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Authors

Donald, Kirsten A
Ipser, Jonathan C
Howells, Fleur M
et al.

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Interhemispheric Functional Brain Connectivity in Neonates with Prenatal Alcohol Exposure: Preliminary Findings

Kirsten A. Donald,
Division of Developmental Paediatrics, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and University of Cape Town, Cape Town, South Africa

Jonathan C. Ipser,
Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Fleur M. Howells,
Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Annerine Roos,
MRC Unit on Anxiety & Stress Disorders, Stellenbosch University, Cape Town, South Africa

Jean-Paul Fouche,
Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Department of Psychiatry, Stellenbosch University, Cape Town, South Africa

Edward P. Riley,
Department of Psychology, San Diego State University, San Diego, California

Nastassja Koen,
Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Roger P. Woods,
Department of Neurology, University of California, Los Angeles, California

Bharat Biswal,
Department of Biochemical Engineering, New Jersey Institute of Technology, Newark, New Jersey

Heather J. Zar,
Department of Paediatrics and Child Health & MRC Unit on Child & Adolescent Health, Red Cross War Memorial Children's Hospital and University of Cape Town, Cape Town, South Africa

Katherine L. Narr, and
Department of Neurology, University of California, Los Angeles, California

Reprint requests: Kirsten A. Donald, MPhil, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, Klipfontein Road/Private Bag, Rondebosch, 7700/7701 Cape Town, South Africa; Tel.: +27-21-658-5535; Fax: +27-21-658-5530; kirsty.donald@uct.ac.za.

CONFLICT OF INTEREST

Authors report no conflict of interest with respect to this study.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary material.

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Dan J. Stein
Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

MRC Unit on Anxiety & Stress Disorders, University of Cape Town, Cape Town, South Africa

Abstract

Background—Children exposed to alcohol in utero demonstrate reduced white matter microstructural integrity. While early evidence suggests altered functional brain connectivity in the lateralization of motor networks in school-age children with prenatal alcohol exposure (PAE), the specific effects of alcohol exposure on the establishment of intrinsic connectivity in early infancy have not been explored.

Methods—Sixty subjects received functional imaging at 2 to 4 weeks of age for 6 to 8 minutes during quiet natural sleep. Thirteen alcohol-exposed (PAE) and 14 age-matched control (CTRL) participants with usable data were included in a multivariate model of connectivity between sensorimotor intrinsic functional connectivity networks. Seed-based analyses of group differences in interhemispheric connectivity of intrinsic motor networks were also conducted. The Dubowitz neurological assessment was performed at the imaging visit.

Results—Alcohol exposure was associated with significant increases in connectivity between somatosensory, motor networks, brainstem/thalamic, and striatal intrinsic networks. Reductions in interhemispheric connectivity of motor and somatosensory networks did not reach significance.

Conclusions—Although results are preliminary, findings suggest PAE may disrupt the temporal coherence in blood oxygenation utilization in intrinsic networks underlying motor performance in newborn infants. Studies that employ longitudinal designs to investigate the effects of in utero alcohol exposure on the evolving resting-state networks will be key in establishing the distribution and timing of connectivity disturbances already described in older children.

Keywords

Blood Oxygen Level-Dependent; Functional MRI; Newborn; Intrinsic Brain Activity; Resting-State MRI; Alcohol Exposure; Fetal Alcohol Spectrum Disorders

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DOCUMENTATION OF THE physical and neurodevelopmental effects of prenatal alcohol exposure (PAE) has a long tradition, extending back in the medical literature for the last 40 years (Jacobson et al., 2011; Jones et al., 1973; Mattson et al., 2013; Riley et al., 2011). The complexity of the brain's structural and functional networks increases rapidly in the prenatal period and early months of life, representing rapid development across motor, sensory, and cognitive areas (Geng et al., 2012; Gilmore et al., 2012; Hagmann et al., 2012; Pannek et al., 2012). The brain is particularly vulnerable during this developmental window to environmental influences, including maternal alcohol consumption during pregnancy, but also including postnatal insults, and these may have long-term effects on its structure and function (Rodier, 1994, 2004).

