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ABSTRACT

Background: Selectively bred rodent lines are valuable tools for investigating gene x environment interactions related to risk for alcoholism in humans. Early maternal environment is one particular factor known for critically influencing neural, hormonal, and behavioral outcomes in adulthood. Cross-fostering is a procedure that may be used to explore the role of genotype-dependent maternal influences on phenotypic variability in adulthood. The purpose of these experiments was to examine the effects of cross-fostering on free-choice alcohol drinking and correlated responses to selection for alcohol preference in mice selectively bred for high- (HAP2) and low- (LAP2) alcohol preference.

Methods: Mice were assigned to one of the following treatments: SHAM (pups that were fostered to their original biological mother), IN (pups that were fostered to a different mother of the same line), and CROSS (pups that were fostered to a mother of a different line). Mice were tested in adulthood for (1) free 24-hr access to alcohol for a period of 28 days; (2) expression of the acoustic startle response and fear-potentiated startle (FPS) and (3) handling-induced convulsions (HICs) during acute alcohol withdrawal.

Results: Overall, the expression of the alcohol preference selection phenotype was robust in all groups (HAP2>LAP2). Cross-fostering produced a moderate but significant reduction in g/kg alcohol drinking and preference scores in HAP2 mice (CROSS<SHAM) but had no effect in LAP2 mice. Cross-fostering did not affect the expression of correlated responses to selection: acoustic startle response (HAP2>LAP2), FPS (HAP2>LAP2), HICs (LAP2>HAP2).

Conclusions: It appears that maternal environment can modify the expression of the high alcohol preference phenotype in HAP2 selectively bred mice. These results suggest a gene x environment interaction with respect to the expression of the high alcohol preference selection phenotype but not correlated responses to selection.

Key Words: alcohol drinking, cross-fostering, fear-potentiated startle, alcohol withdrawal, selected lines

INTRODUCTION

Alcoholism is a complex psychiatric disorder influenced by both genetics and environment (Sher et al., 2010; van der Zwaluw and Engels, 2009). The investigation of gene-environment interactions related to the risk for alcoholism has been facilitated through the use of genetic rodent models of alcohol drinking behavior (see review by Crabbe et al., 2010). Bidirectional selective breeding projects have successfully produced many high- and low-drinking rodent lines ever since the first pair of selected rat lines were developed over 30 years ago (Mardones and Segovia-Riquelme, 1983). These selectively bred rodent lines have served as useful tools to identify specific genes (e.g., Bice et al., 2011), as well as biological and behavioral traits (Crabbe et al., 2010; Murphy et al. 2002), known as genetically correlated responses to selection, that are associated with the alcohol drinking phenotypes in these lines.

As with any selectively bred model, differences between the lines are primarily attributed to changes in gene frequencies at loci relevant to the selection phenotype (Falconer and MacKay, 1996). Further, any identified correlated responses to selection provide evidence that both the selected trait and the correlated trait(s) are regulated by a set of shared genes, which is commonly referred to as pleiotropy. The interpretation that correlated responses to selection reflect pleiotropic influence is strengthened when the correlated responses are replicated in more than one pair of lines selected for the same phenotype (Crabbe et al., 1990). However, as with any genetically-influenced trait, the expression of a selection phenotype, as well as its correlated responses, can be influenced by environmental variables (Phillips et al., 2002). Thus, selected rodent lines provide an excellent model to explore how genetic predisposition toward high or low alcohol drinking and their correlated traits may be modified by environmental factors (e.g., Chester et al., 2004a; 2004b; 2005).

Early pre-weaning environmental factors, such as those associated with mother-pup contact, have been shown to critically influence neural, hormonal and behavioral functions in rodents (Meaney, 2001). Some of the best evidence for these influences comes from studies in

which brief, daily separation of pups from their mother during the pre-weaning period produced changes in a variety of measures, including reactivity to stress (Meaney et al., 1989), hypothalamic-pituitary-adrenal (HPA)- axis functioning (Meaney et al., 1992), neurotransmitter receptor levels (Ognibene et al., 2008), and propensity to consume alcohol and other drugs in adulthood (e.g., Jaworski et al., 2005; van der Veen et al., 2008b).

