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A Mouse Model for Adolescent Alcohol Abuse: Stunted Growth and Effects in Brain

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Abstract

Background: Adolescent alcohol abuse remains a serious public health concern, with nearly a third of high school seniors reporting heavy drinking in the previous month.

Methods: Using the high ethanol-consuming C57BL/6J mouse strain, we examined the effects of ethanol (3.75 g/kg, IP, daily for 45 days) on body weight and brain region mass (cerebral cortex, cerebellum, corpus callosum) during peri-adolescence (postnatal day [P]25 to 70) or adulthood (P180 to 225) of both males and females.

Results: In control peri-adolescent animals, body weight gain was greater in males compared with females. In the peri-adolescent exposure group, ethanol significantly reduced body weight gain to a similar extent in both male and female mice (82 and 84% of controls, respectively). In adult animals, body weight gain was much less than that of the peri-adolescent mice, with ethanol having a small but significant effect in males but not females. Between the control peri-adolescent and adult cohorts (measurements taken at P70 and 225, respectively), there were no significant differences in the mass of the cerebral cortex or the cerebellum from either male or female mice, although the rostro-caudal length of the corpus callosum increased slightly but significantly (6.1%) between these time points.

Conclusions: Ethanol treatment significantly reduced the mass of the cerebral cortex in peri-adolescent (−3.1%), but not adult, treated mice. By contrast, ethanol significantly reduced the length of the corpus callosum in adult (−5.4%), but not peri-adolescent, treated mice. Future studies at the histological level may yield additional details concerning ethanol and the peri-adolescent brain.

Keywords

Body Weight; Cerebral Cortex; Cerebellum; Chronic; Ethanol; Gender

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ADOLESCENT AND YOUNG adult alcohol abuse continue to be major problems for modern society as indicated in a recent white paper from the Research Society on Alcoholism (Research Society on Alcoholism, 2009). This is supported by continued survey findings from the Monitoring the Future project (Johnston et al., 2003, 2008) as well as being highlighted in the *Surgeon General's Call to Action to Prevent and Reduce Underage Drinking* (National Institute on Alcohol Abuse and Alcoholism, 2007). Youths are initiating alcohol use earlier and experiencing more alcohol-related problems than before (Miller et al., 2001; Quine and Stephenson, 1990; Winters, 2001), with a closing of the "gender gap" (Nelson et al., 1998). Approximately 80% of American high school seniors report consuming alcohol, with half of these individuals drinking alcohol before the eighth grade (Johnston et al., 1999). In addition, approximately 30% of high school seniors report binge or heavy drinking (Johnston et al., 1991, 1993), with more than 70% of college students reporting binge drinking during high school (Wechsler et al., 2000).

The prevalence of alcohol use and misuse among adolescents and young adults have brought on strong interests for experimental animal models of adolescent alcohol exposure to better understand the neurobiological mechanisms underlying adolescent alcohol abuse and its long-range consequences (Spear, 2000; Witt, 1994). Research since these seminal reviews indicates that animal models continue to provide a major tool for expanding our knowledge of peri-adolescent brain development and its associated vulnerabilities (Spear, 2004a,b, 2007; Spear and Varlinskaya, 2006; Witt, 2006). An important consideration in animal models is the more rapid pace of development and maturation compared with humans. In particular, Spear (2000) indicated that the temporal boundaries of the adolescent "window" of neurobehavioral development for rats may differ given the parameters examined. For example, neurochemical and neurobehavioral differences between postweaning and adult rats support the hypothesized adolescent developmental window of postnatal day (P) 28 to 42 (Spear, 2000, 2007; Spear and Brake, 1983). Assessing the effects of pharmacological pretreatment during adolescence on adult behaviors in rats, however, Spear (2000, 2004a, 2007) has suggested that this conservative window (P28 to 42) could be extended to P60 in order to accommodate both the earliest maturational changes in the female rat as well as the latest ones in the male rat. The present study examined the effect of chronic high dose ethanol in mice from P25 to 70 in order to evaluate the effects of ethanol exposure from the juvenile to the early adulthood.

Throughout adolescence and into early adulthood, the human brain continues to mature with prominent changes of white matter in cortical areas associated with synaptic pruning and myelination (Bava et al., 2010; Giedd, 2004, 2008). Chronic ethanol abuse/dependence leads to disruption of white matter in general (Pfefferbaum et al., 2006c), with consistent findings of disruption found in the corpus callosum (Kashem et al., 2008, 2009; Pfefferbaum and Sullivan, 2005; Pfefferbaum et al., 2006b) and frontal cortex (Harris et al., 2008; Liu et al., 2006, 2007; Mayfield et al., 2002; Sullivan, 2003). Chronic ethanol abuse induces profound degeneration in the cerebellum as well (Fitzpatrick et al., 2008; Jaatinen and Rintala, 2008; Sullivan, 2003). Peri-adolescent brain development is particularly sensitive to the deleterious effects of ethanol, with evidence indicating that ethanol-induced alterations in and/or disruptions of this development predisposes an individual to continued ethanol use and abuse as well as subsequent dependence (Clark et al., 2008; Crews et al., 2007; Spear, 2000,

2004a, 2007; Witt, 2010). Therefore, the present study examined 3 brain regions which are strongly affected by ethanol and critically dependent on white matter in function—the cerebral cortex, corpus callosum, and the cerebellum. We also monitored the body weight of mice. Using a between-subjects design, the effects of ethanol were examined during peri-adolescence (P25 to 70) versus adulthood (P180 to 225) in both female and male high alcohol-consuming C57BL/6J mice.

