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High-alcohol-drinking rats exhibit persistent freezing responses to discrete cues following Pavlovian fear conditioning

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Abstract

We previously reported that high-alcohol-drinking (HAD) rats exhibited selective deficits in active avoidance learning and that those deficits were partially reversed by moderate doses of ethanol under certain training conditions [Pharmacol. Biochem. Behav. 75 (2003) 89]. In that study, we hypothesized that HAD deficits resulted from exaggerated fear in the conditioning context and that the anxiolytic properties of ethanol, along with prior exposure to the conditioning apparatus, were responsible for the facilitated avoidance learning that was observed in HAD rats following moderate doses of ethanol. The current study was designed to test whether HAD rats exhibit behaviors consistent with increased fear in aversive learning contexts. We used a standard Pavlovian fear conditioning paradigm to assess behavioral freezing in HAD (HAD-1 and HAD-2) and low-alcohol-drinking (LAD; LAD-1 and LAD-2) rats. No significant differences were observed between HAD-1 and HAD-2 or between LAD-1 and LAD-2 rats, indicating that the replicate lines performed similarly in this study. Both HAD and LAD rats exhibited robust fear conditioning during training. Although no differences were observed between HAD and LAD rats during fear training, HAD rats failed to extinguish freezing behavior in response to the discrete tone conditional stimulus during subsequent fear retention tests. Thus, HAD rats demonstrated prolonged cue-elicited fear that was resistant to extinction.

Keywords: Alcohol-preferring rats; Pavlovian fear conditioning; Freezing; Aversive learning

1. Introduction

Overwhelming evidence suggests that alcohol abuse and alcoholism are at least partially genetically mediated (Cloninger, 1987; Finn and Pihl, 1988). Lines of rats selectively bred for alcohol preference, such as the alcohol-preferring (P) and high-alcohol-drinking (HAD; HAD-1 and HAD-2) rats, serve as genetic models of alcoholism and have been studied extensively to determine important characteristics associated with alcohol preference so that a greater understanding of the development and maintenance of alcohol-related problems may be obtained (for a review, see McBride and Li, 1998; Murphy et al., 2002). Although much is known about the neurochemical and pharmacological correlates of alcohol preference, less is known about the behavioral characteristics of these lines of rats.

We have attempted to systematically characterize the behavioral tendencies of these rats and subsequently determine how these behaviors may relate to alcohol preference. Using a standard within-subjects design of appetitive and aversive conditioning in which rats learn to press a lever during a tone signal to receive a food pellet or to avoid a mild footshock (Steinmetz et al., 1993), we have demonstrated that the behavioral characteristics of different lines of P rats (e.g., P and HAD) may differ (Blankenship et al., 1998, 2000; Steinmetz et al., 2000; Rorick et al., 2003). In a study comparing P rats with alcohol-nonpreferring (NP) and Wistar control rats, we found that P rats learned the conditioned barpress response in both tasks when appetitive conditioning preceded aversive conditioning (Blankenship et al., 1998). However, P rats performed more poorly in both tasks when aversive conditioning preceded appetitive conditioning. In another study, P rats took significantly longer to learn to refrain from stepping off a small platform in a passive avoidance step-down task and had difficulty suppressing barpressing during transition periods in a differential reinforcement of low rate (DRL) task (Steinmetz et al., 2000). Because both of these tasks required response inhibition for successful performance, these results, together with the barpressing results, suggested that P rats may be in

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general disinhibited behaviorally and that perhaps their alcohol preference may be related to this general behavioral disinhibition. Consistent with this, most of the alcohol consumed by P rats in a 24-h period occurs in bingelike drinking patterns, early in the dark portion of the light–dark cycle (Kampov-Polevoy et al., 2000). Of note, this is quite similar to a pattern exhibited by many alcoholics, which has been termed "loss-of-control" or binge drinking (Cloninger, 1987). These individuals often refrain from drinking for long periods of time but demonstrate an inability to stop drinking once a binge has begun.

