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Alcohol exposure in utero is associated with decreased gray matter volume in neonates

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Abstract

Neuroimaging studies have indicated that prenatal alcohol exposure is associated with alterations in the structure of specific brain regions. However, the temporal specificity of such changes and their behavioral consequences are less known. Here we explore the brain structure of infants with in utero exposure to alcohol shortly after birth. T2 structural MRI images were acquired from 28 alcohol-exposed infants and 45 demographically matched healthy controls at 2–4 weeks of age on a 3T Siemens Allegra system as part of large birth cohort study, the Drakenstein Child Health Study (DCHS). Neonatal neurobehavior was assessed at this visit; early developmental outcome assessed on the Bayley Scales of Infant Development III at 6 months of age. Volumes of gray

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Compliance with ethical standards

Conflicts of interest The authors report no conflict of interest with respect to this work.

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matter regions were estimated based on the segmentations of the University of North Carolina neonatal atlas. Significantly decreased total gray matter volume was demonstrated for the alcohol-exposed cohort compared to healthy control infants ($p<0.001$). Subcortical gray matter regions that were significantly different between groups after correcting for overall gray matter volume included left hippocampus, bilateral amygdala and left thalamus ($p<0.01$). These findings persisted even when correcting for infant age, gender, ethnicity and maternal smoking status. Both early neurobehavioral and developmental adverse outcomes at 6 months across multiple domains were significantly associated with regional volumes primarily in the temporal and frontal lobes in infants with prenatal alcohol exposure. Alcohol exposure during the prenatal period has potentially enduring neurobiological consequences for exposed children. These findings suggest the effects of prenatal alcohol exposure on brain growth is present very early in the first year of life, a period during which the most rapid growth and maturation occurs.

Keywords

Alcohol; FASD; MRI; Infant; Neuroimaging; Dubowitz

Introduction

Four decades have passed since the physical and neurodevelopmental effects of prenatal alcohol exposure were first formally documented in the seminal reports of Jones and colleagues (Jones et al. 1973, 1976) Documentation of the adverse effects of alcohol on the structure of the brains of children exposed in the prenatal period has grown exponentially since the introduction of magnetic resonance imaging (MRI) technology in the early 1990's. Smaller whole brain volumes were reported in exposed children in addition to reduced global volumes of both white and gray matter (Donald et al. 2015a; Riley et al. 2011). In subsequent studies, frontal, parietal and temporal alterations in cortical volume and thickness as well as alterations in the volume of deep gray structures have been reported (Lebel et al. 2012; Sowell et al. 2002, 2008; Yang et al. 2012; Zhou et al. 2011). Associations reported between gray matter alterations and functional outcomes (both cognitive and behavioral) (Lebel et al. 2012; Sowell et al. 2008; Treit et al. 2013; Gautam et al. 2015), underscores the functional and clinical consequences of damage to these areas after prenatal alcohol-exposure.

Though the effects of prenatal exposure to alcohol on central nervous system (CNS) development are now well-documented, prior studies leave several questions unanswered. Literature on neurobehavioral and developmental assessments in the very young infant suggests that infants with heavy prenatal alcohol exposure have poor habituation, low levels of arousal (including to acute painful stimuli), abnormal reflexive behavioral clusters and motor abnormalities compared to unexposed peers (Chiriboga 2003; Streissguth et al. 1983; Oberlander et al. 2010; Coles et al. 1987). Behavioral disturbances in the first weeks of life are considered to have poor predictive validity for long-term cognitive outcomes, but a number of groups have described between group differences on formal early developmental assessments and measures of information processing and play complexity (Coles et al. 2000; Jacobson et al. 1993) and these may be better at predicting later outcomes (Molteno et al.

2010). Neuroimaging reports to date, however, have focused largely on ages 5 years through to young adulthood with the majority in late childhood or adolescence (Lebel et al. 2012; Treit et al. 2013). There is a paucity of neuroimaging data in children with prenatally alcohol-exposure during the first years of life, a period during which the most rapid brain growth occurs (Moore et al. 2014; Riley et al. 2011). Nevertheless, a small study reporting changes in white matter microstructure in the association tracts (Donald et al. 2015a) and one reporting alterations in structural connectivity (Taylor et al. 2015) provide emerging evidence that the effects of prenatal alcohol exposure can be identified using quantitative neuroimaging techniques. Questions remain regarding the impact of alcohol doses and timing of exposure. Finally the effects of prenatal alcohol exposure on brain structure, controlling for important contextual factors such as smoking, have not been widely reported.

South Africa is one of the regions in the world where fetal alcohol spectrum disorders (FASD) are highly prevalent (May et al. 2014). We used a South African birth cohort sample to investigate whether the impact of prenatal alcohol exposure on early brain structure may be discernible in neonates and whether associations with neurobehavior and early development are present. We assessed alcohol doses and timing of exposure, as well as controlling for additional exposures such as smoking. Based on the literature in children and adolescents with FASD, we predicted that gray matter volume alterations would occur in neonates exposed to alcohol prenatally even before formal diagnosis of FASD (Donald et al. 2015a, b).

