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Effects of short deprivation and re-exposure intervals on the ethanol drinking behavior of selectively bred high alcohol-consuming rats

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Abstract

Alcoholics generally display cycles of excessive ethanol intake, abstinence and relapse behavior. Using an animal model of relapse-like drinking, the alcohol deprivation effect (ADE), our laboratory has shown that repeated 2-week cycles of ethanol deprivation and re-exposure, following an initial 6 week access period, result in a robust ADE by alcohol-preferring (P) and high alcohol-drinking (HAD-1 and HAD-2) rats. These rat lines have been selectively bred to prefer a 10% ethanol solution over water. The present study examined whether P and HAD rats would display an ADE using much shorter ethanol deprivation and re-exposure intervals. Rats were given either continuous or periodic concurrent access to multiple concentrations [10%, 20%, and 30%, volume/volume (vol./vol.)] of ethanol. The periodic protocol involved access to ethanol for 12 days followed by 4 cycles of 4 days of deprivation and 4 days of re-exposure to ethanol access. HAD rats displayed a robust 24 hour ADE upon 1st re-exposure (HAD-1: ~ 5 vs. 8 g/kg/day; HAD-2: ~ 6 vs. 9 g/kg/day, baseline vs. re-exposure), whereas P rats (~ 7 vs. 8 g/kg/day) displayed a modest, nonsignificant, increase in 24 hour intake. In a separate group of rats, ethanol intake and blood alcohol concentrations (BACs) after the 1st hour of the 4th re-exposure cycle were HAD-1: 2.0 g/kg and 97 mg%, HAD-2: 2.3 g/kg and 73 mg%, and P: 1.2 g/kg and 71 mg%; with all three lines displaying a robust 1st hour ADE. These findings suggest that (a) an ADE may be observed with short ethanol deprivation and re-exposure intervals in HAD rats, and (b) the genetic make-up of the P and HAD rats influences the expression of this ADE.

Keywords

Alcohol deprivation effect; High-alcohol-consuming rats; Selectively bred rats; Adult

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1. Introduction

Binge drinking during high school and college is becoming more prevalent and is also a strong predictor of future alcohol-related problems in both men and women in North America (Presley et al., 1994; Wechsler et al., 2000) and Europe (Kuntsche et al., 2004). Patterns of drinking, and total volume consumed are important diagnostic criteria for the onset of alcoholism (e.g., Heather et al., 1993; Lancaster, 1994). Recent research from our laboratory has indicated that different selectively bred, high alcohol-consuming lines of rats may display different types (e.g., binge-like versus more continuous-like) of drinking under free-choice, home-cage conditions (e.g., Bell et al., 2003, 2004b), which has been reported for selectively bred, high alcohol-consuming rats under operant conditions as well (Files et al., 1998; Samson et al., 1998). Moreover, as discussed below, different selectively bred high alcohol-consuming lines of rats display, to varying degrees, the alcohol deprivation effect (ADE), a transient increase in ethanol intake after a period of ethanol deprivation (see below).

Relapse is a ubiquitous problem for individuals “recovering” from alcoholism (c.f., Barrick and Connors, 2002; Chiauzzi, 1991; Dawson et al., 2005; Jaffe, 2002; Weiss et al., 2001). The alcohol deprivation effect (ADE) has been proposed as a model of “loss of control” and “relapse-like” drinking (c.f., Bell et al., 2005; McBride and Li, 1998; Murphy et al., 2002; Rodd et al., 2004; Sinclair and Li, 1989). The ADE is defined as a temporary increase in the ratio of ethanol/total fluid intake and volume of ethanol intake over baseline drinking conditions, when ethanol is reinstated following a period of alcohol deprivation (Sinclair and Senter, 1967). The ADE can be observed following short (12-hr or less; Agabio et al., 2000; Sinclair and Li, 1989) or long (up to 75 days; Sinclair et al., 1973) deprivation intervals. Regarding the expression of an ADE, with a single prolonged (2 weeks or more) deprivation, P rats display robust relapse-like drinking when re-exposed to ethanol access (Rodd-Henricks et al., 2000b; 2001). In the latter study (Rodd-Henricks et al., 2001), concurrent access to multiple concentrations (10%, 20%, and 30%) of ethanol increased the magnitude and duration of the ADE, compared to that displayed by P rats given access to a single concentration of 10% ethanol (Rodd-Henricks et al., 2000b). This increased magnitude in the ADE appears to have been due to a shift in ethanol concentration preference from lower concentrations (10% and 20%) to higher concentrations (20% and 30%) of ethanol across deprivation cycles (c.f., Rodd et al., 2008; Rodd-Henricks et al., 2001). HAD-1 and HAD-2 rats also display a robust ADE when given access to a single concentration of ethanol and experience repeated deprivation cycles (Oster et al., 2006; Rodd-Henricks et al., 2000a). A recent study (Rodd et al., 2008) indicated that when HAD-1 and HAD-2 rats were given concurrent access to multiple concentrations (10%, 20%, and 30%) of ethanol a robust ADE was displayed after the first 2 week deprivation cycle, and multiple deprivation cycles increased the magnitude and duration of the ADE.

In comparison with their male counterparts, adult female rodents reportedly consume more ethanol, in grams per kilogram of body weight (e.g., Adams, 1995; Juárez and De Tomasi, 1999; Lancaster and Spiegel, 1992; Li and Lumeng, 1984). This effect, although modest, has also been found in peri-adolescent and post-weaning P (Bell et al., 2003; McKinzie et al., 1998a; 1998b) and post-weaning HAD (McKinzie et al., 1998a) rats. Results from previous studies have revealed that concurrent access to multiple concentrations of ethanol increased ethanol intake in adult outbred rats (Holter et al., 1998; Wolffgramm and Heyne, 1995), periadolescent P (Bell et al., 2003), adult P (Rodd-Henricks et al., 2001), periadolescent HAD-1 and HAD-2 (Bell et al., 2004b) and adult HAD-1 and HAD-2 (Rodd et al., 2008) rats, and, as discussed above, increases the magnitude and duration of the ADE in P (Rodd-Henricks et al., 2001) and HAD rats (Rodd et al., 2008), when employing two-week deprivation and re-exposure cycles.

