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Review Article

Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review

Donald KA, Eastman E, Howells FM, Adnams C, Riley EP, Woods RP, Narr KL, Stein DJ. Neuroimaging effects of prenatal alcohol-exposure on the developing human brain: a magnetic resonance imaging review.

Objective: This paper reviews the magnetic resonance imaging (MRI) literature on the effects of prenatal alcohol exposure on the developing human brain.
Method: A literature search was conducted through the following databases: PubMed, PsycINFO and Google Scholar. Combinations of the following search terms and keywords were used to identify relevant studies: 'alcohol', 'fetal alcohol spectrum disorders', 'fetal alcohol syndrome', 'FAS', 'FASD', 'MRI', 'DTI', 'MRS', 'neuroimaging', 'children' and 'infants'.
Results: A total of 64 relevant articles were identified across all modalities. Overall, studies reported smaller total brain volume as well as smaller volume of both the white and grey matter in specific cortical regions. The most consistently reported structural MRI findings were alterations in the shape and volume of the corpus callosum, as well as smaller volume in the basal ganglia and hippocampi. The most consistent finding from diffusion tensor imaging studies was lower fractional anisotropy in the corpus callosum. Proton magnetic resonance spectroscopy studies are few to date, but showed altered neurometabolic profiles in the frontal and parietal cortex, thalamus and dentate nuclei. Resting-state functional MRI studies reported reduced functional connectivity between cortical and deep grey matter structures.
Discussion: There is a critical gap in the literature of MRI studies in alcohol-exposed children under 5 years of age across all MRI modalities. The dynamic nature of brain maturation and appreciation of the effects of alcohol exposure on the developing trajectory of the structural and functional network argue for the prioritisation of studies that include a longitudinal approach to understanding this spectrum of effects and potential therapeutic time points.

Kirsten Ann Donald[1], Emma Eastman[1], Fleur Margaret Howells[2], Colleen Adnams[2], Edward Patrick Riley[3], Roger Paul Woods[4], Katherine Louise Narr[4], Dan Joseph Stein[2]

[1]Division of Developmental Paediatrics, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa; [2]Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa; [3]Department of Psychology, San Diego State University, San Diego, CA, USA; and [4]Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

Keywords: foetal alcohol spectrum disorders (FASD); foetal alcohol syndrome (FAS); magnetic resonance imaging (MRI); neuroimaging

Kirsten Ann Donald, Division of Developmental Paeditrics, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa.
Tel: +27 21 658 5535
Fax: +27 21 658 5530
E-mail: kirsty.donald@uct.ac.za

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Summations

- Prenatal alcohol exposure results in smaller total brain volume, specific grey and white matter and cortical regions as well as abnormalities in neurochemistry and functional connectivity.
- There is a gap in the literature of neuroimaging studies in alcohol-exposed children under 5 years of age across all the modalities of neuroimaging.
- The dynamic nature of brain maturation calls for the prioritisation of studies that include a longitudinal approach to understanding the effects of alcohol exposure on the developing human brain.

Considerations

- Differing methodologies make inter-study comparisons difficult.
- Reporting of structural or microstructural alterations in single regions of interest (ROI) has resulted in the effects on specific regions being very well-documented at the expense of a more exploratory approach.
- Controlling for polysubstance abuse, environmental factors and age is required to assess consistency of results and inter-study comparisons.

Introduction

The term foetal alcohol syndrome (FAS) was only formalised in the 1970s when Jones and Smith described abnormalities and disabilities in children born to mothers with alcoholism (1,2). The World Health Organisation's (3) global status report on alcohol and health states that harmful alcohol usage ranks in the top five contributors to disease, disability and mortality. FAS is further recognised as one of the major disease and disability categories within alcohol-related disorders (4–6). Studies indicate that the global prevalence of FAS ranges between two and seven per 1000 births, and foetal alcohol spectrum disorders (FASD) between 20 and 50 per 1000 births (7–9), with the highest rates in South Africa (10). However, May et al. (11–14) argue that the current prevalence estimates of FASD represent an underestimate, and FASD represents a much larger public health issue than what has been previously recognised, especially in emerging economies such as South Africa.

FASD animal studies using MRI modalities demonstrate a spectrum of changes in the developing brain structure (15). Findings in rodent and mouse FASD models include reports of enlarged ventricles (16), diffuse reductions in brain volumes, including whole brain volume (17–19), cortical grey matter volume (19), caudate-putamen volume (16), hippocampal volume (20) and cerebellar volume (20), whereas the pituitary and septal regions of the brain showed increased volumes (20). Lipinski et al. (21) have described the correlation between dysmorphology and brain abnormalities in mice models with prenatal alcohol exposure at different stages of gestation. A single rat FASD diffusion tensor imaging (DTI) study showed increased fractional anisotropy (FA) in cortical tissue (22). In addition, a single spectroscopy FASD rat study has shown that, postnatal day 4–9, neonatal alcohol exposure results in reduced *N*-acetyl aspartate (NAA) and taurine in the striatum and cerebellum and increased myoinositol in the cerebellum (23).

Early investigations into the effects of alcohol on the structure of the human brain have reported on autopsies of children who had died in infancy, although likely to have been on the extreme severe end of the spectrum of alcohol-exposure effects. Findings included microcephaly (most consistently), but also agenesis of the corpus callosum, ventriculomegaly, a small cerebellum as well as a few further malformations due to neuronal and glial migration abnormalities (24,25). Advances in neuroimaging methods over the last decade have allowed researchers to study structural, metabolic and functional abnormalities, resulting from prenatal exposure to drugs of abuse more closely.

Aims of the study

The aim of this paper was to review the current magnetic resonance imaging (MRI) literature on the effects of prenatal alcohol exposure in children.