In contrast to P rats, alcohol preference in HAD rats appears to be associated with excessive fear reactivity. This is suggested by a number of observations. First, HAD rats exhibited no deficits in appetitive learning tasks compared to low-alcohol-drinking (LAD) rats, regardless of task order, but they exhibited selective deficits in active avoidance learning that could not be explained by auditory, motivational, or performance deficits (Blankenship et al., 2000; Rorick et al., 2003). Rather, the evidence suggested that HAD rats showed abnormally high cue-elicited conditioned fear in the avoidance learning context. A second line of evidence suggesting that alcohol preference in HAD rats may be associated with excessive fear reactivity is the observation that moderate doses of ethanol partially reversed avoidance learning deficits in HAD rats. Specifically, HAD (HAD-1 and HAD-2) rats systemically administered with 0.5 or 1.0 g/kg ethanol prior to aversive conditioning and trained previously in an appetitive learning task under the influence of ethanol made more conditioned avoidance responses than HAD rats administered with saline (Rorick et al., 2003). In that study, evaluation of performance factors suggested that the ethanol-induced reversal of learning deficits could not be attributed to increased general motor activity or stimulus salience. Rather, it was hypothesized that the anxiolytic properties of ethanol may have reduced fear in the conditioning context, thus allowing HAD rats to learn the task.

If HAD rats do in fact experience exaggerated levels of fear in the conditioning context compared to LAD rats, it is expected that HAD rats would exhibit increased levels of fear-related behaviors in standard fear conditioning tasks. The Pavlovian fear conditioning paradigm is a standard method of assessing fear in laboratory rats (Maren et al., 1996; Maren, 1999; Wallace and Rosen, 2001). Previous work using this paradigm has shown that paired presentations of a previously novel tone conditioned stimulus (CS) with a footshock unconditioned stimulus (US) rapidly elicits species-typical defense responses in rats, including behavioral freezing, tachycardia, and release of corticosteroids (Bouton and Bolles, 1980; Davis, 2000). Typically, fear has been assessed using measures of behavioral freezing, as this is a robust and easy-to-obtain measure and it is observed in response to both contextual (complex, multimodal) and auditory (discrete, unimodal) cues. Moreover, this task affords the possibility of separately assessing contextual and cue-elicited fear responses (Anagnostaras et al., 1999). The present study was conducted in order to test our hypothesis that HAD rats exhibit higher levels of contextual- or cue-elicited fear in the aversive conditioning context during aversive conditioning tasks.

2. Method

2.1. Subjects

Initially, six HAD-1 (three female, three male), six HAD-2 (three female, three male), six LAD-1 (three female, three female), and six LAD-2 (three female, three male) rats were obtained from the Alcohol Research Center at the Indiana University School of Medicine in Indianapolis, IN. For comparison purposes, another group of six HAD-2 and six LAD-2 rats (all males) were obtained as a tone-alone control group. Rats weighed at least 180 g at the beginning of the study (or at least 90 days of age) and were allowed at least 1 week to adapt to the animal colony prior to testing. All rats were individually housed with food and water available ad libitum. Animal husbandry was provided 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 protocol used in this study was approved by the Indiana University Bloomington Animal Care and Use Committee. All procedures were conducted during the light phase of a 12:12-h light–dark cycle.

2.2. Apparatus

For half of the rats, fear conditioning and contextual fear testing occurred in context A, whereas tone fear testing occurred in context B. Contexts were reversed for the other half of the rats. Context A consisted of a standard operant box with three stainless steel walls and a clear Plexiglas front wall housed within a sound attenuating chamber in a separate room. The floor grid was comprised of stainless steel bars, 0.5 cm in diameter, placed 1.5 cm apart. A speaker and 5-W house lamp were attached to the ceiling of the chamber approximately 10–15 cm above the operant box. Prior to each session, the chamber was cleaned with 5% ammonium hydroxide (v/v) solution and a thin film of the solution was placed under the floor grid. Context B was designed to provide different contextual cues than those experienced in context A. Context B consisted of an operant box constructed of three opaque white Plexiglas walls and a clear Plexiglas front wall. The two side walls were slanted inward at the top to form the shape of an inverted "V." The floor of this operant box consisted of 17 vertically staggered stainless steel bars, 0.5 cm in diameter, spaced 1.5 cm apart (center to center). Prior to each session, the chamber was cleaned with 1% acetic acid (v/v) solution and a thin film of

the solution was placed under the floor grid. The apparatus and stimuli used in this experiment were replications of those used in previous fear conditioning experiments, which reported very little generalization between the contexts and no adverse effects related to the odors (Anagnostaras et al., 1999; Maren, 1999).