Materials and methods

Study design and participants

This is a nested sub-study that included infants enrolled in a larger population-based birth cohort study, the Drakenstein Child Health Study (DCHS). This DCHS is located in the Drakenstein region of the Western Cape, South Africa, in a low to middle-income community of approximately 200,000 people in which there is limited migration. This area has a well established, free primary health care service. Approximately 90 % of women in this area seek public sector antenatal care and child health services. The birth cohort comprises a community cohort of children from a peri-urban area in South Africa. Although the Western Cape is traditionally a high-risk area for alcohol use disorders, this community was chosen primarily for its relative stability and representativeness of the general population and not for any particular risk profile or vulnerability.

The DCHS has enrolled more than 1000 pregnant women to date, and is following them prospectively through childbirth until children reach 5 years of age (Zar et al. 2015). In this nested sub-study, data from 73 infants were included: 28 infants with significant alcohol exposure and 45 infants with no significant history or biological evidence of substance exposure.

Mothers were recruited at 20–24 weeks gestation, written informed consent obtained, and background data collected for the DCHS (Stein et al. 2015). All questionnaires on the DCHS were administered by an interviewer (study nurse or research assistant trained in the questionnaire and in interviewing technique) because of low levels of literacy in the

population as a whole. The questionnaires were offered in all of the locally spoken languages in the Western Cape (English, Afrikaans and Xhosa) where this study is situated. Interviewers were available at each site who were able to conduct the interview in the chosen language. For the group with alcohol exposure, mothers were identified based on a minimum score of 11 (moderate to high risk of experiencing severe problems as a result of their current pattern of use) on the alcohol questions on the ASSIST questionnaire – a widely validated World Health Organization scale to assess comorbid substance use (Humeniuk et al. 2008; Jackson et al. 2010). Mothers were further required to give a positive history of alcohol use in any of the 3 trimesters of pregnancy at levels consistent with WHO moderate-severe alcohol use (either drinking 2 or more times a week or 2 or more drinks per occasion; Table 1). After birth, infants from mothers identified through this approach were included for study unless the mothers had a positive urine screen at 28–32 weeks gestation, for any other drug abuse (opiates, marijuana, cocaine, methamphetamine, barbiturates) (Lozarno et al. 2007), the infant was <36 weeks or had a low Apgar score (<7 at 5 min) and/or admission for hypoxic ischemic encephalopathy or other significant neonatal complication (such as neonatal jaundice requiring phototherapy). Infants were also excluded if they had an identified genetic syndrome or congenital abnormality.

Ethical approval for human subjects research was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences of University of Cape Town (HREC UCT REF 40½009) for the Drakenstein Child Health Study. This sub-study protocol was independently reviewed and approved by the same institutional ethics committee (HREC UCT REF 525/2012). Informed consent was signed by the mothers on behalf of herself and her infant for participation in this study.

Measures

Two to four week old infants underwent brain magnetic resonance imaging. They were wrapped, fed and then imaged in quiet, natural (unsedated) sleep. Earplugs and mini-muffs were used for double ear protection; a pulse oximeter was used to monitor pulse and oxygenation, and a qualified neonatal nurse or pediatrician was present with the infant in the scanner room for the duration of the imaging session. At the time of scanning, basic anthropometry was acquired including weight, occipito-frontal head circumference and length. The Dubowitz neurologic scale, a well-validated measure of neonatal neuromotor and neurobehavioral status, was used to study early neurological and behavioral state. This tool includes an optimality score allowing it to be used for quantitative analysis of potential associations with neuroimaging findings. The score is based on the distribution of the scores for each item in a population of low-risk term infants. The total optimality score in a symptom complex is the sum of the optimality scores of individual items. A higher optimality score represents a healthier neurology and behavioral state, though the actual score depends on the number of items in the symptom complex. The highest possible score for each symptom group is indicated in Table 2 where the between group results are presented. For this study, specific item clusters were chosen as being of particular interest in this population. As defined by the Dubowitz scale authors, the “behavior” cluster includes items scoring irritability, cry, consolability, alertness, visual and auditory orientation and eye movements. The “abnormal signs” cluster has focus on posture, tremor and startle items

(Noble and Boyd 2012; Dubowitz et al. 1998). Infant developmental function was assessed using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) (Bayley 2006). Either of two trained physiotherapists or a registered nurse administered this assessment, at the age of 6 months. Assessors had background experience in pediatric clinical and research environments and were blinded to the exposure status of the infants. Assessors were trained by a pediatric neurologist (KD) and directly observed pilot administrations took place prior to formal data collection to ensure fidelity to specific instructions for test administration and data scoring. KD performed regular site visits to ensure compliance with test administration and technique, and completed protocols were checked systematically to ensure scoring accuracy.

