Developmental Cognitive Neuroscience 16 (2015) 155–165

Contents lists available at ScienceDirect

Developmental Cognitive Neuroscience

journal homepage: http://www.elsevier.com/locate/dcn

Executive function and cortical thickness in youths prenatally exposed to cocaine, alcohol and tobacco

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 19 January 2015
Accepted 23 January 2015
Available online 2 February 2015

Keywords:
Prenatal alcohol exposure
Adolescent brain development
Socio-economic status (SES)
Cortical thickness
Executive functions
Brain-behaviour relationships

ABSTRACT

Small and detrimental, albeit inconsistent, effects of prenatal cocaine exposure (PCE) during early childhood have been reported. The teratogenic effects of prenatal alcohol (PAE) and tobacco exposure (PTE) on neurobehavior are more firmly established than PCE. We tested if co-exposure to all three drugs could be related to greater differences in brain structure than exposure to cocaine alone. Participants ($n = 42$, PCE = 27; age range = 14–16 years) received an executive function battery prior to a T1-weighted 3 T structural MRI scan. Cortical thickness was measured using FreeSurfer (v5.1). Fetal drug exposure was quantified through maternal self-reports usage during pregnancy. Using general linear modeling, we found no main effects of PCE on cortical thickness, but significant main effects of PAE and PTE in superior and medial frontal regions, after co-varying for the effects of age, sex, and each drug of exposure. Significant alcohol-by-tobacco interactions, and significant cocaine-by-alcohol interactions on cortical thickness in medial parietal and temporal regions were also observed. Poly-drug exposure and cognitive function also showed significant interactions with cortical thickness: lower cortical thickness was associated with better performance in PCE-exposed adolescents. Results suggest that although children with PCE have subtle but persistent brain cortical differences until mid-to-late adolescence.

1. Introduction

1.1. Prevalence of substance use during pregnancy

Substance use during pregnancy is highly prevalent in the US and internationally.

As prenatal substance use is associated with poorer developmental outcomes during infancy and also during adolescence and into adulthood, it is an important public health issue based on disability (Bandstra et al., 2010; Lupton et al., 2004; Spohr, 2007). Based on combined data from 2011 to 2012, it is estimated that 5.9% of pregnant women are illicit substance users in the U.S. out of which 0.2% of all pregnant women reported using cocaine (SAMHSA, 2012). In the same report, 8.5% of women reported drinking alcohol while pregnant, with 2.7% reporting binge drinking; and almost 16% of women reported tobacco use when pregnant. Women who use drugs during pregnancy are typically poly-substance users (Havens et al., 2009; Muhuri and Gfroerer, 2009; Keegan

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http://dx.doi.org/10.1016/j.dcn.2015.01.010

et al., 2010). Given that exposure to one drug is known to cause harm to the developing fetus, (Valenzuela et al., 2012; Williams et al., 2012; Zugno et al., 2013) exposure to more than one substance, combined with their potential interactive effects could elevate the risks to the developing fetal brain and cause changes to the developing brain.

1.2. Prenatal cocaine exposure

Several studies in children have shown a small, but consistent detrimental effects of prenatal cocaine exposure (PCE) during infancy and early childhood. Smaller head circumferences, preterm birth, and increased subependymal hemorrhages (Singer et al., 2002; Frank et al., 1999; Buckingham-Howes et al., 2013; Ackerman et al., 2010) are more common in children with PCE. However, meta-analyses suggest reduced effect sizes of cocaine exposure with age (Held et al., 1999), such that effects of PCE exposure are smaller in childhood compared to the effects seen at birth (Ackerman et al., 2010), and are much harder to detect by adolescence (Buckingham-Howes et al., 2013; Hurt et al., 2008). For instance, extensive behavioral and neurocognitive batteries have found no effects of PCE on executive functions, language, memory, or behavior (Singer et al., 2002; Hurt et al., 2009) in adolescents with PCE. Nonetheless, despite smaller than expected behavioral differences, differences in brain structures of the deep gray matter (Roussotte et al., 2010; Avants et al., 2007) and white matter (Lebel et al., 2013; Li et al., 2009) have been reported in children and adolescents. Therefore, it is possible that subtle brain structural differences might persist during adolescence in those with PCE.

1.3. Prenatal alcohol and tobacco exposure

The effects of prenatal alcohol exposure (PAE) on neurobehavioral outcomes are more firmly established than cocaine. Neurobehaviorally, poorer working memory and executive functioning (Mattson et al., 2012; Astley et al., 2009), as well as high rates of conduct disorders cause lifelong disability (Lupton et al., 2004). A dose-response relationship has also been found in IQ with increasing maternal alcohol-intake (Goldschmidt et al., 1996). Effects on brain structure due to PAE include smaller and reduced gray matter (Archibald et al., 2001; Lebel et al., 2012); and reduced white matter volumes (Gautam et al., 2014) in children and adolescents. Abnormalities in cortical thickness (Zhou et al., 2011; Sowell et al., 2008) as well differential longitudinal trajectories of brain activation (Gautam, in press) of the frontal, temporal, and parietal regions have also been documented in those with Fetal Alcohol Spectrum Disorders (FASD).

Similar to PAE, prenatal tobacco exposure (PTE) is associated with increased risk for developing behavioral and psychiatric problems in adolescence (Ekblad et al., 2010). Higher risks of spontaneous abortions in smokers (Blanco-Munoz et al., 2009), lower birth weights and smaller head circumferences in children born to smokers (Kallen, 2000), and differences in cortical thickness in those with PTE (Toro et al., 2008; Liu et al., 2013) have been reported. A meta-analysis has also found lower academic achievement in children of mothers who smoked while pregnant (Clifford et al., 2012).

