EFFECTS OF CHRONIC ALCOHOL TREATMENT ON ACOUSTIC STARTLE REACTIVITY DURING WITHDRAWAL AND SUBSEQUENT ALCOHOL INTAKE IN HIGH AND LOW ALCOHOL DRINKING RATS

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Abstract — Aims: The purpose of the present study is to determine whether the inverse genetic association between alcohol withdrawal magnitude and genetic propensity for alcohol drinking that we have previously identified in alcohol-naive rats given alcohol acutely, would also be seen following chronic alcohol exposure. The effect of forced, chronic alcohol treatment on subsequent voluntary alcohol drinking was also examined. Methods: Male rats from the high alcohol drinking (HAD2) and low alcohol drinking (LAD2) lines received two intragastric (IG) infusions of alcohol (3.0 g/kg BW; 25% v/v) or an equal volume of water, separated by 5 h, every day for 20 consecutive days (chronic alcohol treatment). Acoustic startle reactivity was assessed at 10, 14, and 18 h after the second infusion on days 1, 5, 10, 15, and 20. After acoustic startle testing was completed, all rats received two IG infusions of 3.0 g alcohol/kg BW, separated by 5 h, and blood alcohol content was assessed at 10, 14, and 18 h after the second alcohol infusion. All rats were then given a 24 h free-choice between alcohol and water for 8 weeks. Results: Startle magnitude to a 120 dB tone was suppressed during alcohol withdrawal in both alcohol-treated HAD2 and LAD2 rats after 5, 10, and 15 days of alcohol treatment. Forced, chronic alcohol treatment produced metabolic tolerance in both the HAD2 and LAD2 lines and significantly suppressed subsequent voluntary alcohol intake in rats of the HAD2 line. Conclusions: Reduced acoustic startle reactivity during alcohol withdrawal in both HAD2 and LAD2 lines and significantly suppressed voluntary alcohol intake in rats of the HAD2 line. Understanding the mechanisms underlying alcohol withdrawal are of interest owing to their potential role in influencing alcohol drinking behaviour and alcohol relapse (Wall et al., 2000; Chester et al., 2002, 2003, 2004a; Koob, 2003).

The alcohol withdrawal syndrome consists of overt, physical signs, and subjective symptoms that occur when blood alcohol levels are falling and after blood alcohol levels have reached 0 mg%. Physical signs of alcohol withdrawal, ranging from mild to severe, are similar in both humans and rodents (Kalant, 1977) and include tremors, convulsions, increased heart rate, and increased body temperature (Majchrowicz, 1975; Holloway et al., 1993). Subjective symptoms of alcohol withdrawal in humans include irritability, nausea, headache, anxiety, and craving.

We have hypothesized that innate increased sensitivity to alcohol withdrawal is associated with a genetic propensity to avoid alcohol. Using various experimental paradigms and several pairs of alcohol-naive rat lines selectively bred for high or low alcohol drinking, we have found that a genetic propensity towards low alcohol drinking is associated with an increased magnitude of alcohol withdrawal following forced acute exposure to alcohol (Chester et al., 2002, 2003). This inverse genetic association between alcohol withdrawal magnitude and propensity towards alcohol drinking in rat lines selectively bred for differences in alcohol preference agrees with results from prior studies using genetic mouse models which have reported that an inverse genetic relationship exists between magnitude of alcohol withdrawal and alcohol drinking behaviour. Alcohol-naive mice that normally drink low amounts of alcohol show strong signs of alcohol withdrawal following termination of forced acute alcohol exposure (Rodgers, 1966; Crabbe, 1983; Metten et al., 1998).