MATERIALS AND METHODS

We selected a younger and an older group of C57BL/6J mice (P25 and 180 at the beginning of ethanol treatment). All mice were acquired from the Jackson Laboratory (Bar Harbor, ME) and were timed to arrive at least 3 days prior to ethanol treatment. Upon arrival, all mice were housed 3 to a cage in the University of Missouri-Kansas City (UMKC) laboratory animal center and maintained on standard lab chow with a 6 AM to 6 PM light cycle. We selected 45 days for the treatment so that the P25 mice could be exposed to ethanol during the entire peri-adolescent period (P27 to 55; Odell, 1990; Spear, 2000). We selected intraperitoneal (IP) injections (carried out daily at noon) which could quickly elevate blood alcohol concentrations (BAC). Forty-five consecutive days of such IP ethanol administration, therefore, should be considered as a model for chronic heavy exposure. To gauge the severity of this chronic ethanol exposure and select a dosage for our ethanol treatment, we carried out a preliminary study to monitor the acute effects of ethanol on mice balance and movement coordination following ethanol delivery in 3 additional groups of P25 mice at 1, 2, and 3.75 g/kg ($n = 3 \times 6$). At 3.75 g/kg, mice regained righting reflex in ~30 to 40 minutes. Within another 10 minutes, ethanol-treated mice appeared to move about in their home cage as well as their controls. A rotorod test at 1 hour postinjection, however, revealed that control mice could stay on the rod for 15.2 ± 5.84 seconds whereas ethanol-treated mice fell off within 5 seconds. Only at 120 to 150 minutes postinjection did the ethanol-treated mice perform at a level comparable to control mice. Accordingly, we selected 3.75 g/kg as the experimental dosage in the present study for the length of its effect on movement (~2 hours, roughly matching the typical bouts of acute intoxication in humans). At this dosage, BAC in our mice should reach levels between 300 and 350 mg/dl within 10 minutes and remain at or greater than 300 mg/dl for ~30 minutes and then decrease gradually (Linsenbardt et al., 2009). We avoided higher doses (e.g., 5 g/kg) for possible immediate liver pathology (Kim et al., 2009). On the 46th day, all mice were sacrificed via cervical dislocation and were immediately perfused intracardiacally with buffered formalin (4%). We coded the brains to avoid human bias and stored all 48 brains (12 P25 to 70 controls, 12 P25 to 70 ethanol mice, 12 P180 to 225 controls, 12 P180 to 225 ethanol mice, with equal number of males and females in each of the group of 12) in osmo-balanced phosphate buffer for 2 weeks. To ensure that our 3-step brain dissection protocol was carried out consistently in all 48 brains, so as to minimize the amount of experimental error in brain weight data, we relied upon clear anatomical landmarks to guide our dissection. First, the cerebellum was dissected free by cutting the cerebellar peduncles (Fig. 1). Second, to free the cerebral cortex, a cut was made from just rostral to the superior colliculi on the dorsal surface of the brainstem extending to the ventral surface of the brainstem just caudal to the pons (Fig. 1). The dissected cerebral cortex (with some midbrain tissue) and cerebellum were carefully

blotted to remove extra water and weighed. The mean weight of the cerebral cortex (regardless of age, gender, or ethanol treatment, ~300 mg) was typically 6 times greater than the cerebellum (~50 mg) with the coefficient of variation or the standard deviation as a fraction of the mean at ~6% for the cerebellum and ~3% for the cerebral cortex. This variation was attributed to errors inadvertently introduced in dissection and additional measurement or weighing errors as well as intrinsic individual differences. The third and the last step of the dissection was a vertical midsagittal cut in the brain piece containing the cerebral cortex in order to expose the corpus callosum. We carried out 6 measurements on each of the mouse corpus callosum including its overall rostro-caudal length with the aid of a dissecting microscope (Fig. 2). The overall rostro-caudal length of the mouse corpus callosum (regardless of age, gender, or ethanol treatment) was ~4 mm with a standard deviation of0.11 mm (or 2.6% of the mean value). Thus the coefficient of variation for the overall rostro-caudal length of the corpus callosum was lower than but still comparable to those of brain weights (3 to 6%). This suggested that additional experimental errors introduced by our dissection may be of the order of to 3.5%—the difference between 2.6% for the overall length of the corpus callosum and 3 to 6% for the brain weights. The entire laboratory protocol was approved by the Institutional Animal Care and Use Committee at UMKC.

The 4 major outcome measures as response variables in the present study are (i) body weight, (ii) cerebral cortex mass, (iii) cerebellar mass, and (iv) rostro-caudal length of the corpus callosum in midsagittal sections. Since each of these response variables could vary under the combined effect of age, gender, and the treatment (control or ethanol), we carried out a 2 × 2 × 2 ANOVA to ascertain the age–gender–treatment interactions for each of the response variables. Adjustments for multiple comparisons were made according to the Tukey–Kramer protocol (SPSS, Chicago, IL). No significant interactions were found for any of the 4 response variables ($p > 0.05$). In the sections to follow, we therefore examined each response variable in more detail with a pair-wise or 2-sample analysis in order to focus on the effect of ethanol, age, or gender. Our statistical analyses were performed with the 2-tailed Student's *t*-test. Data were represented as mean ± SD. Cases in which *p*-values < 0.05 were considered statistically significant.