In addition to maternal separation techniques, cross-fostering is a procedure used to explore the role of genotype–dependent maternal influences on phenotypic variability in adulthood (Bartolomucci et al., 2004; Caldji et al., 2000). There are very few reports of cross-fostering effects on alcohol drinking behavior in genetic animal models. This paucity of data is somewhat curious given that the cross-fostering technique can provide valuable information about gene x environment interactions related to alcohol drinking behavior that have been difficult to disentangle in human adoption studies (Sher et al., 2010). An early study by Rodgers and McClearn (1962) found no effect of cross-fostering on alcohol preference in C57BL (a relatively high alcohol-consuming strain) and A (a relatively intermediate alcohol-consuming strain) mice. Subsequent studies reported increased alcohol intake in low-consuming mouse strains cross-fostered to C57BL dams (Komura et al., 1972; Randall and Lester, 1975a). In the Komura et al. (1972) study, DBA/2 and KR mice cross-fostered to C57BL dams showed increased alcohol intake but cross-fostered C57BL mice showed no change in alcohol intake. Randall and Lester (1975a) used a split-litter design and found similar findings to that of Komura et al. (1972). It should be noted that, in the Randall and Lester (1975a) study, mothers had access to alcohol solutions during lactation and it is not clear if this was the case in the Komura et al. (1972) study. However, using an ova transfer procedure and alcohol-naïve dams, Randall and Lester (1975b) again found increased alcohol intake in DBA/2 mice raised by C57BL dams but no intake change in C57BL mice raised by DBA/2 dams. Finally, there are two other reports in which reciprocal F1 hybrid mice were used to explore maternal strain effects on alcohol consumption in the genetically identical pups. Bachmanov et al. (1996) showed that F1 hybrids

reared by high alcohol preferring C57BL/6ByJ mothers drank more alcohol than pups reared by low alcohol-preferring 129/J mothers. Gabriel and Cunningham (2008) found no effects of maternal strain on free-choice alcohol consumption in F1 hybrids from DBA/2J and C57BL/6J strains but did find that the C57-reared pups drank more alcohol than DBA-reared pups under a forced alcohol exposure condition.

The purpose of the present study was to examine the effects of cross-fostering on free-choice alcohol drinking behavior and correlated responses to selection in mice selectively bred for high (HAP2 line) or low (LAP2 line) alcohol preference. The replicate HAP/LAP mouse lines have been tested for many different correlated responses to alcohol preference, including sensitization to the locomotor-stimulant effects of alcohol (Grahame et al., 2000), alcohol-induced conditioned-taste aversion (Chester et al., 2003) and place preference (Grahame et al., 2001), acoustic startle responses and prepulse inhibition during acute alcohol withdrawal (Chester and Barrenha, 2007), acoustic startle responses and fear-potentiated startle (FPS) (Barrenha and Chester, 2007; Barrenha et al., 2011), delay discounting/impulsivity (Oberlin and Grahame, 2009), and handling-induced convulsions (HICs) during withdrawal from chronic alcohol exposure (Lopez et al., 2011). In the current study, in addition to alcohol drinking behavior, we examined cross-fostering effects on the expression of acoustic startle responses, FPS, and HICs during acute alcohol withdrawal in HAP2 and LAP2 mice.

MATERIALS AND METHODS

Subjects

Subjects were adult male and female replicate 2 HAP and LAP mice from the 27th and 35th (LAP2 only) generation of selection. HAP and LAP lines were produced by mass selection from outbred HS/Ibg mice (Boulder, CO, USA) at the Indiana Alcohol Research Center (IARC) in Indianapolis, IN, USA (Grahame et al. 1999). HAP2 and LAP2 breeder pairs used in the current study were experimentally naïve at the time of breeding and were generated at Purdue

University from breeder pairs that were originally obtained from the IARC. One male and one female of each line were paired for a total of 7 days. On the 8th day, males were removed from cages and females were kept individually-housed and checked for pregnancies during cage-change procedures (once per week until parturition and fostering procedures occurred).

After fostering procedures occurred (described next), mice were housed in polycarbonate cages (29.2 x 19.0 x 12.7 cm) with aspen wood shavings. Ambient temperature in the colony rooms ranged from 20.2-21.9ºC and animals had free-access to food (Rodent Lab Diet 5001, Purina Mills Inc., St. Louis, MO, USA) and water in the home cage. Experimental procedures were conducted during the light phase of a 12:12 light/dark cycle.

Experiments were carried out in accordance with the principles of laboratory animal care and all procedures were approved by the Purdue Animal Care and Use Committee.

Fostering Procedures

After births were recorded, entire HAP2 and LAP2 litters (litters were not split) were assigned to one of the following treatments: a sham-fostered (SHAM) group that consisted of pups that were fostered to their original biological mother; an in-fostered group (IN) that consisted of pups that were fostered to a different mother of the same line; and a cross-fostered group (CROSS) that consisted of pups that were fostered to a mother of a different line. SHAM and IN groups were included as control groups for possible non-specific effects of cross-fostering (e.g., brief maternal separation, development with a non-biological parent of the same line) on behavior, as recommended by Randall and Lester (1975a).

