ELSEVIER

Alcohol 38 (2006) 155–164

ALCOHOL

Effects of multiple alcohol deprivations on operant ethanol self-administration by high-alcohol-drinking replicate rat lines

Scott M. Oster[a], Jamie E. Toalston[a], Kelly A. Kuc[b], Tylene J. Pommer[b], James M. Murphy[a,b], Lawrence Lumeng[c], Richard L. Bell[b], William J. McBride[b,d], Zachary A. Rodd[b,*]

[a]*Department of Psychology, Purdue School of Science, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46202, USA*
[b]*Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202-4887, USA*
[c]*Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[d]*Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Received 8 March 2006; received in revised form 1 June 2006; accepted 1 June 2006

Abstract

Previously, we reported that the expression of an alcohol deprivation effect (ADE) under 24-h free-choice alcohol-drinking access in high-alcohol-drinking (HAD) replicate lines of rats is dependent upon repeated cycles of alcohol access and forced abstinence. In the present study, operant techniques (including progressive ratio measures) were used to examine the effects of initial deprivation length and number of deprivation cycles on the magnitude and duration of the ADE in HAD rats to test the hypothesis that repeated deprivations increase the reinforcing effects of ethanol. Adult male HAD-1 and HAD-2 rats were trained in two-lever operant chambers to concurrently self-administer 15% ethanol (v/v) on a fixed-ratio (FR)-5 schedule and water on an FR-1 schedule of reinforcement in daily 1-h sessions. Following 10 weeks of daily 1-h sessions, the HAD-1 and HAD-2 rats were randomly assigned to one of four groups ($n = 6–8$/group/line): nondeprived, or deprived of alcohol for 2, 5, or 8 weeks. Following this initial period, the deprived groups were given 15% ethanol again in the operant chambers for a 2-week period, following which they were deprived again for 2 weeks (all three deprived groups). Following the fifth deprivation, the rats underwent a progressive ratio test to determine the breakpoints for the nondeprived and deprived groups. The expression of an ADE under operant conditions in HAD rats was dependent upon exposure to repeated cycles of ethanol access and abstinence. Additionally, repeated deprivations increased both the magnitude and the duration of the ADE as indicated by increased responding on the ethanol lever for more sessions. Breakpoint values for the deprived groups were 1.5-fold and twofold higher than the value for the nondeprived group for the HAD-1 and HAD-2 rats, respectively. The results suggest that repeated alcohol deprivations increased the expression of an ADE and the reinforcing effects of ethanol in both HAD replicate lines of rats, and these effects were more pronounced in the HAD-2 line than the HAD-1 line.

Keywords: Alcohol deprivation effect; Operant self-administration; High-alcohol-drinking rats; Repeated deprivations; Progressive ratio

1. Introduction

The alcohol deprivation effect (ADE) is defined as a temporary increase in the voluntary intake of ethanol solutions and the ratio of ethanol to total fluid intake over baseline drinking conditions, when ethanol is reinstated following a period of alcohol deprivation (Sinclair & Senter, 1967, 1968). The ADE has been hypothesized to be an animal model of alcohol craving (Heyser et al., 1997; Sinclair & Li, 1989) and has been used to examine the efficacy of pharmacological agents to prevent relapse drinking (Heyser et al., 1998; Kornet et al., 1991; Spanagel & Zieglgansberger, 1997).

The ADE phenomenon has been studied in several lines of rats. Alcohol-preferring (P) rats given continuous access to free-choice ethanol for approximately 1 month demonstrated an ADE after intervals of 12 h or 1 week (Sinclair & Li, 1989). In addition, with daily 4-h operant scheduled access sessions, P rats exhibited an increase in responding for ethanol compared to baseline after 2 weeks of alcohol deprivation (McKinzie et al., 1998). In contrast, other rat lines that were selectively bred for high alcohol consumption did not exhibit an ADE after the initial deprivation following 24-h free-choice drinking conditions. The Alko

This study was supported in part by NIAAA grants AA07611, AA10721, and AA11261.

* Corresponding author. Tel.: +1-317-278-3003; fax: +1-317-274-1365.
E-mail address: zrodd@iupui.edu (Z.A. Rodd).

0741-8329/06/$ – see front matter
doi: 10.1016/j.alcohol.2006.06.001

Alcohol accepting (AA) line of rats does not show an ADE with deprivation periods that exceed 5 days (Sinclair & Li, 1989; Sinclair & Tiihonen, 1988). The Sardinian alcohol-preferring rat did not display an ADE during the initial 24-h period of ethanol reexposure after being alcohol deprived for periods between 3 and 30 days (Agabio et al., 2000). In addition, both replicate lines of the high-alcohol-drinking (HAD) rats did not exhibit an ADE after a 2-week deprivation period that followed 6 weeks of continuous free-choice alcohol drinking (Rodd-Henricks et al., 2000a). Furthermore, a direct comparative study examining the expression of an ADE in AA, P, HAD, and Wistar rats indicated that a period of forced abstinence only increased ethanol intake in P and Wistar rats (Vengeliene et al., 2003).

Although most animal studies examining the ADE have used a single deprivation period, research has shown that the drinking patterns of human alcoholics are segmented by multiple periods of abstinence and intake (Burish et al., 1981; Hilbrom, 1990; McMillen, 1997). Therefore, the effects of multiple deprivations on expression of an ADE were examined in P and HAD rats under 24-h free-choice alcohol-drinking conditions. We reported that expression of an ADE in the selectively bred P and HAD lines of rats is modified by exposure to repeated cycles of alcohol availability and deprivation (Rodd-Henricks et al., 2000a, 2000b, 2001). In P rats given 24-h free choice between 10% (v/v) ethanol and water, repeated deprivations prolonged the expression of the ADE, but did not alter its magnitude (Rodd-Henricks et al., 2000b). In the HAD replicate lines, the expression of an ADE was dependent upon repeated deprivations, and in the HAD-2 line, the ADE was also prolonged with repeated deprivations (Rodd-Henricks et al., 2000a). The increase in duration of the ADE in the P rat with repeated deprivations and the expression of an ADE in HAD rats only after repeated deprivations suggested that alterations in the reinforcing properties of ethanol may be taking place with repeated deprivation cycles. In addition, three low alcohol-consuming lines demonstrated a modest (nonpreferring [NP], LAD-2) to significant (LAD-1) ADE after repeated cycles of ethanol drinking and deprivation, suggesting that selective breeding for low alcohol consumption is not associated with the inability to display an ADE (Bell et al., 2004). One approach toward examining the effects of repeated deprivations on the reinforcing properties of ethanol is the use of operant techniques. Alterations in the amount of work a subject will do to obtain a reinforcement can be inferred to indicate changes in the intrinsic reward of the reinforcer (Hodos, 1961). Previously, we showed that repeated alcohol deprivations increased the number of responses and the duration of elevated responding across sessions on the ethanol lever by P rats, and that P rats repeatedly deprived of ethanol had higher breakpoint ratios than P rats given uninterrupted access to ethanol (Rodd et al., 2003).