RESULTS

Body Weight

Growth Patterns of P25 Mice: Similarities Between Male and Female Mice.— Figure 3 shows the time course of body weight gain for our mice. Figure 4 shows that ethanol treatment significantly attenuated body weight gain in peri-adolescent mice compared with adult mice. The main features of the data in these 2 figures are: (i) At P25, male control mice were slightly lighter than females although the difference was not significant (11.47 ± 1.21 g for males, vs. 11.64 ± 1.26 g for females, $p > 0.05$), also see C_{25} bars for males and females in Fig. 4). (ii) Control male and female adolescent mice grew significantly heavier and nearly doubled their weight between P25 and 70 (24.81 ± 1.19 g for males vs. 20.06 ± 0.83 g for females (also see C_{70} bars for males and females in Fig. 4). This weight gain should be viewed as the mouse peri-adolescent growth spurt. (iii) In our

P25 mice, the rate of weight gain was the fastest at or near P25 and then appeared to taper off with age from that point on. (iv) In Fig. 3, growth curves for male and female control mice began to separate shortly after P25, confirming P25 to be a suitable choice for the starting age of mice concerning the gender-specific effect of ethanol on peri-adolescent weight gain. (v) Ethanol treatment significantly reduced this peri-adolescent growth spurt in both male and female mice (also compare bars E_{70} with C_{70} for males and females in Fig. 4). (vi) In the younger mice (male or female), the values of body weight in control mice were already markedly larger than those for ethanol-treated mice after 2 to 3 weeks of ethanol treatment. (vii) In the older mice (male or female), the values of body weight in control and ethanol treatment mice were more stable throughout the 45-day period (than the younger group).

Peri-Adolescent Growth Spurt is Gender-Specific in Both Control Mice and Ethanol Mice

Peri-adolescent weight gain in control mice was gender-specific. The pair of bars on the left side in the upper left panel of Fig. 5 shows that control males at P70 were significantly heavier than control females (24.81 ± 1.19 g for males vs. 20.06 ± 0.83 g for females, $p < 0.01$, $t = 8.47$, df = 13). The pair of bars on the left side in the upper right panel of Fig. 5 shows that males gained significantly more weight than females (13.85 ± 1.1 g vs. 8.74 ± 0.8 g for females, $p < 0.01$, $t = 14.57$, df = 9). The pair of bars on the left side in the lower left panel of Fig. 5 shows that at P70, 56 ± 3% of the body weight in males was gained during the 45 day period, whereas for females it was significantly less at 44 ± 1% ($p < 0.01$, $t = 12.32$, df = 10). To estimate individual growth rates, we assigned a weight gain ratio (a ratio of body weight at P70 vs. P25) for each mouse. The pair of bars on the left side in the lower right panel of Fig. 5 shows that the growth ratio (P70 body weight/P25 body weight) was 2.27 ± 0.17 for males versus a significantly smaller value at 1.77 ± 0.2 for females ($p < 0.01$, $t = 9.32$, df = 9). Thus, whether one examined absolute weight in grams or relative measures as growth ratios or weight gain as a fraction of body weight, our data showed that male control mice grew significantly more than females from P25 to 70, indicating that the underlying peri-adolescent growth process in control mice was gender-specific. We next focused on analyzing whether the effect of ethanol on peri-adolescent mice growth pattern is gender-specific.

Peri-adolescent weight gain in ethanol-treated mice was gender-specific. The pair of bars in the right side of the upper left panel of Fig. 5 shows that ethanol-treated males were significantly heavier than ethanol females at P70 (21.77 ± 0.85 g for males vs. 17.25 ± 0.40 g for females, $p < 0.01$, $t = 12.37$, df = 18). The pair of bars in the right side of the upper right panel of Fig. 5 shows that ethanol-treated males gained significantly more weight (9.94 ± 1.1 g) than females (5.41 ± 1.48 g; $p < 0.01$, $t = 7.58$, df = 18). The pair of bars in the right side of the lower left panel shows that at P70, 46 ± 5% of the body weight in ethanol males was gained during the 45 days period, whereas it was significantly less for females (31 ± 8%; $p < 0.01$, $t = 4.56$, df = 18). Finally, the pair of bars in the right side of the lower right panel of Fig. 5 shows that weight gain ratios were significantly larger for males (1.86 ± 0.20) than for females (1.48 ± 0.20; $p < 0.01$, $t = 3.91$, df = 18). As in control mice, whether one examined absolute weight in grams or relative measures as growth ratios or weight gain as a fraction of body weight, our data showed that male ethanol treated mice grew significantly

more than female ethanol treated mice from P25 to 70, indicating that the underlying peri-adolescent growth process in ethanol treated mice was also gender-specific.

Is the effect of ethanol on peri-adolescent weight gain gender-specific? Even though the patterns of body weight gain between P25 and 70 for both the control mice and the ethanol-treated mice were gender-specific, the ethanol-induced effect on body weight growth could either be gender-specific or independent of gender. Comparing all 4 bars in the upper left panel of Fig. 5, both male and female ethanol-treated mice were significantly lighter than their controls at the end of the 45-day period (21.77 ± 0.85 g for ethanol-treated males vs. 24.81 ± 1.19 g for control males, $p < 0.01$, $t = -7.33$, df = 22; 17.25 ± 0.45 g for ethanol-treated females vs. 20.06 ± 0.83 g for control females, $p < 0.01$, $t = -10.28$, df = 9). The same conclusion can be drawn for the amount of weight gain, the weight gain at P70 as % body weight, or the growth ratio. From the 4 panels in Fig. 5, the degree of disruption in body weight gain by ethanol in mice of different gender and the intrinsic gender-specific differences in control or ethanol-treated mice were of the same order of magnitude. To address the question of whether the ethanol effect on weight gain was gender-specific, we now turn to Fig. 4. Comparing the C_{70} and E_{70} bars for males and females in Fig. 4, ethanol caused more reduction in body weight in males (3.04 g, from 24.81 to 21.77 g) than in females (2.81 g, from 20.06 to 17.25 g). But this reduction accounted for a larger percentage of body weight in females (14%) than in males (12.3%). Differences between 3.04 and 2.81 g (0.23 g) and between 14 and 12.3% (1.7%) were small. Here, conventional statistics could not be applied to derive a measure of significance for either 0.23 g or 1.7% because these measures were derived from the differences between 2 mean values rather than from individual pairs which contained the critical information on variance. On the other hand, a rough estimation on the significance of values such as 0.23 g or 1.7% could be made. For example, these differences (e.g., 0.23 g or 1.7%) were smaller than or of the same magnitude as the standard deviations for weight gain, which were generally in the range of 0.8 to 1.48 g and 1 to 8%, respectively (from the standard deviations in Figs 4 and 5). We therefore estimated that it was not likely that these differences (e.g., 0.23 g or 1.7%) would be statistically significant at the $p = 0.01$ level.

