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Adolescent C57BL/6J (but not DBA/2J) Mice Consume Greater Amounts of Limited-Access Ethanol Compared to Adults and Display Continued Elevated Ethanol Intake into Adulthood

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Abstract

Background—Alcohol use is common during the adolescent period, a time at which a number of crucial neurobiological, hormonal, and behavioral changes occur (Spear, 2000). In order to more fully understand the complex interaction between alcohol use and these age-typical neurobiological changes, animal models must be utilized. Rodents experience a developmental period similar to that of adolescence. Although rat models have shown striking adolescent-specific differences in sensitivity to ethanol, little work has been done in mice despite the fact that the C57BL/6J (B6) and DBA2/J (D2) mice have been shown to markedly differ in ethanol preference drinking and exhibit widely different sensitivities to ethanol.

Methods—The current study examined ethanol intake in adolescent and adult B6 and D2 mice using a limited access alcohol exposure paradigm called Drinking in the Dark (DID). Additionally, the effect of adolescent (or adult) ethanol exposure on subsequent adult ethanol intake was examined by re-exposing the mice to the same paradigm once the adolescents reached adulthood. We hypothesized that adolescent (P25–45) mice would exhibit greater binge-like alcohol intake compared to adults (P60–80), and that B6 mice would exhibit greater binge-like alcohol intake compared to D2 mice. Moreover, we predicted that relative difference in binge-like alcohol intake between adolescents and adults would be greater in D2 mice.

Results—Adolescent B6 mice consumed more ethanol than adults in the DID model. There was no difference between adolescent and adult D2 mice.

Conclusions—This work adds to the literature suggesting that adolescents will consume more ethanol than adults and that this exposure can result in altered adult intake. However, this effect seems largely dependent upon genotype. Future work will continue to examine age-related differences in ethanol intake, preference, and sensitivity in inbred mouse strains.

Keywords

Adolescent; Mice; Ethanol; Drinking in the Dark; Development

Alcohol (ethanol) use often begins during the teenage years, a sensitive period during which a number of neurobiological, hormonal, and behavioral changes occur. A recent survey

suggests that approximately 26% of high school seniors have had 5 or more drinks in a row (i.e., have engaged in binge drinking) during the previous 2 weeks (Johnston et al., 2008). Moreover, clinical reports indicate that adolescents frequently exhibit the symptoms of alcohol dependence. For example, some adolescents have difficulty controlling their alcohol intake and display signs of tolerance (Hawkins et al., 1997; Spear, 2000; Stewart and Brown, 1995). Such alcohol use during adolescence may have profound effects on brain development maturation, and may even influence the propensity to use the drug in later life.

The development of useful animal models is crucial if we are to understand the complex interaction between alcohol sensitivity and the neurobiological changes that occur during the period of adolescence. Although human adolescents undergo developmental changes unparalleled in lower organisms, other mammalian species (including rodents) also experience a developmental period similar to adolescence (Spear, 2000). In rats and mice, the term “adolescence” is used to describe animals aged from weaning (P21) to adulthood (P60); animals that are no longer infants, but that are not yet adults (Laviola et al., 2003). The period of adolescence has also been subdivided into more discrete intervals described as early (P21–34), middle (P34–46), and late (P46–59) adolescence (Spear, 2000). Adolescent rodents model many of the behaviors believed to be hallmarks of human adolescence, including elevated levels of social play and affiliative behaviors (Meaney and Stewart, 1981; Panksepp, 1981). These behaviors peak at P32 and display an ontogenetic shift toward more aggressive and competitive behaviors, including the establishment of dominant and subordinate relationships (Terranova et al., 1993, 1998).

A number of studies have demonstrated that adolescent rats also exhibit altered behavioral sensitivity to ethanol. Adolescent rats have been documented to display reduced sensitivity to the sedative/hypnotic (Little et al., 1996; Silveri and Spear, 1998; Silveri and Spear, 1999), motor impairing (White et al., 2002), hypothermic (Silveri and Spear, 2000), and anxiolytic (Varlinskaya and Spear, 2002) effects of ethanol. Adolescent rats have demonstrated increased sensitivity to still other ethanol-related behavioral traits. Adolescent rats are more sensitive to the amnesic (Markwiese et al., 1998) and social facilitatory (Varlinskaya and Spear, 2002) actions of ethanol. Moreover, recent evidence suggests that adolescent rats may develop greater acute (within session) ethanol tolerance (Silveri and Spear, 1998; Varlinskaya and Spear, 2006), and consume greater amounts of ethanol (Doremus et al., 2005; Maldonado et al., 2008; Vetter et al., 2007), compared to their adult counterparts. Taken together, the above evidence indicates that rodents might provide a powerful model system for investigating the neurobiological underpinnings of ethanol’s actions during the critical developmental period of adolescence.