Investigators have increasingly sought to document correlations between the functional deficits reported in children who have been exposed to alcohol in utero and underlying neurobiology. These endeavors have been given added impetus by the observation of

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functional deficits associated with PAE, even in cases where children do not exhibit the facial features required for a diagnosis of fetal alcohol syndrome (FAS) (Riley et al., 2011). The wide range of physical, behavioral, and developmental abnormalities in children exposed to alcohol in utero is recognized by their inclusion in the umbrella term fetal alcohol spectrum disorders (FASD). Indeed, animal work (An and Zhang, 2013; Burke et al., 2009) and human imaging studies in school-age children with FASD have demonstrated that in utero exposure to alcohol alters brain morphology and reduces white matter microstructural integrity (Archibald et al., 2001; Fryer et al., 2009; Lebel et al., 2008, 2012; Leigland et al., 2013; O'Leary-Moore et al., 2011; Sowell et al., 2008; Wozniak and Muetzel, 2011; Wozniak et al., 2006).

However, little data exist regarding the impact of PAE in early infancy, before higher-level brain networks have become established and before confounding postnatal environmental factors come into play. Two preliminary studies have reported altered white matter microstructural integrity in the association tracts (Donald et al., 2015) and structural connectivity (Taylor et al., 2015) in neonatal populations, suggesting that the effects of PAE are measurable at this early stage. This paucity of data is particularly striking with regard to the study of functional brain connectivity, described in the imaging literature as dependencies among observed neurophysiological responses or "temporal correlation between spatially remote neurophysical events" (Biswal et al., 1995, p. 537). Evidence that the ability of the brain to coordinate these areas of activity follows a developmental trajectory, reflected in increased functional network connectivity with age in childhood and early adulthood (Hagmann et al., 2012), suggests that the developmental impact of PAE on the brain may manifest in anomalous patterns of functional brain connectivity.

Two preliminary reports have described interhemispheric and global functional connectivity abnormalities in older children (10 to 17 years) with FASD. Wozniak and colleagues (2009) demonstrated that children with PAE had abnormalities in white matter microstructural connectivity in the posterior corpus callosum compared to healthy unexposed controls (CTRL). They further reported reductions in functional connectivity between hemispheres in the paracentral lobule thought to be anatomically mediated by these callosal tracts in alcohol-exposed children (Wozniak et al., 2011). A subsequent report demonstrated abnormalities in global measures of network connectivity using a graph theory approach. The authors reported significantly higher characteristic path length and lower global efficiency in the brains of those children with PAE (Wozniak et al., 2013). Roussotte and colleagues (2012) subsequently reported between-group abnormalities in frontostriatal connectivity in both alcohol- and polydrug-exposed children aged 7 to 15 years, during a working memory task. In both exposed groups, functional connectivity between the dorsal caudate and frontal executive network decreased while increases were noted in frontal cortical coupling with the posterior putamen, a brain region typically more strongly synchronized with the motor network (Roussotte et al., 2012). Santhanam and colleagues (2011) reported decreased functional connectivity between the major nodes of the default mode network at rest in young adults with PAE.

Nevertheless, despite this literature in older children, the effects of alcohol exposure on the longitudinal structural development of the brain in later childhood are still poorly

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characterized (Lebel et al., 2012), with few human data on the onset of these effects, where they are located at this initial stage, and how the complex early behavioral milestones relate to functional and structural changes of the underlying neural substrate. More specifically, while preliminary studies have shown altered connectivity in the more mature brains of school-age children and young adults, the specific effects of alcohol exposure on the establishment of intrinsic connectivity in early infancy have not been explored. Based on the literature reviewed, the connectivity of regions in the brain that are key to early neurodevelopmental functional integration, including the thalamus and the motor cortex as well as the integration and coordination of left and right hemisphere function, would seem to be promising candidate markers of the neuropathological effects of alcohol exposure in the human infant (Doria et al., 2010).

This prospective study was conducted in a South African community with high prevalence of alcohol use disorders, in order to address gaps in the current literature concerning the presence, timing, and regional specificity of altered functional network integrity associated with PAE. We hypothesized that there would be differences in functional brain networks in the first weeks of life as a result of gestational alcohol exposure. We also hypothesized that neonatal quantitative abnormalities associated with maternal alcohol use in pregnancy and detected using resting-state functional magnetic resonance imaging (RS-fMRI) would correlate with early indicators of neurobehavioral health.

MATERIALS AND METHODS

Participants

The current investigation is a nested substudy that includes infants enrolled in a larger population-based birth cohort study, the Drakenstein Child Health Study (Stein et al., 2015; Zar et al., 2015). This larger study 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. Prevalence of FAS and FASD has been reported to be as high as 63/1,000 and 155/1,000, respectively, in this community (May et al., 2013), compared to globally quoted prevalence estimates of between 2 and 7 per 1,000 for FAS and 20 to 50 per 1,000 for FASD (May et al., 2009). In addition, access to women in maternity care was facilitated by the well-established, free primary healthcare service in the Drakenstein area, with approximately 90% of women in this area seeking public sector antenatal care and child health services.