The results of these studies suggest that increased sensitivity to alcohol withdrawal may be a genetic trait that, when inherited, protects against subsequent high alcohol drinking. The effect that the alcohol withdrawal experience may have on subsequent alcohol drinking behaviour probably depends, in large part, on the drinking history of the animal. In our earlier work (Chester et al., 2002, 2003, 2004a), we proposed a motivational role for alcohol withdrawal that depends on both the extent and type of prior experience with alcohol. While the alcohol withdrawal experience may deter subsequent drinking in the alcohol-inexperienced organism, it is likely to play a very different motivational role in the experienced drinker. During the course of chronic alcohol drinking, the experienced drinker learns that alcohol attenuates withdrawal symptoms and hence withdrawal is likely to motivate the experienced drinker to consume alcohol in order to alleviate the aversive effects of alcohol withdrawal (Wikler, 1961). This view is supported by evidence that
increased alcohol self-administration is more likely to be seen if the reinforcing effects of alcohol are well-established prior to the withdrawal experience (Meisch, 1983; Schulteis et al., 1996; Roberts et al., 2000). Although both physical signs and subjective symptoms of alcohol withdrawal have been reported to be aversive in humans (Tiffany, 1990; Swift and Davidson, 1998), the relationship between alcohol withdrawal severity and probability of subsequent alcohol drinking has been difficult to assess in humans, depending, as it does, on self-reports of events not always remembered reliably.

Several genetic association studies suggest that the degree of alcohol withdrawal experienced by an individual may be related to their genetic risk for alcoholism. Individuals with a genetic predisposition toward alcoholism have reported more severe alcohol withdrawal symptoms following alcohol exposure than individuals without a genetic predisposition towards alcoholism (Newlin and Pretorius, 1990; McCaul et al., 1991; Span and Earleywine, 1999). However, it is not clear whether increased sensitivity to alcohol withdrawal was present prior to alcohol exposure in these studies. Genetic association studies in humans have not shed light on this question because the relationship between alcohol withdrawal severity and alcohol drinking behaviour depends on several aspects of drinking history such as pattern of drinking, duration of drinking, and amount of alcohol consumed and these variables are difficult to definitively assess in humans. In addition, changes in sensitivity to the aversive effects of alcohol withdrawal may occur over repeated episodes of alcohol drinking and withdrawal and these changes may occur at different rates in people with and without a genetic predisposition toward alcoholism.

Genetic association studies using rodent lines selectively bred for high voluntary alcohol consumption are useful for examining the relationship between alcohol withdrawal and alcohol drinking behaviour because alcohol history can be controlled. The purpose of the present study is to determine whether genetic differences in propensity for alcohol drinking are associated with differences in innate sensitivity to alcohol withdrawal following forced chronic alcohol exposure and whether forced chronic alcohol exposure and withdrawal alters subsequent voluntary alcohol intake.

**METHODS**

**Subjects**
Subjects were alcohol-naive, adult male rats selectively bred for high alcohol drinking (HAD2 line, n = 20) or low alcohol drinking (LAD2 line, n = 18) from the 37th generation of selection for high or low alcohol drinking. Body weight (BW) ranged from 348 to 486 g in the HAD2 line and from 340 to 480 g in the LAD2 line at the start of the experiment.

The selectively bred HAD2/LAD2 rat lines were derived at Indiana University School of Medicine from a foundation stock of outbred N/NIH rats. Selection of breeders for the HAD2/LAD2 lines was based on the outcome of an alcohol preference test, as previously described (Li et al., 1993). The parental generation of the HAD2 and LAD2 rats used in the present study consumed an average of 7.61 ± 0.45 and 0.27 ± 0.08 g alcohol/kg BW per day, respectively, in the alcohol preference test. Rats in the present study were alcohol-naive at the start of the study.

All rats were individually housed in a colony room maintained at 21 ± 1°C with a 12 h light/dark cycle (lights on at 0700 hours). Food (standard adult rat chow, Harlan Teklad #7001) and water were freely available throughout the experiment. All experimental procedures were conducted in a room separate from the colony room that contained the sound attenuated acoustic startle apparatus. The intragastric (IG) infusions of alcohol or water occurred between 1600 and 2000 h and again between 2100 and 0100 h on each infusion day and acoustic startle testing occurred between 0700 and 1500 on each acoustic startle test day.

**Apparatus and acoustic stimuli**
The acoustic startle response was measured in a Coulbourn Instruments Animal Acoustic Startle System (Coulbourn Instruments, Allentown, PA). The system consisted of four weight-sensitive platforms inside a sound attenuated chamber, as previously described (Chester et al., 2003).

