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LONG TERM EFFECTS OF PRENATAL ALCOHOL EXPOSURE (PAE) ON THE SIZE OF THE WHISKER REPRESENTATION IN JUVENILE AND ADULT RAT BARREL CORTEX

Tyson D. Chappell, **Cecilia P. Margret**[1], **Cheng X. Li**, and **Robert S. Waters**
Department of Anatomy and Neurobiology University of Tennessee Health Science Center College of Medicine 855 Monroe Avenue Memphis, Tennessee 38163, USA

1*Department of Psychiatry and Behavioral Sciences University of Washington 1959 NE Pacific Street, Box 356560 Seattle, WA 98195-6560*

Abstract

Children of mothers that abused alcohol during pregnancy are often reported to suffer from growth retardation and central nervous system (CNS) abnormalities. The use of prenatal alcohol exposed (PAE) animal models has revealed reductions in body and brain weights as well as regional specific brain deficits in neonatal pups. Recently, we and others reported reductions in the size of the posteromedial barrel subfield (PMBSF) in first somatosensory cortex (SI) associated with the representation of the large mystacial vibrissae in neonatal rats and mice that were exposed to alcohol at various times during gestation. While these reductions in barrel field size were reported in neonates, it was unclear whether similar reductions persisted later in life or whether some catch-up might take place in older animals.

In the present study, we examined the effect of PAE on measures of barrel field size in juvenile (6 weeks of age) and adult (7 months of age) rats; body and brain weights were also measured. Pregnant rats (Sprague-Dawley) were intragastrically gavaged during gestational days 1 to 20 with alcohol (6 g/kg) to simulate a binge-like pattern of alcohol consumption (Alc); 6 g/kg alcohol produced blood alcohol levels ranging between 207.4 and 478.6 mg/dl. Chow-fed (CF), pair-fed (PF) and cross-foster (XF) groups served as normal, nutritional/stress, and maternal controls, respectively for juvenile rats; an XF group was not included for adult rats. The major findings in the present study are: (i) PAE significantly reduced the size of the total barrel field in Alc juvenile rats (13%) and adult rats (9%) compared to CF controls, (ii) PAE significantly reduced the total averaged sizes of individual PMBSF barrels in juvenile (14%) and adult (13%) rats, (iii) PAE did not significantly alter the septal area between barrels or the barrel pattern, (iv) PAE significantly reduced body weight of juvenile rats but only in comparison to PF controls (18%), (v) PAE significantly reduced whole brain (8%) and forebrain (7%) weights of juvenile rats but not adult rats, (vi) no differences were observed in forebrain/PMBSF body ratios nor was forebrain weight correlated with PMBSF area, and (vii) PAE resulted in a greater reduction in anterior barrels compared to posterior barrels. These results suggest that the effects of PAE previously reported in neonate PMBSF areas persist into adulthood.

Offprint requests to: Robert S. Waters Department of Anatomy and Neurobiology University of Tennessee Health Science Center 855 Monroe Avenue Memphis, TN 38163 Phone: (901) 448-5796 FAX: (901) 448-71943 Email: rwaters@utmem.edu.

Keywords

fetal alcohol exposure; FASD; somatosensory cortex; PMBSF, vibrissae; birth defects

1. Introduction

Gestational alcohol exposure damages the developing brain and often leads to life-long behavioral and cognitive deficits. In the most extreme cases, prenatal alcohol exposure (PAE) results in children with growth retardation, craniofacial abnormalities, and central nervous system dysfunction and has led to the clinical diagnosis of fetal alcohol syndrome (FAS) (Jones & Smith, 1973; Jones & Smith, 1975). However, children of mothers that consumed alcohol during pregnancy may not meet all of the criteria for a diagnosis of FAS, but nonetheless suffer from permanent damage to brain structures that may result in a variety of dysfunctions including deficits in central nervous system processing. The term, fetal alcohol spectrum disorder (FASD), has been reserved to describe the range of deficits resulting from early alcohol exposure (Goodlett, Horn & Zhou, 2005; Riley & McGee, 2005).

PAE children often have sensorimotor processing deficits as demonstrated by their poor performance on higher-order cognitive motor tasks (Adnams, et al., 2001), difficulties in maintaining postural balance (Roebuck, et al., 1998), slower premotor and motor reaction times (Simmons, et al., 2002), and deficits in fine motor control (Connor, et al., 2006), and timing (Wass, et al., 2002). Many reported sensorimotor disturbances may be due, in part, to altered visual and auditory capacity (Church, 1987; Church & Abel, 1998; Stromland, 1985; Stromland, 2004), reduction in overall brain size and shape (Roebuck, Mattson & Riley, 1998; Sowell, et al., 2001), corpus callosum (Riley, et al., 1995), basal ganglia (Mattson, et al., 1994), and parietal cortex (Archibald, et al., 2001).

Many of the sensorimotor deficits seen in PAE children are mirrored in PAE animal models, the use of these models may be helpful in uncovering potential deficits that can be further explored in PAE children. Similar to children, animals exposed to prenatal alcohol may also exhibit impaired balance (Kelly, Hulsether & West, 1987), disrupted righting reflexes (Lopez-Tejero, et al., 1986), delayed motor (Molina, et al., 1987; Norton, et al., 1988), and altered corticospinal development (Miller, 1987). Furthermore, PAE has been shown to result in regional specific deficits in cerebellum (Maier, et al., 1999; Maier, Miller & West, 1999; Maier & West, 2001), locus coeruleus (Maier & West, 2003), and somatosensory cortex (Margret, et al., 2005a; Margret, et al., 2005b; Miller & Potempa, 1990; Mooney & Miller, 1999; Powrozek & Zhou, 2005).

We, (Margret, et al., 2006a; Margret, et al., 2006b; Margret, et al., 2005b), and others (Powrozek & Zhou, 2005), have used the rodent barrel field cortex to study the effects of gestational alcohol exposure on the organization of somatosensory cortex. Clusters of cells, called barrels, located within layer IV of the primary somatosensory cortex (SI) are associated with the representation of the body surface. These barrels are readily amenable to quantitative methods that allow comparisons of aerial measurements between alcohol and non-alcohol treatment groups. One subfield of barrels, the posterior medial barrel subfield (PMBSF), is associated with the representation of the large mystacial whiskers on the contralateral face. Recently, Powrozek and Zhou (2005) examined serotonin labeling in mice at postnatal day 7 (P7), and reported that PAE reduced the overall size of serotonin-labeled barrels in the PMBSF as well as the sizes of some of the individual barrels within the PMBSF. Furthermore, PAE not only reduced the number of cells within selected barrels, but in some cases, entire barrels were often reported missing. Similarly, in P9 rats, PAE reduced the size of the overall PMBSF,

along with the sizes of individual barrels and septal areas lying between barrels, but did not alter barrel pattern or result in missing barrels (Margret, et al., 2005b).

In the present study, we asked whether area reductions in total PMBSF barrel area and sizes of individual barrels observed in neonatal rats would also be present in six-week-old juvenile rats and seven-month-old adult rats. The results of the present study indicated that PAE exerts a long-term effect on the size of barrel cortex while leaving the barrel pattern unperturbed.

2. Methods

2.1 Animals

A total of 109 Sprague-Dawley pups were used in this study (84 animals were used for the 6-week-old rat experiments, and 25 animals were used for the 7-month-old rat experiments). Pups were produced by placing adult female rats (n=32, 250–300 g) with adult male rats (300–350 g). All females were handled and habituated to the dry gavage procedure 4 days prior to breeding.

2.2 Breeding and treatment groups

Breeding occurred by placing 2–3 adult female rats in a cage overnight with an adult male breeder. Vaginal smears were examined the next morning under a microscope. Sperm positive slides were marked as gestational day one (G1). At that time, pregnant females were weighed, and separated into one of three treatment groups: alcohol (Alc, n=11), pairfed (PF, n=10), and chowfed (CF, n=11). PF mothers were matched in weight to Alc mothers. Pregnant females were separated into individual cages during pregnancy and pup rearing.