The umbrella study enrolled more than 1,600 pregnant women and is following them through childbirth until children reach 5 years of age. In this nested substudy, RS-fMRI scans were acquired for 30 healthy unexposed infants and 30 alcohol-exposed infants at 2 to 4 weeks of age. Mothers were recruited at 20- to 28-week gestation, written informed consent obtained, and background data collected for the umbrella study. For the group with alcohol exposure, mothers were screened antenatally based on a minimum score of 11 on the alcohol questions of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) questionnaire (indicating moderate to high risk of substance abuse)—a widely validated World Health Organization (WHO) scale to assess comorbid substance use (Humeniuk et al., 2008; Jackson et al., 2010). In addition to this initial screen, mothers were

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interviewed and required to have a positive history of alcohol use in any trimester of pregnancy at levels consistent with WHO moderate–severe alcohol use. After birth, infants from mothers identified through this approach were included for study unless the mothers also had a positive urine screen for other drugs of abuse (including methamphetamines, cannabis, methaqualone) (Lozarno et al., 2007), the infants were significantly premature (<36 weeks), or infants had low apgar scores (<7 at 5 minutes) and/or history of neonatal intensive care unit admission for hypoxic ischemic encephalopathy or other significant neonatal complications (such as neonatal jaundice requiring phototherapy). Infants were also excluded if they had an identified genetic syndrome. Mothers also completed formal screening antenatally for depression (Beck Depression Inventory II) as well as posttraumatic stress disorder (Modified PTSD Symptom Scale).

Measures

Two- to 4-week-old infants underwent brain imaging. Basic anthropometry, including weight, occipito-frontal head circumference, and length, was acquired at the imaging visit. The Dubowitz neurobehavioral scale, used to study early neurological and behavioral changes, was also administered at this time. The Dubowitz is a well-validated measure of neonatal neuromotor and neurobehavioral status (Noble and Boyd, 2012). The tool includes an optimality score which enables it to be used for quantitative analysis of potential associations with neuroimaging findings in this study (Dubowitz et al., 1998). The score is based on the distribution of the scores for each item in a population of low-risk terminfants. The total optimality score was the sum of the optimality scores of individual items. However, for this study, specific item clusters were chosen as being of particular interest. As defined by the authors of the tool, the “behavior” cluster includes items scoring irritability, cry, consolability, alertness, visual and auditory orientation, and eye movements.

Intrinsic functional brain connectivity MRI scans were acquired on a 3T Siemens Allegra system at the Cape Universities Brain Imaging Centre, using a single-channel head coil. Infants were in quiet natural (unsedated) sleep during the course of the scan, after being wrapped and fed. 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. Quality control procedures identified poor tissue specificity in T1-weighted MPRAGE images that were acquired to facilitate the anatomical localization of group differences in functional brain connectivity. Accordingly, 5-minute T2-weighted image sequence (TR 3,500 ms; TE (echo time) 354 ms; slice thickness 1 mm; 128 slices; voxel size 1.0 × 1.0 × 1.0 mm) was used for localization instead. Whole-brain 3T gradient echo T2-weighted echoplanar images (EPI) were acquired for 6 minutes (TR 2,000 ms; TE 30 ms; flip angle = 77° slice thickness 4 mm; 33 slices; voxel resolution = 2.5 × 2.5 × 4.0 mm). The EPI sequence was extended to 8 minutes (238 volumes) toward the end of the study, while holding all other scanning parameters the same.

Analysis Approach

Image preprocessing was conducted using AFNI (Cox, 1996). The first 4 volumes of the EPI sequence were removed, to allow for stabilization of the magnetic field, and outlier signal

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intensities in each voxel's time series were truncated using 3dDespike. The blood oxygen level-dependent (BOLD) data were subsequently motion-corrected (via rigid-body alignment of each of the EPI volumes to the third volume), resampled to 2.5 mm in the 3 spatial dimensions, and registered into University of North Carolina (UNC) neonatal atlas space (via displacement parameters obtained through the intermediary registration with the brain-extracted T2 anatomical images; Shi et al., 2011), all in a single step. As the final step, the data were spatially smoothed with a 5-mm full width at half-maximum Gaussian kernel. Only participants for whom more than two-thirds of their BOLD data would have been retained after removing time points with excessive movement, defined as 0.3 mm relative to the preceding time point, were included in the analysis. This corresponded to a minimum of 4 and 5.2 minutes of data for infants with the shorter and longer EPI sequences, respectively.