Each acoustic startle test session began with a 5 min acclimation period during which time no acoustic stimuli were presented. A ventilating fan provided continuous background noise (70 dB). Following the 5 min acclimation period, four different tone stimuli (90, 100, 110, 120 dB) were randomly presented throughout the session for a total of 40 trials (each dB was presented 10 times) to avoid habituation to any single dB. The frequency of the tone bursts was 100, 100, 130, and 240 Hz for 90, 100, 110, and 120 dB, respectively. Each tone burst had a 5 ms rise and fall gate time and 50 ms duration. Tone bursts were presented with a random intertrial interval that ranged from 10 to 20 s. These acoustic stimuli parameters are identical to those used previously in these lines to assess genetic differences in alcohol withdrawal magnitude following acute alcohol treatment (Chester et al., 2003).

The dB intensity of each startle stimulus was verified prior to the onset of the experiment using a Digital Sound Level Meter (Radio Shack, Ft Worth, TX). Each acoustic startle session lasted for ~15 min. Subjects were tested in a balanced order so that one subject from each of the four experimental groups (line/treatment) was tested in each test session.

**Drugs**
Alcohol was diluted from a 95% (v/v) solution to a concentration of 25% (v/v) with tap water. Alcohol was administered IG via oral gavage with a metal feeding tube (Harvard Apparatus, Holliston, MA).

**Acoustic startle testing procedure**
A 3-day acclimation phase preceded the onset of drug treatment and acoustic startle testing. The acclimation phase was introduced in order to reduce the stress associated with handling and infusions and to reduce novelty effects via exposure to the acoustic startle apparatus and tone stimuli. On the first day of the acclimation phase, all rats were handled in the colony room and received a sham IG infusion that consisted of insertion of the feeding tube without delivery of fluid. On the second day, all rats were transported on a cart from the colony room to the experimental room that contained the acoustic startle testing apparatus. Rats were handled, received a sham IG infusion, were placed inside the acoustic startle apparatus for 15 min with only background noise present, and were returned to the colony room. On the third...
day, all rats were transported to the experimental room where they remained for the duration of the experiment. Rats received a ‘baseline’ session during which they were handled, received a sham IG infusion, and were placed inside the acoustic startle apparatus for 15 min during which time they were exposed to one session of the acoustic tone stimuli that was identical to the sessions presented during alcohol withdrawal testing. This baseline session was conducted at the same time of day as was the subsequent 14 h time point following alcohol or water treatment. The purpose of exposing rats to the acoustic startle stimuli during the baseline session prior to drug treatment was to reduce initial startle reactivity, as between-session habituation can occur in the rat (e.g. Leaton, 1976). Mean startle magnitude during this baseline session did not significantly differ between rats subsequently assigned to water or alcohol treatment groups within each line (HAD2 alcohol: 91.4 ± 33.6, HAD2 water: 98.2 ± 26.4, LAD2 alcohol: 223.7 ± 46.7, LAD2 water: 149.6 ± 28.2).

Following completion of 3 days of acclimation, rats were counterbalanced within each line on BW and assigned, in matched pairs, to either an alcohol or water treatment group. Each rat received two IG infusions of alcohol (3.0 g/kg BW; 25% v/v), or an equal volume of water, with infusions separated by 5 h, every day for 20 consecutive days. Acoustic startle reactivity was assessed at 10, 14, and 18 h after the second infusion on days 1, 5, 10, 15, and 20. The rationale for choice of these time points to assess alcohol withdrawal following chronic alcohol treatment was based on results of prior studies that have shown that acoustic startle magnitude is enhanced between 4 and 72 h after termination of prior studies that have shown that acoustic startle magnitude is enhanced between 4 and 72 h after termination of chronic alcohol treatment (Pohorecky and Roberts, 1992; Rassnick et al., 1992; Macey et al., 1996). Our prior work in the HAD2/LAD2 rat lines indicated that blood alcohol concentrations (BACs) are still near the peak level at 6 h after an IG infusion of 4.0 g/kg alcohol (Chester et al., 2003). Therefore, in the present study, acoustic startle testing began at 10 h after the second IG infusion of alcohol in order to test for signs of withdrawal when blood alcohol levels were falling.

BAC analyses

BAC was examined in all rats in order to determine the BAC at the times of alcohol withdrawal testing and to assess potential changes in alcohol metabolism in the HAD2 vs LAD2 rat lines over the course of 20 days of alcohol treatment. Immediately after the last acoustic startle test session on day 20, HAD2 (n = 20) and LAD2 (n = 16) rats from both the alcohol-treated and water-treated groups received two IG infusions of 3.0 g alcohol/kg BW, separated by 5 h, and blood samples were taken, and BAC assessed, at 10, 14, and 18 h after the second alcohol infusion. Blood samples (150 µl) were obtained from the tip of the tail and blood was collected in heparin-coated capillary tubes, placed on ice, and immediately centrifuged at 5000 r.p.m. The plasma was collected and stored at −20°C until analysed for BAC content using gas chromatography, as previously described (Chester et al., 2003).

