Genetic Factors are More Predictive of Adult Drinking Behavior than Early Exposure to Alcohol in Mice Genetically Selected for Alcohol Preference

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Abstract

Research shows that people age 14 or younger who drink alcohol are more likely to become dependent. For these individuals as adults, past-year stressors were highly correlated with increased alcohol intake. Evidence also suggests that children of alcoholic parents are more sensitive to alcohol's effects. The present study was designed to test these relations in a mouse model by examining whether alcohol exposure during adolescence versus adulthood would increase alcohol consumption following exposure to stress as adults, and whether this effect was dependent on a family history of high or low alcohol preference. Adolescent and adult male alcohol-naïve mice selectively bred for high or low alcohol preference received injections of either 2 g/kg alcohol or an equal volume of saline for five consecutive days. As adults, all subjects received a mild footshock stressor for five consecutive days and were subsequently given free-choice access to alcohol and water for 30 days. Results indicated no group differences in alcohol consumption, suggesting early alcohol exposure under these conditions does not affect subsequent alcohol consumption following stress in either line.

Introduction

Adolescence is an impressionable time when environmental stimuli can have profound and lasting effects on neurological functioning. There are several critical periods in the development of the cortex that fall within the period of time defined as adolescence, during which the cortex may be very susceptible to environmental insult (Crews et al., 2007). One important characteristic of adolescence is novelty-seeking or risk-taking behavior (Doremus et al, 2005; Spear, 2000*b*). In keeping with this type of risk-taking behavior, it is very common for human adolescents to experiment with alcohol or other drug use (Spear, 2000a; Andrucci et al, 1989; Baumrind, 1987; Wills et al, 1996). As a result, alcohol use and abuse has become

prevalent in today's adolescent population (Heitzeg et al., 2008; Dawson et al., 2007; Hingson et al, 2006; DeWit et al, 2000; Spear, 2000*b*; Grant, 1998).

Early onset drinking may have long-lasting effects and can be a strong predictor of alcohol dependence later in life (Fullgrabe et al, 2007; Bell et al, 2006; Siegmund et al, 2005; Spear, 2000*b*; DeWit et al, 2000), and this effect can be regardless of family history. Grant and Dawson (1997) report that people who were exposed to alcohol at 14 years old or younger could be as much as four times more likely to become dependent as people who were exposed after 20 years old. For individuals who begin drinking before age 14, early onset drinking was positively correlated with adult stress levels (Dawson et al., 2007). In addition, for every year the onset of use was delayed, the likelihood of alcohol abuse decreased by approximately 5% (Grant and Dawson, 1998). These findings would suggest that early initiation of alcohol drinking may increase subsequent drinking following exposure to a stressor in adulthood.

Family history, however, is also a strong predictor of alcohol use (Bennett et al, 2006; Enoch, 2006; Vengeliene et al, 2003). A long-term study on the sons of alcoholics reinforced the importance of family history as a predictor of alcohol use disorders (Schuckit, 1995). Evidence also suggests that children of alcoholic parents are more sensitive to alcohol's effects (Heitzig et al., 2008). Additionally, stressful life events are another important predictor for alcohol-use disorders; it is known that stress during adolescence is a strong risk factor for alcohol-use disorders (Enoch, 2006; Arnsten and Shansky, 2004, 1999; Koehl et al., 2002). This leads to the question of how family history, and early onset drinking, and stress interact with each other to increase the risk for alcohol use disorders.

Animal models can be a useful tool to investigate alcohol drinking behaviors while controlling for possible confounds. By using animal models, we can control for life experiences, family history, and age of onset of alcohol use. In mice, adolescence is defined as approximately the range of post-natal day (PND) 24-55, during which a gradual transition occurs from immaturity to adulthood (Hefner and Holmes, 2007; Adriani et al., 2002; Spear, 2000*a*).

The interaction between stress and drinking in the rodent model is not fully understood. As mentioned above, research suggests a strong correlation between stress and drinking in humans. However, the results of studies conducted in rodent lines are mixed. Vengeliene et al (2003) found that foot shock increased alcohol consumption in several different lines of rats, but with the most robust effects in high preferring lines. However, Brunell and Spear (2005) showed that chronic stress suppressed drinking in adolescent rats. It has been demonstrated that restraint stress may increase alcohol consumption in some lines of mice (Yang et al, 2008). To further demonstrate the complexity of stress-induced drinking, Chester et al (2004) demonstrated inhibited drinking in alcohol preferring rats

during a stress phase, but an increase in drinking following the stress phase. Additionally, non-preferring rats showed no effect during or immediately after stress, but drinking behaviors increased after a few weeks (Chester et al, 2004). Stress-induced drinking refers to a drinking behavior that is not present before the application of stress, but develops following stress. Stress-induced drinking has also been shown in high alcohol preferring mice as well. Chester et al (2006) demonstrated increased alcohol intake after the termination of stress in male mice. In human studies, such as the study by Dawson et al (2007), it is difficult to disentangle the interactions between stress and drinking. For example, does stress produce drinking, or does drinking cause stress, which can promote further drinking? In animal studies, the order of exposure to stress and drinking can be controlled to try to shed light on these questions.

The purpose of the present study was to compare the effects of early alcohol exposure on stress-related drinking in adulthood in male mice selectively bred for high alcohol preference (HAP2 selected line) and low alcohol preference (LAP2 selected line). The use of selectively bred mouse lines allows control over a variety of variables that would otherwise confound the study. Furthermore, only males where used in the present study because prior studies have suggested that stress-induced drinking in HAP2 mice may depend on sex (Chester et al, 2006). To our knowledge, there are no studies that have investigated the effects of early alcohol exposure on adult stress-induced drinking. It is hypothesized that mice genetically bred for high alcohol preference that are exposed to alcohol as adolescents will exhibit greater alcohol preference after a stressor in adulthood than mice not exposed to alcohol or exposed to alcohol as adults. It is also hypothesized that this effect will not be seen in mice bred for low alcohol preference.

Methods

Subjects

Experiment 1. Subjects were 20 adolescent (PND 34-37) male alcohol-naïve mice selectively bred for high alcohol preference (HAP2 line; generation 27) and 20 adolescent male alcohol-naïve mice selectively bred for low alcohol preference (LAP2 Line; generation 27). The selectively bred HAP2 mouse line was derived from a foundation stock of outbred Hs/Ibg mice (Boulder, Colorado, USA) at the Indiana Alcohol Research Center (IARC). In every generation of selection, high alcohol preference was established during a 4-week, 24-hr, free-choice preference test (Grahame et al., 1999). During the preference test, mice were given free-access to food and two 25-ml graduated cylinders, one that contained 10% alcohol (v/v) in distilled water and the other that contained distilled water. Mice that drank

more than 5.0 g/kg alcohol per day (averaged over the 4-week test period) were selected to be breeders for the next generation of HAP2 mice (Grahame et al., 1999).

Subjects were generated at Purdue University from HAP2 breeders obtained from the IARC. Subjects in the present experiments were derived from 18 different HAP2 families (9 for Experiment 1 and 9 for Experiment 2). Pregnant dams were checked for births daily and day of birth was considered PND 1. Mice were weaned at 21-23 days of age (in order to avoid individually housing weaned mice) and were immediately housed in groups of 2-4 in polycarbonate cages (11.5 x 7.5 x 5.0 in) with aspen wood shavings. Mice remained group- housed until the free-choice alcohol consumption phase of the experiments.

