A Longitudinal Study of the Long-Term Consequences of Drinking during Pregnancy: Heavy In Utero Alcohol Exposure Disrupts the Normal Processes of Brain Development

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Introduction

Brain development, a dynamic process of progressive and regressive changes influenced by complex genetics and experience-dependent plasticity, may be altered in children with neurodevelopmental disorders such as prenatal alcohol exposure (PAE). Concomitant reductions in synaptic density (Huttenlocher, 1979; Huttenlocher and de Courten, 1987) and increases in axonal myelination (Yakovlev and Lecours, 1967) are hallmarks of experience-based neural plasticity and are consistent with selective specialization. It is thought that increasing efficiency, via myelination and synaptic pruning, occurs at the expense of extended plasticity, as underused connections are eliminated and are no longer available for use. While the study of maturation at the cellular level is limited to animal and postmortem human studies, in vivo magnetic resonance imaging (MRI) studies demonstrate similar patterns. Cortical volume and thickness increase initially, peak during late childhood or adolescence, and decline thereafter (Giedd and Rapoport, 2010). These changes vary regionally and, in general, parallel cognitive maturation.

The shape of developmental trajectories may be a better indicator of atypical neurodevelopment than cortical differences at any single time point (Shaw et al., 2006; Giedd et al., 2008), and these shapes have demonstrated differences in children and youth with attention deficit hyperactivity disorder (Shaw et al., 2006). Concomitant reductions in synaptic density (Huttenlocher, 1979; Huttenlocher and de Courten, 1987) and increases in axonal myelination (Yakovlev and Lecours, 1967) are hallmarks of experience-based neural plasticity and are consistent with selective specialization. It is thought that increasing efficiency, via myelination and synaptic pruning, occurs at the expense of extended plasticity, as underused connections are eliminated and are no longer available for use. While the study of maturation at the cellular level is limited to animal and postmortem human studies, in vivo magnetic resonance imaging (MRI) studies demonstrate similar patterns. Cortical volume and thickness increase initially, peak during late childhood or adolescence, and decline thereafter (Giedd and Rapoport, 2010). These changes vary regionally and, in general, parallel cognitive maturation.

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2007), in children with child-onset schizophrenia (Thompson et al., 2001; Vidal et al., 2006), and even in healthy children with differing levels of intelligence (Shaw et al., 2006). Thus, studying maturational trajectories in neurodevelopmental disorders may lend new insight into these disorders and potential treatment strategies, which could vary depending on the cognitive and behavioral deficits and the age of the child.

Children and adolescents with PAE often exhibit facial dysmorphology, growth restriction, and a variety of cognitive, behavioral, and/or neurological problems (Riley et al., 2011), including cortical gray matter abnormalities (Archibald et al., 2001; Sowell et al., 2008a; Zhou et al., 2011; Chen et al., 2012; Yang et al., 2012a). However, little is known about longitudinal brain development in this population. Two cross-sectional studies examining subcortical gray matter volumes (Nardelli et al., 2011) and cortical thickness (Zhou et al., 2011) found no significant differences in linear development trajectories between alcohol-exposed subjects and controls, although qualitative observations suggest group differences. Longitudinal studies have more power to detect change and may reveal abnormalities not apparent in cross-sectional studies.

The purpose of this study was to examine trajectories of cortical change over time in children with and without PAE, and to test relationships between cortical development and cognitive/behavioral function. Given that higher intelligence is associated with later peaks of cortical thickness (Shaw et al., 2006), we hypothesized that children and adolescents with PAE would reach peak cortical volume earlier than their unexposed peers, and that individuals with greater facial dysmorphology and intellectual deficits would show more aberrant cortical maturation during the interscan interval.

Materials and Methods

Subjects. This study was part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) and included data from 133 subjects studied at three sites: Los Angeles, California (n = 36: 17 male/19 female, 13 control/23 alcohol-exposed, 33 right-handed/3 left-handed); San Diego, California (n = 18: 12 male/6 female, 10 control/8 alcohol-exposed, 15 right-handed/3 left-handed); and Cape Town, South Africa (n = 79: 45 male/34 female, 40 control/39 alcohol-exposed, 76 right-handed/3 left-handed). Subjects were aged 5.7–15.9 years (12.4 ± 2.6 years) at the time of their first MRI scan, and were all scanned again the age of the child.

