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Alcohol place preference conditioning in high- and low-alcohol preferring selected lines of mice

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Abstract

High- and low-alcohol preferring (HAP and LAP) selected lines of mice diverge greatly in free-choice alcohol consumption. This study investigated whether the lines differ in a measure of alcohol reward not dependent on drinking, specifically place conditioning. Mice were subjected to a differential conditioning procedure in which four alcohol-paired CS+ trials on one floor cue (0, 1.5, 3, or 4 g/kg; *n*s = 20–24) alternated with four saline-paired CS − trials on a different floor cue. Testing was on a split floor, half CS+ and half CS − . HAP and LAP mice showed no preference at 0 g/kg, and equivalent, moderate preference at 1.5 and 3 g/kg alcohol. At 4 g/kg, LAP, but not HAP mice showed an increase in preference. The present findings imply greater efficacy of alcohol preference conditioning in LAP mice, but do not speak for line differences in sensitivity. Results do not support the hypothesis that selection for high drinking yields greater efficacy of alcohol as a reinforcer when reward is measured using a technique that does not rely on drinking. Low drinking in LAP mice may emerge from innate taste avoidance of alcohol as a result of selective breeding for low preference, which prevents them from encountering alcohol's rewarding, pharmacological effects.

Keywords: Genetic differences; Drinking; HS/Ibg; Selective breeding; Reinforcement

1. Introduction

Selection for differences in free-choice alcohol consumption has frequently been used to study the genetic and neurobiological mechanisms underlying high alcohol drinking behavior, resulting in numerous lines of rats differing greatly in alcohol drinking (Crabbe et al., 1992; Li et al., 1993). One question often arising in the interpretation of these studies is whether high-drinking lines show greater alcohol-reinforced behavior than low-drinking lines. Most such studies utilize the operant oral self-administration model. Perhaps not surprisingly, given that the selection phenotype involves oral consumption of alcohol, high-drinking lines of rats, such as alcohol-preferring (P) rats, high alcohol drinking (HAD) rats, and Alko alcohol (AA) rats respond at higher rates for alcohol-containing solutions than do low-drinking lines such as nonpreferring (NP), low alcohol drinking (LAD), and Alko nonalcohol (ANA) rats (Files et al., 1998; Gauvin et al., 1998; Ritz et al., 1989a,b, 1994; Samson et al., 1989). These differences have been interpreted as indicative of greater alcohol reinforcement in high drinking lines than in low drinking lines.

Important in the interpretation of such studies is whether differences in drinking behavior are driven by preingestive (e.g., taste) or postingestive (e.g., pharmacological effects) factors. Numerous studies have shown that alcohol has reinforcing effects when its taste is not a factor, such as in intragastric (Waller et al., 1984), intracranial (Gatto et al., 1994; Rodd-Henricks et al., 2000), or intravenous self-administration models (Grahame and Cunningham, 1997). However, studies involving drinking of alcohol indicate that taste is an important factor in ethanol's reinforcing effects. Experience with alcohol drinking can influence taste reactivity to alcohol differentially in high- vs. low-drinking lines of rats, with P but not NP rats acquiring a hedonic taste response (Bice and Kiefer, 1990). Moreover, experienced P

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rats do not show robust changes in volume or pattern of alcohol drinking during limited access sessions when a sham drinking procedure is used, in which alcohol has no postingestive consequences (Rowland and Morian, 1994). These data suggest that taste alone is sufficient to regulate alcohol-intake patterns. Additionally, following experience in which taste cues are concurrently ingested with alcohol, taste cues alone can serve as a conditioned reinforcer (Cunningham and Niehus, 1997). These data suggest that line differences in operant oral self-administration of alcohol-containing solutions may be affected by differences in the conditioned taste cues associated with alcohol, or the taste of alcohol itself. Therefore, differences in operant oral self-administration of alcohol cannot be explained by differences in responsiveness to alcohol's pharmacological properties alone.

Besides intragastric, intravenous, and intracranial self-administration studies, place conditioning following parenteral administration of alcohol is another method that allows assessment of line differences in the motivational effects of alcohol in the absence of conditioned or unconditioned taste factors. Indeed, one study did indicate line differences in alcohol place conditioning between P and NP rats (Schuckit and Smith, 1996). However, as with most studies assessing alcohol place conditioning in rats (e.g., Bormann and Cunningham, 1997, 1998; Stewart and Grupp, 1989; van der Kooy et al., 1983), this one found avoidance, rather than preference for the alcohol-paired location. Although P rats showed less avoidance of alcohol-paired cues than NP rats, interpretation of this study in terms of differences in the rewarding effects of alcohol is difficult. One recent study did find preference for alcohol-paired cues in selectively bred Sardinian P rats (Ciccocioppo et al., 1999). However, that study did not compare this line to the selectively bred Sardinian NP rats, preventing interpretation terms of genetic differences in the rewarding effect of alcohol. That study also did not use an unpaired control group, making it unclear as to whether change in preference for the alcohol-paired side was mediated by Pavlovian conditioning. Unlike rats, mice typically show a conditioned preference for alcohol-paired cues under a wide variety of dose, route of administration, and genetic conditions (e.g., Cunningham, 1995; Cunningham et al., 1992; Kelley et al., 1997; Risinger and Oakes, 1996).

Recently, high- and low-alcohol preferring (HAP and LAP) lines of mice have been generated using selective breeding techniques (Grahame et al., 1999a). These animals have been selected for differences in free-choice alcohol drinking over a 1-month period, and now show large differences in chronic (i.e., 24-h access over a 4-week period; Grahame et al., 1999a) and limited access (i.e., 2 h daily; Grahame et al., 1999b) alcohol intake. Because HAP mice show significant free-choice alcohol drinking while LAP mice do not, it seemed reasonable to expect that HAP mice would show greater alcohol conditioned place preference than LAP mice, as would be consistent with a view ascribing greater alcohol reward to a line that drinks more alcohol.