2.3 Treatment procedures

The Alc group was gavaged with 25% (w/v) alcohol solution at a 6 g/kg dose from G1–G20. This period is equivalent to the first and second trimesters in humans (Dobbing & Sands, 1973; Dobbing & Sands, 1979). The PF group was gavaged with 25% maltose dextran solution being isocaloric and isovolumetric to the alcohol given the Alc group. PF mothers received only the amount of food consumed a day earlier by a previously matched Alc female. The PF group served as a control for nutrition and stress. The CF group served as a normal control with no diet restriction or gavage treatment (excepting for the initial gavage acclimation given to all females). A cross-foster group (XF) was used in the 6-week-old rat experiment to test for maternal rearing effects. These pups received equal prenatal treatment as the Alc group, however, following delivery, pups were fostered to a CF mother whose own recently delivered pups had been removed. All groups were provided with ad libitum water.

2.4 Daily procedures

Food hoppers were removed daily between 0830 and 0930 hours. Daily dam weight and food consumption were recorded. Three hours following food hopper removal, Alc and PF dams were gavaged with their respective solutions. Stainless-steel gavage needles, lubricated with canola oil, were used on virgin females during the habituation period prior to mating. Stainless steel tubes were replaced by plastic oral feeding tubes (Instech Solomon) at the time of gestation. The gavage procedure occurred at the end of a 3-hour fast in order to maximize solution absorption. Each day, Alc and CF dams were given ad libitum access to 50 g of chow (Harlan).

2.5 Parturition and rearing

Gavage treatment continued until G20, at which time dams were transferred to clean cages; two nesting pads were added to the cage. Dams were weighed daily, but otherwise were left

undisturbed until parturition, having free access to chow and water. On G22, cages were checked twice daily at 0800 and 1700 hours until delivery. On the day of birth, designated postnatal day 0 (P0), litters were culled to 8 pups. The weight of the dams as well as their food consumption, were noted until P10. Pups were weaned on P28, separated into sex specific and litter specific cages where they remained with ad libitum food and water until testing during the 6th postnatal week.

All animals were maintained in UT animal facility rooms at temperatures of 78–80° F, 35–40% humidity, and a 12-hour light/dark cycle. The experiments conformed to the *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985) and were approved by the Animal Care and Use Committee, University of Tennessee Health Science Center. The Animal Care Facility is AAALAC approved.

2.6 Blood Alcohol Concentration

Blood samples were collected from Alc and PF groups on G13 and G20 at 60, 120, 180, and 240 minutes post-gavage. PF blood samples were taken to match for stress of handling Alc animals during the blood sampling procedure, although the PF samples were not analyzed. Blood was taken by placing the tail in warm water for 5 seconds, wiping off excess water with a paper towel, and cutting the tip of tail with a sharp razor blade. Blood was collected in 20 μl heparinised tubes, centrifuged to separate plasma, and stored at 4° C. Blood alcohol concentration (BAC) was measured using an ANALOX G5 alcohol analyzer.

2.7 Tissue processing

Juvenile rats in their 6th post-natal week were weighed and given a lethal injection of Nembutal (50 mg/kg, i.p.). Rats were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.15M sodium phosphate buffer saline (NaPBS, pH 7.4, 21° C). The brain was removed from the skull and weighed (whole brain), followed by removal of olfactory bulbs and cerebellum then weighed again (forebrain). The forebrain was hemisected, the white matter of each hemisphere was removed and the remaining gray matter of each hemisphere was flattened between two Plexiglas plates. The hemispheres of juveniles were flattened between two Plexiglas plates that were compressed together with rubber washers fitted around rivet posts. In contrast, hemispheres of adult rats were flattened between two Plexiglas plates by adjusting hexagonal nuts fitted to fine-threaded machine bolts holding the plates together. In the latter case, flattening was more uniform across sections, but it appeared that the adult tissue was less compressed than the juvenile tissue. Therefore, we were unable to make direct comparisons of PMBSF measures between juveniles and adults. The flattened hemispheres were then placed in paraformaldehyde and refrigerated overnight. The following day the flattened tissue was sectioned in the sagittal plane at 120 μm on a Vibratome and placed in test tubes containing 0.01M potassium phosphate buffer solution (KPBS, pH 7.4, 21° C). Sections were washed in KPBS (3 × 10 min) at room temperature. The tissue was stained with cytochrome oxidase using a method modified from Wong-Riley and Welt (Wong-Riley & Welt, 1980). Following final KPBS rinse, test tubes with tissue sections were filled with a solution of 30 ml 0.01M KPBS, 22.5 mg diaminobenzidine (DAB, Sigma), 12 mg cytochrome oxidase (Sigma), and 1.5 mg sucrose (Fisher Chemicals). Test tubes were placed in a 37° C water bath for 2 hrs or until barrels were clearly discernible from interbarrel septal regions. Sections were then rinsed 3 ×10 min in 0.01M KPBS, mounted on gelatin coated slides, and cover-slipped using permount/xylene.

2.8 Quantitative morphometric and data analyses

Tissue sections were viewed with a light microscope using the 2 × objective and images were captured with a digital camera. A blue gelatin filter (Kodak, Wratten gelatin filter #47A) was used to visualize the barrel field. Digitized sections containing the PMBSF were aligned,

stacked, and reconstructed using Photoshop 7.0. The PMBSF consists of 27 well-demarcated barrels consisting of five rows (Row A–E) and four posteriorly located straddler barrels (α,β,γ,δ). In addition to the PMBSF, other barrel subfields were present and include the anterolateral barrel subfield (ALBSF) representing the upper lip and sinus hairs of the snout, lower lip barrel subfield (LLBSF), forepaw barrel subfield (FBS), hindpaw barrel subfield (HBS), and a nebulously-stained trunk subfield (TKS). The present study focused exclusively on measurements of the total averaged area of the PMBSF, the total averaged area of individual barrels, and the total averaged area of the septal areas lying between barrels. These aerial measurements are illustrated in the line drawings in Fig. 1. The total PMBSF area was drawn by placing a boundary line around the reconstructed PMBSF and this is shown in Fig. 1A′. The total area of the individual barrels is shown in Fig. 1B′, and the septal area is shown by the blackened region in Fig. 1C′. Digital reconstructions of total barrel area, individual barrels, and septal regions were measured using Image J (NIH, Wayne Rashband).

When two hemispheres from the same animal were available, both hemispheres were measured and averaged. All treatment groups were compared using ANOVA with a P-value set at ($P<0.05$). All comparisons that resulted in a significant main effect were further analyzed using Tukey-Kramer's post-hoc test, also set at a significance level of ($P<0.05$). Statview (5.1) or DataDesk (6.1) were used to perform all statistical analyses. To examine possible differential region effects following PAE, Alc data was normalized, and expressed as a percentage change from CF. The brain/body ratio was determined by dividing the whole brain weight by the body weight of each animal respectively. This ratio examines possible brain/body differential effects following PAE. Forebrain weight and total PMBSF area were compared using a Pearson-Product-Moment Correlation and linear regression analyses (t-ratio).

3. Results

3.1 Maternal Blood Alcohol Concentrations

Blood samples were taken on G13 and G20. On G13, Alc dams had an average peak blood alcohol concentration of 285.8 ± 12.8 (range; 210.4–327.6 mg/dl) whereas peak BAC levels measured on G20 were 329.1 ± 21.0 (range; 207.4–478.6 mg/dl).

3.2 Effects of PAE measured in juvenile rats at 6-weeks of age

3.2.1 Body weight—A significant main effect in body weight occurred between treatment groups [$F(3,80) = 4.348$, $P = 0.0069$]. The body weight of Alc rats was significantly reduced compared to PF alone. No significant differences in body weight were observed between other treatment group comparisons. These data are shown in Table 1. When the Alc data was normalized, by taking the mean body weight and expressing this as a percent change from the CF group, the Alc body weight was reduced 13.6 ± 3.3% compared to the CF group.

3.2.2 Effects of PAE on PMBSF pattern—The PMBSF pattern is illustrated in Fig. 1 for CF, PF, and Alc juvenile rats. Note that the five-row barrel arrangement and the presence of the posteriorly located straddler barrels are seen in each treatment group. The PMBSF barrel pattern, consisting of 27 barrels, likely reflects a common plan of organization that is independent of treatment. A similar PMBSF barrel pattern was also seen in adult rats (data not shown). Thus, PAE does not appear to alter the overall PMBSF pattern in juvenile and adult rats.