1.4. Co-exposure to prenatal substances

Co-exposure to poly-drugs might have interactive effects in the fetus, over and beyond the effects of each drug taken alone. Rivkin et al. (2008) found that while PCE, PAE, and PTE were each related to smaller brain volumes, newborns with the most co-exposure had the smallest brain regions. A potential mechanism for detrimental effects in metabolism is production of cocaethylene, which could have a higher potential for toxicity than the ingestion of each of these drugs alone (McCance-Katz et al., 1998). Poly-substance use is also related to highest overall incidences of lowered fetal birth weights compared to individual substances in human studies (Janisse et al., 2013; Singer et al., 2002) while animal studies suggest that the effects of prenatal exposure might cause heritable changes in the genetic makeup (Vassoler et al., 2014), with synergistic effects of ethanol and cocaine also being reported by others (Tallarida et al., 2014). Finally, while a previous study has also suggested that co-exposure to cocaine and tobacco are related to differences in brain microstructural properties and poorer behavioral outcomes in children (Warner et al., 2006), the effects of poly-drug exposure and their interactions on cortical surfaces are not fully known.

1.5. Effects of socio-economic status and maternal health/education

The use of substances during pregnancy is related to additional health risks for children, above and beyond the direct risks of drug exposure. Previous studies have shown that mothers of children with PCE tend to be of lower socioeconomic status (SES) and have lower levels of education – factors that have been associated with poorer visuo-motor co-ordination and poorer intellectual functioning in the offspring (Singer et al., 2002; Arendt et al., 2004; Bandstra et al., 2002). Similar results have been found for children with PAE and PTE: mothers who drink while pregnant are significantly more likely to be of lower SES and have poorer health behaviors (May et al., 2011; Connor and McIntyre, 1999). In contrast, better health behaviors by mothers and higher family income have been previously associated with better health outcomes in typically developing children (Adler and Rehkopf, 2008; Hackman et al., 2010). For instance, mothers with higher education levels were also more likely to stop smoking while pregnant compared to mothers with lower education levels (Connor and McIntyre, 1999; Yu et al., 2002). Therefore, studies of prenatal drug exposure effects on children need to consider the socio-economic status, maternal education, and income levels to rule out confounding effects of these variables on brain and behavior.

The aim of the current study was to investigate the effects of PCE on cortical thickness in adolescents and how this is affected by co-exposure to alcohol and tobacco in children with PCE. A secondary aim was to test the effects of exposure to cocaine and other drugs on cognitive function in relation to cortical thickness. Given the well-known

effects of alcohol and tobacco on adolescent neurobehavior, we hypothesized that effects of PCE on cortical thickness would be smaller than the effects of PAE and PTE. We also predicted that there would be significant interactive effects of cocaine exposure with alcohol and tobacco exposure (i.e., PCE vs PTE and PCE vs PAE) on cortical thickness and in brain–cognition relationships.

2. Methods

51 subjects between the ages of 14 to 16 years were drawn from a prospective, longitudinal study on the effects of prenatal cocaine to participate in the current study. A complete, detailed description of the longitudinal study has been published previously (Eyler et al., 1998). Institutional Review Board approval of the study was obtained at University of Florida. After an explanation of procedures, the parents/legal guardians provided written informed consent and the adolescent participants provided written assent to participate in the study.

All youth participants were consented the following way: Subjects who lived within 2 h of the study site were invited to participate using a letter that was accompanied by a study-designed brochure explaining magnetic resonance imaging (MRI). Follow-up phone calls to participants were made as part of the recruiting process. For subjects who showed interest, the purpose of the study and study procedures were explained to both the parent/legal guardian and the adolescent subject. A screening questionnaire was also completed by telephone to ensure that subjects met inclusion criteria (e.g., right-handed, no metal in the body, etc.) On the day of study participation, the informed consent form was read and reviewed. Youth were provided a $50 Walmart gift card for participation.

Subjects underwent a brief neurocognitive battery administered by TDW, a licensed neuropsychologist who was blinded to the PCE exposure status of each subject followed by a structural scan. Of these 51 subjects, 4 subjects were excluded due to the following reasons: obvious cognitive impairment (1), metal artifacts in body (1), developed claustrophobia (1), history of head injury with loss of consciousness for >5 min (1) and structural data was lost for 5 subjects. The final sample size was 42 with 27 prenatally exposed and 15 non-PCE exposed age-matched controls. Maternal cocaine use in the longitudinal study was determined using self-report as well as through unanticipated urine tests, and drug usage were quantified for the same time period. The amount and frequency of maternal drug use was obtained during private, structured interviews conducted at the end of each trimester or after birth if the woman had no prenatal care. The frequency of cocaine exposure was quantified by calculating the total number of weeks during gestation and 3 months prior to pregnancy then dividing by the total number of 12 weeks plus the number of weeks of the entire pregnancy. The decision to use gestational week ratio for cocaine use stemmed from the fact that the primary mode of ingestion of cocaine was "rocks" which varied widely by size, shape and quality. Hence, quantity and dosage could vary widely and could not be pinpointed with accuracy. Thus, the proportion of actual use of cocaine was obtained. In contrast, prenatal alcohol and tobacco use could be measured with better accuracy in regards to dosage. Alcohol use was measured as the average number of absolute ounces of alcohol consumed per day during pregnancy (same time period as cocaine). Women who consumed greater than 1.5 ounces of alcohol per day were excluded to minimize the number of children with fetal alcohol effects. Tobacco exposure and marijuana exposure were, respectively, defined as the average number of cigarettes per day and the average number joints per day that were smoked during pregnancy (same time period as cocaine). Only 10 mothers in the PCE group and 1 control reported marijuana exposure, and of these, 6 reported consumption of less than 0.03 joints/per week. Hence, marijuana exposure was not further explored in the study due to small sample size and very low levels of use.