Recent literature, however, provide relatively little evidence for genetic overlap between free-choice alcohol intake and magnitude of alcohol-induced conditioned place preference in mice. In a study of 25 BXD recombinant inbred strains of mice (Cunningham, 1995), alcohol drinking and alcohol conditioned place preference were found to be substantially genetically independent, although some overlap of quantitative trait loci was seen following mapping of these two traits. It is unclear, however, whether these findings of genetic independence between drinking and preference conditioning would apply to HAP and LAP mice. These lines are selectively bred from HS/Ibg mice, a genetically defined, outbred stock, and a more outbred progenitor population than the C57BL/6J and DBA/2J inbred strains used to derive the BXD RI mice. A comparison between HAP and LAP mice is a good way to determine whether alcohol drinking and alcohol reward as measured by conditioned place preference are genetically related, because HS/Ibg mice can have many more alleles at each locus than populations (such as BXD RI) derived from just two strains. Results from comparison among a larger number of alleles are more likely to be generalizeable to wider populations (Crabbe, 1989).

2. Materials and methods

2.1. Subjects

Subjects in the alcohol place conditioning study were 71 HAP and 68 LAP male and female mice bred at the Indianapolis VAMC animal care facility. They were alcohol-naive mice from generation 13 of selection (in the alcohol conditioning study). An additional 24 HAP and 24 LAP mice from generation 16 were used in the saline control study. Mice were moved to the Institute for Psychiatric Research at approximately 30 days of age, and were group housed (four to a cage) in polycarbonate cages ($27.9 \times 9.5 \times 12.7$ cm) with Harlan Sani-Chip bedding, at an ambient temperature of $21 \pm 1^{\circ}$C. Lights were on from 0700 to 1900 h daily, and mice were tested between 1000 and 1500 h. Water and lab chow were available at all times in the home cage. All animals were bred and maintained in an AALAC-approved facility, and all experimental procedures were approved by the Indiana University School of Medicine IACUC. Mice were approximately 90 days of age at the beginning of the studies. Mice were tail-marked for identification initially on the day before the first day of the studies. They were then re-marked on day 7.

2.2. Apparatus

A set of eight identical activity monitors were used. These boxes measured $25.0 \times 13.75 \times 15.0$ cm ($l \times w \times h$),

with Plexiglas long walls and aluminum side walls, and were housed in dark MedAssociates sound attenuating cubicles model ENV 021M ($17 \times 14 \times 12$ in., $w \times h \times d$) equipped with a fan for ventilation and background noise. A row of eight equally spaced photo emitter receiver pairs 2.5 cm above the floor along the long walls (interbeam interval of 3.25 cm) recorded activity. An activity count was defined as a beam interruption by any part of the mouse, followed by a 50-ms timeout on that beam during which additional interruptions were not counted. Tactile floor cues for place preference conditioning were interchangeable halves. During conditioning, they were either entirely "grid" (13-gauge stainless steel rods mounted with centers 0.65 mm apart, in Plexiglas rails) or "hole" (perforated stainless steel with 6.4-mm round holes mounted on 9.5-mm staggered centers). On the test day, these were split, so that half the floor was hole and half grid, counterbalanced for which was the left side and which was the right side. These textures were chosen based on previous studies (Cunningham et al., 1993) indicating that these floors are effective for conditioning, but yield no unconditioned preference for either floor type. On the test day, the position of the mouse (hole or grid side of the activity box) was determined, with a 50-ms resolution, by a program written locally. A side change was indicated when any part of the mouse crosses a line 4.9 cm to one side of the center; this distance roughly corresponds to the distance required for the head and forepaws to be past the center of the chamber.

2.3. Drugs and experimental design

The details of the experimental design and conditioning procedure used in the present study, including the composition of the textural floor cues (grid floor or hole floor), are taken from that used by Cunningham and colleagues (e.g., Cunningham et al., 1993). This is a differential conditioning procedure, in which one cue (grid or hole floor) is paired with alcohol to become the paired conditioned stimulus (CS)+, and the other cue (hole or grid floor) is paired with saline and unpaired with alcohol (CS −). During each conditioning day, only one of the CSs is present. On the test day, mice are placed on a split floor, and choose between the CS+ floor and the CS − floor. Three doses of alcohol were used in the present study to construct a dose–response curve for preference conditioning: 1.5, 3 and 4 g/kg. These doses were chosen, in part, to bracket modest preference seen following 3 g/kg alcohol in pilot experiments. The highest dose also matches the route of administration and dose often used to assess differences in acute alcohol withdrawal (e.g., Buck et al., 1997; Metten et al., 1998). In both lines, one might expect a monotonic relationship between dose of alcohol and magnitude of preference, as has been seen in other place preference conditioning findings with alcohol over this dose range (Risinger and Oakes, 1996), and with other rewarding drugs such as morphine (Barr et al., 1985). The relatively short duration of conditioning trials (5 min) was chosen based both on pilot experiments indicating that 10-min conditioning sessions resulted in poor preference behavior, and on the mouse place conditioning literature (Risinger and Cunningham, 1992). If alcohol drinking and alcohol conditioned place preference tap into the same reward mechanisms, selection for high alcohol drinking might be expected to result either in greater potency of alcohol reward (defined here as greater preference at the low dose[s] of alcohol), or greater efficacy of alcohol reward (defined here as a greater maximal preference at the highest alcohol dose).

