessions, one session per day, with 100 trials in each session. Trial onset was signaled by a 2-kHz tone presented at 85 dB for 6 s. Bar presses made during the tone were recorded as rewarded responses, the tone was terminated, and a pellet was dropped into the food tray. A 20-s intertrial interval was then initiated, followed by a 2–8 s variable pre-CS period and onset of the next trial. Responses made during the pre-CS period resulted in the initiation of a new pre-CS period and subsequently delayed trial onset. The variable pre-CS period was included in the paradigm to prevent time-dependent responding.

2.3.3. Aversive training

Rats were shaped to press the lever to terminate a mild footshock delivered through the floor grid (250 ms pulses, 0.7 mA, 500 ms between pulses). Rats reached criterion when they terminated the footshock at least 50 times in 30 min. To prevent the rats from continuously pressing the bar, continuous footshock was initiated if the bar was depressed for 3 s. The "off-the-bar" shock was terminated when the rat released the bar. Rats were transferred to aversive conditioning when criterion was reached on two consecutive days. The mean number of sessions required to meet this criterion was 3.8. No statistical differences between HAD and LAD rats were apparent during aversive shaping, as HAD rats required a mean of 3.1 sessions and LAD rats required a mean of 4.5 sessions to reach the criterion ($P>.05$).

Aversive conditioning consisted of 15 sessions, one session per day, with 100 trials in each session. Trial onset was signaled by the same 2-kHz, 85-dB tone that was used in appetitive conditioning. The tone was presented for 9 s and footshock was presented concurrently for the last 3 s of the trial. The intertrial interval was 20 s. Bar presses made during the 2–8 s variable pre-CS period delayed the onset of the next trial by initiating a new pre-CS period. Although responses made during this period can technically be defined as avoidance responses because they delay onset of the next trial, these responses rarely, if ever, occurred during conditioning, as observed during this and previous experiments in our laboratory. Bar presses made during the first 6 s of the tone were recorded as avoidance responses, the tone was discontinued, and the next intertrial interval was initiated. Bar presses made during the last 3 s of the tone (i.e. during shock administration) were recorded as escape responses, the shock and tone were terminated, and the next intertrial interval was initiated.

2.4. General experimental design and overview

We initially set out to replicate the poor avoidance learning in HAD1 and HAD2 rats observed by Blankenship et al. (2000) and to extend these results by assessing whether or not administration of various doses of ethanol would alter rates of appetitive and/or aversive instrumental learning in HAD and LAD rats. To accomplish this, 28 HAD1 (11 females, 17 males), 9 HAD2 (5 females, 4 males), and 24 LAD1 (12 females, 12 males) rats were given saline or one of three doses of ethanol injections during appetitive signaled bar press conditioning and during subsequent aversive signaled bar press conditioning. After these groups were run, it was noted that moderate doses of ethanol partially reversed the active avoidance conditioning deficits that had been previously observed in HAD1 and HAD2 rats (Blankenship et al., 2000). However, the HAD1 and HAD2 rats that showed the improvements in avoidance learning also had received repeated ethanol injections and appetitive training prior to the aversive conditioning. It was therefore possible that the improvement in learning was due to the repeated exposure to ethanol, prior appetitive training, or prior exposure to the training context. Additional groups were run to explore these possibilities.

First, 24 HAD1 (13 females, 11 males), 12 HAD2 (11 females, 1 male), and 24 LAD1 (10 females, 14 males) rats were used to determine whether or not the improved avoidance responding could be seen if the aversive signaled bar-pressing task was not preceded by appetitive signaled bar press conditioning. These three groups of rats were given one of three doses of ethanol or saline and trained first in the aversive task and then in the appetitive task. Second, we used 10 HAD1 (7 females, 3 males) and 10 HAD2 (5 females, 5 males) rats to evaluate whether or not exposure to ethanol alone or appetitive conditioning alone over several days prior to aversive conditioning was sufficient to improve HAD avoidance learning. For one group of rats, daily ethanol injections of a moderate dose of ethanol (1.0 g/kg) were given over 15 days. The ethanol injections were continued for another 15 days during which time aversive conditioning was given. For a second group of rats, 15 days of appetitive training (preceded by saline injections) were given before 15 days of aversive training (preceded each day by 1.0 g/kg EtOH injections).

2.5. Data analysis and statistics

Initial statistical analyses were undertaken to determine whether any differences in learning or performance were observed between the HAD1 and HAD2 replicate lines. For these analyses, two-way mixed-design ANOVAs were used in which replicate (HAD1, HAD2) was the between-subject factor and session was the within-subject factor with 15 levels.

Data from rats given ethanol and appetitive-then-aversive training, or ethanol and aversive-then-appetitive train-

ing, were analyzed separately for each response measure using three-way mixed-design ANOVAs, in which line (HAD, LAD) and dose (saline, 0.5, 1.0, or 1.5 g/kg EtOH) were the between-subject factors, while session served as the within-subject factor with 15 levels. While significant line and session effects could be found after these three-way ANOVAs were conducted, dose responses were not found. However, the possibility remained that only a relatively restricted range of doses of ethanol were effective in altering HAD avoidance behavior. That is, avoidance performance of HAD and LAD rats were nearly identical at most doses, thus preventing statistically significant dose effects from being obtained with the three-way analysis. To further explore this possibility, we conducted two-way mixed-design ANOVAs at each dose level. For these ANOVAs, line (HAD, LAD) was the between-subject factor, while session served as the within-subject factor with 15 levels. In rats given either ethanol only or appetitive training only before aversive training, data for the avoidance conditioning task were analyzed using a two-way mixed-design ANOVA, in which group (EtOH–control, Novelty–control) was the between-subject factor and session was the within-subject factor with 15 levels. Statistical results were adjusted using the more conservative values associated with the Greenhouse–Geisser method. Post hoc mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test, except where otherwise noted.

3. Results

3.1. Replicate line comparisons

A major advantage in using the HAD and LAD lines of alcohol-preferring rats is that replicate lines (HAD2 and LAD2) are available for comparison with the original lines (HAD1 and LAD1). Before proceeding with complete analyses of the data, we first statistically compared the performance of the various groups of HAD1 rats with HAD2 rats to determine if behavioral performances either with or without alcohol were similar. Statistical analyses revealed no significant difference in performance on any of the measures taken (P's>.05) when the HAD1 and HAD2 rats were compared. Most notably, similar to a previous study (Blankenship et al., 2000), both HAD1 and HAD2 rats failed to learn the active avoidance response when given saline or high levels of alcohol and in certain training situations (as detailed below), and both HAD1 and HAD2 rats showed improvements in avoidance learning when given moderate doses of ethanol. Thus, it appears that the selective breeding process that established both lines of HAD rats resulted in similar patterns of appetitive and aversive signaled bar press conditioning and similar effects of ethanol injections on learning. Given the comparable behavioral performances seen in the HAD1 and HAD2 rats, these groups were combined into one group for all further statistical analyses that follow.