Ambient temperature in the colony room was maintained at 21±1ºC and mice had free-access to food (Rodent Lab Diet 5001, Purina Mills Inc., St. Louis, MO) and water in the home cage. The colony room was maintained on a 12:12 light:dark cycle (lights on at 0700). Experiments were conducted in accordance with the NIH guidelines for animal care and use and the experimental procedures were approved by the Purdue Animal Care and Use Committee.

Experiment 2. Subjects were 19 adult (PND 62-64) male alcohol-naïve mice selectively bred for high alcohol preference (HAP2 line; generation 27) and 19 adult male alcohol-naïve mice selectively bred for low alcohol preference (LAP2 Line; generation 27).

Experimental Timeline

Table 1 and Figure 1 below illustrate the age ranges at the start of the experimental phase for both adolescent and adult mice. Experiment 1 and experiment 2 each consisted of three phases. During Phase 1, all mice were exposed to 5 doses of intraperitoneal (IP) alcohol for five consecutive days. Phase 2 consisted of five consecutive days of electric footshock. Phase 2 started twenty-two days after the last injection for adolescents and two days after the last injection of ethanol for adults to eliminate any effects of alcohol withdrawal. The onset of stress was controlled between adults and adolescents to fall within a similar age range to control for any interaction of age on response to stress. Phase 3 was initiated two days after the stress session. Phase 3 measured the alcohol preference for about four weeks (30 days)

Table 1. Experimental Timeline for Experiments 1 and 2					
Phase of Experiment	1	Interim	2	Interim	3
Days of Experiment (Days)	5	Adolescent 23 days Adult 2 days	5	2 days	30
Age of Mice (PND) at start of phase					
Experiment 1 (Adolescent Exposure)	34-37	39-42	62-65	67-70	69-72
Experiment 2 (Adult Exposure)	62-64	67-69	69-71	74-76	76-78

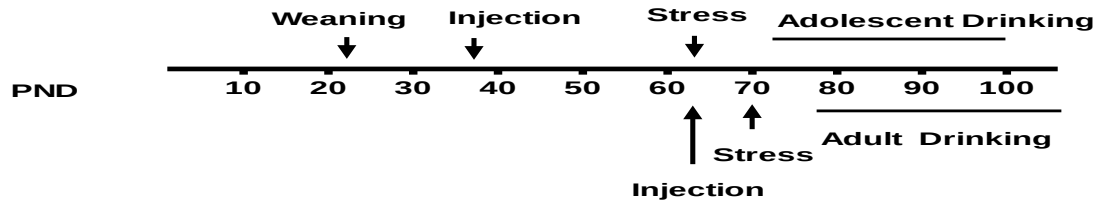


Figure 1 Experimental timeline illustrating starting points of experimental conditions relative to adolescents and adults.

Apparatus and Stimuli Parameters

Application of footshock stress sessions occurred in a Coulbourn Instruments Animal Acoustic Startle System (Coulbourn Instruments, Allentown, PA). The system consisted of eight weight-sensitive platforms located inside a sound attenuated chamber connected to an interfaced computer. The eight platforms were equidistant from a speaker located in the ceiling of the chamber. Subjects were placed individually into open-air holders (8 x 8 x 16 cm) with metal rod floors (rod diameter 0.19 in with each rod separated by 0.39 in). Scrambled electric footshock was administered through the metal rod floors of each holder using Coulbourn Shocker Units. All experimental sessions were run in the alternating current coupled mode, which produces output data in absolute grams of force and does not include subjects' body weight in the force measurement. Each 30-min footshock stress session consisted of 15 shock stimuli (0.6 mA, 0.5 sec

duration) presented every 2 min. A ventilating fan provided continuous background noise (~75 dB).

Procedure

Phase 1: IP Injection. All mice housed within a particular home cage were assigned to either the experimental (ethanol) or control (saline) groups. Groups were counterbalanced so that an equal number of animals from a particular family (litter) were assigned to the EtOH or saline groups to the best extent possible. On day of testing, mice were removed individually from the home cage, weighed, and injected in colony room. The mouse was then returned to its home cage and the next mouse was removed in the same manner. This was done over five consecutive days starting at PND 36 for adolescents (Experiment 1) and PND 62 for adults (Experiment 2). Each mouse was injected with either a 2g/kg of 20% ethanol in saline (v/v) solution or saline only solution.

Phase 2: Stress. All mice were subjected to footshock stress (PND 63 for Experiment 1 and PND 69 for Experiment 2). This occurred 22 days after the last injection in adolescents (Experiment 1), and two days after the last injection in adults (Experiment 2). The age at the onset of stress was an important factor to control for since age may play an important role in the stress response. It is unclear how age and stress interact, and for this reason the age range was made as close together as possible. The eight individual open-air holders were brought into the colony room prior to the experiment. During the five consecutive days of stress, all mice were placed one at a time into an open-air holder with a metal rod floor and the holder was immediately carried into the adjacent room and placed inside the Coulbourn apparatus. The order of stress sessions was counterbalanced based on treatment group. To the best extent possible, each stress session contained four mice that had been injected with saline, and four that had been injected with ethanol. Mice were weighed prior to being placed into the open air-holder each day of footshock stress.

Phase 3: Free-choice Alcohol Consumption. Mice were individually housed following the final stress session. Standard water bottles were removed and replaced with two 25 ml plastic graduated cylinders, fitted with steel sipper tubes. On the first two days after the last stress session, both bottles were filled with water to allow the mice to acclimate to the new bottles. On the third day, one cylinder was filled with a 10% alcohol solution. Fluid levels were checked and recorded every two days, and old fluid was discarded and replaced with fresh fluid once a week. Cages were changed every six to eight days. The left-right position of the alcohol and water-filled cylinders was rotated after each reading to control for a potential positional preference.

Data Analyses

All data were analyzed using analysis of variance (ANOVA). Between-group factors included age (Adolescent, Adult), treatment group (Alcohol, Saline) and line (HAP2, LAP2) and within-group factors included day. To simplify presentation of the results, only significant main effects and highest order interactions from the ANOVA outputs are reported. Significant interactions were followed first with lower order two-way and one-way ANOVAs (Keppel, 1991). Probability values equal to or less than 0.05 were considered significant.

Alcohol intake was expressed as grams of 10% alcohol per kilogram of body weight (g/kg/BW) and as percent alcohol preference [milliliters (ml) of 10% alcohol solution/ml of 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, alcohol and water drinking scores on individual days were examined for outliers. The majority of these outliers were on the high end of the normal distribution curve and were most likely due to accidental fluid loss from mouse activity in the cage (e.g., playing with the plastic tubes). A score was considered an outlier if it passed several conservative criteria. The score first had to exceed by two standard deviations the mean drinking score for that animal and the mean drinking score for the group. If the score passed these criteria, it was then subjected to the Dixon Extreme Score Test (Dixon, 1950). Scores that passed all criteria were replaced with an average intake score, which was calculated by averaging fluid intake on the day before and the day after the outlier occurred. For Experiment 1, valid outliers occurred five times in LAP2 mice and once in HAP2 mice. In Experiment 2, valid outliers occurred three times in LAP2 mice.

Results

Body Weight

Table 2 shows the mean (±sem) body weight for HAP2 and LAP2 mice in Experiments 1 and 2.