3.2.3 Whole brain and forebrain weights—A significant main effect was observed for whole brain [$F(3,80) = 19.804$, $P < 0.0001$] and forebrain [$F(3,80) = 17.094$, $P < 0.0001$] weights for treatment groups. The mean Alc whole brain and forebrain weights were significantly less than controls and these results are presented in Table 1. No significant

differences in brain weight were found between Alc and XF rats or between CF and PF rats. Alc whole brain and forebrain weights were reduced by 8.3 ± 1.6% and 6.9 ± 1.5%, respectively compared to CF rats.

3.2.4 Total area of the PMBSF—A significant main effect was observed for total PMBSF area [$F(3,80) = 6.357$, $P = 0.0006$] for treatment groups. Total averaged PMBSF area was significantly reduced in Alc rats compared to CF. These data are illustrated in Table 2. No significant differences were observed between Alc and XF rats or between CF and PF. Normalization of the data showed that the total Alc PMBSF area was 13.1 ± 1.9% smaller than in CF rats.

3.2.5 Total individual PMBSF barrel area—Analysis of total individual barrel area revealed a significant main effect [$F(3,80) = 7.589$, $P < 0.0002$] for treatment groups. The averaged area of the total individual barrels was significantly reduced in Alc rats compared to non-alcohol controls. These data are shown in Table 2. No significant differences were seen between Alc and XF rats or between CF and PF rats. Normalization of the data indicated that the total individual barrel area of Alc rats was 14.3 ± 1.9% smaller than CF rats.

3.2.6 Total septal area of PMBSF—A significant main effect for treatment groups was not observed for the septal region [$F(3,80) = 2.546$, $P = 0.0618$]. The averaged septal area is shown in Table 2 for each treatment group. While no significant differences were observed between alcohol and non-alcohol treatment groups, normalization of the data showed that the septal region in Alc rats was nonetheless 10.4 ± 2.4% smaller than in CF rats.

3.2.7 Forebrain weight and PMBSF area—PAE significantly reduced both forebrain weight and PMBSF area. A forebrain/PMBSF ratio was constructed to determine whether alcohol played a greater role on either brain weight or PMBSF area, or whether PAE affected weight and area equally. A significant main effect was not observed between treatment groups since the ratios were nearly identical between groups, suggesting that PAE equally affected both brain weight and PMBSF area. Ratio data are shown in Table 2 for each treatment group.

We next addressed whether a relationship existed between forebrain weight and PMBSF area. Forebrain weight was compared with total PMBSF area for each treatment group using a Pearson Product-Moment Correlation (r) and no significant correlations were found for any of the treatment groups. These data are presented in scatter-plot format and a simple regression line (least-squares line) through the data points are shown in Fig. 2.

3.2.8 Regional vulnerability—To determine whether individual barrels within the PMBSF were uniformly reduced following PAE, or whether regional differences in vulnerability exist, as previously reported in P10 pups (Margret, et al., 2005b) we measured and compared the averaged barrel area for each of the 27 barrels for alcohol and non-alcohol treated rats. Individual barrels for Alc and CF rats were compared on a barrel-by-barrel basis and 74% (20/27) of the barrels were significantly smaller in Alc rats compared to CF rats. These data are shown in Fig. 3; asterisks show significant differences between individual Alc and CF barrels. No significant differences in barrel sizes were observed for Alc and XF comparisons, or between PF and CF comparisons. Mean areas of Alc barrels were then normalized and expressed as a percent difference from the mean area of the corresponding CF barrel, and categorized as follows: (i) severely reduced (>20%), (ii) greatly reduced (16–20%), (iii) moderately reduced (11–15%), (iv) slightly reduced (≤10%). Inspection of Fig. 3 showed that anterior barrels in Alc rats showed a greater reduction in size compared to CF, while posterior barrels in Alc rats exhibited a modest reduction in size compared to CF rats.

We next compared barrel rows (A–E) and arcs (1 to 5 and straddlers) between treatment groups. Normalization of the Alc row data as a percent change from CF showed little difference in percent reductions between lateral (A1–A4) to medial (E1–E5) rows (12.1%, 15.8%, 13.8%, 15.3%, and 15.7%, respectively), while normalization of arc data showed a lesser reduction in posterior arcs compared to anterior arcs (10.8%, 12.2%, 14.3%, 15.8%, 16.2%, and 16.3%, respectively). These results are shown in Fig. 4. All row and arc comparisons resulted in significant main effects between treatment groups. Post-hoc analysis (Tukey-Kramer's) revealed that all arcs (except the straddler arc) and all rows (except Row A) were significantly reduced in Alc compared to CF (Fig. 4).

3.3 Effects of PAE measured in adult rats at 7-months of age

3.3.1 Body and brain weights—No significant main effects were observed in body or brain weights between treatment groups. Mean and SEM for each group are presented in Table 3. Normalization of body and brain weights indicated that Alc rats weighed 4.1 ± 10% less than CF rats, whole brain weights were 4.7 ± 2.8% lower than CF rats, and forebrain weights were 3.3 ± 3.2% less than CF rats.

3.3.2 Total PMBSF area—A significant main effect was observed for total PMBSF area [$F(2,22) = 5.017$, $P = 0.016$] for treatment groups. Total PMBSF area was significantly smaller in Alc rats compared to PF and CF rats. These data are shown in Table 4. No significant differences were observed between CF and PF rats. Normalization of the total PMBSF area showed a 8.9 ± 2.0% reduction in Alc rats compared to CF rats.

3.3.3 Total individual PMBSF barrel area—A significant group main effect was found for the total individual PMBSF barrel area [$F(2,22) = 5.938$, $P = 0.0087$]. The total averaged individual barrel area of Alc rats was significantly smaller compared to PF and CF rats; these data are illustrated in Table 4. No significant differences were found between non-alcohol groups. PAE reduced mean total individual barrel area by 12.7 ± 1.7% in Alc rats compared to CF.

3.3.4 Total septal area of PMBSF—No significant group main effect was observed for the total septal area [$F(2,22) = 2.741$, $P = 0.0865$]. Results are shown in Table 4. The septal region in Alc rats is 0.7 ± 4.5% larger than in CF rats.

3.3.5 Regional vulnerability—Similar to results from juvenile rats, total individual PMBSF barrel areas were significantly smaller in adult Alc rats compared to CF and PF rats. When the areas of individual barrels in Alc rats were compared to the areas of identical barrels in juvenile rats, 74% (20/27) of the barrels were smaller in Alc rats compared to 48% (13/27) in adult rats. Asterisks in Fig. 5 indicate significantly smaller barrels. No significant differences resulted between individual PF and CF PMBSF barrels. We next normalized barrels in Alc rats as a percent change from CF rats and categorized the results as shown in Fig. 5. Again, anterior barrels showed greater reduction in size compared to posterior barrels. Examination of rows and arcs revealed little change in percent reduction across rows (A row = 12.2%, B row = 14.4%, C row = 11.9%, D row = 12.1%, E row = 15.6%). In contrast, clear reductions were observed between posterior and anterior (straddlers = 8.3%, Arc 1 = 9.2%, Arc 2 = 10.9%, Arc 3 = 12.4%, Arc 4 = 18.2%, Arc 5 = 19.1%). Tukey-Kramer's post-hoc analysis revealed that all Alc rows and arcs (excepting the straddler arc) were significantly smaller than PF or CF, respectively as shown in Fig. 6.

4. Discussion

We, and others, have reported that PAE altered the size of the PMBSF in neonatal rats (Margret, et al., 2006b; Margret, et al., 2005b) and mice (Powrozek & Zhou, 2005), but did not disrupt the barrel pattern with the exception that an occasional barrel was reported missing in mice. One goal of the present study was to determine whether the effects of similar gestational alcohol exposure from G1–G20 were restricted to neonatal rats or whether PAE exerted long-term reductions in barrel field size in juvenile rats and adult rats. The mitochondrial stain, cytochrome oxidase, was used to visualize the PMBSF; barrels in this subfield are large with wide intervening septal spaces and are easily distinguishable from neighboring barrels. The important findings in the present study are: a) PAE significantly reduced the total averaged PMBSF area in juvenile and adult rats compared to non-alcohol treated rats, b) PAE significantly reduced the sizes of individual PMBSF barrels in juvenile and adult rats, c) PAE did not effect the area of the inter-barrel septal area of PMBSF in either juvenile or adult rats, d) intra-barrel subfield differences were observed within the PMBSF whereby anterior located barrels had a greater reduction in size compared to CF controls than did posterior barrels. e) PAE significantly reduced body weight in juvenile rats but only in comparison with PF controls, f) PAE significantly reduced brain weights of juvenile rats but not the brain weights of adult rats. These findings support the notion that the effects of PAE on the cortical barrel field persist into adulthood, while the effects of PAE are less apparent on body and brain weights in older animals.