Fostering procedures occurred within 12-24 hrs of birth. During this period, entire litters were separated from their biological mother and rolled in bedding that contained urine and feces of their prospective foster dam. This procedure lasted no more than 3 min per cage. Litters were not culled or split across treatment groups due to the short time frame to foster litters following birth as well as limitations in the number of pups available for fostering that were born

within the given time range. Pups were weaned with their littermates at 21-23 days old into same-sex cages in groups of 2-4 per cage.

Analysis of variance (ANOVA) revealed that HAP2 litters were larger than LAP2 litters [$F(1,56)=4.3$, $p<0.05$]. The average number of pups for HAP2 dams was 9.1±0.4 and for LAP2 dams was 7.7±0.6. Litter size ranged from 3 to 13 for HAP2 mice and 1 to 14 for LAP2 mice. There were, on average, 4.0±0.3 male and 4.2±0.4 female pups per HAP2 litter and 3.3±0.5 male and 3.6±0.4 female pups per LAP2 litter. Numbers of dams and average number of pups per dam represented in each fostering group (combined for all experiments) are as follows: for HAP2 mice, SHAM=10 dams/7.4±0.9 pups; IN=8 dams/9.9±0.5 pups; CROSS=9 dams/8.4±0.7 pups, and, for LAP2 mice, SHAM=6 dams/10.0±0.4 pups; IN=11 dams/5.4±0.8 pups; CROSS=9 dams/6.8±0.8 pups. There were no differences in pup survival rate between lines [Fs>0.1, NS] or fostering groups [Fs>4.2, NS] at postnatal day 7 or weaning.

Drugs

For the alcohol drinking study, alcohol was diluted from a 95% (v/v) solution to a concentration of 10% with tap water. For the HICs study, alcohol was diluted from a 95% (v/v) solution to a concentration of 20% (v/v) with physiological saline (0.9%). Alcohol was administered with an intraperitoneal (IP) injection in a dose of 4.0 g/kg of body weight (BW) and in a volume of 25.3 milliliters/kg/BW.

Startle Apparatus

FPS was assessed using two dark, sound-attenuated Coulbourn Instruments (Allentown, PA, USA) Animal Acoustic Startle System chambers; each startle chamber contains four weight-sensitive platforms. Startle stimuli consisted of 100 dB, 40 ms white noise bursts of frequency range 20 Hz-20 kHz. All subjects were placed individually into open-air holders (8 x 8 x 16 cm) with metal rod floors (rod diameter 0.5 cm, each rod separated by 1.0 cm). The holders rested

on top of the weight-sensitive platforms during acoustic startle test sessions. Startle responses were measured as the amount of force in grams exerted against a weight-sensitive platform during the 200 ms after the onset of each acoustic stimulus. The force measurement does not include the subject's BW. A ventilating fan provided continuous 70-71 dB background noise. The holders were cleaned with a 70% alcohol solution between each mouse.

Study Procedures

Experiment 1: Effects of cross-fostering on free-choice alcohol drinking

Forty-two HAP2 male (SHAM, n=14; IN, n=14; CROSS, n=14), 46 HAP2 female (SHAM, n=16; IN, n=14; CROSS, n=16), 40 LAP2 male (SHAM, n=14; IN, n=12; CROSS, n=14), and 41 LAP2 female (SHAM, n=13; IN, n=14; CROSS, n=14) mice were exposed to a 24-hr free choice drinking procedure for 28 days. Experiment 1 was conducted across 3 balanced replications. Before alcohol access began, mice were individually-housed for 7 days with two 25-ml graduated cylinders fitted with stainless steel sipper tubes containing tap water on the home cage. Mice were 61-78 days old at the time of individual-housing. On day 1 of alcohol access, one of the water cylinders was replaced with a cylinder containing the 10% alcohol solution. Cylinders were read while on the cage and fluid intake was measured to the nearest 0.5 ml every 24 (days 1-8) or 48 (days 10-28) hrs, after which mice were weighed and fluid replaced. Cylinders were alternated every day that fluids were recorded to avoid the influence of a possible positional preference.