The BSID III assesses infant development across five scales - cognitive, language, motor, socio-emotional and adaptive behavior (Albers and Grieve 2007; Bayley 2006). The language scale is further subdivided into receptive and expressive communication subtests; the motor scale into gross and fine motor subtests; and the adaptive behavior scale into ten subtests, seven of which are applicable to infants younger than 1 year – communication, health and safety, leisure, self-care, self-direction, social and motor. The cognitive, language and motor assessments are conducted using items administered directly to the infant, while the socio-emotional and adaptive behavior domains were assessed via a questionnaire completed by the primary caregiver (but administered by study staff as per DCHS protocol). This tool has been used widely across low and middle income country settings such as South Africa (Ballot et al. 2012), and is considered a gold-standard measure of development in infants and toddlers. Bayley-III provide four types of norm-referenced scores across the subscales: scaled scores, composite sores, percentile ranks, and growth scores (Bayley 2006). For our purposes, scaled scores were calculated from captured total raw scores on each subtest using the specialised software *Bayley-III Scoring Assistant Update Version 2.0.2 with Bayley-III PDA conduit* (*BayleyIII_PDA_2_0_2.exe)*. Scaled scores represent a child's performance on a subtest relative to his or her same-age peers and are sensitive to subtle differences in developmental outcomes (Bayley 2006). Composite scores are based on the composite equivalents for the scaled scores of the Cognitive and Social-Emotional Scales, and the various sums of subtest scaled scores for the Language, Motor, and Adaptive Behavior Scales. The composite scores have a mean of 100 and standard deviation of 15, and range from 40 to 160. While there is no universally accepted definition of developmental delay (Bayley 2006), criteria based on standard deviations (SDs) below the mean of a reference group is the most widely-used approach. A difference of one standard deviation would be considered clinically significant.

Image acquisition

Magnetic resonance imaging (MRI) was acquired at the Cape Universities Brain Imaging Centre (CUBIC), Tygerberg, Cape Town. Images were acquired on a Siemens Magnetom 3T Allegra MRI system (Erlangen, Germany) with a RF transmit/receive head coil. To overcome limitations with scanning smaller volumes of tissue, voltage was reduced to optimize signal, and the head coil was loaded with a wet clay inlay (40×40 cm with a thickness of 2 cm, standard sculpting clay commercially bought – white stoneware clay with grog).

A 3D T2 image was acquired on the 3T Siemens Allegra in the sagittal direction with the following parameters: FOV = 160×160 mm, TR = 3500 ms, TE = 354 ms, 128 slices, in-plane resolution = 1.3×1.3 mm and a slice thickness of 1.0 mm. The sequence scan time was 5 min 41 s.

Image processing

Images were brain extracted with FSL 5.0 brain extraction tool (BET) (Smith et al. 2006) and exported to SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8) for further processing. Within SPM8, the T2 images were co-registered to a custom infant T2 template in MNI standard space created by a group at the University of North Carolina (UNC) (Shi et al. 2011). In short, the template and gray matter priors were created from 95 infants (56 males, 39 females). The authors have demonstrated that the UNC atlas and custom gray matter priors produced better spatial normalization and labeling of subject segmentations than other comparable infant and adult atlases available at the time of the template creation, as well as better performance compared to manual segmentation (Shi et al. 2011; Tzarouchi et al. 2014; Brown et al. 2014). After co-registration, images were inspected for proper alignment before nonlinear registration to the same infant template. After non-linear registration, images that displayed excessive warping or other problems with registration were discarded. Of the original 85 control and 62 alcohol-exposed infants, 45 controls and 27 cases were included. Due to poor spatial normalization to the infant template, 22 controls and 22 alcohol-exposed infants were removed from the analysis. In addition 17 controls and 13 alcohol-exposed infants were excluded because there were no T2 scans for these participants. T2 images were then segmented into gray and white matter maps by utilizing infant gray matter probabilistic maps or priors designed by UNC (Shi et al. 2011). The T2 images were modulated during the transformation to the gray matter priors. This modulation step ensures that the change in volume due to spatial transformation/warping is accounted for by preserving the volumetric information even if the brain is shrunk or enlarged during the process.

Volumetric information was extracted from the gray matter segmented images for 90 anatomical regions as defined by the automated anatomical labeling atlas (Tzourio-Mazoyer et al. 2002). The volumes were then exported to SPSS 22.0 for further statistical analysis.