Although much is known about the behavioral actions of ethanol in adolescent rats, little work has been done in adolescent mice despite the fact that mice have been one of the most widely used animal models in ethanol research for decades. For example, the adult members of the C57BL/6J (B6) and DBA/2J (D2) inbred mouse strains have been shown to differ widely in ethanol behavioral sensitivity; B6 animals readily consume more ethanol using 2-bottle choice procedures (McClearn and Rodgers, 1959) and are less sensitive to ethanol’s locomotor stimulant (Phillips et al., 1994), hangover (Metten and Crabbe, 1994), and reinforcing actions (Cunningham et al., 1992). Because the ethanol behavioral sensitivities of adult B6 and D2 mice are so extensively characterized, examination of adolescent sensitivity in these strains may provide a useful picture of how ethanol behavioral sensitivity during adolescence relates to that of adulthood.

Several recent studies reported that B6 mice will drink significant amounts of a 20% ethanol solution if it is presented from 2 to 4 hours, 3 hours into the dark cycle (Moore et al., 2007; Rhodes et al., 2005, 2006). Called Drinking in the Dark (DID), B6 mice consume large amounts

of ethanol (4 to 8 g ethanol/kg body weight), often reaching blood ethanol concentrations of 100 mg ethanol/dl plasma, or greater. Blood ethanol concentrations are physiologically relevant, producing significant motor incoordination on the rotarod (Rhodes et al., 2006) and balance beam motor tasks (Moore et al., 2007; Rhodes et al., 2006). However, the mice are not water deprived at any time except during the ethanol access period and are therefore not “artificially” motivated to drink the ethanol solution, and lengthy periods of sucrose-fading or ethanol concentration ramping are not required. This gives the model an advantage over classical 2-bottle choice tests when attempting to assess ethanol intake during the relatively short period of adolescence in mice.

Interestingly, DID appears to mimic the kind of binge-type ethanol intake that is characteristic of ethanol intake in human adolescents. Indeed, binge drinking, consumption of ≥ 5 drinks during a single occasion with blood ethanol concentrations reaching 80 mg/dl (NIAAA National Advisory Council, 2004) accounted for an estimated 56% of drinking among youth aged 12 to 17 years old in 2003, and this estimate increased to 69% in adolescents aged 18 years old and older (Miller et al., 2008). Thus, use of DID to investigate ethanol intake among adolescent mice may provide a more valid approach to investigating whether adolescents do indeed consume more ethanol than their adult counterparts. In the current study, we assess ethanol intake in adolescent and adult B6 and D2 mice using DID procedures. We show that consistent with the literature, B6 adults exhibit greater ethanol intake compared to D2 adults. We also show that although adolescent mice consume more ethanol than their adult counterparts, and that such adolescent ethanol exposure increases ethanol intake in adulthood, both effects depend on genotype.

MATERIALS AND METHODS

Animals

Male and female C57BL/6J (B6) and DBA/2J (D2) inbred mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and shipped to the AAALAC accredited animal research facility at Binghamton University. Animals were housed one mouse per cage in clear polycarbonate shoebox mouse cages with a short segment of PVC pipe (approximately 7.6 cm long, with a 3.8 cm diameter opening) as environmental enrichment and food and water available ad libitum except as indicated below. Mice were either 21 (P21) or 56 (P56) days old at the time of shipping and were allowed to acclimate to the facility for 1 week prior to the initiation of experiments. Therefore, the mice tested in the current work were 28 (P28, adolescent) or 63 (P63, adult) days old at the start of testing. Mice were maintained on a reverse 12-hour light–dark cycle (lights out at 10 AM) with the temperature and humidity of the vivarium kept at approximately 20°C and 50%, respectively. Red incandescent lamps were continuously left on so that experimenters could navigate the procedure room after lights out, and an adjacent anti-room allowed experimenters to enter the dark procedure room without outside light flooding the room. All procedures were approved by the Binghamton University Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

Drinking in the Dark

Limited-access ethanol intake in adolescent and adult mice was assessed using DID procedures ($n = 8$ to 12 per genotype, sex, and age). Our procedure is similar to that of Rhodes and colleagues (2005), and some of our initial work with the procedure was recently published in Moore and colleagues (2007). In this procedure, the water bottle is replaced with a single bottle containing a 20% unsweetened ethanol solution. Mice were given a 2-hour access to this ethanol solution 3 hours after lights out (beginning at 1 PM). A 10 ml plastic tube affixed to a ball-bearing sipper filled with the ethanol solution was placed in the cage where the water bottle was normally located. To prevent the mice from moving the sipper tube and producing potential

leakage, the animals' regular water bottle was placed on top of the sipper tube to hold it steady. Adolescent and adult mice were given daily access to the ethanol-filled sipper tubes for 14 consecutive days (experimental phase 1). Sipper tube volumes were recorded both before and after the 2-hour access period each day except on phase 1 ethanol access days 1, 7, and 14 when ethanol intakes were recorded at 30, 60, 90, and 120 minutes following sipper presentation. On the final day (day 14), retro-orbital sinus blood samples were taken immediately after ethanol access for assessment of ethanol content (see detailed methods below). This 14-day procedure was repeated in both groups of mice after a 3-week period without ethanol access, once the adolescents reached P63 (adults P98), to determine whether adolescent exposure to DID procedures alters subsequent adult DID (experimental phase 2). Sipper tube volumes were again recorded both before and after the 2-hour access period each day except on phase 2 ethanol access days 15, 21, and 28 when ethanol intakes were recorded at 30, 60, 90, and 120 minutes following sipper presentation. A retro-orbital sinus blood sample was again sampled upon conclusion of the 2-hour ethanol access period on the final day of the second phase of the experiment.