Table 2. Body weight (± SEM) at the start of each experimental phase for Experiments 1 and 2

Phase		1	2	3
Experiment 1 (Adolescent Exposure)				
HAP2	EtOH	22.2 ± .5	24.8 ± .4	24.0 ± .4
	Saline	21.8 ± .5	25.0 ± .4	24.5 ± .4
LAP2	EtOH	19.7 ± .5	24.9 ± .3	24.0 ± .6
	Saline	19.5 ± .5	24.5 ± .5	24.6 ± .3
Experiment 2 (Adult Adult)				
HAP2	EtOH	24.6 ± .3	24.4 ± .5	24.4 ± .4
	Saline	24.6 ± .3	25.0 ± .3	24.9 ± .2
LAP2	EtOH	24.6 ± .6	23.8 ± .5	23.7 ± .5
	Saline	25.3 ± .5	24.7 ± .5	234.9 ± .4

For Experiment 1 (adolescent exposure) there was no significant effect of line on body weight. The ANOVA (Age X Block X Treatment X Line) indicated a main effect of Block [$F(14,938)=310.3$, $p<0.01$], a Block X Treatment interaction [$F(14,938)=2.9$, $p<0.01$], and a Block X Age interaction [$F(14,938)=2.5$, $p<0.05$]. Follow-up one-way ANOVA within each line yielded significant main effects of Block for both HAP and LAP mice ($p<0.01$).

Free-Choice Alcohol Consumption

Alcohol Intake and Preference. The ANOVA (Age X Block X Line X Treatment) yielded no significant main effects for treatment in HAP or LAP mice for intake or preference. The ANOVA for g/kg alcohol intake revealed a main effect by line [$F(1,67)=139.7$, $p<0.01$; HAP>LAP] and Block [$F(14,938)=14.8$, $p<0.01$], and a Block X Age X Line interaction [$F(14,938)=3.2$, $p<0.01$]. A follow-up ANOVA yielded a main effect of Block for both HAP and LAP mice, in both age groups ($p<0.01$).

The ANOVA for alcohol preference yielded a main effect of Block [$F(14,938)=20.1$, $p<0.01$] and Line [$F(1,67)=123.4$, $p<0.01$], as well as a Block X Age X Line interaction [$F(14,938)=2.8$, $p<0.01$]. A follow-up one-way ANOVA indicated a main effect of Block for both HAP and LAP mice in both age groups ($p<0.01$).

Figure 2. Mean (±sem) alcohol intake in g/kg/BW in adolescent-injected (top panel) and adult-injected (bottom panel) HAP2 and LAP2 mice exposed to stress averaged across 2-day blocks during the entire 30-day period.

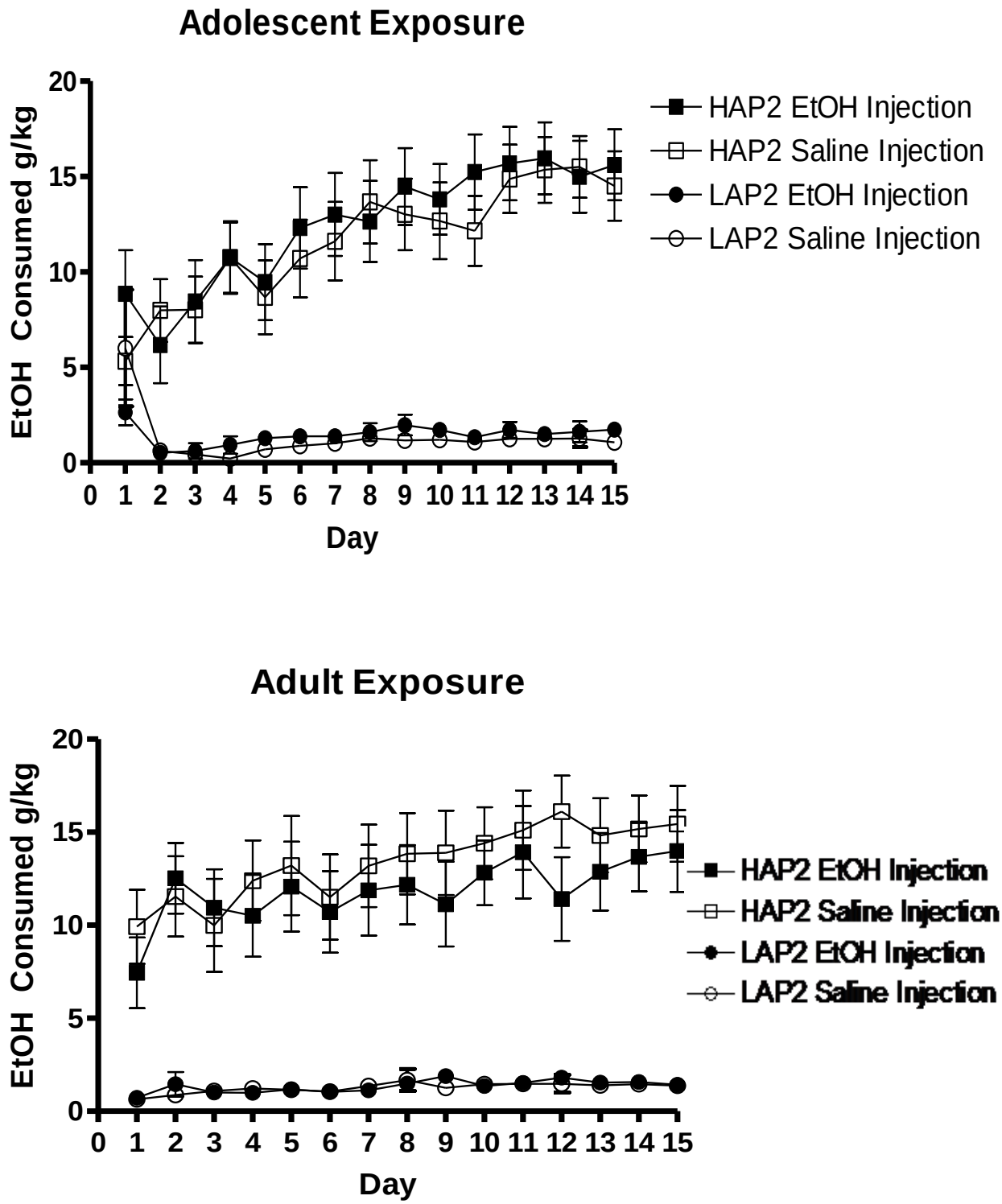
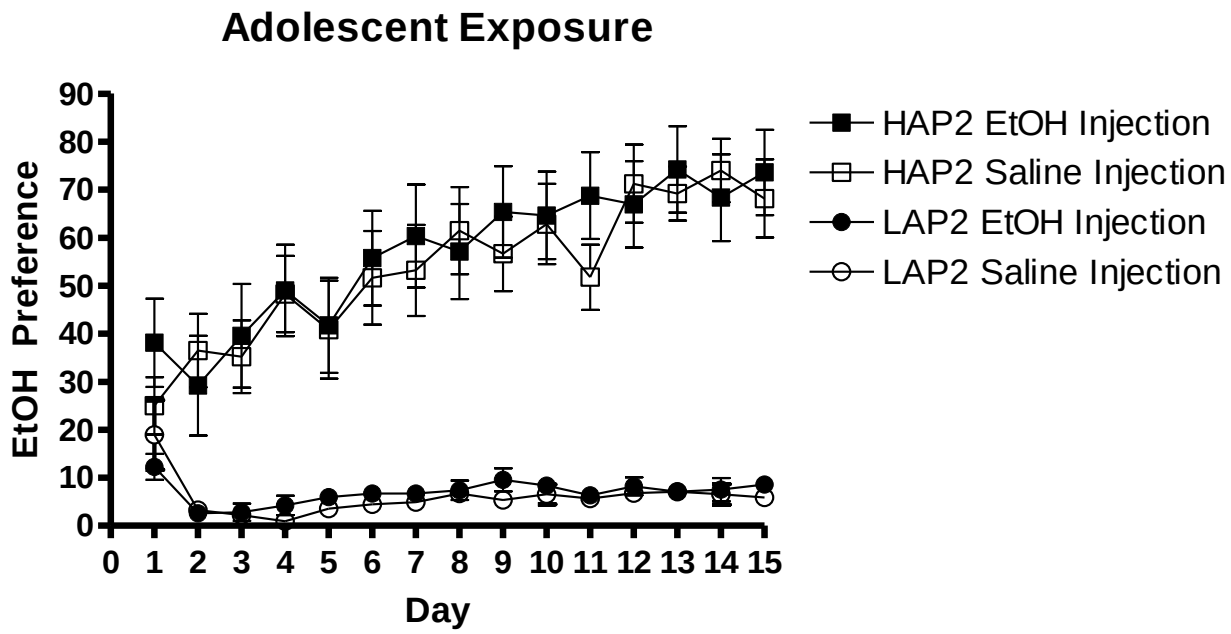


Figure 3. Mean (±sem) alcohol preference in adolescent-injected (top panel) and adult-injected (bottom panel) HAP2 and LAP2 mice exposed to stress averaged across 2-day blocks during the entire 30-day period.