For all testing, a custom computer program (Chen and Steinmetz, 1998) triggered stimulus presentations. A video camera placed in front of the chamber allowed each subject's behavior to be observed via a closed circuit camera located outside the room and recorded for offline analysis of freezing behavior.

2.3. Procedures

Rats received three sessions spaced 24 h apart: a fear conditioning (training) session, a contextual fear test session, and a tone fear test session (which occurred in a separate context). For the training session, rats were placed in the conditioning context and the experimenter immediately left the room and closed the door. After a 1-min pretraining baseline period (BL), rats received a series of 10 tone CS presentations (8 s, 85 dB, 2 kHz) paired with a coterminating mild footshock US (2 s, 0.7 mA) at a 6-s interstimulus interval and a 64-s intertrial interval. Two minutes after the last footshock, rats were returned to their home cages. Twenty-four hours later, rats were tested for fear conditioning to the context (contextual fear test session). For this test, rats were again placed in the original conditioning context and behavior was observed for 8 min. No stimuli were presented during the contextual fear test session. Twenty-four hours later, fear conditioning to the tone was assessed in the second context (tone fear test session). One minute after placement in the chamber (pretone period), rats were presented with the CS for 7 min while behavior was recorded for offline analysis. No shocks were delivered during the tone fear test session (i.e., it was essentially an extinction session). All procedures were identical for the tone-alone control group except that no shocks were administered during the training session. This group was included to dissociate freezing responses to the tone from freezing responses associated with CS–US pairings.

Freezing behavior was coded offline from the videotapes by an independent observer who was blind to the line identity of the rats. Freezing was operationalized as the absence of all movement except that required for breathing. Instantaneous judgments were made every 8 s, as signaled by a custom-made timing device. The judgments were then grouped into approximately 1-min periods (i.e., eight observations per minute) for data analysis.

2.4. Data analysis and statistics

Freezing scores were transformed to the percentage of time spent freezing over a total of eight observations per 1-min period for data analysis. For the training session, this consisted of a 1-min pretraining BL and ten 1-min periods immediately following each footshock. For the single trial of the contextual fear test session, freezing percentages were divided into eight 1-min periods. For the single trial of the tone fear test session, freezing percentages were divided into a 1-min pretone BL and seven 1-min periods during the tone CS.

2.4.1. Fear conditioning

Fear conditioning and retention of contextual and cue-based fear were assessed separately in HAD and LAD rats. The *t* tests comparing freezing during the pretraining baseline of the training session with the 1-min period following both the first and last paired trials of the training session determined whether HAD and LAD rats exhibited acquisition of conditioned fear. The *t* tests were used to compare freezing behavior upon initial reexposure to the context with the preshock freezing behavior prior to the training session in order to determine whether HAD and LAD rats exhibited retention of fear to the context during the contextual fear test session. The *t* test comparisons of freezing behavior in the novel context before and after onset of the conditioned tone stimulus during the tone fear test session were used to determine whether HAD and LAD rats exhibited retention of fear to the tone stimulus.

2.4.2. Line comparisons

Differences between HAD and LAD rats were assessed separately for each session using mixed design ANOVAs in which line (HAD, LAD) was the between-subjects factor and minutes was the within-subjects factor. For each animal, freezing scores in the training and tone fear test sessions were converted to a percentage of freezing relative to the BL at the beginning of the session (difference scores). For the contextual fear test session, freezing scores for each minute were converted to difference scores relative to the first 1-min period for that session (e.g., Minute 2 − Minute 1). Repeated measures ANOVAs computed on the difference scores determined whether freezing behavior differed between lines during training, contextual fear test, and tone fear test sessions. Statistical results were adjusted using the conservative values associated with the Greenhouse–Geisser method. Post hoc comparisons were performed using Tukey's honestly significant difference test.

An analysis of gender differences with regard to the acquisition and retention of cue and contextual fear conditioning was not conducted in this experiment because relatively low statistical power was available for these analyses and, perhaps more important, because previous studies from our laboratory involving aversive instrumental conditioning produced no significant gender differences in HAD and LAD rats in any experiment under alcohol-naïve conditions (Blankenship et al., 2000; Rorick et al., 2003).

2.5. Alcohol preference testing

Following the completion of the behavioral assessment, alcohol preference was confirmed in a subset of the animals ($N=21$). Rats were provided with food available ad libitum. For the initial 48 h of the preference test, 10% v/v ethanol solution was the only available liquid source. For the next 21 days, both ethanol and water consumption were recorded daily in a two-bottle choice situation. Placement of the ethanol and water bottles was pseudorandom and switched occasionally to avoid place-preference bias.