The ADE protocol used by our laboratory (e.g., Bell et al., 2004a; Oster et al., 2006; Rodd et al., 2003, 2008; Rodd-Henricks et al., 2000a, 2000b, 2001) generally involves giving rats 5-1/2, or more, months of periodic access to ethanol. The present study assessed whether repeated, very short deprivation and re-exposure cycles (4 days versus the 2 weeks used in previous studies) would result in the expression of an ADE in adult HAD-1, HAD-2, and P rats. The effects of sex-of-animal on ethanol intake were also examined. Ethanol intake was maximized by giving the rats concurrent access to multiple concentrations (10%, 20%, and 30%) of ethanol, as has been done in previous studies from our laboratory (Rodd et al., 2008; Rodd-Henricks et al., 2001). In summary, we hypothesized that (a) P, HAD-1, and HAD-2 rats would display a robust ADE when given concurrent access to multiple concentrations of ethanol with short deprivation and re-exposure cycles, (b) female rats would consume more ethanol than their male counterparts; and (c) pharmacologically relevant blood alcohol concentrations (BACs) would be achieved upon re-exposure to ethanol access.

2. Materials and methods

2.1. Animals and procedures

Animals used for this project were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine (Indianapolis, IN) and are in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Adult, ethanol-naive, male and female rats ($n=9-11$ /line/sex/ethanol condition), from the S49-S51 generations of P, S37-S39 generations of HAD-1, and S34-S36 generations of HAD-2 selectively bred rats, were obtained from the Indiana University School of Medicine/Veterans Affairs Medical Center (Indianapolis, IN) breeding colonies at 60 days of age and pair housed in plastic tubs, with wood chip bedding, by line and sex. Two squads were run for each condition, with overlap between groups (i.e., males and females and subjects from each line were run at the same time). To reduce litter effects, at least four litters were represented in each condition. After reaching adulthood (>90 days of age), the animals were transferred to hanging wire-mesh cages with water and food freely available throughout the experiment. After 3 to 5 days of habituation to the hanging wire mesh cages, half of the rats of each sex, within each line, were given continuous concurrent access to multiple concentrations of ethanol [10%, 20%, and 30%, volume/volume (vol./vol.)] for 52 days. The other half of the rats of each sex, within each line, received periodic concurrent access to multiple concentrations of ethanol [10%, 20%, and 30% (vol./vol.)], such that the rats had 12 days of initial access followed by 4 cycles of 4 days of deprivation from and 4 days of re-exposure to ethanol access, with the last re-exposure period extended to 12 days. Each re-exposure cycle was initiated at light offset (1900h). The vivarium was maintained on a 12-h light/12-h dark cycle (lights off at 1900h), which was temperature (21°C) and humidity (50%) controlled.

The animals had 24 hr access to their respective solutions except during the collection of body and bottle weights each day. Starting on the 1st day of ethanol access, body weight, water bottle weight, and ethanol solution weights were obtained, to the nearest 0.1 g, by using a Sartorius Balance BP 6100 and Sartorius Interface V24/V28-RS232C(-S)/423 (Sartorius Instruments, McGaw Park, IL) and recorded by a personal computer program (SoftwareWedge, Professional Edition v 5.0 for DOS; Sartorius Instruments) at least 6 days per week. Data for day 7, when missing, were taken as the average of values obtained from the previous and subsequent days. All weights were obtained at the same time each day during the light cycle (1100-1300).

Initially, the lowest concentration [10% (vol./vol.)] was placed next to the water bottle away from the food hopper, with the highest concentration of ethanol [30% (vol./vol.)] placed farthest from the food hopper. Periodically the order of the ethanol concentrations was randomly changed. The water bottle was a standard glass bottle holding approximately 300 ml of fluid, with a stopper (no. 10) holding an angled (~135°) stainless steel sipper tube. Ethanol solutions were maintained in 25-ml serologic pipettes that were cut at both ends and sealed with a polyurethane (no. 13) stopper and a rubber stopper fitted with an angled (~135°) stainless steel sipper tube. Sipper tubes in the present study did not have a ball-bearing tip. Spillage was calculated by employing a “ghost-cage,” with the ethanol pipettes and water bottle weighed daily, although a rat was not present. Approximately 0.5 ml of solution was spilled per weighing and this amount was subtracted from daily intake values for each respective solution. The ethanol pipettes were refilled at least twice a week, and water bottles were refilled at least once a week. Bottles and pipettes were replaced every 2 weeks.

Previous studies with Wistar rats (Bell et al., 2000) and P and NP rats (Bell et al., unpublished observations; Lumeng et al., 1982) indicated that tail blood sampling did not give accurate assessment of changing brain alcohol levels at low to moderate doses, at least during the 1st hr post ethanol exposure. Therefore, a 3rd squad of rats ($n = 7-9$ /line/sex/ethanol condition), from the same generations as indicated above, was run to assess BACs from trunk blood. A review of the drinking data from these animals did not reveal any significant differences from the data obtained from the 1st two squads. For the repeatedly deprived animals, trunk blood samples were obtained 1 hr after the initiation (1900h) of ethanol access on the 1st day of the 4th re-exposure cycle. For the continuous access animals, trunk blood samples were obtained at the same time (2000h). Blood samples were collected in heparinized tubes and centrifuged in a Microfuge (Model B, Beckman: Palo Alto, CA) for 45 sec. Plasma BAC was measured using an Analox Analyser (model GL5: Analox Instruments USA, Lunenburg, MA).

