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Effects of ethanol during adolescence on the number of neurons and glia in the medial prefrontal cortex and basolateral amygdala of adult male and female rats

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Abstract

Human adolescents often consume alcohol in a binge-like manner at a time when changes are occurring within specific brain structures, such as the medial prefrontal cortex (mPFC) and the basolateral nucleus of the amygdala (BLN). In particular, neuron and glia number are changing in both of these areas in the rat between adolescence and adulthood (Markham et al., 2007; Rubinow and Juraska, 2009). The current study investigated the effects of ethanol exposure during adolescence on the number of neurons and glia in the adult mPFC and BLN in Long-Evans male and female rats. Saline or 3 g/kg ethanol was administered between postnatal days (P) 35–45 in a binge-like pattern, with 2 days of injections followed by 1 day without an injection. Stereological analyses of the ventral mPFC (prelimbic and infralimbic areas) and the BLN were performed on brains from rats at 100 days of age. Neuron and glia densities were assessed with the optical disector and then multiplied by the volume to calculate the total number of neurons and glia. In the adult mPFC, ethanol administration during adolescence resulted in a decreased number of glia in males, but not females, and had no effect on the number of neurons. Adolescent ethanol exposure had no effects on glia or neuron number in the BLN. These results suggest that glia cells in the prefrontal cortex are particularly sensitive to binge-like exposure to ethanol during adolescence in male rats only, potentially due to a decrease in proliferation in males or protective mechanisms in females.

Keywords

alcohol; sex differences; stereology; cell death; cell proliferation

1. Introduction

Approximately 85% of people within the United States have had their first drink by the legal age of 21 (Grant and Dawson, 1997). Furthermore, binge-drinking rates steadily increase in adolescence from 7% to 34% between the ages of 14 and 20, a time period when heavy alcohol consumption is prevalent in both males and females (Johnston et al., 2008;

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Substance Abuse and Mental Health Services Administration, 2011). Given the prevalence of binge-drinking in humans during this time as well as reports of adolescent alcohol use increasing the susceptibility for future alcohol dependence (Grant and Dawson, 1997), it is important to evaluate the cellular changes associated with adolescent ethanol exposure in rodent models.

In adolescent rats, neural damage has been reported after exposure to high levels of ethanol. For example, ethanol compromises neurogenesis in the hippocampal dentate gyrus in adolescents to a much greater degree than in adults (Crews and Nixon, 2003; Crews et al., 2006). Silver staining, which is an indicator of cellular stress that may lead to cell death, immediately increases in the olfactory tubercle, hippocampal dentate gyrus, and the piriform, perirhinal, and entorhinal cortices after several days of high doses of ethanol administered during adolescence (Crews et al., 2000; Obernier et al., 2002). Obernier et al. (2002) showed evidence of neuronal death with Fluoro-Jade B, a pyknotic cell death marker, but not with the apoptotic marker, TUNEL, in the dentate gyrus. In addition, Pascual et al. (2007) found evidence of increased cell death through changes in DNA fragmentation and caspase-3 activity in the neocortex, hippocampus, and cerebellum of rats that had been exposed to high doses of ethanol from the juvenile through the adolescent period. This work makes it pertinent to investigate the long-term effects of adolescent ethanol exposure on the number of neurons in adults.