Behavioral deficits would show more aberrant cortical maturation during the peak cortical volume earlier than their unexposed peers, and that higher intelligence is associated with significant head injury with loss of consciousness for >30 min, significant physical or psychiatric disability that would prevent participation in the imaging and/or cognitive assessments, or other known causes of mental deficiency, such as chromosomal abnormalities.

After an explanation of procedures, all subjects provided assent and their parent/guardian provided written informed consent. The institutional review boards at UCLA, SDSU, and the University of Cape Town approved all procedures.

Cognitive assessments and facial evaluations. Participants were assessed with the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV), to obtain a Full Scale IQ score (FSIQ); the neurocognitive aspects of the CIFASD project have been described in more detail previously (Mattson et al., 2010). Most participants at each site were also examined by a trained dysmorphologist for the facial features associated with fetal alcohol syndrome (Jones et al., 2006); 65 alcohol-exposed subjects and 56 control subjects received facial evaluations. Palpebral fissure length (PFL) was measured in millimeters using a rigid ruler against the lower eyelid (Hoyme et al., 2005). The philtrum and upper lip were assessed and ranked separately by a blinded rater using the philtrum index score, which ranks indicate greater dysmorphology. Of 37 alcohol-exposed subjects who received the full physical examination, 23 were classified as having fetal alcohol syndrome based on the Hoyme criteria (Hoyme et al., 2005).

Image acquisition. High-resolution T1-weighted MRI data were collected in Los Angeles, California, on a 1.5 T Siemens Sonata (TR, 1900 ms; TE, 4.38 ms; flip angle, 15°; matrix, 256 × 256 × 160; FOV, 256 × 256 mm; total acquisition time, 8 min 8 s). Data were collected in Cape Town, South Africa, on a 3 T Siemens Allegra (TR, 2200 ms; TE, 5.16 ms; flip angle, 12°; matrix, 256 × 256 × 160; FOV, 256 × 256 mm; total acquisition time, 7 min 4 s). Data were collected in San Diego, California, on a 3 T General Electric Signa Excite (TR, 8 ms; TE, 3.0 ms; flip angle, 12°; matrix, 256 × 256 × 192; FOV, 240 × 240 mm; total acquisition time, 7 min 4 s). Final voxel sizes were 1 × 1 × 1 mm for Los Angeles and San Diego, and 0.94 × 0.94 × 1 mm for Cape Town.

Image processing. All data were processed through the longitudinal stream in FreeSurfer v5.1, using robust, inverse consistent registration (Reuter and Fischl, 2011). Information from each subject template was used to initialize the longitudinal image processing, increasing repeatability.
Results

Cognitive assessments, facial evaluations, and exposure histories

Demographics for each group are shown in Table 1. Subjects with PAE had significantly greater facial dysmorphology than controls (shorter PFL, higher lip and philtrum ranks). Lip and philtrum ranks of 4 and 5 indicate the most severe dysmorphology. Among the 55 control subjects with philtrum ranks, 15 (27%) and 1 (2%) had ranks of 4 and 5, respectively; of the 65 alcohol-exposed subjects, 31 (48%) and 6 (9%) had ranks of 4 and 5, respectively. For lip rank, 13 (21%) and 2 (3%) control subjects ranked 4 or 5, respectively, while 26 (37%) and 8 (11%) subjects with PAE ranked 4 and 5, respectively. Two controls (2%) and 30 exposed subjects (46%) had PFL below the 10th percentile. Groups did not differ on age or gender distribution. WISC-IV FSIQ scores were significantly lower in the alcohol-exposed group than in controls, although the mean IQ of control subjects was still considerably below the normal population mean of 100. Among alcohol-exposed individuals, PAE was significantly higher during all three trimesters compared with control subjects, who had very minimal exposure.

Trajectories of overall brain volumes

Total cortical gray matter volume followed an inverted U-shaped trajectory in both subject groups. However, the quadratic portion was more pronounced in controls, while those with PAE exhibited a more linear decline (Fig. 1). White matter volume linearly increased across the age range in both groups, and the alcohol-exposed group had consistently less white matter than controls. In control subjects, total cerebral volume slightly increased and then decreased. Meanwhile in the PAE group, total cerebral volume changed very little. No age–group interaction terms were significant for gray matter, white matter, or total cerebral volume, indicating no significant differences between group trajectories.

