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Genetic correlation between alcohol preference and conditioned fear: Exploring a functional relationship

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ABSTRACT

Post-traumatic stress disorder (PTSD) and alcohol-use disorders have a high rate of co-occurrence, possibly because they are regulated by common genes. In support of this idea, mice selectively bred for high (HAP) alcohol preference show greater fear potentiated startle (FPS), a model for fear-related disorders such as PTSD, compared to mice selectively bred for low (LAP) alcohol preference. This positive genetic correlation between alcohol preference and FPS behavior suggests that the two traits may be functionally related. This study examined the effects of fear conditioning on alcohol consumption and the effects of alcohol consumption on the expression of FPS in male and female HAP2 and LAP2 mice. In experiment 1, alcohol consumption (g/kg) under continuous-access conditions was monitored daily for 4 weeks following a single fear-conditioning or control treatment (foot shock and no shock). FPS was assessed three times (once at the end of the 4-week alcohol access period, once at 24 h after removal of alcohol, and once at 6–8 days after removal of alcohol), followed by two more weeks of alcohol access. Results showed no change in alcohol consumption, but alcohol-consuming, fear-conditioned, HAP2 males showed increased FPS at 24 h during the alcohol abstinence period compared to control groups. In experiment 2, alcohol consumption under limited-access conditions was monitored daily for 4 weeks. Fear-conditioning or control treatments occurred four times during the first 12 days and FPS testing occurred four times during the second 12 days of the 4-week alcohol consumption period. Results showed that fear conditioning increased alcohol intake in both HAP2 and LAP2 mice immediately following the first conditioning session. Fear-conditioned HAP2 but not LAP2 mice showed greater alcohol intake compared to control groups on drinking days that occurred between fear conditioning and FPS test sessions. FPS did not change as a function of alcohol consumption in either line. These results in mice help shed light on how a genetic propensity toward high alcohol consumption may be related to the risk for developing PTSD and co-morbid alcohol-use disorders in humans.

1. Introduction

Alcohol-use disorders (AUDs) have a high incidence of co-morbidity with post-traumatic stress disorder (PTSD) (Brady, Killeen, Brewerton, & Lucerini, 2000; Kessler et al., 1996). These disorders produce a tremendous impact on the individual and society both in terms of human suffering and economics. A recent study evaluated the incidence of co-morbid AUDs (including both alcohol abuse and alcoholism) and PTSD in Iraq and Afghanistan veterans that utilized the Department of Veterans Affairs health care system from 2001 to 2009 (Seal et al., 2011). Approximately 10% of the veterans had an AUD diagnosis, of which 63% were also diagnosed with PTSD. People with co-morbid disorders represent a special population that suffer greater negative consequences than those with either disorder alone, such as more severe anxiety symptoms, greater alcohol drinking relapse rates, incurrence of higher medical costs, and poorer treatment outcomes (McCarthy & Petrakis, 2010).

There is accumulating evidence that common genetic factors play an important role in the risk for developing co-morbid alcoholism and PTSD. Genetic correlation studies in humans have reported significant overlap in genetic factors contributing to both disorders (Sartor et al., 2011; Scherrer et al., 2008; Xian et al., 2000). Risk for psychiatric disease is determined by complex gene/environment interactions. Exposure to environmental stress (including psychological trauma or a chemical stressor such as alcohol) is

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thought to increase the risk for developing both AUDs and PTSD (McEwen, 2000). A number of studies have reported increased rates of both PTSD and alcohol-use disorders in people exposed to PTSD-related trauma (Engel et al., 1999; Ikin et al., 2004). PTSD has been reported to occur prior to (Davidson, Kudler, Saunders, & Smith, 1990; Forbes et al., 2015; Lopez, Turner, & Saavedra, 2005; McFarlane, 1998), or simultaneously with (Bremner, Southwick, Darnell, & Charney, 1996; Engdahl, Dikel, Eberly, & Blank, 1998), the onset of AUDs, suggesting that trauma may interact with genetic vulnerability to increase risk for developing AUDs. Recent investigations have focused on genes that regulate the hypothalamic-pituitary-adrenal (HPA) axis, such as corticotropin releasing factor type 1 receptor (Crfr1), as important candidates for determining how environmental stress may interact with genotype to influence vulnerability or resiliency to AUDs (Clarke et al., 2008) and PTSD (Mehta & Binder, 2012). Relevant animal models are necessary tools to help define the source and influence of gene/environment interactions on the expression of co-morbid disorders in humans.

We have been utilizing mouse lines selectively bred for high (HAP) and low (LAP) alcohol preference to explore common genetic risk factors for alcohol drinking- and anxiety-related behaviors. Alcohol-naïve HAP lines (1 and 2) show greater fear potentiated startle (FPS), a model for fear-related disorders such as PTSD, compared to LAP lines (Barrenha & Chester, 2007; Barrenha, Coon, & Chester, 2011; Chester, Kirchhoff, & Barrenha, 2014). Fear-conditioning models such as FPS are commonly used to study mechanisms that contribute to fear-related disorders such as PTSD (Kim & Jung, 2006). We also reported that HAP but not LAP lines show an anxiolytic response to alcohol using the FPS procedure (Barrenha et al., 2011). This finding, which is similar to results reported in humans with a family history of AUDs (Sher & Levenson, 1982; Sinha, Robinson, & O'Malley, 1998), suggested that greater sensitivity to the anxiolytic, and therefore, reinforcing, effects of alcohol in people with a genetic propensity toward AUDs may be one of the mechanisms that contributes to the high rate of co-morbidity between AUD and PTSD. These mouse lines also show differences in the HPA axis response to stress (lower corticosterone levels after foot shock and fear-related cues) that correlate with their genetically influenced divergent alcohol-drinking behavior (Chester et al., 2014), and match data reported in humans with PTSD (Yehuda, 2001) and in other rodent models of PTSD-like behavior (Cohen et al., 2006). Overall, our data in these lines suggest they are a unique model for identifying genetic and biological factors that contribute to AUD and PTSD comorbidity in humans.

Genetic correlations suggest that there may be a causal, or functional, relationship between the correlated traits (Crabbe, Phillips, & Belknap, 2010). The purpose of this study was to test this idea in naïve HAP and LAP selected lines, and to explore questions about genetic and environmental interactions that are difficult to control for in humans. For example, if fear conditioning in HAP but not LAP mice increases subsequent alcohol drinking, this result would suggest that exposure to trauma may be a specific risk factor for developing co-morbid PTSD and AUD in people with a family (genetic) history of AUD. This result would also support the previously mentioned human data indicating that PTSD often precedes the onset of AUDs (e.g., McFarlane, 1998), but would also suggest that people with a family history of AUD are especially vulnerable to develop an AUD if they experienced PTSD-inducing trauma.

This study involved two main experiments conducted in male and female HAP2 and LAP2 mice. In the first experiment, we examined 1) the effects of one fear-conditioning session on subsequent continuous-access alcohol drinking, 2) whether alcohol drinking altered the expression of FPS at various times during an alcohol abstinence period, and 3) whether alcohol drinking changed after repeated exposure to FPS testing (exposure to fear-related cues). We hypothesized that alcohol drinking would increase after fear conditioning and testing and that the increased alcohol drinking would subsequently reduce the expression of FPS in HAP2 but not LAP2 mice. In the second experiment, we examined 1) effects of repeated fear-conditioning sessions on the acquisition of limited-access alcohol drinking and 2) effects of repeated FPS testing on the maintenance of limited-access alcohol drinking. We hypothesized that alcohol drinking would increase after repeated exposures to fear conditioning/FPS testing and the increased alcohol drinking, in turn, would reduce the expression of FPS over time.