All injections were intraperitoneal. Alcohol injections were 20% v/v, diluted from 100% ethanol with 0.9% saline. Volume of 0.9% saline injections were matched to the volume of alcohol injections for each mouse. Injection volumes were 9.50 ml/kg for the 1.5 g/kg group, 19 ml/kg for the 3 g/kg group, and 25.3 ml/kg for the 4 g/kg group. Mice in the saline control study received a 19 ml/kg saline injection on all days. The alcohol study was conducted as a factorial design of Dose (1.5, 3, or 4 g/kg alcohol) × CS+ conditioning floor (grid or hole) × Line (HAP or LAP). The separate saline control study was conducted as a CS+ conditioning floor × Line study. Mice in the alcohol study were randomly assigned to one of the six conditioning groups counterbalanced across sex and box number. Simultaneously counterbalanced assignments were made for whether the mouse received alcohol on the first or second conditioning day, and whether the grid or hole floor was on the left on the test day. That alcohol dose response study was conducted as two balanced replications, while the saline control study was run in a single replication.

Magnitude of preference is determined by a between-groups comparison of mice for whom the grid floor is the CS+ floor, and mice for whom the hole floor is the CS+ floor. In other words, within each alcohol dose group in the present study, test day magnitude of preference is determined by a between-groups comparison between paired and unpaired groups. Such a paired–unpaired, between-groups comparison has advantages relative to studies comparing preference back to a preconditioning baseline preference for one floor or the other, because it excludes the possibility that changes in behavior are due to exposure to the drug alone, or to the CS alone (i.e., it excludes pseudoconditioning). A between-groups difference between a paired and unpaired control uniquely indicates that the behavior results from the pairing of the drug with a specific CS (see Rescorla, 1967 for a discussion of control procedures in Pavlovian conditioning and Cunningham, 1993 for a discussion of controls for Pavlovian Drug studies like place conditioning). An added advantage of not assessing "baseline preference" for the floors before conditioning is that there is no preexposure to the to-be-conditioned stimuli, which would be expected to undermine conditioning due to latent inhibition. The present study assesses unconditioned preference in a saline control group, in which both floors were paired with a saline injection during conditioning. In these subjects, any uncon-

ditioned differences in preference for one floor or the other can be directly observed in animals matched with experimental groups for experience with each floor type.

2.4. Statistics

All analyses were first conducted with sex as an added factor; when sex did not interact with any other factor, subsequent analyses collapsed across sex. All ANOVAs conducted with conditioning floor type as a factor compare H+ to G+ groups to determine whether there is an effect of place conditioning training (i.e., this compares paired and unpaired groups as an index of whether conditioning occurred). Initial analyses were conducted as Line × Dose × Reinforced floor × Minutes; a stepwise process of following up with additional ANOVAs with one of the factors removed was conducted when significant higher order interactions were seen.

2.5. Procedure

On each day of the experiments, all mice to be tested were placed on a cart and wheeled from the colony room to the experimental room 45 min to 1 h prior to the beginning of the first session. Immediately before each session, each mouse was weighed and then injected with the weight-appropriate volume. Mice were then immediately placed in the middle of the activity enclosure. On Day 1 (habituation), all mice were given a saline injection and were placed directly on a paper towel floor for a 5-min session. Neither of the floor CSs were used to avoid CS preexposure. Training began on Day 2, and continued for 10 subsequent days. Days 2–5 and 8–11 were conditioning days, while days 6–7 were spent in the home cage. On conditioning days, mice were placed on the appropriate conditioning floor (grid or hole) for a 5-min session. Mice received four conditioning days of each type (CS+ or CS−); on CS+ days mice received alcohol, and on intervening CS− days, mice received saline. In the saline control study, mice received saline injections on both CS+ and CS− conditioning days. On Day 12, the place conditioning test session, all mice received a saline injection immediately prior to a 60-min session; the volume of the saline injection was matched to the conditioning volumes in order to match cues present during conditioning. Mice were placed into a monitor with a half-grid and half-hole floor.

To determine the peak blood alcohol levels present during exposure to the CS+ during conditioning, alcohol-naive mice from the saline control study (12 mice from each line) were injected 4 days after the end of the study. Mice received either 1.5 or 4.0 g/kg alcohol, ip (ns = 6). Line/dose groups were counterbalanced for sex. Five minutes after injection, about 30 μl of retroorbital sinus blood was taken from each mouse. Plasma was separated from whole blood by centrifugation, and was stored in sealed capillary tubes in a −20°C freezer until analysis for alcohol content via gas chromatography (see Lumeng et al. for procedural details).

3. Results

Three subjects were lost from each line during conditioning, due to health reasons, and were excluded from all analyses.

3.1. Conditioning trial activity

Because conditioning trials were short, data from each 5-min session was averaged to create mean activity counts per minute. Additionally, data from CS+ (following alcohol administration) and CS− sessions (following saline administration) were examined separately, because the range of activity seen following saline was quite different from that seen following alcohol injections.