Similar to P rats, the HAD-1 and HAD-2 replicate lines of rats have been selectively bred on the basis of their preference for a 10% (v/v) ethanol solution with water and food concurrently available (Li et al., 1993). Although the replicate lines of HAD rats are not as well characterized as the P line, HAD rats voluntarily consume similar amounts of ethanol as the P line during adolescence (McKinzie et al., 1996) and adulthood (Li et al., 1993), and will emit an operant response for oral ethanol self-administration (Ritz et al., 1994; Samson et al., 1998). Additionally, both P and HAD rats display an ethanol-induced enhancement of locomotor activity, which is not observed in their companion low alcohol-preferring selected lines (Rodd et al., 2003).

Enhanced operant responding for ethanol after multiple deprivations and reinstatements has been shown in P rats (Rodd et al., 2003), but has yet to be examined in the HAD lines. The hypothesis tested in the present study was that repeated cycles of ethanol drinking and long-term deprivation would increase the reinforcing effects of ethanol, as indicated by a marked increase in responding for ethanol, and hence greater ethanol consumption. Additionally, following the last deprivation period, the reinforcing properties of ethanol will be assessed quantitatively through the use of a progressive ratio paradigm (Roberts et al., 1989) to test the hypothesis that the reinforcing properties of ethanol were enhanced following repeated cycles of alcohol deprivation.

2. Methods

2.1. Animals

Adult male HAD-1 rats ($n = 30$) from the 25th–26th generation and HAD-2 rats ($n = 29$) from the 23rd–24th generation, weighing 250–325 g at the start of the experiment, were used. Rats were maintained on a 12-h reversed light–dark cycle (lights off at 0900 h). Food and water were available ad libitum throughout the experiment, except during operant testing. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional 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, 1996).

2.2. Operant apparatus

Ethanol self-administration was conducted in standard two-lever experimental chambers (Coulbourn Instruments) contained within ventilated, sound-attenuated enclosures. Two operant levers were located on the same wall and were placed 15 cm above a grid floor and 13 cm apart. Directly beneath each lever was a trough through which a dipper cup

(0.1 ml) was raised to deliver response-contingent fluid. Upon a reinforced response, a small light cue was illuminated in the drinking trough during the 4-s dipper cup access. A personal computer controlled all operant chamber functions, and recorded lever responses and dipper presentations.

2.3. Operant training and repeated cycles of deprivation and ethanol access

Initially, HAD-1 and HAD-2 rats were given continuous access to 15% (v/v) ethanol as their sole fluid for 2 days, followed by 24-h free-choice access to ethanol and water for 2 weeks. The rats were then given a daily 4-h schedule access to ethanol (with water always available) for another 3 weeks. Ethanol operant self-administration was autoshaped during the initial 4 weeks of operant access. Autoshaping consisted of the rats receiving one noncontingent reinforcer (alternating between ethanol and water) once every 2 min. Also, there were HAD-1 and HAD-2 rats that failed to acquire operant ethanol self-administration under the current paradigm (approximately 30% and 20%, respectively). These rats were removed from the experiment.

Water was always reinforced on a fixed-ratio (FR)-1 schedule, whereas ethanol was gradually increased to an FR-5 schedule. The response requirement on the ethanol lever was increased by 2 every other week (7th week, FR-3; 9th week, FR-5) for a total of 10 weeks concurrent responding for ethanol and water. Levers associated with ethanol or water were counterbalanced among rats but remained constant for each animal. The daily 1-h operant sessions provided the only access to ethanol for the remainder of the study.

Following the initial 10-week operant period, HAD-1 and HAD-2 rats were randomly assigned to one of four groups. Three groups of rats were initially deprived for 2, 5, or 8 weeks ($n = 6–8$/group/line). During the deprivation period, rats were maintained in their home cage and were not given access to the experimental chambers. All rats were then reexposed to the experimental chambers, and allowed to respond on a concurrent FR-5/FR-1 for ethanol versus water for 2 weeks. Following this reexposure period, all previously deprived rats, regardless of initial deprivation length, were deprived of ethanol and access to the operant chamber for 2 weeks. This cycle of 2-week deprivation/2-week ethanol access was continued for a total of five deprivation and reexposure periods. The fourth group (nondeprived; $n = 7–8$/line) received daily 1-h operant sessions for an additional 21 weeks (thus matching the 5-week deprivation group for the duration of ethanol access). The concurrent FR-5/FR-1 schedule for 15% ethanol versus water was maintained during these operant sessions.

2.4. Progressive ratio

In the three deprived groups, following the fifth deprivation period, rats were assessed in a modified progressive ratio test to determine the effects of repeated deprivations on the breakpoint value. The nondeprived rats were tested under the progressive ratio procedure after 31 weeks of continual ethanol operant exposure. The response requirement in the progressive ratio was as follows, as previously described (Rodd et al., 2003): all rats began at an FR-2 schedule of reinforcement on the ethanol lever, and after three reinforcements (six lever presses), the schedule was increased by 2 to FR-4; after receiving another three reinforcements (12 lever presses), the schedule was increased by 2 to FR-6. This pattern of increasing the response requirement by 2 after each three reinforcements was continued until the rat did not meet the FR requirement within 7 min. The lowest FR value that the rat could not attain was defined as the breakpoint. This progressive ratio schedule was selected in an attempt to balance the multiple factors involved in oral ethanol self-administration (i.e., slow delivery of the drug [0.1 ml/reinforcement], slow rate of absorption of ethanol, and the sedative effects of ethanol). Therefore, this progressive ratio paradigm was used to sufficiently increase the required workload before the sedative effects of ethanol began to interfere with operant performance. The water lever was maintained on an FR-1 throughout the progressive ratio paradigm.