2.1. Image acquisition

MRI scans were acquired on a 3 T Philips Achieva, TR = 8.1 ms, TE = 3.7 ms, flip angle = 8°, matrix size = 240 × 240 × 234; total acquisition time = 10 min 14 s, voxel size = 1 mm × 1 mm × 1 mm.

2.2. Image processing

Images were processed with FreeSurfer software (v5.1, http://surfer.nmr.mgh.harvard.edu/). FreeSurfer allows for semi-automatic reconstruction of the cortical surface using T1-weighted MRI images. Major steps during image analyses include motion correction, removal of non-brain tissue, automated Talairach transformation, subcortical and cortical matter segmentation, intensity correction and delineation of gray/white/pial boundaries (Dale et al., 1999; Fischl et al., 1999). After the formation of cortical models, deformable procedures are applied including cortical inflation, registration to a spherical atlas and parcellation of the cerebral cortex into gyral and sulcal units (Desikan et al., 2006). For the participants with longitudinal data, the T1 scans were additionally processed through the longitudinal stream (Reuter et al., 2010). Cortical thickness maps are created using both signal intensity and continuity information from the 3D volume from MR images where thickness is calculated as the closest distance from a pial to white matter boundary at each vertex (Dale et al., 1999). Reliabilities for cortical thickness measures obtained using FreeSurfer have been previously described (Han et al., 2006). After initial processing by FreeSurfer, all MRI scans were visually checked slice-by-slice to ensure there was no misclassification of gray and white matter voxels; scans were reprocessed if errors were detected and rechecked visually a second time. Thickness maps were spatially smoothed with a Gaussian kernel with a half maximum width of 10 mm. Maps were then averaged across participants using a spherical aligning method for cortical folding patterns (Fischl et al., 1999).

2.3. Cognitive battery

Executive functioning was assessed using the Trail Making Test Parts A and B, the Stroop Color and Word Test,

and the Iowa Gambling Task. The Trail Making Test is a timed test which measures processing speed, attention, sequencing, visuo-spatial skills, and motor skills. The second part of the test (TMT-B) also requires mental flexibility as examinees must rapidly draw lines between letters and numbers in sequence (e.g., 1 to A, A to 2, 2 to B) (Reitan and Wolfson, 1992). The Stroop Color Word Test (STCW) is a test of inhibitory control (Golden et al., 2003). For this study, scores related to the 3rd part of the test of the STCW where participants have to rapidly name colors of the ink, while inhibiting their response to reading color names, have been used. The Iowa Gambling Task (IGT) examines decision-making and is sensitive to planning and goal-setting impairments (Bechara et al., 2000). For the IGT, participants have to choose and switch between decks of cards which, unknown to the participants, have inherent losses or gains related to the decks. Longer time-to-completion for the Trail Making Test denotes poorer performance while lower scores in Iowa Gambling Task reflect poorer performance. Higher scores in the Stroop task denote better performance.

2.4. Analyses

Analyses were run in FreeSurfer (v5.1) using General Linear Modeling. Both exposed and comparison groups did not differ on socio-economic status. Main effects for cocaine exposure were modeled after controlling for alcohol and tobacco exposure, as well as for age and sex. Interactions between cocaine and alcohol, cocaine and tobacco, as well as alcohol and tobacco exposure, were modeled separately. Next, main effects of each drug on the each of the cognitive variables were investigated after controlling for drug exposure, age, and sex. Interactions of cognitive variables with each of the three substances were also modeled. All analyses were run for the whole group, while co-varying for various levels of exposure to cocaine, alcohol, and tobacco. Because controls had scores of zero for PCE, to verify that that the findings are driven by those with PCE, only results that were significant for the whole group and verified in the cocaine-exposed group have been reported for effects between drug interactions and cognitive variables. All analyses investigating the main effects and interactions co-varied for remaining drugs of exposure. Analyses for the right and left hemispheres were conducted separately. All analyses were then corrected for multiple comparisons using permutation testing with 1000 iterations and thresholded at $p < 0.01$. As both prenatal alcohol and tobacco exposure has previously been linked with abnormalities of cortical thickness (Sowell et al., 2008; Yang et al., 2012) and brains structural abnormality (Janisse et al., 2013), we did not think it was appropriate to control the analyses for average cortical thickness, as controlling in this way might inflate or deflate actual biological differences between groups. Hence, analyses were conducted on smoothed cortical thickness measures at 10 FWHM, taking a vertex-wise approach and controlling for exposure amounts, age, and sex for all of the cohort.

3. Results

Sample demographics are presented in Table 1. There were no significant group differences in cognitive performance on any of the tests. Groups also did not differ by subject age, mother's education, socioeconomic status, or gestational age by design. Maternal age was significantly higher in those with PCE than in controls ($p < 0.001$), as was the case in the entire cohort at enrollment (Eyler et al., 1998). By design, average cocaine exposure was significantly higher in PCE group ($p < 0.001$). Furthermore, average tobacco exposure was also significantly higher in PCE group compared to controls ($p < 0.001$ for both), and a trend for higher alcohol exposure in PCE group ($p = 0.057$) was also observed. Marijuana exposure did not differ significantly between the two groups. There were no significant group differences between the number of youth who had hair samples tested positive for cocaine use at 14 years of age. Furthermore, IQ as measured through the Weschler Intelligence Scale for Children-III was not significantly different between controls and PCE group [Mean (S.D.): Controls = 83(20), PCE = 86(14); $p = 0.57$]; thus analyses were not controlled for Global IQ.