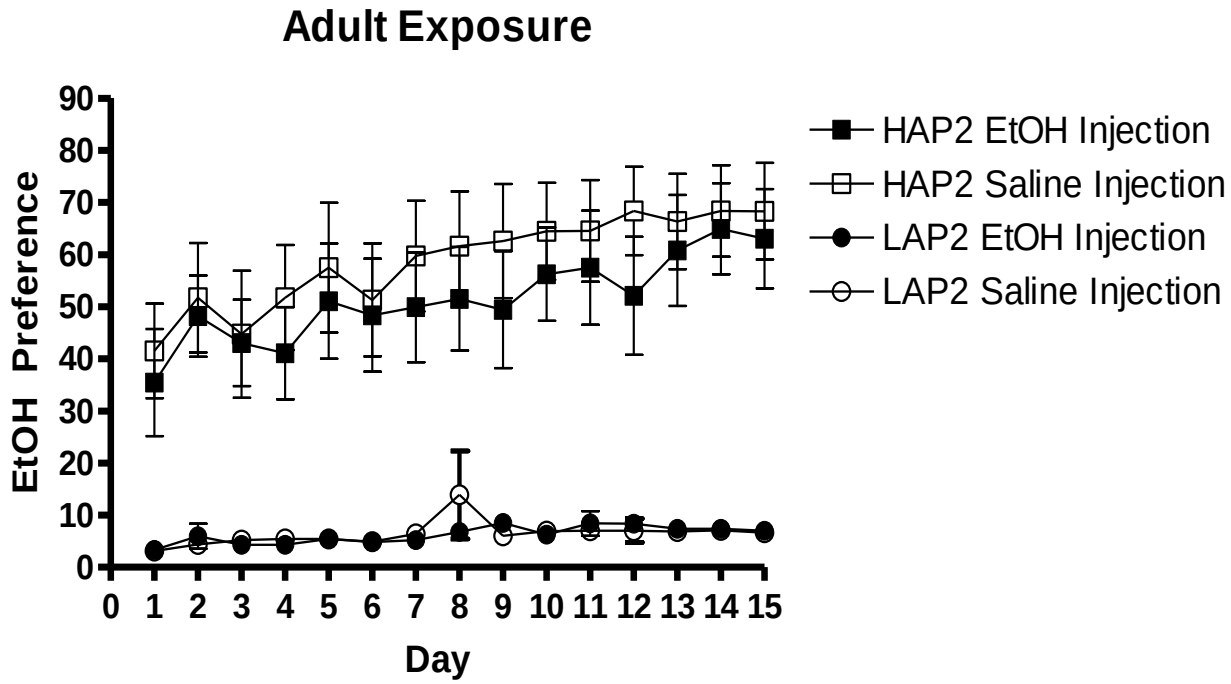


Figure 4. Mean (±sem) water intake in ml/kg/BW in adolescent-injected (top panel) and adult-injected (bottom panel) HAP2 and LAP2 mice exposed to stress averaged across 2-day blocks during the entire 30-day period.