2.5. Statistical analysis

Overall operant responding (60 min) data were analyzed with a mixed factorial analysis of variance (ANOVA) with a between-subject factor of group (initial deprivation length) and repeated measures of ‘‘session’’ and ‘‘cycle’’ when applicable. The baseline measure for the factor ‘‘session’’ was the average number of responses on the ethanol lever for the three sessions immediately prior to deprivation. Operant responding data were also analyzed in 10-min blocks, which required the additional repeated measure of time. The progressive ratio data were analyzed with an ANOVA performed on the dependent measure of breakpoint (FR value) and total responses on the ethanol lever with a between-subject factor of group. Post hoc Tukey's *b* comparisons were performed to determine individual differences.

3. Results

At the end of the 10-week period of operant access, there were no differences among any of the four groups in either line with regard to responses/session on either the ethanol lever (Figs. 1–3: HAD-1 $F_{3,26} = 0.7$; $P = .56$; HAD-2 $F_{3,25} = 1.1$; $P = .37$) or the water lever (water lever data for deprived groups not shown: HAD-1, range 10 ± 2 to 13 ± 3 responses/session; $P = .58$; HAD-2, range 11 ± 2 to 14 ± 4 responses/session; $P = .47$). For HAD-1 and HAD-2 rats in the nondeprived group, responding on the ethanol lever remained relatively constant throughout the course of the experiment (Fig. 1). Analysis of the weekly

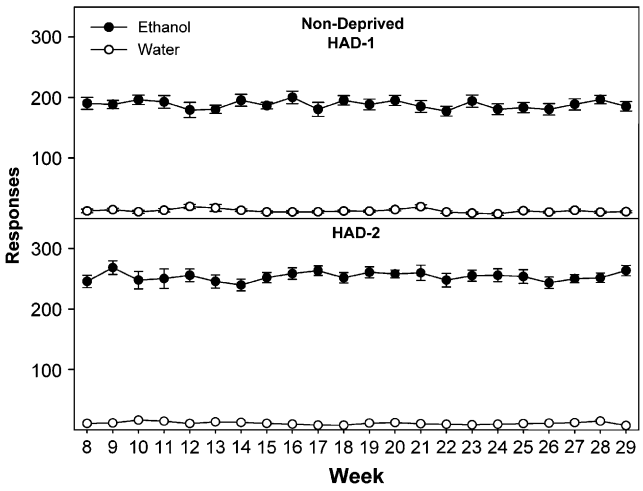

Fig. 1. The mean (±S.E.M.) responses/session on the ethanol levers for HAD-1 (top) and HAD-2 (bottom) rats ($n = 6–8$/line) in the nondeprived group given daily 60-min access to 15% ethanol and water for 29 consecutive weeks. Responses on both the ethanol and water levers did not change significantly across sessions. HAD, high alcohol drinking.

averages for responses/session on the ethanol or water lever indicated that responding on each lever was fairly stable across the final 21 weeks of the experiment for both HAD-1 and HAD-2 rats (P values $> .43$). In general, HAD-2 rats responded more than HAD-1 rats (approximately 250 vs. 200 responses/session), which resulted in more reinforcements/session for the HAD-2 rats (50 vs. 40 reinforcements). Statistically, examination of the baseline average for all HAD-1 and HAD-2 rats showed that the HAD-2 rats responded significantly more than HAD-1 rats ($F_{1,42} = 12.4$; $P < .0001$).

3.1. Responses/session on ethanol lever after a single deprivation

In HAD-1 rats, responding on the ethanol lever by the three deprived groups after the initial deprivation period (Fig. 2) was significantly reduced from baseline levels, with the reduction being most pronounced following 8 weeks of deprivation (session: $F_{5,15} = 5.0$; $P = .006$; session × group: $F_{10,32} = 5.3$; $P < .0001$). Examination of the number of responses during the first reexposure session revealed that there was a significant effect of group ($F_{2,19} = 3.5$; $P = .04$), and post hoc comparisons indicated that responding in the 2- and 5-week groups was significantly higher than that in the 8-week group. Additionally, t-test comparisons between baseline level of responding and responding during the initial reexposure session indicated that for all groups, responding was significantly lower (P values $< .016$).

In HAD-2 rats, responses on the ethanol lever by the three deprived groups after the initial deprivation period (Fig. 3) were not significantly changed in the 2- and 5-week groups but were reduced from baseline levels in the 8-week group (session: $F_{5,15} = 4.1$; $P = .016$; session × group:

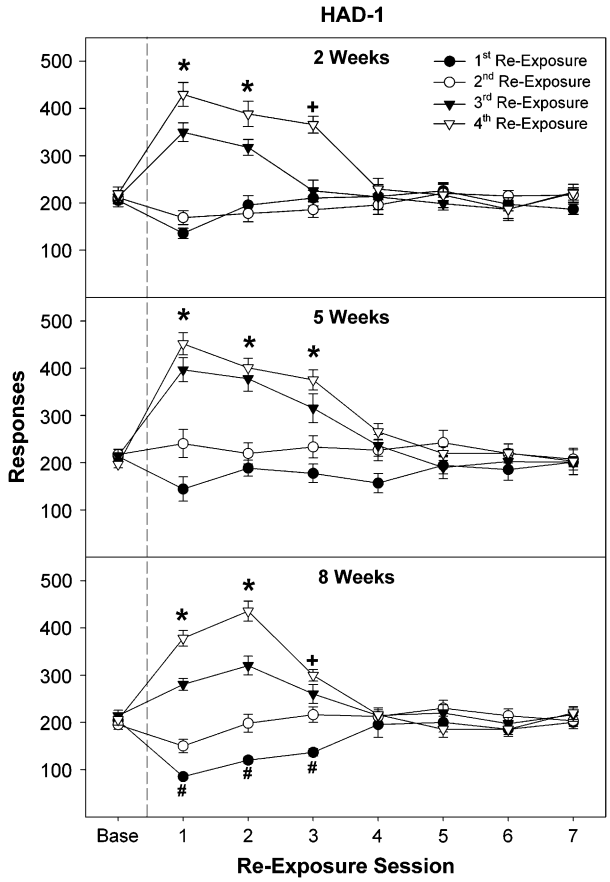

Fig. 2. The mean (±S.E.M.) responses/session on the ethanol lever during 60-min access sessions by HAD-1 rats initially deprived of ethanol for 2, 5, or 8 weeks ($n = 6–8$/group), and then subjected to three additional cycles of 2 weeks of ethanol drinking and 2 weeks of deprivation (second, third, and fourth reexposures). Baseline values are the average responses for each rat on the last three sessions prior to deprivation. *$P < .05$ (values for the third and fourth reexposures being higher than baseline); +$P < .05$ (values during the fourth reexposure being significantly higher than baseline); #$P < .05$ (values for the first reexposure being lower than baseline). HAD, high alcohol drinking.