4.1 PAE has long-term effects on the somatosensory cortex

Previously we used intragastric gavage to administer a high dose of alcohol (6 g/kg) to pregnant rat dams during the first twenty days of gestation and reported reductions in body and brain weight as well as a reduction in overall PMBSF size, sizes of individual barrels in PMBSF, and septal regions between barrels in neonatal pups examined at P10 (Margret, et al., 2005b). Since body, brain, and cortical representation were all reduced it could not be ruled out that PAE exerted a global reduction rather than a specific effect on somatosensory cortex. However, a recent study in mice, showed that alcohol administered over a more limited period of gestation (G8 to G18; typical gestation length is 19 days) reduced the size of the PMBSF but did not significantly reduce body or brain weights although the averaged body and brain weights of Alc mice were less than the nutritional controls (Powrozek & Zhou, 2005). These results suggested that PAE had a direct effect on barrel field cortex that was not confounded by concomitant reductions in body and brain weight. In the present study body and brain weights and PMBSF area were compromised in juvenile rats, while only the overall area of the PMBSF and the areas of individual barrels were significantly reduced in adult rats. Interestingly, the septal regions between barrels were not significantly reduced in either juvenile or adult rats, in contrast to previous findings in neonates (Margret, et al., 2005b), and this may reflect differences in afferent input between septae (Gil, Needleman & Huntley, 2002) and barrels (Molnar, Higashi & Lopez-Bendito, 2003; Senft & Woolsey, 1991) as well as possible differences in cortical circuitry (Kim & Ebner, 1999). Dendrites and spines of layer V pyramidal neurons in SI cortex are particularly affected by PAE (al-Rabiai & Miller, 1989; Galofre, et al., 1987). However, it remains to be determined whether dendrites and spines of barrels and/or septae are similarly affected by PAE, that could possibly account for PAE-related changes in the PMBSF reported here.

Effects of PAE are also seen in the somatosensory cortex of rats examined at approximately 12 weeks of age. These include reduction in overall cortical volume, reductions of neurons and glial cells (Miller & Potempa, 1990), ultrastructural changes in corticospinal somata (al-Rabiai & Miller, 1989), and altered glucose utilization following whisker stimulation (Miller & Dow-Edwards, 1993). Reductions in cortical packing density and thickness of layer V were found

following PAE, while thickness of layers II/III increased in 6-week-old juvenile rats (Fakoya & Caxton-Martins, 2006). These findings, along with the present results, support the conclusion that PAE has long-term effects on somatosensory cortex, and specifically on the cortical barrel field.

The present results are relevant for understanding deficits in fine motor control and higher-order cognitive functions (Adnams, et al., 2001) reported in sensorimotor systems in humans following gestational alcohol exposure. PAE children often have difficulties with fine motor control (Connor, et al., 2006) and timing (Wass, et al., 2002), exhibit slower reaction times (Simmons, et al., 2002), and perform poorly on higher-order cognitive motor tasks (Adnams, et al., 2001). It is well established that the motor cortex controls voluntary movements but more recently has been shown to play a role in motor learning and possibly even in cognitive functioning (Sanes & Donoghue, 2000). It is also well known that the somatosensory cortex modulates the motor cortex output through direct corticocortical connections. A somatosensory cortex that is smaller in overall size with fewer neurons and glia likely communicates a compromised sensory message to the motor cortex that may help explain, in part, the deficits in sensorimotor behavior observed in children with FASD.

4.2 Regional and intraregional vulnerability

It is well established that PAE results in regional vulnerability whereby some brain regions are affected while others are spared. Previous studies have indicated that PAE administered specifically during neurogenesis of locus coeruleus (LC), cerebellar purkinje cells or inferior olive cells only reduced the number of cells in LC (Maier & West, 2003). PAE significantly reduced CA1 hippocampal pyramidal cells without a concomitant reduction in CA2 through CA4 pyramidal cells or in dentate gyrus granule cells (Barnes & Walker, 1981; Gibson, et al., 2000). Cell number and volume of ventrobasal thalamus (Mooney & Miller, 1999), and ventral lateral thalamus (Livy, et al., 2001) do not appear vulnerable in pups exposed to alcohol during neurogenesis even though thalamocortical afferents were reported delayed in reaching barrel cortex (Margret, et al., 2005a). In contrast, cortical thickness (Fakoya & Caxton-Martins, 2006), neuronal number (Miller, 1987), glucose metabolism (Miller & Dow-Edwards, 1988), and neuronal migration (Miller, 1993) are disrupted in rats exposed to gestational alcohol.

Here we report that while the area of individual PMBSF barrels is reduced in PAE rats, normalization of individual Alc barrel areas in comparison to CF controls indicated intraregional differences in the amount of reduction between posterior and anterior barrels. Anterior PMBSF barrels have a greater reduction in area compared to CF rats than do the posterior barrels. Several potential mechanisms may be responsible for this regional gradient in barrel area first identified in neonates (Margret, et al., 2005b) and now in juvenile and adult rats. Previous examinations of the mystacial whiskers and follicle-sinus complexes (FSC) indicated that not only are posterior whiskers longer and thicker than anterior whiskers (Brecht, Preilowski & Merzenich, 1997), but posterior FSCs are innervated by a greater number of axons than the more anteriorly located follicles (Welker & Van der Loos, 1986). Vibrissal Merkel cell development also progresses in a posterior to anterior gradient during embryonic and early postnatal development (Nurse & Farraway, 1988). Within the PMBSF, large posterior vibrissae are represented by larger and more cell dense cortical barrels than their anterior counterparts (Lee & Woolsey, 1975; Welker & Van der Loos, 1986). During whisking, not only do posterior whiskers palpate objects more frequently than anterior whiskers (Carvell & Simons, 1990), but posterior whiskers have distinctly lower fundamental resonance tuning frequencies (60-100Hz) than do anterior (~750Hz) whiskers which may reflect differential tactile encoding properties at anterior and posterior locations across the whisker pad (Neimark, et al., 2003), and across anterior and posterior regions of PMBSF (Andermann, et al., 2004). 2-deoxyglucose uptake levels also appear greater in posterior barrels following whisker

stimulation than uptake levels in anterior barrels (McCasland, et al., 1991; Shin, et al., 2005) which may reflect differences in levels of inhibitory interaction in posteriorly and anteriorly located barrels (McCasland, et al., 1991). While possible differential usage of anterior and posterior whiskers may play some part in establishing the gradient, other factors must also contribute since a similar gradient was observed in P10 pups (Margret, et al., 2005b), and whisking behavior is not reported until approximately P15 (Landers & Philip Zeigler, 2006).

4.3 PAE effect on body weight

In the present study, a main treatment effect was found for body weight in juvenile rats with a significant effect only between Alc and PF rats, while normalization of the data showed that Alc body weight was 13.6% reduced from CF and 18.7% reduced from PF rats. In contrast, no significant differences in body weight were found for any treatment group comparisons in adult rats, suggesting a catch-up in older animals. Other investigators that used a similar dose and administration regiment reported a reduction in body weight at 4 weeks of age (McMechan, et al., 2004), while liquid-diet administration of alcohol during gestation did not result in differences in body weight at 3 weeks (Christie, et al., 2005), 12 weeks (Miller & Potempa, 1990), or 16 weeks (Miller & Dow-Edwards, 1988; Mooney, Napper & West, 1996), or between 21 and 28 weeks (Savage, et al., 2002) of age. On the otherhand, when alcohol (4 g/kg) was administered to pregnant guinea pigs by gavage throughout gestation, no differences in body weight of alcohol and non-alcohol treated offspring were found at 8 weeks of age (Bailey, Brien & Reynolds, 2001; Bailey, Brien & Reynolds, 2004). These results are interesting in light of reported reductions in body weight, height (Streissguth, et al., 1991), and body-mass index (Day, et al., 2002; Klug, et al., 2003) in adolescents. However, as adolescents reach adulthood differences become less apparent and may be somewhat ameliorated by environmental circumstances (Nordstrom-Klee, et al., 2002).