Experiment 2: Effects of cross-fostering on FPS

Fifty-seven HAP2 male (SHAM, n=18; IN, n=25; CROSS, n=14), 66 HAP2 female (SHAM, n=19; IN, n=23; CROSS, n=24), 44 LAP2 male (SHAM, n=9; IN, n=19; CROSS, n=16), and 48 LAP2 female (SHAM, n=22; IN, n=13; CROSS, n=13) mice were exposed to a fear-conditioning (FC) session followed 24 hrs by a FPS test session. Experiment 2 was conducted

across 4 balanced replications. Mice were 62-78 days old at time of FC. Fear-conditioning sessions began with 5-min of habituation followed by 10 trials [2-min inter-trial interval (ITI)] of 100 dB (40 msec) startle stimuli and then by 40 conditioning trials, as previously described in Barrenha et al. (2011). Briefly, each conditioning trial consisted of a 30-sec, 7 W light stimulus paired with a 0.5-sec, 0.8 mA foot shock that occurred during the last 0.5 sec of the light stimulus presentation. The FPS test session occurred 24 h after FC. Mice were weighed and placed in the apparatus for 5-min followed by 36 total trials (2-min ITI) presented on a random schedule (range: 12-108s) to reduce habituation to any single trial type. Twelve of the trials were blank (no stimuli), 12 were noise-alone (100dB, 40ms), and 12 were light (7W, 30 s) + noise (100 dB, 40ms). On light + noise trials, the noise stimulus was presented immediately after the light stimulus ended.

Experiment 3: Effects of cross-fostering on HICs

Fifty-three HAP2 male (SHAM, n=16; IN, n=22; CROSS, n=15), 56 HAP2 female (SHAM, n=14; IN, n=21; CROSS, n=21), 38 LAP2 male (SHAM, n=7; IN, n=18; CROSS, n=13), and 42 LAP2 female (SHAM, n=16; IN, n=13; CROSS, n=13) mice were assessed for HICs (baseline) and immediately afterwards weighed prior to an IP 4.0 g/kg IP alcohol injection. Subjects used in this study were alcohol-naïve and had been previously tested for FPS 14 days prior in experiment 2. Mice were 77-93 days old at the time of HIC testing. HIC scores were assessed at 4, 6, 8, 10, and 12 hrs following the alcohol injection using a 0-7 point rating scale, as previously described (Kosobud and Crabbe, 1986). HIC scores for this study did not exceed a score of 4. Scores were averaged from two raters who were blinded to fostering group assignments. The inter-rater reliability coefficient for this study was r=0.94.

Statistical Analyses

Alcohol intake was expressed as g of 10% alcohol per kg of BW and as percent alcohol preference (ml 10% alcohol solution/ml total fluid consumed). Alcohol and water intakes were averaged across 2-day blocks to reduce day-to-day variability in drinking patterns. Prior to averaging data across days, scores on individual days were examined for outliers, most likely due to accidental fluid loss. A value was considered an outlier if it was more than two standard deviations away from 1) the mean intake for that subject across the entire 28-day drinking period and 2) the mean intake for that day across subjects in a group. If these two conditions were satisfied, the value was then subjected to the Dixon Extreme Score Test (Dixon, 1950). If the value passed the Dixon test it was replaced with an intake value obtained by averaging intake of that individual subject on the days before and after the outlier value. There was one instance where an alcohol drinking tube leaked on the first day of alcohol access and this missing value was replaced by the mean intake for all animals in that subgroup on that day. Valid outliers occurred 12 times (8 alcohol values and 4 water values) during the entire 28-day drinking period, representing approximately 0.1% of the entire data set.

Acoustic startle responses for each mouse on the 12 noise-alone and light + noise trials were averaged. FPS was analyzed using a proportional change score, termed % FPS, calculated with the following formula: [((average startle amplitude on light + noise trials - average startle amplitude on noise-alone trials)/average startle amplitude on noise-alone trials) x 100]. The % FPS measure adjusts for individual and group differences in startle reactivity and is an accurate and sensitive measure of FPS (Walker and Davis, 2002). Fourteen of 229 mice were removed from Experiment 2 (HAP2: 2 SHAM, 3 IN, 6 CROSS; LAP2: 0 SHAM, 1 IN, 2 CROSS) because their startle responses across all noise-alone and light + noise trials did not reach a minimum startle response criterion of 11 grams of force.

HIC scores were analyzed as area under the withdrawal curve (AUC) that was calculated using GraphPad Prism software version 5.30 (GraphPad Software, San Diego, California, USA) for the 6 scores measured during the entire 12-hr period.

All data were analyzed using ANOVA with the significance level set at $p<0.05$. Between-group factors included Line, Sex, Fostering Group and within-group factors included 2-Day Blocks (2-day drinking averages). Significant main effects, highest order interactions, and interactions with Fostering Group are reported. Interactions with Fostering Group were followed using lower-order ANOVAs, t-tests and Tukey's multiple comparison tests (Keppel, 1991), where applicable.