3.2. Appetitive conditioning preceding aversive conditioning: a dose–response analysis

The data showed that although HAD and LAD rats in each dose group acquired the conditioned appetitive response, ethanol dose-dependently affected appetitive responding in all rats. Most importantly, in the aversive task that followed the appetitive training, moderate doses of ethanol (0.5 and 1.0 g/kg EtOH) partially reversed the active avoidance learning deficits previously observed in HAD rats. Moreover, the results indicated that the largest dose of ethanol (1.5 g/kg EtOH) abolished avoidance responding altogether in the HAD rats.

3.2.1. Appetitive training

3.2.1.1. Learning assessment. To assess conditioned response learning in the appetitive task, the percentages of rewarded responses made by each group were analyzed. Because none of the Line × Session interactions were significant (P's>.05), the data from HAD and LAD rats receiving each dose of ethanol were combined for further analysis. These data are depicted in Fig. 1. Whereas rats receiving saline during conditioning reached asymptotic levels of responding at greater than 90% by Session 5 and continued to respond at high levels, ethanol impaired conditioned response acquisition. The mixed-design ANOVA confirmed this observation, revealing only a main effect of Dose [$F(3,93) = 12.56$, $P < .001$]. Post hoc analyses indicated that rats receiving 1.5 g/kg EtOH performed significantly fewer conditioned responses than rats in all other groups ($P < .05$) and that rats receiving 1.0 g/kg EtOH made significantly fewer conditioned responses than rats receiving saline. In summary, we found that both HAD and LAD rats performed conditioned appetitive responses at relatively high rates, but ethanol decreased responding in a dose-dependent manner.

3.2.1.2. Performance assessment. Performance of the bar press response during the appetitive task was evaluated by analyzing response onset latencies and total number of bar presses made during each session. A significant Dose × Session interaction was observed for response onset latency [$F(16,502) = 1.70$, P=.042]. Post hoc analyses revealed that animals receiving 1.5 g/kg EtOH showed a general slowing of motor performance during the first two sessions (P's < .05). A main effect of Line was also obtained for response onset latency [$F(1,93) = 5.31$, P=.023], indicating that LAD rats responded later in the CS period than HAD rats overall. Analysis of the total number of bar presses made during the appetitive task revealed a significant Dose × Session interaction [$F(13,394) = 6.15$, $P < .001$], with post hoc tests indicating that rats receiving 1.5 g/kg EtOH

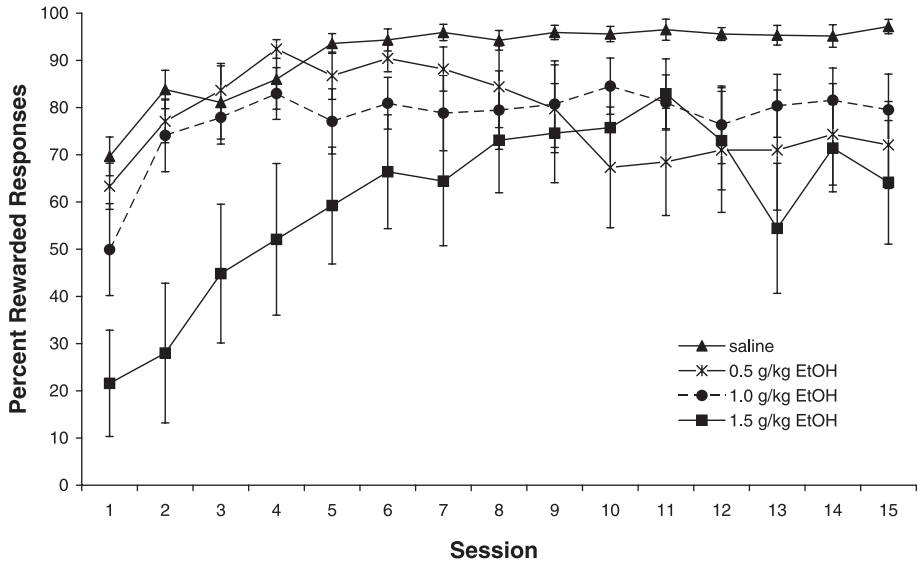

Fig. 1. Mean (±S.E.M.) percentage of rewarded responses made during the appetitive conditioning task. Ethanol dose-dependently impaired acquisition of the conditioned appetitive response in both HAD and LAD rats. Rats receiving 1.5 g/kg EtOH made significantly fewer conditioned appetitive responses than all other groups ($P < .05$). Rats receiving 1.0 g/kg EtOH made significantly fewer conditioned responses than rats receiving saline ($P < .05$).

made significantly fewer total bar presses than all other groups during the first two sessions (P's $< .05$). These results suggest that rats receiving 1.5 g/kg EtOH exhibited significant motor impairment during the initial two sessions, but eventually all animals performed at comparable levels.

3.2.2. Aversive training

3.2.2.1. Learning assessment. Fig. 2 depicts the percentage of avoidance responses made by HAD and LAD rats in each dose group after the animals had received appetitive training. A mixed-design ANOVA performed on the per-