2.2. Statistical analyses

To facilitate presentation of the data and decompose the significant interaction terms (3-way and higher interactions for ethanol intake and ethanol concentration preference), daily ethanol intake values and ethanol concentration preference scores were collapsed into 4-day blocks and the five 4-day blocks associated with the deprivation and re-exposure cycles [i.e., the block before the 1st deprivation (baseline) and the blocks after each of the four deprivation cycles] evaluated within each line. Note: the data from the 1st and last blocks of ethanol access did not differ significantly from the 1st and last blocks of data analyzed and depicted in the graphs. Appropriate simple effect analyses were conducted following significant interactions and main effects. To help control for alpha-error, alpha was set at 0.025 for these analyses. Due to multiple 3-way and higher interactions, the water intake and body weight data were analyzed separately within each line, with the data collapsed into 4-day blocks. Appropriate simple effect analyses were conducted following significant interactions and main effects. To help control for alpha-error, alpha was set at 0.025 for these analyses. To confirm a significant association between the amount of ethanol consumed and BAC levels achieved, during the initial hr of the 4th re-exposure, separate regression analyses were conducted for each line by sex by ethanol condition. Alpha was set at 0.05 for these analyses.

3. Results

3.1. Ethanol intake (4 day averages, g/kg/day)

The three selectively bred lines (P, HAD-1 and HAD-2) were evaluated separately, i.e., the line factor had 3 levels, because substantial evidence from previous research indicates that these three lines display differences in their ethanol drinking behavior (e.g., Bell et al., 2003,

2004b; Files et al., 1998; Oster et al., 2006; Rodd et al., 2008; Rodd-Henricks et al., 2000a, 2000b, 2001, 2003; Samson et al., 1998).

The $2 \times 2 \times 5$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the ethanol intake data from the HAD-1 rats revealed a significant ethanol condition by block interaction and significant main effects for block and ethanol condition (F -values > 6.62 , and P -values < 0.025). As seen in Figure 1 (top panel), HAD-1 rats experiencing deprivation periods significantly increased their ethanol intake upon re-exposure. The data were collapsed across sex, which did not have a significant effect for the HAD-1 rats, and the significant increases in ethanol intake detected by the mixed ANOVA were confirmed by significant (P -values < 0.025) ethanol condition (between-subject) effects during the 1st three re-exposure cycles.

The $2 \times 2 \times 5$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the ethanol intake data from the HAD-2 rats revealed a significant ethanol condition by block interaction and significant main effects for block and ethanol condition (F -values > 5.64 , and P -values < 0.025). As seen in Figure 1 (middle panel), HAD-2 rats experiencing deprivation periods displayed significant increases in ethanol intake upon re-exposure. The data were collapsed across sex, which did not have a significant effect for the HAD-2 rats, and the significant increases in ethanol intake detected by the mixed ANOVA were confirmed by significant (P -values < 0.025) ethanol condition (between-subject) effects during the 1st three re-exposure cycles.

The $2 \times 2 \times 5$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the ethanol intake data from the P rats did not reveal any significant effects for the ethanol condition factor within the P line. However, there was a main effect of sex-of-animal (F -values > 5.57 , and P -values < 0.025). As seen in Figure 1 (bottom panel), in general, female P rats drank more ethanol than male P rats, with no significant differences between the ethanol conditions.

3.2. Preference for ethanol concentration (4-day averages, % of total ethanol volume consumed/day)

The $2 \times 2 \times 3$ (sex-of-animal by ethanol condition by ethanol concentration) mixed ANOVA on the preference for ethanol concentration data for the HAD-1 line revealed a significant 3-way interaction and a significant main effect of ethanol concentration (F -values > 8.9 , and P -values < 0.025), with the main effect of sex-of-animal having a trend towards significance ($P = 0.048$). There was a strong preference for the 20% over both the 10% and 30% concentrations in the deprived male and continuous access female HAD-1 rats, whereas the continuous access male and deprived female HAD-1 rats displayed similar preferences for the 20% and 30% concentrations over that shown for the 10% concentration of ethanol (data not shown). Evaluation of the ethanol concentration preference data from the HAD-2 and P lines of rats, revealed no significant effects of ethanol concentration for either of these lines.

3.3. Water intake (4-day averages, ml/kg/day)

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the HAD-1 rats' water intake data revealed significant 3-way and 2-way (ethanol condition by block) interactions and significant main effects of sex-of-animal and block (F -values > 2.40 , and P -values < 0.025). As seen in Figure 2 (upper panel), overall, female HAD-1 rats drank more water than their male counterparts. Also, female deprived HAD-1 rats drank slightly more water than female continuous access HAD-1 rats at the beginning of the experiment, whereas female deprived HAD-1 rats drank similar or lower amounts of water than the female continuous access HAD-1 rats after the fourth 4-day block. On the other hand, male deprived HAD-1 rats drank similar or lower amounts of water than male continuous access HAD-1 rats.

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the HAD-2 rats' water intake data revealed that all interactions with block were significant as well as the main effects of sex-of-animal and block (F -values > 2.10 , and P -values < 0.025). As seen in Figure 2 (middle panel), overall, female HAD-2 rats drank more water than their male counterparts. Also, in general, female and male deprived HAD-2 rats drank as much, if not significantly more, water than their male and female continuous access HAD-2 counterparts, with this effect being stronger in male HAD-2 rats and the most dramatic differences occurring during the 4-day blocks associated with ethanol deprivation for the deprived rats.

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the P rats' water intake data revealed that both 2-way interactions with block (sex-of-animal and ethanol condition) were significant as well as the main effects of sex-of-animal, ethanol condition and block (F -values > 3.80 , and P -values < 0.025). As seen in Figure 2 (lower panel), overall, female P rats drank more water than their male counterparts. Also, female and male deprived P rats drank significantly more water than their male and female continuous access P counterparts during the 4-day blocks associated with the ethanol deprivation intervals.

3.4. Total fluid intake (4-day averages, ml/kg/day)

Because there were substantial increases in water intake by the deprived P rats, and to a lesser degree deprived HAD-2 rats, during the 4-day blocks associated with ethanol deprivation, we also analyzed total fluid intake to see if the high levels of water intake during ethanol deprivation intervals were due to maintenance of fluid balance or not (i.e., when total fluid intake was evaluated, were the differences between the deprived and continuous access rats maintained and did total fluid intake for the deprived P rats change across 4-day blocks).

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the total fluid intake data from the HAD-1 line revealed significant 3-way and 2-way (ethanol condition by block) interactions, as well as significant main effects of sex-of-animal, ethanol condition and block (F -values > 2.10 , and P -values < 0.025). As seen in Figure 3 (upper panel), overall, female HAD-1 rats drank more fluids than their male counterparts. Also, in general, continuous access HAD-1 rats drank more fluids than deprived HAD-1 rats, with this effect occurring primarily during the ethanol deprivation intervals.