3. Results

3.1. Behavioral results

We first tested whether or not differences in the replicate lines could be observed. For this analysis, two-way mixed design ANOVAs were used in which replicate (HAD-1 vs. HAD-2 or LAD-1 vs. LAD-2) was the between-subjects factor and minutes was the within-subjects factor for each session. Results indicated no statistical differences in freezing between HAD-1 and HAD-2 rats or between LAD-1 and LAD-2 rats during the three training sessions (all Ps>.05). Based on these results, the replicate lines were combined into HAD and LAD groups for statistical analysis.

3.1.1. Fear conditioning

Fig. 1 depicts the level of fear conditioning and fear retention exhibited by HAD and LAD rats during each phase of the study.

3.1.1.1. Training session. Fig. 1 (top panel) shows the percentage of time spent freezing by HAD and LAD rats during the pretraining period and during the 1-min periods following the first and last CS–US trials of the training session. Statistical analysis revealed that the percentage of time spent freezing by HAD rats was significantly increased during the 1-min periods following both the first and last paired trials, as compared to the pretraining baseline, $t(11)=7.39$, $P<.001$ and $t(11)=5.49$, $P<.001$, respectively (Fig. 1, top panel). Similarly, LAD rats exhibited a significant increase in freezing behavior during the 1-min periods following both the first and last CS–US pairings as compared to the pretraining baseline, $t(11)=4.08$, $P=.002$ and $t(11)=4.47$, $P=.001$, respectively (Fig. 1, top panel). Thus, as reported in previous studies, the fear-conditioned freezing response was robust and rapidly acquired in both HAD and LAD rats (i.e., one-trial learning; Bouton and Bolles, 1980). No differences in freezing were observed between HAD and LAD rats during either the pretraining period or the 1-min periods following both the first and last paired trials (Ps>.05).

3.1.1.2. Contextual fear test. The t tests comparing the percentage of time spent freezing in the conditioning chambers prior to fear conditioning with the percentage of time spent freezing upon reexposure to the conditioning chambers during the contextual fear test session demonstrated that HAD rats exhibited robust retention of fear to the conditioning context, $t(10)=4.62$, $P=.001$ (Fig. 1, middle panel). Likewise, LAD rats also exhibited robust retention of fear to the conditioning context, $t(11)=5.33$, $P<.001$ (Fig. 1, middle panel). That is, both HAD and LAD rats exhibited retention of fear to the conditioning context during the contextual fear test session. As observed in the training session, no differences in freezing were observed between HAD and LAD rats during the first 1-min period of the contextual fear test session (P>.05).

3.1.1.3. Tone fear test. Fig. 1 (bottom panel) shows the percentage of time spent freezing by HAD and LAD rats during the pretone baseline and the first 1-min period following CS onset during the tone fear test session. The t test comparisons revealed that freezing in HAD rats was significantly increased during the tone CS as compared to the pretone BL, $t(10)=4.62$, $P=.001$ (Fig. 1, bottom panel). Similarly, LAD rats exhibited increased freezing in response to the tone CS during the first minute following CS onset as compared to the pretone period, $t(11)=5.33$, $P<.001$ (Fig. 1, bottom panel). That is, both HAD and LAD rats exhibited robust retention of fear to the tone CS during the tone fear test session.

Both HAD and LAD rats exhibited significantly more freezing during the pretone period of the tone fear test session (i.e., in the novel context) than during the pretraining period of the training session when that context was also novel (see Fig. 1, top and bottom panels). The mean ($\pm$S.E.M.) level of freezing in HAD rats during the pretone period of the tone fear test session was 25.00% ($\pm$13.18), whereas the mean ($\pm$S.E.M.) level of freezing in LAD rats was 53.13% ($\pm$11.22). When compared to the pretraining levels of freezing prior to the training session, the increase in freezing was significant for both HAD and LAD rats, $t(10)=2.23$, $P=.05$ and $t(11)=4.47$, $P=.001$, respectively. Interestingly, the difference between HAD and LAD rats was also significant, $t(21)=-2.15$, $P=.044$ (Fig. 1, bottom panel, **). LAD rats froze at significantly higher levels than HAD rats during the pretone period of the tone fear test session, which involved exposure to a new context.