$F_{10,32} = 5.2$; $P < .0001$). Examining the number of responses during the first reexposure session revealed there was no significant effect of group ($P = 0.47$). A priori t-test comparisons between baseline level of responding and responding during the initial reexposure session indicated that responding was reduced in reexposure sessions 1 and 2 for the 8-week group ($P = .04$).

3.2. Responses on the ethanol lever after repeated deprivations

In HAD-1 rats, responding on the ethanol lever for the three deprived groups returned to baseline levels by the end of each reexposure period (Fig. 2). Responding on the ethanol lever was not significantly altered following a second deprivation period (P values $> .36$). Following the third and fourth exposures to cycles of alcohol access and deprivation, responding on the ethanol lever increased

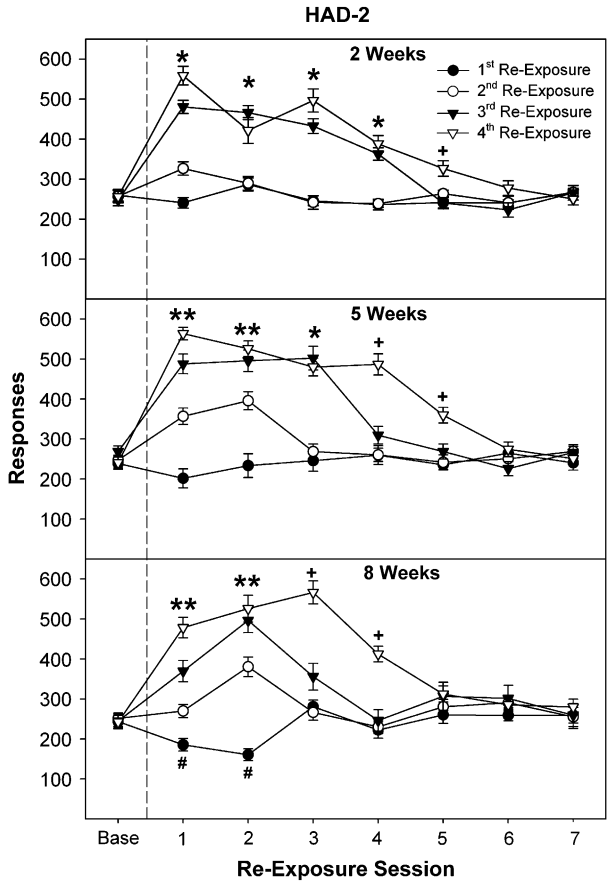

Fig. 3. The mean (±S.E.M.) responses/session on the ethanol lever during 60-min access sessions by HAD-2 rats initially deprived of ethanol for 2, 5, or 8 weeks ($n = 6–8$/group), and then subjected to three additional cycles of 2 weeks of ethanol drinking and 2 weeks of deprivation (second, third, and fourth reexposures). Baseline values are the average responses for each rat on the last three sessions prior to deprivation. $*P < .05$ (values for the third and fourth reexposures being higher than baseline); $+P < .05$ (values for the fourth reexposure being higher than baseline); $**P < .05$ (values for the second, third, and fourth reexposures are higher than baseline); $P < .05$ (values during the fourth reexposure being significantly higher than baseline); $\#P < .05$ (values during the first reexposure being lower than baseline). HAD, high alcohol drinking.

(cycle: $F_{3,17} = 66.6$; $P < .0001$; cycle × session × group: $F_{24,18} = 7.1$; $P < .0001$).

Following the third and fourth deprivation cycles, there was a significant effect of session (P values $< .0001$). During the first session following the third deprivation period, all groups responded higher than baseline (P values $< .02$), with post hoc comparisons indicating that rats initially deprived for 2 and 5 weeks responded more than rats initially deprived for 8 weeks. Following the third deprivation period, responding remained elevated during the second reexposure session in all groups, with no significant differences between the groups. However, during the third reexposure session following the third deprivation period, rats initially deprived for 2 and 5 weeks displayed higher ethanol responding compared to baseline levels ($P = .003$). Following the fourth deprivation period, all deprived groups showed elevated responding on the ethanol lever for at least four sessions (P values $< .02$).

In HAD-2 rats, responding on the ethanol lever was significantly increased following repeated cycles of ethanol abstinence and access (cycle: $F_{3,17} = 75.3$; $P < .0001$; cycle × session × group: $F_{24,18} = 3.5$; $P < .0001$). During the second reexposure cycle, HAD-2 rats initially deprived for 5 weeks increased responding on the ethanol lever during the first and second reexposure sessions compared to baseline ($P < .01$). Although HAD-2 rats, initially deprived for 2 or 8 weeks, did not display an increase in responding during the first session of the second reexposure ($P = .56$ and $P = .82$, respectively), rats initially deprived for 8 weeks did respond more on the ethanol lever during the second session of the second reexposure ($P < .01$).

Responses on the ethanol lever following the third and fourth reexposure cycles were increased compared to baseline and earlier cycles. In all deprived groups, responding on the ethanol lever during the first, second, and third sessions following the third and fourth reexposures was increased compared to baseline levels or levels observed following the first and second deprivation periods (all P values $< .05$). Additionally, the prolongation of the ADE was increased as the number of exposure cycles of ethanol access/abstinence was increased. For example, in HAD-2 rats initially deprived for 5 weeks, the increase in responding on the ethanol lever following the third deprivation cycle was evident for three consecutive sessions; ethanol lever responding was increased compared to baseline levels for five consecutive sessions following the fourth deprivation cycle. Similar patterns were observed for rats initially deprived for 2 or 8 weeks.

Water lever responding by both HAD-1 and HAD-2 rats was low and was not altered throughout the experiment (data not shown; HAD-1: 15 ± 4; HAD-2: 17 ± 4 responses/session). In fact, water lever responses tended to be lower during reexposure sessions, e.g., HAD-1 rat responses on the water lever during the first session of reexposure after the third and fourth deprivations were 13 ± 3 and 11 ± 2, respectively.