3.1. Cortical thickness and substance use: main effects and interactions of drug exposure

There were no main effects of PCE on cortical thickness in the whole group. There was a significant main effect of PAE with higher PAE associated with lower cortical thickness in the left orbitofrontal region (Fig. 1a). Significant relationships were also observed with PTE, such that more exposure was associated with thicker cortices in the right superior frontal region (Fig. 1b).

There were significant interactions related to exposure to both cocaine and alcohol, in the right precuneus region (Fig. 2a) and similarly, between alcohol and tobacco co-exposure in the left superior temporal and the right middle and inferior temporal regions (Fig. 2b). While higher tobacco and cocaine exposure were related to greater cortical thickness in these regions, higher exposure to alcohol was related to lower cortical thickness in the whole group.

3.2. Cortical thickness and cognitive function: interactions between cognitive variables and drug exposure

For these sets of analyses, interactions with cognitive measures were repeated after splitting the sample into controls and PCE, to further confirm that significant results with prenatal substances were predominant in those with cocaine exposure. Hence, analyses were re-run only in those with prenatal cocaine exposure and only the results that remained significant in this subset of adolescents are reported.

None of the interactions between the prenatal substances and cortical thickness were significant for IGT. For all other cognitive variables, only significant negative correlations (poorer cognition related to thicker cortices) were

Table 1
Demographic and cognitive variables.

	Controls		Cocaine exposed		*t*-Stat	*p*-Value
	Mean (S.D.)	Range	Mean (S.D.)	Range		
Age (years)	15.184 (0.613)	14.06 to 15.95	15.379 (0.455)	14.5 to 16.29	−1.030	0.290
Current cocaine use[a]	3 yes/12 no	–	5 yes/22 no	–	1.233	0.228
Sex	8 girls/7 boys	–	18 girls/9 boys	–	0.822	0.418
Gestational age (weeks)	38.870 (1.302)	37 to 42	38.480 (2.082)	33 to 42	0.736	0.466
Birth weight (kg)	3.291 (0.542)	1.913 to 4.02	3.023 (0.464)	2.174 to 3.96	1.613	0.119
Head circumference at birth (cm)	34.113 (1.439)	31 to 36.5	33.811 (1.218)	32 to 36.5	0.688	0.498
Maternal age (years)	21.530 (3.603)	18 to 29	27.740 (4.679)	19 to 36	−4.795	**<0.001**
Maternal education (years)	11.130 (1.060)	9 to 12	11.630 (1.334)	10 to 14	−1.322	0.195
Hollingshead SES at birth[b] (1 = highest SES; 5 = lowest SES)	4 (n = 1) 5 (n = 14) –	– – –	3 (n = 1) 4 (n = 6) 5 (n = 20)	– – –	2.397[b]	0.302[b]
Cocaine exposure (number of weeks of exposure/total gestational weeks)	0	–	0.411 (0.254)	0.037 to 1	−8.389	**<0.001**
Alcohol exposure (ml/weeks)[c]	0.382 (0.712)	0 to 2.18	1.443 (2.630)	0 to 10.83	−1.97	0.057
Tobacco exposure (cigarettes/weeks)[c]	2.936 (10.091)	0 to 39.40	57.005 (55.565)	0 to 150.89	−4.912	**<0.001**
Marijuana exposure (joints/weeks)[c]	0.0167 (0.064)	0 to 0.25	0.905 (2.615)	0 to 10.40	−1.765	0.089
Iowa gambling task (raw score)	−5.23	−30 to 38	−3.12	−46 to 70	−0.285	0.777
Stroop color-word task (raw score)	42.47	21 to 61	39.15	23 to 60	1.11	0.274
Trail Making Test A (s)	12	6 to 21	12.81	6 to 27	−0.545	0.590
Trail Making Test B (s)	28.33	14 to 61	29.96	13 to 62	−0.404	0.688

Statistically significant differences are presented in bold font.
[a] Hair tested positive for cocaine use at 14 years.
[b] Chi-squared test of significance was performed and Pearson's Chi-squared statistic and *p*-value has been reported.
[c] While cocaine has been reported in weekly fraction use over the pregnancy period, the other three drugs are reported as ingested amounts/week during pregnancy.

observed between cognitive variables and cognitive function in those with PCE, and are described below.

3.2.1. Interactions with cocaine amount

Significant interactions were observed for TMT-B in left hemisphere in the posterior parietal/pericalcarine region and the right precuneus, fusiform and lateral occipital regions (Fig. 3a) with cocaine exposure. Interactions with cocaine exposure were not significant for STC and TMT-A. The interactions revealed that higher cocaine exposure was related to positive relationships between test scores and cortical thickness. In contrast, in those with lower cocaine exposure thinner cortices were related to better test scores.

3.2.2. Interactions with alcohol amount

Similarly, there were significant interactions between STC and alcohol exposure bilaterally in the precuneus and also in the left superiorparietal, middlefrontal, and the right post-central regions. Interactions were significant in the left lateral occipital and post-central regions for TMT-A (Fig. 3b) and in the right precuneus for TMT-B (Fig. 3c). Similar to interactions with cocaine, higher alcohol exposure was related to positive relationships between test scores and cortical thickness, but lower alcohol exposure was related with thinner cortices and better test scores.