Methods

Search methods for identification of studies

An initial electronic search was conducted to identify studies. A search was conducted through the following databases: PubMed, PsycINFO and Google Scholar. Combinations of the following search terms and keywords were used to identify relevant studies: 'alcohol', 'fetal alcohol spectrum disorders', 'fetal alcohol syndrome', 'FAS', 'FASD', 'MRI', 'DTI', 'MRS', 'neuroimaging', 'children' and 'infants'. No starting date limits were enforced to restrict the search; however, the search extended up to 15 August 2014 and papers were restricted to those that included human subjects <18 years of age as a proportion of their cohort and that were published in English language journals. Abstracts were manually examined in order to confirm relevance. Further studies were identified by searching the reference lists of the studies identified in the initial database search. This was carried out to ensure that studies that were missed in the initial search were identified. A total of 64 relevant articles were identified and included in this review.

A qualitative approach was taken for this review instead of a quantitative comparison such as meta-analysis. Reasons for this decision included the

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following: data required to compute effect size were not always available; the methodological detail to define ROI in different studies varied considerably, preventing clear comparisons; there were important differences in secondary variables across different studies (in particular age, gender, extent and timing of alcohol exposure and polysubstance exposure); and, finally, different analytic methods were used to report the imaging findings (e.g. voxel-based vs. ROI-based) across studies. As a result, a qualitative approach seemed more appropriate for this particular review, which covers over two decades of neuroimaging studies on the effects of prenatal alcohol exposure on the brain.

Results

Structural magnetic resonance imaging (sMRI) in prenatally alcohol-exposed children

Since the early 1990s, MRI technology has been used to report quantitative effects on the brains of children exposed to alcohol in the antenatal period. Structural neuroimaging studies of prenatal alcohol exposure have been most widely reported and 32 relevant studies were identified for this review and are listed alphabetically in Table 1.

DTI in prenatally alcohol-exposed children

White matter in the brain provides the connections that comprise the brain's structural neural networks. Its integrity is essential for the effective functioning of a wide spectrum of complex cognitive processes. In particular, white matter integrity has been demonstrated to play a critical role in normal executive function, attention and processing speed (59–61).

White matter microstructure can be measured *in vivo* with DTI, as well as other diffusion MRI approaches, and can estimate the overall directional diffusion of water molecules along fibre pathways (62,63). Analysis of this data allows the degree of structural and organisation of areas within the brain tissue to be determined. Traditional scalar metrics derived from DTI data include FA, which quantifies the overall directionality of diffusion, and may represent variations in axon integrity and/or packing density. Mean diffusivity (MD) provides a measure of the average diffusivity and may primarily reflect myelin breakdown, decreased cellular density or increased extra- or intra-cellular volumes, although these relationships are less clearly defined in the developing brain. High FA and low MD values are typically associated with healthier neural microstructure in adults, whereas low FA and high MD values may indicate white matter pathology. However, it is also relevant to note that during brain maturation in healthy children and adolescents, axonal pruning and other biological processes may also lead to reduced FA (64–66).

Studies reporting the effects of prenatal alcohol exposure on the white matter microstructure in children extend back 10 years. Seven studies have been reported using this modality and are detailed in Table 2.

Proton magnetic resonance spectroscopy (^{1}H-MRS) in prenatally alcohol-exposed children

Both basic and clinical studies have begun to implicate a number of neurometabolic processes that may underlie the association between maternal alcohol abuse and subsequent negative outcomes in offspring (72,73). ^{1}H-MRS is a non-invasive magnetic resonance technique that measures the concentration of several brain metabolites. Levels of individual brain metabolites may suggest abnormalities in the neuronal microstructure and/or neurometabolism. The most commonly reported metabolites include NAA, which is an indicator of neuronal integrity and or viability, choline metabolites (Cho), an indicator of neuronal-membrane turnover and myelination, creatine metabolites (Cr), energy metabolites and glutamate with its precursor glutamine (Glx), the brain's major neuroexcitatory neurotransmitter (74,75). Published ^{1}H-MRS studies include only four studies that were performed on children and adolescents, these data are presented in Table 3.

Functional MRI (fMRI) in prenatally alcohol-exposed children

Increasingly, over the recent years, investigators have sought to document correlations between the functional deficits reported in children with FASD and the underlying neurobiology. Animal work (79,80) and human imaging studies in school-age children have demonstrated that *in utero* exposure to alcohol alters brain morphology and reduces white matter microstructural integrity (15,26,36,37,59,67,81–85). However, there are few data on functional connectivity in these children. Functional connectivity in the imaging literature has been defined as dependencies among observed neurophysiological responses or 'temporal correlation between spatially remote neurophysical events' (86). In this case, intrinsic brain activity is measured in the absence of an experimental task or 'at rest'. The ability of the brain to co-ordinate areas of activity in specific functional networks follows a developmental trajectory, reflected in the increased functional network connectivity with age in childhood and early adulthood (87). Two preliminary reports from a single cohort

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Table 1. Structural magnetic resonance imaging (MRI) in prenatally alcohol-exposed children