Beyond Adolescent Growth Spurt: Smaller Differences.—The peri-adolescent growth spurt in body weight from P25 to 70, although impressive, accounted for only 77.4 and 85.6% for the total amount of weight gain between P25 and 225 for male and female mice, respectively (Table 1, also see Fig. 3). More growth therefore occurred after P70, although at a progressively reduced rate with age. Thus, the body weight of the P180 mice were at 97 to 98% of the P225 mice for both males and females. At P180, control males were already significantly heavier than females at the beginning of the ethanol treatment (30.94 ± 1.24 g for males vs.22.92 ± 1.38 g for females, $p < 0.01$, $t = 12.25$, df = 15). This was also the case at P225 (32.05 ± 3.12 g for males vs.23.44 ± 1.99 g for females, $p < 0.01$, $t = 6.61$, df = 13, also compare bars C_{180} with C_{225} for males and females in Fig. 4). No significant weight gain occurred, however, from P180 to 225 in either control males or females ($p > 0.05$). The E_{225} bars in Fig. 4 showed that ethanol caused a small but significant decrease in body weight in the P180 mice for males (28.47 ± 1.17 g vs. controls at 30.94 ± 1.24 g, 8% decrease, $p < 0.01$, $t = -3.45$, df = 13) and no significant difference in

females (21.73 ± 3.13 g vs. controls at 23.44 ± 1.99 g, $p > 0.05$). We concluded that ethanol interferes with peri-adolescent growth spurt during maturation but had a progressively smaller effect on body weight as the mice became older.

Brain Mass

In the present study, ethanol treatment was associated with significant differences in body weight of the adolescent mice. The differences were clearly visible during much of the 45 days ethanol treatment period (Fig. 3). Brain mass measurements were, however, invasive and therefore were carried out only at P70 and 225. The mass of the cerebellum and the cerebral cortex remained essentially constant between P70 and 225 (Tables 2 and 3). There was no clear correlation between the data on mice body weight and (i) cerebellum or cerebral cortex mass (also the overall rostro-caudal length of the corpus callosum) between P70 and 225, (ii) gender, and (iii) ethanol treatment (Pearson's correlation coefficients were < 0.5). It was possible that our invasive brain weight measurements contained too few time points for the examination of such correlations. It was also possible that between P70 and 225, age-related differences in brain mass were too small, statistically insignificant, and hence did not correlate well with body weight differences. We therefore did not consider mouse body weight as a covariate for brain measurements. The correlation of body weight and brain mass remains an important and interesting question. More detailed probes on this will have to be made in future studies.

Age-Related Differences in Control Mice.—In a marked deviation from the data on body weight, gender difference and ethanol treatment were not associated with large and significant differences in the mass of the cerebral cortex or the cerebellum (Table 2, $p > 0.05$). We therefore pooled the data from males and females into a single entry in Table 3. With the larger sample size (now $n = 12$), the cerebellum of the P225 controls was 4.6% heavier than the P70 controls (0.0545 g vs. 0.0521 g, $p > 0.05$); the cerebral cortex of the P225 controls was 1.7% heavier than the P70 controls (0.300 g vs. 0.295 g, $p > 0.05$). These small differences, however, did not reach statistical significance at $p = 0.05$.

Effects of Ethanol.—The effect of ethanol treatment on the cerebral cortex mass was more pronounced and statistically significant in the younger group of mice (3.1% decrease from0.295 to 0.286 g, $p < 0.01$, $t = 3.96$, df = 28); ethanol caused smaller differences that did not reach statistical significance in the older group of mice. We did not detect significant effects of ethanol on the cerebellum in either age group ($p > 0.05$). The lack of significant differences in the cerebellum must be considered in light of its smaller mass and the resultant larger coefficients of variation (~3% for the cerebral cortex and 6% for the cerebellum).