3.2.3. Interactions with tobacco amount

Significant interactions were observed for TMT-A in left postcentral and lateraloccipital, as well as the right supramarginal, inferior parietal, paracentral, and superior parietal regions (Fig. 3d). For TMT-B, significant interactions were observed for the left superior frontal and fusiform and right inferior frontal and lateral occipital regions (Fig. 3e). Finally, for STC, interactions were observed bilaterally in the temporal regions (Fig. 3f). In those with higher tobacco exposure, thinner cortices were related to better performances, but in those with lower tobacco exposure, better performances were related to thicker cortices.

4. Discussion

Results suggest that while behavioral effects of prenatal cocaine by itself on adolescents between the ages of 14–16 are not significant in our sample, co-exposure to alcohol and tobacco *are* related to differences in cortical thickness. In the current study, there were limited main effects of cocaine, but higher cortical thickness was related to more tobacco consumption, and lower cortical thickness was found for those with alcohol exposure. Interestingly, in those with cocaine exposure, there were also significant alcohol-by-tobacco interactions, and significant

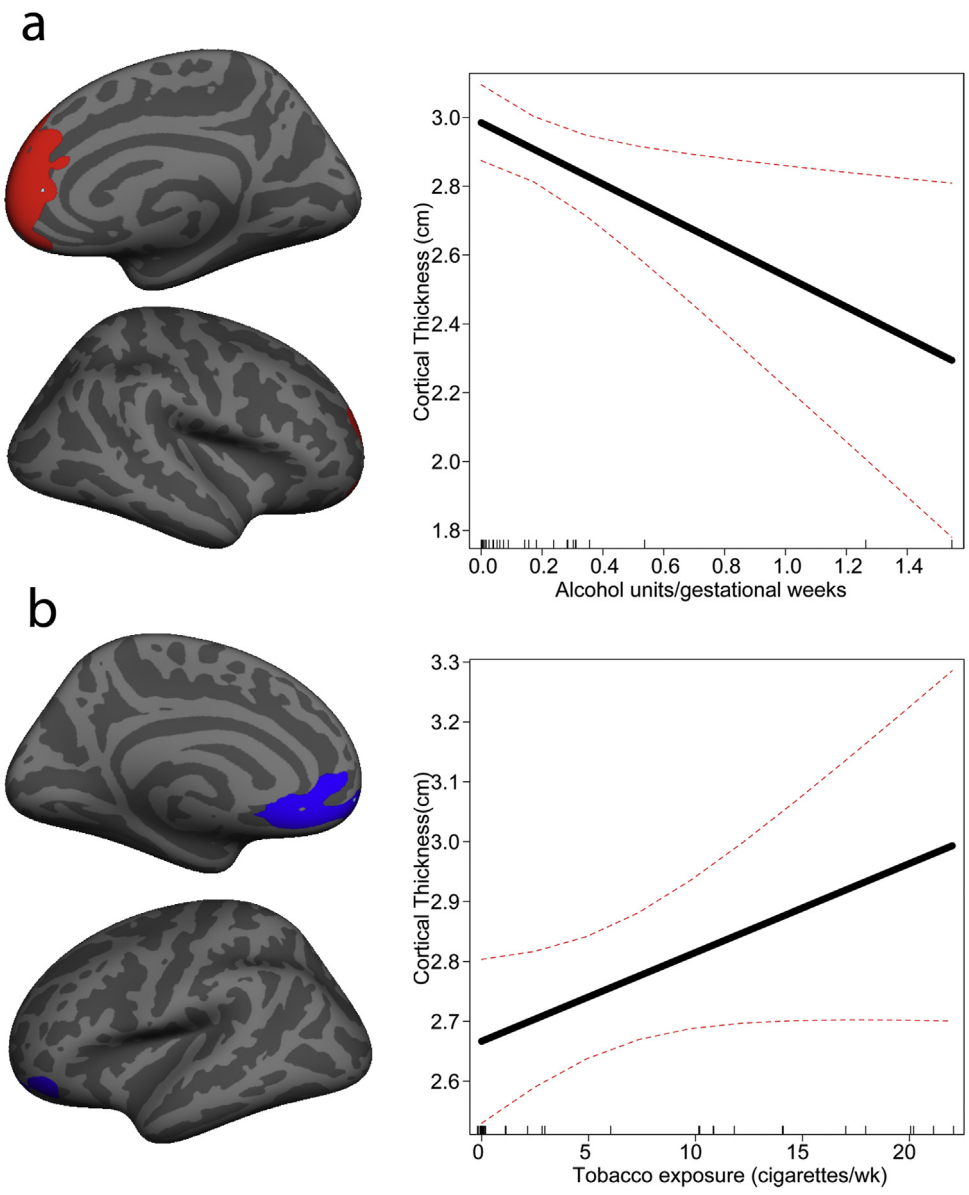

Fig. 1. Main effects of (a) alcohol and (b) tobacco exposure after controlling for the effects of co-exposed drugs, age, and sex. Results corrected at $p < 0.01$. Peak mean voxels have been plotted to illustrate the interactive effects.

cocaine-by-alcohol interactions in cortical thickness. Similarly, all cognitive variables showed significant interaction effects due to poly-drug exposure, where co-exposure to *both* alcohol and tobacco was related to abnormalities in cortical thickness. These differences were significant even after taking socioeconomic status, gestational age, and mother's education into account. Our results are important, as they show that despite similar neurobehavioral performance, there are subtle structural effects of poly-drug exposure on cortical thickness in mid-to late adolescence. Studied at age ~10, a similar sample of children with prenatal cocaine exposure showed significantly poorer performance in TMT-B tasks compared to non-cocaine exposed children (Warner et al., 2006). Present results suggest that these cognitive differences may not persist until mid-adolescence. Instead, there are more subtle brain structural differences present in those who both have PCE *and* were co-exposed to either alcohol or tobacco.