Blood EtOH Concentration Determination

For determination of blood ethanol content, a retro-orbital sinus blood sample (25 μ l) was collected and spun down, and the plasma fraction siphoned off and stored in a -20° freezer until the time of assay. Determination of blood ethanol content was achieved using an Analox Alcohol Analyzer (Analox Instruments, Lunenburg, MA).

Statistical Analysis

Data were analyzed by a 4-way mixed factor analysis of variance (ANOVA) with genotype, sex, and age as between groups factors, and day (or time) as the within groups factor. However, the initial 14 days of ethanol exposure (phase 1) were analyzed separately from the latter 14 days of ethanol access (phase 2). Additional analyses with the average intake over each 14-day ethanol access period were conducted to determine the influence of adolescent ethanol exposure on adult intake. Additionally, when the pattern of ethanol intake (time) was analyzed on ethanol access days 1, 7, 14, 15, 21, and 28, each day was analyzed separately. Subsequent Newman-Keuls posthoc comparisons were used to isolate significant effects when appropriate. Differences were considered significant at $p < 0.05$.

RESULTS

Daily Ethanol Intake During Experimental Phase 1

Daily limited-access ethanol intakes for adolescent and adult B6 and D2 mice can be seen in Fig. 1A,B and Table 1. The 4-way analysis indicated significant main effects of genotype [$F(1,67) = 431.5, p < 0.001$], sex [$F(1,67) = 6.1, p < 0.02$], age [$F(1,67) = 13.1, p < 0.001$], and day [$F(13,871) = 6.2, p < 0.001$], and significant interactions of genotype $\times$ age [$F(1,67) = 6.2, p < 0.02$], and genotype $\times$ day [$F(13,871) = 2.7, p < 0.001$]. The 4-way interaction was not significant. Follow-up Newman-Keuls post-hoc analysis of the interaction between genotype and age revealed that B6 adolescents consumed significantly greater amounts of ethanol compared to all other ages/strains (p 's < 0.001). Additionally, B6 adults consumed more ethanol than D2's of either age (p 's < 0.001). Thus, both age and genotype are important influencers on ethanol consumption. Adolescent B6 mice appear to consume more ethanol than their adult counterparts, while there is no apparent age effect in the D2 inbred strain.

Daily Ethanol Intake During Experimental Phase 2

The 4-way analysis of the second 14-day period was performed to determine whether adolescent limited-access ethanol exposure alters subsequent intake in adulthood. This analysis

indicated significant main effects of genotype [$F(1,67) = 562.5, p < 0.001$], sex [$F(1,71) = 28.4, p < 0.001$], and day [$F(13,871) = 9.8, p < 0.001$]. The main effect of age did not quite reach significance, although there was a trend towards a significant effect [$F(1,71) = 3.2, p = 0.08$]. Significant interactions of genotype $\times$ age [$F(1,67) = 45.1, p < 0.03$], and day $\times$ genotype [$F(13,871) = 12.6, p < 0.001$] were also detected. Newman–Keuls post-hoc analysis of the genotype $\times$ age interaction indicated that B6 mice with adolescent ethanol exposure consumed the most ethanol (p 's < 0.01), while B6 mice with prior adulthood ethanol exposure consumed more ethanol than D2's regardless of age of prior ethanol exposure (p 's < 0.001). Age of prior ethanol exposure did not affect ethanol consumption during adulthood in D2 mice. Thus, adolescent exposure to limited-access ethanol appears to elevate ethanol exposure during adulthood in B6 mice, but similar adolescent exposure does not alter adult intake in D2 animals.

Blood Ethanol Concentration

Two separate 2-way ANOVAs were conducted for the analysis of blood ethanol concentrations (BECs). Analysis of the BECs on ethanol exposure day 14 revealed only a main effect of genotype [$F(1,72) = 49.9, p < 0.001$]. Analysis of the BECs on ethanol exposure day 28 also found only a main effect of genotype [$F(1,72) = 92.6, p < 0.001$]. Although B6 mice had higher BECs than D2 mice (corresponding to the intake data), no differences in BEC were observed between adolescents and adults of either strain (Table 2).