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the total fluid intake data from the HAD-2 line revealed that all interactions with block were significant as well as the main effects of sex-of-animal and block (F -values > 2.00 , and P -values < 0.025). As seen in Figure 3 (middle panel), overall, female HAD-2 rats drank more fluids than their male counterparts. Also, in general, female deprived HAD-2 rats drank less fluids than their continuous access counterparts during the ethanol deprivation intervals. In the male HAD-2 rats, this latter effect was seen primarily during the last deprivation interval (i.e., block 10).

The $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the total fluid intake data from the P line revealed that all interactions with block were significant, as well as the sex-of-animal by ethanol condition interaction and the main effects of sex-of-animal and block (F -values > 2.20 , and P -values < 0.025). As seen in Figure 3 (lower panel), female P rats drank more fluids than their male counterparts. Also, whereas male deprived P rats drank significantly less total fluids than their continuous access counterparts during the ethanol deprivation intervals, this effect was seen in the female P rats only during the first ethanol deprivation period (i.e., block 4). As a caveat, the ethanol condition main effect had a trend towards significance ($P = 0.060$).

3.5. Body weight (4-day averages, g)

Because of the sex-of-animal differences in body weight and, in the case of male HAD rats, ethanol condition-associated differences in body weight, all intake levels discussed above had been converted to grams (ethanol) or milliliters (water and total fluid) per kg body weight per day. This was done to limit the effects of overt body weight differences on these dependent measures.

For the HAD-1 rats, the $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the body weight data revealed all interactions with block, as well as the main effects of sex-of-animal and block were significant (F -values > 8.60 , and P -values < 0.025). As seen in Figure 4 (upper panel), male HAD-1 rats weighed more than female HAD-1 rats; the continuous access HAD-1 rats weighed slightly less than the deprived HAD-1 rats at the beginning of the experiment, with these differences absent after the 3rd 4-day block. This latter effect indicates the rate of growth was slightly higher in the continuous access HAD-1 rats.

For the HAD-2 rats, the $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA revealed that the 2-way interactions involving the block factor and the sex-of-animal by ethanol condition interactions were significant, as well as the main effects of sex-of-animal, ethanol condition, and block (F -values > 6.90 , and P -values < 0.025). As seen in Figure 4 (middle panel), male HAD-2 rats weighed more than their female counterparts, and, whereas the male repeatedly deprived HAD-2 rats weighed more than the male continuous access HAD-2 rats across all 13 blocks, this ethanol condition difference was not present in the female HAD-2 rats. In addition, the male HAD-2 rats displayed a higher growth rate than the female HAD-2 rats.

For the P rats, the $2 \times 2 \times 13$ (sex-of-animal by ethanol condition by block) mixed ANOVA on the body weight data revealed that the sex-of-animal by block interaction as well as the sex-of-animal and block main effects were significant (F -values > 12.40 , and P -values < 0.025). As seen in Figure 4 (lower panel), male P rats weighed more than female P rats, and male P rats gained body weight to a greater extent than female P rats, but there were no ethanol condition effects.

3.6. Association between ethanol intake and BACs

Separate squads of rats were tested to determine the association between ethanol intake during the 1st hr of the 4th re-exposure cycle and trunk BACs obtained at the end of this hr of access (Table 1). There were significant ($P < .05$) associations (correlations) between ethanol intake and BACs achieved at the end of the 1st hr of the 4th re-exposure cycle for repeatedly deprived HAD and P rats (Table 1). However, except for the male HAD-1 rats, there were poor correlations between ethanol intake and BACs achieved for the continuous access rats (Table 1). In addition, repeatedly deprived rats displayed significantly ($P < .05$) greater ethanol intake and BACs than their continuous access counterparts, within each respective line (Table 1). Overall ranking of BACs achieved for the repeatedly deprived/periodic access animals revealed HAD-1 rats had greater BACs than HAD-2 rats, which in turn had BAC levels approximating those seen in P rats (Table 1).

4. Discussion

In general, the results indicate that HAD-1 and HAD-2 rats display a robust 24-hr ADE using the present protocol (Fig. 1), with P rats displaying nonsignificant increases in ethanol intake upon re-exposure. All three lines displayed a robust 1-hr ADE, which resulted in pharmacologically relevant BACs approximating 65 mg% or greater (Table 1). Regarding preference for ethanol concentrations, although there was an overall preference for the higher

concentrations of ethanol (data not shown), which did not change significantly across re-exposure cycles, this effect was driven primarily by the HAD-1 data. Analyses of sex-of-animal effects revealed that only the P line displayed a difference in ethanol intake (g/kg/day), with female rats consuming more ethanol than their male counterparts (Fig. 1). However, female rats from all three lines consumed more water (ml/kg/day) and total fluid (ml/kg/day) than their respective male counterparts (Fig. 2 and Fig 3).

The present finding that HAD-1 and HAD-2 rats displayed a robust ADE during the 1st re-exposure period (Fig. 1) parallels a recent report (Rodd et al., 2008) indicating these rat lines display a robust ADE upon 1st re-exposure when given concurrent access to multiple concentrations of ethanol and an extended initial deprivation interval (2 weeks or longer). However, this finding differs from studies where HAD-1 and HAD-2 rats were given access to a single concentration of ethanol irregardless of initial deprivation length (Oster et al., 2006; Rodd-Henricks et al., 2000a). This suggests that the concurrent availability of multiple concentrations of ethanol allows HAD rats to consume sufficient amounts of the higher ethanol concentrations to display an ADE after both short and long deprivation intervals.