4.1. Comparisons with previous studies

Our results are largely consistent with previous studies. Mixed findings have been reported in regards to prenatal cocaine exposure in adolescents. For instance, while abnormal brain activations were observed in those with PCE, no performance differences were observed between groups (Li et al., 2009, 2011). Similarly, while some studies

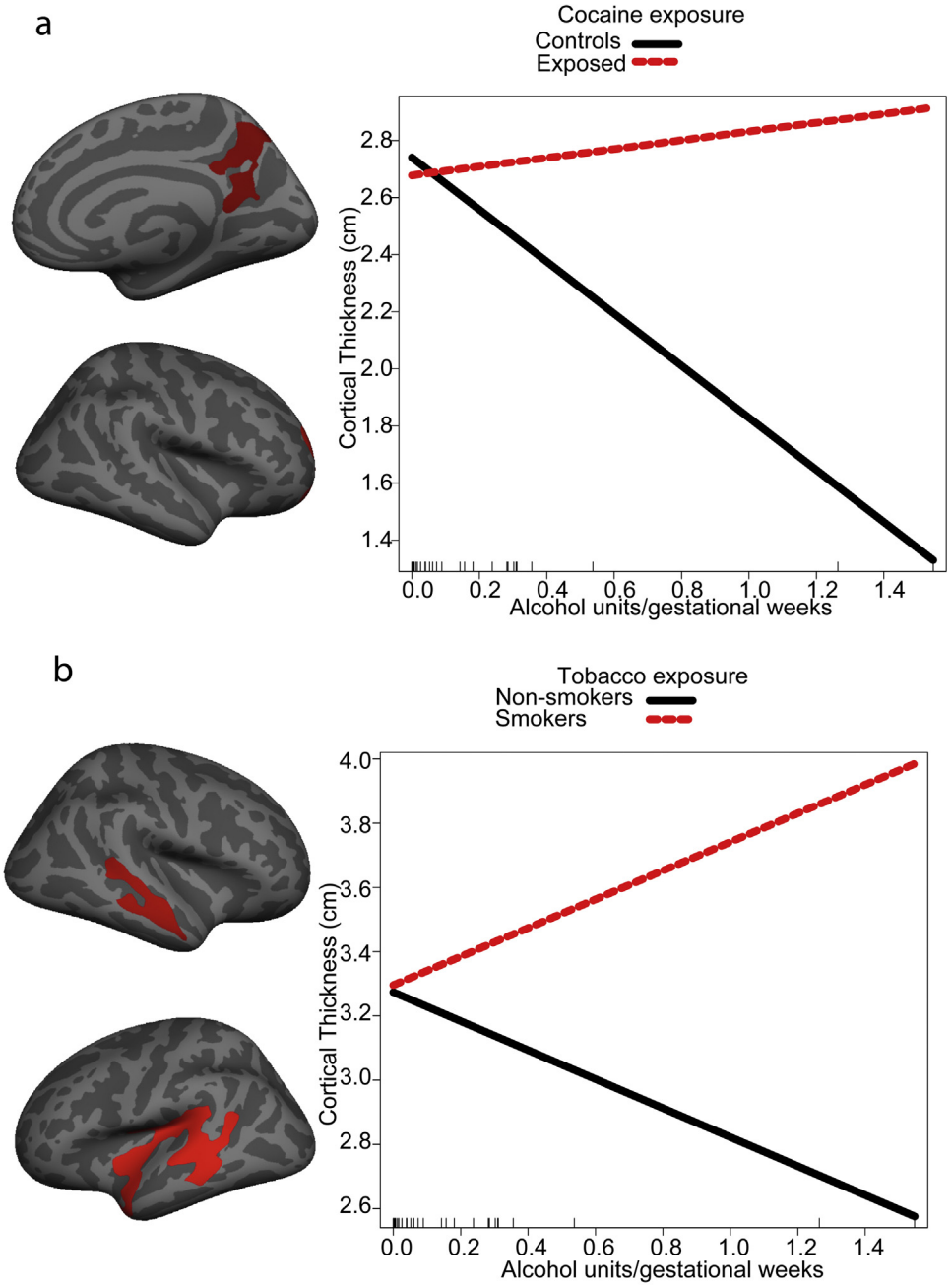

Fig. 2. Cortical thickness interactions for (a) cocaine-by-alcohol and (b) tobacco-by-alcohol exposure after controlling for the effects of co-exposed drugs, age, and sex in the whole group. Results corrected at $p < 0.01$. Peak mean voxels have been plotted to illustrate the interactive effects.

have reported structural differences in the corpus callosum between those with or without PCE (Liu et al., 2013), and smaller brain structures in exposed children (Avants et al., 2007; Rivkin et al., 2008), others have only found minute differences in brain volume (Roussotte et al., 2012). However, meta-analytic reviews support a small but significant detrimental effect of PCE on brain structure and behavior (Buckingham-Howes et al., 2013; Ackerman et al., 2010), with smaller group differences detected during adolescence, compared with during childhood (Held et al., 1999). Finally, only one previous study has specifically examined cortical thickness differences in PCE adolescents with controls (Liu et al., 2013), and reported thinner cortices only in medial prefrontal areas in PCE compared to controls. This study also did not find any subcortical volume differences as an effect of cocaine exposure. In the current study,