2. Materials and methods

2.1. Subjects

Subjects were alcohol-naïve adult male and female HAP and LAP mouse lines from replicate 2. The selectively bred HAP2/LAP2 mouse lines were derived from a progenitor population of outbred HS/Ibg mice (Institute of Behavioral Genetics, Boulder, CO) at the Indiana Alcohol Research Center in Indianapolis, IN (Grahame, Li, & Lumeng, 1999). HAP2 and LAP2 mice were from the 27th and 29th (experiment 1) and 31st (experiment 2) generation of selection. Subject representation in each replication was balanced across replicate, line, sex, and litter of origin to the best extent possible. For the purposes of alcohol-drinking measurements, all mice were singly housed in polycarbonate cages (29.2 × 19.0 × 12.7 cm) with aspen wood shavings for 5 (experiment 2)–7 (experiment 1) days prior to the start of fear conditioning. In experiment 1, mice were between 56 and 122 days old at the start of fear conditioning and in experiment 2, mice were 73–86 days old at the start of fear conditioning. Ambient room temperature was maintained at 21 ± 2 °C. Mice had free access to food (Rodent Lab Diet 5001, Purina Mills Inc., St. Louis, MO) and water in the home cage at all times, except when testing procedures took place. Experimental procedures were conducted during the light phase of a 12:12 light:dark cycle (lights off at 19:00).

All experimental procedures were approved by the Purdue Animal Care and Use Committee and were conducted in accordance with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Fear conditioning/startle apparatus

FPS was assessed using two dark, sound-attenuated Coulbourn Instruments (Allentown, PA, USA) Animal Acoustic Startle System chambers, as previously described (Barrenha & Chester, 2007). Foot shock was delivered through a metal rod floor at the base of the open-air animal holders that rested on top of weight-sensitive platforms. Startle stimuli consisted of 100-dB, 40-msec white noise bursts (frequency range: 20 Hz–20 kHz). Subjects' startle responses were measured as the amount of force in grams exerted against the weight-sensitive platform during the 200 msec after the onset of each acoustic stimulus. The force measurement does not include the subject's bodyweight. A ventilating fan provided continuous 71–75 dB background noise.

2.3. General fear conditioning/startle procedures

Mice received fear conditioning (paired light + shock), unpaired light + shock, or no shock treatments. Fear-conditioning sessions began with a 5-min habituation period followed by 10 startle trials of 100-dB [40-msec; 20-sec intertrial interval (ITI)] white noise

bursts (frequency range: 20 Hz–20 KHz) to acclimate mice and reduce their initial startle reactivity. Two minutes later, the first of 40 conditioning trials began with the onset of a 30-sec, 7-W light stimulus paired with a 0.5-sec, 0.8-mA foot shock during the last 0.5 s of the light stimulus presentation [2-min intertrial interval (ITI)]. Mice exposed to unpaired light + shock received the same number of light and shock presentations as the fear-conditioned groups, but these stimuli were explicitly unpaired during each of the 40 2-min intervals (interstimulus range 13–118 s). Mice exposed to no shock treatments received the same number and sequence of light stimuli on the conditioning day and to the light + noise stimuli on the FPS test day.

The FPS test consisted of a 5-min habituation period followed by 36 total trials (2-min ITI) presented on a random schedule (range: 12–108 s) to reduce habituation to any single trial type. Twelve of the trials were blank (no stimuli), 12 were noise alone (100 dB, 40 ms), and 12 were light (7 W, 30 s) + noise (100 dB, 40 ms). On light + noise trials, the noise stimulus was presented immediately after the light stimulus ended. These stimuli parameters were chosen based on our previous work in HAP/LAP replicate lines (Barrenha & Chester, 2007).

2.4. General alcohol-drinking procedures

Alcohol was diluted from a 95% (v/v) solution to a concentration of 10% (v/v) with tap water. Mice were given free choice between tap water and a 10% alcohol solution in tap water under 24-h access conditions. Mice were acclimated to drinking water from two graduated cylinders, and then one water cylinder was removed in an alternating sequence from the left or right position in the cage top and was replaced with a cylinder filled with 10% alcohol solution. Fluid levels were read while the cylinders were on the cage in order to minimize fluid/cage disturbances. All mice were then weighed and fluids were replaced. The left/right position of the water and alcohol cylinders was rotated at each cylinder reading to avoid a location preference.

2.5. Experimental groups and study procedures

Both experiment 1 and 2 had four different experimental groups; the names refer to type of conditioning treatment [paired (FEAR), unpaired (CONTROL), light only (NO SHOCK)] and type of subsequent fluid access (ALCOHOL or WATER)]. The FEAR/WATER and FEAR/ALCOHOL groups received fear conditioning, the CONTROL/ALCOHOL group was exposed to the same number of light and shock presentations except that they were presented randomly during the 2-min ITI, and the NO SHOCK/ALCOHOL group was exposed to the same light and light + noise stimuli except without the shock stimulus. Comparisons between the FEAR/WATER group and the FEAR/ALCOHOL group indicated whether changes in FPS in FEAR/ALCOHOL group were due to alcohol exposure (hypothesized effect) vs. general extinction of conditioned responses due to repeated exposure to the CS during FPS testing sessions. Comparisons between the NO SHOCK/ALCOHOL group and the CONTROL/ALCOHOL and FEAR/ALCOHOL groups indicated whether subsequent alcohol intake changed as a function of fear conditioning specifically or general stress (foot shock) exposures.

Mice were assigned to groups and conditioned/tested in a counterbalanced fashion based on line, sex, litter, and startle platform. Experiments were run in balanced replications due to constraints imposed by the number of subjects to be tested, especially during the drinking portion of the study.

2.5.1. Experiment 1

Mice received one conditioning/control/no-shock session followed 24 h later by one FPS test session. Immediately after the FPS test session, water bottles were replaced with two water-filled 25-mL plastic graduated cylinders for 6–8 days to allow acclimation to the tubes. Then, one water bottle was replaced with a cylinder filled with a 10% v/v alcohol solution and free-choice, 24-h consumption was monitored for 30 days. Fluid intake was measured at the same time on a daily basis for the first 8 days, in order to detect transient effects of FPS conditioning/testing on alcohol intake, and then every other day thereafter until the end of the 30-day period. All mice were then tested for FPS three times: once at the end of the 30-day alcohol-drinking period (Test 2) while alcohol was still continuously available, once at 24 h after removal of alcohol bottles (Test 3), and once at 6–8 days after removal of alcohol bottles (Test 4). Tests 3 and 4 were designed to examine the expression of FPS during both “early withdrawal” and “protracted withdrawal” phases after removal of alcohol bottles. One to three days after the last FPS test, all mice again received free-choice, 24-h access to alcohol and water for 14 days. Fluid intake was again measured daily for 8 days and then every other day until the end of the 14-day period (see Fig. 1).

2.5.2. Experiment 2

This experiment was separated into two phases. During phase 1, mice were exposed to paired (fear-conditioned groups) or unpaired (control groups) presentations of light and shock or to the light alone (no shock group) immediately before 2-h access to alcohol and water. This fear-conditioning/drinking procedure occurred four times over the course of 12 days in phase 1. In between each fear-conditioning/drinking day, mice received two consecutive days of 2-h access to alcohol and water or water only. Phase 2 began the next day after the end of phase 1. During phase 2, mice in all groups received exposures to light and noise (fear cues via FPS tests) immediately before 2-h access to alcohol and water; this procedure occurred four times over the course of the 18 days in phase 2. After each FPS test/drinking day, mice received two consecutive days of 2-h access to alcohol and water only. Limited-access drinking was then monitored daily for an additional 6 days (see Fig. 2). Drinking was measured at 30 min and at the end of the 2-h drinking session.

