Published in final edited form as:

Neurotoxicol Teratol. 2012 January ; 34(1): 128–135. doi:10.1016/j.ntt.2011.08.002.

Selective Effects of Perinatal Ethanol Exposure in Medial Prefrontal Cortex and Nucleus Accumbens

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Abstract

Ethanol exposure during development is the leading known cause of mental retardation and can result in characteristic physiological and cognitive deficits, often termed Fetal Alcohol Spectrum Disorders (FASD). Previous behavioral findings using rat models of FASD have suggested that there are changes in the nucleus accumbens (NAC) and medial prefrontal cortex (mPFC) following ethanol exposure during development. This study used a rat model of FASD to evaluate dendritic morphology in both the NAC and mPFC and cell number in the NAC. Dendritic morphology in mPFC and NAC were assessed using a modified Golgi stain and analyzed via three dimensional reconstructions with Neurolucida (MBF Bioscience). Cell counts in the NAC (shell and core) were determined using an unbiased stereology procedure (Stereo Investigator (MBF Bioscience)). Perinatal ethanol exposure did not affect neuronal or glial cell population numbers in the NAC. Ethanol exposure produced a sexually dimorphic effect on dendritic branching at one point along the NAC dendrites but was without effect on all other measures of dendritic morphology in the NAC. In contrast, spine density was reduced and distribution was significantly altered in layer II/III neurons of the mPFC following ethanol exposure. Ethanol exposure during development was also associated with an increase in soma size in the mPFC. These findings suggest that previously observed sexually dimorphic changes in activation of the NAC in a rat model of FASD may be due to altered input from the mPFC.

Keywords

Fetal Alcohol Spectrum disorder; prefrontal cortex; nucleus accumbens; ethanol; development; Golgi stain; stereology

1. Introduction

Teratogenic effects of alcohol have been documented for nearly forty years since Smith and Jones (1973) first defined the clinical features of Fetal Alcohol Syndrome (FAS). Ethanol consumption during pregnancy continues to be a problem; 12.5 % of women drink while pregnant (CDC, 2009) with the prevalence of FAS estimated at 1% of live births (May and Gossage, 2001, Sampson et al., 1997) and associated care costs estimated at nearly 4 billion

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Conflict of Interest Statement

The authors have no conflicts of interest.

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dollars annually in the US alone (Lupton et al., 2004). It is now recognized that ethanol exposure during development can produce a spectrum of behavioral and cognitive deficits, referred to as Fetal Alcohol Spectrum Disorders (FASD) (Manning and Hoyme, 2007, Riley and McGee, 2005, Streissguth et al., 1996). Executive functioning deficits commonly reported in FASD patients (Connor et al., 2000, Kodituwakku, 2009, Rasmussen, 2005) can in turn result in deficits in social behaviors and increases in high risk behaviors such as drug use (Connor et al., 2000, Olson et al., 1997). Consistent with this relationship, individuals with FASD are impaired in social behavior to a greater extent than can be explained by IQ deficits alone (Connor et al., 2000, Thomas et al., 1998) and are at high risk for drug and alcohol dependence (Alati et al., 2006, Baer et al., 1998, 2003, Yates et al., 1998). The constellation of deficits in executive function, social behavior, and drug addiction suggests that there may be changes in brain regions important to these types of behavioral changes, namely the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC) (Gass et al., 2007, Lawrence et al., 2008, Mihalick et al., 2001).

Animal models of FASD have indeed suggested that both the mPFC and NAC are altered by ethanol exposure during development. Ethanol exposure in rodent models of FASD occurs during the prenatal period, postnatal period or both the prenatal and postnatal period, the latter of which comprises the period equivalent to all three trimesters of brain growth in humans (Bayer et al., 1993). Prenatal ethanol exposure via liquid diet (35% Ethanol derived calories) resulted in significant reductions in cell number in layers II and V in mPFC in adult males (Mihalick et al., 2001). The mPFC cell loss was significantly correlated with executive function deficits (Mihalick et al., 2001). Complexins I and II, regulators of Ca^{2+} neurotransmission, were significantly reduced in the mPFC of adult rats exposed to ethanol during the prenatal period via liquid diet (36% EDC) (Barr et al., 2005). Zink et al. (2009) demonstrated similar reductions in complexin II mRNA in mPFC of young (postnatal day (PD) 8) rats exposed to ethanol from gestational day (GD) 1 to PD 8 via housing in an inhalation chamber. Given that Complexin II is localized to axospinal and axodendritic synapses (Yamada et al., 1999), these findings imply that ethanol exposure during development induced a reduction in synapses.