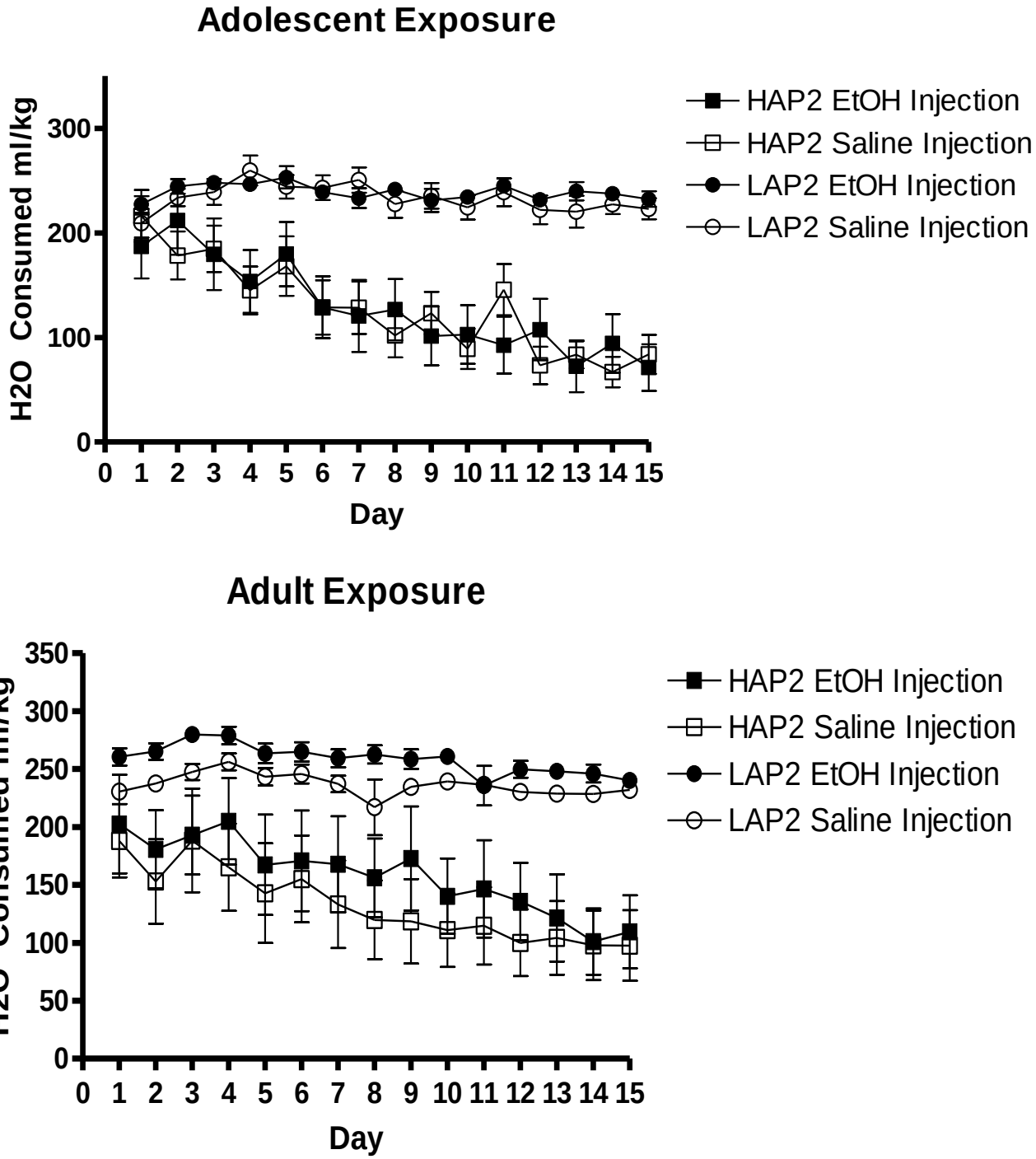
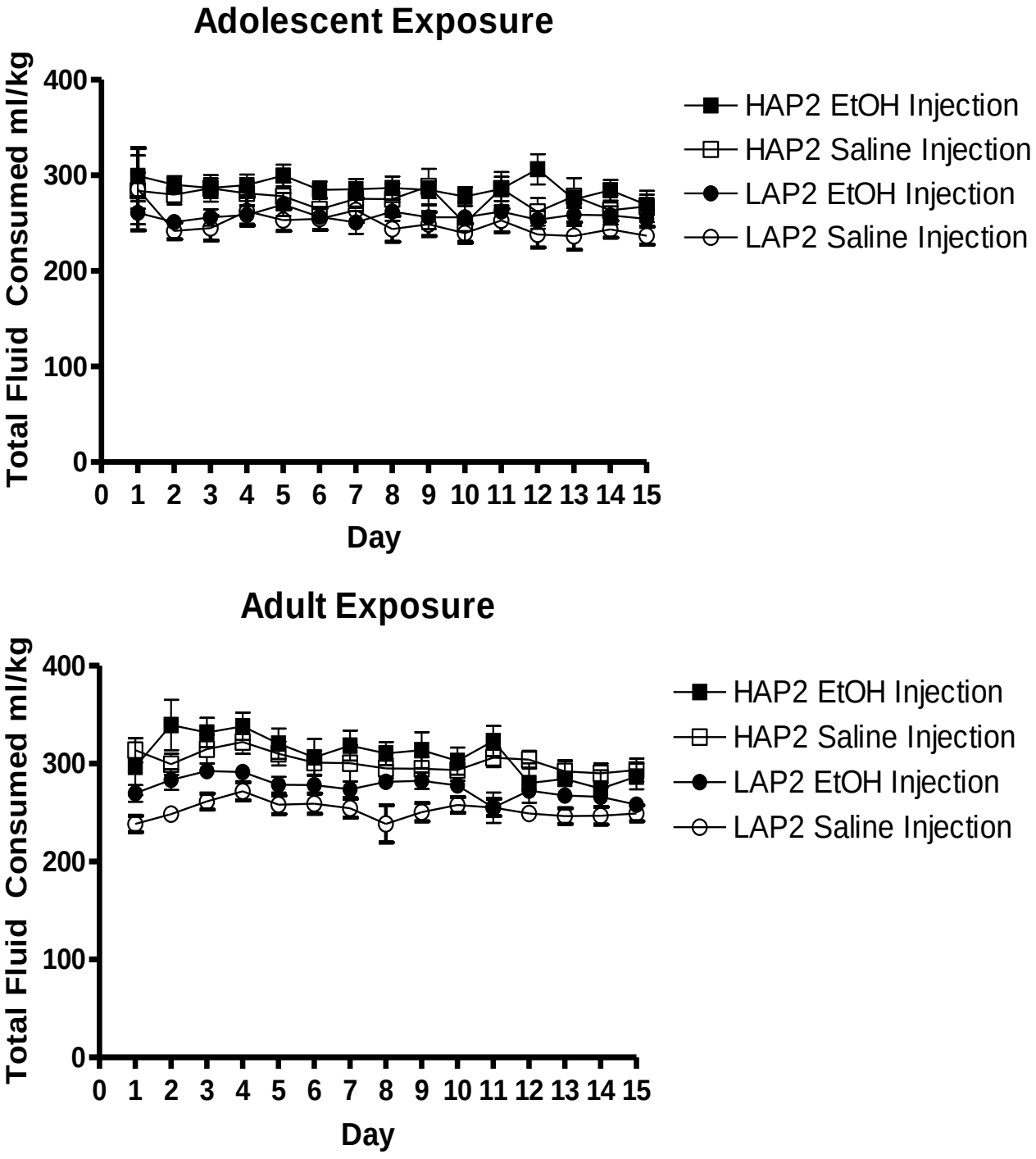


Figure 5. Mean (±sem) total fluid intake in ml/kg/BW in adolescent-injected (top panel) and adult-injected (bottom panel) HAP2 and LAP2 mice exposed to stress averaged across 2-day blocks during the entire 30-day period.



Water and Total Fluid Intake. The ANOVA (Age X Block X Line X Treatment) yielded no significant main effects for treatment in HAP or LAP mice for intake. The ANOVA for ml/kg water intake revealed a main effect by line [$F(1,67)=49.8$, $p<0.01$; LAP>HAP] and Block [$F(14,938)=23.5$, $p<0.01$], and a Block X Line interaction [$F(14,938)=13.7$, $p<0.01$]. A follow-up one-way ANOVA yielded a main effect of Block for both HAP and LAP mice ($p<0.01$).

The ANOVA for fluid intake yielded a main effect of Block [$F(14,938)=5.8$, $p<0.01$], Age [$F(1,67)=5.7$, $p<0.05$] and Line [$F(1,67)=22.5$, $p<0.01$], as well as a Block X Age X Treatment interaction [$F(14,983)=1.8$, $p<0.05$]. Follow-up analysis found a main effect of Block [$F(14,462)=2.3$, $p<0.01$] and Line [$F(1,33)=6.4$, $p<0.05$] for adolescents, and a main effect of Block [$F(14,476)=6.5$, $p<0.01$] and Line [$F(1,34)=18.1$, $p<0.01$], as well as a Block X Treatment interaction [$F(14,476)=1.9$, $p<0.05$] for adults. Follow-up analysis revealed a main effect of Block for mice in both the Saline and Alcohol groups ($p<0.01$)

(Table 3 on next page. Water and total fluid intake (ml/kg/BW) during the 30-day drinking period)