Alcohol drinking procedure

Immediately after the final blood sample was collected for BAC analysis (18–20 h after all rats received their final infusion of alcohol), all rats were transferred from plastic tubes to hanging wire-mesh cages in the colony room and were provided with free access to food, a 10% alcohol solution, and distilled, de-ionized water (D2H2O) for 8 weeks. The 10% alcohol solution and D2H2O was presented in 200 ml calibrated drinking tubes and fluid intake was recorded daily. BW was recorded twice per week. The tubes containing alcohol and water were rotated after each fluid measurement to avoid confounds that could arise from a potential positional preference. Average daily intake of alcohol was converted from ml of fluid per kg BW to g alcohol/kg BW and is reported as such.

Data analyses

The magnitude of the acoustic startle response was determined for each rat by calculating the average of the five highest startle responses (in grams of force) to each tone stimulus. At least 20 g of force had to be exerted on the startle platform in order for the response to be considered a true startle response (Chester et al., 2003).

Acoustic startle, BAC, and alcohol and water intake data were analysed using mixed factor, three-way repeated-measures analysis of variance (ANOVA) with line (HAD2 vs LAD2) and treatment (alcohol vs water) as between-groups factors and dB, day, and hour or week as within-groups factors. Significant interactions were followed with lower-order ANOVAs to determine the source of the interaction (Keppel, 1991). Probability values ≤0.05 were considered significant.

RESULTS

Acoustic startle analyses

The 90 dB (100 Hz) and the 100 dB (100 Hz) did not produce startle responses in either the HAD2 or the LAD2 line, which is consistent with what we have previously found when using these same acoustic tone stimuli to assess genetic differences in alcohol withdrawal magnitude following acute alcohol treatment (Chester et al., 2003). Initial analysis of the 110 dB and 120 dB startle data using a Line × Treatment × dB × Day × Hour ANOVA showed no effects of dB or interactions with dB. Therefore, only startle responses to the 120 dB (240 Hz) tone are reported, as in our prior work (Chester et al., 2003).

Figure 1 shows mean (±SEM) startle magnitude (force in grams) in response to the 120 dB tone stimulus in HAD2 (left panels) and LAD2 (right panels) rats at 10, 14, and 18 h after the second infusion of 3.0 g/kg BW alcohol or water on days 1, 5, 10, 15, and 20. Analysis of startle magnitude (Line × Treatment × Day × Hour ANOVA) yielded significant main effects of Line [F(1,34) = 7.6, P < 0.01], indicating that startle magnitude in LAD2 rats in both treatment groups was greater overall than in HAD2 rats in both treatment groups, and Day [F(4,272) = 8.1, P < 0.01], indicating that startle magnitude decreased in all groups over the course of the five testing days. Significant Treatment × Hour [F(2,272) = 4.9, P = 0.01] and Day × Hour [F(8,272) = 2.5, P = 0.01] interactions were also found. To investigate these interactions, Treatment × Hour ANOVAs were conducted on each testing day, which showed no significant effects or interactions on Day 1. Main effects of
Fig. 1. Mean (±SEM) startle magnitude (force in grams) in response to the 120 dB tone stimulus in HAD2 (left panels) and LAD2 (right panels) rats at 10, 14, and 18 h after the second infusion of 3.0 g/kg BW alcohol or water on days 1, 5, 10, 15, and 20.
Treatment, but no interactions, were found on Days 5, 10, and 15 \( [Fs > 4.4, Ps < 0.05] \), indicating that startle magnitude was suppressed during alcohol withdrawal in both HAD2 and LAD2 rats. A main effect of Hour was also found on Day 10 \( [F(2,72) = 3.6, P < 0.05] \), indicating that startle magnitude decreased in all groups over the three testing time points on this day.