An analysis contrasting the effects of repeated alcohol deprivations between HAD-1 and HAD-2 indicates an overall separation between the two rat lines. The overall analysis revealed a significant line × session × cycle × group interaction ($F_{30,50} = 10.5$; $P < .00001$), an overall effect of line ($F_{1,38} = 22.6$; $P < .0001$), and other significant line factor interactions (P values $< .002$). Briefly, collapsing the interaction by holding group constant revealed that for each deprivation group there was a significant effect of line (cycle × line × group: $F_{15,30}$ values $= 6.4$; P values $< .0001$). For example, an analysis contrasting the effects of repeated deprivation cycles in HAD-1 and HAD-2 rats initially deprived for 2 weeks (Figs. 2 and 3, top panels) revealed a significant session × line interaction for the third and fourth reexposure periods ($F_{3,11}$ values $= 10.6$; P values $< .001$). Further decomposing the

interaction revealed that HAD-2 rats responded more during the third and fourth reexposure sessions following the third deprivation period than HAD-1 rats (P values $< .05$). During the fourth reexposure period, HAD-2 responded significantly more than HAD-1 rats during the first, third, fourth, and fifth reexposure periods (P values $< .05$). For brevity, similar findings were obtained during a thorough analysis contrasting HAD-1 and HAD-2 rats that were initially deprived for 5 (Figs. 2 and 3, middle panels) and 8 (Figs. 2 and 3, bottom panels) weeks.

3.3. Cumulative responses on the ethanol lever in session 1 after repeated deprivations

An examination of the cumulative responses on the ethanol lever revealed that, in both HAD-1 and HAD-2 rats, there were no significant changes in the response pattern during the last session prior to deprivation (all Ps $> .37$). Under baseline conditions, approximately 90% of the responses on the ethanol lever occurred within the first 20 min (Fig. 4 shows data for the 2-week groups for HAD-1 and HAD-2 rats). A comparison of the cumulative response pattern (mixed factor ANOVA) between the average of the three sessions immediately prior to deprivation and the three initial reexposure sessions for each group indicated a significant effect of cycle ($F_{3,17} = 96.2$; $P < .0001$), time ($F_{5,15} = 33.3$; $P < .0001$), time × group × cycle interaction ($F_{30,12} = 3.1$; $P = .021$). In HAD-1 rats, responding during the first reexposure session following the first and second deprivation periods indicated that rats reduced the amount of responding during the initial 20 min of the operant session; a similar trend was observed in HAD-2 rats (Fig. 4). With exposure to repeated cycles of ethanol access/abstinence, HAD-1 and HAD-2 rats increased responding during the initial 20 min of the operant session (the time when the vast majority of responses occurred). HAD-2 rats responded more than HAD-1 rats in general, and HAD-2 rats either increased or maintained ethanol lever responding during the second reexposure cycle compared to baseline (Fig. 4).

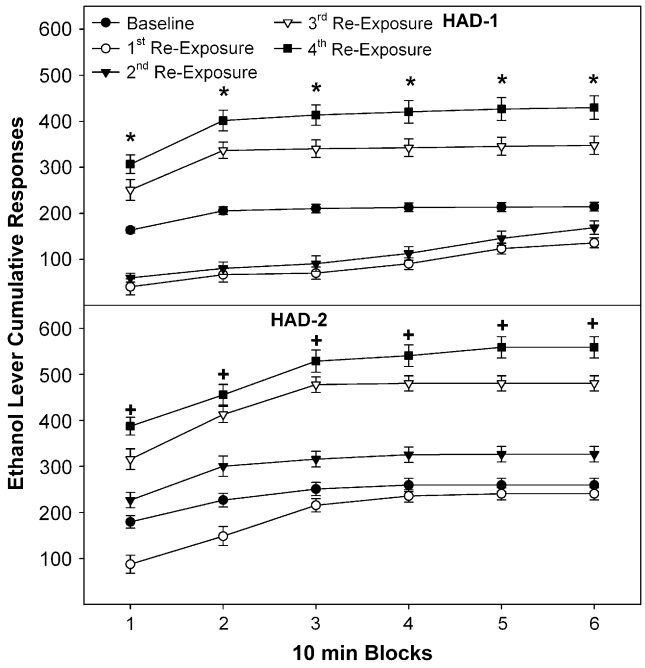

Fig. 4. The mean (±S.E.M.) cumulative responses (in 10-min blocks) on the ethanol lever in the first session after each reexposure for HAD-1 and HAD-2 rats initially deprived for 2 weeks ($n = 6–8$/group), and then subjected to four additional cycles of 2 weeks of ethanol access and 2 weeks of deprivation. Data for the 5- and 8-week groups are not shown but are similar to data for the 2-week group. Baseline values are the average responses for each rat on the last three sessions prior to the initial deprivation. *$P < .05$ (all reexposure cycles significantly different from each other, with the first and second reexposure period being significantly lower than beseline and the third and fourth reexposure period being significantly higher than beseline). +$P < .05$ (values for the second, third, and fourth reexposures being greater than baseline and values for the first reexposure, and values for the third and fourth reexposures being greater than values for the second reexposure). HAD, high alcohol drinking.

3.4. Progressive ratio

After the fifth deprivation, a progressive ratio session was undertaken to determine if repeated deprivation cycles altered breakpoint values (Fig. 5). In HAD-1 rats, during the last session prior to the fifth deprivation, or in the nondeprived animals, the last session prior to progressive ratio testing, the responses/session on the ethanol lever were between 206 ± 17 and 220 ± 16, whereas responses/session on the water lever were between 8 ± 5 and 14 ± 3 (HAD-2: ranged from 256 ± 13 to 267 ± 15 with little water responding). There was a significant effect of group for both the breakpoint value and the total number of responses on the ethanol lever during the progressive ratio session for HAD-1 rats ($F_{3,26}$ values > 12.2; P values $< .01$). Post hoc comparisons revealed that rats exposed to repeated cycles of ethanol access/deprivation had higher breakpoints (FR values 15–18 for deprived groups vs. 9 for the nondeprived group) and ethanol lever responses than the nondeprived group (200–300 responses for deprived groups vs. 100 responses for the nondeprived group).

In general, HAD-2 rats had higher breakpoint values than HAD-1 rats (Fig. 5). Nondeprived HAD-2 rats had a breakpoint for ethanol approximately 60% higher than nondeprived HAD-1 rats (15 vs. 9), and values for the repeatedly deprived HAD-2 groups were twofold higher than those for nondeprived HAD-2 rats. Exposure to repeated cycles of ethanol access/abstinence greatly increased the breakpoint for ethanol reinforcement (group $F_{3,25}$ values > 46.9; P values $< .0001$). Post hoc comparisons revealed that rats exposed to repeated cycles of ethanol access/deprivation had higher breakpoints (FR values 27–33 for deprived groups vs. 15 for the nondeprived group) and ethanol lever responses than the nondeprived group (590–800 responses for deprived groups vs. 200 responses for the nondeprived group).

