HEAVY PRENATAL ALCOHOL EXPOSURE RELATED TO SMALLER CORPUS CALLOSUM IN NEWBORN STRUCTURAL MRI SCANS

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INTRODUCTION

BACKGROUND

- Autopsy and MRI studies have consistently demonstrated disproportionately smaller corpus callosa in children and adults with a history of heavy prenatal alcohol exposure (PAE)
- Incidence of partial or complete corpus callosum (CC) agenesis among children with PAE may be as high as 6.8%, as compared to a normal population rate of 0.3% and a developmentally delayed population rate of 2.3%
- Imaging studies in children and adults have documented:
  - reduced callosal size
  - callosal shape abnormalities
  - anterior and inferior displacement of the posterior CC regions
  - reduced CC thickness
- Alterations in the antenatal growth of CC and other brain structures as a consequence of PAE may result in profound effects on subsequent integration of neural activity.
- Only a few studies have implemented MRI segmentation techniques for cerebral morphometry in neonates. None have previously examined feasibility of detecting this effect in alcohol-exposed newborns or infants
- Scanning nonsedated newborns is challenging
  - small head size (which requires scanning at a higher resolution)
  - availability of shorter scanning period provides less opportunity for reduction of motion artifacts
- Segmentation of the newborn brain is extremely time-consuming because
  - analysis techniques developed for the adult or child brain are not directly transferable
  - incompletely myelinated brain is difficult to segment
  - contrast-to-noise ratio is poor due to regional variation in degree of myelination, in which gray-white matter contrast may be inverted

METHODS

SAMPLE

- 43 pregnant mothers and their infants were recruited from a Cape Coloured (mixed ancestry) community in Cape Town, South Africa in 2011-2013
- Heavy alcohol consumption including weekend binge drinking is a major source of recreation for many in this community
- Prevalence of fetal alcohol syndrome (FAS) in this community is among the highest in the world

Prenatal alcohol exposure

- Timeline follow-back interviews were administered to the mother twice during pregnancy and at 1-month postpartum to reflect latter part of the pregnancy
- Volume of each type of beverage (beer, wine, liquor, cider) consumed each day during the pregnancy was converted to oz of absolute alcohol (AA)—0.5 oz AA≈1 standard drink
- 31 children whose mothers drank 6-12 drinks/occasion comprise the prenatal alcohol exposure group
- 10 children whose mothers abstained and 2 who drank 2-3 drinks during pregnancy were included as controls

FASD diagnosis

- September 2013, we organized a clinic during which each infant was examined for growth and FAS dysmorphic features by two expert FASD dysmorphologists (HEM and GDJ) using a standard protocol (Hoyme et al., 2005). Case conferences were held to reach consensus regarding diagnosis:
  - FAS–distinctive craniofacial dysmorphology, including short palpebral fissures, thin upper lip, and flat or smooth philtrum; small head circumference; and growth retardation
  - Partial FAS (PFAS)–maternal alcohol history and at least 2 of 3 key facial anomalies + small head circumference, growth retardation, or cognitive/behavioural deficits + documented maternal alcohol exposure
  - Heavily exposed (HE) nonsyndromal–heavily exposed children who lack distinctive alcohol-related facial features but may demonstrate significant neurobehavioral and cognitive deficits
  - The FAS, PFAS and HE groups together make up the PAE group
  - 7 children were diagnosed with FAS, 5 with PFAS, 17 as HE, 2 were alcohol exposed but were not diagnosed, and 12 were controls.