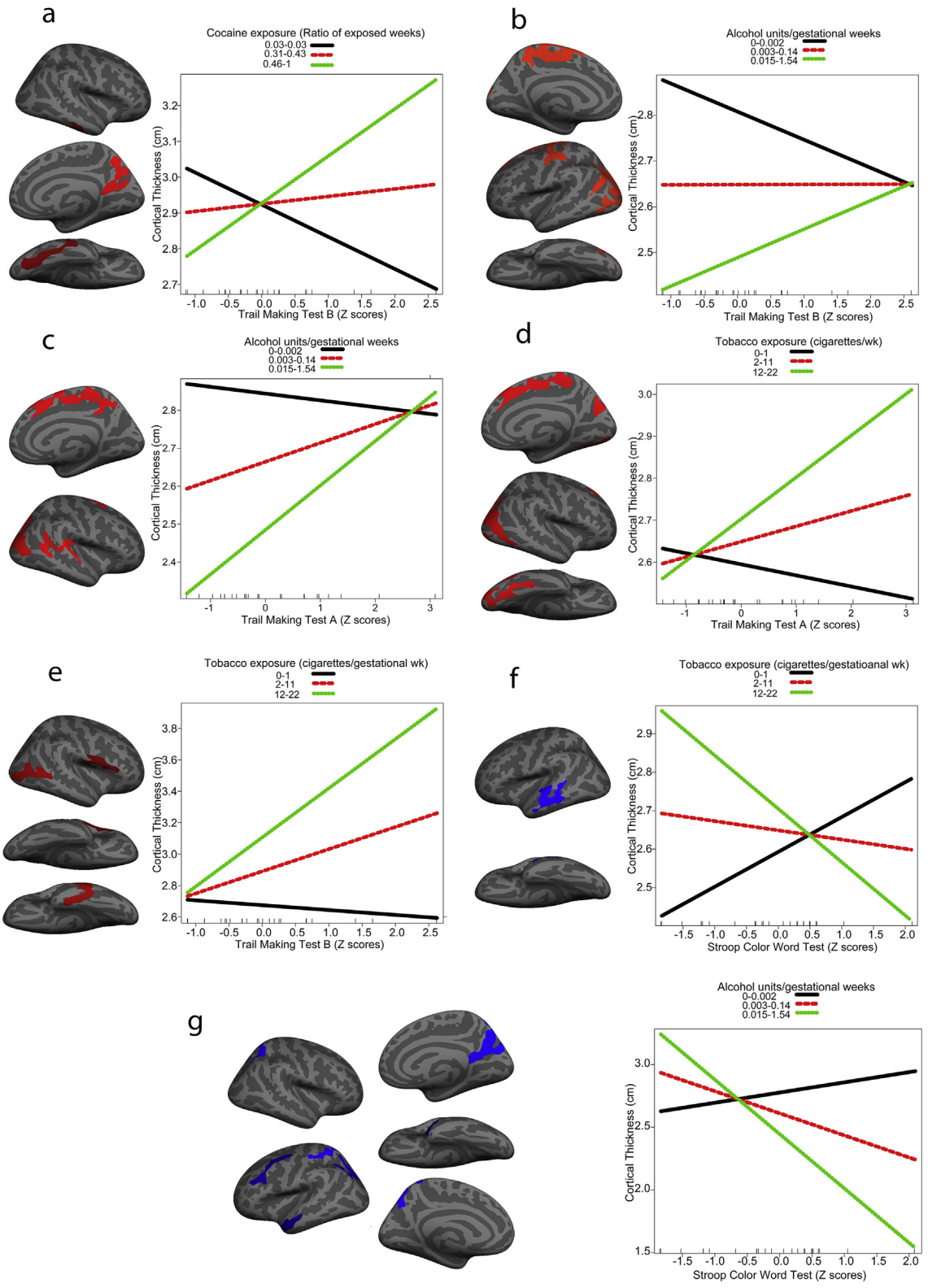

Fig. 3. Cortical thickness interactions for (a)cocaine-by-Trail Making Test B, (b)alcohol-by-Trail Making Test B, (c)alcohol-by-Trail Making Test A, (d)tobacco-by Trail Making Test A, (e) tobacco-by-Trail Making Test B, (f) tobacco-by-Stroop Color Word Test, (g)alcohol-by-Stroop Color Word Test after controlling for effects of co-exposed drugs, age, and sex only in those with prenatal cocaine exposure. Results corrected at $p < 0.01$. Higher values represent poorer performance for TMT_A/B, but better performance for STCW. Peak mean voxels from clusters have been plotted to illustrate the interactive effects. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

we similarly did not find a main effect of cocaine, however observed cortical thickness abnormalities in relation to co-exposure with both alcohol and tobacco.