Effect of Adolescent Ethanol Exposure

Although prior analyses suggested that adolescent ethanol exposure increased ethanol intake in adulthood, the analysis compared 63- to 76-day-old mice (that drank ethanol during adolescence at ages 28 to 41 days) and 98- to 111-day-old mice (that previously drank ethanol during early adulthood at days 63 to 76 of life). We therefore conducted an analysis to compare mice of the same age: P63–76 adult mice from pass 1 (with no prior ethanol exposure) with P63–76 adult mice of pass 2 (with prior adolescent ethanol exposure). Because it was not of interest to compare the specific intakes on each of the 14 days of ethanol access, we averaged the daily ethanol intake data and analyzed it by a 3-way between subjects ANOVA (with genotype, sex and prior adolescent ethanol exposure as between subjects factors). The analysis revealed main effects of genotype [$F(1,67) = 586.3, p < 0.001$], sex [$F(1,67) = 18.4, p < 0.001$], prior adolescent ethanol exposure [$F(1,67) = 23.8, p < 0.001$], and an interaction of genotype and prior ethanol exposure [$F(1,67) = 20.0, p < 0.001$]. Newman–Keuls post-hoc tests indicated that B6 adult mice with access to ethanol during adolescence consumed more ethanol during adulthood than B6 adult mice with no prior ethanol exposure ($p < 0.001$). Additionally, B6 mice consumed more ethanol than D2 mice (regardless of prior ethanol exposure; p 's < 0.001). Adolescent ethanol exposure had no influence on ethanol intake during adulthood in D2 mice. Thus, even when controlling for age at testing, adolescent ethanol exposure increases ethanol intake in adulthood in B6 (but not D2) mice.

Pattern of Ethanol Intake

Intake was assessed at 30, 60, 90, and 120 during ethanol exposure days 1, 7, 14, 15, 21, and 28 to determine whether the pattern of ethanol intake differed by genotype, sex, or age. Intake pattern data can be seen in Fig. 2A–F. The 4-way analysis indicated significant main effects of genotype [$F(1,67) = 316.9, p < 0.001$], sex [$F(1,67) = 14.3, p < 0.001$], age [$F(1,67) = 7.3, p < 0.01$], and time [$F(23,1541) = 20.8, p < 0.001$], and significant interactions of genotype $\times$ time [$F(23,1541) = 17.4, p < 0.001$], and age $\times$ time [$F(23,1541) = 1.7, p < 0.02$], and genotype $\times$ age $\times$ time [$F(23,1541) = 1.6, p < 0.03$]. The 4-way interaction was not significant. Similar to the results reported above, sex did not interact with any other factor. Therefore, because of our a priori interests in determining whether ethanol intake patterns differed between adolescent and adult mice and whether previous exposure to ethanol alters ethanol intake

patterns, the decision was made to collapse on sex and reanalyze the data to reduce the complexity and redundancy of the analyses of the pattern of ethanol intake. The pattern of ethanol intakes on days 1, 7, 14, 15, 21, and 28 were each analyzed separately. Therefore, the following analyses are 3-way repeated measures ANOVAs, with genotype and age as the between subjects factors, and time as the within-subjects factors.

Pattern of Ethanol Intake on Day 1

The 3-way analysis of the pattern of ethanol intake on the first day of ethanol access revealed main effects of genotype [$F(1,71) = 54.5, p < 0.001$], and age [$F(1,71) = 9.3, p < 0.01$], however there was no main effect of time and no significant interactions. Although B6 mice consumed more ethanol than D2 mice, and adolescents consumed more ethanol than adults, there was no genotype or age-dependent difference in ethanol intake among any of the 30-minute time bins during the 2-hour testing period (Fig. 2A).

Pattern of Ethanol Intake on Day 7

The 3-way analysis of the pattern of ethanol intake on the seventh day of ethanol access revealed main effects of genotype [$F(1,71) = 125.6, p < 0.001$], and time [$F(3,213) = 17.9, p < 0.001$], as well as a genotype $\times$ time interaction [$F(3,213) = 13.8, p < 0.001$]. Post-hoc analysis of the genotype $\times$ time interaction revealed that B6 mice consumed more ethanol than D2 mice at each 30-minute time bin (p 's < 0.001). Additionally, it was revealed that B6 mice consumed the most ethanol during the first 30-minute time bin (p 's < 0.03 ; Fig. 2B).

Pattern of Ethanol Intake on Day 14

The 3-way analysis of the pattern of ethanol intake on the fourteenth day of ethanol access revealed main effects of genotype [$F(1,71) = 138.2, p < 0.001$], and time [$F(3,213) = 39.3, p < 0.001$], as well as a genotype $\times$ time interaction [$F(3,213) = 29.4, p < 0.001$]. Post-hoc analysis of the genotype $\times$ time interaction revealed that like the pattern on Day 7, B6 mice consumed the most ethanol intake during the first 30-minute time bin in the 2-hour ethanol access period (p 's < 0.001). However, although B6 mice consumed significantly more ethanol than D2 mice in the first and second time bins (p 's < 0.001) there was no significant difference between B6 and D2 mice in their ethanol consumption during the third and fourth 30-minute time bins (Fig. 2C).