					Findings		
References	N = PAE	N = Control	Site	Age (years)	Global	Focal	Comment
Archibald et al. (2001) (26)	26	41	California, San Diego, USA*	7–24	Smaller volumes in the cranial, cerebral, cerebellar vaults and cerebral and cerebellar grey (GM) and White matter (WM)	Smaller volume of the parietal lobe in WM&GM and caudate nucleus. Relative sparing of hippocampal volume and cerebellar hypoplasia greater than cerebral	Cerebellar hypoplasia greater than cerebral – approaching statistical significance $p = 0.069$
Astley et al. (27)	61	20	Washington, Seattle, USA**	8–15	Smaller total brain volume	Smaller volume of the frontal lobe, caudate, hippocampus, corpus callosum (CC)	Controlled for overall brain size
Autti-Rämö et al. (28)	17	0	Helsinki, Finland	13–15	Smaller volume of cerebral area and skull surface area	Smaller volume in CC area, posterior fossa and mesencephalon areas and in length of splenium	No control subjects
Bookstein et al. (29)	23	21	Washington, Seattle-Tacoma area, USA**	1–16 weeks	Ultrasound study only, showing a 'hook' (obtuse angle) in the splenium of CC^{++}		
Bookstein et al. (30)	30	15	Washington, Seattle, USA**	18–36	MR study demonstrated variation in shape of the CC^{++}		
Bookstein et al. (31)	120	60	Washington, Seattle-area, USA**	14–37	MR study demonstrated variation in shape of the CC^{++}		
Chen et al. (32)	67	27	Atlanta, Georgia, USA#	Mean: 22	Smaller total brain volume	Smaller volume of the superior and inferior parietal lobule, precential gyrus, pars opercularis (frontal lobe), superior temporal gyrus bank, pericalcarine cortex, lingual gyrus and isthmus of cingulate cortex	
Cortese et al. (33)	11	4	Detroit, USA	9–12	Smaller total brain volume	Smaller volume of left caudate nucleus	Controlled for overall brain size
Johnson et al. (34)	9	0	South Dakota, Vermillion, USA	4.5 months–20 years	Total brain volume reduced	Agenesis of the CC and cavum septi pellucidi, mild micrencephaly, hypoplastic CC, small cavum vergae, basal nasal meningocoele, disproportionately small brain stem and hypoplasia of the inferior olivary eminences	No control subjects
Joseph et al. (35)	12	19	Cape Town, South Africa	Mean: 11	Not indicated	Deformations in the hippocampus and caudate nucleus	Controlled for overall brain size
Lebel et al. (36)	24	95	Alberta, Canada	5–13	Smaller GM, WM and total brain volume	Not applicable	Focus on diffusion imaging
Lebel et al. (37)	70	63	California, Los Angeles and San Diego, USA and Cape Town, South Africa*	5–16	Smaller total cerebral and lesser WM volume	Longitudinal changes in volume relative to controls across many parietal and temporal cortical regions	

Table 1. (Continued)

References	N = PAE	N = Control	Site	Age (years)	Findings		
					Global	Focal	Comment
Li et al. (38)	7	7	Atlanta, USA[#]	18–24	Smaller total brain volume	Smaller volume of occipito-temporal area in both WM&GM	
Mattson et al. (39)	2	9	California, San Diego, USA*	13–14	Smaller cerebral and cerebellar volume	Agenesis of the CC and hypoplastic CC, enlarged lateral ventricles, temporal horns and caudate, volume reduction in thalamus, increased sub-arachnoid space and cerebral atrophy	
Mattson et al. (16)	2	20	California, San Diego, USA*	16	Smaller volume in cranial and cerebellar vaults	Smaller volume of basal ganglia	
Mattson et al. (40)	6	7	California, San Diego, USA*	8–19	Smaller cerebral volume	Smaller volume of the basal ganglia and caudate nucleus	Controlled for overall brain size
Meintjes et al. (41)	39	16	Cape Town, South Africa	9–11	Smaller total brain volume	Smaller volumes in particualr brain regions were no longer significant after being controlled for overall brain size	
Nardelli et al. (42)	28	56	Alberta, Canada	6–17	Smaller volumes of the intracranial vault, total WM and deep cortical GM	Bilaterally smaller volume in the hippocampus, thalamus, putamen, caudate, and globus pallidus	Controlled for overall brain size
O'Hare et al. (43)	21	21	California, Los Angeles and San Diego, USA*	8–25	Not indicated	Displacement and smaller volume of the anterior vermal region[++]	Controlled for overall brain size
Rajaprakash et al. (44)	36	52	Toronto, Ontario, Canada	8–16	Smaller overall brain volume and GM volume	Narrower cortical thickness and smaller GM volume in frontal, L parietal and R temporal lobes and smaller cortical surface area in frontal, temporal and R occipital lobes	
Riikonen et al. (45)	11	6	Kymenlaakso, Kotka and Kupio Finland	3–13	Not indicated	Slightly dilated right ventricle (fronto-temporal), cortical (frontal) and cerebellar atrophy, delayed myelination, arnold–chiari type 1 malformation, atrophic cerebellum, slight reduction of WM (occipital), large cortical and frontotemporal post-infarctal damage (left), atrophic basal ganglion (left), hypoplasy of CC and a larger left–right asymmetry of the hippocampus	

Table 1. (Continued)