3.1.2. Within-session effects

To assess differences between HAD and LAD rats within each session, line comparisons were made using repeated measures ANOVA. Fig. 2 depicts the within-session effects in HAD and LAD rats for each phase of fear conditioning.

3.1.2.1. Training session. The top panel in Fig. 2 depicts freezing behavior exhibited by HAD and LAD rats during

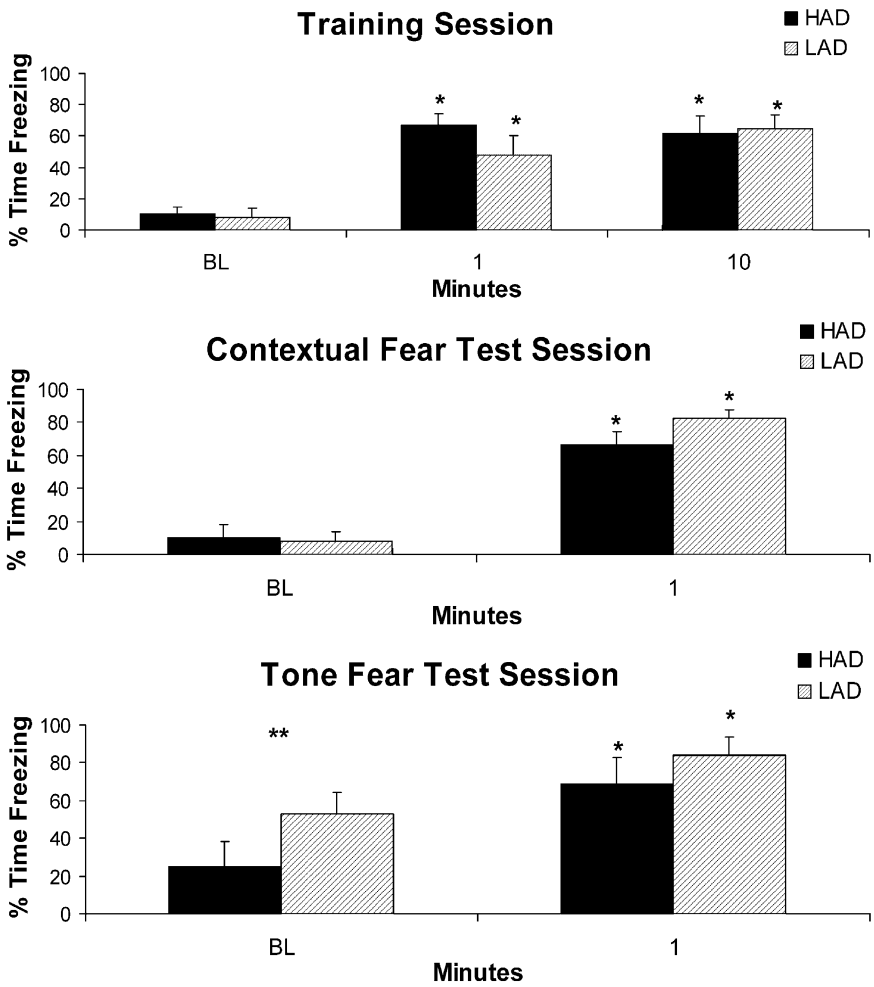

Fig. 1. Mean (± S.E.M.) percentage of time spent freezing in HAD and LAD rats during the conditioned fear training session (top panel) and contextual fear test and tone fear test sessions (middle and bottom panels, respectively). * Significantly higher levels of freezing, as compared to baseline (pretrain and pretone) levels of freezing in HAD and LAD rats, respectively (Ps < .05). * * LAD rats exhibited significantly higher levels of freezing during the pretone period of the tone fear test session than HAD rats (P < .05). Both HAD and LAD rats demonstrated robust acquisition and retention of fear conditioning.

the 1-min periods following each tone-shock pairing calculated as the percentage change from pretraining baseline. Statistical analysis revealed that no significant differences were observed between HAD and LAD rats upon initial exposure to the conditioning chambers (i.e., prior to fear conditioning; P>.05). Similarly, no differences in fear conditioning were observed between HAD and LAD rats (P>.05; Fig. 1, top panel). Thus, both HAD and LAD rats demonstrated robust fear conditioning during the training session. Functionally, no learning curve was observed because HAD and LAD rats learned the conditioned response very rapidly (i.e., one-trial learning; Bouton and Bolles, 1980).