	HAP2/EtOH		HAP2/Saline		LAP2/EtOH		LAP2/Saline	
Experiment 1	**Water**	**Total**	**Water**	**Total**	**Water**	**Total**	**Water**	**Total**
2	187.1 ± 30.6	299.4 ± 21.3	216.3 ± 24.9	283.9 ± 18.8	227.2 ± 8.0	260.8 ± 12.0	209.3 ± 13.6	285.6 ± 42.9
4	211.9 ± 32.5	290.2 ± 9.1	178.5 ± 23.0	279.7 ± 10.2	244.5 ± 7.0	251.2 ± 7.3	233.9 ± 8.4	241.8 ± 8.6
6	179.6 ± 34.2	286.8 ± 10.2	184.7 ± 22.1	286.4 ± 13.9	248.2 ± 6.4	256.1 ± 8.4	239.1 ± 12.5	244.8 ± 13.0
8	153.6 ± 30.1	290.0 ± 10.8	145.0 ± 23.0	281.2 ± 12.3	246.6 ± 6.8	258.5 ± 9.1	259.7 ± 14.3	262.5 ± 15.0
10	179.8 ± 30.7	299.8 ± 11.4	168.2 ± 28.5	278.2 ± 14.3	253.1 ± 10.6	269.6 ± 12.0	244.2 ± 11.2	253.2 ± 11.4
12	128.9 ± 29.5	284.9 ± 8.5	128.7 ± 25.9	264.4 ± 11.0	238.9 ± 6.0	256.5 ± 7.6	243.2 ± 11.8	254.4 ± 11.6
14	120.6 ± 34.4	285.5 ± 10.5	128.5 ± 25.2	275.6 ± 9.9	233.3 ± 9.4	250.9 ± 12.0	250.6 ± 12.1	263.6 ± 12.4
16	126.6 ± 29.4	287.0 ± 12.0	101.8 ± 20.9	275.1 ± 15.9	241.8 ± 6.3	262.1 ± 9.7	227.6 ± 13.0	244.0 ± 13.4
18	101.5 ± 28.1	284.9 ± 8.8	123.3 ± 20.3	288.3 ± 18.6	231.2 ± 11.2	256.3 ± 12.4	235.6 ± 12.2	248.9 ± 12.5
20	102.8 ± 28.0	277.8 ± 9.7	89.0 ± 19.3	249.8 ± 18.4	234.3 ± 5.9	256.2 ± 7.6	224.3 ± 11.6	239.6 ± 10.0
22	92.7 ± 27.3	285.8 ± 12.7	145.6 ± 24.5	286.1 ± 17.6	245.2 ± 6.3	262.2 ± 7.4	238.9 ± 13.3	252.8 ± 12.3
24	107.6 ± 29.3	306.4 ± 15.8	73.3 ± 17.9	261.9 ± 14.3	231.7 ± 6.2	253.5 ± 9.4	222.1 ± 13.7	238.0 ± 13.5
26	72.5 ± 24.8	274.7 ± 9.0	83.4 ± 13.1	278.0 ± 19.3	239.9 ± 8.8	258.9 ± 11.7	220.4 ± 15.1	236.5 ± 14.3
28	94.4 ± 28.1	284.4 ± 10.9	67.0 ± 14.6	263.7 ± 15.1	237.6 ± 6.7	258.1 ± 9.4	227.5 ± 9.5	243.6 ± 8.8
30	71.2 ± 22.4	269.1 ± 10.7	83.8 ± 18.7	267.6 ± 16.3	232.4 ± 7.4	254.6 ± 8.4	223.1 ± 10.0	236.8 ± 9.2
Experiment 2	**Water**	**Total**	**Water**	**Total**	**Water**	**Total**	**Water**	**Total**
2	202.6 ± 42.6	296.8 ± 29.0	188.1 ± 31.6	313.78 ±12.5	260.5 ± 7.5	269.7 ± 8.4	230.5 ± 6.3	238.6 ± 8.4
4	180.6 ± 33.8	339.4 ± 25.7	153.1 ± 36.5	299.5 ± 10.8	265.3 ± 7.1	283.6 ± 10.6	237.6 ± 5.9	248.7 ± 6.6
6	193.1 ± 33.9	331.7 ± 15.0	188.3 ± 44.8	314.9 ± 14.7	279.7 ± 6.6	292.6 ± 7.6	247.4 ± 6.9	261.3 ± 8.0
8	205.0 ± 37.3	338.2 ± 14.0	164.3 ± 37.5	320.9 ± 11.5	279.1 ± 7.6	291.6 ± 7.7	256.2 ± 7.3	271.7 ± 9.3
10	167.4 ± 43.4	320.4 ± 15.5	142.9 ± 43.1	310.3 ± 12.2	263.6 ± 8.4	278.6 ± 7.9	243.4 ± 7.5	258.1 ± 9.4
12	170.7 ± 43.5	306.5 ± 18.7	155.1 ± 37.4	301.1 ± 12.1	264.8 ± 8.3	278.2 ± 7.6	245.7 ± 8.3	259.2 ± 10.3
14	167.9 ± 41.4	318.6 ± 15.2	133.2 ± 37.8	300.4 ± 18.6	259.3 ± 7.7	273.7 ± 7.9	237.2 ± 7.1	254.4 ± 9.6
16	156.1 ± 34.0	310.4 ± 11.8	119.8 ± 34.0	295.2 ± 10.0	262.6 ± 7.8	281.6 ± 7.2	217.1 ± 24.1	238.4 ± 19.0
18	172.7 ± 44.9	313.8 ± 18.5	118.7 ± 36.5	294.7 ± 12.1	258.5 ± 8.5	282.5 ± 8.4	234.6 ± 6.7	250.6 ± 9.4
20	140.3 ± 32.5	302.6 ± 13.8	110.8 ± 31.7	293.4 ± 10.9	260.8 ± 6.6	277.9 ± 4.3	239.4 ± 6.2	258.0 ± 8.0

Experiment 2	Water	Total	Water	Total	Water	Total	Water	Total
22	146.5 ± 41.9	323.0 ± 15.3	114.7 ± 33.4	306.3 ± 9.6	235.7 ± 17.1	255.1 ± 15.5	236.5 ± 6.1	255.3 ± 8.8
24	135.6 ± 33.4	280.3 ± 20.2	100.0 ± 28.7	304.1 ± 8.9	249.8 ± 7.3	272.6 ± 6.0	230.3 ± 4.4	249.9 ± 6.9
26	121.5 ± 37.7	284.6 ± 17.8	104.2 ± 31.8	292.2 ± 11.3	248.0 ± 6.3	267.6 ± 5.5	228.8 ± 5.9	246.5 ± 7.9
28	101.1 ± 28.6	274.3 ± 18.8	97.9 ± 30.0	290.3 ± 9.7	246.1 ± 7.7	266.1 ± 9.3	228.4 ± 6.0	246.9 ± 9.0
30	109.5 ± 31.5	286.9 ± 13.2	97.7 ± 30.5	293.5 ± 11.5	240.2 ± 6.0	258.2 ± 5.2	231.9 ± 5.7	249.3 ± 8.1

Discussion

The present results showed that alcohol exposure did not increase subsequent alcohol drinking behavior in mice selectively bred for high or low alcohol preference. This was contrary to our hypothesis. These results suggest that genetic predisposition is a stronger predictor of future alcohol drinking than early alcohol exposure under the present conditions. These findings support the studies that find family history is strongly correlated with adult drinking (Bennett et al, 2006; Enoch, 2006; Vengeliene et al, 2003; Schuckit, 1995).

In addition to these results, there was a trend for adult mice that were injected with saline to show higher preference for alcohol than mice injected with alcohol as adults. However, this trend was not statistically significant. Forced alcohol intake has been shown to reduce the consumption of alcohol in rats bred for high alcohol intake (Chester et al, 2005). The findings of the present study would support the theory that forced alcohol exposure constitutes a stress, that when associated with drinking inhibits alcohol consumption. However, this only partially explains the differences between adult and adolescent drinking.

Varlinskaya and Spear (2006) demonstrated acute tolerance in adolescent rats. In that study, adolescents, but not adults, injected with 1g/kg ethanol showed reduced social inhibition 30 minutes after injection. It was argued that this was a sign of acute tolerance in the adolescent, but not adult, rat (Varlinskaya and Spear 2006). The possibility remains that adolescent mice in the present study also demonstrated an acute tolerance to ethanol, reducing the effect of early alcohol exposure. This theory may partially explain the present results, but still leaves questions to be answered.

Waldrop et al (2007) finds that people with childhood traumas drink at an earlier age and begin heavy drinking earlier. Additionally, a prior study done in our laboratory showed an increase in drinking behavior in stressed adolescent mice selectively bred for high alcohol preference (Chester et al, under editorial review). These results would suggest that adolescents are more susceptible to stress-

induced drinking. However, Brunell and Spear (2005) demonstrate a suppression of adolescent drinking in stressed rats. These data contradict our previous study; however, because the age at which the mice were given free-access to alcohol was viewed as the most important factor to control for, there was a difference in experimental time frames between these two studies. Both studies do, however, support the theory that stress during adolescence has some effect on drinking behavior. The present study used IP injections during adolescence. The stress of the injection may have had an effect on the drinking behavior of all mice, given the fact that all mice received the same amount of injections.