References	N = PAE	N = Control	Site	Age (years)	Findings		
					Global	Focal	Comment
Riikonen et al. (46)	12	10	Kupio, Finland	5–16	Smaller volume of the intracranial vault	Controlled for overall brain size. No significant differences between groups after normalisation	Left hippocampus was smaller than right in FASD groups
Riley et al. (47)	13	12	California, San Diego, USA*	8–18	Smaller total brain volume	Agenesis and smaller volume of the overall callosal area	When corrected for brain size, three of the five callosal regions were still smaller in the alcohol-exposed (ALC) group, although overall area of the CC was no longer significantly different
Roussotte et al. (48)	56	43	Los Angeles and San Diego, USA and Cape Town, South Africa*	8–16	Smaller total brain volume and lesser total cortical GM	Smaller volume of some regions of the basal ganglia, diencephalon, left putamen and right pallidium	Controlled for overall brain size
Sowell et al. (49)	9	24	California, San Diego, USA*	8–24	Not indicated	Smaller volume of the anterior vermal regions (vermal lobules I-V)$^{++}$	
Sowell et al. (50)	21	21	California, San Diego, USA*	8–25	Smaller total intracranial volume, and lesser total WM, GM and CSF volume	Left posterior tempero-parietal area showing relatively too much GM and too little WM	Controlled for overall brain size
Sowell et al. (51)	21	145	California, San Diego (and Yale) USA*	7–25	Not applicable	Cortical surface and grey matter asymmetry in the posterior inferior temporal lobes (rightward-asymmetry)	
Sowell et al. (52)	21	21	California, Los Angeles and San Diego, USA*	8–25	Smaller brain volume	Greater cortical thickness over lateral temporal, frontal and parietal lobes bilaterally. No significant change from controls over dorsal frontal and parietal lobes	Authors comment that this MRI measurement likely reflects decreased myelination of WM rather than actual increase of GM constituents (i.e. indicate difficulty of establishing the WM&GM boundary on imaging)
Swayze et al. (53)	10	119	Iowa City, Vermillion, USA	4–26	Not indicated	Microcephaly, midline developmental anomalies, agenesis of and a hypoplastic CC, thinning of posterior callosum body and inferior olivary eminences, cavum septa pellucidi and cavum vergae	

Table 1. (Continued)

References	N = PAE	N = Control	Site	Age (years)	Findings		
					Global	Focal	Comment
Treit et al. (54)	17	27	Alberta, Canada	5–15	Lesser total brain volume, WM and cortical GM	Smaller volumes of the basal ganglia (globus pallidus and putamen), thalamus and hippocampus	Corrected for overall brain size
Willoughby et al. (55)	19	18	Toronto, Ontario, Canada	9–15	Smaller intracranial volume	Smaller left hippocampal volumes++	Corrected for overall brain size
Yang et al. (56)	82	71	Los Angeles and San Diego, USA and Cape Town, South Africa*	8–16	Not applicable	Lesser callosal thickness, anterior regions and the splenium++	
Yang et al. (57)	69	58	Los Angeles and San Diego, USA* and Cape Town, South Africa	8–16	Not applicable	Thicker cortices in several frontal, temporal and parietal regions	
Zhou et al. (58)	33	33 + 66	Alberta, Canada	6–30	Smaller total brain volume	Lesser cortical thickness in the bilateral middle frontal lobe, bilateral pre- and post-central gyri, bilateral superior parietal lobe, left lateral temporal lobe, bilateral inferior temporal lobe and bilateral occipital lobe	Additional 66 controls recruited and matched for secondary analysis. Controlled for brain size

CSF, cerebrospinal fluid; FASD, foetal alcohol spectrum disorder; ROI, region of interest.

The following codes denote sample overlap from specific regions: *California/San Diego; #Atlanta; **Washington. The following codes denote report on a single ROI: ++

Table 2. Diffusion tensor imaging (DTI) in prenatally alcohol-exposed children

					Findings		
References	N = PAE	N = Control	Site	Age (years)	MD	FA	Comment
Fryer et al. (67)	15	12	California, San Diego, USA*	8–18	↑ (In ALC group) Left sub-cortex and superior frontal lobe	↓ (In ALC group) Bilateral occipital, inferior parietal lobe and superior frontal lobe, right temporo-parieto-occipital junction, parietal lobe and lateral frontal lobe and left superior frontal lobe, temporo-occipital junction and frontal lobe and CC body	
					↓ (In ALC group) Right inferior frontal lobe and temporo-occipito-parietal junction	↓ (In control group) Right sub-cortex and cingulate gyrus	
Lebel et al.(36)	24	95	Alberta, Canada	5–13	↑ (In ALC group) Bilateral Inferior fronto-occipital fasciculus (IFO), left Inferior longitudinal fasciculus (ILF), right corticospinal tracts (CST), globus pallidus, right putamen and thalamus	↓ (In ALC group) Right cingulum, bilateral ILF and superior longitudinal fasciculus (SLF), splenium of CC and left thalamus	
					↓ (In ALC group) Genu of CC	↑ (In ALC group) Bilateral globus pallidus	
Li et al. (68)	57	25	Atlanta, USA#	19–27	↑ (In ALC groups) Isthmus of CC++	↓ (In ALC groups) Isthmus and splenium of the CC to lateral callosal fibres	
Ma et al. (69)	9	7	Atlanta, USA#	18–25	↑ (In FAS group) Genu and splenium of CC++	↓ (In FAS group) Genu and splenium of CC	
Sowell et al. (60)	17	19	California, Los Angeles, USA*	7–15		↓ (In FASD group) Lateral splenium (medial superior parietal WM), posterior cingulate WM bilaterally, deep WM of right temporal lobe	MD was lower in the FASD group in some, but not all regions where FA was affected. Lower MD was observed in the lateral splenium of corpus callosum bilaterally and in right temporal association fibres
						↓ (In FASD group) Right internal capsule and brainstem regions	
Spottiswoode et al. (70)	13	12	Cape Town, South Africa	9–14		↓ (In FASD group) Middle cerebellar peduncle++	Association with poor eye-blink conditioning in prenatally alcohol-exposed children
Treit et al.(54)	17	27	Alberta, Canada	5–15	↓ (In FASD group) Genu of CC	↓ (In FASD group) Superior Fronto-occipital Fasciculus (SFO)	Atypical developmental trajectories in superior and inferior fronto-occipital fasciculus and superior longitudinal fasciculus. Primarily MD findings (steeper decline in MD than controls)
Wozniak et al. (59)	14	13	Minneapolis, Minnesota, USA^	10–13	↑ (In FASD group) Isthmus of CC++		
Wozniak et al. (71)	33	19	Minneapolis, Minnesota, USA^	10–17		↓ (In FASD group) Posterior midbody, isthmus, splenium of CC++	

CC, corpus callosum; FA, fractional anisotrophy; FASD, foetal alcohol spectrum disorder; GM, grey matter; MD, mean diffusivity; ROI, region of interest; WM, white matter.
The following codes denote sample overlap from specific regions: *California/San Diego; #Atlanta; ^Minnesota;. The following code denotes report on a single ROI: ++.