4.4 PAE effect on brain weight

Our data showed significant differences in whole brain and forebrain weights in juvenile rats, but these differences did not persist in adult animals even though the mean brain weights of Alc rats remained lower than the non-alcohol controls. In a similar study where alcohol (5.8 g/kg) was delivered by gavage, a reduction in brain weight was also reported in 6-week-old juvenile rats (Fakoya & Caxton-Martins, 2006). Liquid-diet delivery of alcohol during gestation only reduced brain weight at 12 (Miller & Potempa, 1990) and 16 (Miller, 1987; Mooney, Napper & West, 1996) weeks of age but not after 16 weeks (Allan, et al., 1998; Savage, et al., 2002). These results suggest that the significant differences in brain weight reported in neonates (Maier, et al., 1997; Maier, Miller & West, 1999; Margret, et al., 2005b) disappear in adult rats. One hallmark feature, of children whose mothers consumed alcohol during pregnancy is microcephaly (Clarren, et al., 1978; Ferrer & Galofre, 1987) that persists through adolescence and into adulthood (Sowell, et al., 2001; Sowell, et al., 2002). In the present study, even though the mean brain weights of PAE adults was not significantly different from controls, the mean brain weights of both juvenile and adult rats were always lower than brain weights in non-alcohol controls suggesting that PAE likely influences overall brain weight. Nonetheless, the finding that brain weights between Alc and non-alcohol controls were significantly different for juvenile but not for adult rats is puzzling since housing conditions and food and water availability were equivalent for all treatment groups. Interestingly, body weighs were also not significantly different between adult treatment groups. Normalizations of both body and brain weights in adult Alc rats as a percent reduction from CF rats showed similar reductions for each weight comparison; noteworthy is the fact that the reductions seen in adults were two to three times less than those observed for similar measures in juvenile rats. If adult Alc rats consumed proportionally more chow than control rats then nutrition may play some role in the body and brain catch-up.

In conclusion, the present results suggest that deficits in the size of the vibrissae barrel field in SI cortex previously reported in neonatal pups exposed to alcohol during gestation persist in juvenile and adult rats. In contrast, body and brain weights of adult Alc rats were not significantly different from non-alcohol controls, although averaged body and brain weights of adult Alc rats were always lower than for controls. Our findings are relevant for understanding deficits in information processing reported for children with FASD who often perform poorly on behavioral tasks requiring sensorimotor integration.

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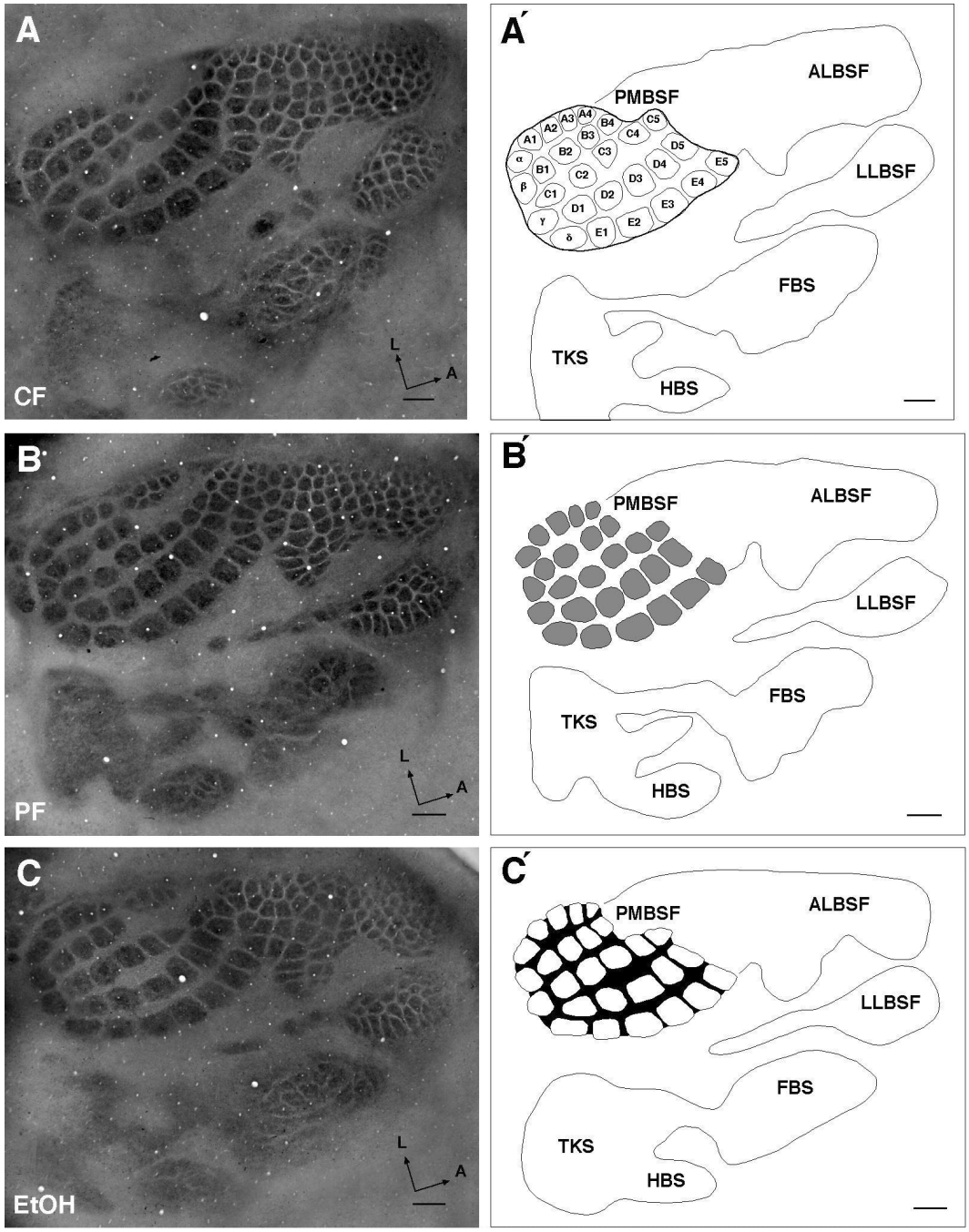

Figure 1.

Barrel field pattern and measures in 6-week-old (juvenile) chow-fed (CF), pair-fed (PF) and alcohol-treated (Alc) rats. **A** Digital image of the barrel field in layer IV in a CF rat. **A′** Barrel field reconstruction delineating the major subfields of the rodent barrel cortex (posterior medial barrel subfield, PMBSF; anterolateral barrel subfield, ALBSF; lower lip barrel subfield, LLBSF; forepaw barrel subfield, FBS; hindpaw barrel subfield, HBS; trunk subfield, TKS). Line surrounding the 27 barrels of PMBSF indicates the measured area defining the total PMBSF barrel area. Nomenclature within PMBSF barrels indicates the orientation of the anterior-posterior running barrel rows (A through E) and the locations of the lateral-medial running arcs (barrels 1 through 5). The most posterior barrels comprising the straddler barrels

lie between barrel rows; these are designated as α, β, γ, δ barrels). **B** Digital image of barrel cortex in PF rat. **B′** The shaded region within the PMBSF shows the area of each barrel and is used to define the total individual barrel area. **C** Digital image of barrel field in Alc rat. **C′** The blackened inter-barrel region within the PMBSF denotes the septal area; measurement of the septal area was made by subtracting total individual barrel area from total PMBSF barrel area. Note that the general barrel field pattern is not noticeably different between the treatment groups. Scale bar indicates 500 μm.