In addition to the hippocampus, the cerebral cortex and the amygdala may be particularly vulnerable to ethanol during adolescence and they are part of a circuit that is important for addiction. The BLN and the mPFC, in particular, receive dopamine innervations from the ventral tegmental area, a region that also supplies dopamine to the nucleus accumbens (Sesack et al., 2003). In humans, structural magnetic resonance imaging studies have shown that both the mPFC and BLN are continuing to develop throughout adolescence. The cerebral cortex decreases in volume during adolescence in a region- and sex-specific manner, with the largest decreases occurring in the prefrontal cortex (De Bellis et al., 2001; Giedd, 2004; Sowell et al., 1999). Conversely, the volume of the amygdala increases between childhood and adulthood with more change occurring in males than in females (Giedd et al., 1996; Merke et al., 2003). The cellular basis for these changes has not been thoroughly investigated (Guillery, 2005), but there is evidence for a loss of synapses in the human and non-human primate mPFC (Anderson et al., 1995; Huttenlocher, 1979). In rats, our laboratory demonstrated multiple cellular alterations in both the mPFC and the BLN between the early adolescence period (P35) and adulthood (P90). In the mPFC and BLN, neuron number significantly decreases during this time period, whereas glia number decreases in the BLN of both sexes and in the mPFC a sex difference appears at day 90 due to a slight decrease of glia in females and an increase in males (Markham et al., 2007; Rubinow and Juraska, 2009). Additionally, spine density in the mPFC is decreasing in both sexes between adolescence and adulthood, but is unchanged in the BLN (Koss et al., 2009; Koss et al., 2010). At the same time, there are increases in BLN innervation to the mPFC (Cunningham et al., 2002), whereas mPFC efferents to the BLN are being pruned (Cressman et al., 2010). Given that these brain structures are undergoing such significant changes that are concurrent with the increase of ethanol consumption in humans, it is important to investigate how ethanol alters these brain structures. Ethanol sharply increases naturally occurring neuronal death throughout the brain during the prenatal and early postnatal period in rats (Goodlett and Eilers, 1997; Ikonomidou et al., 2000; Miller and Potempa, 1990), including in the mPFC (Mihalick et al., 2001). The BLN is far less studied in the effects of ethanol; however ethanol has been shown to affect the glutamatergic and GABAergic neurotransmission in 4–6 week old rats in the BLN (Christian et al., 2012; Silberman et al., 2009), which may have consequences to long-term stress and anxiety levels. Furthermore, ethanol has effects on many types of glia including astrocytes and microglia (Evrard et al.,

2006; McClain et al., 2011). The normal occurrence of neuronal death and alterations in the number of glia during adolescence may render the mPFC and BLN particularly vulnerable to alcohol exposure.

Work on the neural effects of alcohol exposure has been restricted to males, but there are indications that females might not exhibit the same effects. For example, humans display sex differences in the pattern of drinking and abuse (Greenfield and Rogers, 1999; Hasin et al., 2007; Witt, 2007) and in rats, sex differences appear in alcohol consumption during both adolescence and adulthood (Lancaster et al., 1996; Vetter-O'Hagen et al., 2009). Sex differences in consumption during adulthood also occur following binge-like exposure to alcohol during adolescence (Maldonado et al., 2008; Sherrill et al., 2011b), and these differences do not occur if females are ovariectomized before puberty (Sherrill et al., 2011b). We have also found sex differences in the effects of ethanol during adolescence on ethanol-induced conditioned taste aversion in adults (Sherrill et al., 2011a). Although few studies examine sex-dependent cellular changes in response to ethanol exposure during adolescence, binge-like exposure alters the expression of stress-related genes in the hypothalamus of male, but not female rats (Przybycien-Szymanska et al., 2010).

The present study examines the long-term effects of binge-like exposure to alcohol during adolescence on the number of neurons and glia in the rat mPFC and BLN. Both males and females were examined in light of sex differences in the response to alcohol, as well as the sex-specific development of the mPFC that we have previously demonstrated (Markham et al., 2007).

2. Results

2.1 Body and Brain Weights

As previously reported in our laboratory (Sherrill et al., 2011b), at the last day of injection (P45), ethanol administration significantly decreased body weight in males and females ($F(1,27)=8.0$, $p=0.009$); however this effect was not permanent (Table I). All animals compensated for this weight loss and were not significantly different at P100. There were significant sex differences in body weights at both ages with males weighing more than females (P45, $F(1,27)=24.1$, $p<0.001$; P100, $F(1,28)=74.0$, $p<0.001$). Brain weights also differed between the sexes with males being greater ($F(1,17)=16.6$, $p=0.001$), but ethanol treatment had no effect on brain weight (Table I).