Pattern of Ethanol Intake on Day 15 (Phase 2, Day 1)

The 3-way analysis of the pattern of ethanol intake on the fifteenth day of ethanol access revealed main effects of genotype [$F(1,71) = 35.8, p < 0.001$], age [$F(1,71) = 5.0, p < 0.001$], and time [$F(3,213) = 4.2, p < 0.01$], as well as a genotype $\times$ age $\times$ time interaction [$F(3,213) = 2.8, p < 0.04$] [and a trend towards an age $\times$ time interaction, $F(3,213) = 2.2, p = 0.09$]. Post-hoc analysis of the genotype $\times$ age $\times$ time interaction revealed that B6 mice that were previously exposed to ethanol during adolescence consumed significantly more ethanol than their D2 counterparts during the first 30-minute period on Day 36 ($p = 0.001$); however no other interesting significant effects were detected (Fig. 2D).

Pattern of Ethanol Intake on Day 21 (Phase 2, Day 7)

The 3-way analysis of the pattern of ethanol intake on the twenty-first day of ethanol access revealed main effects of genotype [$F(1,71) = 99.5, p < 0.001$], age [$F(1,71) = 4.8, p < 0.04$], and time [$F(3,213) = 39.6, p < 0.001$], as well as a genotype $\times$ time interaction [$F(3,213) = 40.2, p < 0.001$]. Post-hoc analysis of the genotype $\times$ time interaction revealed that B6 mice consumed significantly more ethanol than D2 mice during the first 30-minute period ($p = 0.001$). Additionally, the post-hoc analysis revealed that B6 mice consumed the most ethanol during the first 30-minute time period (p 's < 0.001 ; Fig. 2E).

Pattern of Ethanol Intake on Day 28 (Phase 2, Day 14)

The 3-way analysis of the pattern of ethanol intake on the twenty-eighth day of ethanol access revealed main effects of genotype [$F(1,71) = 99.1, p < 0.001$], and time [$F(3,213) = 30.6, p < 0.001$], as well as interactions of genotype $\times$ time [$F(3,213) = 34.6, p < 0.001$], age $\times$ time [$F(3,213) = 4.9, p < 0.01$], and genotype $\times$ age $\times$ time [$F(3,213) = 4.8, p < 0.01$]. Post-hoc analysis of the genotype $\times$ age $\times$ time interaction revealed that both B6 mice that were previously exposed to ethanol during adulthood and adolescence consumed significantly more ethanol than D2 mice previously exposed to ethanol during adulthood and adolescence (respectively) during the first 30-minute period (p 's < 0.001). Additionally, B6 mice previously exposed to ethanol during both adulthood and adolescence consumed the most ethanol during the first 30 minutes of ethanol access (p 's < 0.001). Interestingly, although B6 mice that were previously exposed to ethanol during adolescence consumed more ethanol than B6 mice previously exposed during adulthood during the first 30-minute access period ($p < 0.001$); a switch occurred during the second 30-minute time period where the B6 mice exposed during adulthood consumed more ethanol than the B6 mice exposed during adolescence ($p < 0.01$; Fig. 2F).

DISCUSSION

Adolescent C57BL/6J (B6) mice consume more ethanol than their adult counterparts, however there is no difference between adolescent and adult DBA/2J (D2) mice in their consumption of ethanol. Furthermore, ethanol intake during adolescence increases subsequent ethanol intake during adulthood in the B6 but not D2 mice. One important caveat to note here is that because ethanol intakes differed between adolescent B6 and D2 mice (where B6 mice consumed significantly more ethanol than D2 mice) it is not possible to know if D2 mice provided with the same amount of ethanol exposure during adolescence would increase subsequent ethanol consumption in adulthood. The B6 and D2 mice have long been shown to differ in their ethanol intake (McClern and Rodgers, 1959; Rhodes et al., 2005). However, to our knowledge this is the first report to compare B6 and D2 mice in their ethanol intake during adolescence as well as their subsequent adult ethanol intake.

Ethanol sensitivity is likely dependent upon both age and genotype. Further, it is possible that these age- and genotype-related differences in ethanol sensitivity may also underlie the observed differences in ethanol drinking. For example adolescent mice may, in general, be more sensitive to the rewarding properties of ethanol while at the same time being less sensitive to the aversive properties of the drug. This might reduce the adolescent mouse's capacity to determine when to stop drinking, resulting in increased adolescent consumption of ethanol (as observed in B6 mice). However, that behavioral sensitivity to ethanol is also dependent upon genotype may explain why no differences are observed between adolescent and adult D2 mice.