2.6. Statistical analyses

All 12 startle responses on each trial type (noise alone, light + noise) were averaged for each mouse. Mice that did not

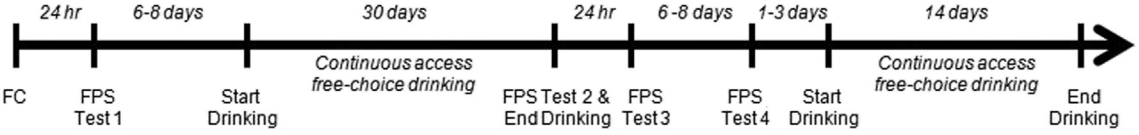

Fig. 1. Schematic illustration of the timeline for experiment 1. Mice had one fear-conditioning session (FC) and four test sessions, and two phases of continuous-access free-choice drinking (water and alcohol or water alone). The first phase of drinking was a 30-day period that started 6–8 days after FPS Test 1 and ended before FPS Test 2. The second stage of drinking was a 14-day period that started 1–3 days after FPS Test 4. Each tick mark represents the start/end of each experimental phase.

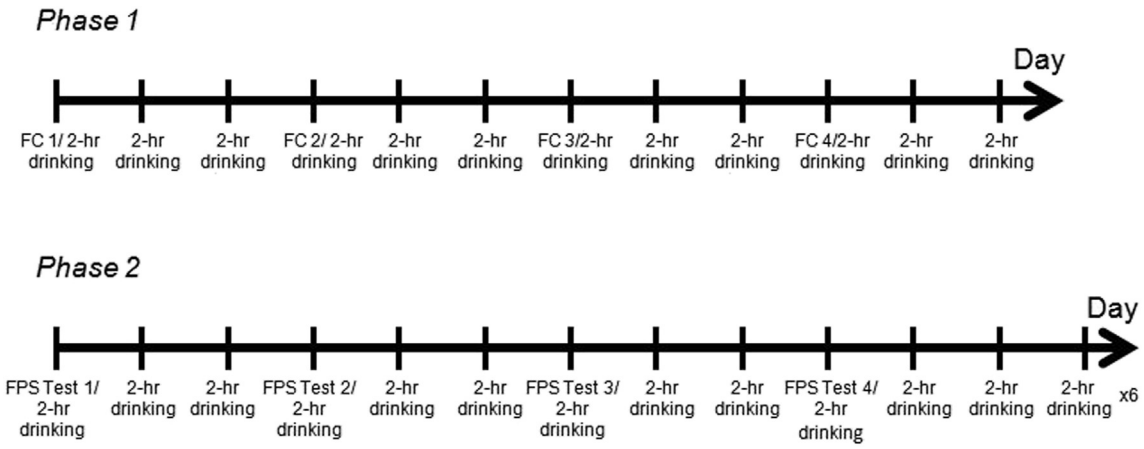

Fig. 2. Schematic illustration of the timeline for experiment 2. In phase 1, mice had four fear-conditioning (FC) sessions immediately followed by 2-h limited access to fluids (water and alcohol or water alone). Two consecutive days of limited-access drinking occurred after each of the four FC sessions. Phase 2 began the next day after the end of phase 1. In phase 2, mice had four FPS test sessions immediately followed by 2-h limited access to fluids. Two consecutive days of limited-access drinking occurred after each of the four FPS test sessions, and six additional days of limited-access drinking were given at the end of phase 2. Each tick mark represents one day.

meet the minimum startle response criterion of 11 g of force were removed from all analyses. In experiment 1, 19 mice [FEAR/WATER group: 4 HAP2 (1M, 3F), 2 LAP2 (F); FEAR/ALCOHOL group: LAP2 (2M, 1F); CONTROL/ALCOHOL group: 2 HAP2 (F), 3 LAP2 (F); NO SHOCK/ALCOHOL group: HAP2 (3M, 1F), 1 LAP2 (F)] were removed because they did not meet the minimal startle response criterion. One LAP2 male from the CONTROL/ALCOHOL group was removed because of a procedure error. No mice were removed from experiment 2. To explore a potential confounding effect of body weight on the magnitude of the acoustic startle reflex, we conducted Pearson correlations between body weight taken on FPS test days and average grams of force measured during the blank, noise alone, and light + noise trials during the FPS tests. No significant correlations were found.

The % FPS measure was obtained using proportional change scores calculated using the following formula: [([startle amplitude on light + noise trials − startle amplitude on noise-alone trials]/ startle amplitude on noise-alone trials) × 100]. The % FPS measure adjusts for individual and group differences in startle reactivity. It also adjusts for potential non-specific drug treatment effects on startle reactivity and thus is indicated to be an accurate and sensitive way to detect selective effects of pharmacological compounds on FPS (Walker & Davis, 2002).

Alcohol intake was expressed as grams of alcohol per kilogram of body weight (g/kg BW). Daily alcohol intake values were examined for outliers using several conservative criteria. A value first had to exceed the mean alcohol intake for that animal and that animal's group by 2 standard deviations. If the value passed these criteria, it was then subjected to the Dixon Extreme Score Test (Dixon, 1950). An alcohol intake value that was considered an outlier was replaced by the mean intake value of the particular group if it was on the first or last day of drinking. If the outlier was not on the first or last day of drinking, it was replaced by the mean intake value of the day before and after the outlier occurred. In experiment 1, 14 data points qualified as outliers, composing 0.17% of the total data points. In experiment 2, 6 of the data points were outliers, composing 0.41% of the total data points.

Data were analyzed using analysis of variance (ANOVA) with Line (HAP2, LAP2), Sex (male, female), and Treatment Group (FEAR/ALCOHOL, FEAR/WATER, CONTROL/ALCOHOL, and NO SHOCK/ALCOHOL) as between-group factors and Test and Days as within-group factors, where applicable. Highest order interactions are reported. Lower-order ANOVAs and Tukey's *t*-test were used to explore interactions and main effects; in some cases, only interactions with the treatment factor were further explored. Probability values less than 0.05 were considered significant.

3. Results

3.1. Experiment 1

3.1.1. FPS data across the 4 tests (Fig. 3)

We first conducted an ANOVA (Line × Sex × Treatment Group) on the % FPS data on Test 1 to ensure that repeated-measures analyses on subsequent tests would not be biased by any initial group differences. As such, it was important to show that % FPS did not differ between the FEAR/WATER and FEAR/ALCOHOL groups prior to the initiation of drinking, as comparison of these two groups was of primary interest. The ANOVA showed a main effect of Treatment Group [$F(3,211) = 22.6$, $p < 0.01$, $\eta_p^2 = 0.241$] and Tukey's *post hoc* indicated this was due to significantly greater % FPS in fear-conditioned groups (FEAR/WATER: $M = 81.3$, $SD = 64.0$; FEAR/ALCOHOL: $M = 61.7$, $SD = 83.1$) compared to the CONTROL/ALCOHOL ($M = -0.6$, $SD = 30.7$) and NO SHOCK/ALCOHOL ($M = 22.7$, $SD = 39.3$) groups, (p values < 0.05), as expected.

Overall ANOVA [Line × Sex × Treatment Group (4) × Test (4)] yielded main effects of Test [$F(3,633) = 5.2$, $p < 0.01$, $\eta_p^2 = 0.024$; Test 1 ($M = 41.2$, $SD = 66.2$), 2 ($M = 37.5$, $SD = 68.5$), 3 ($M = 42.7$, $SD = 74.1$) > Test 4 ($M = 23.5$, $SD = 38.7$)] and Treatment Group [$F(3,211) = 20.8$, $p < 0.01$, $\eta_p^2 = 0.228$]. Tukey's comparisons of treatment groups (collapsed over the four tests) showed greater % FPS (p values < 0.05) in the FEAR/WATER ($M = 57.2$, $SD = 38.0$) and FEAR/ALCOHOL ($M = 51.6$, $SD = 48.0$) groups compared to the CONTROL/ALCOHOL ($M = 16.5$, $SD = 24.4$) and NO SHOCK/ALCOHOL groups ($M = 19.5$, $SD = 22.8$). The ANOVA also yielded interactions: Treatment Group × Test [$F(9,633) = 3.8$, $p < 0.01$, $\eta_p^2 = 0.051$], Line × Sex [$F(1,211) = 4.3$, $p < 0.05$, $\eta_p^2 = 0.020$], and Line × Treatment Group [$F(3,211) = 3.8$, $p = 0.01$, $\eta_p^2 = 0.051$].