2.2 Medial Prefrontal Cortex

An example of a mPFC parcellation used to quantify volume is shown in Figure 1a. Ethanol administration had no detectable effect on the volume of the whole mPFC or on individual layers. There was a weak trend ($F(1,25)=3.1$, $p=0.09$) towards a sex difference in volume, with females having a smaller volume than males. Neurons and glia were separately counted (Figure 3), and there was a trend towards a sex difference ($F(1,25)=3.7$, $p=0.07$) in total number of neurons in all layers, with females having 10% fewer neurons than males (Figure 3a), similar to previous results (Markham et al., 2007). The sex difference reached significance in the upper layers (Figure 3b; $F(1,25)=5.1$, $p=0.034$), but not in the lower layers (Figure 3c). There were no significant effects of treatment and no treatment by sex interaction for neuron number in all layers combined or in individual layers (Figure 3).

Analysis of glia number across all layers resulted in a strong trend ($F(1,25)=4.1$, $p=0.053$) towards a sex by ethanol-treatment interaction. Further analysis revealed that this interaction was based on a significant decrease ($F(1,11)=8.0$, $p=0.02$) of 14% in male rats that received ethanol compared to those that received saline, while no difference occurred between treatment groups in females (Figure 3d). This pattern was present in both the upper

($F(1,11)=5.6$, $p=0.04$) and lower ($F(1,11)=7.2$, $p=0.02$) layers with males, but not females, showing differences between treatments (Figure 3e and 3f). As demonstrated previously by our lab (Markham et al., 2007), a trend towards a lower number of glia was found in female controls compared to male controls in combined layers ($F(1,12)=4.1$, $p=0.07$) and in the lower layers ($F(1,12)=4.6$, $p=0.053$).

2.3 Basolateral Nucleus of the Amygdala

A representation of a BLN parcellation used to quantify volume is shown in Figure 1b. Neither sex nor ethanol treatment had a significant effect on volume of the BLN. The lack of sex difference in controls replicates our prior findings in the BLN reported by Rubinow and Juraska (2009). Similar to the mPFC, total neuron number was not significantly affected by alcohol (Figure 4a). As previously found there was also no sex difference in neuron number in the BLN (Rubinow and Juraska, 2009). In contrast to the mPFC, there was no significant treatment effect in total glia number in the BLN (Figure 4b). There also was no sex difference in glia number confirming prior results (Rubinow and Juraska, 2009).

3. Discussion

The present study showed that repeated binge-like exposure to alcohol during adolescence did not alter the number of neurons in the mPFC or the BLN in adulthood in either male or female rats. There was, however, an effect of ethanol exposure on glia number in the mPFC, but not in the BLN. In the mPFC, ethanol-treated males had fewer glial cells in comparison to controls while females were unchanged. Given the interval between the last dose of ethanol (P45) and the time the brains were collected (P100), these differences are not due to the immediate effects of alcohol exposure but are instead long-term, and perhaps permanent, changes in the brain.

While the lack of effects of ethanol on neuron number was not predicted given the neuronal losses normally occurring in the mPFC and the BLN during adolescence (Markham et al., 2007; Rubinow and Juraska, 2009), there is support for the observed effects of ethanol on glia in the mPFC. Human alcoholics display a substantial decrease in glia density in the prefrontal cortex (Miguel-Hidalgo et al., 2002; Miguel-Hidalgo et al., 2006b) and glia number in the hippocampus (Korbo, 1999). Furthermore, in one of these studies neuron number was not altered (Korbo, 1999) but in another study neuronal density was decreased (Miguel-Hidalgo et al., 2006b). In rodent models, ethanol impedes glia proliferation in cell cultures (e.g., Guerri et al., 1990; Kennedy and Mukerji, 1986), and prenatal exposure results in fewer glia cells (but also neurons) in adult somatosensory cortex (Miller and Potempa, 1990). Unfortunately, the neural effects of exposure to ethanol during adolescence in rats are limited. One report has found a short-term decrease in glia density in the hippocampus (Oliveira-da-Silva et al., 2010) which is consistent with the current study. However, in the frontal cortex a decrease in cytoplasmic optic density of S100 β immunoreactive astrocytes was found along with an increase in GFAP-immunoreactivity within astrocytes immediately after ethanol administration (Evrard et al., 2006). Also in the same study it was reported that after 10 weeks both levels either returned to control levels (S100 β) or trended closer to the level of controls (GFAP-ir) (Evrard et al., 2006). However, how both of these measures relate to the number of glia rather than changes in expression of the marker is unknown. An additional study has found no immediate change in GFAP immunoreactive cell density but an increase after a 3-day withdrawal in alcohol-preferring rats (Miguel-Hidalgo, 2006a). Comparing this study with the current study is also difficult because of the differences in the strain of rat, the timing of withdrawal, and the exposure of ethanol differed greatly (11 days in the current study versus 2 months in the previous study).