Several labs have recently published reports describing behavioral sensitivity to ethanol in B6 and/or D2 mice which are consistent with the idea that adolescents may exhibit increased sensitivity to the rewarding properties of ethanol and/or decreased sensitivity to the aversive properties of ethanol. For example, Lopez and colleagues (2003) demonstrated that adolescent B6 mice were less sensitive to the ethanol's aversive locomotor sedative and hypothermic effects. Further, Hefner and Holmes (2007) showed that whereas adolescent B6 mice are more sensitive to ethanol's rewarding locomotor stimulant and anxiolytic effects, they are less sensitive to ethanol's aversive hypnotic effects and may absorb and metabolize ethanol faster than their adult counterparts. Contrary to findings using rats (Doremus et al., 2005; Vetter et al., 2007), it was also shown that early adolescent B6 mice might exhibit reduced ethanol preference drinking (classic 2-bottle choice) compared to adults (Hefner and Holmes, 2007). A recent report by Strong and colleagues (2009) has demonstrated that using a schedule high alcohol consumption (SHAC) procedure, adolescent B6 mice exhibit greater ethanol intakes during the initial ethanol sessions compared to adults, and this adolescent binge-like ethanol

exposure increased subsequent 24-hour access ethanol (but not limited access ethanol) intake in adulthood. Fewer published reports are available regarding the D2 mice, however Stevenson and colleagues (2008) have demonstrated that adolescent D2 mice are more sensitive to the low-dose rewarding locomotor stimulant actions of several ethanol doses. However, Dickinson and colleagues (2009) reported that adolescent D2 mice are less sensitive to the rewarding effects of ethanol compared to adults as assessed by conditioned place preference, which is consistent with the idea that genotype may over-ride the age-related alterations in ethanol sensitivity.

Only one study to date has directly compared adolescent and adult B6 and D2 inbred mice in their sensitivity to ethanol. Linsenhardt and colleagues (2009) assessed adolescent and adult sensitivity to the aversive hypnotic and motor-incoordinating effects of ethanol in the B6 and D2 inbred mouse strains. Adolescents of both inbred mouse strains were found to regain their righting reflex more quickly than adults after a 4 g/kg ethanol injection; though upon regain the blood ethanol concentrations (BECs) between the ages were not different, which may indicate that adolescents may exhibit differing rates of ethanol metabolism. Further, although B6 adult mice regained their righting reflex faster than D2 adult mice, there was no difference between the genotypes in the adolescent mice. Linsenhardt and colleagues (2009) also indexed genotype dependent differences in low-dose ethanol induced motor-incoordination and found that adolescent mice of both genotypes were generally more sensitive to the ataxic effects of ethanol compared to adult mice, although again BECs after the test seemed to indicate that there may be differences in ethanol absorption/metabolism between the ages.

An alternative explanation for the increased ethanol consumption seen in the B6 adolescents may simply be a result of adolescent-typical hyperphagia (Spear, 2000). The current work only assessed ethanol intake, and not general food or water intake. Previous work from our laboratory has measured 24-hour food and fluid intake in adolescent and adult B6 and D2 mice and found that adolescents typically consume more food (but not water) compared to adults (Moore et al., unpublished observation). Considering that adolescents are undergoing a rapid phase of growth, this is not surprising. It is therefore not possible to exclude a general hyperphagic response of the adolescents as the underlying cause of the increased ethanol intake. However, since no difference in ethanol intake was observed between D2 adolescents and adults, if the adolescent mice are displaying hyperphagia it is limited by genotype.

Additionally, various perturbations have been shown to affect adolescent rodents differentially than their adult counterparts (Brunell and Spear, 2005; Ristuccia et al., 2007; Varlinskaya and Spear, 2002) and may also explain age-related differences in ethanol intake. It is important, therefore, to note that there were a number of potential perturbations present in the current investigation that may have affected our results. First, our animals were shipped from Jackson Laboratory to the facilities at Binghamton University. Various unknown conditions that the animals may have encountered during this shipping process could potentially have affected the data, and therefore future investigations utilizing animals bred in-house are warranted. Secondly, in order to assess any potential differences in the pattern of ethanol intake between adolescents and adults across the ethanol access paradigm we carefully removed and replaced ethanol drinking bottles every 30 minutes on ethanol access days 1, 7, 14, 15, 21, and 28. Although leak was accounted for via control cages, there is the potential that the disruption from removing the ethanol bottles may have altered drinking on those days. Finally, retro-orbital bloods were sampled on the final day of each phase of the experiment (ethanol access days 14 and 28). Because the animals were on a reverse-light dark schedule, we had to take these blood samples in the room while some of the animals were still drinking their ethanol solution. Therefore, it is possible that the smell of the blood and/or any sounds that were emitted by animals from which bloods were being sampled may have created a stressful atmosphere, and potentially could have altered ethanol drinking behaviors on access days 14 and/or 28.

Interestingly, ethanol intakes and corresponding BECs were not different between the ages on either of these days (unlike the preceding days). Thus, it is quite possible that the sampling of bloods may have reduced ethanol intake in the younger mice and/or increased ethanol intake in the older mice.

Despite the possible perturbations, the current data is the first to demonstrate alterations in ethanol intake between C57BL/6J and DBA/2J adolescent mice. Further, it adds to the literature suggesting that adolescents have differential ethanol intake behavioral patterns compared to adults and that adolescent ethanol access/exposure may alter subsequent ethanol intake in adulthood. Importantly though, the present data suggests that genotype is an important factor in the expression of not only adolescent ethanol intake but also subsequent adult ethanol intakes.