Data from CS+ conditioning trials are shown in Fig. 1. Both lines showed a similar effect of alcohol on the first trial, with higher doses producing lower levels of locomotor

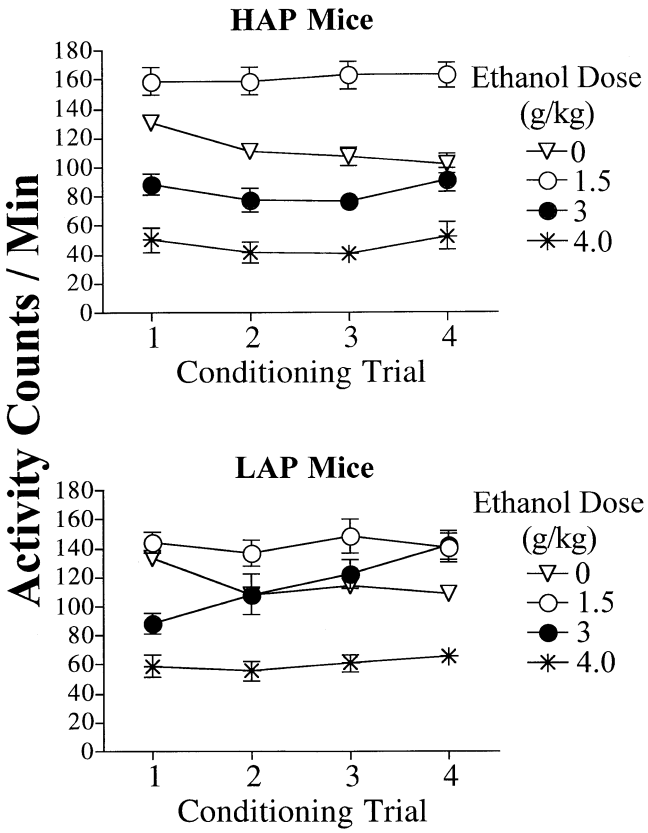

Fig. 1. The locomotor response to 0, 1.5, 3 or 4 g/kg alcohol injections given immediately prior to 5-min CS+ place preference conditioning trials. Both lines show dose-dependent changes in locomotor activity on the first trial. There was no change in the alcohol response over trials, except in LAP mice at 3 g/kg, which do not differ from 1.5 g/kg-treated animals by the fourth conditioning trial. Bars indicate S.E.M.s.

activity. Anecdotally, mice in the 4-g/kg treatment group had typically lost their righting reflex by the end of the session. With repeated trials, LAP mice treated with 3 g/kg shifted from locomotor sedation to locomotor activation, while HAP mice appeared to develop tolerance to the sedative effect of this dose. At other doses, LAP mice and HAP mice did not show changes in locomotor activity over trials. To determine whether the response to alcohol changed between the first and last trials, CS+ data were subjected to a 2 (line) × 4 (dose) × 2 (Trials 1 and 4) mixed factorial ANOVA, with repeated measures on trials. This showed a three-way interaction, $F(3,174) = 2.70$, $P < .05$. To find the source of this interaction, follow-up Dose × Trial ANOVAs were done for each line. Both LAP mice, $F(3,85) = 18.52$, $P < .001$ and HAP mice, $F(3,89) = 3.06$, $P < .05$ showed a significant Dose × Trials interaction, showing that changes in the alcohol response over trials depended on dose. This interaction was followed up by assessing simple main effects of trial within each dose group. In LAPs, both saline and 3 g/kg alcohol-treated mice changed activity levels between Trials 1 and 4 (a decrease and increase, respectively, Ps < .001). In HAP mice, only saline-treated mice changed over the same period, showing a decrease in locomotor activity, $P < .01$. Interestingly, in LAPs, 3 g/kg caused a decrease in locomotor activity relative to saline on Trial 1 (Tukey's HSD $P < .001$) and an increase in locomotor activity relative to saline on Trial 4 (Tukey's HSD $P = .015$). In HAP mice, 3 g/kg decreased locomotor activity relative to saline on Trial 1 (Tukey's HSD $P < .001$), but had no effect on activity by Trial 4, $P > .5$. Together, these findings indicate sensitization, or sensitization and tolerance to 3 g/kg alcohol in LAP mice, but only tolerance to the same dose in HAP mice.

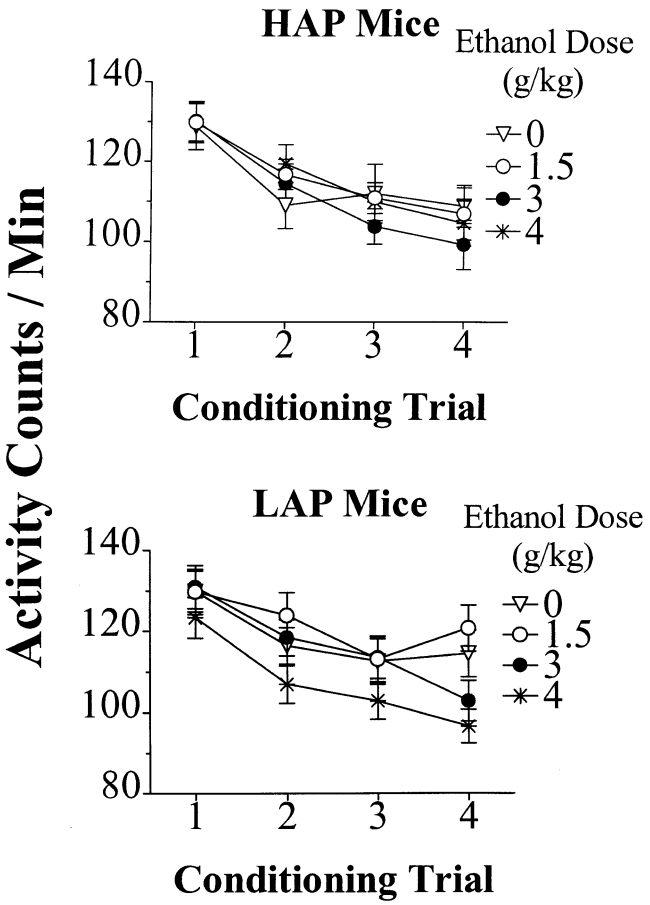

Fig. 2. The locomotor response to saline injections given immediately prior to CS − place preference conditioning trials. LAP, but not HAP mice show an alcohol dose-dependent decrease in locomotor activity over trials, based on their experience with alcohol on alternate trials.