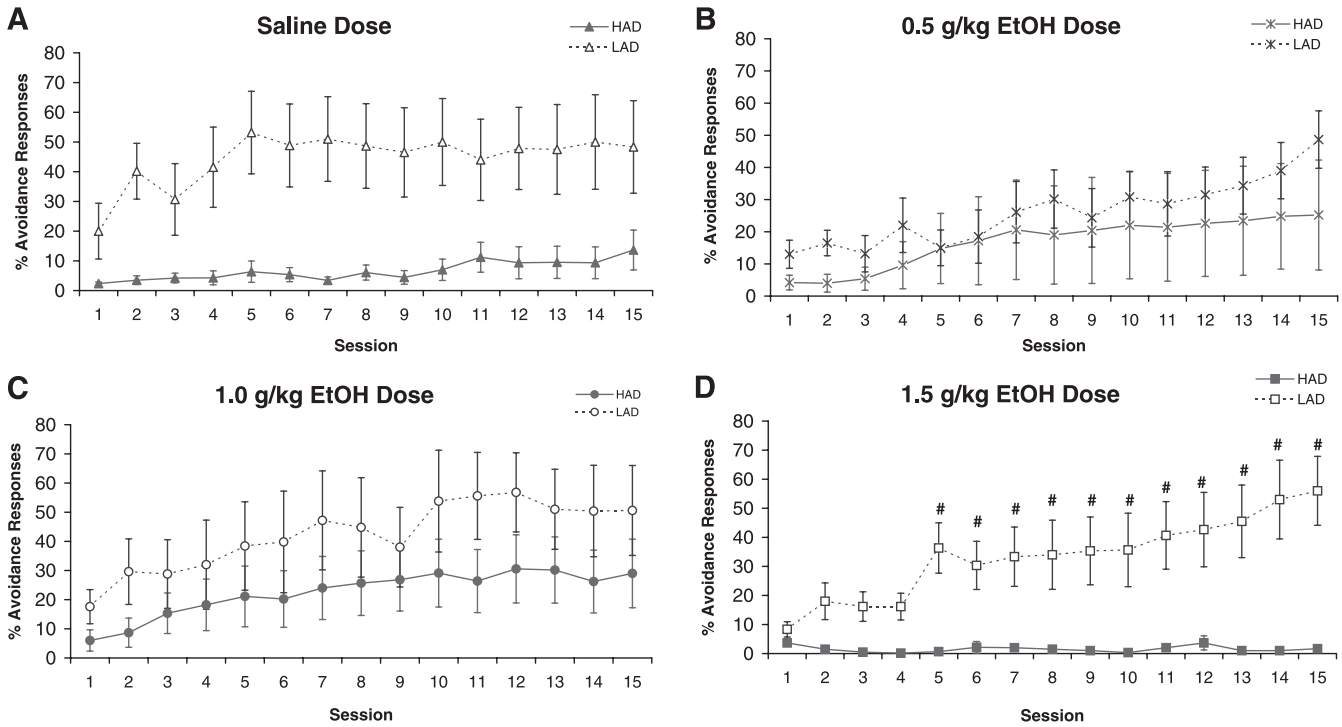

Fig. 2. Mean (±S.E.M.) percentage of avoidance responses made during the aversive conditioning task following prior appetitive training in HAD and LAD rats administered saline (A), 0.5 g/kg EtOH (B), 1.0 g/kg EtOH (C), and 1.5 g/kg EtOH (D). When appetitive training preceded aversive training, moderate doses of ethanol partially reversed avoidance learning deficits previously observed in HAD rats. #: HAD rats receiving 1.5 g/kg EtOH made significantly fewer conditioned responses than LAD rats receiving 1.5 g/kg EtOH (P's $< .05$).

centage of avoidance responses made by HAD and LAD rats administered saline during the aversive task revealed a significant main effect of Line [$F(1,18) = 14.17$, $P = .001$], indicating that HAD rats receiving saline performed significantly fewer conditioned avoidance responses than LAD rats receiving saline (Fig. 2A), replicating a previous study (Blankenship et al., 2000).

No differences in avoidance learning were observed between HAD and LAD rats receiving 0.5 g/kg ethanol (P's>.05, Fig. 2B), but a significant main effect of Session [$F(2,15) = 3.97$, $P = .04$] indicated that both lines learned the avoidance response. As indicated in Fig. 2B, HAD rats administered 0.5 g/kg EtOH exhibited a partial reversal of learning deficits. However, the improvement in avoidance learning was not significant when compared to HAD rats receiving saline (P>.05). Although LAD rats receiving 0.5 g/kg EtOH performed fewer avoidance responses than LAD rats receiving saline, this difference was not significant (P>.05).

HAD rats receiving 1.0 g/kg EtOH also exhibited a partial reversal of avoidance learning deficits, as shown in Fig. 2C. No differences were observed between HAD and LAD rats receiving 1.0 g/kg EtOH (P>.05), but a main effect of Session indicated that both lines learned the task [$F(3,59) = 9.29$, $P < .001$]. Although HAD rats receiving 1.0 g/kg EtOH performed more conditioned responses than HAD rats administered saline, this difference was only marginally significant (P=.06). To explore this relationship further, we divided the data into two parts representing acquisition (Sessions 1 through 7) and performance (Sessions 8 through 14). The mean performance of HAD rats in each dose group during the acquisition and performance phases of conditioning is depicted in Fig. 3. A mixed-design ANOVA revealed a significant Dose × Session interaction during the acquisition phase of conditioning [$F(2,58) = 3.21$, $P = .05$], indicating that HAD rats administered 1.0 g/kg EtOH learned the conditioned avoidance response while HAD rats administered saline did not (Fig. 3A). Post hoc analyses revealed that HAD rats administered 1.0 g/kg EtOH performed significantly more avoidance responses than HAD rats administered saline during Sessions 3 through 7 (P's < .05). Furthermore, comparison of acquisition in HAD and LAD rats administered saline revealed a significant Line × Session interaction [$F(2,42) = 6.06$, $P = .003$], confirming that LAD rats administered saline learned the task whereas HAD rats administered saline did not. No differences in acquisition were observed between HAD and LAD rats administered 1.0 g/kg EtOH (P>.05). A mixed-design ANOVA performed on the data from the performance phase of conditioning revealed only a significant main effect of Dose [$F(1,30) = 3.99$, $P = .05$], indicating that HAD rats administered 1.0 g/kg EtOH performed more avoidance responses than HAD rats administered saline during this later phase of conditioning and that performance was asymptotic (Fig. 3B). A significant main effect of Line indicated that LAD rats administered saline performed significantly more conditioned responses during the performance phase of conditioning than HAD rats administered saline [$F(1,18) = 11.03$, $P = .004$]. No significant differences were observed during the performance phase of conditioning between HAD and LAD rats administered 1.0 g/kg EtOH. Unlike the HAD rats, LAD rats given either saline or 1.0 g/kg ethanol learned the aversive task at the same rate. Mixed-design ANOVAs revealed that LAD rats administered saline did not differ from LAD rats administered 1.0 g/kg EtOH during either the acquisition or the performance phases of conditioning (P's>.05). A main effect of Session during the acquisition phase [$F(2,19) = 6.34$, $P = .008$] indicated that LAD rats in both dose groups learned the task.

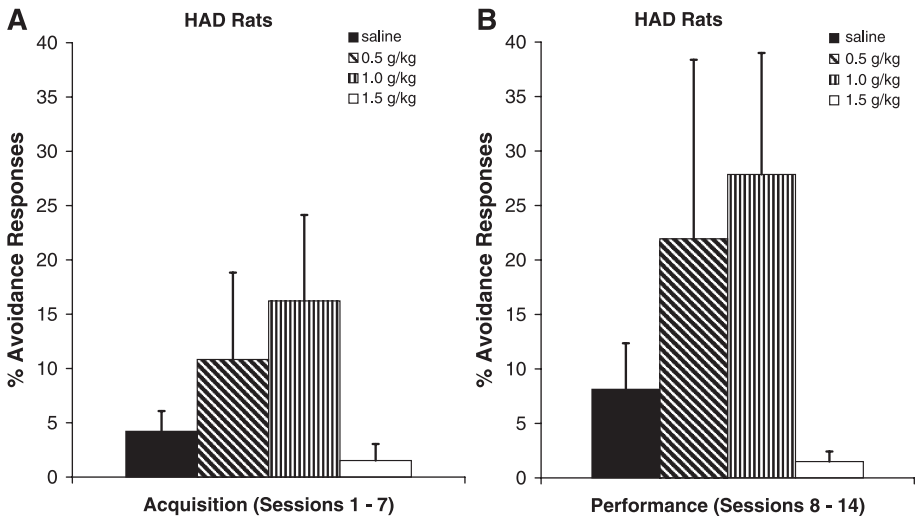

Fig. 3. Mean (±S.E.M.) percentage of avoidance responses made by HAD rats given prior appetitive training under ethanol during acquisition (A) and performance (B) phases of conditioning. When appetitive training preceded aversive training, 1.0 g/kg EtOH significantly improved acquisition of conditioned avoidance responses in HAD rats and this facilitation was maintained throughout the performance phase of conditioning.