**BAC analyses**

Figure 2 shows mean (± SEM) BAC (mg/100 ml) in HAD2 \( (n = 20) \) and LAD2 \( (n = 16) \) rats that were previously treated with alcohol (alcohol history) or with water (water history) for 20 consecutive days during the acoustic startle portion of the study. Two IG infusions of 3.0 g/kg BW, separated by 5 h, were given to all rats immediately after the last acoustic startle test session on day 20. Asterisks indicate significant differences between alcohol- and water-treated groups as determined by one-way ANOVA.

![BAC Graph](https://example.com/bac-graph.png)

**Table 1.** Mean (±SEM) daily water intake in ml/kg BW during the 8 weeks of free-choice access to a 10% alcohol solution and water in HAD2 and LAD2 rats with a history of alcohol or water treatment.

<table>
<thead>
<tr>
<th>Line/history</th>
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<td>78.6 ± 8.8</td>
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<td>62.1 ± 9.5</td>
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<tr>
<td>HAD2/water</td>
<td>62.8 ± 9.5</td>
<td>49.9 ± 9.1</td>
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<td>24.6 ± 9.6</td>
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<td>81.6 ± 7.1</td>
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<td>83.7 ± 3.8</td>
<td>75.6 ± 4.9</td>
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<td>74.8 ± 4.2</td>
<td>72.1 ± 2.5</td>
<td>55.8 ± 8.5</td>
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Figure 2 shows mean (±SEM) BAC (mg/100 ml) in HAD2 \( (n = 20) \) and LAD2 \( (n = 16) \) rats that were previously treated with alcohol (alcohol history) or with water (water history) for 20 consecutive days during the acoustic startle portion of the study. Two IG infusions of 3.0 g/kg BW, separated by 5 h, were given to all rats immediately after the last acoustic startle test session on day 20. Asterisks indicate significant differences between alcohol- and water-treated groups as determined by one-way ANOVA.

**Table 1.** Mean (±SEM) daily water intake in ml/kg BW during the 8 weeks of free-choice access to a 10% alcohol solution and water in HAD2 and LAD2 rats with a history of alcohol or water treatment.

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[\(F(1,35) = 9.1, P < 0.01\)]. These analyses indicate that metabolic tolerance developed in both the HAD2 and LAD2 lines over the course of 20 days of forced alcohol treatment.

**Alcohol drinking analyses**

Figure 3 shows mean (±SEM) daily alcohol intake in g/kg BW and Table 1 shows mean (±SEM) daily water intake in ml/kg BW during the 8 weeks of free-choice between the 10% alcohol solution and water in HAD2 \( (n = 20) \) and LAD2 \( (n = 18) \) rats previously treated with alcohol (alcohol history) or with water (water history) for 20 consecutive days during the acoustic startle portion of the study. All rats had also received one additional dose of alcohol (2 infusions of 3.0 g/kg BW alcohol separated by 5 h) for BAC testing. Hence rats in the alcohol history groups received a total of 20 plus 1 forced exposures to alcohol and those in the water-history groups received 1 forced exposure to alcohol.

Daily intake of alcohol and water was averaged over 7 day intervals and alcohol intake was analysed using three-way ANOVAs \( (Line \times Treatment \times Week) \). The analysis of alcohol intake yielded a significant effect of Line \( [F(1,34) = 25.2, P < 0.01] \), Treatment \( [F(1,34) = 7.2, P = 0.01] \), Week \( [F(7,238) = 13.7, P < 0.01] \), and Line × Treatment \( [F(1,34) = 6.7, P = 0.01] \) and Line × Week \( [F(7,238) = 12.4, P < 0.01] \) interactions. The Line × Treatment × Week interaction was not significant \( [F(7,238) = 1.8, P = 0.09] \). To determine the source of the two-way interactions, follow-up one-way
ANOVA of Treatment within each Line and Line within each Week were conducted. A significant Treatment effect in HAD2 rats \( F(1,18) = 7.9, P = 0.01 \) but not in LAD2 rats was found, indicating that alcohol intake was suppressed in HAD2 rats with a history of alcohol treatment vs water treatment throughout the 8 week period. Significant Line effects at weeks 3–8 \( F_s(1,36) > 7.4, P_s < 0.01 \) were found, indicating that overall alcohol intake was higher in HAD2 vs LAD2 rats in both treatment groups during weeks 3–8 but overall alcohol intake was not different between HAD2 vs LAD2 rats during the first 2 weeks of alcohol access.