Follow-up ANOVAs of the Treatment Group × Test interaction indicated a main effect of Test in the FEAR/WATER [$F(3,162) = 6.2$, $p < 0.01$, $\eta_p^2 = 0.102$], FEAR/ALCOHOL [$F(3,174) = 3.7$, $p < 0.05$, $\eta_p^2 = 0.060$], and CONTROL/ALCOHOL [$F(3,168) = 7.5$, $p < 0.01$, $\eta_p^2 = 0.119$] groups. Fig. 3 shows that the main effects were due to a reduction in % FPS across the four tests in the FEAR/WATER and FEAR/ALCOHOL groups, likely due to extinction. Interestingly, the % FPS measure in the CONTROL/ALCOHOL group increased across the

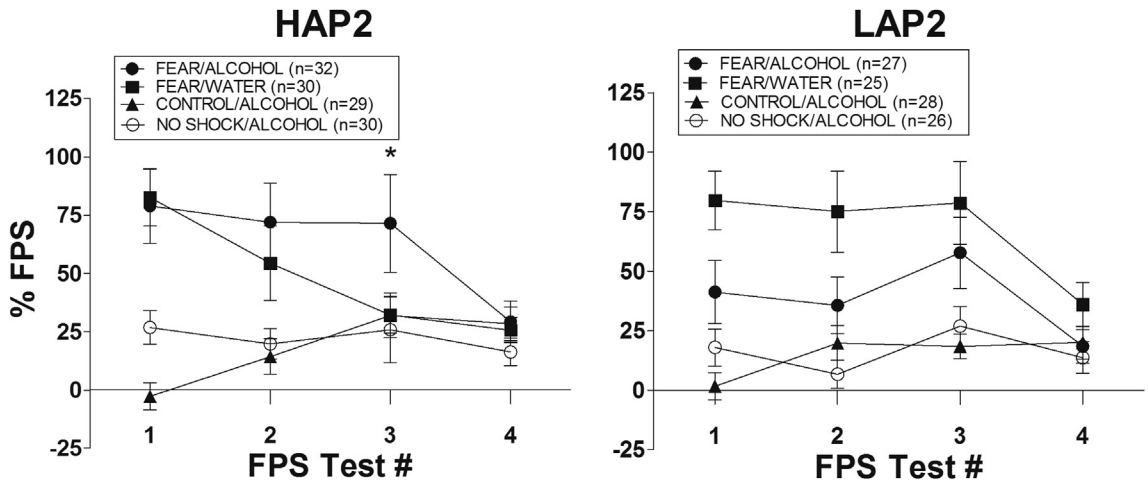

Fig. 3. Mean (±SEM) % FPS across four tests in male and female HAP2 (left panel) and LAP2 (right panel) mice in experiment 1 (collapsed across sex). *% FPS significantly greater in the FEAR/ALCOHOL groups compared to the CONTROL/ALCOHOL and NO SHOCK/ALCOHOL groups (p values < 0.05). HAP2: FEAR/ALCOHOL (16 males and 16 females), FEAR/WATER (15 males and 15 females), CONTROL/ALCOHOL (15 males and 14 females), NO SHOCK/ALCOHOL (14 males and 16 females); LAP2: FEAR/ALCOHOL (11 males and 16 females), FEAR/WATER (12 males and 13 females), CONTROL/ALCOHOL (12 males and 16 females), NO SHOCK/ALCOHOL (13 males and 13 females).

four tests, which could reflect a contextual conditioning effect that produces enhanced startle on the light + noise trials.

Follow-up analyses of Treatment Group within each test yielded Treatment Group effects on Test 1 [$F(3,223) = 22.7$, $p < 0.01$, $\eta_p^2 = 0.234$], Test 2 [$F(3,223) = 8.8$, $p < 0.01$, $\eta_p^2 = 0.106$], and Test 3 [$F(3,223) = 4.3$, $p < 0.01$, $\eta_p^2 = 0.055$], but not on Test 4. Tukey's comparisons of the treatment group effects on Tests 1 and 2 showed greater % FPS in FEAR/WATER (Test 1: $M = 81.3$, $SD = 64.0$; Test 2: $M = 63.8$, $SD = 86.5$) and FEAR/ALCOHOL groups (Test 1: $M = 61.7$, $SD = 83.1$; Test 2: $M = 55.3$, $SD = 82.7$) compared to the CONTROL/ALCOHOL (Test 1: $M = -0.6$, $SD = 30.7$; Test 2: $M = 17.0$, $SD = 39.4$) and NO SHOCK/ALCOHOL (Test 1: $M = 22.7$, $SD = 39.3$; Test 2: $M = 13.7$, $SD = 33.3$) (p values < 0.05). On test 3, % FPS was significantly greater in the FEAR/ALCOHOL groups ($M = 65.2$, $SD = 101.3$) compared to the CONTROL/ALCOHOL ($M = 25.3$, $SD = 41.5$) and NO SHOCK/ALCOHOL ($M = 26.3$, $SD = 62.8$) groups (p values < 0.05). Follow-up ANOVAs of the Line × Sex interaction resulted in a Line effect (HAP2 > LAP2) in males only [$F(1,106) = 4.3$, $p < 0.05$, $\eta_p^2 = 0.039$].

Follow-up ANOVAs of the Line × Treatment Group interaction (collapsed across the four tests) yielded main effects of Treatment Group in both HAP2 [$F(3,117) = 10.5$, $p < 0.01$, $\eta_p^2 = 0.213$] and LAP2 [$F(3,102) = 15.4$, $p < 0.01$, $\eta_p^2 = 0.312$] lines. In HAP2 mice, Tukey's comparisons showed greater % FPS in the FEAR/WATER ($M = 48.7$, $SD = 31.7$) and FEAR/ALCOHOL ($M = 62.9$, $SD = 52.9$) groups compared to the CONTROL/ALCOHOL ($M = 18.0$, $SD = 28.4$) and NO SHOCK/ALCOHOL ($M = 22.2$, $SD = 25.1$) groups (p values < 0.05). However, in LAP2 mice, % FPS was significantly greater in the FEAR/WATER group ($M = 67.3$, $SD = 42.9$) compared to the FEAR/ALCOHOL ($M = 38.3$, $SD = 38.2$), CONTROL/ALCOHOL ($M = 15.0$, $SD = 19.7$), and NO SHOCK/ALCOHOL ($M = 16.3$, $SD = 19.8$) groups. Also in LAP2 mice, % FPS in the FEAR/ALCOHOL group was significantly greater than in the CONTROL/ALCOHOL group (p values < 0.05).

Line analyses within each treatment group showed a Line effect (HAP2 > LAP2) in the FEAR/ALCOHOL group only [$F(1,57) = 4.1$, $p < 0.05$, $\eta_p^2 = 0.067$].