The decrease in glia in the male mPFC can be a result of either an increase in cell death or a decrease in proliferation. There are indications that ethanol interferes more potently with proliferative processes than cell death once the organism is beyond early development (Miller, 2003). For example, in the hippocampal dentate gyrus, exposure to ethanol decreases the proliferation of neurons that is normally observed during adulthood (Nixon and Crews, 2002), and this effect is even more severe in adolescence (Crews et al., 2006). Likewise, the subventricular cells that give rise to the olfactory granule neurons, which proliferate throughout life, are decreased by ethanol exposure (Hansson et al., 2010). Effects of ethanol on proliferation would have made glia in the mPFC more vulnerable than neurons, which are postmitotic after birth. More specifically, ethanol blocks a mitogenic growth factor (PDGF), which regulates astrocytic proliferation *in vitro* (Luo and Miller, 1999). In the mPFC, Markham et al. (2007) found no sex differences in the number of glia at P35, the first day of ethanol exposure in the present study, but males had significantly more glia than females by adulthood (P90) because the number of glia increased in males between these ages, while the absolute number of glia decreased in females. Therefore, ethanol exposure appears to have interfered with the developmental trajectory of proliferation of glia that occurs specifically in males. In contrast, glia number in the BLN decreased in both sexes between the adolescence and adulthood (Rubinow and Juraska, 2009), indicating that cell death of glia in the BLN may be greater than the proliferation of glia, and that proliferation in the BLN is attenuated compared to the mPFC. Thus, the differential effects of ethanol on glia in the mPFC and BLN may reflect contrasting proliferation rates of glia between these two brain regions.

The changing properties of neurons and their receptors during development may account for the present finding that ethanol exposure during adolescence did not cause neuronal death in the mPFC or BLN as it does early in development (Ikonomidou et al., 2000; Ikonomidou et al., 2001). It has been suggested that both the NMDA antagonist and GABA_A agonist properties of ethanol are responsible for triggering cell death (Olney et al., 2002). Likewise, there is widespread attenuation of many cortical effects of prenatal ethanol in NMDA-NR1 knockout mice (Deng and Elberger, 2003). Both glutamate and GABA_A receptors change in their properties and number between the early neonatal period and adolescence, which may alter the apoptotic response to ethanol or the degree of this response (Insel et al., 1990; Wang and Gao, 2010; Yu et al., 2006).

Although sex differences in ethanol pharmacokinetics are well established, it is unlikely that similar sex differences in adolescents are the source of sex differences that we observed in mPFC anatomy. Notably, while females have been shown to exhibit faster blood ethanol elimination rates than males, it has also been demonstrated that there are no difference in pharmacokinetic profiles for brain ethanol concentrations (BrECs; Crippens et al., 1999). Moreover, a recent study found that although BrEC and blood ethanol concentrations (BECs) differed between adult male and female animals given 2 g/kg ethanol, but neither BrEC nor BECs differed in adolescents (Morales et al., 2011). In our previous study, which utilized the same adolescent treatment procedure, we found that intoxication ratings were not significantly different between male and female rats (Sherrill et al., 2011b). Thus, although we did not measure BrECs or BECs in the present study and therefore cannot definitively determine the role of metabolism in our findings, it is unlikely that this would explain the reductions in mPFC glia in ethanol-exposed males, but not females.