Statistical analysis

Group effects and age-by-group effects were investigated for gray matter volumes of the 45 Automated Anatomical labelling regions of interest per hemisphere in SPSS 22.0 by utilizing a general linear model that included age, gender, ethnicity and maternal smoking status as covariates of no interest. Therefore a total of 90 regressions were performed in the context of the multivariate analysis. Main effects were tested between groups, with a follow-up analysis investigating the interaction term between age and group. The gray matter volumes for each region were corrected by dividing each volume by the total gray matter volume (Mathalon et al. 1993). A twotailed p-value of .01 was used at the threshold of statistical significance. To investigate associations of gray matter volume with behavior, partial correlational analyses were performed between the corrected neonatal gray matter volumes and total brain matter volume and Dubowitz optimality scores, as well as between

corrected volumes and total brain matter volume and BSID III scores acquired from the same infants at 6 months of age.

Results

The final sample for analysis included 28 alcohol-exposed and 45 healthy control infants (Table 2). There was no significant difference in length of neonates, gestation, socio-economic status, age, gender or weight of the infants between groups. However alcohol-exposed infants showed a significantly smaller head circumference compared to controls. In addition, maternal smoking status was significantly different between the groups, where the ratio of smokers to non-smokers was higher in the alcohol-exposed group compared to the control group. There was also a significant difference in ethnicity distribution between the two groups with higher proportion of infants of African ancestry in the control group compared to the alcohol-exposed infants where the proportion of infants of mixed ancestry was higher. For the Dubowitz scores, no significant differences were apparent between the two groups. BSID III scores differed significantly on the socio-emotional scale between groups with the infants with prenatal alcohol exposure performing more poorly that their unexposed peers. Distribution of alcohol exposure by trimester in terms of frequency and intensity is presented in Table 1 and highlights that, though the majority of the infants in this study were exposed to alcohol in the 1st trimester of pregnancy, a significant proportion were also exposed during the later trimesters.

Volumetric analyses controlled for age, gender, ethnicity, maternal smoking status and total gray matter volume. Significantly lower total gray matter volume was demonstrated for the alcohol-exposed cohort compared to healthy control infants ($p<0.001$). Prior to correction for total gray matter volume, a huge majority of areas across all regions of the brain were significantly different between infants with prenatal alcohol exposure and unexposed control infants with p values ranging from $p=0.01$ to $p=0.001$ (supplementary Table 2). Regions that displayed lower volume (after correction for overall gray matter volume) at a threshold of $p<0.01$ were the left hippocampus, amygdala (left and right) and left thalamus, (Table 3, Figs. 1 and 2).

Significant associations between early neurobehavioral outcomes and developmental outcomes at 6 months and specific brain regions corrected for total gray matter volume are presented in Tables 4 and 5.

Discussion

To our knowledge this is the first report of the effect of prenatal alcohol exposure on the volume of brain regions in the first weeks of life. Neonates in this study were found to have reduced overall gray matter volume when comparing those with prenatal alcohol exposure to unexposed control neonates. Further analysis revealed lower volumes in the left hippocampus, bilateral amygdala and left thalamus when controlling for total gray matter volume. These findings persisted even when correcting for infant age, gender, ethnicity and maternal smoking status. We also report correlations between early infant neurobehavior and 6-month developmental outcomes and altered gray matter volumes in the neonatal period.

Lower total gray matter volume in children with prenatal exposure to alcohol has been well described in school-age children (Nardelli et al. 2011; Archibald et al. 2001; Rajaprakash et al. 2014). Our finding of reduced total gray matter volume in the first weeks of life suggests that these structural alterations represent the teratogenic effects of prenatal alcohol exposure. The contribution of postnatal environment and stimulation to the findings of reduced gray matter volume described in older children exposed to prenatal alcohol is therefore likely to be mostly secondary.

Alterations in volume of the deep gray structures have been reported in studies of older children with prenatal alcohol exposure. The hippocampus in particular, but also the thalamus, caudate and globus pallidus, has been singled out as a region affected in children with FASD. The hippocampus is a small c-shaped structure is part of the limbic system and plays a key role in consolidation of memory and spatial organization (Duvernoy 2005). These are functions which have been implicated in prior literature documenting the multidimensional cognitive effects of prenatal alcohol exposure in older children (Mattson et al. 2011, 2013). There remains little consistency in the extant literature with respect to whether volume reductions in the hippocampus remain after correction for total gray matter volume. Some authors describe the volume loss over and above total gray matter volumes (Willoughby et al. 2008; Nardelli et al. 2011) and others report volume changes proportional to overall brain size (Archibald et al. 2001). The findings of lower hippocampal volume in this young cohort supports the evidence that not only is the hippocampus an important area differentially affected by alcohol exposure, but that alcohol exposure effects in this region are identifiable very early in life.