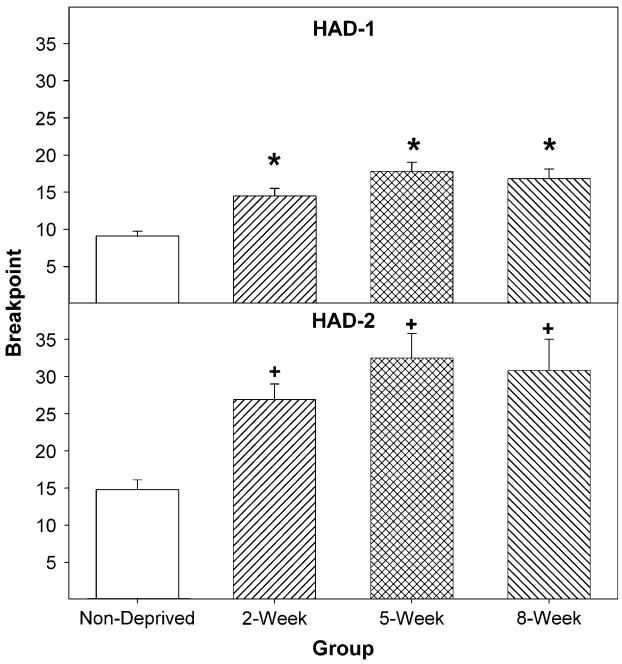

Fig. 5. The mean (±S.E.M.) breakpoint values on the ethanol lever during the progressive ratio session after the fifth deprivation by HAD-1 (top panel) and HAD-2 (bottom panel) rats in the nondeprived group and in the three deprived groups (2-, 5-, and 8-week initial deprivation). The breakpoint is the lowest fixed-ratio requirement that the HAD rat did not meet. *$P < .05$ (values for all three deprived groups being greater than values for the nondeprived group); +$P < .05$ (all breakpoint values for HAD-2 rats were significantly higher than values for HAD-1 rats and values for the nondeprived group). HAD, high alcohol drinking.

4. Discussion

The major findings of the present study are that three or more cycles of alcohol deprivation increased the magnitude and the duration of responding on the ethanol lever by HAD-1 and HAD-2 rats (Figs. 2 and 3), and that both replicate lines of rats repeatedly deprived of ethanol had higher breakpoint ratios than rats given continuous access to ethanol (Fig. 5). These results support our hypothesis that multiple deprivations can increase the reinforcing effects of ethanol in the HAD lines of rats.

The progressive ratio test is a quantitative measure of the amount of work a subject will perform to obtain a reinforcer (Hodos, 1961). The strength, or saliency, of a reinforcer is inferred from the amount of work a subject will perform until a point (breakpoint) where the work effort is not matched by the intrinsic reward of the reinforcer and the animal terminates responding (Hodos, 1961). Therefore, the 1.5- (HAD-1) and twofold (HAD-2) higher breakpoint values for the deprived groups compared to the nondeprived group (Fig. 5) suggest that the reinforcing strength of ethanol has increased with repeated deprivations. This effect is not merely a result of ethanol exposure alone because the nondeprived and the 5-week group had the same number of operant sessions and exposure to ethanol. However, the 5-week deprived group had 2-week periods of abstinence followed by several sessions of twofold higher ethanol intakes (Figs. 2 and 3). The combination of prolonged deprivation followed by sessions of high alcohol intake could be producing neuronal alterations that strengthen the reinforcing effects of ethanol. Additionally, the validity of the modified progressive ratio used is supported in that both nondeprived and repeatedly deprived rats received about half the number of reinforcers typically earned during a regular FR operant session. However, interpretation of the current progressive ratio data must be tempered by the fact that only a single concentration of ethanol was used during progressive ratio testing. To completely assess the reinforcing properties of ethanol in nondeprived and repeatedly deprived rats, an ethanol dose–response experiment would need to be conducted (Hodos, 1961; Roberts et al., 1989).

In contrast to the amount of research using progressive ratio paradigms on other drugs of abuse, e.g., cocaine, amphetamine, and morphine (Covington & Miczek, 2001; Roberts et al., 1989), there has been limited use of this technique in the study of alcohol. One of the reasons is that increasing ethanol intakes can produce sedation, whereas the other drugs of abuse are generally stimulating under self-administration conditions. A second reason is that nonselected rats will not demonstrate robust operant responding for ethanol. The HAD replicate lines of rats have been selectively bred for HAD behavior and will self-administer ethanol under operant conditions. In Lister rats, which had repeated training with progressive ratio testing, withdrawal from an ethanol diet (11 g/kg/day) increased the breakpoint determinant (Brown et al., 1998). P rats maintained on an FR-1 schedule of reinforcement for 10% v/v ethanol, and trained to lever press using the saccharin-fade procedure, displayed a higher breakpoint determinant than comparably trained alcohol NP rats (Ciccocioppo et al., 2001), although the number of reinforcements/session obtained by both the P and the NP rats was relatively low (approximately 18 and 8, respectively). With the current procedure, the HAD-1 and HAD-2 rats received a higher number of reinforcements at baseline (HAD-1: 38 ± 2 reinforcements/session; HAD-2: 49 ± 2 reinforcements/session) and repeated deprivations significantly increased the number of reinforcements (HAD-1: 90 ± 4 reinforcements/session; HAD-2: 113 ± 6 reinforcements/session) in the first session of the fourth reexposure.

The presence of an ADE under operant conditions has been previously reported. In nonselected Wistar rats, a modest increase (60 responses/session; FR-1 schedule of reinforcement) in responding was observed following a single deprivation period of between 5 and 28 days (Heyser et al., 1997). Additionally, Heyser et al. (1998) reported that chronic acamprosate treatment (200 mg/kg/day for 5 days) inhibited responding on the ethanol lever during ADE testing. Furthermore, an ADE-like phenomenon was observed in ethanol vapor–exposed rats that were abstinent for 2

or 4 weeks, and repeatedly withdrawn (Roberts et al., 2000). An operant ADE in P rats was observed following a single deprivation of 2 weeks (McKinzie et al., 1998; Rodd-Henricks et al., 2002a, 2002b).