3.1.2. Alcohol drinking after FPS test 1 (Fig. 4)

Separate analyses of days 1–8 and 9–30 showed similar results. Thus, the first eight daily readings were averaged into four 2-day scores and included in the overall repeated-measures ANOVA with the subsequent 11 2-day scores across the 30 days of drinking. The ANOVA of g/kg alcohol intake [Line × Sex × Treatment Group

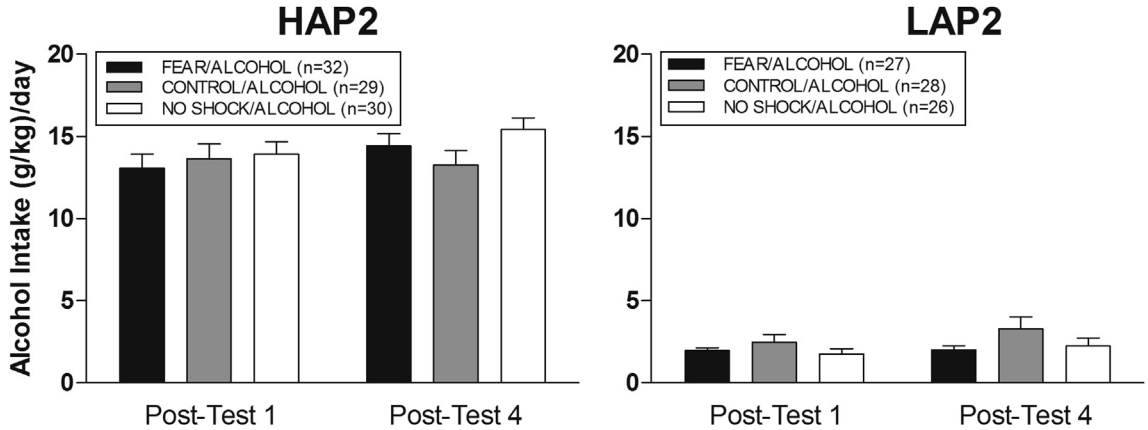

Fig. 4. Mean (±SEM) g/kg alcohol intake per 24 h (g/kg/day) in male and female HAP2 and LAP2 mice (collapsed across sex). Data are averaged across the 30 days after the first FPS test (Post-test 1) and across the 14 days after the fourth FPS test (Post-test 4). See Fig. 3 legend for number of males and females within each line and treatment group.

(3) × Day (15)] yielded main effects of Line [$F(1,160) = 528.6$, $p < 0.01$; $\eta_p^2 = 0.768$; HAP2 > LAP2], Sex [$F(1,160) = 13.9$, $p < 0.01$; $\eta_p^2 = 0.078$; F > M], Day [$F(14,2240) = 42.7$, $p < 0.01$; $\eta_p^2 = 0.211$] and a three-way interaction [$F(14,2240) = 2.0$, $p < 0.05$; $\eta_p^2 = 0.012$]. Follow-up ANOVAs (Line × Sex on each Day) indicated Line × Sex interactions [$Fs(1,168) \geq 5.6$, p values < 0.05; $\eta_p^2 = 0.032–0.076$] during the last 16 days (eight data points). The interactions were due to greater alcohol intake in female than male HAP2 ($Fs(1,89) \geq 9.4$, p values < 0.01; $\eta_p^2 = 0.096–0.220$) mice. Fig. 4 shows data collapsed across the 30-day period due to the absence of interactions with treatment group.

3.1.3. Alcohol drinking after FPS test 4 (Fig. 4)

As was done with alcohol drinking data after FPS test 1, the first eight daily readings were averaged into four 2-day scores and included in the overall repeated-measures ANOVA with the subsequent three 2-day scores across the 14 days of drinking. The ANOVA of g/kg alcohol intake [Line × Sex × Treatment Group (3) × Day (7)] yielded main effects of Line [$F(1,160) = 501.1$, $p < 0.01$; $\eta_p^2 = 0.758$; HAP2 > LAP2], Sex [$F(1,160) = 20.0$, $p < 0.01$; $\eta_p^2 = 0.111$; F > M], Day [$F(6,960) = 6.1$, $p < 0.01$; $\eta_p^2 = 0.036$] and a three-way interaction [$F(6,960) = 2.8$, $p = 0.01$; $\eta_p^2 = 0.017$]. Follow-up ANOVAs (Line × Sex on each Day) indicated Line × Sex interactions [$Fs(1,168) \geq 6.3$, p values < 0.05; $\eta_p^2 = 0.036–0.053$] on days 5–12 (four data points). The interactions were due to greater alcohol intake in female than male HAP2 ($Fs(1,89) \geq 18.1$, p values < 0.01; $\eta_p^2 = 0.169–0.228$) mice. Fig. 4 shows data collapsed across the 14-day period due to the absence of interactions with treatment group.

3.2. Experiment 2

3.2.1. FPS data across the four tests (Fig. 5)

As was done in experiment 1, we conducted an ANOVA on data from the first FPS test to ensure that repeated-measures analyses on subsequent tests would not be biased by group differences. The ANOVA showed a main effect of Treatment Group [$F(3,50) = 3.8$, $p < 0.05$, $\eta_p^2 = 0.185$]. Tukey's *post hoc* comparisons between specific groups failed to reach statistical significance (FEAR/WATER: $M = 97.4$, $SD = 100.9$; FEAR/ALCOHOL: $M = 103.5$, $SD = 160.1$ CONTROL/ALCOHOL: $M = 7.1$, $SD = 40.6$; NO SHOCK/ALCOHOL: $M = 21.8$, $SD = 32.6$).

Overall ANOVA [Line × Sex × Treatment Group (4) × Test (4)] yielded main effects of Treatment Group [$F(3,50) = 5.5$, $p < 0.01$, $\eta_p^2 = 0.248$] and Test [$F(3,150) = 2.8$, $p < 0.05$, $\eta_p^2 = 0.053$]; Test 1 ($M = 60.4$, $SD = 108.0$) and Test 2 ($M = 46.3$, $SD = 84.6$) > Test 3 ($M = 32.9$, $SD = 54.9$) and Test 4 ($M = 19.0$, $SD = 33.8$), due to a reduction in % FPS across the testing sessions. Tukey's comparisons of treatment groups (collapsed over the four tests) showed greater % FPS in the FEAR/WATER ($M = 55.0$, $SD = 70.1$) and FEAR/ALCOHOL ($M = 42.4$, $SD = 57.5$) groups compared to the NO SHOCK/ALCOHOL group ($M = 13.2$, $SD = 22.7$). In addition, % FPS in the FEAR/WATER group was greater than in the CONTROL/ALCOHOL group ($M = 10.6$, $SD = 34.0$) (p values < 0.05).

Limited-access alcohol drinking behavior during each study phase was analyzed separately. We only present the data from the 30-min reading because these analyses revealed more group differences than the 2-h reading measure.

3.2.2. Alcohol drinking immediately after fear-conditioning sessions (phase 1)

Overall ANOVA [Line × Sex × Treatment Group (3) × Day (4)] revealed a main effect of Day [$F(3,111) = 8.0$, $p < 0.01$, $\eta_p^2 = 0.178$] and Line × Day [$F(3,111) = 3.1$, $p < 0.05$, $\eta_p^2 = 0.077$] and Sex × Treatment Group × Day [$F(6,111) = 2.9$, $p = 0.01$, $\eta_p^2 = 0.134$] interactions. Follow-up of the Sex × Treatment Group × Day interaction (Treatment Group × Day within each sex) showed a Treatment Group × Day interaction in males only [$F(6,66) = 3.8$, $p < 0.01$, $\eta_p^2 = 0.256$]. One-way ANOVAs of Treatment Group within each day in males indicated a main effect of Treatment Group on day 1 (first conditioning session day) only [$F(2,22) = 3.7$, $p < 0.05$, $\eta_p^2 = 0.251$]. Tukey's *post hoc* comparisons of Treatment Group within day 1/males indicated significantly greater alcohol intake in both HAP2 and LAP2 FEAR/ALCOHOL groups ($M = 3.3$, $SD = 3.5$) compared to the CONTROL/ALCOHOL ($M = 0.6$, $SD = 1.1$) and NO SHOCK/ALCOHOL ($M = 0.8$, $SD = 1.1$) groups. (p values < 0.05) (Fig. 6).

Follow-up of the Line × Day interaction showed a main effect of Day in LAP2 [$F(3,78) = 8.3$, $p < 0.01$, $\eta_p^2 = 0.241$] but not HAP2 mice. There was also a significant line difference in alcohol intake (HAP2 > LAP2) on the drinking session after the 4th fear-conditioning session [$F(1,47) = 12.7$, $p < 0.01$, $\eta_p^2 = 0.213$] (Fig. 6). Taken together, these analyses suggest that fear conditioning increased alcohol intake in both HAP2 and LAP2 male mice only after the first of the four conditioning sessions.