Table 3. Proton magnetic resonance spectroscopy (^{1}H-MRS) in prenatally alcohol-exposed children

References	N = PAE	N = Control	Site	Age (years)	Findings		
					Neurometabolite	Site	Comment
Astley et al. (76)	61	20	Washington, Seattle, USA	8–15	wCho	↓ Right frontal-parietal WM voxel	
					hCho	↓ Hippocampal voxel	Near significant $p = 0.07$
					Cho/Cre ratio	↓ WM and hippocampus voxel	Overall ANOVA not significant, but important to report from exploratory standpoint. WM $p = 0.058$ and hippocampal $p = 0.061$.
					wCho	↓ Volume of frontal lobe WM and midsagittal area of cerebellar vermis lobules 1–5	Fixed-size voxel will capture more surrounding tissue in a smaller brain than in a larger brain. Referred to as partial voluming
					wCho	↓ Midsagittal area of CC	
					wNAA	↓ Midsagittal area of CC	
					hCho	↓ Hippocampus, frontal lobe and CC cerebellar vermis lobules 6–7, putamen and total brain	
Cortese et al. (33)	11	4	Detroit, USA	9–12	NAA/Cr	↑ Left caudate nucleus	Associated with FAS facial dysmorphology
					NAA	↑ Left caudate	
Fagerlund et al. (77)	10	10	Turku and Helsinki, Finland	14–21	NA/Choline & NAA/Cr	↓ Cerebral cortex (anterior cingulate and parietal cortex)	
					NAA/Cho	↓ Cerebral cortex (lateral frontal cortex)	
					NA/Cho & NAA/Cr	↓ Cerebral white matter (frontal white matter)	
					NAA/Cr	↓ Cerebral white matter (CC)	
					NAA/Cho	↓ Cerebral nuclei (thalamus)	
					NAA/Cho & NAA/Cr	↓ Cerebellum (dentate nucleus)	
Lindie du Plessis et al. (78)	37	17	Cape Town, South Africa	8–12	NAA	↓ Deep cerebellar nuclei	Associated with alcohol consumption at conception
					Cho	↓ Deep cerebellar nuclei	Associated with alcohol consumption throughout pregnancy
					Glx	↑ Deep cerebellar nuclei	Associated with alcohol consumption at conception and during pregnancy

Cho, choline containing metabolites; Cr, creatine-containing metabolites; Glx, glutamate with its precursor glutamine; NAA, N-acetyl-aspartate.

have described inter-hemispheric and global functional connectivity abnormalities in older children (10–17 years) with FASD.

Task-based fMRI is a direct method for investigating the function of the brain in humans. The technique measures the dynamic distribution of blood flow to specific regions of the brain during a defined motor or cognitive activity. The choice of tasks investigated has largely focussed on functional deficits previously described in children prenatally exposed to alcohol and include working memory (88–95), sustained attention (38), response inhibition (96,97), verbal learning (98) and mathematical tasks (99,100). Rousotte and colleagues have also reported connectivity in alcohol-exposed and in polydrug-exposed children, aged between 7 and 15 years, during a working memory task (Table 4).

Discussion

The neuroimaging literature of the past 20 years has documented the deleterious effects of prenatal alcohol exposure on many important systems in the developing brain. These findings in children are consistent with the animal neuroimaging literature, which has demonstrated the teratogenic effects of alcohol on the immature nervous system via a number of cellular and molecular mechanisms leading to structural, functional and metabolic abnormalities in a wide spectrum of brain regions.

sMRI

Early reports on structural neuroimaging effects of prenatal alcohol exposure focussed largely on global effects and on the severe end of the FASD spectrum. Studies consistently reported smaller whole-brain volume as well as more specifically smaller volumes of both white and grey matter across the whole brain. A small minority found proportionally greater volume differences in overall deep grey matter (42) or white matter (26) when controlling for overall brain volumes. In addition, although cortical thickness alterations have been reported, the direction of change remains mixed across studies (37,44,48,54,56–58). However, these findings were not specific to alcohol exposure and research focus moved on to the identification of volume and shape differences of more defined regions.

The most consistent finding across studies of structural MRI in alcohol-exposed children remains alterations in both the shape and area of the corpus callosum. Given that this structure represents the largest white matter tract in the brain, an important midline structure as well as the primary connection between the hemispheres, alterations in its shape and volume might be reasonably hypothesised in the context of alcohol exposure. Reported variations range from total absence of the structure or partial agenesis in individual cases (45) to more subtle between-group differences in volume (47,48,56), area or length (27,28,47,103) and position (103) relative to unexposed children. These findings are likely to represent in part the well-described vulnerability of midline structures to alcohol-induced damage in exposed individuals, but may also represent downstream effects of alterations in other areas of the brain, resulting in reduction in connections between these regions across the hemispheres. Finally, although these studies confirm that the corpus callosum is a heavily affected region during prenatal alcohol exposure, as can be seen from Table 1, the region has frequently been singled out for discrete ROI analysis, and thus effects on the brains of these exposed children may in fact be more extensive than they would seem from these reports.

Differences in grey matter volumes and/or thickness have been reported for cortical regions as well as for the deep grey matter. Frontal, parietal and temporal cortices have been implicated, although the direction of effect is not consistent between studies (37,51,52,57,58). In particular, the relationships between the abnormalities of the cortical structure have been related to cognitive functional outcomes, and the differences in cortical development over time in alcohol versus control groups have been reported by Sowell and colleagues (37,52,54). This argues for the clinical importance of damage to these areas after alcohol exposure.