The difference in expression of an ADE between P rats (ADE shown after the first deprivation in 2- and 5-week groups; Rodd et al., 2003) and the HAD lines (robustly expressed after third and fourth deprivation in 2- and 5-week groups; Figs. 2 and 3) may be due to differences in neuronal alterations that occurred as a result of chronic ethanol self-administration followed by a prolonged deprivation period. Under baseline conditions, HAD-1 rats consume approximately 1.2 g/kg/session and HAD-2 rats consume around 1.6 g/kg/session. These amounts are equivalent to baseline ethanol intakes of P rats (1.3 g/kg/session) previously reported using the same operant paradigm (Rodd et al., 2003), suggesting that baseline differences alone cannot account for the differences in expression of an ADE after the first deprivation. One possibility to explain the difference between the P and HAD rats in the expression of an ADE after the first deprivation is that, in the P rat, the neuronal alterations associated with the expression of an ADE are more readily established, whereas in the HAD lines, it takes repeated deprivations to establish these alterations. In addition, these results suggest that selective breeding for alcohol preference (in a two-bottle choice paradigm) is not associated with the expression of an ADE after a single deprivation under operant conditions. The differences between P and HAD rats in the expression of an ADE after a single deprivation in the operant paradigm are also evident between these rat lines in a 24-h free-choice ethanol-drinking paradigm (Rodd-Henricks et al., 2000a, 2000b).

In comparison to the 2- and 5-week groups, HAD-1 and HAD-2 rats in the 8-week group had lower responding during the first reexposure in the first session, which extended to the second session for HAD-2 rats (Fig. 3) and to the third session for the HAD-1 rats (Fig. 2). These results suggest that, in the 8-week group, loss of memory associating the lever response with ethanol presentation may have occurred.

The HAD-1 and HAD-2 rats in the current experiment would not have had sufficient alcohol exposure to establish physical dependence (no overt withdrawal symptoms were noted in any of the rats during the current experiment). Under baseline ethanol self-administration, estimated intakes of 1.2–1.6 g/kg would produce blood alcohol concentrations (BACs) in the range of 75–125 mg% (Murphy et al., 1986, 2002). These BACs are considerably lower than the 200 mg% BACs sustained in the vapor chamber studies (Becker et al., 1997). However, estimated intakes of 3.0–3.5 g/kg could be attained in the third and fourth reexposures and persist for more than one session. These intakes would be expected to produce BACs in the range of 200 mg% (Rodd-Henricks et al., 2001) and could produce dependence and tolerance. However, with the minimum of a 2-week deprivation period between ethanol reexposures, it is not known whether tolerance or dependence may be playing a role in the expression of an ADE in the HAD lines observed after multiple deprivations. In the P rats, tolerance and dependence do not appear to be factors contributing to the expression of an ADE, because signs of physical dependence (Waller et al., 1982) and tolerance (Gatto et al., 1987) have been reported to dissipate within 2 weeks.

Repeated alcohol withdrawals have been postulated to increase the vulnerability and susceptibility of future withdrawal episodes (Becker et al., 1997). Thus, it is possible that, in genetically vulnerable populations, such as the P and HAD rat lines, neurobiological adaptations may occur with multiple cycles of alcohol drinking and abstinence, which enhance the reinforcing effects of ethanol. This cyclic pattern of consumption and deprivation may have severe consequences in humans since multiple previous detoxifications are associated with a reduction in the response to treatment of withdrawal symptoms and heavier drinking during outpatient detoxification (Malcolm et al., 2000).

Differences between the HAD-1 and HAD-2 lines in the expression of an ADE were evident in the operant paradigm. In the 2-week group, the HAD-1 line had lower responding on the ethanol lever in the first reexposure session after the first and second deprivations (Fig. 2), whereas the HAD-2 line did not show lower responding on the ethanol lever after the first deprivation and had slightly elevated responding after the second deprivation (Fig. 3). This difference is more pronounced when the cumulative responding on the ethanol lever is examined (Fig. 4). For the HAD-1 rats, responses on the ethanol lever remained below baseline levels throughout the 60-min session (Fig. 4), whereas for the HAD-2 line, responses on the ethanol lever had reached baseline levels by 30 min after the first deprivation and were above baseline values within 10 min in the first reexposure session after the second deprivation (Fig. 4). These results suggest that neuronal alterations associated with the development and expression of an ADE were occurring more readily in the HAD-2 than the HAD-1 line. This conclusion is supported by the observation that the HAD-2 rats in the 5-week group express a robust ADE after the second deprivation (Fig. 3), whereas the HAD-1 line did not show an ADE until after the third deprivation (Fig. 2).

In conclusion, the present study demonstrated that repeated alcohol deprivations increased the reinforcing effects of ethanol in both replicate lines of HAD rats, and that these effects appeared to be more pronounced in the HAD-2 than the HAD-1 line. The results of this study provided additional evidence supporting the idea that repeated cycles of alcohol drinking and abstinence produce neuronal alterations that promote progressive increases in the magnitude and duration of high alcohol intake associated with relapse drinking. Understanding the mechanisms underlying these neuronal alterations would greatly aid the

development of pharmacological agents to reduce alcohol relapse.

References

Agabio, R., Carai, M. A. M., Lobina, C., Pani, M., Reali, R., Vacca, G., Gessa, G. L., & Colombo, G. (2000). Development of short-lasting alcohol deprivation effect in Sardinian alcohol-preferring rats. *Alcohol 21*, 59–62.

Becker, H. C., Diaz-Granados, J. L., & Weathersby, R. T. (1997). Repeated ethanol withdrawal increases the severity and duration of subsequent withdrawal seizures in mice. *Alcohol 14*, 319–326.

Bell, R. L., Rodd, Z. A., Boutwell, C. L., Hsu, C. C., Lumeng, L., Murphy, J. M., Li, T.-K., & McBride, W. J. (2004). Effects of long-term episodic access to ethanol on the expression of an alcohol deprivation effect in low alcohol–consuming rats. *Alcohol Clin Exp Res 28*, 1867–1874.

Brown, G., Jackson, A., & Stephens, D. N. (1998). Effects of repeated withdrawal from chronic ethanol on oral self-administration of ethanol on a progressive ratio schedule. *Behav Pharmacol 9*, 149–161.

Burish, T. G., Maisto, S. A., Cooper, A. M., & Sobell, M. B. (1981). Effects of voluntary short-term abstinence from alcohol on subsequent drinking patterns of college students. *J Stud Alcohol 42*, 1013–1020.