Clark et al (2008) postulates that researchers expect negative developmental effects to occur in the adolescent brain following early alcohol exposure. For this reason, many results may be over-interpreted. Small interactions may be reported as evidence to support the claim that early alcohol exposure has profound negative effect. The absence of interactions may not be reported at all. Further supporting this claim is the lack of definitive results in the literature. While it is highly unlikely that early alcohol exposure has no effect on the developing brain, the effects may not be as large as expected. The findings of the current study may support the theory that early alcohol exposure does not have as dramatic an effect on a developing brain as once believed. With the small effects found in the present study, one could question the interpretation of prior research findings. In fact, there is evidence to suggest that adolescence may be less sensitive to alcohol's effects.

One question that remains unanswered is the individual differences that occur in the development of alcohol dependency. In a study of children of alcoholics (COAs), it was shown that COAs could be broken into two groups, vulnerable and resilient to problem drinking (Heitzeg et al., 2008). After analyzing individual drinking patterns of HAP2 mice, there were individual differences in the amount of ethanol consumed. It would be logical for all animals of a certain line within a group that are treated in the same manner to exhibit similar drinking patterns. This does not appear to be the case upon further examination of the data. As shown in Figure 12, adult HAP2 mice injected with ethanol, as compared to adolescent mice (Figures 10 and 11) and adult mice injected with saline (Figure 13), did not show any consistent trend between subjects. These data could support the theory of resiliency and vulnerability among individuals with a family history of alcohol use, or the existence of these characteristics within mouse lines of high alcohol preference.

Figure 6. Daily mean alcohol intake in g/kg/BW (left panel) and % alcohol preference (right panel) in HAP2 mice injected with ethanol during adolescence averaged across the entire 30-day period.

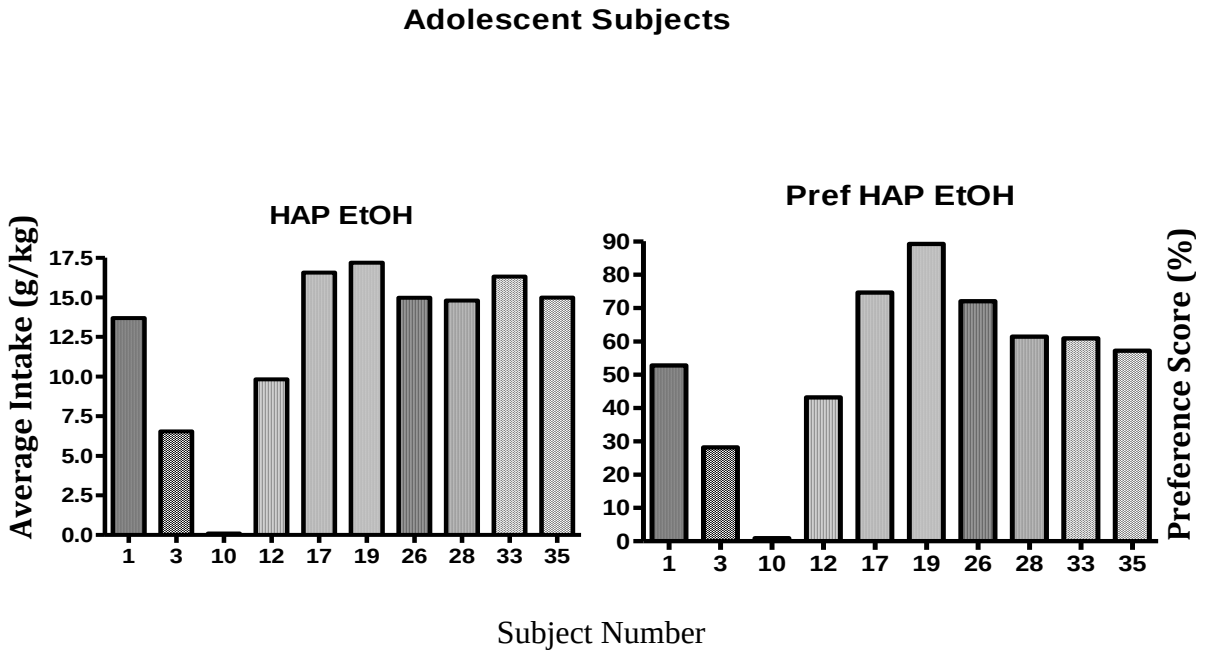


Figure 7. Daily mean alcohol intake in g/kg/BW (left panel) and % alcohol preference (right panel) in HAP2 mice injected with saline during adolescence averaged across the entire 30-day period.

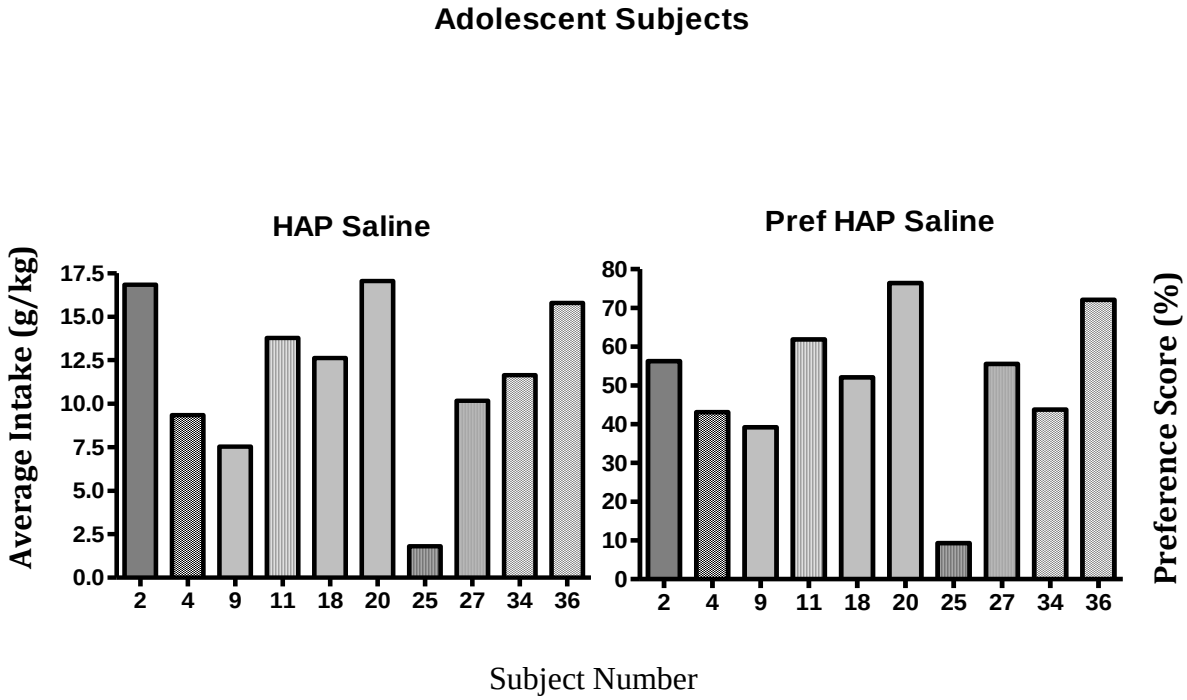


Figure 8. Daily mean alcohol intake in g/kg/BW (left panel) and % alcohol preference (right panel) in HAP2 mice injected with ethanol during adulthood averaged across the entire 30-day period.