Examination of dendritic morphology in the mPFC has also revealed changes induced by ethanol exposure during development. Postnatal ethanol exposure from PD 2 to 6 via inhalation resulted in decreased basilar dendritic endpoints and length in layer II pyramidal neurons resulting in a simplification of the dendritic arbor (Granato et al., 2003). Similarly, postnatal exposure from PD 4 to 9 via intragastric intubation resulted in reductions in basilar length and branching as well as an increase in mature spines in both sexes (Whitcher and Klintsova, 2008). Apical dendrites in the same set of animals exposed to ethanol from PD 4 to 9 demonstrated significant reductions in spine density without changes in dendrite length (Hamilton GF et al., 2010). These latter two studies examined spines on limited portions of the dendrites and so conclusions regarding distribution of spines cannot be drawn. Together, these findings suggest reduced synaptic targets and decrements in function in the mPFC after exposure to ethanol during development.

Importantly, mPFC neurons project directly to the NAC with regional and laminar specificity (Basar et al., 2010, Ding et al., 2001, Brog et al., 1993) and affect NAC neuronal activity (Ishikawa et al., 2007). There have been no published studies examining neuronal structure in NAC following ethanol exposure during early development although there are findings that exposure to alcohol (Zhou et al., 2007) and other drugs of abuse (Robinson and Kolb, 1997, 1999) during adulthood reduce spine density in the NAC. Moderate ethanol exposure throughout gestation and the first two weeks postnatally failed to alter NAC volume (Rudeen, 1986) which is suggestive of no ethanol-induced changes in neuronal structure. However, there clearly are changes in the neurochemical nature of the NAC after

exposure to ethanol during development. For example, perinatal ethanol exposure resulted in an increase in Met-enkephalin levels in the nucleus accumbens of female but not male rats (Lugo et al., 2006b). Male rats exposed to ethanol during the prenatal period show elevated dopamine (DA) in the NAC following self-administered ethanol compared to controls; this effect was not seen in females (Blanchard et al., 1993). In rats exposed to ethanol during the prenatal period, there are reductions in spontaneously active DA neurons in the ventral tegmental area (VTA), which project to the NAC. Furthermore, stimulation of the NAC normalizes this reduction, suggesting an alteration in connectivity between these two structures (Choong and Shen, 2004). We have previously demonstrated alcohol exposure during development induces alterations in NAC activation in juveniles following a period of play (Lawrence et al., 2008). Additionally, social motivation, which involves activation of the NAC, has been suggested to be impaired by ethanol exposure during development (Gass et al., 2007, Kelly et al., 2009). Taken together, these findings suggest the strong possibility of altered neuronal survival and/or morphology in the NAC.

Because of the previous findings suggesting changes in the function of both the mPFC and NAC after ethanol exposure, we hypothesize that exposure to ethanol during development will reduce cell number and/or result in a decrease in dendritic branching and spines in the NAC and reduce dendritic branching and spines and changes the dendritic spine distribution in the mPFC. The effects are predicted to be sexually dimorphic as differential activity in the NAC was previously reported between male and female subjects exposed to ethanol during development (Lawrence et al., 2008).

2. Materials and Methods

2.1 Animal Model

Animal facilities were accredited by the American Association of Laboratory Animal Care (AALAC), and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina. All animals were housed in the animal colony of the Department of Psychology at the University of South Carolina. The colony was maintained at 22°C with a 12:12 hr light: dark cycle, with the light beginning at 0700 h.

The three trimester model of FASD was used (Cronise et al., 2001, Tran et al., 2000) which entails alcohol exposure via intragastric intubation during the prenatal and early postnatal period (PD 2–10) in the rat. The intubation procedure takes less than 2 minutes in both dams and pups and every effort is made to minimize the separation time of the pup from the dam.