Ciccocioppo, R., Angeletti, S., & Weiss, F. (2001). Long-lasting resistance to extinction of response reinstatement induced by ethanol-related stimuli: role of genetic ethanol preference. *Alcohol Clin Exp Res 25*, 1414–1419.

Covington, H. E. III, & Miczek, K. A. (2001). Repeated social-defeat stress, cocaine or morphine: effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology 158*, 388–398.

Gatto, G. J., Murphy, J. M., Waller, M. B., McBride, W. J., Lumeng, L., & Li, T.-K. (1987). Persistence of tolerance to a single dose of ethanol in the selectively bred alcohol-preferring P rats. *Pharmacol Biochem Behav 28*, 105–110.

Heyser, C. J., Schulteis, G., Durbin, P., & Koob, G. F. (1998). Chronic acamprosate eliminates the alcohol deprivation effect while having limited effects on baseline responding for ethanol in rats. *Neuropsychopharmacology 18*, 125–133.

Heyser, C. J., Schulteis, G., & Koob, G. F. (1997). Increased ethanol self-administration after a period of imposed ethanol deprivation in rats trained in a limited access paradigm. *Alcohol Clin Exp Res 21*, 784–791.

Hilbrom, M. E. (1990). Alcohol withdrawal seizures and binge versus chronic drinking, In R. J. Port, R. H. Mattson, J. A. Cramer, & I. Diamond (Eds.), *Alcohol and Seizures: Basic Mechanisms and Clinical Concepts* (pp. 206–215). Philadelphia, PA: FA Davis.

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science 134*, 943–944.

Institute of Laboratory Animal Resources. (1996). *Guide for the Care and Use of Laboratory Animals.* Washington, DC: National Academy Press.

Kornet, M., Goosen, C., & Van Ree, J. M. (1991). Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. *Psychopharmacology 104*, 367–376.

Li, T.-K., Lumeng, L., & Doolittle, D. P. (1993). Selective breeding for alcohol preference and associate responses. *Behav Genet 23*, 163–170.

Malcolm, R., Roberts, J. S., Wang, W., Myrick, H., & Anton, R. F. (2000). Multiple previous detoxifications are associated with less responsive treatment and heavier drinking during an index outpatient detoxification. *Alcohol 22*, 159–164.

McKinzie, D. L., Eha, R., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T.-K. (1996). Effects of taste aversion training on the acquisition of alcohol drinking in adolescent P and HAD rat lines. *Alcohol Clin Exp Res 20*, 682–687.

McKinzie, D. L., Nowak, K. L., Yorger, L., McBride, W. J., Murphy, J. M., Lumeng, L., & Li, T.-K. (1998). The alcohol deprivation effect in the alcohol-preferring P rat under free-drinking and operant access conditions. *Alcohol Clin Exp Res 22*, 1170–1176.

McMillen, B. A. (1997). Toward a definition of a valid animal model of alcoholism: multiple animal models for multiple diseases. *Alcohol 14*, 409–419.

Murphy, J. M., Gatto, G. J., Waller, M. B., McBride, W. J., Lumeng, L., & Li, T.-K. (1986). Effects of schedule access on ethanol intake by the alcohol-preferring P line of rats. *Alcohol 3*, 331–336.

Murphy, J. M., Stewart, R. B., Bell, R. L., Badia-Elder, N. E., Carr, L. G., McBride, W. J., Lumeng, L., & Li, T.-K. (2002). Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet 32*, 363–388.

Ritz, M. C., Garcia, J. M., Protz, D., & George, F. R. (1994). Operant ethanol-reinforced behavior in P, NP, HAD, and LAD rats bred for high versus low alcohol preference. *Alcohol Clin Exp Res 18*, 1406–1415.

Roberts, A. J., Heyser, C. J., Cole, M., Griffin, P., & Koob, G. F. (2000). Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacology 22*, 581–594.

Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. (1989). Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. *Psychopharmacology 97*, 535–538.

Rodd, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., Lumeng, L., Li, T.-K., & McBride, W. J. (2003). Effects of repeated alcohol deprivations on operant ethanol self-administration by alcohol-preferring (P) rats. *Neuropsychopharmacology 28*, 1614–1621.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T. K. (2001). Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. *Alcohol Clin Exp Res 25*, 1140–1150.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T. K. (2002a). Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats. I. Periadolescent exposure. *Alcohol Clin Exp Res 26*, 1632–1641.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T. K. (2002b). Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats. II. Adult exposure. *Alcohol Clin Exp Res 26*, 1642–1652.

Rodd-Henricks, Z. A., McKinzie, D. L., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T.-K. (2000a). The expression of an alcohol deprivation effect in the high-alcohol-drinking replicate rat lines is dependent on repeated deprivations. *Alcohol Clin Exp Res 24*, 747–753.

Rodd-Henricks, Z. A., McKinzie, D. L., Shaikh, S. R., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T.-K. (2000b). Alcohol deprivation effect is prolonged in the alcohol preferring (P) rat after repeated deprivations. *Alcohol Clin Exp Res 24*, 8–16.

Samson, H. H., Files, F. J., Denning, C., & Marvin, S. (1998). Comparison of alcohol-preferring and nonpreferring selectively bred rat lines. I. Ethanol initiation and limited access operant self-administration. *Alcohol Clin Exp Res 22*, 2133–2146.

Sinclair, J. D., & Li, T.-K. (1989). Long and short alcohol deprivation: effects on AA and P alcohol-preferring rats. *Alcohol 6*, 505–509.

Sinclair, J. D., & Senter, R. J. (1967). Increased preference for ethanol in rats following deprivation. *Psychon Sci 8*, 11–12.

Sinclair, J. D., & Senter, R. J. (1968). Development of an alcohol-deprivation effect in rats. *Q J Stud Alcohol 29*, 863–867.

Sinclair, J. D., & Tiihonen, K. (1988). Lack of alcohol-deprivation effect in AA rats. *Alcohol 5*, 85–87.

Spanagel, R., & Zieglgansberger, W. (1997). Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. *Trends Pharmacol Sci 18*, 54–59.

Vengeliene, V., Siegmund, S., Singer, M. V., Sinclair, J. D., Li, T.-K., & Spanagel, R. (2003). A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. *Alcohol Clin Exp Res 27*, 1048–1054.

Waller, M. B., McBride, W. J., Lumeng, L., & Li, T.-K. (1982). Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. *Pharmacol Biochem Behav 16*, 501–507.