RESULTS

Experiment 1: Effects of cross-fostering on free-choice alcohol drinking

BW

Table 1 shows BW data taken on the first day of drinking prior to alcohol exposure. Cross-fostered HAP2 mice showed decreased BW compared to sham-fostered HAP2 mice and males showed greater overall BW than females. There was no effect of fostering procedures on BW in LAP2 mice.

A Line x Sex x Fostering Group ANOVA yielded a main effect of Sex [$F(1,157)=202.9$, $p<0.01$; male > female] and a significant Line x Fostering Group interaction [$F(2,157)=7.1$, $p<0.01$]. Follow-up one way ANOVAs showed a significant main effect of fostering group in HAP2 [$F(2,85)=4.1$, $p<0.05$] but not LAP2 mice. Tukey's follow-up analyses in HAP2 mice revealed lower BW in the CROSS than SHAM group ($p<0.05$).

Insert Table 1 about here

Alcohol Intake and Preference

Figure 1 shows g/kg alcohol intake and alcohol preference scores in HAP2 and LAP2 mice collapsed across sex and 2-day blocks because these factors did not interact with Fostering Group. Cross-fostered HAP2 mice showed decreased g/kg alcohol intake and alcohol

preference scores compared to sham-fostered HAP2 mice. In addition, in-fostered LAP2 mice showed significantly greater alcohol preference scores compared to cross-fostered LAP2 mice.

The ANOVA on g/kg alcohol intake (Line x Sex x Fostering Group x 2-Day Blocks) yielded main effects of Line [$F(1,157)=576.9$, $p<0.01$; HAP2>LAP2], Sex [$F(1,157)=20.3$, $p<0.01$; female>male], Fostering Group [$F(2,157)=4.3$, $p<0.05$], 2-Day Blocks [$F(13,2041)=49.9$, $p<0.01$], and a significant Line x Sex x Fostering Group x 2-Day Blocks [$F(26,2041)=1.6$, $p<0.05$] interaction. This 4-way interaction was followed up by lower-order 3-way ANOVAs (Sex x Fostering Group x 2-Day Blocks) conducted within each line. Main effects of 2-Day Blocks, due to an increase in drinking over the 28-day period [$Fs>7.9$, $Ps<0.01$], and Sex [$Fs>6.2$, $Ps<0.05$; female>male], were found for both HAP2 and LAP2 lines. In addition, a main effect of Fostering Group was found for HAP2 [$F(2,82)=3.8$, $p<0.05$] but not LAP2 mice. Tukey's post-hoc analyses in HAP2 mice revealed lower g/kg alcohol intake in CROSS than SHAM groups ($p<0.05$).

The ANOVA on preference scores (Line x Sex x Fostering Group x 2-Day Blocks) yielded main effects of Line [$F(1,157)=590.2$, $p<0.01$; HAP2>LAP2], Sex [$F(1,157)=7.7$, $p<0.01$; female>male], Fostering Group [$F(2,157)=6.0$, $p<0.01$], 2-Day Blocks [$F(13,2041)=61.3$, $p<0.01$], and significant interactions of Line x Fostering Group [$F(2,157)=3.6$, $p<0.05$] and Line x Sex x 2-Day Blocks [$F(13,204)=2.1$, $p=0.01$]. The Line x Fostering Group interaction was investigated with analyses of Fostering Group within each line, revealing significant main effects of Fostering Group for both HAP2 and LAP2 lines [$Ps<0.05$]. Tukey's post-hoc analyses in HAP2 mice revealed decreased alcohol preference in CROSS than SHAM groups ($p<0.01$). In LAP2 mice, the IN group showed greater alcohol preference than the CROSS group ($p<0.05$).

Total Fluid Intake

The ANOVA (Line x Sex x Fostering Group x 2-Day Blocks) for ml/kg total fluid intake (data not shown) revealed significant main effects of Sex [$F(1,157)=30.8$, $p<0.01$; female>male]

and 2-Day Blocks [F(13,2041)=14.6, p<0.01] and a Line x Sex x 2-Day Blocks interaction [F(13,2041)=2.1, p=0.01]. This analysis indicates that the fostering group differences in alcohol intake and preference are not due to non-specific changes in total fluid intake as a consequence of fostering manipulations.