Other areas that have been reported as consistently affected include the deep grey matter structures. Most consistently reported was volume reduction in the hippocampi, even when corrected for total brain volume (26,42,55). Additional studies found the hippocampi to have smaller volumes in proportion to overall brain volume reduction (27,48). Basal ganglia volume reductions in alcohol-exposed children in comparison with their non-exposed peers have been reported by a number of authors (16,26,33,40,42,46,48,76). Alcohol exposure effects on the caudate have been most frequently cited (16,26,33,40,42,46,48,76), but also discrete changes in the globus pallidus (42,48,54), putamen (42,46,48,54,76) or the lenticular nucleus as a whole (40). The implication of alcohol targeting on these sub-cortical nuclei is not well-established due, in part, to the paucity of functional outcome data linked to these changes. However, their role in critical networks regulating behaviour and impulse control among other functions could be considered a consistent hypothesis in this group of affected children (104).

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Table 4. Functional MRI (fMRI) in prenatally alcohol-exposed children

References	N = PAE	N = Control	Site	Age (years)	Brain function	Findings		
						Brain activity	Site	Comment
Astley et al. (94)	61	20	Washington, Seattle, USA**	8–15	Working memory	↓	(2-back tasks in FASD group) Right inferior frontal gyrus, R posterior parietal lobe, R dorsolateral prefrontal cortex (DLPFC) and R middle frontal gyrus	Overall, performance on 1-back and 2-back tasks decreased in FASD groups, but activation levels only decreased on 2-back tasks and not on 1-back tasks
Diwadkar et al. (93)	30	17	Cape Town, South Africa	8–10	Working memory	↑	(In syndromatic PAE group) Right inferior parietal cortex and left cerebellum (Crus I/lobule VI) and L inferior cerebellum (lobule VIIB–VIIIA). (In non-syndromatic PAE group) Bilateral dorsal, prefrontal cortex, L caudate and L putamen	
Fryer et al. (97)	13	9	California, San Diego, USA*	8–18	Go/No-Go	↑	(In FASD group) Right middle frontal gyrus, left middle, medial and superior frontal gyri	
						↓	(In FASD group) Right caudate nucleus	
Li et al. (38)	7	7	Atlanta, USA#	8–24	Sustained attention	↑	(In PAE groups) Occipital-temporal region, in the Z (inferior-superior) direction	Brain networks that were activated were more widespread in the PAE groups than in CON group
Malisza et al. (90)	24	25	Monitoba, Winnipeg, Canada	7–12 18–33	Spatial working memory	↑	(In FASD group) Inferior middle frontal gyrus	The CON groups showed an overall increase in frontal activity with increasing task difficulty, whereas the FASD group showed decreased activity
Meintjes et al. (99)	15	18	Cape Town, South Africa	8–12	Number processing	↑	(In FAS groups) Angular gyrus, cuneus, posterior cingulate gyrus, anterior horizontal intraparietal sulcus (HIPS), superior frontal gyrus, R basal operculum, L sub callosal stratum, L deep precentral gyrus, L inferior temporal gyrus, R occipital gyrus, L anterior insula, R putamen, L thalamus and red nucleus	
Norman et al. (88)	18	17	California, San Diego, USA*	12–18	Spatial working memory	↑	(In PAE groups) Bilateral middle and superior frontal gyrus, lingual gyrus, cuneus, lentiform nucleus, insula and precuneus	PAE group showed fewer regions of activation, and limited frontal activation, in comparison to CON groups
O'Brien et al. (96)	20	15	California, San Diego, USA*	8–18	Go/No-Go	↑	(In No-Go tasks, in PAE group) Left precuneus, cingulate gyrus, anterior cingulate and right medial frontal gyrus	PAE group showed activation in more widespread areas, especially in the left hemisphere
						↓	(In No-go, cue-dependent tasks, in PAE group) Left pre and post-central gyri	
O'Hare et al. (95)	20	20	California, Los Angeles, USA	7–15	Verbal working memory	↑	(In PAE group) Left dorsal frontal, L inferior parietal, and bilateral posterior temporal regions	

Table 4. (Continued)

References	N = PAE	N = Control	Site	Age (years)	Brain function	Findings		
						Brain activity	Site	Comment
Roussotte et al. (91)	32	18	California, Los Angeles, USA	7–15	Working memory	↓	(In PAE group) Bilateral anterior cingulate, right orbito-frontal, R frontal pole, R insula, R caudate and R putamen	
Roussotte et al. (92)	32	18	California, Los Angeles, USA	7–15	Working memory	↑	(In functional connectivity, in PAE group) Between caudate and frontal and prefrontal areas (only in lateral subregions: inferior frontal gyrus, in the right hemisphere) and right medial temporal lobe	
						↓	(In functional coupling, in PAE group) Between putamen and superior and inferior frontal subregions	
Santhanam et al. (100)	37	17	Atlanta, USA#	20–26	Number processing	↓	(In dysmorphic PAE group) Left superior and right inferior parietal regions and medial frontal gyrus	
Sowell et al. (98)	11	16	California, Los Angeles, USA	7–15	Verbal learning	↑	(In PAE group) Left dorsal prefrontal cortices, right ventral and lateral frontal and superior parietal cortices	
						↓	(In PAE group) Left medial temporal region	
Spadoni et al. (89)	10	12	California, San Diego, USA*	10–18	Spatial working memory	↑	(In PAE group) Frontal, insular, superior, middle temporal, occipital and sub-cortical regions	
Wozniak et al. (101)	24	31	Minnesota, Minneapolis, USA^	10–17		↑	Characteristic path length Global	All measures are global. Study set out to specifically measure global connectivity rather than specific region to region connectivity
						↓	Global efficiency (GE) Global	GE positively correlated with cortical thickness in frontal, temporal and parietal regions
						↑	Local efficiency Global	
Wozniak et al. (102)	21	23	Minnesota, Minneapolis, USA^	10–17		↓	(In functional connectivity, in FASD group) Medial parietal (paracentral) regions	The authors used the paracentral area as the region of interest as they had previously shown that children with FASD have microstructrual abnormalities in these regions and the posterior CC that connects these regions

ACER, Alcohol Clin Exp Res; FASD, foetal alcohol spectrum disorders.