The Corpus Callosum.—While the overall rostro-caudal length of all mice (regardless of age, gender, or ethanol treatment) was typically 4 mm, the other 5 width measurements of the corpus callosum were typically between 0.15 and 0.45 mm (Fig. 2, Table 4). The values for the standard deviation of the 5 smaller width measurements in Table 4 were much smaller (0.03 to 0.08 mm) but the coefficients of variation of these 5 measurements of the corpus callosum were greater than those for the overall length of the corpus callosum (8.6 to

20.9% of the mean value). We did not detect significant gender-specific differences in any of the 6 measurements of the corpus callosum from control mice in the same age group ($n = 6$, p-values ranging from 0.052 to 0.922, data not shown). After combining the data from male and female mice into a single entry, the rostro-caudal length of the corpus callosum in control mice (now $n = 12$) showed significant age-related difference between P70 and 225(6.09% increase, from 4.058 ± 0.135 mm to 4.305 ± 0.111 mm, $p < 0.01$, $t = -5.16$, df = 29). The effect of ethanol on the length of the corpus callosum was significant only in the older group of mice (5.04% decrease, from 4.305 ± 0.111 to 4.088 ± 0.153 mm, $p < 0.01$, $t = 3.59$, df = 17). None of the other 5 smaller parameters of the corpus callosum were significantly affected by ethanol or age (p-values ranging from 0.122 to 0.954, data not shown).

DISCUSSION

The basic and clinical literature provides strong support for the contention that adolescents consume more ethanol and/or respond differently to ethanol than their adult counterparts (Chin et al., 2010; Doremus et al., 2005; Lallemand et al., 2009; Nixon et al., 2010; Riley et al., 2010; Ristuccia and Spear, 2008; Rodd-Henricks et al., 2002a,b; Truxell et al., 2007; Vetter et al., 2007; Vetter-O'Hagen et al., 2009). The existing literature also indicates that females often consume more ethanol and/or respond differently to ethanol than their male counterparts (Bell et al., 2003, 2008; Devaud et al., 1999; Devaud and Prendergast, 2009; Fox et al., 2009; Kelly et al., 2009; Lallemand et al., 2009; Maldonado et al., 2008; Maldonado-Devincci et al., 2010; Miller et al., 2009; Ridge et al., 2009; Truxell et al., 2007; Vetter-O'Hagen et al., 2009). Thus, the present study examined the effects of peri-adolescent (P25 to 70) and adult (P180 to 225) ethanol exposure in both male and female C57BL/6J mice. The combined choice of the duration of the ethanol treatment (45 days) and the age of our younger group of mice (started at P25) provided ethanol exposure for our mice during their entire peri-adolescence period (Odell, 1990; Spear, 2000; but see Moore et al., 2010, for P21 to 60). Over this period, the adolescent, gender-specific weight gain in mice (from P25 to 70) was impressive. Ethanol caused a dramatic reduction in this peri-adolescent growth spurt. These results on body weight are in general agreement with a recent study in which data were reported on body weight gain of male adolescent mice in a chronic alcohol consumption model over a 6-week period (starting age P28, ~2 g/kg/d in a 2-bottle free-choice paradigm) (Zou et al., 2009). In particular, they also observed that the body weight values for control mice and ethanol mice became significantly different after 2 to 3 weeks of ethanol treatment. Here we have additionally determined that the patterns of peri-adolescent body weight gain remained strongly gender-specific in a statistically significant manner for both control and ethanol-treated mice. We could only estimate, however, that the gender-specificity of the intrinsic process of ethanol-induced attenuation in peri-adolescent weight gain was weak at best and possibly not significant (but see Emanuele et al., 2002, for the effect of ethanol on developing females). Although it is not clear whether the ethanol-treated peri-adolescent mice would have remained smaller throughout their life span, consequences of perturbations on development and maturation can be irreversible.

A rodent model that mimics alcohol consumption and dependence in humans is the alcohol-preferring (P) rat bred from a closed colony of Wistar rats (Bell et al., 2006; McBride and

Li, 1998; Murphy et al., 2002). The P rat model is of considerable interest not only because they voluntarily consume large amounts of alcohol, but also because ethanol can produce similar abnormalities in their telencephalon as in human drinkers. Results from a recent longitudinal brain imaging study of male P rats (from P88 to 578) have revealed that free-choice ethanol consumption attenuated the growth and expansion of brain structures, including the corpus callosum and hippocampus (Pfefferbaum et al., 2006a; Sullivan et al., 2006). Direct comparisons between results of the P rat study (Pfefferbaum et al., 2006a; Sullivan et al., 2006) and those of the present study, however, are difficult. Differences exist not only in the different experimental parameters but also in additional considerations. For example, both the cerebral cortex and the cerebellum in control P rats have been found to increase in volume continuously between P88 and 578 although the increases are not likely to involve increases in the number of neurons. Control P rats also gained weight continuously without signs of asymptote between P88 and 578 and became obese. Mice exhibited a significant peri-adolescent growth spurt and then gained weight slowly and perhaps only up to 180 days (Table 1, Fig. 3). Modern human weight gain patterns are partly similar to that of the P rat and partly similar to that of mice. Further studies are needed, as fat metabolism in adults and maturational growth involve different cellular mechanisms which can interact with ethanol differently.

Significant maturational changes are known to occur in the mammalian cerebral cortex between P25 and 225, including synaptic pruning (Rakic et al., 1994). In the cerebellum, significant changes in parallel fiber length and the number of synapses have also been reported for mice and rats (Huang et al., 1999, 2006). In humans, maturational changes occur in both white and gray matters in the cerebral cortex (Ashtari et al., 2007; Bamea-Goraly et al., 2005; Giedd et al., 1999; Lenroot and Giedd, 2006; Pfefferbaum et al., 1994; Rakic et al., 1994; Rubia et al., 2000; Segalowitz and Davies, 2004; Sowell et al., 1999, 2003, 2004). At present, the detailed effects of ethanol on these changes at the cellular level are largely unclear. Although Hommer (2003) has suggested that women may be more vulnerable to alcohol-induced brain damage in a review, detailed evidence on the influence of gender on the effect of ethanol on brain structures is still incomplete.