Insert Figure 1 about here

Experiment 2: Effects of cross-fostering on FPS

BW

Table 1 shows BW data taken immediately prior to the FPS test session. Cross-fostered mice weighed less than both sham- and in-fostered mice. HAP2 mice weighed more than LAP2 mice and males weighed more than females. ANOVA (Line x Sex x Fostering Group) yielded main effects of Line [F(1,203)=8.7, p<0.01; HAP2>LAP2], Sex [F(1,203)=134.9, p<0.01; male > female], and Fostering Group [F(2,203)=4.1, p<0.05]. Tukey's follow-up analyses revealed lower BW in CROSS than both SHAM and IN groups [Ps<0.05].

% FPS

Figure 2 shows mean (±SEM) % FPS in HAP2 and LAP2 mice, collapsed by sex because no interactions with this factor were found. HAP2 mice showed greater FPS than LAP2 mice, as previously reported (Barrenha and Chester, 2007; Barrenha et al., 2011). The ANOVA (Line x Sex x Fostering Group) indicated a significant main effect of Line [F(1,203)=7.3, p<0.01; HAP2>LAP2].

There were no effects of fostering conditions on magnitude of the acoustic startle response (data not shown). ANOVAs (Line x Sex x Fostering Group) conducted on pre-conditioning startle trials and noise-alone trials revealed increased startle magnitude in HAP2 compared to LAP2 mice [Fs>13.0, Ps<0.01].

Insert Figure 2 about here

Experiment 3: Effects of cross-fostering on HICs

BW

Table 1 shows BW data taken immediately prior to alcohol injection. All mice used in this experiment were previously tested for FPS in experiment 2. There were 13 days between BW measurements collected in experiment 2 and those reported here in experiment 3. As reported in experiment 2, cross-fostered mice weighed less than in-fostered mice and males weighed more than females. ANOVAs (Line x Sex x Fostering Group) yielded main effects of Sex [$F(1,177)=128.1$, $p<0.01$; male > female], and Fostering Group [$F(2,177)=4.1$, $p<0.05$]. Tukey's follow-up analyses revealed lower BW in the CROSS than in the IN group ($p<0.01$).

HIC AUC

Figure 3 shows AUC for HIC scores in HAP2 and LAP2 mice. AUC was greater in LAP2 than in HAP2 mice. Analysis of AUC (Line x Sex x Fostering Group) revealed a significant main effect of Line [$F(1,177)=8.1$, $p<0.01$; LAP2>HAP2] only.

Insert Figure 3 about here

DISCUSSION

Selectively bred rodent models are powerful tools to facilitate the identification of genetic determinants of alcohol drinking behavior (Crabbe et al., 2010; Grahame, 2000). Data from these models indicate pleiotropic influences of alcohol drinking genes on other traits, known as correlated responses to selection, which have provided many clues about the underlying mechanisms that contribute to alcohol drinking behavior (e.g., Murphy et al., 2002). It is well known that the expression of alcohol-related phenotypes reflects a complex interplay between

genetic and environmental variables (Phillips et al., 2002, Sher et al., 2010). A major source of environmental variance can occur during the pre-weaning period and various factors, such as quality and quantity of mother-pup contact, have been linked with propensity to consume alcohol in adulthood (e.g., Jaworski et al., 2005). The present study examined the effects of maternal environment, using a cross-fostering procedure, on the expression of genetic predisposition toward free-choice alcohol drinking behavior and genetically correlated responses in a selectively bred mouse model. The main finding of this study is that cross-fostering reduced alcohol drinking/preference in HAP2 mice but did not change alcohol drinking behavior in LAP2 mice. No significant effects of cross-fostering were found on the expression of correlated responses to selection: acoustic startle responses, FPS, and HICs during acute alcohol withdrawal.

To our knowledge, this is the first report in which fostering manipulations were tested in a selectively bred mouse model for genetic predisposition toward alcohol drinking behavior. The finding that cross-fostering decreased alcohol drinking in the high alcohol-preferring HAP2 line but did not increase drinking in the LAP2 line is inconsistent with prior studies in which mouse pups fostered by high-alcohol- preferring C57 mothers drank more alcohol compared to relevant control groups (see introduction). It is well known that mouse maternal behavior is strongly influenced by genes (Carlier et al., 1982) and genetic differences in maternal behavior across inbred mouse strains can influence the expression of the pups' phenotypes (Caldji et al., 2000). The reduced drinking behavior in HAP2 mice suggests that perhaps some aspect of maternal behavior in the LAP2 dams serves to protect against high alcohol drinking behavior. For example, higher levels of licking and grooming were shown to be associated with reduced alcohol and cocaine intake in rats (Francis and Kuhar, 2008). The mechanism for this effect remains unclear but much evidence suggests that maternal behavior may alter pups' behavior through epigenetic mechanisms (Hager et al., 2009; Meaney, 2001). Future studies that include

assessments of maternal behavior are necessary to elucidate the basis for the observed effects in this study.