3.2.3. Alcohol drinking on non-fear-conditioning days (phase 1)

Overall ANOVA [Line × Sex × Treatment Group (3) × Day (8)]

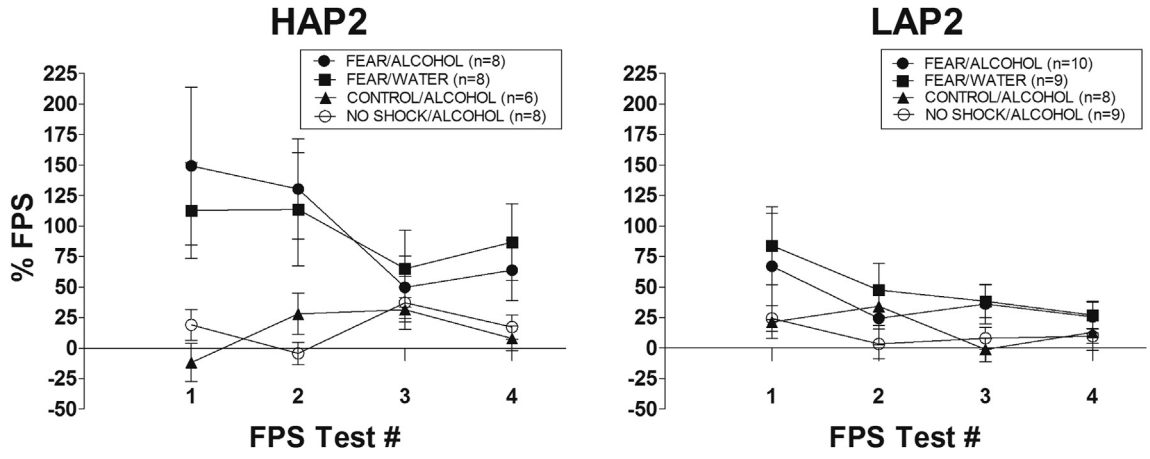

Fig. 5. Mean (±SEM) % FPS across four tests in male and female HAP2 (left panel) and LAP2 (right panel) mice in experiment 2 (collapsed across sex). FEAR/WATER: HAP2 (4 males and 4 females); LAP2 (4 males and 5 females).

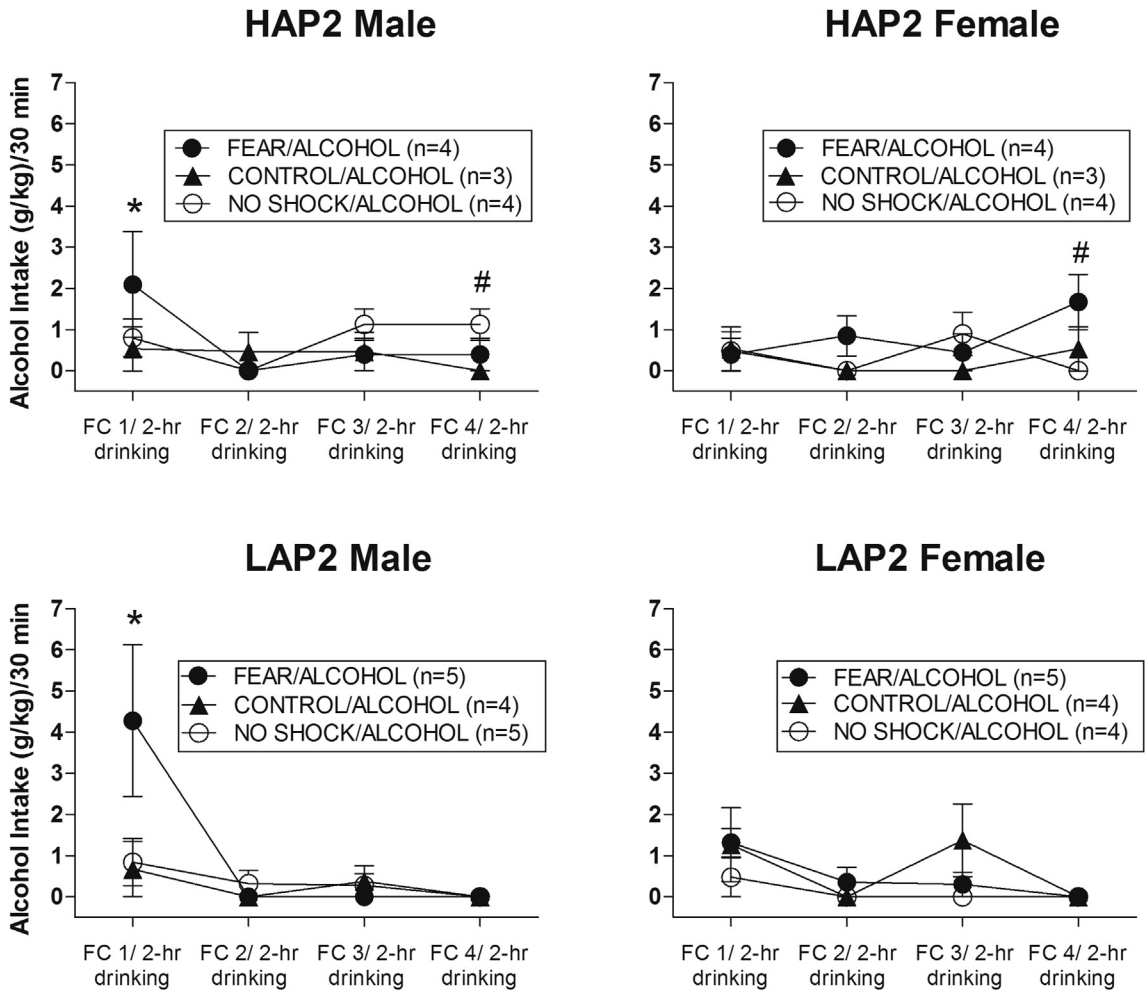

Fig. 6. Mean (±SEM) g/kg alcohol intake in male and female HAP2 and LAP2 mice during the first 30 min of the 2-h free-choice drinking sessions that occurred immediately after each fear conditioning session (1–4). $^{*}p < 0.05$ FEAR/ALCOHOL groups > CONTROL/ALCOHOL and NO SHOCK/ALCOHOL groups; $^{\#}p < 0.01$ main effect of Line (HAP > LAP). See Fig. 5 legend for number of males and females within each line and treatment group.

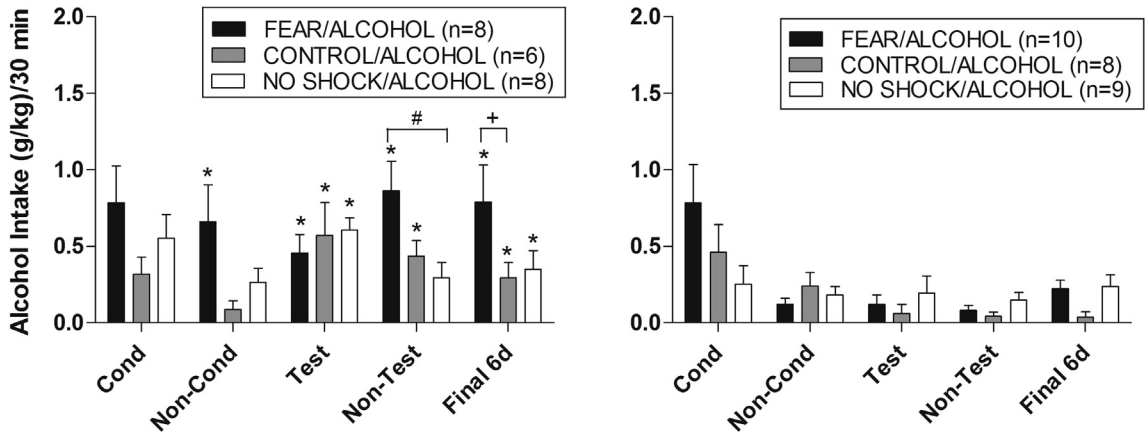

Fig. 7. Mean (±) g/kg alcohol intake in male and female HAP2 and LAP2 mice (collapsed across sex) during the first 30 min of the 2-h free-choice drinking sessions across the five phases of experiment 2. Cond: mean alcohol intake across the 4 days of data (shown in Fig. 6); Non-Cond: mean alcohol intake over 8 non-fear conditioning days; Test: mean alcohol intake over 4 FPS testing days; Non-Test: mean alcohol intake over 8 non-FPS testing days; Final 6d: mean alcohol intake over the final 6 days. $^{*}p < 0.05$ main effect of Line (HAP2 > LAP2); $^{\#}p < 0.05$ FEAR/ALCOHOL > NO SHOCK/ALCOHOL; $^{+}p < 0.05$ FEAR/ALCOHOL > CONTROL/ALCOHOL. See Fig. 5 legend for number of males and females within each line and treatment group.