3.1.2.2. Contextual fear test session. The middle panel in Fig. 2 depicts freezing behavior during the contextual fear test session calculated as a percentage of freezing during the first minute of context reexposure in both HAD and LAD rats. A repeated measures ANOVA conducted to compare lines revealed only a significant main effect of minutes, $F(4,84) = 6.53$, $P < .001$. This indicates that after several minutes in the conditioning context without the presentation of any stimuli, both HAD and LAD rats demonstrated a reduction of fear to the contextual stimuli (Fig. 2, middle panel).

3.1.2.3. Tone fear test session. The bottom panel in Fig. 2 depicts the percentage of time spent freezing by HAD and LAD rats during the tone fear test session relative to pretone baseline freezing. A significant Line × Minutes interaction was obtained, $F(3,69) = 3.06$, P=.032, indicating that LAD rats demonstrated a reduction of fear to the tone after a few minutes in the new context whereas HAD rats did not. Post hoc analyses showed that the difference between HAD and LAD rats was significant during Minutes 3 through 7 (Fig. 2, bottom panel). A main effect of line was also observed in the tone fear test session, $F(1,22) = 9.04$, P=.007, indicating that HAD rats exhibited higher levels of freezing overall, as compared to LAD rats, in the tone fear test session. In other words, unlike LAD rats, HAD rats did not show a reduction

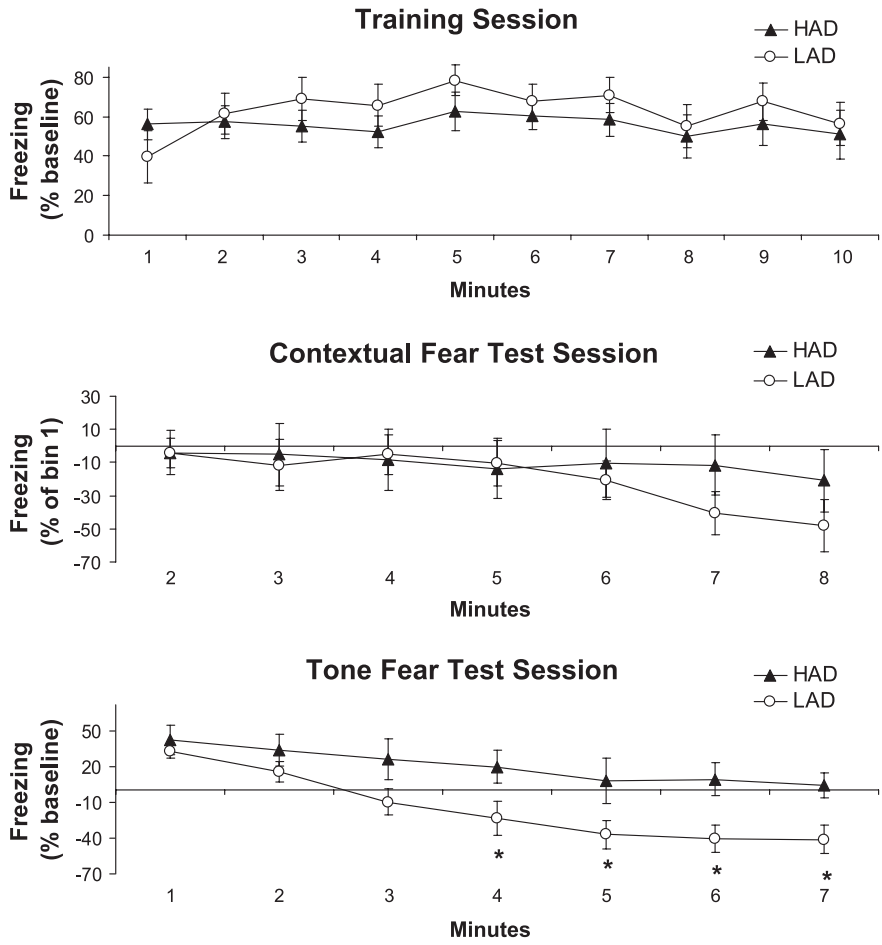

Fig. 2. Mean (± S.E.M.) percentage of time spent freezing, relative to baseline, during training, contextual fear test, and tone fear test sessions in HAD and LAD rats. * HAD rats exhibited significantly higher levels of freezing than LAD rats (*P*s < .05). No differences in fear-conditioned freezing were observed between HAD and LAD rats during the training session or the contextual fear test session. Whereas LAD rats showed extinction of cue-elicited fear during the subsequent tone fear retention test, HAD rats did not.

in cue-elicited freezing over the course of the tone-alone session.