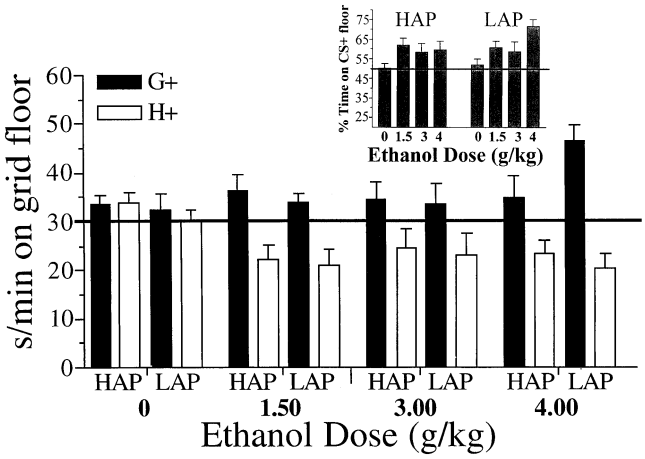

Fig. 3. Conditioned place preference, averaged over the 60-min drug-free test session, in HAP and LAP mice following conditioning with the doses of alcohol indicated. The magnitude of preference is indicated by the size of the difference between equivalently treated G+ and H+ conditioning groups (ns = 10–12). The line at 30 s indicates the chance level preference for the grid floor that would be expected in the absence of either conditioned or unconditioned floor preferences. No conditioning was seen following saline injections (0 g/kg), while LAP mice treated with 4 g/kg show greater preference than other alcohol-treated groups. The inset indicates the data transformed into percent time spent on the alcohol-paired (i.e., CS+) floor, collapsing across G+ and H+ groups.

Data from CS − conditioning trials are shown in Fig. 2. Immediately prior to these trials, all mice received saline injections, but there were modest effects of experience with alcohol on alternate sessions. Alcohol-treated LAP mice showed an alcohol dose-dependent decrease in locomotor activity during saline sessions, while HAP mice showed no such dose-dependent behavior. A Line × Dose × Trials ANOVA showed a marginally nonsignificant Line × Dose interaction, $F(2,127) = 2.98$, $P = .054$. A Trial × Dose interaction $F(3,381) = 2.29$, $P < .05$, indicated that during later trials, the effect of alcohol injections on alternate trials increased. Based on these interactions, follow-up comparisons indicated that in LAP mice by Trial 4, the simple main effect of dose was strong, $F(2,62) = 6.16$, $P < .005$, but was not present in HAP mice, $P > .5$. Mice in the saline control study showed a decrease in activity over trials consistent with habituation, as indicated by a main effect of trials, $F(3,138) = 8.54$, $P < .001$. There was no effect of line or a Line × Trials interaction, $F < 1.0$.

3.2. Place preference

Fig. 3 shows the outcome of the 1-h drug-free place preference test; magnitude of preference is determined by comparing each of the G+ groups to the comparably treated H+ group. A line indicates the 30-s point, at which preference for grid and hole floors would be equivalent. No place conditioning was seen in the 0 g/kg groups, as indicated by the lack of a difference between G+ and H+ groups. At 4 g/kg alcohol, LAP mice showed a larger preference than did HAP mice. HAPs and LAPs receiving 1.5 and 3 g/kg alcohol prior to CS+ trials showed moderate and comparable preference for the alcohol-paired floor.

Statistical analysis confirmed these observations. Analysis of preference data used 5-min sample periods to reduce the severity of Greenhouse–Geisser correction required. A 2 (line) × 3 (dose) × 2 (conditioning floor) × 12 (5-min blocks) ANOVA on the preference data showed an overall 4-way interaction, $F(22,1331) = 1.925$, $P < .01$ (with Greenhouse–Geisser correction, this was adjusted to $F(11.5,697) = 1.925$, $P < .05$). Based on this finding, analysis of Line × Condi-Conditioning floor × 5-min blocks was assessed within each dose group to determine the source of the interaction. No place conditioning occurred following saline administration, as indicated by a lack of differences between G+ and H+ conditioned groups on the test day. This was confirmed by a Line × Conditioning floor × 5-min blocks ANOVA on saline-treated subjects that showed no three-way interaction, $F(11,484) = 1.31$, $P > .20$, no Line × Con-Conditioning floor interaction nor any main effects of conditioning floor or line, Fs < 1.0. Mice treated with 1.5 and 3 g/kg alcohol did show preference for the alcohol-paired side, as indicated by main effects of conditioning floor at each of these doses, $F(1,38) = 20.85$, $P < .001$, and $F(1,42) = 6.32$, $P < .05$, respectively. However, mice in these dose groups did not show Line × Conditioning floor × Mi-Minutes interactions, Ps > .4, nor were there Line × Condi-Conditioning floor interactions, Ps > .80, indicating that at lower doses, lines did not differ in the magnitude of place conditioning, or in the rate of extinction of place conditioning during the test. No extinction was seen during the entire session in 1.5 and 3 g/kg-treated groups, as indicated by a lack of any conditioning Floor × Time interactions in any of these groups, Ps > .13.