Female Long-Evans rats were housed with an experienced male overnight and gestational day (GD) 1 was defined by the presence of sperm in the vaginal smear on the following morning. No handling other than routine cage changing occurred prior to breeding. Pregnant female rats were singly-housed and assigned to one of three treatments: ethanol-treated (ET), intubated control (IC), and non-treated control (NC). Prenatal treatments occurred from GD 1 through GD 22. ET dams were intubated using a stainless steel gavage tube with 4.5 g/kg of ethanol in 20 ml/kg of distilled water on a daily basis between 3:00 and 5:00 PM; food and water was available *ad libitum*. IC dams were pair-fed to an ET dam of similar weight and were intubated daily with maltose-dextrin made isocaloric to the ethanol solution in 20 ml/kg of distilled water. Inclusion of the IC group is to control for both nutritional and stress effects of the intubation procedure (Kelly et al., 2008). ET and IC dams were weighed daily, while NC dams were not handled in any manner. The day of birth was GD 23 and was considered PD 1 and no treatments occurred on this day.

Litters were culled to eight animals, four animals of each sex when possible, on PD 2. Postnatal treatments occurred from PD 2 through 10. ET pups were intubated using Intramedic PE 10 tubing with 3.0 g/kg of ethanol in 27.8 ml/kg of enriched milk formula (West et al., 1984) at 11 AM followed two hours later with 27.8 ml/kg of milk alone. IC pups were intubated twice daily but no solutions were administered. NC pups were weighed daily. Rats were weaned on PD 21 and housed in same sex pairs from the same litter. No more than one male and one female from each litter were used for a particular dependent measure. Animal numbers are listed in the figure legends and Tables 1 and 2.

2.2 Blood Ethanol Concentration

Blood samples (10 μ l) were taken from the dams on GD 20 three hours after ethanol or control intubation and from pups on PD 10 two hours after ethanol or control intubation via a nick to the end of the tail. The times were chosen because they assess maximum blood alcohol concentrations (BACs) in this model (Marino, et al., 2002). Blood samples along with standards generated at the time of blood sampling were frozen at -80°C until time of assay. BACs were analyzed using an enzymatic procedure (Dudek and Abbott, 1984).

2.3 Unbiased Stereology

On PD 90, animals were deeply anesthetized via sodium pentobarbital and perfused intracardially with phosphate-buffered saline followed by 4% paraformaldehyde in phosphate-buffered saline. Brains were removed, post-fixed overnight in the 4% paraformaldehyde solution and then incubated in a 15% followed by a 30% sucrose solution. Brains were frozen at -80°C until sectioned. Brains were sectioned coronally (40 μ m) beginning anterior to the rostral NAC (Plate 9, Paxinos and Watson, 2005) and continuing through the hippocampus (Plate 57, Paxinos and Watson, 2005). First section was randomly selected and every 6th succeeding section throughout the caudal extent of the NAC was mounted on glass slides and cover slips were attached with Permount. Serial sets were stained using the classical Nissl stain. Glia and neurons in both core and shell were counted using the optical fractionator method using Stereo Investigator (MBF Bioscience). Glial cells were defined by dense heterogeneous staining in the nucleus only compared to neurons which had staining throughout the soma and dense nuclear staining (see Cotter et al., 2002). Separate contours were used for NAC core and shell based on matched sections from the atlas of Paxinos and Watson (2005).

2.4 Golgi Stain

On PD 21, animals were deeply anesthetized via isoflourane anesthesia and rapidly decapitated. Brains were removed, bisected coronally and immersed in rapid-Golgi impregnation solution (FD Rapid Golgistain kit, FD Neurotech) for up to 2 weeks in the dark. After impregnation, brains were flash frozen in isopentane on dry ice and stored at -80°C until sectioned. Brains were sectioned coronally at 100 μ m on a cryostat. Sections were mounted on slides allowed to dry overnight and processed according to kit directions. Briefly, sections were rinsed in distilled water, fixed in proprietary solutions, rinsed in distilled water, then dehydrated through increasing alcohol concentrations and cleared with Histochoice (AMRESCO). Glass coverslips were attached with Permount and slides were allowed to dry in the dark until analyzed.