3.1.3. Tone-alone control group results

In order to dissociate the freezing responses due to fear conditioning from the freezing responses to the tone alone, a separate group of HAD and LAD rats was included in this experiment. The mean (± S.E.M.) percentage of time spent freezing across the 10 tone-alone trials of the training session, including the BL, was 1.14% (± 1.04) in HAD rats and 0.95% (± 0.86) in LAD rats (data not shown). Statistical analysis revealed that neither HAD nor LAD rats exhibited a significant increase in freezing in response to tone presentations, as compared to pretraining baseline (all *P*s>.05). Similarly, neither HAD nor LAD rats displayed significant freezing responses during either the contextual fear test session or the tone fear test session (data not shown, all *P*s>.05). In summary, none of the rats in the tone-alone control group demonstrated significant acquisition or retention of cue- or contextual-based conditioned fear.

3.1.4. Preference test results

The alcohol preference test confirmed the phenotype of HAD and LAD rats after completion of the behavioral portion of the experiment. No differences were observed between HAD-1 and HAD-2 or between LAD-1 and LAD-2 rats (*P*s>.05). Therefore, they were combined into HAD and LAD groups for analysis. HAD rats consumed a mean (± S.E.M.) of 3.57 (± 2.24) g/kg ethanol per day. LAD rats consumed a mean (± S.E.M.) of 0.65 (± 0.19) g/kg ethanol per day. Analysis of variance revealed a significant main effect of line, $F(1,19) = 16.47$, $P = .001$, showing that HAD rats consumed significantly more ethanol per day than LAD rats.

4. Discussion

We previously reported that alcohol preference in HAD rats may be associated with excessive fear reactivity in an aversive conditioning context and that the anxiolytic properties of ethanol, combined with preexposure to the condi-

tioning stimuli, may allow HAD rats to overcome excessive fear during subsequent aversive conditioning tasks (Rorick et al., 2003). The current study was conducted in order to test the hypothesis that HAD rats exhibit increased fear responses in the conditioning context, as compared to LAD rats, during an aversively motivated associative learning task. We demonstrated here that there were no differences in fearful reactivity (i.e., freezing) between HAD and LAD rats upon initial exposure to the conditioning chambers. Moreover, both HAD and LAD rats exhibited robust acquisition of Pavlovian fear conditioning, as measured by behavioral freezing, which was similar to reports from previous studies (Anagnostaras et al., 1999; Maren, 1999; Wallace and Rosen, 2001). The fear-conditioned freezing observed in HAD and LAD rats in this study was associative in nature, resulting specifically from the CS–US pairings, as demonstrated by the lack of freezing observed in HAD and LAD rats in the tone-alone control group. Following fear conditioning, both HAD and LAD rats demonstrated robust retention of fear to the conditioning context and they did not differ from each other. However, HAD rats exhibited a resistance to extinction of cue-elicited fear during subsequent fear retention tests, as compared to LAD rats. That is, relative to the LAD rats, the HAD rats showed elevated fear to the conditioned tone stimulus that persisted for the duration of the tone.

Although no differences were observed between HAD and LAD rats during training or during the contextual fear test session, substantial differences between the lines emerged during the tone fear test session. First, upon initial exposure to the novel context during the tone fear test, both HAD and LAD rats showed elevated freezing as compared to the levels observed prior to fear conditioning. Thus, both lines were more fearful, overall, following fear conditioning. This likely resulted from the formation of an association in both HAD and LAD rats between novel contexts and aversive stimulation since the conditioning context was also novel to the rats at the beginning of the experiment. Surprisingly, LAD rats showed more initial freezing in the second context than HAD rats (approximately 50% and 25%, respectively). However, this initial difference was no longer observed after tone onset, as both lines froze at significantly higher levels to the tone. This freezing response was quickly extinguished in LAD rats after 2 min passed without further aversive stimulation. In fact, by the sixth minute, freezing behavior in LAD rats had returned to the level of freezing observed prior to fear conditioning. These data suggest that LAD rats display an initial exaggerated fear or anxiety response when placed in a new conditioning context after receiving tone-shock pairings. However, the LAD rats quickly extinguish this fear response when no aversive stimuli are encountered in the new context.