Interestingly, although the highest dose of ethanol did not impair avoidance responding in LAD rats, the same dose prevented conditioned avoidance responding altogether in HAD rats. The ANOVA revealed a significant Line × Session interaction [$F(2,20) = 5.10$, $P = .014$]. HAD rats receiving 1.5 g/kg EtOH made significantly fewer avoidance responses than LAD rats receiving 1.5 g/kg EtOH during Sessions 2 through 15 (P's < .05, Fig. 2D). Overall, the results indicated that administration of moderate doses of ethanol before conditioning significantly increased avoidance response acquisition in HAD rats to levels near the LAD rats. This facilitation was evident early in the acquisition phase of conditioning and persisted throughout the 15 sessions.

3.2.2.2. Performance assessment. In order to assess the effects of ethanol on performance of the bar-press response in the aversive task, the rate of escape responses on non-avoidance trials, response onset latencies, and the total number of bar presses were analyzed. Escape responses were defined as bar presses made during shock delivery, thus allowing the rat to terminate (escape) the shock. Mixed-design ANOVAs performed on the percentage of escape responses and the total number of bar presses made by HAD and LAD rats administered saline revealed no significant differences (P's>.05). However, a significant main effect of Line was obtained for response onset latencies [$F(1,10) = 5.95$, $P = .04$], indicating that HAD rats administered saline responded significantly later in the CS period than LAD rats administered saline. No significant differences were observed between HAD and LAD rats administered 0.5 g/kg EtOH on any performance variable. Mixed-design ANOVAs performed on the performance variables for HAD and LAD rats receiving 1.0 g/kg EtOH revealed a main effect of Line for the percentage of escape responses [$F(1,21) = 10.38$, $P = .004$], indicating that LAD rats made fewer escape responses than HAD rats. Also, a main effect of Line indicated that HAD rats receiving 1.0 g/kg EtOH made more total bar presses than LAD rats receiving the same dose [$F(1,21) = 5.39$, $P = .03$]. Mixed-design ANOVAs performed on the performance variables for HAD and LAD rats administered 1.5 g/kg EtOH revealed a significant main effect of Line for response onset latencies [$F(1,9) = 8.68$, $P = .016$], indicating that HAD rats responded later than LAD rats.

To assess whether the improvement in performance after moderate doses of ethanol could have resulted from general motor stimulatory effects, HAD rats receiving each dose of ethanol were compared to HAD rats receiving saline using mixed-design ANOVAs. No differences in escape responding, response onset latencies, or total number of bar presses were observed between HAD rats receiving saline and HAD rats receiving 0.5 g/kg EtOH (all P's>.05). A significant main effect of Dose was observed between HAD rats administered saline and HAD rats administered 1.0 g/kg EtOH for the total number of bar presses made [$F(1,22) = 5.42$, $P = .03$], indicating that HAD rats administered 1.0 g/kg EtOH made more total bar presses. Thus, the possibility exists that the improvement in performance in HAD rats administered 1.0 g/kg EtOH could have been caused by a general motor stimulation effect of ethanol injections. Other data argue against this possibility, however. First, increased bar-pressing rates were not seen in rats given 0.5 g/kg ethanol, a dose which also produced a facilitation of avoidance learning. Second, if ethanol at moderate doses produced a general stimulatory effect that increased bar-pressing rates, one would expect that this effect would be seen during appetitive training. This effect was not seen. Third, an increase in bar pressing was not seen in 1.0 g/kg EtOH rats given aversive training before appetitive training (see below). These data suggest that the enhanced performance seen in HAD rats given moderate doses of ethanol was not due to a general stimulatory motor effect of the ethanol. No differences were observed when performance variables of saline HAD rats and 1.5 g/kg HAD rats were compared, but this likely resulted from a floor effect. Assessment of performance factors in LAD rats administered saline and each dose of ethanol yielded no significant differences (P's>.05).

3.3. Aversive conditioning before appetitive conditioning: a dose–response analysis

Overall, and in contrast to the results obtained when aversive training was preceded by appetitive training, our results showed that when aversive training with ethanol was not preceded by appetitive training with ethanol, administration of moderate doses of ethanol failed to alleviate avoidance learning deficits in HAD rats. In fact, HAD rats failed to learn the avoidance response at any dose of ethanol. We found that although HAD and LAD rats in each dose group acquired the conditioned appetitive response after the aversive training, ethanol dose-dependently affected appetitive responding in all rats.

3.3.1. Aversive training

3.3.1.1. Learning assessment. In order to assess conditioned avoidance response learning in HAD and LAD rats in groups of animals given aversive training initially, the percentages of avoidance responses made during the aversive task were analyzed using mixed-design ANOVAs. These data are depicted in Fig. 4. A significant main effect of Line was obtained for rats receiving saline [$F(1,16) = 9.97$, $P = .006$], indicating that HAD rats receiving saline performed significantly fewer conditioned avoidance responses than LAD rats receiving saline. Contrary to the results found in animals given appetitive training before aversive training, LAD rats receiving 0.5 g/kg EtOH performed significantly more avoidance responses than HAD rats receiving the same dose. The Line × Session interaction confirmed this result [$F(3,32) = 3.49$, $P = .025$]. Post hoc