2.5 Dendritic Morphological Analyses

Three-dimensional analysis of dendritic morphology was performed using Neurolucida (MBF Biosciences Inc.) with the experimenter blind to treatment. 3–5 neurons per animal per region were traced using Neurolucida at 100x creating a three dimensional reconstruction of each neuron including the full dendritic arbor with spines (Figure 1). Layer

II/III pyramidal neurons were identified by triangular soma, and apical dendrite. Medium spiny neurons were identified by soma size, many dendritic extensions and spines as described previously (Robinson and Kolb 1997). Fully reconstructed neuronal tracings (see Figure 1) were analyzed in the companion software, Neurolucida Explorer.

The full reconstruction of neurons allowed Sholl analyses of dendritic branching (a branch is defined by the point at which one dendrite leaves another) and spine distribution (number of spines in a particular area of the dendritic tree) which means that Sholl rings are used as repeated measures on a single neuron (Sholl, 1953). A Sholl ring is the area between two concentric circles centered on the cell body. The circles are put at 50 μm intervals and Sholl rings are referred to by the distance of the outer circle; thus, a Sholl ring at 150 μm is the area defined by concentric circles drawn 100 and 150 μm from the center of the cell body.

3. Results

For all repeated measures analyses of variance (ANOVA), Greenhouse-Geisser adjusted degrees of freedom were used.

3.1 Blood Ethanol Concentration (BECs)

Average BECs and standard error of the means ($\pm$ SEMs) for the dams (13), male ET pups (9), and female ET pups (10) were 289.36 ± 15.39 , 283.88 ± 17.14 , 309.26 ± 29.24 mg/dl respectively. Separate t-tests comparing male and female pups and between pups and dams revealed no significant differences.

3.2 Body Weights

Repeated measures ANOVA on body weights of the IC and ET dams over gestational days indicated no significant effect of treatment or interaction of gestational day with treatment (data not shown). Pup weights ($\pm$ SEMs) are shown in Table 1. Repeated measures ANOVA on pup body weights with treatment and sex as between factors and day (PD 2, PD 10, and PD 21) as a repeated measure revealed a significant main effect of day ($F(2, 116) = 1549.6$, $p < 0.001$) (see Table 1). There were no effects of treatment or sex. Analysis of body weight on PD 90 indicated a significant main effect of sex ($F(1, 28) = 187.1$, $p < 0.001$) but not treatment (see Table 1).

3.3 NAC Stereology

There were no significant main effects of sex or treatment or interaction between sex and treatment on stereological measurement of neuronal or glial number in the core, shell or combined core and shell of the NAC. Estimated cell counts ($\pm$ SEM) of neurons and glia in the NAC and Coefficients of Error (CE's) are shown in Table 2.

3.4 Dendritic Morphology Analyses

Since layer II/III pyramidal neurons receive different inputs based on branch and distance from the soma, spine density is expected to differ across apical and basilar branches. Thus, Sholl analysis of spine branching and spine distribution in apical, basilar and all branches of neurons in the mPFC were analyzed by separate ANOVAs. Treatment and sex were between measures and Sholl ring (where appropriate) are repeated measures. For spine density (number of spines per micron of dendrite), separate ANOVAs with treatment and sex as between factors were conducted on apical, basilar and all dendrites in the mPFC and on dendrites in the NAC. When spine density differences were detected, spine distribution (spine density/Sholl ring) was analyzed by separate ANOVAs. Soma size was analyzed in the NAC and mPFC by ANOVAs with treatment and sex as between factors. All data presented are collapsed for sex unless indicated.

3.4.1 Nucleus Accumbens—An ANOVA on dendritic intersections within Sholl rings revealed a significant interaction of sex and treatment at the 150 μm Sholl ring ($F(2, 25) = 4.6, p < 0.05$). Tukey's HSD *post hoc* tests indicated the interaction was due to the ET females having more intersections at 150 μm compared to ET males and NCs and ICs of both sexes (see Figure 2; sex difference shown in inset of Figure 2). Separate ANOVA's on spine density and soma size failed to reveal significant effects (see Figure 3).