The amygdala abuts the anterior portion of hippocampus and also comprises part of the limbic system. It is believed to form the integration hub for networks involved in memory, emotional behavior, and motivation and has structural connections to both the hippocampus and the thalamus (Amunts et al. 2005). The amygdala in isolation has not stood out as a region that demonstrated volume differences between children with prenatal alcohol exposure and unexposed controls, though both groups that have specifically focused on this area described absolute volume differences (Nardelli et al. 2011; Archibald et al. 2001). Findings in our cohort reflected robust bilateral amygdala volume reduction in alcohol exposed infants when compared to unexposed infants. However, the limbic structures are known to be highly dynamic in their development for longer periods than many other gray matter regions. The presence of this finding in early infancy suggests that it may be an important region in prenatal alcohol exposure effects. However, future studies investigating the longitudinal trajectory of development in this region may be key in determining the long-term significance.

The thalamus is a bilateral are midline structure lying superior to the midbrain. Its functions are multiple and it operates as a relay center for sensory and motor signals to the cerebral cortex, as well as playing a key role in the regulation of consciousness, sleep and alertness (Steriade and Llinás 1988). Few studies have described the neurobehavioral status of very young infants with prenatal exposure to alcohol and these have reported poor habituation, low levels of arousal and motor abnormalities compared to unexposed peers (Chiriboga 2003; Streissguth et al. 1983; Testa et al. 2003). Previous neuroimaging literature has also

cited the thalamus as being affected by prenatal alcohol exposure (Nardelli et al. 2011; Meintjes et al. 2014). The finding of reduced left thalamus volume in this cohort of prenatal alcohol exposed neonates, along with poorer performance on socio-emotional metrics of development at 6 months of age is therefore congruent with existing understanding of the effects of prenatal alcohol exposure on the function of the developing brain. However, as is true for all the above structures, the deep gray structures are regions with complex connections and functions. Data investigating the developmental trajectory of these regions over time and which employ techniques which address structural and functional connectivity may provide more convincing conclusions on the roles of specific regions in the effects of prenatal alcohol exposure.

In this analysis in addition to controlling for key factors such as age, sex and maternal smoking, we additionally controlled for overall gray matter volume. The rationale for this was for us to be able to highlight the areas that are proportionally the most affected in our cohort with prenatal alcohol exposure compared to unexposed controls. Nevertheless, absolute smaller volume of individual regions and structures may still be important for these children in functional terms and should still be viewed as important.

Neonatal neurobehavioral measures shortly after birth as well as domains in both developmental and adaptive behavioral outcomes at 6 months of age on the BSID III were associated with altered volumes in particularly temporal and frontal regions in the prenatal alcohol exposed infants. These exploratory associations between gray matter volumes, and motor, language and cognitive outcomes, indicate the global nature of the effects of prenatal alcohol exposure on the developing brain. Total gray matter volume at neonatal age was associated with language development at 6 months in the control infants, but not in those with prenatal alcohol exposure. The reported direction of this correlation was a negative one (as were most of the other correlations in the control group). Previous literature has cited thinner cortices associated with better neurocognitive function in typically developing children at school age (Sowell et al. 2004). However, thicker or thinner cortices at different developmental stages in brain development carry different significance (Lebel et al. 2012). Very little is known about the optimal trajectory of cortical volumes during the first weeks of life and so the significance of this finding in this group remains uncertain. The behavioral and developmental subscales where brain behavior correlations were identified for both groups and which can be discussed alongside one another were the Dubowitz reflex subscale and the composite motor score on the BSID III. Both of these functional outcomes reflect elements of the infants' neuromotor development. In the unexposed infants there was a correlation with the supplementary motor cortex, which is coherent with understanding of the function of this region. The inferior temporal gyrus, which was also negatively correlated with both BSID III motor scores as well as scores on the Dubowitz reflex subscale, is primarily reported to play a central role in visual object recognition (faces, patterns and objects) as well as in more general involvement in visual perception and spatial processing and receives processed visual information from the occipital visual cortex (Creem and Proffitt 2001; Kolb and Whishaw 2014). These findings are likely to reflect a typical developmental profile. The infants with prenatal alcohol exposure, however, demonstrated positive correlations with regions in the frontal lobe as well as in the posterior cingulate gyrus, areas not typically associated with motor development.

However, very little has been published on brain behavior relationships in prenatal alcohol exposure in the first weeks and months of life and so interpretation of these findings remains difficult. The interpretation of both the direction of the correlations as well as the expectation that a particular cognitive score will correlate with a defined anatomical region, especially in the largely undifferentiated neonatal brain should at best be considered exploratory. In addition, it should be noted, that in this cohort, both infants with prenatal alcohol exposure as well as the control group performed in the average range on the BSID III.

Our study has a number of limitations. First, our findings were based on a relatively small sample size. Although the sample is adequately powered for the primary outcome analysis, secondary analyses with breakdown of prenatal alcohol exposure severity and timing with respect to gray matter volume outcomes was not possible. Second, an appreciable number of scans needed to be discarded largely due to movement artifact. While the infants whose scans were included and excluded did not differ significantly on background variables, the loss of this large amount of data may have impacted our results. Third, only qualitative methods to evaluate rater reliability on developmental assessments were used. Although assessors were blinded and assessed infants across both exposure and control groups, inter-rater differences may have impacted on these results and potentially masked additional between group findings. Although our study employed a cross-sectional design where infants were matched for age, gender, and maternal smoking during pregnancy, longitudinal study data on this group is going to be key in establishing the trajectory of developing brains exposed to alcohol during prenatal life and relationship with facial characteristics of FAS.