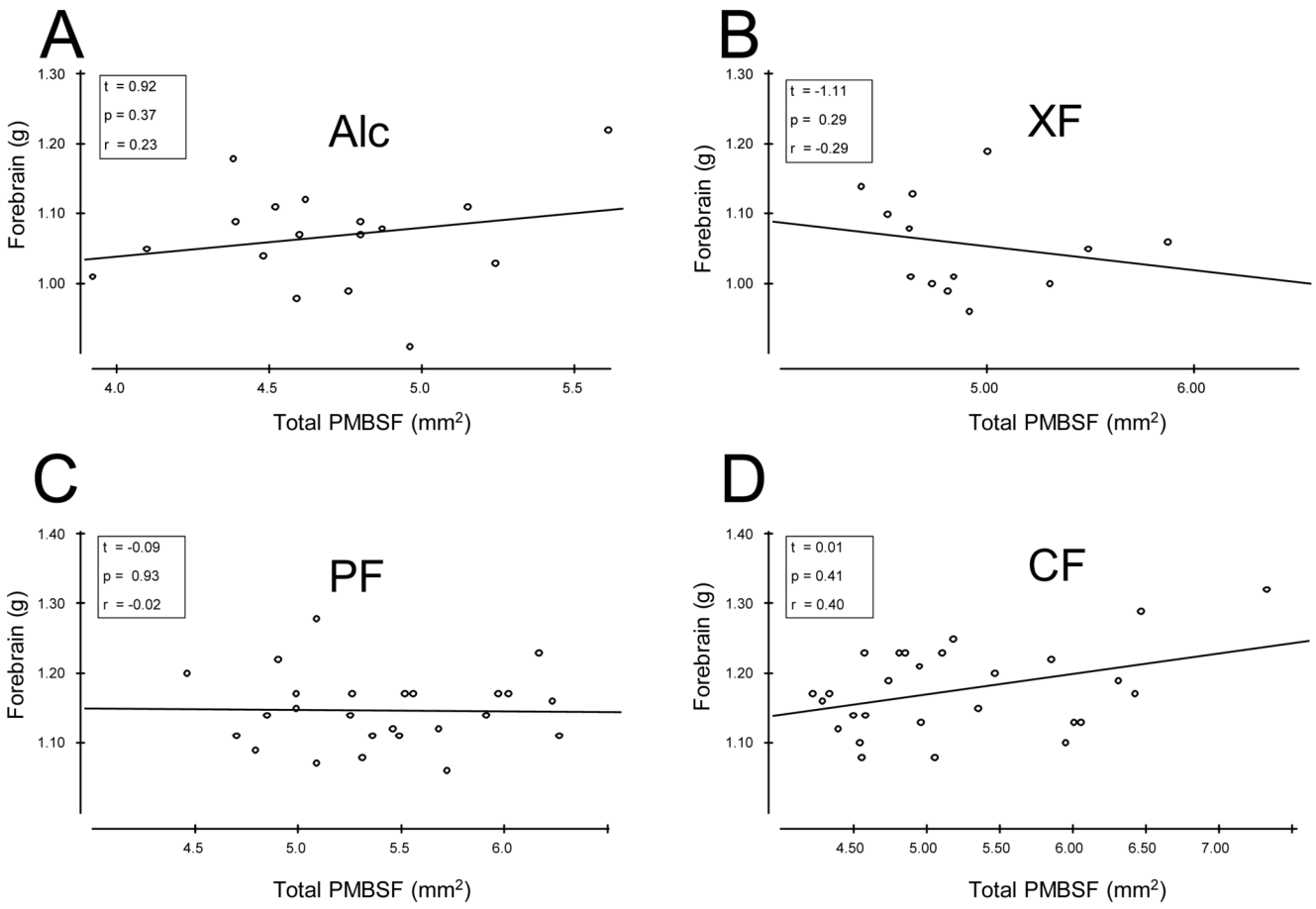

Figure 2.

Pearson Product-Moment Correlation and linear regression analyses [t-ratio] of forebrain weight and total PMBSF area for each treatment group in 6-week-old rats. **A** Forebrain weight and total PMBSF area correlation and regression analyses for Alc treated rats. Inset provides t-ratio, probability (p), and correlation coefficient (r) values. **B**: Measures for XF treated rats, **C**: PF treated rats, and **D**: CF treated rats.

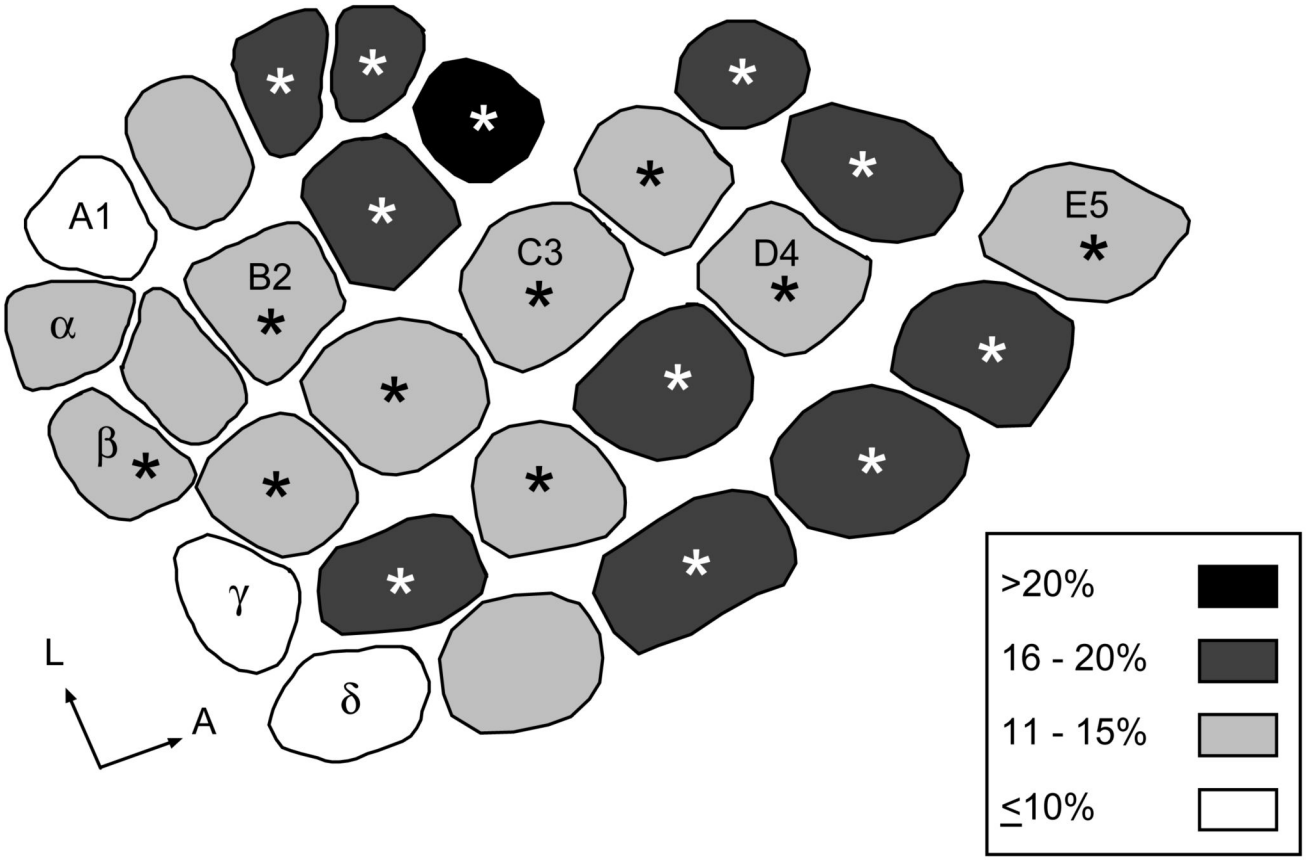

Figure 3.

Comparison of averaged individual barrel area in PMBSF between Alc and CF rats at 6-weeks of age. Shading denotes averaged percent barrel reduction for Alc barrels normalized to CF barrels. Percent reduction was categorized into 4 groups: i) unshaded barrels were ≤10% reduced in Alc rats, ii) light-gray barrels were 11% to 15% reduced in Alc rats, iii) dark-gray barrels were 16% to 20% reduced in Alc rats, iv) blacked-barrels were greater than 20% reduced in Alc rats. Anterior barrels showed a greater reduction in size compared to posterior barrels. Asterisks denote barrels that were significantly reduced in Alc rats compared to CF rats ($P<0.05$).