The developing human brain also displays ethanol-induced changes in the size and shape of the corpus callosum (Bookstein et al., 2002; Riley et al., 1995). In animal models, other than those for fetal alcohol syndrome (Elberger, 2007), details on the effect of ethanol on the corpus callosum in the adolescent brain are largely unclear. The corpus callosum is topographically organized so that the anterior, middle, and posterior portions contain fibers that connect the anterior, middle, and the posterior regions of the cerebral cortex. Detailed topographic features of the corpus callosum at the cellular resolution may provide useful information on region-specific differences in various parts of the cerebral cortex. In the present study, only small differences in mass measurements of the brain, the cerebellum, and the corpus callosum were seen either as a result of age (from P70 to 225), gender, or ethanol treatment. Although greatly increasing the sample size could possibly reveal some small difference in such gross measurements of the brain, it may not be a practical alternative. Detailed studies at the histological level preferably matched by behavioral studies must be considered in future animal model studies to further examine these small but potentially significant effects (Crews et al., 2000; Dlugos and Pentney, 1997; Tabbaa et al., 1999).

Small effects in brain mass measures, however, can still have serious functional consequences if the differences are region-specific (e.g., if all mass differences in the forebrain come from the prefrontal cortex). In the temporal dimension, the results of adolescent exposure to ethanol can be influenced by an array of complex and interacting conditions. First, there may be a latent period with serious functional manifestations taking place only later in adulthood. Second, structural damages in the adolescent brain may also have a better chance for functional recovery compared with the same damage occurring in the adult brain. Third, early onset of alcohol use leads to a higher risk for the development of alcohol dependence in adulthood (Anthony and Petronis, 1995; Chou and Pickering, 1992). Findings from 1 study revealed that almost half of the adult respondents who reported first alcohol use at age 13 met DSM-IV diagnostic criteria for “lifetime” alcohol dependence (American Psychiatric Association, 1994), whereas only one-tenth of those who reported first alcohol use after age 20 met the DSM-IV criteria (Grant and Dawson, 1997). Age of first alcohol use also influences the impact of other psychosocial risk factors for the development of alcohol abuse including parenting and peer influence (Hawkins et al., 1997). These findings reinforce the significance of impact of alcohol on adolescents.

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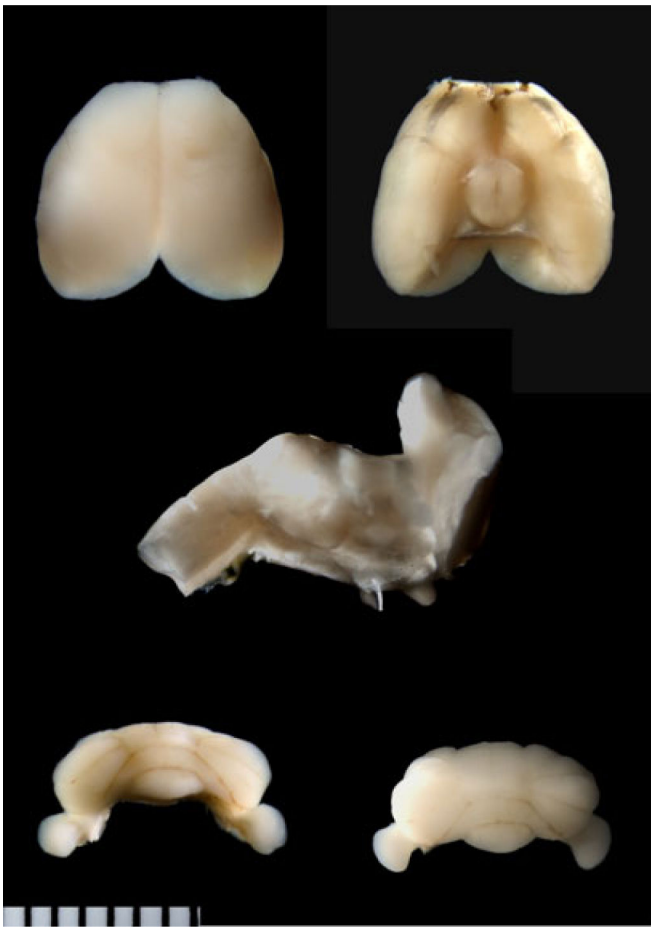

Fig. 1.

The mouse brain. The 2 top photos show dorsal and ventral views of the mouse cerebral cortex prior to the midsagittal cut to expose the corpus callosum. The olfactory appendages are cut flush at the level of the anterior end of the cerebral cortex. A cut mark (that separated the brainstem) is visible in the ventral view. The middle photo shows a sagittal view of the cut-away brainstem. Here, the rostral cut plane is visible, indicating the cerebral cortex was dissected free just rostral to the superior colliculi on the dorsal surface of the brainstem and just caudal to the pons on the ventral surface of the brainstem. The bottom photos show anterior and posterior views of the cerebellum. Each division of the marker is 0.6 mm.