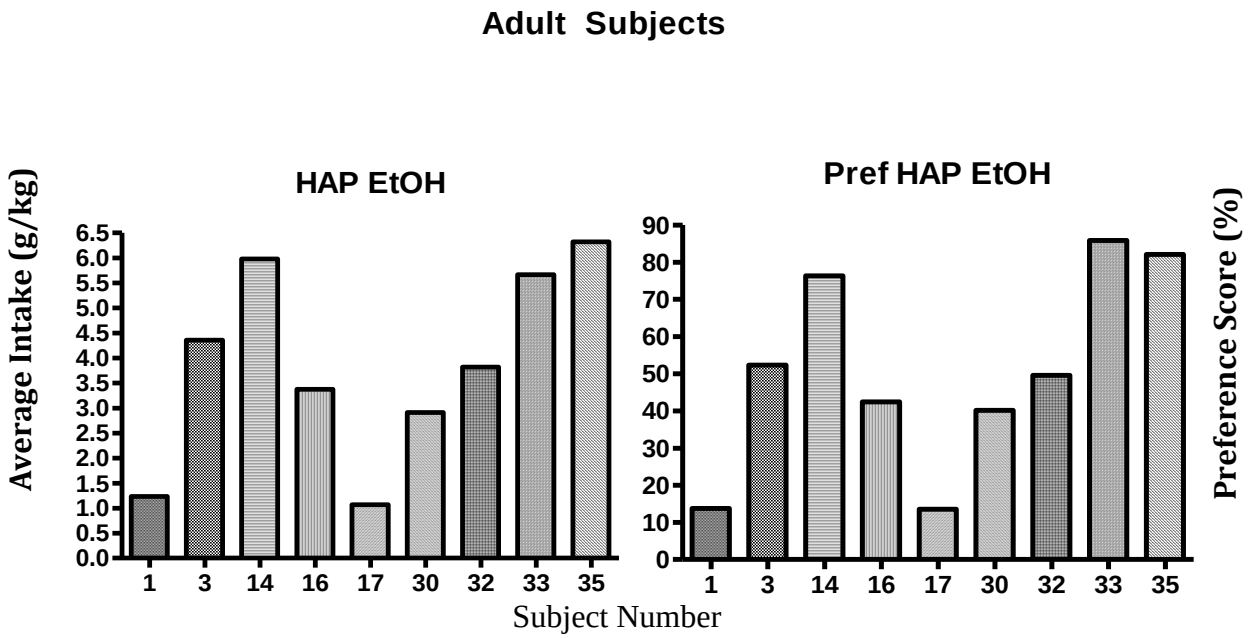
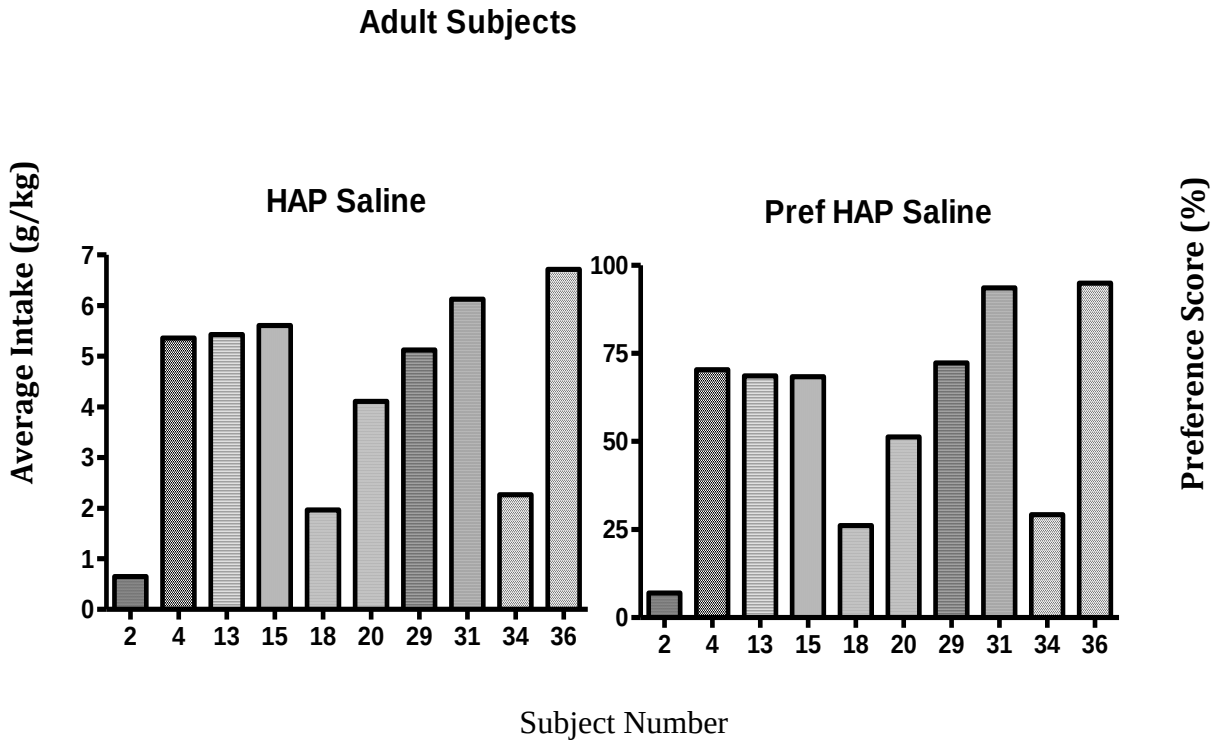


Figure 9. Daily mean alcohol intake in g/kg/BW (left panel) and % alcohol preference (right panel) in HAP2 mice injected with saline during adulthood averaged across the entire 30-day period.



The present study had a couple of important limitations that should be noted. First of all, due to the limitation of the number of animals at our disposal, a no-stress group could not be included. The inclusion of a no-stress group would allow us to differentiate the effects of the alcohol pretreatment and stress on drinking behaviors. The possibility exists that the alcohol injection alone would show a significant effect on drinking behavior, but stress is masking the effect.

The second important limitation is that mice did not self administer the alcohol pre-exposure. Self-administration was not an option for the purposes of the pre-exposure phase of the present study due to the fact that the LAP mice naturally avoid alcohol and HAP mice would drink a higher amount. It was deemed necessary for the present experiment to control the amount of alcohol given during the pre-exposure phase. IP injections were best method available to us.

The method of pre-exposure has been shown to be a factor in drinking behavior. Blizard et al (2004) demonstrated that different types of exposure may affect alcohol preference differently. This study utilized several different types of exposure, and measured alcohol consumption following these exposures. While all forms of exposure increased preference in BALB/cByJ lines of mice, only gradual initiation increased preference in BALB/cJ lines. These findings suggest that not only does the method of exposure have an effect, but that it interacts with line.

The possibility exists that the HAP2 mice in the current study are consuming the highest level of alcohol that they will voluntarily consume. Prior data show that HAP1 mice consume about 18 g/kg in response to line selection (see Subject section for more information), and HAP2 male mice consume slightly less at 16 g/kg (Nicholas Grahame, personal communication). These levels are very close to the levels of intake shown in the present study. This could suggest that these mice will not voluntarily consume more than this level. However, further research is needed in order to substantiate this claim.

While early onset drinking correlates highly with lifetime dependence, it may serve as a marker for later abuse instead of a precursor (Spear 2002). The early initiation of drinking behavior may not be the cause of later alcohol abuse, but, instead, both behaviors may stem from an underlying condition. This idea may be justification for a switch in the focus of research from the impact of early alcohol exposure to the causes of early alcohol exposure. This could help identify potential alcohol abusers early in life, and help to set up an early intervention and treatment program.

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