Age-related changes within regions

Of 68 cortical regions, 34 had significant ($q < 0.05$) quadratic age-related changes, and 27 had linear age-related changes (Fig. 2). In six regions where the linear term met the stringent significance criterion ($q < 0.05$), the quadratic term remained significant at $p < 0.05$. Thus, for 40 regions, both linear and quadratic

### Table 1. Demographic information for the alcohol-exposed and control groups

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<td>Left, 3 left</td>
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The alcohol-exposed group consisted of 70 subjects. The control group consisted of 63 subjects. Because not all subjects had valid measurements for each variable, actual subject numbers are given in left column for each variable

$^{a}$For gender and handedness, the Mann–Whitney U test statistic was used.

and statistical power. Using these processing techniques, we obtained 34 cortical volumes in each hemisphere, as well as the volumes of total cortical gray and white matter and supratentorial volume.

Reliability analysis. To ensure reliability of imaging data across the three sites, one subject (female, aged 41–45 years during the scanning period) was scanned several times at each site (3 times in Los Angeles, twice in San Diego, 4 times in Cape Town) across a period of 4 years. Data from this subject was processed in the same manner described above to determine cortical volume in 68 brain regions. Intraclass correlation coefficients (2-way mixed analysis for consistency) from data within each site were 0.997–0.999, and the intraclass correlation coefficient of the average data from each site was 0.994, indicating excellent agreement both within and across sites. To compensate for any absolute differences in volume, site was included as a random variable in all analyses.

Statistical analysis. Statistical analysis was performed in SPSS v19 (IBM) and run in two stages: (1) a preliminary analysis for age-related volume changes and (2) a subsequent analysis for age–group interactions. The preliminary analysis tested linear and quadratic age changes within each cortical region using mixed models with site and subject as random variables; alcohol-exposed and control groups were tested separately for these age-related changes. False discovery rate correction was used over the linear and quadratic age terms for both groups in all regions. An additional analysis was conducted to test for gender differences among regions with significant age-related changes. Gender was included in the model, along with the relevant interaction terms with group, age, and age-squared.

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interaction terms were tested, and for 21 regions, linear (and no quadratic) age–
group interactions were tested to deter-
mine whether trajectory differences existed between subjects with and without
PAE. In general, control subjects exhib-
ted more quadratic trajectories of volume
change than the subjects with PAE.

Differences in developmental trajectories
Of the regions examined for group differ-
cences in developmental trajectories, six
had significant age-squared– group inter-
actions \( (p < 0.05) \), indicating signifi-
cantly different patterns of maturation.
No regions had significant linear age–
group interactions. The six regions with
significant trajectory differences were pri-
marily located in posterior brain regions,
specifically in the inferior and superior
parietal regions, as well as the banks of the
superior temporal sulcus and the postcen-
tral gyrus (Fig. 3). In all of these regions,
control subjects had more curvature to
their development trajectories than sub-
jects with PAE, who exhibited less over-
all change in cortical volume.

Gender effects
Of the regions with significant age effects,
eight had significant age– gender interac-
tion effects. The right and left medial or-
itofrontal, the right lateralorbitofrontal,
and the right middle temporal regions had significant linear
age– gender interactions. In all of these areas, females exhibited
faster volume declines than males. In the right precentral region,
there was a significant age-squared– gender interaction such that
females showed a more linear volume decline across the age
range, while males had a more curved quadratic trajectory with
increases followed by decreases. Three left-hemisphere areas had
significant age-squared– group– gender interactions: the precen-
tral, superior parietal, and supramarginal regions. In all three of
these regions, male control subjects had more quadratic tra-
citories of volume change than the other three groups, indicat-
ing a longer period of volume increases. The left superior
parietal area was the only one with gender effects that also had
significant age– group interactions; however, the gender inter-
actions here did not remain significant when all terms were
included in the overall model, while the quadratic age– group
interaction remained significant.