Although cross-fostering did not alter alcohol drinking behavior in LAP2 mice, it is interesting to note that the IN group showed significantly higher alcohol preference than the CROSS group. One possibility is that this finding is simply an anomaly, because alcohol preference in the LAP2 CROSS group did not statistically differ from the SHAM group and the similar group differences in g/kg intake did not reach statistical significance (see Figure 1). Another possibility is that LAP2 mothers' behavior towards adopted pups depends on the genotype of the pups. For example, it has been shown that genotype of the pup can influence maternal behaviors and the amount of provisioning provided toward the pups (Hager and Johnstone, 2006; van der Veen 2008a) although other studies in mice have not found such effects (e.g., Bartolomucci et al., 2004). Thus, LAP2 maternal behaviors may have increased alcohol preference in adopted LAP2 pups (LAP2 IN group) and decreased alcohol preference in adopted HAP2 pups (HAP2 CROSS group).

It is important to note that the present findings might have been influenced by effects of cross-fostering procedures or limitations of the experimental design rather than, or in addition to, mechanisms related to maternal strain. For example, variations in litter size within and between lines could have affected behavioral and physiological variables in mothers and pups. One such variable could be changes in mothers' endocrine function related to differences in foster vs. biological litter size or due to general stressful effects of the cross-fostering procedure. Changes in circulating levels of maternal corticosterone, the primary hormone secreted by the adrenal glands in response to physical or emotional stressors, are reflected in milk and can alter developmental outcomes in the offspring. Interestingly, moderately increased levels of maternal corticosterone during lactation in rodents have been shown to improve performance in learning tasks (Catalani et al., 2000), decrease anxiety-related behavior (Catalani et al., 2000), and alter dopaminergic functioning (Moles et al., 2004). We have data in HAP2 and LAP2 mice

indicating that LAP2 mice show greater stress-induced release of corticosterone than HAP2 mice (Chester et al., 2012-abstract). Thus, it is possible that HAP2 pups were exposed to greater amounts of stress-related LAP2 maternal corticosterone which resulted in their decreased alcohol drinking behavior in adulthood. This potential mechanism warrants further study.

Cross-fostering effects on BW were also found in this study. In Experiment 1, cross-fostered HAP2 mice weighed less than sham-fostered HAP2 mice whereas in Experiment 2, cross-fostered mice from both the HAP2 and LAP2 lines weighed less than the sham- and in-fostered control groups. Results of Experiment 3, conducted 13 days later in the same mice from Experiment 2, showed that the cross-fostered HAP2 and LAP2 mice still weighed less when compared to the in-fostered groups but not when compared to the sham-fostered groups. One possible factor that could be related to these effects is that HAP2 litters were significantly larger than LAP2 litters. This difference in litter size might explain reduced BW in cross-fostered HAP2 mice (e.g., lower milk output in LAP2 mothers) but it doesn't fit with the reduced BW in cross-fostered LAP2 mice. Cross-fostering has been shown to produce varied effects on pups' BW, most likely due to the influence of complex interactions between genetic and environmental variables, such as maternal strain (e.g., Gabriel and Cunningham, 2008), body weight of the mother (van der Veen et al., 2008a), and pups ability to obtain milk (Drewett, 1983). It is not entirely clear how our results fit with this complex literature. In general, our data suggest that cross-fostering HAP2 and LAP2 pups to mothers of a different line affect some aspect of their development, as measured by BW.

Another goal of the present studies was to investigate how cross-fostering procedures would affect the expression of correlated responses to selection for high or low alcohol preference. We have previously reported a robust and reliable genetic correlation between alcohol preference and anxiety-related behaviors; specifically, acoustic startle responses and FPS. HAP mice show greater ASR (Barrenha and Chester, 2007; Chester and Barrenha, 2007)

and FPS (Barrenha and Chester, 2007; Barrenha et al., 2011; Powers et al., 2010) than LAP mice. These positive genetic correlations were replicated in the current study in HAP2 and LAP2 mice but cross-fostering did not significantly alter the expression of these behaviors.

This study is the first to report a negative genetic correlation between alcohol preference and acute alcohol withdrawal, as measured by HICs, in the HAP2 and LAP2 lines. This finding agrees with prior unpublished data in HAP1 and LAP1 lines (P. Metten, N.J. Grahame, and J.C. Crabbe) and with the recent report by Lopez et al. (2011) who showed that both replicate 1 and 2 LAP lines display greater HICs during withdrawal from chronic alcohol vapor exposure than HAP lines. These data are also consistent with many findings in other genetic rat and mouse models indicating that rodents with a genetic predisposition toward low alcohol drinking show greater signs of acute alcohol withdrawal (Chester and Barrenha, 2007; Chester et al., 2002, 2003, 2006; Metten et al., 1998). We did not find any effects of cross fostering on the expression of HICs in this study suggesting that maternal environment does not influence the expression of this correlated response to selection.