What is known about the neural effects of ethanol is predominantly based on male animals, but glia both manufacture and respond to the gonadal steroids (Garcia-Segura and Melcangi, 2006), which complicates the effects of ethanol on cell death. Furthermore, it should be noted that the time of ethanol exposure in the current study coincides with the appearance of puberty markers in our vivarium (female vaginal opening = ~day 35; male pubertal

separation = ~40; Koss et al, unpublished data). Estrogen is known to decrease cell death in adult rodents following hypoxia-ischemia (Wise and Dubal, 2000; Zhu et al., 2006). Since cell death markers do not discriminate between neurons and glia, estrogen may decrease the probabilities of glia death with exposure to ischemia or potentially, ethanol. Interestingly, there is also less cell death in organotypic cultures derived from females, compared to males, in response to oxygen and glucose deprivation as well as NMDA excitation (Li et al., 2005). These findings have led to the speculation that in addition to its activational role, estrogen may also have organizational or epigenetic effects that do not require estrogen to be present for its protective influence (Siegel et al., 2010). Obviously, more work is needed on the role of estrogen and other gonadal steroids in influencing the effects of ethanol.

In the current study we observed a significant sex difference in the number of neurons in the upper layers of the mPFC, but unlike Markham et al (2007), we did not demonstrate this when all layers were combined. There were also only trends in overall volume or number of glia. This could be due to increased variability in the present study caused by injection stress during adolescence (Brown et al., 2005) which did not occur in the Markham et al study. The present study also had a smaller sample size of the control animals, and in Markham et al (2007) litter was represented across groups so that litter was used as a factor to decrease variance. Both studies showed similar percent difference between the sexes in the total number of neurons (10% in the present study versus 13%), the total number of glia (20% versus 18%) and volume (13% versus 18%).

Determining the types of glia that were affected in the male mPFC in the current study should be the next step in understanding this effect of ethanol. The cortex contains the major classes of glia, including astrocytes, oligodendrocytes, and microglia. In the current study, astrocytes are one of the likely classes of glia that were decreased in the male mPFC after ethanol exposure. Previous work has shown acute increases in GFAP immunoreactivity with adult or prenatal ethanol exposure, but in the long-term these increases are significantly reduced or not detectable (Franke et al., 1997; Goodlett et al., 1993; Rintala et al., 2001). Also, in cortical cultures, ethanol initiates astrocytic cell death (Blanco et al., 2005; Kane et al., 1996). Additionally, male oligodendrocytes may also be affected by ethanol exposure in adolescence. During normal development of the cortex in male rats, the number of oligodendrocytes, as opposed to astrocytes and microglia, increase dramatically between 1 month and 3.5 months of age in the rat cortex (Ling and Leblond, 1973). Therefore, ethanol may decrease oligodendrocyte proliferation, thereby preventing the increase in glia number that occurs only in males during adolescence (Markham et al., 2007). Opposite to the decrease in glia seen in the cortex of the current study, microglia in males tend to increase in response to ethanol in both the cerebellum of adult rats (Riikonen et al., 2002) and in the hippocampus of adolescent rats (McClain et al., 2011). These studies may help further explain why there was no effect of ethanol on glia in the BLN. The BLN may be comprised of different proportions of subtypes of glia compared to the mPFC. For instance, if the BLN contains more microglia than the mPFC, that would negate the decrease in astrocytes or oligodendrocytes. To date, this kind of comprehensive study of glia cell type proportions in either of these brain regions is unavailable, thus making it difficult to predict which glia cell type is being altered by alcohol. However, it is unlikely that changes in microglia contribute to the decreases of glia seen in the mPFC in the current study. Regardless, the types of glia that are decreased following adolescent ethanol exposure in males needs to be directly established.