Motor networks, as well as networks that previous functional and resting-state connectivity analyses have implicated as being functionally integrated with the motor network, including the somatosensory, thalamic, and striatal networks, were identified using probabilistic independent component analysis (ICA), as implemented in FSL's melodic tool (Beckmann et al., 2005). ICA employs a model-free signal separation algorithm to identify spatially orthogonal components based on voxel time series and allows investigators to identify connectivity patterns resulting from motion and physiological sources of no interest as separate networks that can be excluded from the subject's data prior to conducting between-group analyses. It was with this in mind that ICA was performed individually for each infant's RS-fMRI data set in this study, in order to identify networks using the criteria of Kelly and colleagues (2010) that corresponding to motion artifacts, as well as signals of no interest from white matter and cerebrospinal fluid tissue (please see the Supporting Information for the exact criteria employed). The time series for these components were subsequently regressed from the preprocessed EPI data for that subject, using the fsl_regfilt tool.

Components corresponding to the motor, somatosensory, striatal, and thalamic networks across the entire sample were identified from among the total number of components determined as estimable from the data. These components were obtained by applying the ICA algorithm to the temporally concatenated denoized time series from all participants, after truncating each individual data set at 176 volumes and normalizing the mean intensity of each volume. Voxels with a BOLD time course that mapped onto the component's time course, using a Z-score threshold of 4, were designated as members of this component. Verification of candidate networks of interest was obtained through visual comparison with the published literature on neonate and adult ICA networks (Beckmann et al., 2005; Doria et al., 2010; Smith et al., 2009) and through consulting the Automated Anatomical Labeling segmentation map provided with the UNC infant atlas.

A multivariate general linear modeling (MVM) framework was employed to assess the effect of PAE on connectivity between the time courses for 6 networks identified through ICA as being of interest (see Results section). To prepare the data for this analysis, group component spatial maps were regressed from each infant's preprocessed 4D EPI data set, yielding subject-specific separate time series corresponding to each of the group components. Group differences in Fisher Z-transformed Pearson's correlation connectivity

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estimates between the network time courses were identified using a wrapper to AFNI's 3dMVM tool, provided as part of the FATCAT toolbox (Taylor and Saad, 2013). 3dMVM allows one to treat correlations between each pairwise combination of regions of interest as within-subject repeated-measure factors, while, uniquely among MRI analysis software packages, simultaneously adjusting for the effect of continuous covariates (Chen et al., 2014). A significant effect for an omnibus model of group differences in connectivity between any of the networks can be used to justify the subsequent inspection of the results of post hoc models for each pairwise comparison, to help determine specifically which of the networks are differentially connected as a result of PAE. Subject age in days and gender were included as covariates of no interest for both the omnibus and post hoc models in this study.

The clinical significance of differences in connectivity between the 6 networks was assessed by correlating the networks' time series for each infant with both the total and subscale scores from the Dubowitz neurological examination. In addition, the moderation by group of any association between development and internetwork connectivity was assessed by multivariate modeling of an interaction effect of group status and Dubowitz total score on Fisher *Z*-transformed connectivity scores.

Ethical Issues

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Health Sciences of University of Cape Town (HREC REF 40½009) for the Drakenstein Child Lung Health Study. This substudy protocol was independently reviewed and approved (HREC REF 525/2012).

RESULTS

Description of Sample

RS-fMRI data were acquired from 60 infants, at 2 to 4 weeks of age (30 CTRL, 30 PAE). Thirteen alcohol-exposed (PAE) and 14 age-matched control (CTRL) participants were included in the analysis. A total of 33 participants were excluded (16 CTRL, 17 PAE). Reasons for excluding data included excessive subject motion (7 CTRL, 5 PAE), missing EPI or T2 anatomical data (8 CTRL, 3 PAE), pronounced EPI signal loss (1 CTRL, 7 PAE), and poor registration of the EPI into standard space (2 PAE). Five of the CTRL infants and 2 PAE children were scanned with the longer 8-minute EPI sequences. Low rates of psychopathology were observed in this sample, with a single PAE mother screening positive for depression on a self-report measure.