3.4.2 Medial Prefrontal Cortex—An ANOVA on apical intersections across Sholl rings indicated a significant main effect of treatment in the 150 μm Sholl ring ($F(2, 27) = 3.9, p < 0.05$) (see Figure 4A). Further analysis using Tukey's HSD *post hoc* tests indicated that the main effect was due to decreased intersections in the ET group compared to the IC group (Tukey's HSD, $p < 0.05$). An ANOVA on basilar intersections did not indicate any significant effects. Analysis of soma size in Layer II/III pyramidal neurons indicated a significant increase in the ET group compared to the NC group ($F(2, 28) = 3.5, p < 0.05$, Tukey's HSD, $p < 0.05$) (see Figure 4B).

Assessment of spine density in layer II/III pyramidal neurons indicated a significant main effect of treatment on total ($F(2, 35) = 4.4, p < 0.05$), basilar ($F(2, 36) = 4.2, p < 0.05$), and apical ($F(2, 35) = 5.4, p < 0.01$) dendritic spine density (see Figure 5). Ethanol exposure significantly reduced spine density on all dendritic branches (Apical, Basilar and Combined Apical and Basilar) compared to NC and IC groups (Tukey's HSD, $p < 0.05$).

3.4.2.1 Spine Distribution: Analysis of apical spine distribution across Sholl rings revealed significant main effects of treatment at the 50, 100 and 150 μm Sholl ring ($F(2, 29) = 5.1, p < 0.05$), ($F(2, 29) = 9.3, p < 0.05$), ($F(2, 28) = 3.8, p < 0.05$), respectively) (see Figure 6). Tukey's HSD *post hoc* tests indicated significant reductions in apical spine density in the ET group compared to both IC and NC groups at 50 μm and 100 μm and compared to the IC group only at 150 μm (Tukey's HSD, $p < 0.05$). There was also a significant main effect of sex at the 50 μm Sholl ring ($F(1, 29) = 9.3, p < 0.05$) due to increased spines in females (see Figure 7) but no interaction of sex with treatment. Ethanol exposure did not produce any significant changes in spine distribution on basilar dendrites (data not shown).

4. Discussion

Ethanol exposure during a period equivalent to all three trimesters in the human (Bayer et al., 1993) significantly impacted dendritic morphology in mPFC and had no impact on dendritic morphology or cell number in the NAC. Both basilar and apical spine densities were reduced by ethanol exposure during development in mPFC. The distribution of apical spines was also changed with reductions in numbers in the neuronal regions between 0 and 50 and also 50 and 100 μm from the soma in the ET group compared to both control groups but not in other regions. Other measures of dendritic integrity, including branching patterns and overall length, were unaffected by ethanol treatment in the mPFC. In addition, ethanol exposure during development resulted in a significant increase in cell body size in the mPFC compared to non-treated controls only.

4.1 Findings on the medial Prefrontal Cortex (mPFC)

Using the three trimester equivalent exposure model, we demonstrate significant alterations on dendritic morphology in layer II/III mPFC pyramidal cells similar to those previously reported in the hippocampus (Abel et al., 1983, Davies and Smith, 1981, Durand et al., 1989, Gonzalez-Burgos et al., 2006, Kuge et al., 1993, Saunders et al., 1995, Tarello Acuna et al., 2000), visual cortex (Cui et al. 2010) and sensory cortex (al-Rabiai and Miller 1989, Fabregues et al., 1985, Galofre et al., 1987, Hammer and Scheibel 1981, Stoltenburg-

Didinger and Spohr, 1983) following developmental ethanol exposure. This study is the first to utilize fully reconstructed neurons in the mPFC to quantify spine density and distribution following ethanol exposure during development and shows specific and localized reductions of spines on basilar and apical dendrites proximal to the cell body. A reduction in spines in this particular area is consistent with ethanol-induced reductions in excitatory input arising from area CA1 of the hippocampus (Jay et al., 1992, Parent et al., 2010, Swanson, 1981, Theiry et al., 2000, Heidbreder and Groenewegen, 2003, Spruston 2008). This in turn could lead to reduced neuronal output from the mPFC which would include reduced output to the NAC.