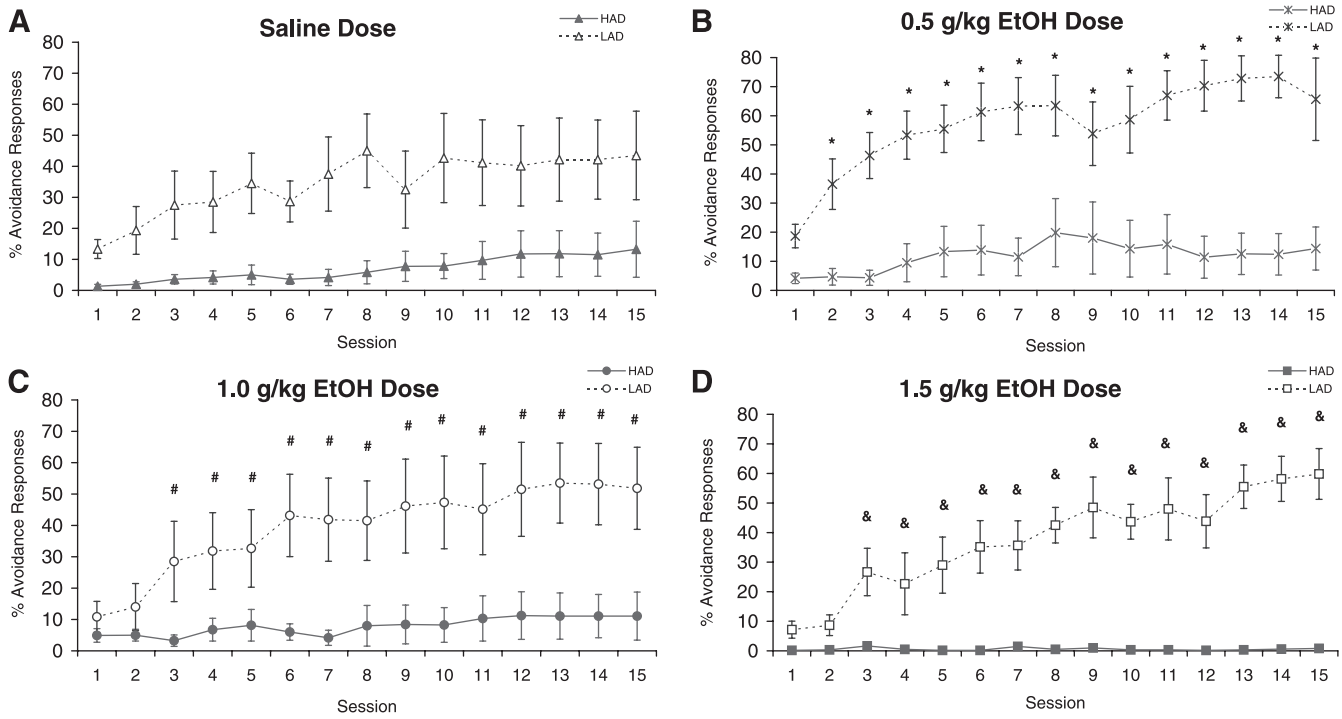

Fig. 4. Mean (±S.E.M.) percentage of avoidance responses made during aversive conditioning (without prior appetitive conditioning) in HAD and LAD rats administered saline (A), 0.5 g/kg EtOH (B), 1.0 g/kg EtOH (C), and 1.5 g/kg EtOH (D). When aversive conditioning preceded appetitive conditioning, ethanol did not significantly improve active avoidance learning deficits in HAD rats at any dose. *, #, &: HAD rats made significantly fewer conditioned avoidance responses than LAD rats (all P's < .05).

analyses revealed that this difference was significant in Sessions 2 through 15 (P's < .05, Fig. 4B). Also contrary to results from animals given prior appetitive training, HAD rats receiving 1.0 g/kg EtOH performed significantly fewer conditioned avoidance responses than LAD rats receiving the same dose [Line × Session interaction: $F(3,43) = 4.77$, $P = .007$]. This difference was significant in Sessions 3 through 15 (P's < .05, Fig. 4C). As observed in rats receiving appetitive conditioning first, avoidance responding in HAD rats receiving the highest dose of ethanol was abolished altogether. HAD rats receiving 1.5 g/kg EtOH performed significantly fewer conditioned responses than LAD rats receiving 1.5 g/kg EtOH [$F(3,26) = 4.88$, $P = 0.011$]. This difference was significant in Sessions 2 through 15 (P's < .05, Fig. 4D). In summary, when aversive conditioning was given before appetitive training, moderate doses of ethanol failed to improve avoidance responding as seen when appetitive training preceded avoidance training.

3.3.1.2. Performance assessment. Performance of the bar press response in HAD and LAD rats was analyzed using a mixed-design ANOVA on the percentage of escape responses on nonavoidance trials, response onset latencies, and the total number of bar presses made during the aversive task. A significant main effect of Dose was revealed for percentages of escape responses [$F(3,52) = 6.45$, $P = .001$], indicating that rats receiving the high dose of ethanol (1.5 g/kg EtOH) escaped the shock at significantly lower rates than rats receiving all other doses. A main effect of Line was revealed for response onset latencies [$F(1,52) = 5.89$, $P = .019$], indicating that HAD rats responded later after the onset of the CS than LAD rats. A main effect of Dose was observed for the total number of bar presses [$F(3,52) = 8.86$, $P < .001$]. Rats receiving 1.5 g/kg EtOH made fewer total bar presses than rats receiving any other dose ($P < .05$). Overall, these results indicate that the high dose of ethanol appeared to impair avoidance response performance similarly for both HAD and LAD rats. No significant differences were observed between HAD rats administered saline and HAD rats administered either 0.5 or 1.0 g/kg EtOH on any response measure (all P's > .05). No significant differences were observed between LAD rats administered saline and LAD rats administered 0.5, 1.0, or 1.5 g/kg EtOH on any response measure (P's > .05).

3.3.2. Appetitive training

3.3.2.1. Learning assessment. To assess appetitive response acquisition in rats given aversive training first, some of the rats from each group ($N = 41$) received ethanol injections and appetitive conditioning after aversive conditioning. Mixed-design ANOVAs were performed on the percentage of rewarded responses made during the appetitive conditioning task at each dose level. A main effect of Session was observed for HAD and LAD rats receiving saline [$F(3,29) = 18.96$, $P < .001$], indicating that both

lines learned the task. Similarly, significant main effects of Session were obtained for HAD and LAD rats receiving 0.5 g/kg EtOH [$F(3,18) = 7.96$, $P = .002$], HAD and LAD rats receiving 1.0 g/kg EtOH [$F(3,25) = 2.83$, $P = .05$], and HAD and LAD rats receiving 1.5 g/kg EtOH [$F(4,32) = 8.22$, $P < .001$; data not shown]. No significant differences were observed between HAD and LAD rats (all P's>.05). As reported in rats given appetitive training first, ethanol dose-dependently impaired appetitive conditioning, with 1.5 g/kg EtOH causing the most significant disruption ($P < .05$). This impairment was expressed as retardation of the acquisition of appetitive responding. Also, the absence of any significant results involving the line variable indicated that ethanol impaired acquisition of appetitive responding similarly in both HAD and LAD rats.