At 4 g/kg, LAP mice showed a larger preference during part of the test session than HAP mice, as indicated by a Line × Conditioning floor × 5-min blocks interaction, $F(11,235) = 3.53$, $P < .001$ (Greenhouse–Geisser corrected $P < .005$), as well as a Line × Conditioning floor interaction, $F(1,41) = 4.38$, $P < .05$. Assessment of time effects in the hour-long test session is important, because animals are effectively tested in the absence of the unconditioned stimulus: persistence of a preference for the alcohol-paired floor indicates resistance to extinction (Tzschentke, 1998). To find the source of the Line × Conditioning floor × Mi-Minutes interaction (and thus when preference extinguished), the session was divided into the first and second 30 min, and average time spent on grid was calculated for each 4-g/kg mouse during the first and second 30 min of the 60-min session (see Fig. 4). During the first 30 min, both lines showed roughly equal preference, as indicated by the main effect of conditioning floor, $F(1,41) = 330$, $P < .001$, and the lack of a Line × Conditioning floor interaction, $F(1,41) = 1.53$, $P = .224$. During the second 30 min of the test session, preference in HAP mice extinguished, while in LAP mice preference remained strong. This was confirmed by a Line × Conditioning floor interaction during this period, $F(1,41) = 7.07$, $P = .01$. Additionally, during the second 30 min of the session, there was a difference between G+ and H+ conditioning floors in LAPs, $F(1,21) = 42.82$, $P < .001$, but not in HAPs $F(1,20) = 1.27$, $P > .25$.

Notably, no unconditioned preference for either tactile cue was seen during the test session. This was confirmed by assessing average time spent on the grid floor, regardless of treatment group, within each line. If mice had preferred or avoided the grid floor, they would have spent greater or less than 30 s out of each minute on that floor, regardless of treatment condition. Mean (± S.E.M.) time on the grid floor for HAP mice was 30.39 ± 1.24 s, and for LAP mice was 30.14 ± 1.40 s. For both these values, 30 s (i.e., no preference for either floor type) fell within the 95% confidence interval.

Final analyses were conducted to ensure that alcohol-treated groups showed greater place preference than saline-treated groups, using separate pairwise comparisons within each line between each alcohol dose group and saline. For simplification, comparisons were performed on the time spent on the CS+ floor (regardless of G+ or H+ condition), during the first 30 min of the test session, when place conditioning was strong in all groups. Overall, greater preference for the CS+ floor was seen in mice receiving alcohol on these floors than in mice receiving saline. In HAP mice, comparing to saline scores, P values for 1.5, 3, and 4 g/kg alcohol, respectively, were $P < .005$, $P < .05$, and

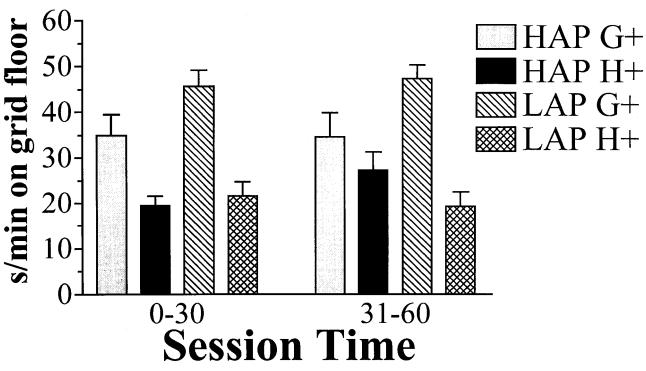

Fig. 4. Conditioned place preference during the first and second 30 min of a 60-min test session in HAP and LAP mice following conditioning with 4 g/kg alcohol. The magnitude of preference is indicated by the size of the difference between G+ and H+ conditioning groups. HAP but not LAP mice show extinction over the course of the test session.

$P < .005$. In LAP mice, comparing to saline scores, P values for 1.5, 3, and 4 g/kg alcohol, respectively, were $P < .005$, $P = .06$, and $P < .001$. Although LAP 3 g/kg was not quite significantly different from LAP saline, these findings indicate that overall, alcohol was responsible for preference for the alcohol-paired floor.

3.3. Test session activity

Activity data from the drug-free test session, shown in Fig. 5, were generally similar to data in saline conditioning trials, in that LAP but not HAP mice showed a dose-dependent decrease in locomotor activity with increasing doses of alcohol administered during CS+ conditioning sessions. A 2 (line) × 3 (alcohol dose) ANOVA indicated an interaction, $F(2,127) = 3.09$, $P < .05$. A follow-up analysis indicated a main effect of dose in LAP mice, $F(2,41) = 4.22$, $P < .05$, but not in HAP mice, $F(2,65) = 2.88$, $P > .05$. Finally, LAP 1.5 g/kg mice differed from LAP 4 g/kg mice, $F(1,41) = 9.00$, $P < .01$ but not from LAP 3 g/kg mice, $P > .25$. No line differences were seen in mice receiving saline during conditioning, $F(1,47) = 1.34$, $P > .25$.

Two blood samples were lost during analysis, both in the LAP 4-g/kg group. Analysis indicated high blood alcohol concentrations, consistent with rapid absorption (5 min) following intraperitoneal injection. Blood alcohol data showed effects of dose, $F(1,22) = 75.9$, $P < .01$, but no effects of line nor a Line × Dose interaction, Fs < 1.0. Although there was no influence of line on blood alcohol concentrations, data are presented separated by line to help indicate that differences in magnitude of place preference conditioning were not secondary to differences in blood alcohol levels. Blood alcohol concentrations were as follows, in mg/dl (± S.E.M.): HAP 1.5 g/kg, 260.8 ± 22.2; LAP 1.5 g/kg, 256.8 ± 20.0; HAP 4.0 g/kg 671.3 ± 60.2; LAP 4.0 g/kg, 667.8 ± 55.6. These values are proportionally similar to values emerging from recent studies using the same alcohol concentration, route of administration, blood sampling procedure, and timepoint, following either a 3 g/kg alcohol injection (Ponomarev and Crabbe, 2000) or a 2 g/kg alcohol injection (Gill and Deitrich, 1999), although the latter showed that blood alcohol concentrations at this timepoint also depended upon genotype.