The following codes denote sample overlap from specific regions: *California/San Diego; #Atlanta; ^Minnesota; **Washington.

Areas where findings have showed greater discrepancy include the thalamus, which has been reported to have smaller volumes even when correcting for brain volume by some groups (39,42,54) and changes only in proportion to overall brain volume by others (26,48). The diencephalon, overall, has also been reported as displaying reduced volume (40) or area (28) or no significant structural changes at all (16).

The body of literature reporting structural brain changes associated with *in utero* alcohol exposure using cross-sectional study designs, described above, is now quite large. The studies have focussed on a range of ages from 5 years through to young adulthood. However, the majority of participants were either in late childhood or adolescence, and very few studies grouped participants in a narrow age-bracket. As a result, although there is reasonable consistency in the location of the PAE effects on the developing brain, there is very little clarity on when these changes have onset, the nature and functional importance of changes in specific areas at particular time points and the developmental trajectory of these changes across childhood or into later adulthood. There is established documentation of age-related changes in the developing brain, both at the structural and microstructural level (64,65,105,106). These changes do not necessarily follow linear trajectories, and different areas of the brain appear to follow different maturational patterns (106). The CIFASD group, in one of the only reported longitudinal studies in alcohol-exposed children, has reported altered trajectory of cortical development compared with non-exposed peers (37,54). In particular, parietal and some temporal regions demonstrated either inverted or flattened volume curves over time. Of interest are the different effects that were noted at different ages in specific areas. For example, a cross-sectional comparison of these groups at 5–6 years of age may have demonstrated increased volume in the parietal cortex in prenatally alcohol-exposed children, whereas at 11–12 years of age the volume relationship may have been reversed. This study supports the view that developmental trajectories may be a better indicator of atypical brain development, and emphasises the conspicuous absence of data in children with prenatal alcohol exposure during the early years of life, which represents the most rapid period of brain growth.

DTI

DTI has proved to be a particularly useful tool in the investigation of the more subtle effects of the spectrum of alcohol and polysubstance exposure on white matter integrity of the developing brain (59). The available data on DTI findings in children exposed to alcohol have consistently demonstrated reduced FA in the corpus callosum. Abnormalities in MD have also been reported, although the nature and specific location of these changes have been different across studies (60,71,107). A recent review of seven DTI studies in older children and young adults exposed to alcohol prenatally reported white matter microstructural abnormalities (lower FA) in the corpus callosum, anterior–posterior fibre bundles and the cerebellum (59,68,69,71,71,82,108). These abnormalities have also been reported in the frontal (36,67), temporal lobe regions in particular (36,60), and sub-cortical structures (globus pallidus, thalamus and putamen) (36,37) (Table 2).

Very few studies have addressed associations between white matter microstructural abnormalities and specific measures of cognitive and behavioural function (60,84). Alhough negative findings exist for several functional domains, Sowell et al. (81) showed associations between reduced performance on a measure of visuomotor integration and reduced FA in the splenium of the corpus callosum and parietal white matter. Lebel et al. reported significant associations between reduced FA in the left parietal lobe, cerebellum and brainstem with mathematical ability in 5- to 13-year-old children (108). These results suggest clinical significance of the DTI findings in these brain regions, at least in school-age children. However, existing studies have generally used relatively low angular resolution data (with between 6 and 35 gradient directions) and spatial resolution ≥2.5 mm. Thus, more sensitive non-tensor-based models have not been applied, which may have greater sensitivity. To date, almost no data exist regarding the impact of prenatal alcohol exposure in early infancy before higher-level brain networks have become established or the confounding postnatal environmental influences to which children from these backgrounds are exposed have come into play. Moreover, there are few data on when changes in white matter structural integrity have onset, regional specificity in early brain development and whether there are any early neurobehavioural associations.

^{1}H-MRS

The first reported ^{1}H-MRS study in children exposed to alcohol *in utero* reported increased NAA concentration in the caudate nucleus in a group of children with full-blown FAS. The group also reported relationships between NAA concentration and facial dysmorphometry (33). A second study in slightly older children identified decreased NAA/Cr and NAA/Cho ratios in multiple regions including the cortical and sub-cortical regions. These included

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parietal and frontal cortices, thalamus and cerebellar dentate nucleus as well as the frontal white matter and corpus callosum (77). These changes in neurometabolite ratios are suggested to reflect changes in glial proliferation (Cho and Cr) rather than decreased neuronal integrity/viability (NAA) in children with FASD. A subsequent study in a larger cohort found reduced choline in the frontal and parietal white matter of children with FASD compared with exposed children without the FAS facial phenotype or cognitive/behavioural dysfunction and unexposed children. Another group has also reported reduced choline, but in the left striatal region, in a group of children with a diagnosis of FASD compared with a control group (109). Finally, a well-characterised group of FASD children in South Africa were found to have lower NAA levels in the deep cerebellar nuclei associated with alcohol exposure around conception. Higher levels of alcohol consumption during pregnancy were related to reduced Cho and with increased concentrations of GLx in the deep cerebellar nuclei (78). Despite discrepencies in site and type of neurometabolites reported in these studies, these individual reports indicate alterations in the neurochemistry across important areas in the grey and white matter regions of children exposed to alcohol *in utero*. However, brain metabolism and associated neurochemistry are dynamic and site-specific. Certainly, age-specific changes in concentrations of these metabolites are documented (74), and the findings described above represent a broad age range as well as differing approaches to clinical classification for comparison groups. In addition to the above, technical factors such as the choice of the location of voxel placement make comparison across these studies largely unhelpful.