3.3.2.2. Performance assessment. Performance of the bar press response in the appetitive task was analyzed using mixed-design ANOVAs on response onset latencies and total number of bar presses. No interactions or main effects involving the line variable were significant for any of the appetitive performance variables during the appetitive task. Thus, no significant differences were observed between HAD and LAD rats for any response measure. In rats given appetitive training after aversive training, significant main effects of Session were obtained at all dose levels for both response onset latencies (all P's < .03), indicating that all rats responded more quickly after CS onset as conditioning proceeded. Also, significant main effects of Session at all dose levels for total bar presses (all P's < .02) indicated that more bar presses were made early in training and that the number of bar presses made declined as animals learned the task. Administration of 1.5 g/kg EtOH resulted in longer response onset latencies and fewer total bar presses early in conditioning in both HAD and LAD rats (P's < .05). Thus, even when appetitive conditioning followed aversive conditioning, motor performance in both lines was impaired similarly in a dose-dependent manner.

3.4. Aversive conditioning after chronic ethanol exposure or after appetitive conditioning in the absence of ethanol

In summary, we found that neither prior exposure to ethanol alone (EtOH-control) nor prior exposure to appetitive conditioning procedures alone (novelty-control rats) was sufficient to account for the reversal of active avoidance deficits in HAD rats by 1.0 g/kg EtOH that was observed in rats given appetitive training while under the influence of ethanol prior to aversive training. This suggests that ethanol can facilitate avoidance response learning in HAD rats, but only if administered in moderate doses and only if appetitive training, while under the influence of ethanol, precedes aversive training in the same context.

3.4.1. Aversive training

3.4.1.1. Learning assessment. To assess the effects of prior experience on subsequent aversive conditioning, avoidance learning in EtOH-control HAD rats was compared to avoidance learning in novelty-control HAD rats. These data are depicted in Fig. 5. A 2 (Group) × 15 (Session) mixed-design ANOVA performed on the data revealed a significant main effect of Session [$F(1,26) = 4.70$, $P = .027$]. No significant group differences were observed (P's>.05). We also compared avoidance responding of EtOH-control and novelty-control HAD rats with avoidance responding of HAD rats given appetitive training with alcohol before avoidance training using a repeated-measures ANOVA on the last three aversive conditioning sessions, when avoidance responding should be asymptotic. For this comparison, Group (EtOH-

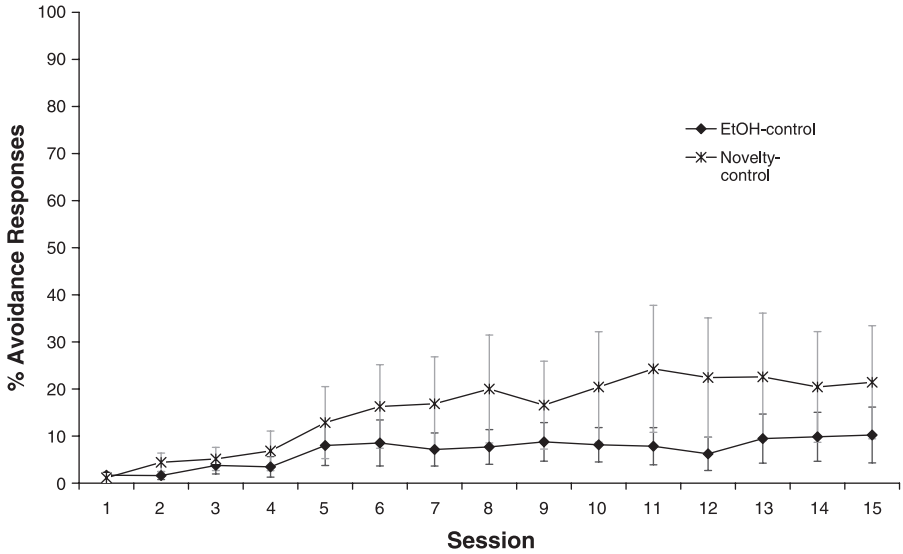

Fig. 5. Mean (±S.E.M.) percentage of avoidance responses made by EtOH-control and novelty-control HAD rats. Neither prior exposure to ethanol alone nor prior exposure to appetitive conditioning procedures alone (in the absence of ethanol) facilitated avoidance learning in HAD rats.

control, novelty-control, HAD–1.0 g/kg EtOH) was the between-subject factor and Session was the within-subject factor. A significant Group × Session interaction was observed [$F(4,43) = 2.71$, $P = .043$]. A Newman–Keuls post hoc test revealed that the mean number of avoidance responses made during the last three sessions by HAD rats administered 1.0 g/kg EtOH, and trained first in the appetitive task, was significantly higher than the mean number of avoidance responses made by both the EtOH-control and novelty-control groups ($P < .05$). The EtOH-control and novelty-control HAD rats were not significantly different from each other.

3.4.1.2. Performance assessment. Performance of the avoidance response was analyzed using mixed-design ANOVAs on the percentage of escape responses, response onset latencies, and the total number of bar presses made in the aversive task. No significant differences were observed between the EtOH-control and novelty-control groups for percentage of escape responses or response onset latencies (P's>.05). A significant main effect of Session was obtained for the total number of bar presses made during the aversive task [$F(6,99) = 2.86$, $P = .016$], indicating that more bar presses were made earlier in training by both groups and that the number of bar presses made declined as rats became more familiar with the task.

3.4.2. Appetitive training

3.4.2.1. Learning assessment. To assess conditioned response learning in the appetitive task, the percentages of rewarded responses made by HAD rats in the novelty-control group were compared to the percentages of rewarded responses made by HAD rats receiving saline in the initial group of rats that were trained in this experiment. Although the interaction was not significant, a main effect of Session was observed [$F(4,44) = 10.12$, $P < .001$]. This indicates that, similar to rats administered saline during appetitive training in the initial groups, HAD rats in the novelty-control group learned the conditioned appetitive response rapidly and performed consistently throughout the rest of the training sessions.

3.4.2.2. Performance assessment. To assess performance of the bar press response in the appetitive task, the response onset latencies and total number of bar presses made were compared between novelty-control rats in the current experiment and the group of HAD rats that received saline during training in our initial study. No significant differences involving the group variable were observed in the mean response onset latencies ($P > .05$). However, a main effect of Session was observed for response onset latencies [$F(4,49) = 19.19$, $P < .001$], indicating that rats responded earlier in the CS period as training proceeded. A main effect of Session was also observed for the total number of bar presses [$F(2,33) = 35.23$, $P < .001$], indicating that more bar presses were made earlier in training by both groups and that the number of bar presses made declined as rats became more familiar with the task.