Despite these limitations, this is a very well characterized group of infants recruited as part of a population-based prospective study design, and the selection of the infant age-group is an additional strength. For this study, 2 to 4 weeks old infants were chosen for imaging because cerebral changes are particularly intense during the last weeks of gestation and the first postnatal months; neuroanatomical abnormalities may be present and identified at this early age that may lead to motor and cognitive dysfunction in the later years. Secondly, imaging during the early postnatal period may more accurately reflect the effects of prenatal alcohol exposure on brain structure before postnatal risk factors known to be highly prevalent in our cohort can compromise brain development.

In summary, this paper addresses a critical gap in the literature. Given progressive as well as regressive developmental processes of gray and white matter, including myelination, synaptogenesis, pruning and synaptic modification (Tau and Peterson 2010), it is important to understand the early impact of prenatal exposure. The findings here are consistent with work in older children, and correlation with neurodevelopmental measures suggests high functional and clinical relevance. Here we have shown that the effects of prenatal alcohol exposure manifest in structural brain changes very early in life and that changes in brain structure in the neonatal period correlate with poorer neurobehavioral and neurodevelopmental outcomes at 6 months of age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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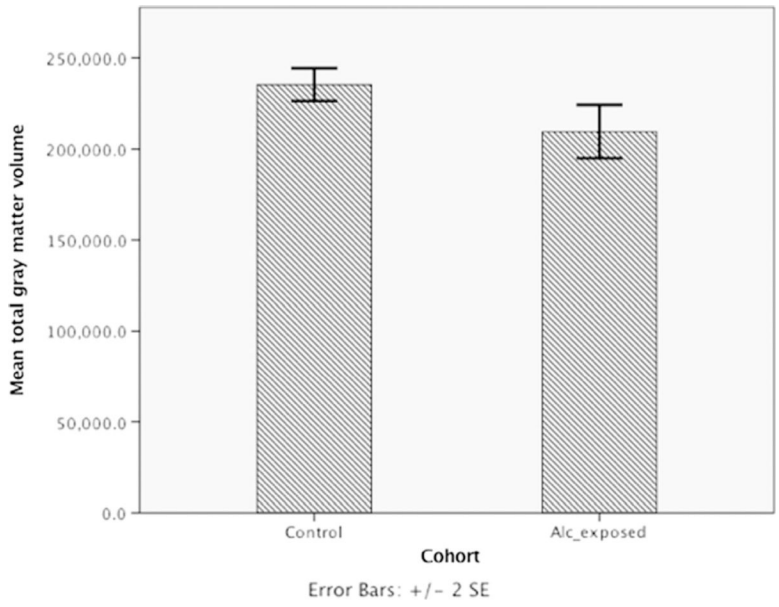

Fig.1.

Mean volume images showing decreased total gray matter volume in alcohol-exposed infants compared to unexposed controls