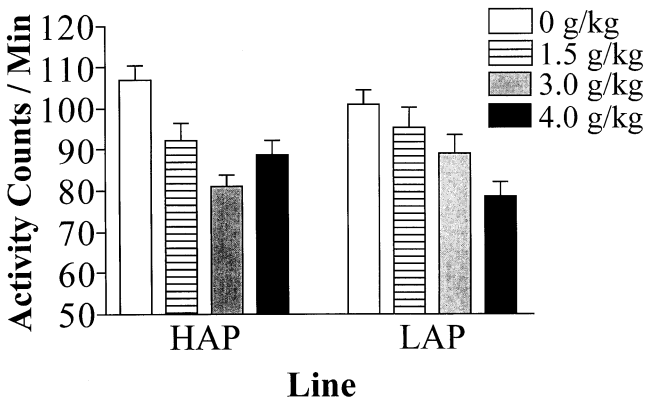

Fig. 5. Locomotor activity during the drug-free 60-min test session. LAP, but not HAP mice showed alcohol-dose dependent activity decreases. Bars indicate S.E.M.s.

4. Discussion

The present findings indicate that bidirectional selection for differences in alcohol preference results in differences in conditioned place preference. This finding is consistent with previous studies indicating that alcohol is a rewarding drug when behavioral assays that bypass taste are used (Gatto et al., 1994; Grahame and Cunningham, 1997; Rodd-Henricks et al., 2000; Waller et al., 1984). However, line differences were not in the expected direction: rather, at 4 g/kg alcohol, LAP mice showed a larger preference than HAP mice. These findings indicate greater rewarding effects of alcohol in LAP mice than in HAP mice. At other alcohol doses, both lines showed equivalent preference conditioning. No conditioning was seen following saline injections, showing that floor preferences in the other in alcohol groups was not secondary to an unconditioned preference for a particular floor texture, or some other effect not related to alcohol administration.

These results are consistent with some previous studies in BXD recombinant inbred strains indicating genetic independence between alcohol drinking and alcohol preference conditioning in mice using a 2-g/kg conditioning dose (Cunningham, 1995). However, there is no way to determine from those data whether an inverse genetic correlation between alcohol drinking and preference conditioning (as seen in the present data) would have emerged at a higher conditioning dose. Overall, HAP mice seemed insensitive to changes in the conditioning dose of alcohol, whereas LAP mice showed greater efficacy of preference conditioning at 4 g/kg alcohol than at other doses. The low dose (1.5 g/kg) appeared sufficient to yield preference conditioning in both lines, making conclusions about line differences in sensitivity to alcohol's reinforcing effects difficult. Line differences were not secondary to alcohol pharmacokinetic differences, because both lines showed equivalent retroorbital blood alcohol concentrations at both the lowest and highest conditioning doses used.

Caution must be used when interpreting line differences in conditioned place preference seen at 4 g/kg as indicative of a correlated response to selection for divergent alcohol consumption in HAPs and LAPs, because replicate lines are not included. Replicate HAP and LAP lines, which are selected from the same foundation population for the same phenotype as the present lines, are currently in a selective breeding program, and can be tested for differences in place conditioning once they show sufficient differences in alcohol intake. Differences seen in the present study could be mediated by genetic drift between the lines, which would yield divergence in responses genetically unrelated to alco-

hol drinking (Crabbe et al., 1990). However, the similarity between the lines in preference at the lower doses used suggests that lines do not differ in genes that would have a general effect on learning, and that the present findings are possibly specific to motivational attributes of a high conditioning dose.

At first glance, these findings are not congruent with data gathered from selectively bred rats on differences in place conditioning, because P rats (as well as NP rats) show conditioned place aversion to alcohol rather than a preference (Stewart et al., 1996). However, in that experiment, the NP rats showed a larger place aversion than the P rats at the higher doses used (1.0 and 1.5 g/kg), but not at the lowest dose (0.5 g/kg), which failed to yield any aversion in either line. Therefore, low-preferring lines showed greater efficacy of alcohol as an unconditioned stimulus for place conditioning both in that study and in the present one, even though the mice show preference conditioning while the rats show aversion conditioning. Interpreted this way, selection for differences in alcohol consumption yields differences in efficacy of alcohol's hedonic effect as measured by place conditioning. It is worthwhile to point out, however, that line differences seen at the highest conditioning doses in the present study are likely mediated through blood alcohol concentrations unlikely to be encountered during oral alcohol consumption in HAP mice (Grahame et al., 1999b), which are considerably lower than the levels seen 5 min after intraperitoneal injection of alcohol in the present study, even at the lowest dose. Therefore, it is perhaps all the more surprising that differences were seen both between P and NP rats and between HAP and LAP mice at the higher doses of alcohol, because selection for differences in drinking likely makes contact with lower blood alcohol concentrations.

The present findings can be interpreted in terms of dose- and line-dependent differences in preference conditioning to the CS+ floor mediated by alcohol's acute reinforcing effect, indicating greater alcohol reward in LAP than in HAP mice. Such an interpretation is consistent with findings in humans. Those who are genetically predisposed to drink excessively show less sensitivity to numerous alcohol effects than those who do not share such a genetic predisposition (Schuckit, 1998; Schuckit and Smith, 1996). According to this theoretical framework, HAP mice may drink more because they need higher doses to obtain alcohol's rewarding effects. Notably, the main line difference was seen in slower extinction of preference in LAP mice at 4 g/kg than in HAP mice at the same dose. Line differences in the rate of extinction can be interpreted as indicating a more robust response to drug-conditioned cues, which might reflect continued maintenance of craving in LAP mice compared to HAP mice (Tzschentke, 1998).