NEWBORN ASSESSMENT

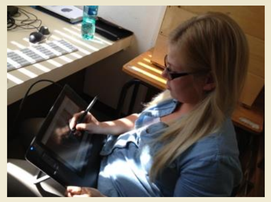
- 43 newborns (*M* scan age=17.8 days; range=6-40) were scanned at the Cape Universities Brain Imaging Centre (CUBIC)
- A detailed protocol (developed by PW and SWJ) was used to acquire the neonatal MRI data without use of sedation
- Scanning procedure included:
  1. Not fed for 3 hr prior to scan
  2. Examined by CDM, a developmental paediatrician
     - Brazelton Neonatal Behavioural Assessment Scale administered—includes newborn's responses to visual and auditory and social and nonsocial stimuli, reflexes, responses to stress, self-calming, motor maturity, and hand-mouth coordination
     - Weight, length and head circumference obtained
     - Tiring – easier for infant to fall asleep after examination and remain asleep during scan without sedation
  3. Newborn undressed, diaper changed, and swaddled (arms wrapped to reduce jerky movements)
  4. Fed and rocked into deep sleep by mother in dark room
  5. Placed on a special pillow containing Styrofoam beads that fits snugly around newborn's body and head
  6. Newborn is fitted with sponge earplugs and foam ear pads to reduce noise
  7. Oxygen saturation monitored by attaching a pulse oximeter to the toe
  8. Air is removed from pillow, which tightly secures newborn's body
  9. Infant moved into scanner on pillow
  10. Newborn's head positioned within a custom-built birdcage coil
- During the scan, newborn was visually monitored for movement, crying, decrease in oxygen saturation, or any other signs of distress by CDM or research nurse seated in the scanner room

DATA ACQUISITION

- No sedation was used
- Scanned using 3T Allegra MR Scanner (Siemens, Erlangen, Germany)
  - A custom-built 170.9 mm (inner diameter) circularly polarized birdcage radiofrequency coil (designed by L. Wald, Ph.D., Director MR Core, Martinos Center, Radiology, MGH; built by A. Hess, Ph.D.) was used
  - T1 scans were acquired at two different flip angles, allowing the images to be synthesized with different relative T1 vs. proton density weightings to optimize contrast across boundaries of adjacent structures (to facilitate manual tracing)
- The following structural protocol was used:
  - Multiecho FLASH (MEF) 30° (3D encoding, 144 x 144 matrix on 144 mm FoV, 128 sagittal slices of 1 mm thickness giving 1 mm isotropic resolution, TR 20 ms, 8 echoes with TE = 1.46 ms + n×1.68 ms where n = 0,..,7, bandwidth 651 Hz/px, non-selective excitation with flip angle 30°, T_{acq} 6 min 9 s)
  - Multiecho FLASH 5° (same as above with flip angle 5°)
  - MEF scans were combined to obtain quantitative proton density, T1 and T2* estimates per voxel by solving the steady-state FLASH equation for two flip angles. Bandwidths were matched across MEF and had high values so that distortions (due to B0 inhomogeneities) were small and matched across scans. These are true physical parameters of the scanned tissue in the 3 T field and not simply weighted intensities that vary with the scanning sequence and system.

IMAGE PROCESSING

- CC was independently traced by 2 graduate research assistants (NML and FW)
- Both initially trained at the MGH Martinos Center and subsequently supervised by a senior neuroanatomist (CW) at UCT
- Each neonatal CC was hand-segmented (using FreeView software) on mid-sagittal slice on an AC-PC aligned T1-weighted image
  - Intraclass correlation = 0.89
  - Median interobserver Dice Index S_D = 0.85
- Average of the 2 tracings used as CC area measure in the analyses