In summary, cross-fostering produced a moderate but significant reduction in g/kg alcohol drinking and preference scores in HAP2 mice but had no effect in LAP2 mice. Cross-fostering did not affect the expression of correlated responses to selection: acoustic startle response (HAP2>LAP2), FPS (HAP2>LAP2), HICs (LAP2>HAP2). These results suggest a gene x environment interaction with respect to the expression of the high alcohol preference selection phenotype but not correlated responses to selection. The HAP/LAP mouse model may be useful for future studies of the relative contributions of maternal environment on shaping the expression of a genetic predisposition toward high alcohol drinking behavior.

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Table 1. BW (g) in SHAM, IN, and CROSS, male and female, HAP2 and LAP2 mice

Fostering Group	SHAM	IN	CROSS
EXPERIMENT 1			
HAP2 Male	27.7±0.6[a,b]	26.7±0.5[a]	25.2±0.6[a]
HAP2 Female	23.0±0.4[b]	22.5±0.2	21.3±0.5
LAP2 Male	25.7±0.8[a]	26.3±0.3[a]	26.4±0.7[a]
LAP2 Female	21.4±0.4	21.9±0.7	22.1±0.3
EXPERIMENT 2*			
HAP2 Male	26.2±0.4[a,b,c]	25.6±0.6[a,c,d]	25.3±0.3[a,c]
HAP2 Female	23.3±0.7[b,c]	22.9±0.3[c,d]	21.2±0.3[c]
LAP2 Male	25.3±0.8[a,b]	25.3±0.5[a,d]	25.2±0.7[a]
LAP2 Female	21.6±0.6[b]	21.4±0.2[d]	20.2±0.5
EXPERIMENT 3*			
HAP2 Male	27.3±0.5[a]	26.4±0.6[a,e]	25.9±0.3[a]
HAP2 Female	23.2±0.7	23.9±0.5[e]	22.1±0.4
LAP2 Male	26.6±0.8[a]	27.2±0.6[e]	26.5±0.6[a]
LAP2 Female	22.9±0.8	22.8±0.4[e]	21.6±0.6

Experiments 2 and 3 utilized the same subjects; BW data collection was separated by 14 days.

Table 2. Total fluid intake (ml/kg BW) in male and female HAP2 and LAP2 mice in Experiment 1

Fostering Group	SHAM	IN	CROSS
HAP2 Male	248.6±8.2	246.5±9.4	272.3±12.3
HAP2 Female	225.3±10.7[a]	247.0±10.8[a]	242.1±10.5[a]
LAP2 Male	275.0±7.1	276.3±8.6	291.1±8.4
LAP2 Female	290.0±15.0[a]	268.2±10.3[a]	284.3±14.2[a]

Figure Legends

Fig 1A. Mean (±SEM) alcohol intake in g/kg BW collapsed across the entire 28-day drinking period in SHAM, IN, and CROSS HAP2 (left panels) and LAP2 (right panels) mice (collapsed by sex). **Fig 1B.** Mean (±SEM) % preference scores collapsed across the entire 28-day drinking period in SHAM, IN, and CROSS HAP2 (left panels) and LAP2 (right panels) mice (collapsed by sex). *p<0.01; SHAM > CROSS; +p<0.05; IN > CROSS

Fig 2. Mean (±SEM) %FPS in SHAM, IN, and CROSS HAP2 (left panels) and LAP2 (right panels) mice, collapsed by sex.

Fig 3. Mean (±SEM) AUC for HIC scores in SHAM, IN, and CROSS HAP2 (left panels) and LAP2 (right panels) mice, collapsed by sex.

Table Legends

Table 1. Mean (±SEM) BW (g) values for SHAM, IN, and CROSS groups within each line and sex for Experiments 1, 2, and 3. [a]p<0.01, male>female; [b]p<0.05, SHAM>CROSS; [c]p<0.01, HAP2>LAP2; [d]p<0.05, IN>CROSS; [e]p<0.01, IN>CROSS

Table 2. Mean (±SEM) total fluid intake (ml/kg BW) scores for SHAM, IN, and CROSS groups within each line and sex collapsed across the entire 28-day drinking period for Experiment 1. [a]p<0.01; female>male