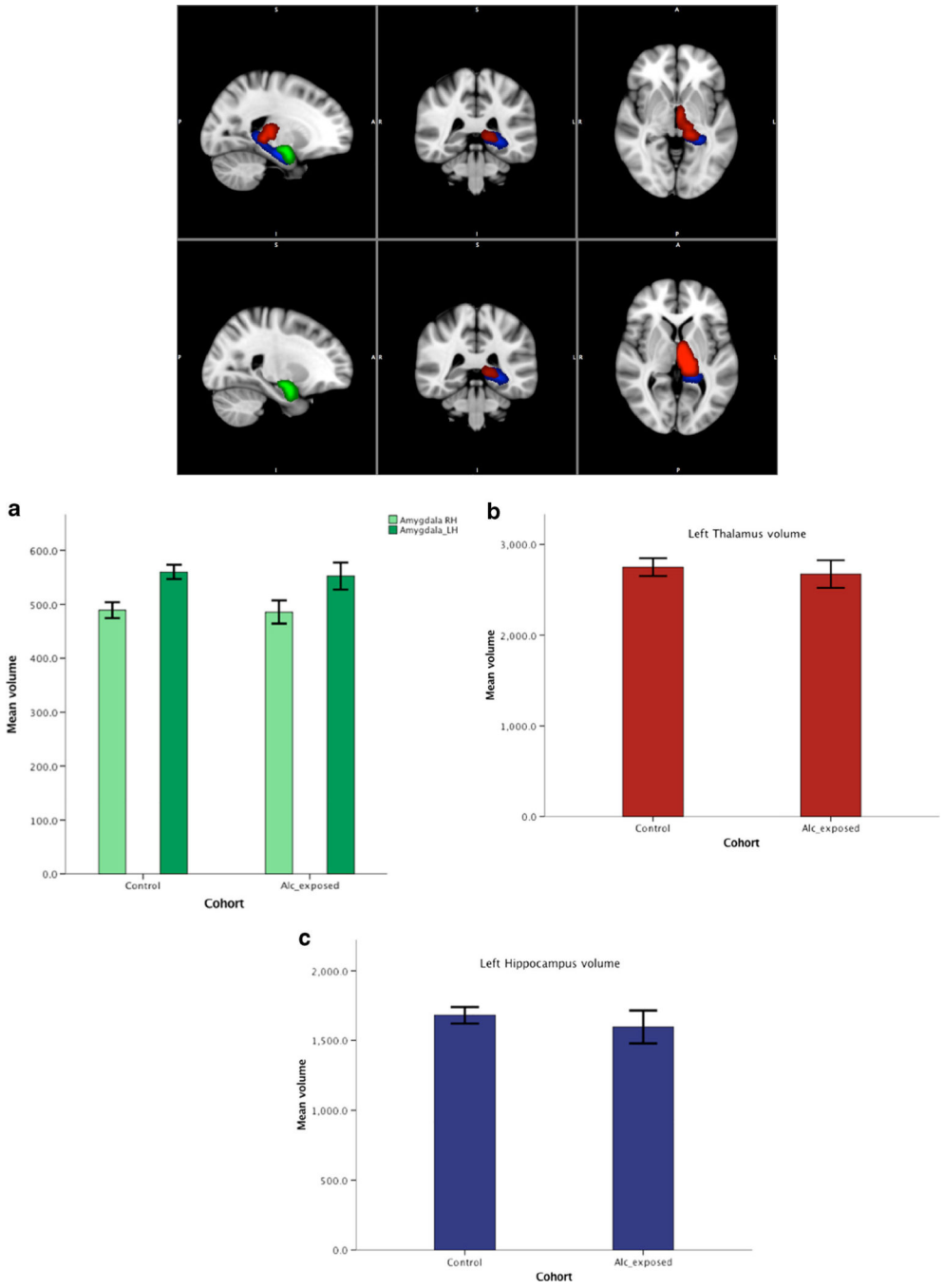

Fig. 2.

Mean volume images showing regions of decreased gray matter▶volume in alcohol-exposed infants compared to unexposed controls corrected for total gray matter volume. Areas showing decreases in (**a**) amygdala volumes (bilateral) are presented in *green* and (**b**) left thalamus volume presented in red (p<0.01) and (**c**) left hippocampus volumes presented in *blue*. Results are superimposed onto a representative atlas image in mid-sagittal, coronal and axial slices (from *left* to *right*). *Bar graph* representation of volume differences in bilateral

amygdala, left thalamus and left hippocampus between controls and in-utero alcohol-exposed infants shown in *red*, *green* and *blue* respectively. Mean volume is shown in mm^3

Table 1

Frequency and quantity of maternal alcohol use by trimester

	Trimester 1	Trimester 2	Trimester 3
Alcohol usage (n, %)	22, 78.6 %	11, 39.3 %	7, 25 %
Once per week or less	15	6	3
2 to 3 times per week	7	5	4
Number of drinks per occasion			
<2	2	0	0
2 to 3	20	11	7

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Table 2

Demographic, anthropometric, Dubowitz and BSID III data of infants

	Alcohol-exposed (*n*=28)	**Controls (*n*=45)**	**Statistics**
Gestation (weeks, SD)	38.75 (1.78)	38.80 (1.83)	t=0.12, p=0.91
Gestation status (36–37 weeks/ >37 weeks)	5/23	5/40	χ^2=0.664, p=0.415
Ethnicity (mixed race/black)	21/7	23/22	χ^2=4.11, p=0.04*
Socio-economic status (low-med/med-high)	14/14	14/31	χ^2=2.61, p=0.11
Maternal smoking (yes/no)	12/16	8/37	χ^2=5.46, p=0.02*
Age (days, SD)	20.54(5.98)	22.24(6.11)	t=1.18, p=0.24
Gender (male/female)	11/17	28/17	χ^2=3.65, p=0.06
Weight (kg, SD)	3.80(0.68)	4.09(0.66)	t=1.76, p=0.08
Head circumference (cm, SD)	35.55(1.37)	36.71(1.62)	t=3.25, p=0.002*
Length (cm, SD)	51.27(5.03)	50.92(3.97)	t=−0.31, p=0.76
Dubowitz optimality scores (mean,(SD))			
Tone (/10)	7.93(2.11)	8.44(1.37)	t=1.25, p=0.22
Reflex (/6)	4.69(0.56)	4.77(0.56)	t=0.60, p=0.55
Spontaneous movement (/2)	1.93(0.30)	1.95(0.18)	t=0.42, p=0.68
Behavior (/7)	4.54(1.32)	4.22(1.26)	t=−1.02, p=0.31
Abnormal signs (/3)	2.86(0.36)	2.60(0.93)	t=−1.37, p=0.18
BSID-III scores (6 months)			
CS general adaptive behavior	103.81 (8.82)	106.26 (8.34)	t=0.87, p=0.39
CS socio-emotional	101.25 (13.15)	113.70 (17.00)	t=2.56, p=0.015*
CS motor	108.19 (13.18)	111.48 (14.45)	t=0.74, p=0.47
CS language	104.13 (15.24)	101.70 (14.49)	t=−0.50, p=0.62
CS cognitive	97.50 (8.76)	101.09 (11.48)	t=1.11, p=0.276