In the present study, P rats did not display a 24-hr ADE (Fig. 1). Previously, Rodd-Henricks and colleagues (2001) showed that female P rats demonstrate a robust 24-hr ADE after an extended deprivation period (2 or more weeks) when given concurrent access to multiple concentrations of ethanol, and the ADE increased in magnitude and duration across repeated re-exposure periods. The observation that a 24-hr ADE was not displayed, in the present study, when P rats experienced repeated deprivations suggests that the duration of ethanol exposure and/or the duration of deprivation periods may influence the expression of an ADE in P rats. It may be that with shorter durations of alcohol exposure and abstinence neuronal and/or behavioral adaptations associated with the 24-hr ADE phenomenon may not have fully developed in P rats of the present study. However, P rats did display a significant ADE in the 1st hr of the 4th ethanol re-exposure (Table 1), indicating that the repeated cycles of ethanol deprivation and re-exposure were producing neuronal and/or behavioral adaptations. The lack of a 24-hr ADE may have also been due to the overall higher intakes of ethanol displayed by P rats under baseline conditions, compared with the HAD rats. Therefore, even though there were instances where female P rats displayed average ethanol intakes, within each re-exposure period, that were as high, if not higher, as those displayed by HAD-1 and HAD-2 rats, the difference between these values and baseline, or for that matter continuous access, levels did not reach statistical significance.

Comparison of ethanol intakes between the continuous access and the repeatedly deprived HAD-1 groups (Fig. 1) indicates that the ethanol intake of the continuous HAD-1 group gradually increased to levels just below that observed for the repeatedly deprived group. This suggests that repeated deprivations may have increased the magnitude and/or rate that tolerance to the more aversive effects of ethanol developed. This development of tolerance is thought to contribute to observed increases in ethanol intake of chronically drinking animals over time (e.g., Stewart et al., 1991). However, data from the HAD-2 rats did not support this interpretation, because, even though the continuous access group showed a modest increase in ethanol intake across blocks, it appears the deprived rats displayed a greater reduction in ethanol intake across blocks. Alternatively, the continuous access HAD-1 rats may have reached a threshold for experiencing ethanol's rewarding effects, and may have subsequently increased their intake across blocks to obtain this effect. Regarding the P rats, there were no significant differences observed between the continuous access and repeatedly deprived rats across blocks. Nevertheless, the increases in ethanol drinking displayed by the continuous access HAD-1 rats across 4-day blocks, and the lack of differences between the repeatedly deprived and continuous access rats across all three lines during the 4th re-exposure period may have been due, at least in part, to different patterns of ethanol intake between rats from the two ethanol

conditions, although this hypothesis remains to be tested under 24-hr access conditions. Some support for this hypothesis comes from the 1-hr ethanol intake and BACs achieved data depicted in Table 1. These observations of line differences in the expression of an ADE and fluid intake are consistent with other studies indicating that ethanol drinking behavior differs in various aspects across these lines of rats (e.g., Bell et al., 2003,2004b,2006;Files et al., 1998;McKinzie et al., 1998a;Rodd et al., 2003,2008;Oster et al., 2006;Rodd-Henricks et al., 2000a,2000b,2001;Samson et al., 1998).

During the 1st hr of the 4th re-exposure, HAD rats are consuming approximately 2 g/kg and attaining pharmacologically relevant BACs of 65 to 98 mg%, which are 2-fold higher than BACs observed during this same period (1st hr of the dark cycle) in continuous access rats (Table 1). The differences in BACs obtained between the HAD-1 and HAD-2 rats are likely due to differences in absorption (e.g., amount of food in stomach will influence absorption) and/or differences in the drinking pattern. In general, 4-day cycles of ethanol abstinence and access result in a robust ADE during the 1st hour of the 4th re-exposure cycle in all 3 selectively bred lines. Also, the BACs attained during ethanol re-exposure, using this protocol, are significantly higher than those observed during continuous access and are more likely to induce pharmacological effects in the central nervous system. However, the BACs reached with the short 4-day cycle protocol (Table 1) do not reach the levels attained by P (~160 mg%: Rodd-Henricks et al., 2001) or HAD (~150 mg%: Rodd et al., 2008) rats using the 2-week cycle protocol, again, suggesting that the use of repeated, longer ethanol drinking and/or deprivation periods enhances a 24-hour ADE in these lines of rats.

The pattern of water intake (ml/kg/day) across days for the repeatedly deprived groups was different for each line of rat (Fig. 2). P rats in the repeatedly deprived group demonstrated markedly higher water consumption during each of the 4 ethanol deprivation periods (Fig. 2). The higher water intakes may be due in part to compensate for the absence of ethanol solutions. However, with the possible exception of male HAD-2 rats, similar robust increases in water intake were not observed for the HAD lines of rats (Fig. 2). This suggests that other factors may be playing a role in altered water intake of the P rats, which is not evident in male and female HAD-1 rats or in female HAD-2 rats. When total fluid intake (ml/kg/day) was examined, the opposite effect was observed, such that, for the most part, deprived female P, and to some extent male P and HAD-2, rats displayed modest changes in fluid intake across 4-day blocks (Fig. 3). Contrarily, deprived male HAD-1 and female HAD-1 and HAD-2 rats displayed marked changes in total fluid intake across 4-day blocks (Fig. 3). Taken together, these results suggest that male HAD-1 and female HAD-1 and HAD-2 rats consumed ethanol over-and-above their water/fluid requirements, whereas fluid balance may have played a role in the ethanol intake of female P and male P and HAD-2 rats. With 2-week ethanol deprivation and re-exposure cycles (Rodd-Henricks et al., 2001), no significant increases in water intake during the ethanol deprivation intervals have been reported. Therefore, it is possible that P, and to some degree male HAD-2, rats need longer ethanol exposure and deprivation periods to stabilize their fluid balance between ethanol and water intakes.

In conclusion, the results of the present study indicate that 4-day cycles of ethanol deprivation and re-exposure can produce an ADE in these three selectively bred lines of rats, with a more robust effect observed in HAD compared to P rats. Moreover, BACs that are pharmacologically relevant and significantly higher than those attained by non-deprived rats were observed. Therefore, the use of these lines of rats and this protocol of short ethanol deprivation and reexposure periods can provide researchers an experimental model that involves the attainment of pharmacologically relevant BACs over a relatively short period of time in animals that have freechoice access to ethanol.