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Fig. 1.

Ethanol intake. Note that there was a 3-week abstinence period between experimental phases 1 and 2. B6 adolescent mice consumed more ethanol than their adult counterparts upon initial exposure ($p < 0.001$), and exposure to ethanol during adolescence resulted in increased ethanol intake during adulthood ($p < 0.01$; **A,B**). There was no difference among D2 adolescents and adults in ethanol intake, nor did adolescent ethanol exposure alter subsequent adult ethanol intake in D2 mice (**C,D**). Asterisks (** $p < 0.01$; $p < 0.001$) indicate significant differences among adolescents and adults within an experimental phase. Number signs (# $p < 0.001$) indicate significant differences among adults (P63–76) with prior adolescent ethanol exposure and adults (P63–76) with no prior ethanol exposure.

Fig. 2.

Pattern of ethanol intake. Note that there was a 3-week abstinence period between experimental phases 1 and 2. Ethanol Exposure Day 1: No specific differences in the pattern of ethanol intake emerged among B6 and D2 or adult and adolescent mice (**A**). Ethanol Exposure Day 7: B6 mice consumed most of their fluid during the first 30 minutes of ethanol access (p 's < 0.03), while no differences in intake patterns emerged in the D2 mice (**B**). Ethanol Exposure Day 14: Again, B6 mice consumed most of their fluid during the first 30 minutes of ethanol access (p 's < 0.001), and there was no difference between B6 and D2 ethanol intakes in the 3rd and 4th 30-minute access periods (**C**). Ethanol Exposure Day 15: B6 mice that were previously exposed to ethanol as adolescents consumed most of their fluid in the first 30 minutes of ethanol access ($p = 0.001$), and no differences in patterns emerged in the B6 mice previously exposed as adults or in the D2 mice (**D**). Ethanol Exposure Day 21: B6 mice consumed most of their fluid during the first 30 minutes of ethanol access (p 's < 0.001), while no differences in intake patterns emerged in the D2 mice (**E**). Ethanol Exposure Day 28: B6 mice previously exposed to ethanol during adolescence consumed more than B6 mice previously exposed to ethanol during adulthood in the first 30 minutes of ethanol access ($p < 0.001$) while in the 2nd 30 minutes of ethanol access the opposite is true ($p < 0.01$; **F**).

Table 1

Ethanol Intake (g/kg)