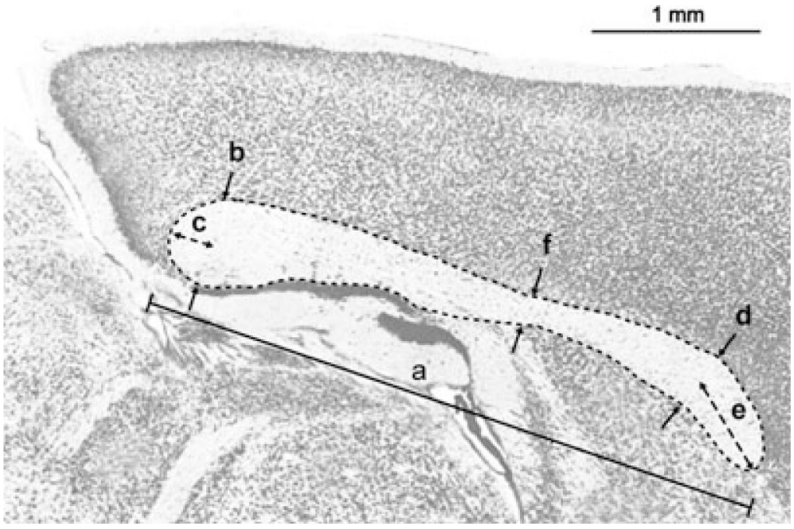

Fig. 2.

A midsagittal view of the corpus callosum of the mouse. We made 6 measurements on the midsagittal view of the corpus callosum. (**A**) The overall rostro-caudal length; (**B**, **C**) the thickness and the protrusion of the posterior corpus callosum; (**D**, **E**) the thickness and the protrusion of the anterior corpus callosum; (**F**) the thickness of the narrowest part of the corpus callosum.

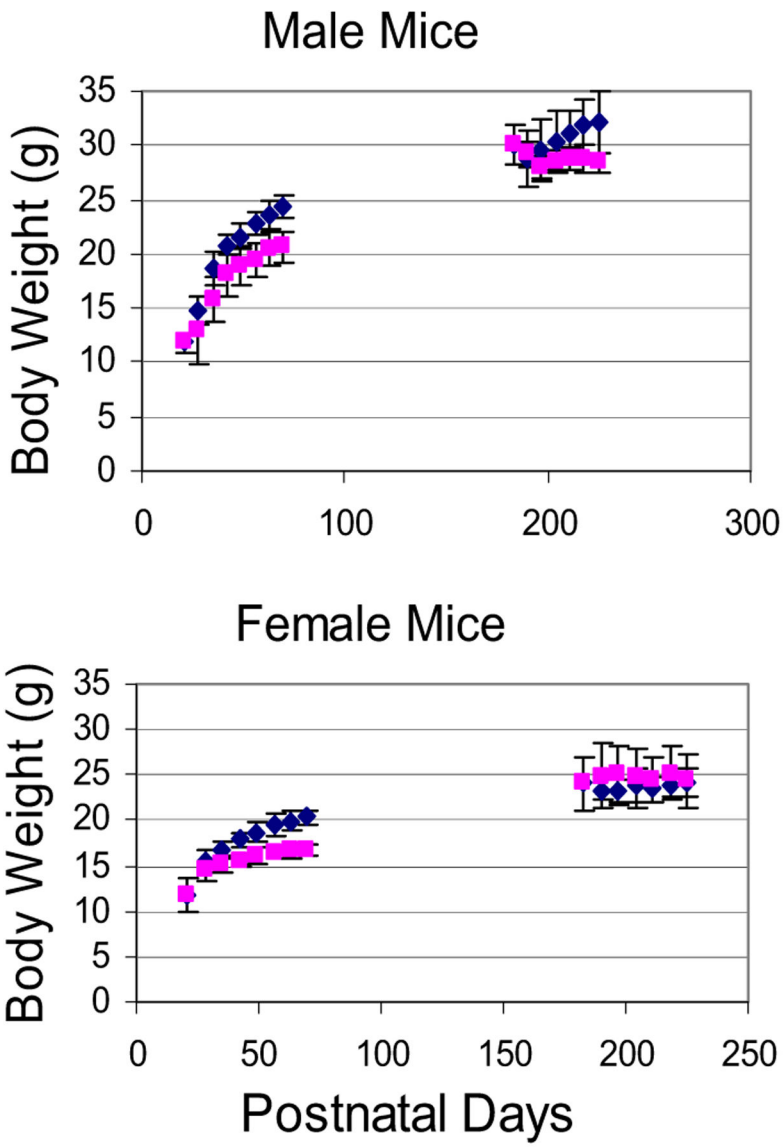

Fig. 3.

Body weight of mice as a function of time in the present study. **Upper panel** shows that control P25 males grew more quickly than ethanol-treated P25 males, whereas control P180 males did not grow more than ethanol-treated P180 males. **Lower panel** shows data on female mice. (Diamonds are control mice and squares are ethanol-treated mice.)

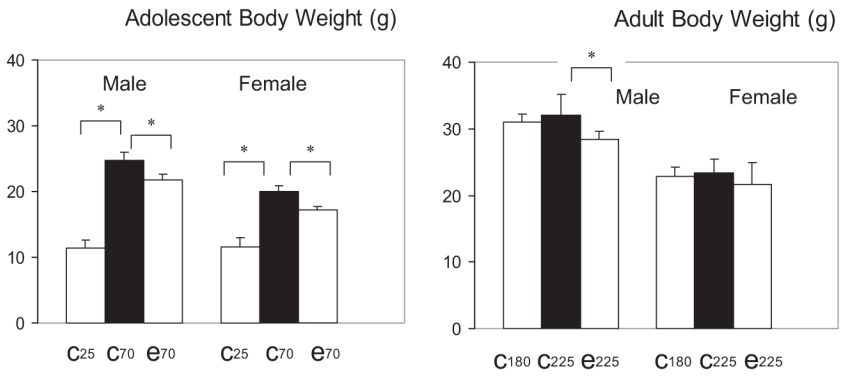

Fig. 4.