Acknowledgments

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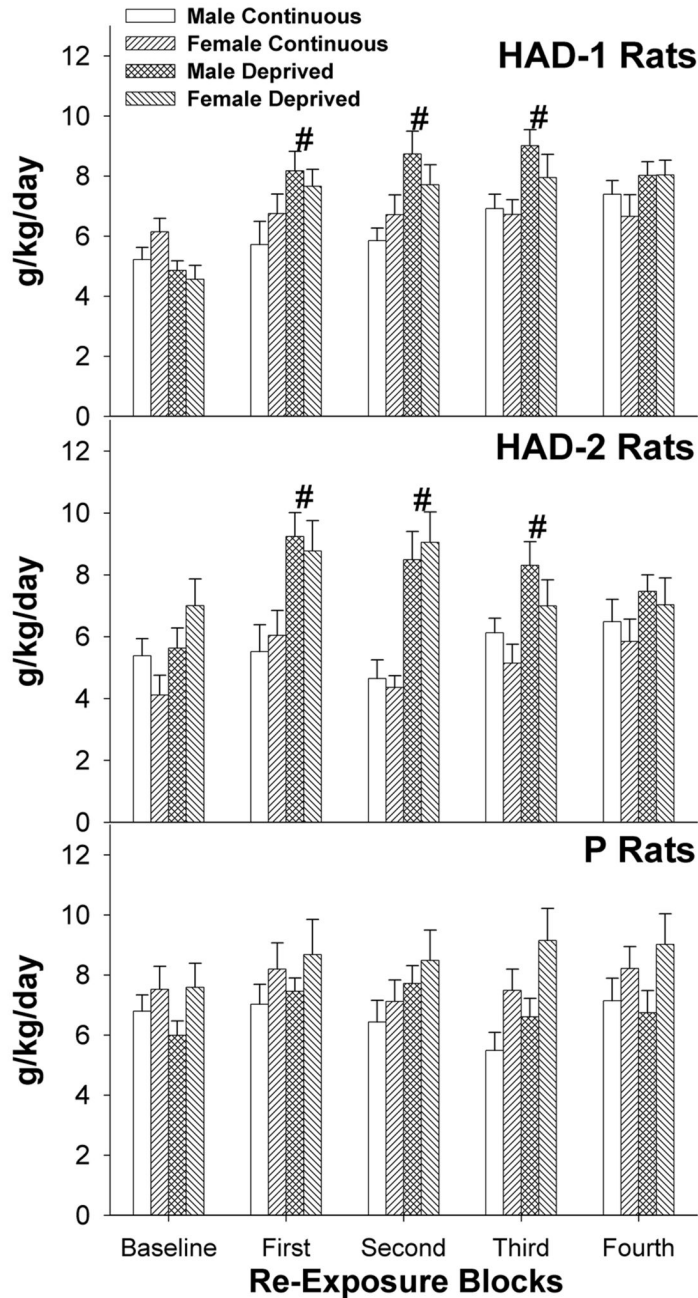


Fig. 1. Effects of line [high-alcohol-drinking (HAD)-1 ($n = 18$, top panel) vs. HAD-2 ($n = 20$, middle panel) vs. P ($n = 19$, bottom panel)], sex-of-animal [male ($n = 28$) vs. female ($n = 29$)] and ethanol condition [continuous access ($n = 61$) vs. repeatedly deprived ($n = 57$)] on the expression of an alcohol deprivation effect [(ADE), ethanol intake as g/kg/day, mean (\pm S.E.M.)]. Baseline refers to the average of the last 4 days before the 1st deprivation. #, indicates the presence of an ADE [significant ($P < 0.025$) increase in ethanol intake] after collapsing across sex of the respective line during the respective re-exposure block. Note: ethanol intake during the first 4-day block did not differ from that displayed during the baseline block for any of the lines. Similarly, ethanol intake during the last 4-day block did not differ from that

displayed during the fourth re-exposure block for any of the lines. Overall, the findings indicate that, whereas HAD rats displayed a robust 24-hr ADE, P rats did not when using the present shortened protocol.