Correlations with facial morphology
Correlations between development rates and facial measures (PFL, lip rank, philtrum rank) occurred in all brain lobes, but
were most prominent in frontal cortices (Fig. 4). Subjects with
PAE demonstrated 32 significant correlations between develop-
ment rates and facial dysmorphology (in 22 distinct regions),
most of which were in frontal lobes. Control subjects had 14
significant correlations (in 10 distinct regions), which were fo-
cused in posterior brain areas. The vast majority of correlations
with facial measures were such that smaller decreases of brain
volume were associated with worse facial morphology. The only

![Figure 1. Developmental trajectories for cortical gray matter, white matter, and total cerebral volumes for control subjects (black) and those with PAE (blue). For PAE subjects, the total brain volume trend was not significant (dashed line). Trajectories were not significantly different between groups for any of these three measures.](image1)

![Figure 2. Colored regions indicate areas with significant (false discovery rate corrected, \( q < 0.05 \)) linear (blue, red, or dark purple) or quadratic (light purple, yellow) age-related changes in the control and/or PAE groups. Yellow regions had significant quadratic age-related changes in controls and significant linear changes in subjects with PAE. The asterisk marks areas that had significant linear changes at \( q < 0.05 \), but in which the quadratic term met uncorrected significance \( (p < 0.05) \). Regions in light purple, yellow, or with an asterisk were analyzed for both age– group and age-squared– group interactions, while other colored regions were analyzed for linear age– group interactions only.](image2)
exceptions to this pattern were in the frontal pole, where controls demonstrated a positive correlation with PFL (right side), and subjects with PAE had negative correlations with lip rank (left side); in both cases, these correlations indicate larger decreases of brain volume are associated with worse facial morphology.

Correlations with cognition and quantities of alcohol exposure

Correlations between WISC-IV FSIQ and annualized cortical volume changes were observed in several posterior brain regions for controls, and in two frontal regions in subjects with PAE (Fig. 5). Most correlations were positive, indicating a relationship between higher IQ and larger volume decreases. The left frontal pole had a positive relationship between IQ and rates of change in the PAE group, such that lower IQ was associated with larger volume decreases. Rates of cortical change were associated with quantities of alcohol exposure in temporal and cingulate cortices (Fig. 5). The temporal lobe regions (right transverse temporal, left parahippocampal) had negative correlations, indicating that greater volumes of prenatal alcohol exposure were associated with larger decreases of volume between scans. In the left posterior cingulate, correlations were positive, indicating that increased alcohol exposure was associated with smaller volume decreases between scans. Because detailed exposure information was primarily available from the subjects in South Africa (n = 32 from Cape Town; n = 6 from California), the relationships observed between exposure amounts and brain development may not be representative of this entire sample.

Discussion

This longitudinal study used structural MRI to demonstrate altered trajectories of brain maturation in children and youth with heavy in utero alcohol exposure. While unexposed control children show a plastic cortex with a prolonged pattern of cortical volume increases followed by equally vigorous volume decreases, individuals with PAE showed only volume loss in most cortical areas; trajectory differences were significant in posterior cortices bilaterally. In both groups, higher overall intellectual functioning and more normal facial morphology were associated with greater volume reductions; these correlations were focused in posterior brain regions for control subjects, but spread throughout the brain in PAE subjects. Finally, within the PAE group, volume

Figure 3. Brain regions with significantly different development trajectories between subjects with PAE and controls are shown in various colors on the brain surface, with accompanying volume–age plots. In general, the PAE trajectories (blue) showed more linear trends with smaller magnitudes of change (both increasing and decreasing volumes) than controls (black), suggesting reduced brain plasticity.
Changes in the temporal and cingulate cortices were negatively and positively associated with the amount of alcohol exposure in utero, indicating that increased alcohol exposure was associated with greater and lesser volume reductions between scans, respectively.

The findings presented here support the notion of trade-offs between plasticity and efficiency, such that children who have a more prolonged period of brain growth and plasticity (marked by cortical volume increases) followed by more synaptic pruning and myelination (which would appear as volume reductions over time on MRI) within posterior cortices process information more efficiently. In contrast, children with PAE exhibit less plasticity and may prematurely prune connections that could ultimately have been useful in developing more efficient functioning.