RESULTS

Sample Characteristics

	PAE (*n* = 31) Mean/%	SD	HC (*n* = 12) Mean/%	SD	*p* value
Infant characteristics					
Sex (% female)	45.2	-	33.3	-	0.492
Gestational age at birth (wk)	38.2	2.0	38.7	2.0	0.418
Postpartum age at scan (wk)	2.8	1.3	1.9	1.2	0.046
Postconception age at scan (wk)	41.0	2.4	40.6	2.1	0.660
Birthweight (g)	2754.7	485.6	2941.7	319.7	0.226
Head circumference (%ile for age)[a]	32.7	1.4	33.4	0.8	0.114
Total intracranial volume (cm^3)	475.1	62.4	504.6	62.2	0.171
Maternal characteristics					
Maternal age at delivery (yr)	28.5	6.0	26.5	5.0	0.318
Parity	2.1	1.8	1.6	1.2	0.334
Education (yr)[b]	9.1	1.7	10.3	1.2	0.031
Marital status (% married)	9.7	-	33.3	-	0.062
Smoking (cig/day)	6.2	4.7	3.5	3.1	0.076
Marijuana (days/wk)	2.1	4.7	3.5	3.1	0.157
Alcohol consumption					
At conception					
oz AA/day	1.7	2.4	0.0	0.1	0.019
oz AA/occasion	3.7	3.2	0.1	0.3	0.000
Frequency (days/wk)	2.45	1.4	0.07	0.28	0.000
Across pregnancy					
oz AA/day	1.2	1.9	0.0	0.0	0.042
oz AA/occasion	4.3	2.7	0.2	0.6	0.000
Frequency (days/wk)	1.4	1.4	0.0	0.1	0.001

[a] Measures from infant follow-up exam 6.5 months after scan; value missing for one PAE newborn.
HC = healthy control; PAE = prenatal alcohol exposed; SD = standard deviation; AA = absolute alcohol

[b] Education missing for one HC mother.

Relation of newborn characteristics and other prenatal exposures to corpus callosum area (*N* = 43)

	r
Gestational age at birth	.02
Sex	-.07
Age at scan	-.18
Prenatal exposure	
Cigarettes	-.13
Marijuana	-.06
Methamphetamine	-.11

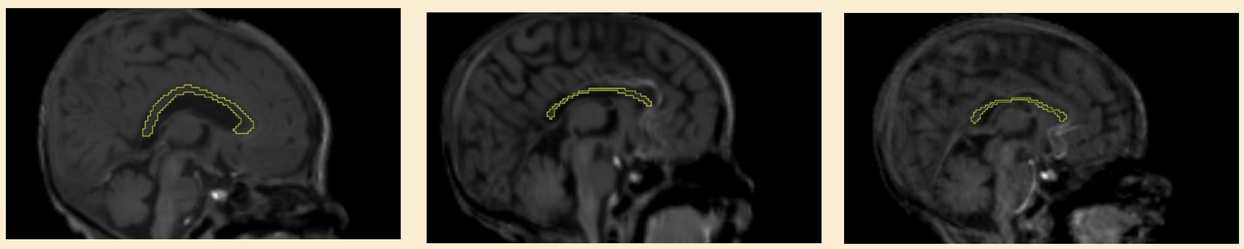
Effect of prenatal alcohol exposure to newborn corpus callosum area controlling for other prenatal exposures (*N*=43)

		Methamphetamine	Marijuana	Smoking
	r	β	β	β
Across pregnancy				
AA/day	-.35*	-0.38*	-.36*	-.35*
AA/drinking day	-.27†	-.26†	-.26†	-.25
Frequency	-.38*	-.37*	-.36*	-.35*

†p < .10; *p < .05

- CC was smaller in alcohol-exposed neonates than in controls
- CC area was unrelated to infant sex, gestational age at birth, or age at scan
- CC was unrelated to maternal smoking, marijuana, or methamphetamine ("tik") use during pregnancy
- Smaller CC area was also seen in heavy exposed newborns after those with FAS were removed from the analyses

Corpus callosum in a healthy control and two newborns with heavy prenatal alcohol exposure



Healthy control | Newborn with FAS | Heavy exposed nonsyndromal

CONCLUSIONS

- Given the rapid and heterochronus nature of early brain growth, volumetric measures based on MRI in the first weeks of life may provide a more sensitive index of future neurological outcome than standard newborn behavioral or neurological examinations.
- Because the craniofacial dysmorphic features that characterize FAS are difficult to detect in infancy, early indicators of effect are needed that will contribute to understanding the ontogeny of impairment as it unfolds across development.

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