3.5. Gender effects in avoidance learning and performance

Due to the small and, in some cases, unequal numbers of male and female rats within each group resulting from the availability of HAD and LAD rats at the time each group was run, the statistical power was too low to conduct any formal analyses of gender effects in this experiment. Nonetheless, we examined the trends observed in male and female rats within each group to determine whether any gender differences could be detected in either the ethanol-naive or ethanol-treated rats with regard to the learning or performance of conditioned responses. Overall, very few gender-related effects were apparent in the data. Of note, we found no significant differences between HAD males and HAD females under ethanol-naive conditions in any response measure during appetitive or aversive learning tasks. Trends toward two ethanol-related effects seemed apparent in the data. First, compared to HAD males, HAD females had difficulty learning the avoidance conditioned response following both saline and ethanol administration, but, similar to males, moderate doses of ethanol did improve this learning somewhat. Second, the improvement in avoidance responding observed in HAD males administered moderate doses of ethanol and appetitive-then-aversive training was accompanied by decreased response onset latencies and increased total bar pressing. We found no differences between LAD males and LAD females under either ethanol-naive or ethanol-treated conditions.

4. Discussion

Previously, we reported that high-alcohol-drinking (HAD1 and HAD2) and low-alcohol-drinking (LAD1 and LAD2) rats acquired conditioned appetitive responses equally well, whether appetitive conditioning preceded aversive conditioning or vice versa (Blankenship et al., 2000). Similarly, in the present study, HAD and LAD rats administered saline during appetitive conditioning acquired the appetitive conditioned response rapidly and performed well throughout the 15 training sessions. Consistent with previous reports that ethanol impairs appetitive learning in the radial arm maze (Devenport et al., 1983; Maier and Pohorecky, 1986; Matthews et al., 1999) and instrumental conditioning tasks (Holloway and Vardiman, 1971), ethanol dose-dependently impaired acquisition of the appetitive conditioned response in both HAD and LAD rats in this study. As expected, 1.5 g/kg EtOH produced the greatest learning deficits early in training, with the effects waning after six or seven sessions, whereas none of the rats receiving saline were impaired. The magnitude of impairment produced by more moderate doses of ethanol was less

substantial. Ethanol, at relatively high doses, impaired the motor performance factors associated with conditioned responses in both HAD and LAD rats, as measured by longer response onset latencies and fewer total bar presses during the first two training sessions. Nonetheless, HAD and LAD rats exhibited robust appetitive learning overall.

Although HAD and LAD rats tended to learn the appetitive task equally well, more careful evaluation of the data revealed some minor differences. Performance of appetitive conditioned responses tended to decline in both HAD and LAD rats administered the small dose of ethanol (0.5 g/kg EtOH, see Fig. 1) during the last several training sessions in rats given appetitive training prior to aversive training. This trend was not statistically significant. Further, this trend was not observed during the appetitive task in rats given aversive conditioning before appetitive conditioning, which precludes the possibility that the decline may be attributed to motor deficits associated with chronic treatment with small doses of ethanol. Performance following 1.0 g/kg ethanol and 1.5 g/kg ethanol administration did not decline significantly in later sessions, nor did it differ in general between HAD and LAD rats.

We demonstrated previously that HAD1 and HAD2 rats exhibited selective deficits in active avoidance learning in comparison to LAD1, LAD2, and N/Nih control rats, regardless of whether or not the aversive training was preceded by appetitive training (Blankenship et al., 2000). The current experiments replicated those results: HAD rats administered saline exhibited retarded acquisition of the conditioned avoidance response, regardless of test order. Importantly, in this study, we found that these deficits could be at least partially reversed by administration of moderate doses of ethanol (0.5 and 1.0 g/kg EtOH, see Fig. 2). This facilitation effect of ethanol appeared to occur relatively early in training—for example, a greater number of avoidance responses were seen during early acquisition sessions when HAD rats given 1.0 g/kg ethanol were compared to HAD rats given saline. It appears that the beneficial effects of ethanol on avoidance learning in HAD rats could only be realized under limited circumstances. Specifically, the beneficial effects of ethanol were only seen when aversive conditioning was preceded by prior exposure to appetitive conditioning in conjunction with ethanol injections.

A number of possibilities may explain these results. First, the selective deficits in avoidance learning, but not appetitive learning, in HAD rats could result from the simple fact that the aversive conditioning task is more difficult to learn than the appetitive conditioning task, as demonstrated by the rates of asymptotic responding in each task (as high as 90% in the appetitive task compared to approximately 50% in the aversive task). Perhaps the deficit observed in HAD rats is related to a general learning or cognitive deficit that is only revealed under relatively complex learning conditions. We have preliminary data that argue against this possibility. When the appetitive task was made more difficult by introducing a required response delay period, no differences were observed between HAD and LAD rats (Villarreal and Steinmetz, 2002). Both HAD and LAD rats performed relatively poorly under this condition (approximately 30%–40% conditioned responses after 15 training sessions). The similar appetitive performance seen in HAD and LAD rats during this relatively difficult delay task suggests that HAD avoidance learning deficits cannot be attributed solely to task difficulty.

A second possible explanation for avoidance learning deficits in HAD rats is a lack of discrimination between the pre-CS period and the CS period during the aversive learning task. This is not a likely explanation, however, as HAD rats should exhibit similar deficits in the appetitive conditioning task if this were true because the same apparatus (context), conditional stimulus (tone), and conditioned response (i.e., bar press) were required in the appetitive learning task. In fact, both HAD and LAD rats performed relatively well in the appetitive learning task, even following administration of ethanol, which demonstrates that avoidance learning deficits in HAD rats cannot be attributed to an inability to discriminate the intertrial interval from the CS period.

Alternatively, the deficits in avoidance conditioning could be related to conditioned fear, and the partial reversal of those deficits could therefore be due to the anxiolytic properties of ethanol. We have proposed that HAD rats may be more fearful in the tone-signaled conditioning context and that the fear is expressed as excessive behavioral freezing (Blankenship et al., 2000). The freezing response is in direct competition with the active response of pressing the lever. Thus, the freezing response predominates over the required bar press because it is a ‘‘natural’’ expression of fear in rats. We have preliminary evidence suggesting that HAD rats may, in fact, exhibit behaviors consistent with excessive fear in an aversive learning context. Although no differences were observed between HAD and LAD rats on baseline measures of heart rate and behavioral freezing in a classical fear conditioning paradigm, HAD rats exhibited facilitated acquisition of fear conditioning as indexed by exaggerated heart rate reactivity to auditory conditioned stimuli during fear conditioning (Rorick et al., 2002). This facilitated acquisition was attenuated by pre-exposure to both the tone and contextual conditional stimuli. Moreover, although both HAD and LAD rats exhibited robust acquisition of conditioned fear, freezing responses in HAD rats, as compared to LAD rats, persisted despite the absence of further shocks. That is, HAD rats failed to extinguish the freezing behavior in response to both contextual and discrete tone conditional stimuli during subsequent fear retention tests (Rorick et al., 2002). Together, these results suggest that HAD rats exhibit behaviors consistent with increased fear and that the fear may be expressed as excessive freezing in the conditioning context. It should also be noted that HAD and LAD rats do not differ in the number of escape responses made during nonavoidance trials. That is, the excessive freezing exhibited by HAD rats

is limited to the preshock CS tone period. It appears that shock onset is sufficiently motivating to provoke escape responses reliably in HAD rats.