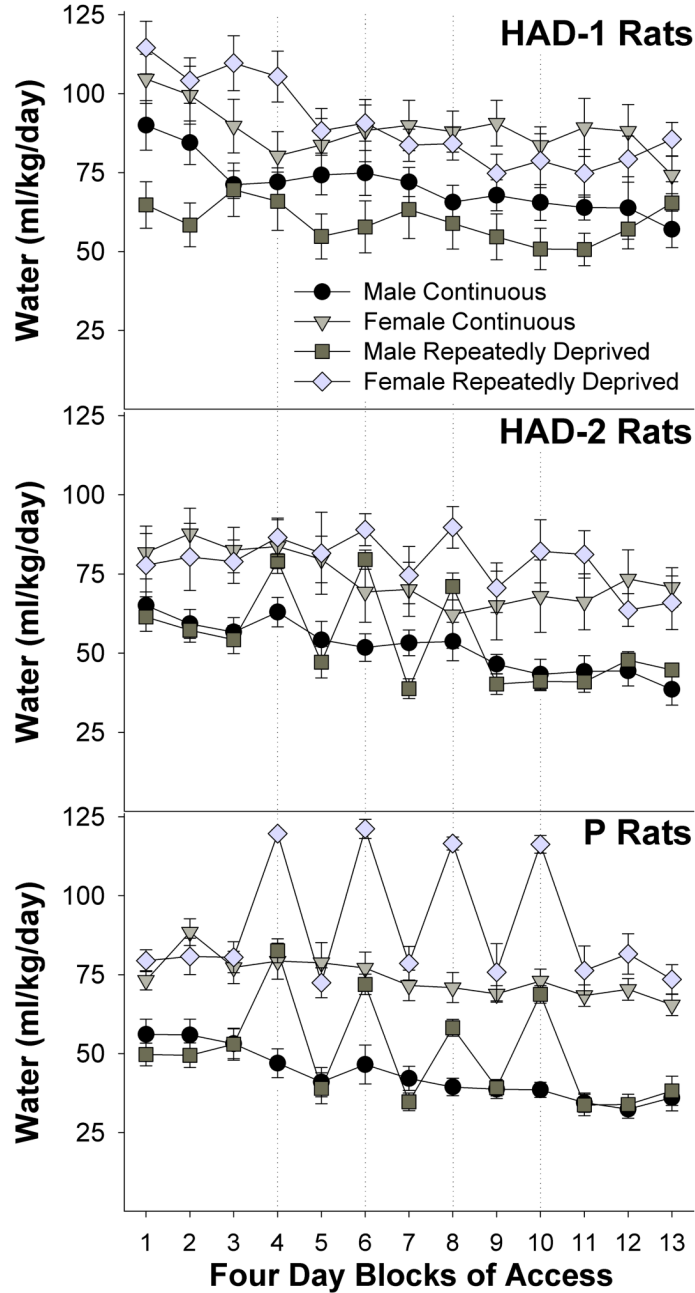


Fig. 2. Effects of line [high-alcohol-drinking (HAD)-1 ($n = 39$, top panel) vs. HAD-2 ($n = 40$, middle panel) vs. P ($n = 39$, bottom panel)], sex-of-animal [male ($n = 58$ vs. female ($n = 60$)] and ethanol condition [continuous access ($n = 61$) vs. repeatedly deprived ($n = 57$)] on water intake [ml/kg/day, mean (\pm S.E.M.)] averaged across 4-day blocks. For ease of presentation, symbols are absent. Overall, HAD-1 rats drank more water than P rats, which, in turn, drank more water than HAD-2 rats. Female rats drank more water than male rats, and, overall, repeatedly deprived animals drank more water than continuous access animals. Note that, in general, both male and female deprived P, and to a lesser extent male deprived HAD-2, rats displayed pronounced 31 increases in water intake during the ethanol deprivation cycles, whereas male and female

deprived HAD-1 and female deprived HAD-2 rats did not display significant increases in water intake during the ethanol deprivation cycles. The vertical dotted lines indicate the 4-day blocks during which ethanol was withheld from the repeatedly deprived rats.

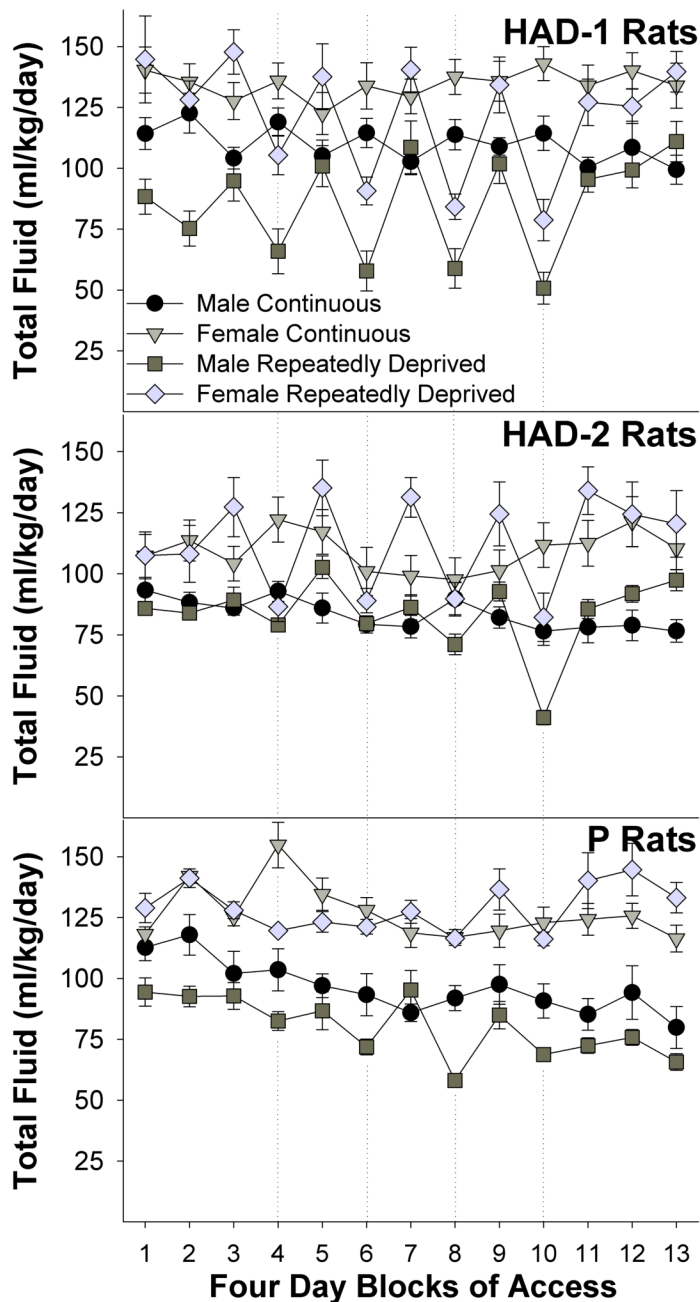


Fig. 3. Effects of line [high-alcohol-drinking (HAD)-1 ($n = 39$, top panel) vs. HAD-2 ($n = 40$, middle panel) vs. P ($n = 39$, bottom panel)], sex-of-animal [male ($n = 58$ vs. female ($n = 60$)] and ethanol condition [continuous access ($n = 61$) vs. repeatedly deprived access ($n = 57$)] on total fluid intake [ml/kg/day, mean (\pm S.E.M.)] averaged across 4-day blocks. For ease of presentation, symbols are absent. Overall, HAD-1 rats consumed slightly more fluids than P rats, which, in turn, drank more fluids than HAD-2 rats. Female rats drank more fluids than male rats, and, in general, repeatedly deprived animals drank more fluids than continuous access animals. The findings for the total fluid intake were, for the most part, opposite to those depicted for water intake in Fig. 2, such that male and female deprived P and, to some degree,

male deprived HAD-2 rats displayed modest, if any, changes in total fluid intake across the 4-day blocks; whereas male and female deprived HAD-1 and female deprived HAD-2 rats displayed dramatic decreases in total fluid intake during the ethanol deprivation intervals. The vertical dotted lines indicate the 4-day blocks during which ethanol was withheld from the repeatedly deprived rats.