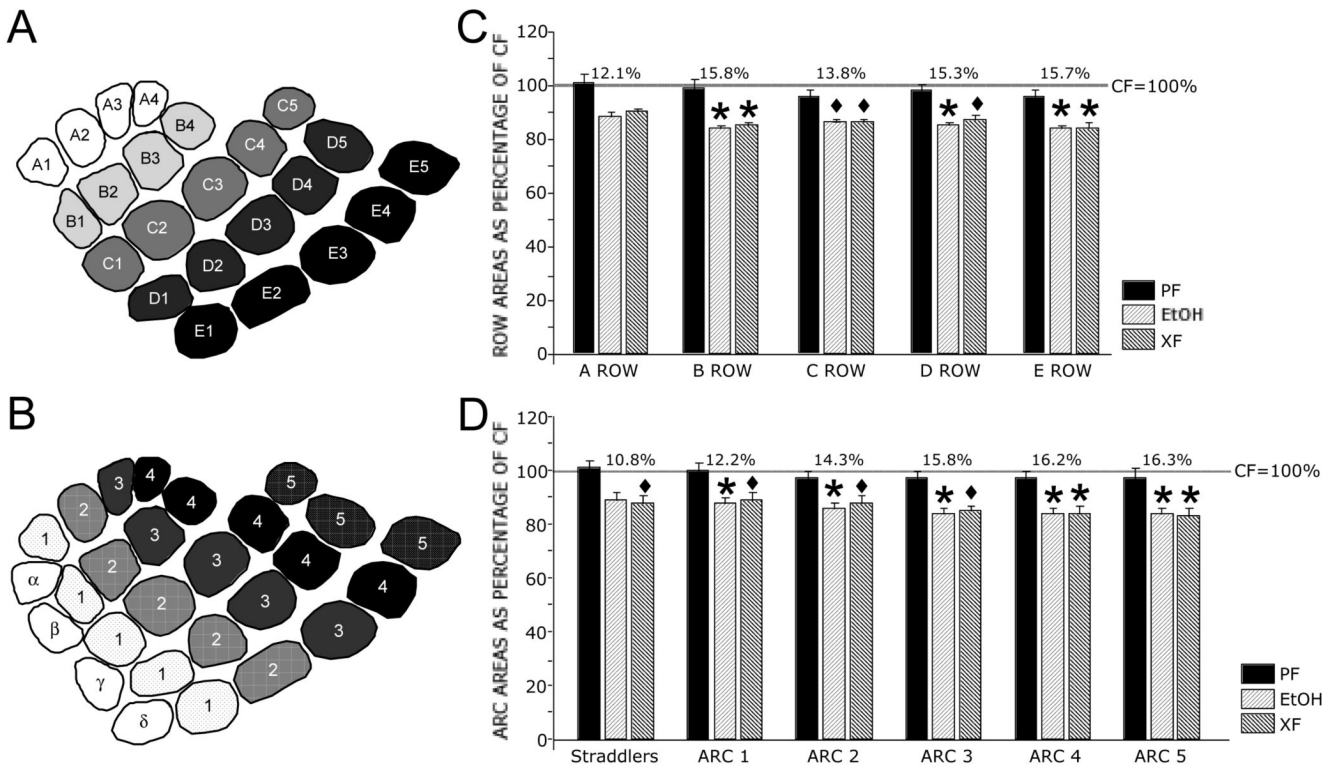

Figure 4.

Averaged PMBSF row and arc areas in Alc, XF, and PF rats compared to CF rats at 6-weeks of age. Schematic diagrams showing barrel rows (**A**) and arcs and straddler barrels (**B**). **C** Graph showing averaged row area for Alc, XF, and PF rats normalized as a percentage change of CF rats. Significant area reductions occurred between Alc and CF rats for rows B–E. No significant row differences occurred between CF and PF or between Alc and XF. Percent reduction in row area between Alc and CF is indicated above the bars for each of the rows. **D** Graph showing averaged arc and straddler areas for Alc, XF, and PF rats normalized as a percentage change of CF rats. The straddler arc showed no significant difference between Alc and CF, while arcs 1–5 were significantly reduced. No significant differences occurred between CF and PF or between Alc and XF arc areas. Percent reduction in arc area between Alc and CF is indicated above the bars of each arc/straddler. Note the gradient moving from posterior to anterior arcs, which is not seen between rows. Gray lines in (**C**) and (**D**) indicate normalized CF values at 100%. Diamonds (◆) indicate significant differences between Alc and CF or between XF and CF. Asterisks (*) indicate significant differences between Alc and both CF and PF groups or between XF and both CF and PF groups.

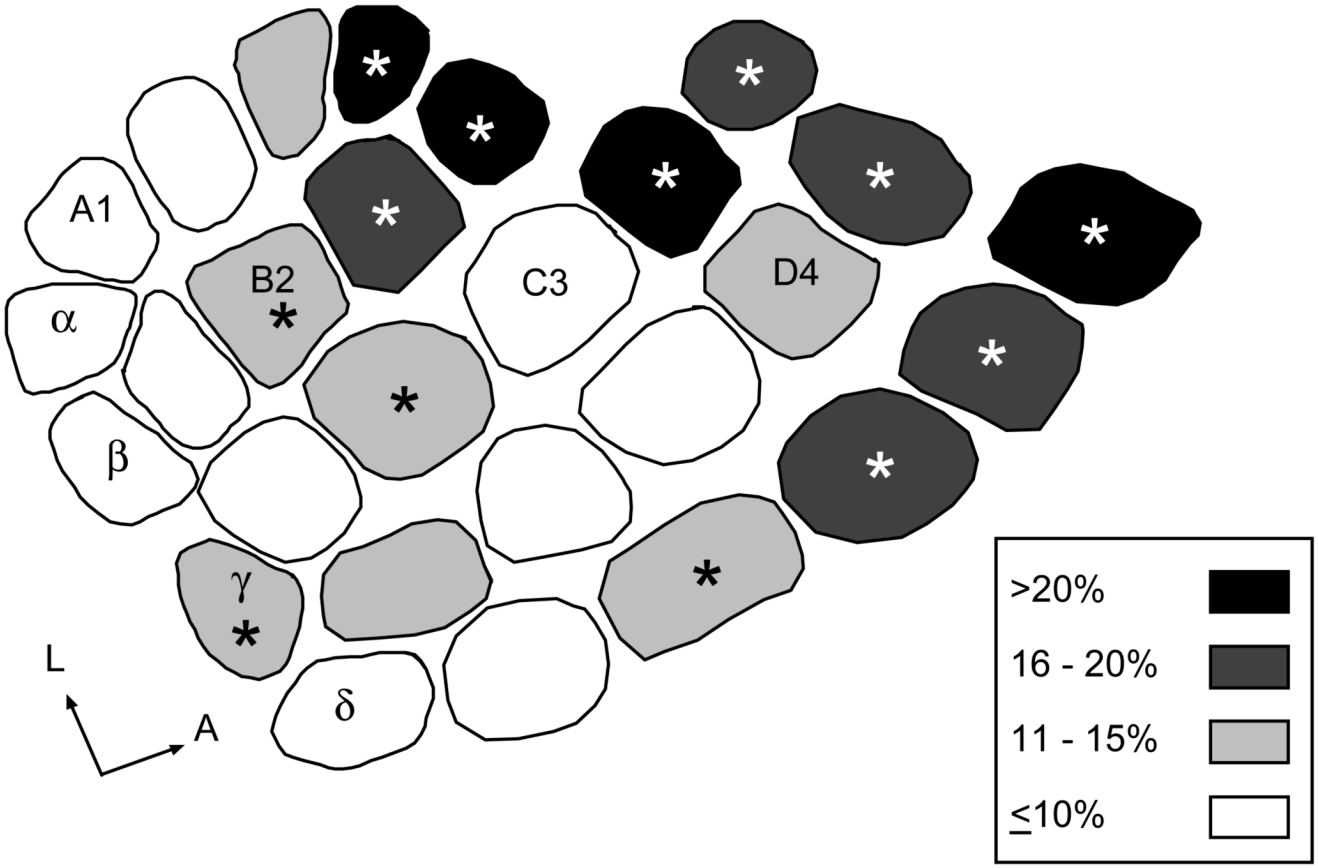

Figure 5.
Comparison of averaged individual barrel area in PMBSF between Alc and CF rats at 7-months of age. Shading denotes averaged percent barrel reduction for Alc barrels normalized to CF barrels. Percent reduction was categorized as in Fig. 3. Anterior barrels showed a greater reduction in size compared to posterior barrels. Asterisks denote barrels that were significantly reduced in Alc rats compared to CF rats ($P<0.05$).