yielded a Line × Treatment Group interaction [$F(2,37) = 4.6$, $p < 0.05$, $\eta_p^2 = 0.200$]. Follow-up analyses of the interaction (Treatment Group ANOVAs within each Line and Line ANOVAs within each Treatment Group) indicated significantly greater alcohol intake in HAP2 than LAP2 mice in the FEAR/ALCOHOL groups only [$F(1,16) = 6.0$, $p < 0.05$, $\eta_p^2 = 0.271$] (Fig. 7).

3.2.4. Alcohol drinking immediately after the four FPS tests (phase 2)

Overall ANOVA [Line × Sex × Treatment Group (3) × Day (4)] yielded only a main effect of Line [$F(1,37) = 22.3$, $p < 0.01$, $\eta_p^2 = 0.376$; HAP2 > LAP2] (Fig. 7).

3.2.5. Alcohol drinking on the non-FPS test days (phase 2)

Overall ANOVA [Line × Sex × Treatment Group (4) × Day (8)] yielded a Line × Treatment Group interaction [$F(2,37) = 5.5$, $p < 0.01$, $\eta_p^2 = 0.231$]. Follow-up analyses (Line ANOVAs within each Treatment Group and Treatment Group ANOVAs within each Line) indicated significant line effects (HAP2 > LAP2) in the FEAR/ALCOHOL [$F(1,16) = 19.8$, $p < 0.01$, $\eta_p^2 = 0.552$] and CONTROL/ALCOHOL [$F(1,12) = 17.7$, $p < 0.01$, $\eta_p^2 = 0.596$] groups but not in the NO SHOCK/ALCOHOL groups. The Treatment Group ANOVAs showed a significant main effect in HAP2 [$F(2,19) = 4.4$, $p < 0.05$, $\eta_p^2 = 0.317$] but not LAP2 mice. Tukey's *post hoc* indicated significantly greater alcohol intake in the FEAR/ALCOHOL vs. the NO SHOCK/ALCOHOL HAP2 groups ($p < 0.05$) (Fig. 7).

3.2.6. Alcohol drinking on six additional consecutive days after last FPS test (phase 2)

Overall ANOVA [Line × Sex × Treatment Group (3) × Day (6)] yielded main effects of Line [$F(1,37) = 8.6$, $p < 0.01$, $\eta_p^2 = 0.189$; HAP2 > LAP2] and Treatment Group [$F(2,37) = 3.6$, $p < 0.05$, $\eta_p^2 = 0.162$]. Tukey's *post hoc* indicated the FEAR/ALCOHOL groups in both lines consumed significantly more alcohol than the CONTROL/ALCOHOL groups ($p < 0.05$) (Fig. 7).

Separate analyses of water intake during each phase indicated that these effects as a function of treatment group were specific to alcohol intake (data not shown).

4. Discussion

Overall, the present experiments indicate evidence for a functional relationship between alcohol-drinking propensity and fear-conditioned behavior under certain conditions. The data from experiment 1 did not support the hypothesis that fear conditioning/testing would increase alcohol drinking under 24-h access conditions. Oppositely, we found that FPS was enhanced in alcohol drinking HAP2 (but not LAP2) mice (FEAR/ALCOHOL group) on Test 3, which occurred during an alcohol abstinence period 24 h after removal of alcohol bottles. The data from experiment 2 provide some evidence in support of the hypothesis that repeated exposures to fear conditioning/FPS testing would increase alcohol drinking under limited-access conditions (discussed below). However, unlike in experiment 1, FPS expression as a function of alcohol drinking did not change over repeated testing. A few sex differences were found in this study. In experiment 1, males showed evidence of greater FPS (a follow-up analysis found a significant line difference only in males) and females drank more alcohol than males, both of which are findings consistent with our prior work in these lines (Chester, Barrenha, Hughes, & Keuneke, 2008; Chester et al., 2014) and the literature (Cohen & Yehuda, 2011; Finn, Beckley, Kaufman, & Ford, 2010). In experiment 2, male but not female HAP2 and LAP2 mice in the FEAR/ALCOHOL groups showed increased alcohol intake after the first fear conditioning session.

It is well known that internal states of arousal influenced by fear, anxiety, and stress can influence the unconditioned acoustic startle reflex (Davis, 1990; Garrick, Morrow, Shalev, & Eth, 2001). Acoustic startle is also a common measure of anxiety-related effects of drug and alcohol withdrawal. Acoustic startle in rodents has been shown to be both increased (Chester, Blose, & Froehlich, 2004, 2003; Macey, Schulteis, Heinrichs, & Koob, 1996; Rassnick, Koob, & Geyer, 1992; Reilly, Koirala, & Devaud, 2009; Vandergriff, Kallman, & Rasmussen, 2000) and decreased (Chester & Barrenha, 2007; Chester, Blose, & Froehlich, 2005; Chester, Blose, & Froehlich, 2003; Gilliam & Collins, 1986; Mejia-Toiber, Boutros, Markou, & Semenova, 2014) during the first 24–72 h in withdrawal from forced alcohol exposure. The differences in direction of the acoustic startle response during withdrawal in these studies are likely due to factors such as species and strain differences in alcohol sensitivity as well as dose and pattern of forced alcohol exposure.

The current findings are the first demonstration, to our knowledge, of enhanced conditioned acoustic startle (FPS) during alcohol withdrawal following a period of voluntary alcohol consumption. The most direct interpretation of the greater FPS in HAP2 mice exposed to alcohol drinking after fear conditioning in experiment 1 is that alcohol drinking and its subsequent withdrawal activates anxiety-related neurocircuitry that was reflected as greater FPS during the period of alcohol abstinence, at 24 h following termination of voluntary drinking. This finding adds to a growing literature demonstrating a variety of physiological and behavioral effects observed during alcohol withdrawal that are thought to be relevant to the development and maintenance of alcohol-seeking behavior and addiction, including anxiety, negative affect, and reactivity to stress (Heilig, Egli, Crabbe, & Becker, 2010; Lee, Coehlo, McGregor, Waltermire, & Szumlinski, 2015). It should be noted that we did not see changes in acoustic startle on noise-alone trials during the FPS tests in either experiment (data not shown), indicating the enhanced FPS seen in experiment 1 was specific to conditioned fear/anxiety-related behavior (FPS).

Another possible explanation for the enhanced FPS in alcohol-drinking HAP2 mice is that alcohol drinking (and withdrawal) slowed the normal extinction of FPS behavior across repeated testing (see Fig. 3, left panel: % FPS in the FEAR/ALCOHOL vs. FEAR/WATER group across the four FPS tests), an effect similar to that reported by Bertotto, Bustos, Molina, and Martijena (2006). Overall, these data suggest that the FPS model can provide an index of changes in anxiety-related/emotional regulation processes during protracted withdrawal that may be particularly relevant for exploring mechanisms that contribute to PTSD and AUD co-morbidity.