The second major difference that emerged during the tone fear test session is the failure of HAD rats to show any significant reduction of freezing to the tone conditional stimulus (Fig. 2, bottom panel). Even by the end of the session, the exaggerated cue-elicited freezing observed in HAD rats never returned to the pretraining level of freezing, as was seen in the LAD rats. That is, after 7 min of exposure to the CS in the novel context without any further aversive stimulation, HAD rats were still freezing nearly 30% of the time. During the same time period, LAD rats were freezing only about 10% of the time. Indeed, the lowest level of freezing in HAD rats obtained by the end of the session was equivalent to the elevated level of freezing observed during the pretone period of the tone fear test session. This failure to extinguish suggests that HAD rats experienced more prolonged fear to the discrete cue in the conditioning context. This result provides preliminary support for our previous contention that selective deficits in avoidance responding in HAD rats may be attributed to increased cue-elicited fear in the conditioning context during the signaled barpress paradigm (Blankenship et al., 2000; Rorick et al., 2003). In these two previous studies, we found that although HAD rats escaped the signaled footshock as well as LAD rats, they could not learn the avoidance response. We speculated that excessive fear in HAD rats resulted in excessive freezing, which interfered with the active barpress response required to avoid the footshock. The results presented here demonstrated that HAD rats may in fact exhibit excessive freezing to the cue used to signal the footshock in the aversive conditioning context. The excessive cue-elicited freezing behavior in turn may prevent execution of barpresses that would lead to shock avoidance. Therefore, it seems reasonable to conclude that a failure to extinguish cue-elicited freezing may be the source of the avoidance learning deficits seen in HAD rats. In sum, our data suggest that the excessive fear or anxiety seen in HAD rats is somewhat specific—they do not show prolonged freezing to the training context, but rather to the cue that was paired with the aversive footshock.

As in previous studies (Blankenship et al., 2000; Rorick et al., 2003), we observed no systematic differences between HAD-1 and HAD-2 or between LAD-1 and LAD-2 rats in this study. These results strengthen the validity of these lines as genetic models of alcohol preference by demonstrating that experimental results can be reproduced in the replicate lines, thus eliminating the possibility that they could be due to chance genetic fixation of alleles during the selective breeding process. Moreover, these results provide strong support for a genetic linkage between alcohol preference and increased cue-elicited anxiety in HAD rats. This is consistent with previous reports that alcohol preference in P rats may be associated with increased anxiety (Stewart et al., 1993; McKinzie et al., 2000). However, other studies have reported less clear results regarding the relationship between alcohol preference and anxiety (Viglinskaya et al., 1995; Overstreet et al., 1997; Moller et al., 1997). This discrepancy could be due to the lack of consensus for any one experimental paradigm or dependent measure as an index of anxiety in rodents. Alcoholism is a heterogeneous disorder

(Cloninger, 1987). It is therefore likely that the mechanisms underlying the development and maintenance of alcoholism may also be heterogeneous. The discovery of different mechanisms underlying alcohol preference in various selectively bred rats (e.g., P and HAD) should not be viewed as a scientific failure, but a confirmation that the lines are valid models for different subtypes or features of such a complicated human disorder like alcoholism.

In summary, the current results provide preliminary support for the contention that alcohol preference in HAD rats is associated with excessive fear reactivity. However, the deficit seen in HAD rats is somewhat specific. It appears that HAD rats do not generate greater levels of behavioral freezing under normal conditions. Rather, following aversive learning, they show more prolonged cue-elicited freezing that is resistant to extinction over the course of a training session. Fear is a complex emotional construct that involves a variety of somatic, autonomic, hormonal, and behavioral responses (Bouton and Bolles, 1980; Davis, 2000). In previous studies, behavioral freezing has proven a robust and sufficiently sensitive measure of fear. However, a complete evaluation of fear in HAD and LAD rats requires examination of other dependent measures of fear, such as heart rate reactivity or ultrasonic vocalization, that have been used to study fear in other strains of rats (Knapp et al., 1997; Kikusui et al., 2000; Lee et al., 2001). We are currently exploring potential differences between HAD and LAD rats using these indices of fear, as well as the effects of ethanol on these behaviors.

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