Previous studies using selectively bred lines of rats support the idea that alcohol preference may be associated with increased fear and that the propensity to consume alcohol in these lines may be related to the anxiolytic properties of ethanol. First, P rats scored higher on measures of fear-like behavior compared to NP rats, and these behaviors were reduced following administration of 0.5, 0.75, or 1.0 g/kg ethanol (Stewart et al., 1993). Second, although no differences in initial startle reactivity were observed between P and NP rats prior to fear conditioning, P rats showed a greater potentiation of the startle reflex after fear conditioning (McKinzie et al., 2000). This potentiation was reduced in P rats, but not in NP rats, by prior treatment with 0.5 g/kg ethanol (Jones et al., 2000). Moreover, another line of P rats, the alcohol-accepting (AA) line, exhibited increased freezing in response to a loud aversive tone stimulus, as compared to the alcohol-non-accepting (ANA) line of rats (Fahlke et al., 1993). Neurochemical evidence suggests an association between alcohol preference in HAD rats and increased fear. Compared to LAD rats, HAD rats have lower levels of neuropeptide Y, an endogenous anxiolytic, in the amygdala, a structure known to be critical for the acquisition and expression of both unconditioned and conditioned fear (Hwang et al., 1999; LeDoux, 2000). Thus, a neural substrate exists through which HAD rats may exhibit increased fear (Hwang et al., 1999). The current results are consistent with the contention that alcohol preference in HAD rats may be associated with increased fear, but the conditions under which ethanol provides anxiolytic effects in HAD rats appear to be rather complicated since reversal of active avoidance deficits by moderate doses of ethanol occurred only after appetitive conditioning, carried out under the influence of ethanol.

So, why is the initial appetitive training such a crucial step in facilitating avoidance learning in HAD rats? Previous research has shown that novel events elicit freezing in laboratory rats (Bronson, 1968; Bolles and Fanselow, 1980). Behavioral freezing is a predominant response to fear in rodents (LeDoux, 2000). Thus, the freezing behavior observed in HAD rats upon initial exposure to the conditioning chambers could be interpreted as an expression of fear to the novel contextual stimuli. Moreover, in rats receiving no prior appetitive training, the animals receive aversive stimuli (shocks) in the novel chambers. It seems plausible that fear reactions to the novel conditioning stimuli and fear reactions to aversive stimuli could have additive effects, thus leading to an exaggerated level of fear and freezing in HAD rats. When HAD rats receive appetitive conditioning prior to aversive conditioning, the contextual and conditional stimuli are no longer novel at the start of aversive training. In addition, at the onset of aversive training, HAD rats given prior appetitive training also have received repeated ethanol injections. The repeated exposure to ethanol injections may make HAD rats more tolerant to the aversive properties of ethanol and more sensitive to the rewarding properties of ethanol (i.e. anxiolysis; Bozarth, 1990). The combination of repeated exposure to the conditioning stimuli during appetitive conditioning and repeated ethanol injections may allow HAD rats to become familiar with all of the conditioning parameters and, thus, less susceptible to novelty-induced freezing, which ultimately results in facilitated avoidance learning (i.e., tone-related bar presses emitted by HAD rats). In this study, we showed that prior appetitive training alone is insufficient to facilitate avoidance learning in HAD rats. HAD rats in the novelty-control group (receiving prior appetitive training without ethanol) exhibited some improvement in performance (see Fig. 5), although this difference was not statistically significant. They did, however, perform better than HAD rats administered saline during both appetitive and aversive training, suggesting that the combination of prior exposure to the conditioning parameters and repeated exposure to ethanol is critical.

Interestingly, learning in HAD rats was particularly sensitive to the dose of ethanol administered. Moderate doses (0.5 and 1.0 g/kg) of ethanol facilitated avoidance learning in HAD rats. Conversely, a relatively higher dose (1.5 g/kg EtOH) abolished avoidance responding altogether in HAD rats. This was unexpected based on previous reports that alcohol preference is associated with increased sensitivity to the motor stimulating effects of ethanol and decreased sensitivity to the aversive properties of ethanol, as measured by taste reactivity, spontaneous motor activity, and loss of righting reflex indices (Waller et al., 1986; Krimmer, 1992; Stewart and Li, 1997). This exaggerated sensitivity to high doses of ethanol was limited to HAD rats, however, as none of the doses of ethanol in this study impaired aversive learning or motor performance significantly in LAD rats. By contrast, HAD rats were adversely affected by the high dose of ethanol in terms of both conditioned learning, as measured by avoidance percentages, and motor performance, as measured by response onset latencies and total number of bar presses. The reason for this is unclear, but it may be related to differential sensitivity to various rewarding and aversive properties of ethanol in HAD and LAD rats, as well as the motivational nature of the learning task (i.e. approach vs. avoidance). Further studies will be required in order to answer this question.

Importantly, avoidance learning deficits, as well as the effects of a moderate dose of ethanol, that were observed in HAD1 rats were also seen in the HAD2 replicate line in each of the experiments included in this study. These findings provide strong support for a genetic linkage between alcohol preference and poor avoidance learning in HAD rats. These data also suggest that the complex circumstances required for the expression of the anxiolytic effects of ethanol may also be genetically mediated, rather than simply the result of chance fixation of irrelevant alleles

during the selective breeding process. More broadly, this study demonstrates that HAD rats may be a reliable model of the negative affectivity/anxiety vulnerability to excessive alcohol use and abuse. Studies with humans clearly demonstrate the important role that negative affectivity, and anxiety in particular, play in the etiology of alcoholism (Zucker, 1986; Kushner et al., 1999).

Further evidence for an association between avoidance learning deficits and their reversal by alcohol consumption under rewarding circumstances may be drawn from studies involving alcohol tolerance and motor-skill learning in human subjects. To our knowledge, no studies have been conducted to determine whether alcohol consumption during rewarding tasks influences subsequent learning in aversive situations. However, studies have indicated that behavioral tolerance to the motor-impairing effects of alcohol has been shown to transfer from one rewarding behavioral task to another (Vogel-Sprott, 1997). Moreover, inebriated subjects who received rewarding feedback for performance that resembled nonimpaired (i.e. sober) behavior have been reported to develop tolerance to alcohol more quickly than those who received nonrewarding feedback of their performance or those who received rewarding feedback that was not contingent on the demonstration of sober-like behavior (Beirness and Vogel-Sprott, 1984). Together, these results suggest that the combination of rewarding feedback and alcohol tolerance may provide subjects with adequate behavioral strategies to compensate for impaired motor skills during subsequent learning tasks. We speculate that this result may generalize to subsequent facilitation of simple associative aversive learning tasks.

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