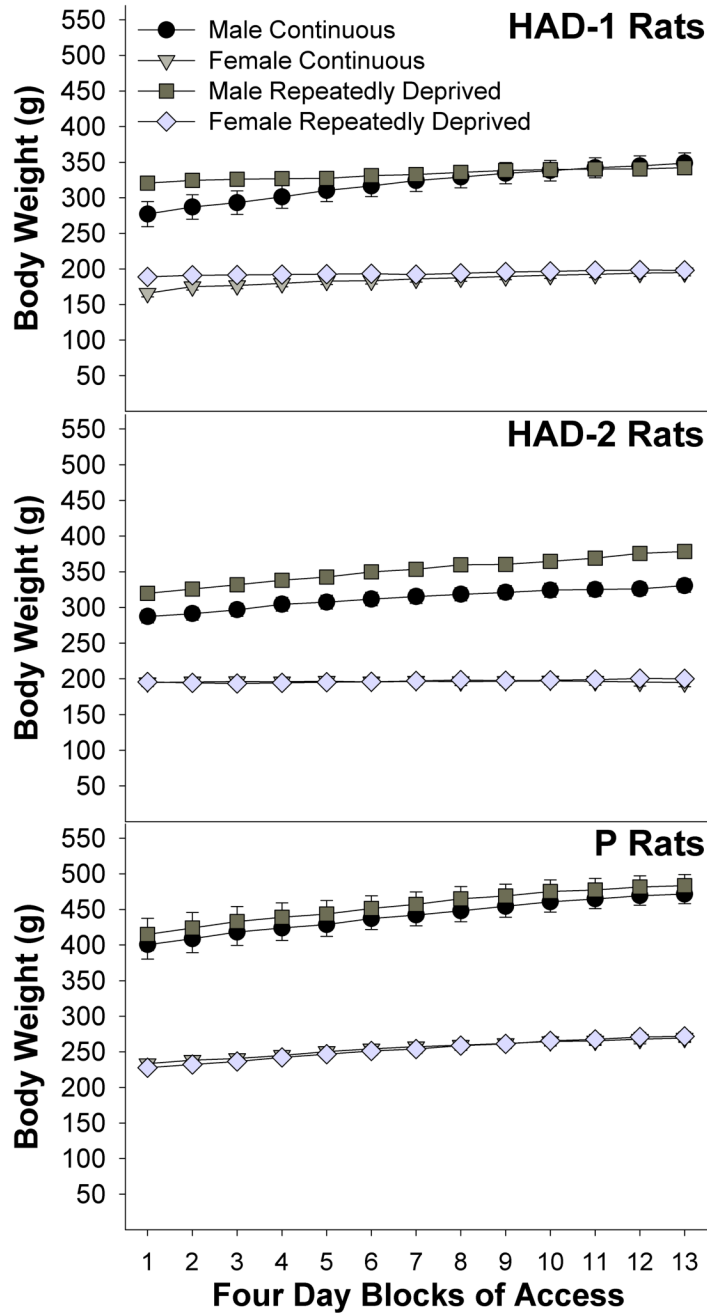


Fig. 4. Effects of line [high-alcohol-drinking (HAD)-1 ($n = 39$, top panel) vs. HAD-2 ($n = 40$, middle panel) vs. P ($n = 39$, bottom panel)], sex-of-animal [male ($n = 58$ vs. female ($n = 60$)] and ethanol condition [continuous access ($n = 61$) vs. periodic access ($n = 57$)] on body weight [g, mean (\pm S.E.M.)] averaged across 4-day blocks. For ease of presentation, symbols are absent. In general, P rats weighed more than HAD rats, male rats weighed more than female rats, and, except for differences between the repeatedly deprived and continuous access male HAD rats, there were no differences between ethanol condition. Because of the differences noted, all individual intake measures (daily ethanol, water, and total fluids) were corrected for the animals' respective body weight.

Association between amount of ethanol consumed and BACs achieved during the 1st hr of the 4th re-exposure cycle. Data obtained from a separate squad of rats.

Table 1

Line	Sex	Condition	Sample Size	Intake	BAC	Correlation	Effect Size
HAD-1	Male	Continuous	8	0.9 ± 0.2 [*]	50.9 ± 8.9 [*]	0.94 ⁺	0.86
HAD-1	Female	Continuous	7	1.0 ± 0.3 ^{**}	43.4 ± 12.2 ^{**}	0.65	0.31
HAD-1	Male	Periodic	9	1.9 ± 0.2	98.5 ± 10.7	0.98 ⁺	0.94
HAD-1	Female	Periodic	8	2.0 ± 0.2	95.0 ± 12.6	0.98 ⁺	0.95
HAD-2	Male	Continuous	7	1.5 ± 0.1 [*]	30.3 ± 10.7 [*]	0.12	0.00
HAD-2	Female	Continuous	7	1.9 ± 0.1	34.0 ± 7.5 ^{**}	0.62	0.26
HAD-2	Male	Periodic	8	2.4 ± 0.2	82.2 ± 9.0	0.97 ⁺	0.93
HAD-2	Female	Periodic	7	2.2 ± 0.3	63.9 ± 10.6	0.98 ⁺	0.95
P	Male	Continuous	7	0.3 ± 0.1 [*]	13.7 ± 3.4 [*]	0.02	0.00
P	Female	Continuous	7	0.5 ± 0.1 ^{**}	26.5 ± 14.1	0.58	0.20
P	Male	Periodic	8	1.0 ± 0.1	66.6 ± 15.4	0.76 ⁺	0.51
P	Female	Periodic	8	1.3 ± 0.2	76.3 ± 21.5	0.90 ⁺	0.77

Ethanol intake in g/kg/hr (mean ± S.E.M.); BAC in mg% (mean ± S.E.M.); Correlation as “r”; Effect size as Adjusted R².

* significant ($P < .05$) difference between male continuous and male periodic access rats for the respective rat line.

** significant ($P < .05$) difference between female continuous and female periodic access rats for the respective rat line.

+ significant ($P < .05$) correlation between amount of ethanol consumed and BAC achieved.