4.2. Substance use in prenatally exposed adolescents and the role of socio-economic status

As the two groups did not differ on their use of illegal substances as when tested at age 14, and a very small proportion of youth were users (3 in controls, 5 in exposed) (Table 1), our findings are more likely due to prenatal exposure and not due to current substance use by youth in the study. Mixed findings have been reported on usage of illicit drugs by those with and without PCE. Some have reported that adolescents with PCE are also more likely to initiate substance abuse compared to controls (Delaney-Black et al., 2011; Frank et al., 2011). In contrast, others have found no such differences in drug use initiation or other problem behaviors (Warner et al., 2011; Gerteis et al., 2011) in youths with PCE compared to controls. Our results of no group differences in either cognitive function or in behavior are consistent when taken in the context of previous studies which have shown a large effect of socio-economic status and mother's education in future behavioral problems in adolescents (Bandstra et al., 2010). This is because, it is possible that some of the main effects of cocaine that have been found previously were confounded due to not taking the mother's SES, education, and/or family income into account. Effects of early-life and ongoing socioeconomic status such as maternal education, and maternal IQ have been found to largely predict a substantial proportion of variance in later cognitive development in children and adolescents (Batty et al., 2006). In the current study, we attempted to carefully match both groups for these confounding variables, hence, by controlling for these factors known to be related to detrimental neurobehavioral outcomes in adolescents, we were able to investigate more subtle brain structural differences in PCE youth compared with control youth.

4.3. Brain structural effects of prenatal exposure to alcohol and tobacco and co-exposure

Both PAE and PTE have been previously shown to significantly relate to abnormalities in cortical morphology. For instance, those with PAE show altered trajectories of gray matter volume development (Lebel et al., 2012), have decreased white matter volumes (Gautam et al., 2014) and also show abnormal cortical thickness compared with typically developing children (Sowell et al., 2008). Abnormalities are also observed in subcortical regions in those with PAE, where smaller volumes of the thalamus, putamen and other structures have been reported (Archibald et al., 2001; Nardelli et al., 2011). In the current study, those with PCE showed differences in correlations with cortical thickness and cognitive function. Given the previous findings of brain structural differences, especially with gray matter, these observed relationships could possibly be related to altered gray matter development during adolescence such as differences in neuronal pruning (Huttenlocher, 1984). Similarly, PTE has been linked with higher mortality, and higher psychiatric illnesses and behavioral problems in children and adolescents (Ekblad et al., 2010). Maternal smoking during pregnancy has also been linked with lowered academic achievements including poorer vocabulary, memory, and executive function, reviewed in more detail here (Clifford et al., 2012; Batty et al., 2006). As it is evident that the neurobehavioral sequelae of PAE and PTE are severe, it is possible that cocaine exposure combined with all three drugs could lead to even more detrimental effects on brain structure. For instance, it is known through animal studies that prenatal cocaine exposure leads to changes predominantly to the dopamine neurotransmitter system (Dow-Edwards, 2011). On the other hand, animal studies show that alcohol acts as a depressant to the central nervous system and affects the fetus through a number of ways: alteration of glutamate levels (Basavarajappa et al., 2008); affecting cell migration and proliferation (Creeley et al., 2013); and can cause long-term effects by changing gene-regulation in the fetus (Hashimoto-Torii et al., 2011). Finally, smoking during pregnancy has been shown to inhibit critical stages of embryonic development (Holloway et al., 2013) both in vitro and in vivo studies in animals.

Our results support the view of combined detrimental effects of co-exposure to drugs compared with single drug exposure, which have been suggested by prior studies (Singer et al., 2002; Janisse et al., 2013). For instance, Rivkin et al. (2008) found that head circumferences of those primarily exposed to prenatal cocaine, but with poly-drug exposure to be smaller than control children. An additive effect of poly-drug exposure were also found, where those with $\geq$2 drugs of exposure had smaller head circumferences than those exposed to only one drug in utero. In the current study, head circumferences were similar between PCE and control groups. Hence, our findings of abnormal cortical thickness in the PCE group hint at subtle, yet persistent cortical structural differences in those with PCE combined with poly-drug exposure.

4.4. Limitations

As this is a cross-sectional study, results need to be treated with caution, as causality between prenatal substances cannot be unequivocally assessed. Another possible limitation of our study is that while those with prenatal cocaine exposure showed different cortical thickness in relation to co-exposure with alcohol and tobacco and cognitive function, we cannot be certain if these effects are stronger only in those with cocaine exposure. This was because there were significantly more youth with prenatal alcohol and tobacco exposure in the PCE group than in the control group. Hence, while we did not detect such interactions in the control sample, our sample size of 15 typically developing children might have lacked sufficient power to detect such differences. Hence, an interaction between alcohol and tobacco even in those with no cocaine exposure cannot be completely ruled out. Longitudinal follow-up of these youth is needed to clarify long-lasting effects of cocaine on these children. Also, some youth were already using cocaine by ages 14–16, this effect could not be disentangled from the rest of the group due to limited numbers

of participants who were using. Future studies with larger sample sizes, and more use of data-sharing consortiums (e.g., ENIGMA consortium, ABIDE consortium etc.) would be needed to investigate this issue further. Finally, we also did not have dosage or frequency information on current cocaine use by youth in the study.

4.5. Conclusions

Although our study is cross-sectional, our results suggest that even when the effects of prenatal cocaine by itself may not be dramatic, interaction between cocaine and tobacco exposure is related to different cortical thickness in affected individuals. All subjects with PCE showed significantly different relationships between cortical thickness and drug exposure compared with unexposed youth. This study highlights the importance of simultaneously examining co-exposure to multiple drugs on brain structure to fully understand the effects of such exposure. This is especially important, when the prenatal effects of secondary drugs of abuse (alcohol and tobacco) on neurobehavior are known to be severe.

Conflicts of interest

The authors declare that there are no known conflicts of interest.

Acknowledgement

This work was supported by a grant from the National Institute on Drug Abuse (NIDA), Grant number R33 DA027561 to ERS and TDW.

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