*p<0.05

Table 3

Differences in gray matter volumes between alcohol-exposed and unexposed control infants corrected for age, gender, ethnicity, maternal smoking status and total gray matter volume

AAL region	Hemisphere	Mean gray matter volume (mm^3)		F-value	p-value	Effect size (Cohen's D)
		Controls (SD)	Alcohol-exposed (SD)			
Controls > alcohol-exposed						
Total gray matter volume		235,237.0 (30,179.4)	209,561.0 (38,659.4)	2.300	0.006	0.740
Hippocampus	Left	1681.4(200.9)	1596.5 (312.1)	2.901	0.004	0.323
Amygdala	Left	559.7 (44.6)	552.0 (65.9)	2.988	0.003	0.137
	Right	489.219 (49.8)	485.8 (57.3)	3.277	0.003	0.064
Thalamus	Left	2748.8 (332.4)	2673.1 (397.9)	2.718	0.002	0.206

AAL Automated anatomic labeling

Table 4

Significant associations between BSID-III and Dubowitz optimality scores and corrected gray matter volumes in control infants (n=22)

AAL region	Hemisphere	R-value	p-value
BSID III CS socio emotional			
Inferior frontal gyrus	Left	−0.703	0.001
Rolandic operculum	Left	−0.598	0.009
Medial superior frontal gyrus	Left	−0.613	0.007
Dorsal superior frontal gyrus	Left	−0.712	0.001
Dorsal superior frontal gyrus	Right	0.710	0.001
Fusiform gyrus	Right	0.680	0.002
Supramarginal gyrus	Left	−0.631	0.005
Supramarginal gyrus	Right	0.614	0.007
Middle frontal gyrus	Left	−0.625	0.006
BSID III CS motor			
Inferior temporal gyrus	Left	−0.603	0.008
BSID III CS language			
Total gray matter volume		−0.714	0.001
Dubowitz spontaneous movement			
Inferior frontal gyrus triangularis	Left	−0.665	0.003
Inferior orbitofrontal cortex	Right	0.647	0.004
Lingual gyrus	Right	0.655	0.003
Posterior cingulate gyrus	Left	−0.702	0.001
Inferior parietal lobule	Left	−0.666	0.003
Supramarginal gyrus	Left	−0.649	0.004
Middle frontal gyrus	Left	−0.711	0.001
Middle frontal gyrus	Right	0.639	0.004
Dubowitz reflex			
Supplementary motor area	Left	−0.637	0.004
Supplementary motor area	Right	0.629	0.005
Inferior temporal gyrus	Left	−0.614	0.007

Results shown at p<0.01. The model included, maternal smoking status, age, sex and ethnicity as covariates of no interest. Values were corrected for total gray matter volume

AAL Automated anatomic labeling, *CS* Composite score

Table 5

Significant associations between BSID-III and Dubowitz optimality scores and corrected gray matter volumes in alcohol-exposed neonates (n=27)

AAL region	Hemisphere	R-value	P-value
BSID III CS general adaptive behavior			
Medial orbitofrontal gyrus	Right	0.849	0.001
Middle frontal gyrus	Right	0.811	0.002
Middle temporal pole	Left	−0.790	0.004
BSID III CS Motor			
Middle orbitofrontal cortex	Right	0.756	0.007
Superior orbitofrontal cortex	Right	0.753	0.008
Dubowitz behavior			
Angular gyrus	Right	0.862	0.001
Dubowitz reflex			
Posterior cingulate gyrus	Left	0.800	0.003
Dubowitz tone			
Inferior frontal gyrus triangularis	Left	0.817	0.002
Rolandic operculum	Left	0.760	0.007
Hippocampus	Right	−0.786	0.004
Parahippocampal gyrus	Right	−0.856	0.001
Lingual gyrus	Right	−0.739	0.009
Superior temporal gyrus	Left	0.932	<0.0001

Results shown at p<0.01. The model included, maternal smoking status, age, sex and ethnicity as covariates of no interest. Values were corrected for total gray matter volume

AAL Automated anatomic labeling, *CS* Composite score