Relationships between alcohol drinking behavior and stress and anxiety are complex. In rodent models, stress effects on alcohol drinking behavior depend on many factors such as sex, pre-exposure to alcohol, timing, duration, and pattern of stress exposure in relation to drinking, type of stressor, and genetic predisposition toward alcohol preference (Becker, Lopez, & Doremus-Fitzwater, 2011; Chester, de Paula Barrenha, DeMaria, & Finegan, 2006; Chester et al., 2004). A large body of literature supports the idea that, for some individuals, AUDs can develop in response to a cyclical pattern of alcohol consumption by individuals consuming alcohol to alleviate anxiety symptoms that were either pre-existing or induced by alcohol (Conger, 1956; Sher, 1987; Sinha et al., 1998; Weiss & Rosenberg, 1985). This theory, often referred to as the "tension-reduction hypothesis", has been explored for many decades and is thought to contribute to the development of co-morbid anxiety and AUDs in some individuals. For example, people with a family history of AUDs report more "stress-relieving" and anxiolytic effects of alcohol consumption (Sher & Levenson, 1982; Sinha et al., 1998). In support of these human findings, we previously found that HAP lines are more sensitive to alcohol's anxiolytic effects on FPS than LAP lines (Barrenha et al., 2011), and others have found a similar greater sensitivity to alcohol-induced anxiolysis in high-alcohol drinking rat lines tested in unconditioned anxiety models (Colombo et al., 1995; Stewart, Gatto, Lumeng, Li, & Murphy, 1993).

The question of "order of onset" of co-morbid disorders is often

difficult to address in human studies, although significant evidence indicates that trauma and PTSD often precede AUDs (Davidson et al., 1990; Forbes et al., 2015; Lopez et al., 2005; McFarlane, 1998). In this study, we designed experiments to examine how exposure to trauma before alcohol exposure, as well as how repeated exposures to trauma and fear-conditioned cues, influenced alcohol drinking behavior in mice with and without a genetic predisposition toward high alcohol drinking. In experiment 1, we did not see changes in alcohol intake in either line after a single exposure to fear conditioning/FPS test. However, in experiment 2, repeated, intermittent sessions of fear conditioning increased alcohol intake in both HAP2 and LAP2 mice during the 30 min immediately following the first fear-conditioning session. Fear-conditioned HAP2 but not LAP2 mice also showed greater alcohol intake compared to control groups on non-fear conditioning days in phase 1 and non-FPS testing days in phase 2 (Fig. 7).

The increased alcohol drinking in the HAP2 FEAR/ALCOHOL groups in experiment 2 but not experiment 1 suggests the importance of repeated, intermittent exposures to a stressor, in this case, fear conditioning specifically, in facilitating increased alcohol intake. Interestingly, we reported a similar effect in a prior study in which 10 consecutive days of repeated restraint stress (applied prior to the start of alcohol access) did not influence the acquisition of 21 days of consecutive limited-access alcohol drinking behavior in naïve male and female HAP2 mice. However, subsequent application of repeated and intermittent stressor exposure to these mice, prior to limited-access alcohol drinking sessions, increased limited-access alcohol intake in male HAP2 mice. Alcohol drinking remained elevated in these mice during a final phase of 8 days of continuous alcohol access (Chester et al., 2006). It should be noted that our ability to interpret line-dependent changes in FPS and alcohol drinking in these two experiments are limited by the fact that 1) we did not collect blood alcohol data and 2) we cannot determine whether the enhanced FPS during alcohol withdrawal in HAP2 but not LAP2 mice (experiment 1) reflects line differences in sensitivity to anxiety during alcohol withdrawal or is simply due to a greater amount of alcohol exposure via higher voluntary alcohol consumption in HAP2 mice.

With respect to the second issue, this question can be difficult to address because it requires matching the lines for alcohol exposure using a forced alcohol consumption procedure or experimenter-administered alcohol. We plan to conduct these experiments in future work to determine whether the lines differ in sensitivity to alcohol withdrawal-induced anxiety-related behaviors. We previously reported that LAP2 mice show greater handling-induced convulsions (HICs) after a single high dose of alcohol compared to HAP2 mice (Barrenha & Chester, 2012). Similarly, LAP2 mice show greater HICs than HAP2 mice following chronic inhalation of ethanol vapor (Lopez, Grahame, & Becker, 2011). These data are consistent with many reports showing that alcohol-naïve rodents with a genetic propensity toward high alcohol drinking/preference show weaker physical alcohol withdrawal signs compared to lower drinking lines and strains (e.g., Chester et al., 2003; Metten et al., 1998). However, it is less clear how genetic propensity toward alcohol drinking may relate to sensitivity to cognitive and affective alcohol withdrawal-induced effects, such as craving and anxiety-related behavior, that are thought to play a primary role in continued alcohol-seeking behavior (Weiss & Rosenberg, 1985). Overstreet, Knapp, and Breese (2005) found that selectively bred alcohol-preferring P rats were more sensitive to alcohol withdrawal-induced anxiety-like behavior compared to Sprague Dawley counterparts. In addition, Lopez et al. (2011) found that, despite having reduced physical signs of withdrawal, male HAP2 mice showed increased 2-h alcohol intake at 72 h after termination of the vapor inhalation, suggesting that HAP2 males are more sensitive to withdrawal-related "relapse" drinking. Findings from both these reports fit with our current finding of greater FPS during withdrawal in HAP2 mice and suggest that greater withdrawal-induced anxiety in HAP2 mice could be a mechanism that serves to increase high alcohol drinking behavior. Overall, more work is needed to understand the gene × environment interactions that contribute to the development of escalated alcohol drinking behavior during alcohol withdrawal in AUD models.

Several possible mechanisms may underlie the increased alcohol drinking behavior in the HAP2 FEAR/ALCOHOL groups in Experiment 2. Repeated fear-conditioning stress can increase anxiety-related behavior (Pickens, Golden, & Nair, 2013), which in turn may promote alcohol drinking in experienced mice, as suggested by the tension reduction hypothesis. Many data indicate that these behaviors emerge due to neuroadaptations in CRF/neuroendocrine mechanisms within the amygdala (AMG), an area of the brain that is a critical mediator of anxiety, alcohol drinking/relapse, and FPS (Davis, 2006; Heilig et al., 2010; Rosenkranz, Venheim, & Padival, 2010; Sandi et al., 2008; Shekhar, Truitt, Rainnie, & Sajdyk, 2005). Crfr1 is an important target to explore in mediating stress/alcohol interactions and line differences in anxiety during alcohol withdrawal. Crfr1 gene expression is upregulated in rat AMG during alcohol withdrawal (Sommer et al., 2008), and Crfr1 antagonists given during alcohol withdrawal block the withdrawal-induced anxiety in rodents (e.g., Breese, Knapp, & Overstreet, 2004; Wills, Knapp, Overstreet, & Breese, 2009). As well, molecular changes in GABA neurotransmission occur in the AMG after fear conditioning (Chhatwal, Myers, Ressler, & Davis, 2005), and alcohol can facilitate GABA neurotransmission via $GABA_A$ receptors within the AMG (Roberto, Madamba, Moore, Tallent, & Siggins, 2003). In light of our past data where HAP lines showed greater alcohol-induced anxiolysis in the FPS procedure than LAP lines (Barrenha et al., 2011), future work should explore line differences in GABA neurotransmission in the AMG. As mentioned in the introduction, there are common genetic factors that influence vulnerability to both AUDs and PTSD in humans (e.g., Scherrer et al., 2008). We suspect that CRF- and GABA-related genes may be some of the common genetic factors that underlie the correlations found between alcohol drinking, FPS, and HPA-axis function in the HAP and LAP lines (Chester et al., 2014).

In summary, results of this study suggest a functional relationship between genetic susceptibility to FPS and alcohol-drinking behavior in the selectively bred HAP2 and LAP2 lines. This work adds to a growing body of evidence that sheds light on how vulnerability to stress and anxiety may increase risk for AUDs in humans. In particular, this genetic animal model is useful for exploring biological targets and behavioral interventions that may lead to treatments for individuals with co-morbid PTSD and AUDs.

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