The differences in maturational trajectories observed may be due to PAE or may be related to prolonged dysfunctional experiences throughout childhood and adolescence. Children with PAE experience more cognitive, behavioral, and social difficulties than their unexposed peers (Mattson et al., 2011; Riley et al., 2011). Experiences within one's environment help build brain connections, and these experiences are highly likely to differ in children with neurodevelopmental disorders because of the deficits such children have. It has long been known from animal studies that environmental enrichment can affect the architecture of the brain, and that there are critical periods during which the brain is primed to adapt to certain types of external sensory input (for review, see Baroncelli et al., 2010). Even in adults, structural brain changes have been observed with MRI after learning new skills (for review, see Fields, 2011). The same could be happening in children with PAE and other neurodevelopmental disorders where the wiring of the brain throughout development is happening either in the absence of functional behaviors within the environment or in the presence of behaviors that are dysfunctional. Future studies with more detailed histories may be able to examine the separate effects of prenatal alcohol exposure and childhood environments, providing valuable insight into the factors contributing to altered development trajectories.

In this report, general intellectual functioning predicted development rates in posterior cortices in control subjects and more widespread areas in individuals with PAE. Relationships between gray matter development trajectories and IQ have been shown previously, with children of superior intelligence demonstrating later peak cortical thickness than children with average or high intelligence (Shaw et al., 2006). The results here suggest a similar relationship in posterior cortices of children of low-to-average IQ but with no prenatal exposure and in the rostral mid-
point in subjects with PAE (Astley et al., 2009; Roussotte et al., 2012; Yang et al., 2012b), including one study that related inferior temporal cortical thickness to PFL in an overlapping group of subjects, cross-sectionally at time 1 (Yang et al., 2012a). Here, correlations with facial measures indicated that larger volume decreases were associated with more normal facial morphology. The only exception was the frontal pole, where smaller volume decreases were observed in subjects with the most normal facial morphology (PFL and lip rank). Correlations between facial characteristics and brain structure are not entirely surprising in control subjects, as the brain and the face develop at similar times in utero, and these relationships are not necessarily indicative of cognitive or behavioral dysfunction. The brain–face relationships were much more widespread in the alcohol-exposed individuals, for whom facial dysmorphology predicted brain changes in multiple brain areas. Except for the frontal pole, worse facial dysmorphology (higher lip or philtrum ranks, shorter PFL) was associated with less change between scans (smaller volume decreases) in subjects with PAE.

It is possible that the timing and quantity of PAE at any given time point, particularly during the early phases of pregnancy, when the face is forming, affect both the brain and the face more negatively than exposure during later stages of pregnancy, when the face is already more fully formed (O’Leary-Moore et al., 2011). While it is difficult to quantify the timing and severity of exposure
alcohol-exposed subjects demonstrated steeper volume drops during adolescence than controls in frontal and temporal cortices, whereas they tended to have less overall change in parietal and occipital areas than control subjects.

Though the mechanisms remain somewhat unclear, the parieto-cortical changes have been consistently implicated as abnormal in children and adolescents prenatally exposed to alcohol, demonstrating reduced cortical volume (Archibald et al., 2001; Chen et al., 2012), increased gray matter density (Sowell et al., 2001), and altered shape (Sowell et al., 2002), in addition to white matter abnormalities in pathways connecting to parietal cortices (Lebel et al., 2008; Sowell et al., 2008b). Cortical thickness is altered in parietal areas, though some studies report thicker cortices in children and youth with PAE (Sowell et al., 2008a; Yang et al., 2012a), and one study found thinner cortices in alcohol-exposed subjects (Zhou et al., 2011). The differing cortical volume trajectories observed here may help explain the apparently conflicting findings in previous studies, as the differences between subjects with PAE and unexposed individuals change over time.

In conclusion, we observed significantly altered development trajectories in children and youth with heavy PAE. These individuals demonstrated less volume change over time than their unexposed peers, suggesting reduced brain plasticity. Furthermore, this reduced plasticity was related to lower IQ and more severe facial dysmorphism. These long-term effects may be due specifically to drinking during pregnancy, but are likely also influenced by dysfunctional childhood experiences. These observations indicate that brain abnormalities in children and youth with PAE are not static, and have important implications for early treatments and interventions within this disorder (Adnams et al., 2007) that may be able to “correct” the developmental trajectories. Finally, these implications may not be limited specifically to in utero alcohol exposure, but are likely also relevant to other neurodevelopmental disorders.

References


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