Table 1 presents the comparison of the PAE and CTRL infants on demographic and developmental variables. The groups were comparable with respect to sex, with boys representing approximately half of the infants in the PAE ($N = 7$) and CTRL ($N = 8$) groups, as well as with respect to physical development ($\alpha > 0.2$ for all comparisons of weight, length, and head circumference). The average age at scan was 21 days (range: 11 to 32) and also did not differ by group. The proportion of infants of mixed race ancestry was slightly higher in the CTRL than PAE infants (50 vs. 62%), although this difference was not

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statistically significant ($\chi^2 = 0.364$, $p = 0.547$). The proportion of mothers of PAE infants who drank, as well as the quantity of alcohol consumed per occasion, was highest in the first trimester, with only a third (4/12) of the PAE mothers who drank during the first trimester still drinking during the third (Table 2). With respect to performance on the Dubowitz scale, no differences were observed between the PAE and CTRL participants for the tone, behavior, and total scores. Very low variability in scores for spontaneous motion, reflex, and abnormal movement prevented any meaningful comparison of group differences on these subscales.

Independent Components Analysis

The number of components from the individual-level ICA ranged from 27 to 103, with more than half (57.5%, mean = 34.61, SD = 14.16) identified as resulting from artifacts or from nongray matter sources, and regressed from the infant's RS-fMRI EPI sequences. A total of 30 group-level components were subsequently extracted from the denoized data. Of the 30 ICA networks, 6 were selected as networks suited to test the hypothesis that intrauterine alcohol exposure will affect motor connectivity (Fig. 1).

Of the 6 networks identified, 2 extended bilaterally within the precentral gyrus, and were designated as representing motor networks. The first was located more anteriorly, and included the supplementary motor area (SMA) and the superior frontal gyrus (Fig. 1A). A more posterior network included the postcentral gyrus, the SMA, and the paracentral lobule (Fig. 1B), as well as a separate small (10 voxel) cluster in the left thalamus. Two networks primarily situated within the postcentral gyrus, and largely mirroring one another in their more lateral position within the left and right hemispheres, were considered to represent somatosensory networks (Fig. 1C,D). The right somatosensory network also included a relatively small (72 voxel) cluster in the left postcentral gyrus. A bilateral network was evident in the striatum, with strongest activation in the left and right putamen, but also including the bilateral caudate and palladium, as well as part of the left anterior insula (Fig. 1E). Finally, a diffuse thalamic network was selected that in addition to the bilateral thalamus also incorporated regions of the brainstem, hippocampus, amygdala, and palladium (Fig. 1F).

The MVM analysis revealed a significant effect of group in connectivity between all 6 networks ($\chi^2 = 11.080$, df = 1, $p < 0.001$), after adjusting for age in days and gender (no significant effects on connectivity were observed for either of these covariates). An MVM approach was employed, as it allows one to take a hierarchical approach to hypothesis testing, while simultaneously controlling for covariates that could otherwise introduce spurious group differences. The null hypothesis that there are no differences in connectivity between the exposed versus control infants (either representing increased or decreased connectivity) for any pairwise combination of the 6 networks was initially tested. Further identification of particular networks whose relationships varied in conjunction with PAE would only have been warranted where the null hypothesis for this omnibus test rejected, as was the case in this study. Support for the null hypothesis would have terminated any further investigation of differences in network connectivity. The significant group difference observed for the omnibus test ($\chi^2 = 11.080$, df = 1, $p < 0.001$) further supports the argument

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that the association of history of PAE with functional connectivity between our networks of interest is unlikely to have occurred by chance.

Examination of the post hoc comparisons of connectivity between particular networks indicated that alcohol exposure increased connectivity between the brainstem and the anterior motor network ($t = 2.426$, df = 23, $p = 0.024$), with a trend-level effect in the same direction observed between the brainstem and the left somatosensory network ($t = 2.0125$, df = 23, $p = 0.056$). Increased connectivity in the PAE infants was also observed between the striatum and both the anterior ($t = 3.045$, df = 23, $p = 0.006$) and posterior motor networks ($t = 3.257$, df = 23, $p = 0.004$). In the only instance of attenuated internetwork connectivity in the PAE relative to CTRL infants, the alcohol-exposed infants displayed preliminary evidence for attenuated interhemispheric connectivity between the left and right somatosensory networks ($t = -1.929$, df = 23, $p = 0.066$).