Ethanol markedly attenuated the body weight gain in the adolescent mice but not in adult mice. **Left**: Adolescent body weight; **right**: adult body weight. The C_{25} bar is the body weight of control mice at the beginning of the 45-day period; C_{70} bar indicates body weight of control mice at the end of the 45-day period; E_{70} bar indicates body weight of ethanol mice at the end of the 45-day period. The C_{180}, C_{225}, and E_{225} bars are for adult mice. * indicates significant change.

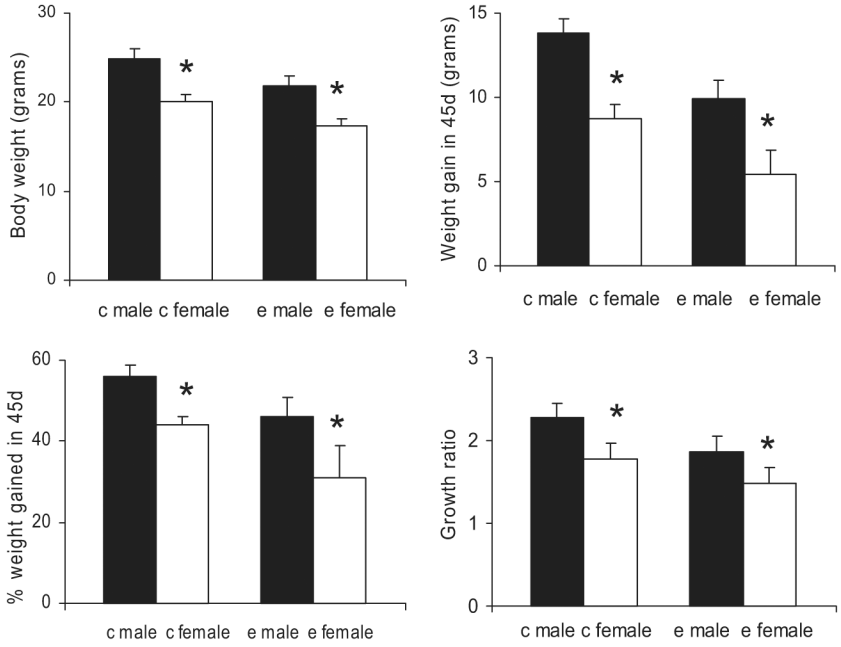

Fig. 5.

Adolescent body weight growth (from P25 to 70) in both control mice and ethanol-treated mice are gender-specific. **Upper left panel**: Mice body weight at P70, control males and females are on left, ethanol males and females are on right; **upper right panel**: amount of weight gain in the 45 days period (between P25 and 70); **lower left panel**: the % of body weight gained in the 45 days period; **lower right panel**: growth ratio. Male mice grew significantly more than females from P25 to 70 regardless of ethanol treatment, indicating that the underlying adolescent growth process remained gender-specific despite the ethanol treatment. The * indicates that the difference between the open bar and the filled bar is statistically significant ($p < 0.01$).

Table 1.

Values of Body Weight (g) and % Body Weight (with P225 Body Weight as 100) of Male and Female Control Mice from P25 to 225

	Male (g)	Male (%)	Female (g)	Female (%)
P25	11.47	34.8	11.64	49.7
P70	24.81	77.4	20.06	85.6
P180	30.94	96.5	22.92	97.8
P225	32.05	100	23.44	100

Between 77 and 86% of the weight gain has occurred at P70 for males and females, respectively, and between 97 and 98% of the weight gain has occurred at P180 for males and females, respectively.

Table 2.

The Weight of the Cerebellum and Cerebral Cortex in Control and Ethanol-Treated Mice (at P70 and 225)

Age group	Cerebellum of control mice (mg)	Cerebellum of ethanol-treated mice (mg)	Cortex of control mice (mg)	Cortex of ethanol-treated mice (mg)
P70 male	51.5 ± 3.8	52.4 ± 2.2	296 ± 7.9	285 ± 5.6
P70 female	53.2 ± 3.3	50.9 ± 2.0	294 ± 12.5	285 ± 5.7
P225 male	54.6 ± 2.6	51.9 ± 3.2	298 ± 5.3	297 ± 5.8
P225 female	54.4 ± 4.0	52.4 ± 2.5	303 ± 12.4	294 ± 11.8

Values for males and females are listed separately ($n = 6$). There are no gender-specific differences here.

Table 3.

The Weight of the Cerebellum and Cerebral Cortex in Ethanol Treated Mice and Their Controls (at P70 and 225)

Age group	Cerebellum weight of control mice (mg)	Cerebellum weight of ethanol treated mice (mg)	Cortex weight of control mice (mg)	Cortex weight of ethanol treated mice (mg)
P70 mice	52.1 ± 3.3	51.9 ± 1.7	295 ± 8.4	286 ± 5.2
P225 mice	54.5 ± 3.0	52.2 ± 2.7	300 ± 8.2	296 ± 8.7

Values for males and females are combined ($n = 12$). There are no age-related differences here. See text for ethanol-related differences.

Table 4.

Mean and Standard Deviation Values of the 6 Measurements of the Corpus Callosum in P225 Control Mice (Male and Female Data Combined, $n = 12$)

Length code	Mean (mm)	SD (mm)	SD/mean (%)
A	4.31	0.11	2.6
B	0.38	0.04	10.3
C	0.42	0.08	18.6
D	0.45	0.04	8.6
E	0.37	0.06	15.3
F	0.14	0.03	20.9

A: Overall length; B: maximal width at posterior end; C: extent of protrusion at the posterior end; D: maximal width at anterior end; E: extent of protrusion at the anterior end; F: minimal width (also see Fig. 2).