The decrease in the number of glia in the mPFC in males could have long-term consequences for neural function and behavior. Glia, and astrocytes in particular, are involved in regulating the extracellular chemical environment, supplying energy to neurons, and participating in several aspects of neuroprotection, such as the production of

neurotrophic factors and the homeostasis of neuronal glutathione (Gonzalez and Salido, 2009; Watts et al., 2005). A decrease in the number of glia may result in slower neural processing when mPFC functions are taxed, and long-term effects may include less protection from drugs that induce oxidative stress, including ethanol itself (Allaman et al., 2011; Watts et al., 2005). Furthermore, even though we found no changes in the BLN after adolescent ethanol exposure, future studies may find changes within specific glia types. Clearly, more work is needed to understand how a decrease in glia may alter other changes occurring during adolescence in males and the mechanism by which the sexes differ in their response to ethanol during adolescence.

4. Experimental Procedures

4.1 Subjects

The male and female Long-Evans rats in this study were offspring of rats obtained from Simonsen Labs (Gilroy, CA) and bred in the Psychology department. Rats were maintained on a 12:12-h light-dark cycle (lights on at 0800h) with free access to food and water. At P25, pups were weaned and then double or triple-housed with same-sex littermates throughout the remainder of the experiment. Subjects came from three separate cohorts of rats, born 2–3 months apart. All animal procedures were in compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign.

4.2 Ethanol Administration

From P35–45, male (7 per group) and female (8 per group) rats received intraperitoneal (i.p.) injections of 3g/kg ethanol (25% v/v saline solution; Ricca Chemical, Arlington, TX) or saline. Injections were administered in a binge-like pattern, with one injection per day for 2 days followed by 1 day without an injection. This cycle was repeated 4 times so that 8 total injections of saline or ethanol occurred across a span of 11 days. This pattern of exposure has previously been utilized in our laboratory and in other studies that find behavioral or physiological effects of adolescent ethanol exposure that persists into adulthood (Pascual et al., 2009; Philpot et al., 2009; Sherrill et al., 2011a; Sherrill et al., 2011b). BECs in alcohol-treated male rats were ~195mg/dl (Pascual et al., 2009). After completion of injections, rats were handled weekly until histology was performed in adulthood.

4.3 Histology

At P100, rats were weighed, injected (i.p.) with 100 mg/kg sodium pentobarbital and then perfused intracardially with 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde in PBS. After removal, a subset of the brains were weighed (males, n=5 per treatment; females, n=6 per treatment) and all brains were stored in the 4% paraformaldehyde solution for three weeks. Brains were then transferred to a 30% sucrose solution for 3 days, and then sectioned on a freezing microtome. Every fourth 60 µm section was mounted on slides and then stained with Methylene Blue/Azure II on the following day.

All tissue was coded such that the experimenter was blind to group. Also, a single experimenter did all of the parcellations and cell counts on each neural area to maintain consistency.

4.4 Volume

Parcellations of both the ventral mPFC (infralimbic and prelimbic regions) and the BLN were performed based on differences in cytoarchitecture as described previously (Krettek

and Price, 1978; Markham et al., 2007; McDonald et al., 1996; Rubinow and Juraska, 2009; Van Eden and Uylings, 1985) (Figure 1a and 1b). Parcellations of the boundaries of the mPFC were conducted from the most anterior mounted section where the underlying white matter appeared and continued on every mounted section until the appearance of the genu of the corpus callosum, resulting in analysis of 4–6 sections for each brain. Within each parcellation, dorsal and ventral boundaries were drawn as well as for the upper layers 2/3 and lower layers 5/6 (the rat mPFC does not have a layer 4). Layer 1 was not included because it contains very few cells. Duplicate drawings of boundaries were done in a subset of animals to confirm consistency within 5% of the original parcellations. The entire extent of the BLN (10–11 sections per brain) was parcellated separating it from the multiple nuclei in the basolateral complex (lateral, basolateral, and basolateral ventral amygdala). In both brain structures, areas were obtained and post-shrinkage thickness was measured during cell counting and used to determine an average thickness. Volumes were calculated with the Cavalieri method (Mouton, 2002) as the product of the areas and the measured tissue thickness between the saved sections. In the mPFC, all of these measurements were calculated for each layer as well as all layers combined.