The three-way ANOVA of water intake yielded a significant effect of Treatment \( F(1,34) = 7.3, P = 0.01 \) but no interactions with Line, indicating that water intake was higher in both HAD2 and LAD2 rats with a history of alcohol-treatment vs those with a history of water-treatment. This analysis also showed significant effects of Line \( F(1,34) = 15.9, P < 0.01 \) and Week \( F(7,238) = 5.8, P < 0.01 \) and a Line \( \times \) Week interaction \( F(7,238) = 10.9, P < 0.01 \), but no Line \( \times \) Treatment \( \times \) Week interaction \( F(7,238) = 1.2, P = 0.3 \). A follow-up one-way ANOVA of Line within each week showed a significant Line effect during weeks 3, 4, 5, 7, and 8 \( F(1,36) > 6.6, P_s < 0.01 \) indicating higher water intake in LAD2 rats vs HAD2 rats during weeks 3, 4, 5, 7, and 8.

A three-way ANOVA of total fluid intake indicated a significant effect of Week \( F(7,238) = 2.8, P < 0.01 \) and Line \( \times \) Week \( F(7,238) = 4.9, P < 0.01 \) and Treatment \( \times \) Week \( F(7,238) = 3.0, P < 0.01 \) interactions but no Line \( \times \) Treatment \( \times \) Week interaction \( F(7,238) = 1.5, P = 0.2 \). Follow-up one-way ANOVAs of Line within each week and Treatment within each week showed greater total fluid intake during week 6 in HAD2 vs LAD2 rats \( P < 0.01 \) and greater total fluid intake during week 1 in rats with a history of alcohol vs water treatment \( P < 0.05 \) (data not shown).

Consumption of 5.0 g alcohol/kg BW/day is the criterion used for selection of breeders in the HAD2 line. Eight out of ten HAD2 rats with a history of water treatment reached the 5.0 g/kg BW criterion during the 8 weeks of free-choice between alcohol and water. In contrast, only 3 out of 10 HAD2 rats with a history of alcohol treatment reached the
DISCUSSION

The first goal of the present study was to determine whether genetic differences in propensity for alcohol drinking in rats of the HAD2/LAD2 lines are associated with differences in innate sensitivity to alcohol withdrawal following forced chronic alcohol exposure when alcohol withdrawal is assessed by acoustic startle reactivity to a tone stimulus. The acoustic startle test parameters included four different tone stimuli (90, 100, 110, and 120 dB) in order to replicate, as far as possible, the acoustic startle paradigm used in our prior investigation of alcohol withdrawal following acute alcohol treatment in these lines (Chester et al., 2003). Although the 90, 100, and 110 dB tones are low in sound intensity and usually below the threshold for producing reliable startle responses (Marsh et al., 1973; Blumenthal and Berg, 1986; Chester et al., 2003), they are within the range of hearing for the rat (Webster, 1995) and inclusion of these tones provided an opportunity to examine whether chronic vs acute alcohol treatment decreases startle threshold during withdrawal. No change in startle reactivity to 90, 100, or 110 dB tones was found during withdrawal.

Startle reactivity to a 120 dB tone stimulus was reduced in both HAD2 and LAD2 rats during alcohol withdrawal following 5, 10, and 15 days of chronic alcohol treatment. This finding is similar to our previous observations in HAD2 rats, but not in LAD2 rats, after acute alcohol treatment (Chester et al., 2003), which suggests that the genetic association between alcohol withdrawal magnitude and alcohol drinking behaviour in these lines varies as a function of prior forced alcohol exposure (single vs repeated or acute vs chronic forced alcohol exposure).

Day 1 of acoustic startle testing constituted a test of withdrawal after a single alcohol exposure and hence is similar to our prior work using 1 day of forced alcohol exposure. No sign of alcohol withdrawal was seen in either the HAD2 or LAD2 lines after 1 day of alcohol treatment. This was surprising given that we have previously seen a suppression in acoustic startle responding in HAD2 rats and an elevation in acoustic startle responding in LAD2 rats during withdrawal following a single alcohol exposure (Chester et al., 2003). This difference may be owing to the type of forced alcohol exposure used in the two studies; in the present study, alcohol was administered as two IG infusions of 3.0 g/kg BW alcohol, whereas, in our prior study alcohol was administered as a single IG infusion of 4.0 g/kg BW alcohol (Chester et al., 2003). In the prior study, changes in acoustic startle reactivity were evident after BAC had reached zero, yet, in the present study, no change in startle reactivity was evident on Day 1 when BAC had reached zero (Fig. 3, water-treated groups, 18 h BAC time-point). Additional studies will be needed to determine whether changes in acoustic startle reactivity emerge at time points later than 18 h after two IG infusions of 3.0 g/kg BW alcohol.