On the basis of the finding of a possible association between PAE and hemispheric asymmetry in the somatosensory network, and the consistency of this finding with reports of reduced interhemispheric connectivity in sensorimotor networks in children and adolescents exposed prenatally to alcohol (Wozniak et al., 2011), a seed-based analysis was conducted to further investigate the effect of PAE on connectivity between the hemispheres for both the anterior and posterior motor networks. A standard preprocessing pipeline was applied to the data set described above, prior to denoizing, and included regression of nuisance parameters, such as signal from a white matter mask, mean, linear, quadratic and cubic temporal trends, as well as the 6 rigid-body motion parameter estimates and their first-order derivatives. Censoring of high motion volumes was also applied to the data. Fisher *Z*-transformed Pearson's correlation coefficients were subsequently calculated between the time series extracted separately from each hemisphere, per network, and compared using multiple linear regression procedures, controlling for infant gender and age in days (please refer to the Supporting Information for additional information).

Although the hemispheric connectivity estimates were lower for the PAE than for the CTRL infants in the anterior motor network (mean; SD = 0.69; 0.1 and 0.75; 0.12), and marginally so for the posterior motor network (mean; SD = 0.80; 0.07 and 0.82; 0.10) networks, these differences did not achieve statistical significance (Mann–Whitney $Z = -1.359$, p-value = 0.174 and Z =0.679, p-value = 0.497, respectively).

Finally, the MVM analysis of the clinical significance of internetwork differences failed to detect evidence for a moderating effect of group status on the association between internetwork connectivity and Dubowitz total, behavior, and tone in this small cohort. Of the 15 comparisons conducted, only connectivity between the striatum and anterior motor network achieved significance ($t = -2.2290$, df = 22, uncorrected $p = 0.0363$). Nonparametric correlation tests revealed that greater connectivity between these networks predicted higher total clinical deficits on the Dubowitz scale in the CTRL infants (Spearman $\rho = 0.609$, $p = 0.027$), with no association detected among the PAE neonates ($\rho = -0.238$, $p > 0.1$). Replicating these analyses for the Dubowitz behavior and tone subscales produced no evidence of a moderating effect of group for behavior ($\chi^2 = 0.165$, df = 1, $p = 0.684$) or tone ($\chi^2 = 3.288$, df = 1, $p = 0.070$). Inspection connectivity between networks suggested that

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prior alcohol exposure had the greatest effect on the association between tone and striatal connectivity, with differences observed between connectivity of this structure and both the anterior motor network ($t = -2.145$, df = 22, $p = 0.043$) and the left somatosensory network ($t = -2.249$, df = 22, $p = 0.035$). In line with the findings for the correlation tests using the total Dubowitz score, positive associations between striatal motor connectivity and tone for these networks ($\rho = 0.549$, $p = 0.052$ and $\rho = 0.529$, $p = 0.062$) were not evident in PAE infants ($\rho = -0.309$, $p = 0.305$ and $\rho = -0.307$, $p = 0.308$, respectively).

DISCUSSION

This study reports preliminary data on functional network connectivity in alcohol-exposed infants compared to controls. In this small sample, there were significant group differences in functional connectivity between networks underlying motor behavior, after correction for gender and age differences. Our results support the possibility that PAE may be associated with altered sensorimotor connectivity.

The only previous group to report resting-state functional network abnormalities in alcohol-exposed children reported robust asymmetry in interhemispheric connectivity of the sensorimotor cortex in exposed versus unexposed children (Wozniak et al., 2011). Additional analyses by the authors revealed associations between hemispheric asymmetry in their cohort and the compromised microstructural integrity of the posterior corpus callosum, as well as impaired perceptual reasoning. We were unable to replicate the significant findings of disrupted functional sensorimotor connectivity across brain hemispheres in this small, relatively immature sample.

Reductions in internetwork connectivity and increased connectivity within networks in adult intrinsic functional networks compared to healthy infants were recently argued as a hallmark of the functional compartmentalization and segregation of brain networks (Wylie et al., 2014). The finding reported in this paper that alcohol exposure was associated with greater connectivity of the striatal and brainstem/thalamic networks to the rest of the sensorimotor system, but with possible reductions in interhemispheric motor and somatosensory connectivity, is therefore consistent with the notion that exposure may play a role in delayed development of maturing brain networks. Apparently contradictory findings of increased interhemispheric connectivity of the motor and somatosensory networks as infants approach term, as well as greater thalamic connectivity to these networks (Doria et al., 2010), may reflect the dynamic nature of brain maturation. Indeed, a possible nonlinear relationship between a child's chronological age and connectivity of the default mode network, considered one of the major hubs of intrinsic functional connectivity in adults, has previously been documented (Gao et al., 2013). Furthermore, there is evidence from other brain imaging modalities indexing brain volume (Treit et al., 2013), white matter microstructural integrity (Donald et al., 2015), and neurometabolite concentrations (Cortese et al., 2006; Fagerlund et al., 2006) that the direction of the difference between PAE and control children may vary as a function of the precise developmental stage at which assessment takes place, underscoring the importance of more finely grained longitudinal analyses of the maturation of functional brain networks in the first years of life.