To date, no studies have reported the use of ^{1}H-MRS to explore the effects of prenatal alcohol exposure on the developing infant brain. This is an important period to investigate, as the neurometabolic milieu at this early stage of development is likely to have a significant impact on subsequent brain development (74) and is less likely to be contaminated with environmental factors. In particular Glutamate (Glu), an excitatory neurotransmitter, also plays a key role in early life in the regulation of cell proliferation, migration and pruning (110), and alcohol exposure has been shown to disrupt this process in animal models (23,72).

Functional magnetic resonance imaging (RS-fMRI)

Functional changes in brain activity relating to specific tasks in children exposed prenatally to alcohol vary across a number of cognitive domains. Working memory is the domain that has been most widely investigated (88–95), although other areas of cognitive function have been described (38,96–100). All these studies reported differences in the distribution of activated brain areas during a working memory task in the prenatal alcohol exposure group compared with unexposed peers even in the absence of between-group differences in the task itself. Alterations in blood flow regulation appear most consistently in the frontal regions in these studies, and, although the particular distribution of these changes are not consistent, there appears to be a more generalised pattern of activation in the prenatal alcohol-exposed children, possibly representing reduced efficiency in activation of specific neural pathways. Whether this relates to delayed functional maturation or more permanent impairment will be addressed by studies tracking these important cognitive functions over time and specifically into early adulthood.

Wozniak et al. (71,102), in their initial study, demonstrated that children with prenatal alcohol exposure had abnormalities in white matter microstructural connectivity in the corpus callosum compared with healthy unexposed controls, as well as a disturbance of functional connectivity in the alcohol-exposed group in this region. A subsequent analysis of the same children demonstrated further abnormalities in global measures of network connectivity using a graph theory approach (101). The authors reported significantly higher characteristic path length and lower global efficiency in the brains of those children with prenatal alcohol exposure. These exploratory findings are an early indication that the dynamic activation of brain regions may provide key insights into the neuropathological basis of functional impairments demonstrated by children with FASD (Table 4).

The development and maturation of the central nervous system require a carefully patterned sequence of events and processes more complex and extending over a longer period than that of any other organ system. The brain is particularly vulnerable to prenatal environmental influences, which may have long-term effects on its structure and function (111,112). The complexity of the brain's structural and functional networks increases rapidly in the early months of life, representing a rapid acquisition of abilities across motor, sensory and cognitive areas (62,65,87,105). To date, little data exist regarding the impact of prenatal alcohol exposure in early infancy before higher-level brain networks have become established and before the confounding postnatal environmental influences to which children from these backgrounds are exposed have come into play. Although information is emerging on the effects of alcohol exposure on the

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longitudinal structural development of the brain in later childhood (37), there are few human data on when changes have onset, where they are located at this initial stage and how complex early behavioural milestones relate to functional and structural changes of the underlying neural substrate. More specifically, although preliminary studies have shown altered connectivity in the more mature brains of school-age children, the specific effects of alcohol exposure on the establishment of intrinsic connectivity in early infancy have not been explored. Characterising the connectivity of regions in the brain that are key to early neurodevelopmental functional integration, including the thalamus and the motor cortex, may, therefore, serve as a sensitive indicator of the neuropathological effects of alcohol exposure in the human infant (113).

Conclusions

The body of evidence documenting the neuroimaging changes associated with children and young adults exposed to alcohol during the prenatal period is now substantial. Limitations common to work in this field and which are likely to have had an impact on the consistency of the results include the issue of polysubstance abuse, even though alcohol exposure was in most cases the predominant reported exposure. Although expensive, the emerging use of biological measures of alcohol exposure such as biomarkers obtained from hair or nails are likely to improve the quantification of alcohol exposure in studies going forward. The exclusion or careful control of subjects with other substance misuse may also be improved with approaches such as urine identification of excreted drugs and cotinine measurements for tobacco exposure.

Differences in age and gender between subjects in neuroimaging studies were noted (Tables 1–4). These differences are unlikely to have affected within-study results, as exposed and control groups were generally carefully matched. However, it is possible that age in particular, but also gender differences within studies, may have affected the sensitivity for detecting changes between these two groups. As has been discussed in relation to the specific imaging modalities above, a critical gap in the extant literature is the lack of neuroimaging studies in prenatally alcohol-exposed children under 5 years of age or those over 25 years of age when brain maturation has occurred across all the modalities of imaging. In addition, the dynamic nature of brain maturation and appreciation of the effects of a significant insult such as alcohol exposure on the developing trajectory of the structural and functional network argue for the prioritisation of studies that include a longitudinal approach to understanding this spectrum of effects. Hypothesis-driven studies that include longitudinal time points as well as an integrated approach using a number of modalities at one age point, neuropsychological and behavioural outcomes and links to genetic vulnerabilities are likely to provide the most robust understanding of the neurobiological effects of prenatal alcohol exposure on the developing brain. Refining our understanding at a neurobiological level is key in developing not only earlier identification of the spectrum of alcohol-exposure effects but also targeted interventions during this important window for early intervention.

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Conflicts of Interest

The authors report no conflicts of interest with respect to this manuscript or relevant financial disclosures.

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