A modest suppression of acoustic startle reactivity was evident in the alcohol-treated HAD2 and LAD2 lines after 5, 10, and 15 days, but not after 20 days of alcohol treatment. This is interesting given that prior reports indicate that acoustic startle responses are enhanced in rats during withdrawal from chronic alcohol treatment (Pohorecky and Roberts, 1992; Rassnick et al., 1992; Macey et al., 1996). This difference may be owing to the fact that these prior studies employed greater exposure to alcohol such as 14 days of forced exposure to 12 g/kg BW/day (Pohorecky and Roberts, 1992), 3–4 weeks of forced alcohol intake via an alcohol-containing liquid diet (Rassnick et al., 1992; Macey et al., 1996), or 13–15 days of continuous exposure to alcohol vapour via an inhalation chamber (Macey et al., 1996). In these prior studies, acoustic startle reactivity was not suppressed at any time point during withdrawal testing. A recent report noted a reduction in acoustic startle magnitude during withdrawal from chronic alcohol exposure in mice, similar to the results in the present study (Fernandez et al., 2003).

The results of the present study suggest that CNS excitability is reduced in both HAD2 and LAD2 rats during alcohol withdrawal following chronic forced alcohol exposure. The analyses of BAC indicate that the suppression in startle reactivity in HAD2 and LAD2 rats is not dependent on the presence of alcohol in the blood at the time of acoustic startle testing. This is supported by the fact that no suppression in acoustic startle reactivity was seen in either the HAD2 or the LAD2 line on day 1 of acoustic startle testing even though alcohol was present in the blood at two of the testing time points (10 and 14 h).

The results of the current study in selectively bred rat lines do not support the finding in humans that a genetic predisposition toward alcoholism is accompanied by more severe alcohol withdrawal symptoms (Newlin and Pretorius, 1990; McCaul et al., 1991; Span and Earleywine, 1999). This may not be surprising in light of the many differences in the human studies compared with the current study including the forced nature of alcohol exposure in the current study and the voluntary nature of alcohol exposure in the human studies. In addition, although rats of the HAD2 line were ‘family history positive’, they were also alcohol-naive at the onset of the study and 20 days of forced chronic alcohol exposure may constitute a very limited and specific type of ‘alcohol history’. In contrast, in the human genetic association studies the family history positive subjects were not alcohol-naive and the pattern, amount, and duration of alcohol drinking, as well as the number and intensity of prior alcohol withdrawal experiences, were not known. These factors are important in determining the intensity of alcohol withdrawal symptoms that can change over the course of repeated cycles of drinking and withdrawal and that may depend on an individual’s inherited risk for alcoholism (Booth and Blow, 1993; Duka et al., 2004). Additional studies, using several different measures of alcohol withdrawal, will be necessary in order to fully characterize the genetic relationship between withdrawal from chronic alcohol treatment and genetic differences in the propensity to drink alcohol.