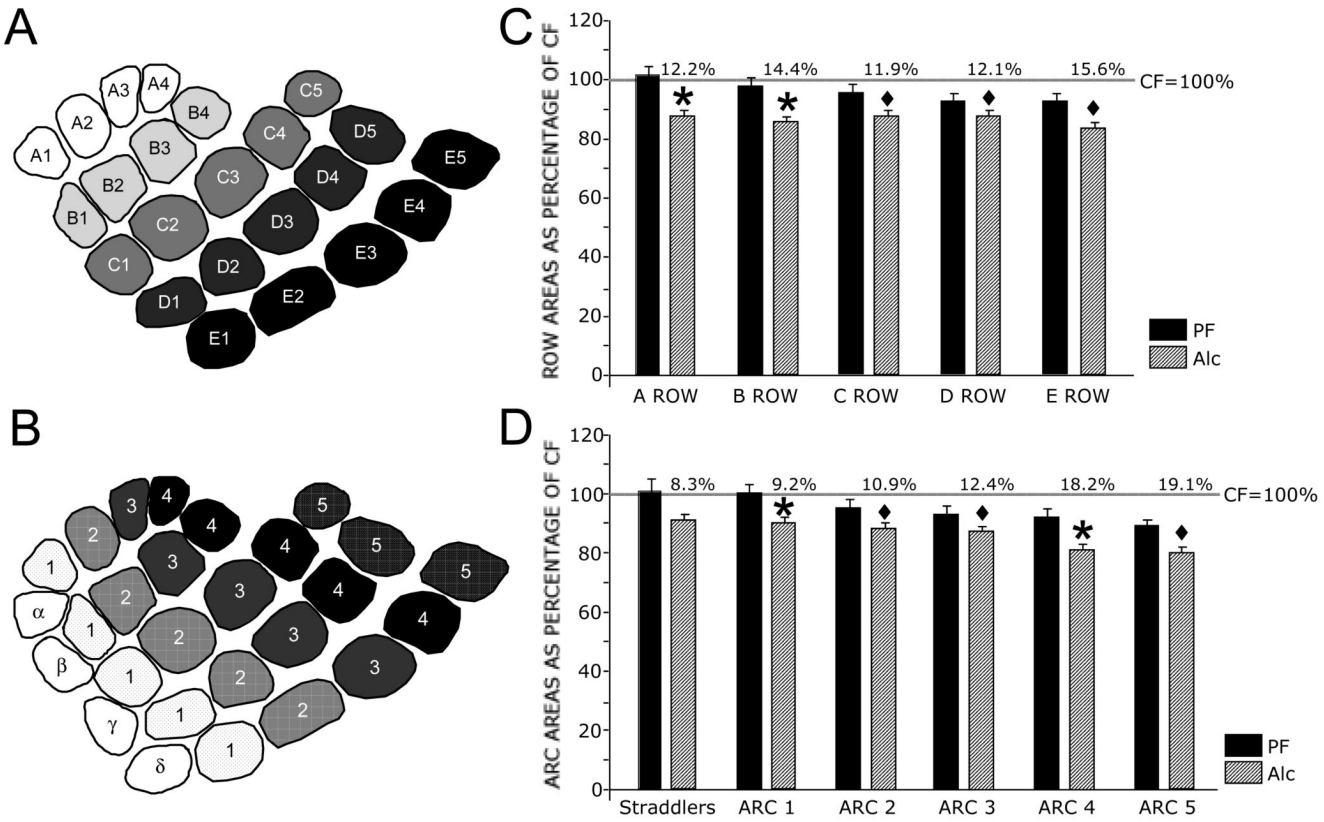

Figure 6.

Averaged PMBSF row and arc areas in Alc and PF rats compared to CF rats at 7-months of age. Schematic diagrams showing barrel rows (**A**) and arcs and straddler barrels (**B**). **C** Graph showing average percent row area for Alc and PF rats normalized as a percentage change of CF rats. Significant reductions in row area were observed between Alc and non-alcohol rats. No significant row differences occurred between CF and PF groups. Percent reduction in row area between Alc and CF is indicated above the bars of each row. **D** Graph showing averaged percent arc and straddler areas for Alc and PF rats normalized as a percentage change of CF rats. The straddler arc showed no significant difference between Alc and CF, while arcs 1–5 were significantly reduced from CF or both CF and PF groups. No significant differences occurred between CF and PF arc areas. Percent reduction in arc area between Alc and CF is indicated above the bars of each arc/straddler. Gray lines in **C** and **D** indicate normalized CF values at 100%. Diamond (◆) indicates significant difference between Alc and CF. Asterisk (*) indicates significant difference between Alc and both non-alcohol groups.

Table 1

The effect of PAE on juvenile body and brain weights (g)

Treatment	Body weight (g)	Whole brain weight (g)	Forebrain weight (g)
Alc (*n =17*)	156.12 (6)[b]	1.48,[ab]	1.07,[ab]
XF (*n =15*)	179.67 (11)	1.47,[cd]	1.06,[cd]
CF (*n =25*)	180.75 (5)	1.61 (0.01)	1.15 (0.01)
PF (*n =27*)	191.86 (7)	1.63 (0.02)	1.18 (0.01)

Data are presented as mean ± S.E.M.

All significant differences (P<0.05)

[a] Alc vs. CF

[b] Alc vs. PF

[c] XF vs. CF

[d] XF vs. PF

Table 2

PAE effect on PMBSF barrels and septal areas (mm^2) and forebrain/PMBSF ratio in juvenile rats

Treatment	Total PMBSF barrel area (mm^2)	Total individual PMBSF barrel area (mm^2)	Total PMBSF septal area (mm^2)	Forebrain/PMBSF ratio
Alc (*n =17*)	4.69,[ab]	3.27,[ab]	1.43 (0.04)	0.23 (0.01)
XF (*n =15*)	4.74,[cd]	3.29,[cd]	1.45 (0.06)	0.23 (0.01)
CF (*n =25*)	5.40 (0.10)	3.81 (0.07)	1.59 (0.04)	0.21 (0.01)
PF (*n =27*)	5.22 (0.16)	3.74 (0.11)	1.48 (0.05)	0.23 (0.01)

Data are presented as mean ± S.E.M.

All significant differences ($P<0.05$)

[a] Alc vs. CF

[b] Alc vs. PF

[c] XF vs. CF

[d] XF vs. PF

Table 3

PAE effect on adult body and brain weights (g)

Treatment	Body weight (g)	Whole brain weight (g)	Forebrain weight (g)
Alc (*n=9*)	383.83 (40)	1.80 (0.05)	1.27 (0.04)
CF (*n=8*)	400.31 (40)	1.89 (0.04)	1.31 (0.03)
PF (*n=8*)	405.94 (37)	1.93 (0.05)	1.35 (0.03)

Data are presented as mean ± S.E.M.

No significant differences between any treatment group comparisons ($P < 0.05$).

Table 4

PAE effect on adult PMBSF and septal areas (mm^2)

Treatment	Total PMBSF barrel area (mm^2)	Total individual PMBSF barrel area (mm^2)	Total PMBSF Septal area (mm^2)
Alc (*n=9*)	4.30,[ab]	2.98,[ab]	1.33 (0.06)
CF (*n=8*)	4.73 (0.12)	3.41 (0.12)	1.32 (0.04)
PF (*n=8*)	4.76 (0.13)	3.28 (0.10)	1.48 (0.06)

Data are presented as mean ± S.E.M.

No significant differences occurred between any treatment groups ($P<0.05$).

[a] Alc vs. CF

[b] Alc vs. PF