Under normal conditions, hippocampal CA1 activation leads to monosynaptic activation of mPFC pyramidal neurons (Jay et al., 1996, Laroche, 1990, Parent et al., 2010). Retrieval and consolidation of hippocampal-dependent memory has been shown to be dependent on mPFC activation (Mameli and Valenzuela, 2006) with coordinated firing increasing with task difficulty (Jones and Wilson 2005). Alcohol exposure during development compromises the hippocampus (Miller 1989; for reviews see Gil-Mohapel et al., 2011, Berman and Hannigan 2000) resulting in inhibited glutamatergic signaling, LTP induction (Puglia et al., 2010) and permanent immature spines (Mameli and Valenzuela, 2006). Together, these findings would predict altered CA1 output in animal models of FASD which could result in adaptive changes in the mPFC including reductions in dendritic spines near the soma of neurons as described in the current study.

Golgi staining is a commonly utilized in neuroscience but it should be noted that it is inherently unpredictable with staining of only 4–6 % of total neurons for reasons which remain unknown. Further, dendritic morphology, spine numbers and shape are dynamic with the ability to change rapidly or remain stable for prolonged periods (Holtmaat et al., 2005). Several studies have failed to find ethanol-induced differences in spine density and morphology using the Golgi procedure (Pentney et al., 1984, Galofre et al., 1987, Ferrer et al., 1988). It should be noted that these studies used manual quantification which may have resulted in more error in the findings. However, our findings on morphology and density in mPFC are consistent with recent studies using computer-based quantification of Golgi staining (Whitcher and Klintsova 2008, Hamilton GF et al., 2010) and similar to the findings of Granato et al. (2003) which used a retrograde tracing method and computer-based quantification (Granato et al., 2003). While results from Golgi staining should always be viewed with caveats due to the inconsistency in staining, the consistency in findings suggests that some confidence in the ability to generalize and replicate of the current findings is warranted.

In the present study, we demonstrated an increase in soma size in mPFC in ethanol-treated subjects relative to non-treated controls. There was no effect on soma size in NAC neurons. Because the difference in soma size is only relative to the non-treated group and not the intubated-control group, it is likely that the increase in soma size is the result of a synergy between the ethanol exposure and the stress of the administration procedures. A previous study identified a reduction in soma size of magnocellular, but not parvocellular, neurons in the lateral geniculate nucleus following moderate exposure to ethanol during the third trimester in monkeys (Papia et al., 2010). One possible mechanism underlying changes in soma size is a change in gene expression levels since Ransdell et al. (2010) recently demonstrated significant positive correlations between 18s rRNA expression in single isolated neurons and soma size. However, the functional significance of changes in soma size after ethanol exposure during development will need more empirical investigation.

4.2 Findings on the Nucleus Accumbens (NAC)

This is the first study to report on dendritic measures in the NAC following developmental ethanol exposure. In contrast to the findings in mPFC, most measures of dendritic morphology were unaffected in the NAC. Ethanol-exposed females did show an increase in dendritic branches in the 150 μ m Sholl ring relative to controls but this is a modest effect that was not shown at any other point. Further, neither the core nor shell of the NAC had any ethanol-induced cell loss. In contrast, chronic ethanol exposure in adults has previously been reported to produce significant reductions in density and an increase in atypical spine morphologies in the NAC (Zhou et al., 2007). However, simple interpretation of current findings suggests that the NAC is not vulnerable to direct effects of ethanol exposure during development.

There is evidence that the activity in the NAC is altered by ethanol exposure during development. Blanchard et al. (1999) previously demonstrated a sexually dimorphic response on ethanol-induced dopamine release with males responding only after a high dose of ethanol when compared to controls. Prenatally exposed females failed to increase dopamine release in response to either dose of ethanol (Blanchard et al., 1999). We have previously demonstrated similar ethanol-induced alterations in behavioral tasks involving NAC activity including cocaine induced conditioned place preference (CPP) and juvenile play (Gass and Kelly, 2006, Lawrence et al., 2008). Females perinatally exposed to ethanol demonstrated cocaine-induced CPP at a lower dose and elevated c-Fos immunoreactivity (IR) in the NAC whereas males failed to demonstrate CPP until the highest dose of cocaine and no effect on c-Fos IR (Gass and Kelly, 2006). Further, male rats exposed to ethanol during both the prenatal and postnatal periods demonstrated reduced c-Fos IR in the NAC after a period of play compared to non-treated control rats. In contrast, ethanol-exposed females showed elevated c-Fos IR in the NAC after a period of play compared to intubated-control rats (Lawrence et al., 2008).