We have previously suggested that genetic differences in sensitivity to alcohol withdrawal may shape the subsequent pattern of alcohol intake and amount of alcohol consumed. The second goal of this study was to explore the relationship between chronic forced alcohol exposure and subsequent
alcohol drinking behaviour in rats of the HAD2 and LAD2 lines. The findings reveal that repeated, experimenter-administered, forced alcohol exposure robustly retarded the expression of a genetic predisposition toward alcohol drinking in rats selectively bred for high alcohol intake. Alcohol intake was negligible during the first 3 weeks of free-choice between alcohol and water in LAD2 rats previously treated with either alcohol or water. This was expected because the LAD2 rat line has been selectively bred for low alcohol intake. However, alcohol intake was also negligible during the first 3 weeks of free-choice between alcohol and water in HAD2 rats previously treated with alcohol even though the HAD2 rat line has been selectively bred for high alcohol intake. Alcohol intake was significantly lower in the alcohol-treated than in the water-treated HAD2 rats. A detailed examination of alcohol intake patterns in individual animals revealed that 5 out of the 10 alcohol-treated HAD2 rats never drank more than 1.0 g/kg BW alcohol/day during the entire 8 weeks of free-choice between alcohol and water. This level of alcohol intake is similar to that seen in LAD2 rats selectively bred for low alcohol drinking. Only 3 out of the 10 alcohol-treated HAD2 rats ever reached the criterion used for selection of the HAD2 line (5.0 g/kg BW/day) during the 8 weeks of free-choice between alcohol and water. These findings are remarkable considering that the average daily alcohol intake in the parental generation of the HAD2 rats used in the present study was >7.5 g/kg BW/day during 3 weeks of free-choice between alcohol and water, the classic alcohol preference test. In addition, acquisition of high alcohol drinking normally occurs quickly in HAD2 rats with elevated alcohol intake seen in the first week of free-choice between alcohol and water. For instance, average daily alcohol intake exceeded 5.0 g/kg BW by the third day of free-choice between alcohol and water in the parental generation of the HAD2 rats used in the present study (data not shown). It is well-known that forced alcohol exposure constitutes a stress. It appears that the stress associated with forced chronic alcohol exposure may have suppressed alcohol intake in the HAD2 rats. This supports prior reports that, under certain conditions, stress results in a decrease in voluntary alcohol intake (Rockman et al., 1987; Van Erp and Miczek, 2001; Roman et al., 2003; Chester et al., 2004b).

The acquisition of alcohol drinking behaviour in the current study was somewhat delayed in the water-treated HAD2 rats given a single forced exposure to alcohol relative to the rate of acquisition of drinking found in the parental generation. Average daily alcohol intake in water-treated HAD2 rats did not reach the 5.0 g/kg BW alcohol selection criterion until approximately the 5th week of free-choice between alcohol and water, as opposed to the third day in the parental generation. It appears that two experimenter-administered infusions of alcohol over a 5 h period, followed by alcohol withdrawal, is sufficient to deter the expression of alcohol drinking behaviour in rats with a genetic predisposition towards high alcohol intake. The complete avoidance of alcohol by many of the alcohol-treated HAD2 rats following chronic forced alcohol treatment is similar to the avoidance reported in monkeys (Myers et al., 1972) and rats (Hunter et al., 1974) that were made physically dependent on alcohol via forced alcohol exposure and then given an opportunity to drink alcohol under free-choice conditions. However, some investigators have reported increased alcohol intake following passive or ‘forced’ chronic alcohol exposure (Deutsch and Koopmans, 1973; Veale, 1973; Marfaing-Jallat and Le Magnen, 1982; Fidler et al., 2004). It has been suggested that allowing animals to learn about the positive and/or negative (relief from withdrawal) reinforcing effects of alcohol, by providing them access to alcohol before or during the course of repeated forced alcohol exposure and withdrawal experiences, may be necessary in order to see an increase in alcohol drinking behaviour following forced exposure to alcohol (Veale and Myers, 1969; Hunter et al., 1974; Meisch, 1983; Schulteis et al., 1996; Brown et al., 1998; Roberts et al., 2000). It will be interesting to determine whether allowing HAD2 rats the opportunity to voluntarily consume alcohol prior to forced alcohol dependence induction and repeated withdrawal experiences results in increased alcohol drinking behaviour.

The results of the present study suggest that forced alcohol exposure and withdrawal produces an aversion to alcohol that can retard the expression of a genetic predisposition toward alcohol drinking in rats selectively bred for high alcohol intake. It is striking that forced alcohol exposure in rats of the HAD2 line was as potent in suppressing the acquisition of alcohol drinking behaviour as was naltrexone, the classic pharmacotherapeutic agent for the treatment of alcoholism, when naltrexone was co-administered with daily opportunity to drink alcohol (Badia-Elder et al., 1999). It appears that further studies to assess the role of alcohol withdrawal in shaping subsequent alcohol drinking behaviour and relapse may need to avoid forced alcohol exposure. Rat lines selectively bred for high voluntary alcohol drinking may be useful for assessing the relationship between voluntary alcohol drinking, alcohol withdrawal, and relapse in paradigms where the amount and duration of alcohol intake is known and systematically varied.

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