4.5 Neuron and Glia Number

Total number of neurons and glia were determined using the optical disector with the Stereoinvestigator program (Microbrightfield; Williston, VT), as described previously (Markham et al., 2007; Rubinow and Juraska, 2009). For both neuron and glia density, the entire anterior-posterior extent of the mPFC and the BLN was quantified including separate quantifications for the upper and lower layers of the mPFC. The computer program chose the location of counting frames, which measured $35\mu\text{m} \times 35\mu\text{m}$ (length $\times$ width), within parcellated boundaries. Guard zones were set at $1\mu\text{m}$ for the mPFC and $1.5\mu\text{m}$ for the BLN at the top and bottom of each section with both brain regions utilizing a disector height of $13\mu\text{m}$. A cell was counted only if the bottom of the cell was within the volume of the counting frame. Both neurons and glia were separately counted within the frame. They were distinguished based on differences in morphological, size, and color characteristics as described by Markham et al. (2007) and Rubinow and Juraska (2009) (Figure 2). At least 200 neurons and 140 glia were counted from the BLN and from the upper and lower layers of the mPFC for each animal. These numbers were divided by the total volume of the counting frames to determine neuron and glia density. The densities for each animal were then multiplied by the volume of the structure for that animal to determine total number of neurons and glia. In the mPFC, this was done for the upper and lower layers separately as well as the all layers combined.

4.6 Statistical Analysis

Two-way analysis of variance (ANOVA), using sex and treatment as factors and cohort as a covariate, was performed for all analyses. Post-hoc tests were performed using one-way ANOVAs of treatment or sex effects so that the cohort covariate could be used.

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Highlights

- * Adolescent rats were exposed to binge-like levels of ethanol.
- * As adults, the number of neurons and glia were quantified in two neural areas.
- * No change in neuron number in the medial prefrontal cortex or basolateral amygdala.
- * No change in glia in the basolateral amygdala.
- * Males, but not females, exposed to ethanol had fewer glia in the medial prefrontal cortex.

Fig. 1.

A representative photograph and parcellation of the mPFC (a) and the anterior BLN (b). In the mPFC, layers 2/3 and 5/6 are distinguished, and in the BLN several surrounding white matter and nuclei are labeled. LA = Lateral Amygdala; BMA = Basomedial Amygdala; ec = external capsule; IM = main intercalated nucleus

Fig. 2.
A photograph illustrating a neuron (N) and glia cell (G) at the high magnification used for counting. Note the distinguishing characteristics in size and shape.

Fig. 3.

The number of neurons (mean + SEM) in adults in all layers (a), layers 2/3 (b), and layers 5/6 (c) of the mPFC. No effects of ethanol on neuron number were found, but a significant sex difference was present in layers 2/3 whereas only a trend was found in layers 5/6 and combined layers. The number of glia (mean + SEM) is also shown in all layers (d), layers 2/3 (e), and layers 5/6 (f). There was a significant effect of ethanol on glia in males only in separate and combined layers. There was also a trend for a sex difference in the controls in combined layers and layers 5/6.

Fig. 5.
The number of neurons (a) and glia (b) in the adult BLN (mean + SEM). There were no significant sex or treatment differences in the BLN.

Table I

	P45 Body Weight (mean grams $\pm$ SEM)	P100 Body Weight (mean grams $\pm$ SEM)	P100 Brain Weight (mean grams $\pm$ SEM)
Males			
Vehicle	176.1 $\pm$ 7.7	375.0 $\pm$ 21.4	1.47 $\pm$ 0.04
ETOH	154.0 $\pm$ 4.8	388.6 $\pm$ 12.1	1.45 $\pm$ 0.01
Females			
Vehicle	143.7 $\pm$ 2.0	257.4 $\pm$ 3.8	1.38 $\pm$ 0.01
Ethanol	138.8 $\pm$ 2.8	266.3 $\pm$ 5.0	1.34 $\pm$ 0.03

At P45 the last day of injections, ETOH treated animals weighed significantly less regardless of sex ($p=0.009$). There were no differences due to treatment later in adulthood (P100). Adult brain weights have a sex difference ($p=0.001$) but no ETOH effects.