Thus, changes in activity without a change in morphology suggest atypical processing in the NAC likely due to a change in input, possibly from the mPFC. The NAC receives significant glutamatergic projections from layer II/III pyramidal neurons in the mPFC (Jay et al., 1992, Vertes, 2006). Cues predictive of reward elicit mPFC activity and glutamate release in the NAC (Ishikawa et al., 2008, Jodo et al., 2000) with coordinated firing (Moscarello et al., 2007). Further, inhibition of mPFC projection neurons prevents NAC firing and eliminates behavioral responding to cues (Ishikawa et al., 2008). Thus, alterations in mPFC spine density in the ethanol-exposed animals may impair processing and integration of cues (as carried in hippocampal afferents) and this is in turn reflected by impaired activation during motivated behavior in the NAC. A direct test of this hypothesis remains to be done.

4.3 Conclusions

Clinical assessments of people with FASD have suggested that executive dysfunction results in impairments in everyday functioning including social behavior and increased risk for drug and alcohol abuse (Connor et al., 2000, Olson et al., 1997). The current findings suggest a disruption of processing in the mPFC which may be relevant to these behavioral impairments. The current data indicate a selective sparing of medium spiny neurons in the NAC from teratogenic effects of ethanol while layer II/III pyramidal neurons of the mPFC had reductions in spine density, changes in spine distribution, and increased soma size. Taken together with previous findings from both our laboratory and others, it suggests that secondary effects, such as impaired social behavior and substance abuse, may be the result of alterations in the morphology of the mPFC and activity but not morphology of the NAC. Further experimental investigation into the impact of ethanol exposure during development

on the NAC and mPFC would provide a greater understanding of mechanisms of complex secondary effects and insights with respect to treatment possibilities in FASD.

Acknowledgments

The project described was supported by Award Number RO1AA121566 from the National Institute on Alcohol Abuse and Alcoholism. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We would like to thank Amy Perkins for her helpful feedback on the manuscript.

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Highlights

Ethanol exposure during the prenatal and early postnatal period did not affect neuronal or glial cell population numbers in the nucleus accumbens.

Ethanol exposure during development produced a sexually dimorphic effect on dendritic branching at one point along the nucleus accumbens dendrites but was without effect on all other measures of dendritic morphology in this region.

Ethanol exposure during development reduced spine density and altered spine distribution in layer II/III neurons of the medial prefrontal cortex.

Figure 1.

A) Representative neuron from a Golgi stained section of the rat mPFC corresponding to plates 8–12 of rat atlas (Paxinos and Watson 2006). This image is from a light microscope at 20X. **B)** This image is a reconstructed tracing resized to match the light microscope image. **C)** Details of the Golgi image and reconstructed image of a portion of the dendrite as defined by the rectangles in A and B.

Figure 2.

Dendritic Branching in the Nucleus Accumbens (NAC). Perinatal ethanol exposure did not alter dendritic morphology in the NAC when sexes were combined. Data are collapsed across sex. Error bars represent SEMs and subject numbers are 12, 13, and 8 for the NC, IC and ET groups respectively. **Inset:** There was a significant increase in intersections in the ET females at 150 μ m compared to NC males and IC females ($p < 0.05$). ET males and NC females did not have any intersections in the 150 μ m Sholl ring. Error bars represent SEMs and subject numbers are 7, 6 and 4 in NC, IC, and ET males respectively and 5, 7, and 4 for NC, IC and ET females respectively. The symbol * indicates significant differences from all other groups (p 's < 0.05).

Figure 3. Soma Size (A) and Spine Density (B) in the Nucleus Accumbens. No significant differences in soma size or spine density were found across treatment groups. Data are collapsed across sex and error bars represent SEMs. Subject numbers are 12, 13, and 8 for the NC, IC and ET groups respectively.

Figure 4. Dendritic Branching (A) and Soma Size (B) in mPFC. Intersections within the 150 μm Sholl rings were significantly decreased in the ET group relative to the IC group ($p < 0.05$). Perinatal ethanol exposure induced an increase in soma size relative to the NC group only (p 's < 0.05). Data are collapsed across sex and error bars represent SEMs. Subject numbers are 12, 13, and 9 for the NC, IC and ET groups respectively. The symbol ** indicates significantly different from the IC group only and the symbol ++ indicates a significant difference from the NC group only (p 's < 0.05).