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Much literature in developmental neuroscience has focused on localizing and classifying the function of specific brain areas and how these regional specializations arise. The sensorimotor cortex is a region that traditionally has been associated with tasks of motor planning and control. However, more recent functional models have explored broader involvement of this region in the integration of sensory stimuli, as well as the dynamic organization of the sensorimotor cortex during the generation of a wide range of complex cognitive and motor functions (Bouchard et al., 2013; Crone et al., 1998). Although very little has been reported with respect to early neurobehavior in alcohol-exposed neonates, studies that have investigated this outcome have identified poor habituation and low levels of arousal along with motor abnormalities in the infants of women who used alcohol heavily during pregnancy (Chiriboga, 2003; Streissguth et al., 1983). Sensitive functional outcomes in this age group remain difficult to define because structural and functional networks are still in their earliest stages. Published work on infants born prematurely suggests that the emerging connectivity of the thalamus at term with the visual, auditory, and motor networks is indicative of its important developmental role in the formation of these networks (Doria et al., 2010). Further, effective development of the structural core connecting the medial cortical regions has been characterized as an integrated system that is critical to the coordination of left and right hemispheres (Hagmann et al., 2012). It is therefore not surprising that previous work has revealed that this core network is particularly susceptible to the damaging effects of alcohol exposure during development (Wozniak and Muetzel, 2011).

Imaging infants remains technically difficult in terms of both imaging acquisition and the inherent challenges in reliably analyzing data from brains that are small and have high water content and poor gray–white matter differentiation (Geng et al., 2012; Gilmore et al., 2012; Hagmann et al., 2012). Subject motion has been identified as a potential source of artifactual group differences in RS-fMRI connectivity, particularly for pediatric samples (Power et al., 2012). Approximately half of the infants scanned for this study were excluded due to factors that included motion-induced imaging artifacts and signal attenuation. Although a series of steps were implemented to adjust for subject motion in the data, it is possible that residual motion may have introduced confounding of the data. Moreover, despite efforts to equate groups with respect to differences in signal intensity, as reflected by the exclusion of a greater proportion of PAE than CTRL participants due to widespread signal loss, it is not possible to entirely discount the effect of signal attenuation on group differences reported in this paper. Accordingly, despite the demonstration of relatively robust evidence of group differences in overall connectivity between the 6 networks assessed using sophisticated statistical methodology, the results of this study should therefore be considered preliminary, particularly with respect to findings of differences in connectivity between particular networks, and warrant replication in larger studies. Further limitations included the fact that infants in this study were exposed to either moderate or severe levels of PAE. The inclusion of the less severely exposed neonates may have masked clearer differences between groups.

This study begins to address gaps in the current literature concerning the feasibility of functional connectivity studies in infants of this age and in particular of brain functional connectivity studies in association with PAE. Studies that employ longitudinal designs to investigate the effects of in utero alcohol exposure on the evolving resting-state networks