Day	B6						D2					
	Male			Female			Male			Female		
	Adolescent	Adult	Adolescent	Adolescent	Adult	Adult	Adolescent	Adult	Adolescent	Adolescent	Adult	Adult
1	5.0 ± 0.6	4.0 ± 0.6	5.1 ± 0.7	4.5 ± 0.7	4.5 ± 0.7	2.4 ± 0.5	1.1 ± 0.2	2.8 ± 0.5	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
2	5.6 ± 0.6	4.6 ± 0.3	6.0 ± 0.5	5.4 ± 0.7	5.4 ± 0.7	1.6 ± 0.4	1.2 ± 0.3	1.9 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
3	4.9 ± 0.8	4.3 ± 0.5	5.0 ± 0.5	3.7 ± 0.7	3.7 ± 0.7	0.8 ± 0.4	1.0 ± 0.2	1.7 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
4	4.7 ± 0.5	4.7 ± 0.4	6.0 ± 0.9	5.2 ± 0.4	5.2 ± 0.4	1.7 ± 0.2	1.4 ± 0.2	2.4 ± 0.4	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3
5	6.1 ± 0.8	4.7 ± 0.5	5.7 ± 0.5	4.1 ± 0.6	4.1 ± 0.6	1.2 ± 0.2	1.2 ± 0.2	2.0 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
6	5.6 ± 0.8	4.0 ± 0.5	5.6 ± 0.6	4.5 ± 0.7	4.5 ± 0.7	1.0 ± 0.3	1.3 ± 0.2	1.5 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
7	5.8 ± 0.9	4.4 ± 0.5	5.8 ± 0.6	5.2 ± 0.5	5.2 ± 0.5	1.0 ± 0.3	1.5 ± 0.4	1.9 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
8	5.7 ± 0.5	4.8 ± 0.4	6.2 ± 0.9	4.8 ± 0.6	4.8 ± 0.6	1.2 ± 0.3	1.3 ± 0.3	1.5 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.3
9	5.7 ± 0.5	4.0 ± 0.6	6.0 ± 0.5	5.0 ± 0.4	5.0 ± 0.4	1.1 ± 0.3	1.4 ± 0.3	1.7 ± 0.5	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
10	5.2 ± 0.4	4.1 ± 0.3	6.1 ± 0.5	4.9 ± 0.5	4.9 ± 0.5	1.2 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3
11	6.9 ± 0.8	4.3 ± 0.4	6.6 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	1.1 ± 0.3	1.3 ± 0.3	2.5 ± 0.4	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3
12	6.4 ± 0.7	3.9 ± 0.5	6.3 ± 0.4	5.8 ± 0.5	5.8 ± 0.5	1.2 ± 0.4	1.3 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
13	6.9 ± 0.5	4.5 ± 0.6	7.5 ± 0.4	5.8 ± 0.4	5.8 ± 0.4	1.6 ± 0.3	1.2 ± 0.3	1.9 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
14	5.1 ± 0.4	4.6 ± 0.6	5.8 ± 0.3	5.9 ± 0.4	5.9 ± 0.4	2.0 ± 0.4	1.6 ± 0.3	1.8 ± 0.4	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3
15	4.1 ± 0.4	3.9 ± 0.4	6.1 ± 0.9	4.8 ± 0.4	4.8 ± 0.4	1.6 ± 0.5	1.8 ± 0.4	3.8 ± 0.7	1.9 ± 0.4	1.9 ± 0.4	1.9 ± 0.4	1.9 ± 0.4
16	4.4 ± 0.3	3.8 ± 0.3	5.0 ± 0.4	4.4 ± 0.2	4.4 ± 0.2	1.3 ± 0.6	1.0 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
17	5.6 ± 0.4	4.4 ± 0.8	7.5 ± 0.7	6.6 ± 0.6	6.6 ± 0.6	0.5 ± 0.3	0.9 ± 0.5	1.3 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	1.0 ± 0.4
18	7.4 ± 0.5	6.3 ± 0.7	9.4 ± 1.2	8.2 ± 0.7	8.2 ± 0.7	0.8 ± 0.3	1.2 ± 0.6	1.4 ± 0.4	1.2 ± 0.5	1.2 ± 0.5	1.2 ± 0.5	1.2 ± 0.5
19	4.8 ± 0.4	4.5 ± 0.4	6.9 ± 1.1	5.2 ± 0.4	5.2 ± 0.4	1.5 ± 0.2	1.1 ± 0.2	2.2 ± 0.5	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.3
20	5.2 ± 0.3	4.2 ± 0.4	6.1 ± 0.5	5.6 ± 0.5	5.6 ± 0.5	0.8 ± 0.3	1.2 ± 0.3	1.6 ± 0.4	2.6 ± 1.2	2.6 ± 1.2	2.6 ± 1.2	2.6 ± 1.2
21	7.3 ± 0.3	5.6 ± 0.5	7.5 ± 0.6	6.6 ± 0.4	6.6 ± 0.4	1.9 ± 0.4	2.6 ± 0.5	4.3 ± 0.6	3.1 ± 0.5	3.1 ± 0.5	3.1 ± 0.5	3.1 ± 0.5
22	7.1 ± 0.7	5.0 ± 0.4	7.1 ± 0.8	6.8 ± 1.0	6.8 ± 1.0	0.7 ± 0.3	1.4 ± 0.5	1.2 ± 0.3	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5
23	6.1 ± 0.4	5.7 ± 0.4	6.9 ± 0.2	6.3 ± 0.5	6.3 ± 0.5	0.8 ± 0.2	1.3 ± 0.3	1.9 ± 0.5	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.5
24	5.1 ± 0.3	4.7 ± 0.4	7.0 ± 0.4	6.4 ± 0.4	6.4 ± 0.4	0.7 ± 0.2	0.7 ± 0.3	1.1 ± 0.3	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
25	5.6 ± 0.4	4.1 ± 0.4	6.4 ± 0.7	5.9 ± 0.5	5.9 ± 0.5	1.8 ± 0.2	1.6 ± 0.3	2.4 ± 0.4	2.8 ± 0.3	2.8 ± 0.3	2.8 ± 0.3	2.8 ± 0.3
26	6.2 ± 0.6	4.9 ± 0.7	7.1 ± 0.4	7.4 ± 0.6	7.4 ± 0.6	0.6 ± 0.2	1.3 ± 0.6	1.3 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.5
27	7.5 ± 0.8	5.1 ± 0.7	7.4 ± 0.7	8.2 ± 1.0	8.2 ± 1.0	0.9 ± 0.3	0.9 ± 0.4	1.1 ± 0.4	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.5

		B6				D2			
		Male		Female		Male		Female	
Day		Adolescent	Adult	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult
28		4.2 ± 0.3	5.0 ± 0.9	5.1 ± 0.4	5.6 ± 0.8	1.0 ± 0.2	1.0 ± 0.2	1.8 ± 0.3	1.7 ± 0.2

Table 2

Blood Ethanol Concentrations (mg/dl)

	B6		D2	
	Adolescent	Adult	Adolescent	Adult
Day 14				
Female	129.9 ± 27.2	140.3 ± 17.4	26.9 ± 3.9	24.0 ± 10.4
Male	61.4 ± 14.4	117.7 ± 29.8	9.9 ± 1.5	10.8 ± 1.7
Day 28				
Female	120.1 ± 23.1	138.2 ± 14.4	14.0 ± 4.1	12.3 ± 2.6
Male	107.2 ± 19.4	137.9 ± 25.8	8.0 ± 1.6	6.0 ± 1.2