There is, perhaps, another explanation that could fit one aspect of the present data. The increase in observed preference in LAP mice at 4 g/kg alcohol could emerge from a conditioned place aversion in addition to a conditioned place preference: specifically, on the test day, when mice had to choose between the CS+ and CS − floor, the greater preference came not from greater preference for the CS+ floor, but rather from *avoidance* of the CS − floor in LAP but not HAP mice. In the present study, that aversive stimulus could have been withdrawal experienced on the CS − floor mediated by alcohol given 24 h previously during CS+ trials. Several aspects of the present data seem, at least, compatible with such an explanation. First, Fig. 4 indicates that the line difference in preference at this dose is not mediated so much by a difference in the peak magnitude of preference, but rather by resistance to extinction of preference. To the extent that LAP mice are avoiding the CS − floor, they minimize extinction by avoiding contact with that floor, thus preserving apparent preference for the CS+ floor. Although the conditions under which avoidance behavior are typically studied are quite different, a similar explanation (i.e., minimal contact with the avoidance CS) has been used to understand why avoidance conditioning is notoriously slow to extinguish (Solomon and Wynne, 1953). Second, there were dose-dependent effects of alcohol given during CS+ trials on locomotor activity during CS − saline trials in LAP mice. One possibility is that high doses of alcohol had delayed after effects that decreased locomotor activity, and that these effects were still measurable 24 h after administration. Decreased spontaneous locomotor activity has been seen following alcohol withdrawal (Files, 1994), including following a 4 g/kg ip alcohol injection in mice selected from the same population as HAPs and LAPs (Crabbe et al., 1994). Although few if any studies have assessed whether these effects are present 24 h after alcohol, the locomotor activity findings during CS − trials in the present study are consistent with some form of alcohol withdrawal in LAP mice. This would be consistent with previous findings of a genetic correlation in mice between low alcohol consumption and high susceptibility to alcohol-mediated handling induced convulsions (often used as an index of alcohol withdrawal), and vice versa (Li et al., 1993). Further studies will need to be performed to investigate the possibility that both aversive after effects and appetitive immediate effects contribute to alcohol motivation in this assay when high doses are used. Notably, this interpretation of the present results would be consistent with theories in the field speculating that both factors contribute to alcohol's reinforcing effects (Koob et al., 1998). However, place preference in both lines at 1.5 g/kg is unlikely to be affected by any interaction with withdrawal-based aversive motivation, because this low dose is unlikely to produce alcohol withdrawal. Therefore, much of the present data can be interpreted uniquely as an assessment of alcohol's rewarding effects.

Along with preference conditioning, the locomotor response to alcohol was also measured in the present study, at least for the first 5 min after injection on each CS+ conditioning trial. The low dose of alcohol, 1.5 g/

kg, initially produced locomotor activation, while both 3- and 4-g/kg doses produced locomotor sedation. Consistent with a previous report using HAPs and LAPs (Grahame et al.), no line differences were seen in either the locomotor activating or sedative effects of alcohol following the first injection. Because the locomotor response to alcohol was measured repeatedly, we were also able to measure changes in the response to alcohol over trials, allowing some assessment of whether mice showed tolerance or sensitization to both the sedative and activating effects of alcohol. Interestingly, the locomotor response of LAP mice receiving 3 g/kg appeared to shift, from locomotor sedation after the first injection to locomotor activation after the fourth injection. Such a shift is consistent with sensitization to the locomotor-activating effect of alcohol, and/or tolerance to the sedative effect. To the extent that this shift is caused by sensitization, it is inconsistent with a previous finding showing that HAP mice are more likely to sensitize to alcohol's activating effects than LAP mice (Grahame et al.). Conclusions based on the present study are limited, however, by the short duration of measurement of alcohol's effects on each trial, and by the fact that all mice were never tested at a common dose of alcohol. Indeed, in the previous study, line differences did not emerge until all dose groups were tested at 2 g/kg (Grahame et al., 2000).

In summary, LAP mice showed greater conditioned place preference than HAP mice at 4 g/kg, but lines did not differ at lower doses. This finding shows that LAP mice may be more rewarded than HAP mice by alcohol's pharmacological effects. The possibility also exists that the greater preference in LAP mice may be mediated by a withdrawal-based aversion of the CS− floor. The present findings cannot distinguish between these explanations. Given that LAP mice do not drink pharmacologically meaningful quantities of alcohol (Grahame et al., 1999b), one might conclude that the present findings are inconsistent with their low alcohol intake. On the other hand, if LAP mice do form a place aversion to the CS− floor, they may avoid alcohol consumption because it induces withdrawal (i.e., low drinking and high withdrawal susceptibility are related (Metten et al., 1998)). Finally, low drinking in LAP mice may be related either to conditioned or unconditioned alcohol taste avoidance, preventing these mice from encountering alcohol's rewarding, postingestive effects. Similar explanations have been proposed previously to explain why the low-drinking DBA/2J inbred strain demonstrates alcohol reward using assays that do not depend on drinking (Grahame and Cunningham, 1997). Future studies would have to address whether differences in alcohol taste hedonics exist between HAP and LAP mice. Together, these data indicate the usefulness of multiple measures of alcohol's rewarding effects when seeking to understand the genetics of alcohol reward.

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