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will be key in establishing the distribution and timing of connectivity disturbances already described in older children. Preliminary study outcomes are starting to elucidate the early neurodevelopmental mechanisms leading to subsequent behavioral and neurological disturbances, which may allow opportunities for targeting interventions when brain plasticity is still relatively fluid.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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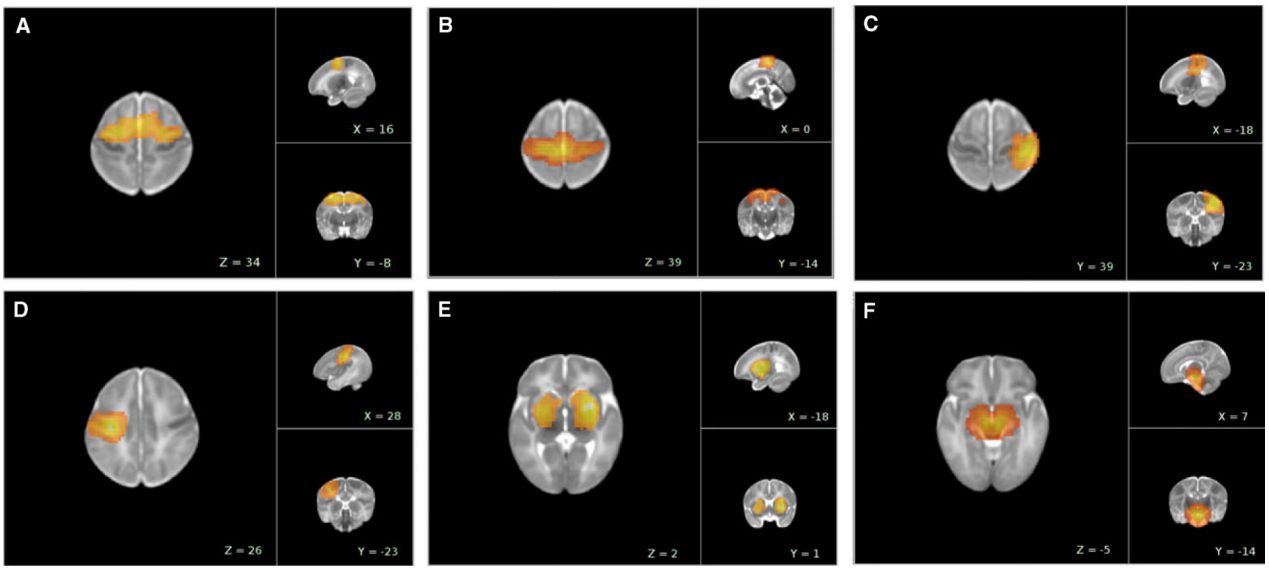

Fig. 1.

Axial, sagittal, and coronal slices of independent component analysis derived networks (shown in orange-yellow), thresholded at $Z = 4$ and superimposed on the University of North Carolina neonatal template (radiological convention: left of display equals right side of brain). Networks: (**A**) anterior motor, (**B**) posterior motor, (**C**) left somatosensory, (**D**) right somatosensory, (**E**) striatal and (**F**) brainstem/thalamic.

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Table 1.

Comparison of Alcohol-Exposed and Un-exposed Neonates on Demographic and Developmental Variables

	PAE (*N* = 13)	CTRLS (*N* = 14)	Test statistic
Gestation in weeks[a]	38.55 2.07	38.11 1.54	$Z = 0.691$, $p = 0.490$
Age (in days)	21.38 (5.5)	20.71 (5.25)	$Z = 0.244$, $p = 0.981$
% Male	53.85	57.14	$\chi^2 = 0.030$, $p = 0.863$
Ethnicity (%)			
Black African	38.5	50	$\chi^2 = 0.364$, $p = 0.547$
Mixed race	61.5	50	
Weight[b] (kg)	3.86 (0.69)	3.98 (0.66)	$Z = -0.489$, $p = 0.625$
Length[b] (cm)	49.88 (4.11)	50.93 (4.39)	$Z = -0.619$, $p = 0.536$
Head circumference[b]	35.66 (1.45)	36.16 (1.6)	$Z = -0.876$, $p = 0.381$
Dubowitz scale			
Tone subscale[c]	8.27 (1.69)	8.54 (1.22)	$Z = -0.104$, $p = 0.917$
Behavior subscale	4.35 (1.3)	4.43 (1.34)	$Z = 0.075$, $p = 0.941$
Total score[b]	22.23 (2.9)	22.42 (2.67)	$Z = -0.283$, $p = 0.777$

[a]Data missing for 3 prenatal alcohol exposure (PAE) and 5 healthy unexposed controls (CTRL) participants.

[b]Data missing for a single PAE participant.

[c]Data missing for a single CTRL participant.

All continuous variables reported as means (SDs). Mann–Whitney tests conducted for group comparisons on all continuous variables.

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Table 2.

Alcohol Use of Prenatal Alcohol Exposure Mothers by Trimester

	Trimester 1	Trimester 2	Trimester 3
Alcohol usage, *n* (%)	12 (92.3)	7 (53.8)	4 (30.8)
Once per week or less	10	5	2
2 to 3 times per week	2	2	2
4 to 5 times per week	0	0	0
Daily	0	0	0
Number of drinks per occasion			
<2	1	2	1
2 to 3	3	1	3
4 or more	8	4	0