Figure 5.

Spine Density in mPFC following perinatal ethanol exposure. Ethanol treatment resulted in a reduction in dendritic spine density compared to both control groups whether the data were separated by apical or basilar dendrites or combined across apical or basilar dendrites. Data are collapsed across sex and error bars represent SEMs. Subject numbers for this measure are 12, 13, and 9 for the NC, IC and ET groups respectively. The symbol * indicates significant differences from both control groups ($p<0.05$).

Figure 6.

Spine distribution in the mPFC as determined by spine density per Sholl ring (A) was significantly reduced at 50μm and 100 μm compared to both control groups. Spines were reduced in the ET group compared to the IC group only at 150 μm. Data are collapsed across sex and error bars represent SEMs. Subject numbers for this measure are NC (12), IC (13), ET (9). * indicates a significant difference from both control groups and the symbol ** indicates a significant difference from the IC group only ($p<0.05$).

Figure 7.

Spine distribution in the mPFC at the 50 μm Sholl ring. At 50 μm, females across all groups had increased spine density relative to males. Error bars represent SEMs and subject numbers are 7, 6, and 4 in NC, IC and ET males respectively and 5, 7, and 5 in NC, IC and ET females respectively.

Table 1Average body weights ($\pm$ SEMs) and total number of subjects

Groups	N	Weight on PD 2	Weight on PD 10	Weight on PD 21	Weight on PD 90 (n)
NC	22	7.06 $\pm$.2	20.53 $\pm$.7	49.9 $\pm$ 1.9	337.8 $\pm$ 33
Males	11	7.22 $\pm$.2	20.65 $\pm$.8	51.1 $\pm$ 2.4	421.0 $\pm$ 20
Females	11	6.91 $\pm$.2	20.40 $\pm$.8	48.8 $\pm$ 2.4	254.5 $\pm$ 5
IC	22	6.26 $\pm$.3	18.27 $\pm$.9	47.1 $\pm$ 1.5	340.3 $\pm$ 31
Males	9	6.60 $\pm$.3	19.06 $\pm$.9	47.5 $\pm$ 2.6	441.8 $\pm$ 24
Females	13	6.03 $\pm$.3	17.72 $\pm$.7	46.8 $\pm$ 2.2	255.7 $\pm$ 9
ET	20	6.53 $\pm$.1	18.64 $\pm$.5	49.7 $\pm$ 1.7	352.2 $\pm$ 37
Males	10	6.63 $\pm$.2	19.71 $\pm$.8	52.5 $\pm$ 2.5	472.8 $\pm$ 22
Females	10	6.42 $\pm$.2	17.57 $\pm$.8	46.9 $\pm$ 2.5	251.7 $\pm$ 10

Table 2

Average estimated cell counts ($\pm$ SEMs), coefficient of errors (CE's) and subject numbers in the stereology study of the NAC

Group	Region	N	Neurons	CE	Glia	CE
	Core					
ET		11	$3.8 \times 10^5 \pm 42,118$	.05	$1.7 \times 10^5 \pm 18,077$	.08
IC		12	$4.5 \times 10^5 \pm 35,750$	.04	$1.8 \times 10^5 \pm 13,500$	.07
NC		12	$3.6 \times 10^5 \pm 32,001$	.05	$1.7 \times 10^5 \pm 14,749$	.07
	Shell					
ET		11	$3.3 \times 10^5 \pm 36,333$	.05	$1.5 \times 10^5 \pm 15,139$	.07
IC		12	$3.9 \times 10^5 \pm 33,830$	.02	$1.7 \times 10^5 \pm 13,000$	.07
NC		12	$3.2 \times 10^5 \pm 33,269$	.05	$1.5 \times 10^5 \pm 15,645$	.07
	Both					
ET		11	$6.8 \times 10^5 \pm 76,989$	.06	$3.2 \times 10^5 \pm 30,700$	.08
IC		12	$8.8 \times 10^5 \pm 68,907$	.05	$3.5 \times 10^5 \pm 25,713$	.07
NC		12	$7.2 \times 10^5 \pm 62,914$	.05	$3.3 \times 10^5 \pm 28,049$	.07