HHS Public Access

Author manuscript

Acta Neuropsychiatr. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

Acta Neuropsychiatr. 2024 April ; 36(2): 87–96. doi:10.1017/neu.2022.34.

Associations between community-level patterns of prenatal alcohol and tobacco exposure on brain structure in a non-clinical sample of 6-year-old children:a South African pilot study

Kristina A. Uban[1,2], **Deborah Jonker**[3,4], **Kirsten A. Donald**[3,5], **Stefanie C. Bodison**[6], **Samantha J. Brooks**[7], **Eric Kan**[8], **Babette Steigelmann**[1], **Annerine Roos**[4,5], **Andrew Marshall**[8], **Shana Adise**[8], **Letitia Butler-Kruger**[4], **Brigitte Melly**[3], **Katherine L. Narr**[9,10], **Shantanu H. Joshi**[9,11], **Hein J. Odendaal**[12], **Elizabeth R. Sowell**[8], **Dan J. Stein**[4,5,13]

[1]Public Health, University of California, Irvine, CA, United States

[2]Center for Neurobiology of Learning and Memory

[3]Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa

[4]Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

[5]Neuroscience Institute, University of Cape Town, Cape Town, South Africa

[6]Department of Occupational Therapy, College of Public Health and Health Professions, University of Florida, Gainesville, Florida, United States

[7]Faculty of Health, Liverpool John Moores University, Liverpool, United Kingdom

[8]Department of Pediatrics, Keck School of Medicine, Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA, United States

[9]Ahmanson-Lovelace Brain Mapping Center, Department of Neurology, Geffen School of Medicine, University of California, Los Angeles, CA, United States

[10]Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA, United States

[11]Department of Bioengineering, University of California, Los Angeles, CA, United States

[12]Department of Obstetrics and Gynaecology, Stellenbosch University, Cape Town, South Africa

[13]South African Medical Research Council (SAMRC), Unit on Risk and Resilience in Mental Disorders, Cape Town, South Africa

Abstract

Corresponding author: Kristina Uban, PhD (kuban@uci.edu), Assistant Professor of Public Health; Phone: 1-949-824-9577; Mail: 653 E. Peltason Drive, Suite 2040, Irvine, CA, 92617, USA.

Authors' contributions. S.J. B. designed the study and participated in data collection. K.A.U, E.K., D.J., B.M. and S.A. assisted with data analysis and interpretation. K.A.U., D.J., S.C.B., and D.J.S. drafted the manuscript. L.B.K. assisted with formatting the manuscript. All authors contributed to the final draft of the manuscript.

Conflict of interest. Authors declare no conflict of interests.

Ethical standards. Ethical approval for human subject research was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences of Stellenbosch University (REF 248/2014). Informed consent and assent were obtained from parents/guardians and participants before enrolment.

The current small study utilized prospective data collection of patterns of prenatal alcohol and tobacco exposure (PAE and PTE) to examine associations with structural brain outcomes in 6-year-olds, and served as a pilot to determine the value of prospective data describing community-level patterns of PAE and PTE in a non-clinical sample of children. Participants from the Safe Passage Study in pregnancy were approached when their child was ~6 years old and completed structural brain magnetic resonance imaging (MRI) to examine with archived PAE and PTE data (n=51 children-mother dyads). Linear regression was used to conduct whole brain structural analyses, with FDR correction, to examine: a) main effects of PAE, PTE and their interaction; and b) predictive potential of data that reflects *patterns* of PAE and PTE (e.g., quantity, frequency, and timing (QFT)). Associations between PAE, PTE and their interaction with brain structural measures demonstrated unique profiles of cortical and subcortical alterations that were distinct between PAE only, PTE only and their interactive effects. Analyses examining associations between patterns of PAE and PTE (e.g., QFT) were able to significantly detect brain alterations (that survived FDR correction) in this small non-clinical sample of children. These findings support the hypothesis that considering QFT and co-exposures is important for identifying brain alterations following PAE and/or PTE in a small group of young children. Current results demonstrate that teratogenic outcomes on brain structure differ as a function PAE, PTE or their co-exposures, as well as the pattern (QFT) or exposure.

Keywords

Teratogen; prenatal alcohol exposure (PAE); prenatal tobacco exposure (PTE); brain structure; children; low-middle income country (LMIC)

Introduction

Prenatal alcohol exposure (PAE) can produce enduring alterations on the developing human brain, which can lead to challenges in a range of physical, behavioural, neurological, and mental health. Significant individual variability is observed in the range of these outcomes, theoretically in part, due to differing patterns of PAE and presence/absence of tobacco exposure (PTE) (Cook et al., 2015; McLachlan et al., 2017). Animal models clearly demonstrate alcohol as a teratogen, with variable PAE-effects on brain and cognitive outcomes that depend on quantity, frequency, and timing (QFT) of PAE (Sulik, 1986; Sulik, 2008). While fetal alcohol spectrum disorder (FASD) is preventable, PAE is in the leading known cause of intellectual and developmental disability (May et al., 2013; Popova et al., 2016; Roozen et al., 2016; Lange et al., 2017), with an estimated 428 comorbidities (i.e., conduct disorder and receptive language disorder; Popova et al., 2016), making it a major global health concern.

There are several key gaps in FASD human literature. First, most published studies examining the impact of PAE on the human brain have relied on retrospective designs for acquisition of prenatal alcohol exposure patterns, introducing caregiver/parent recall bias and error, and poor to no data on QFT. Second, much work has focused on clinical samples recruited for known or strongly suspected with heavy PAE, or completely unexposed participants as a comparison group. Thus, FASD literature often excludes community

patterns of PAE, particularly regarding mild to moderate PAE. Third, prenatal tobacco exposure (PTE) can also cause deleterious effects on brain and cognitive development (El Marroun et al., 2014; Wiebe et al., 2015; El Marroun et al., 2016), as shown in animal studies indicating an interaction between PAE and PTE, (Bhattacharya et al., 2020); however, there is a paucity of human studies that examine this interaction (Odendaal et al., 2020). Fourth, conducting magnetic resonance imaging (MRI) on young children aged 6 years old is challenging due to excessive movement that results in motion artifacts interfering in usable scans; thus, this age range is limited in FASD human brain imaging literature for informing early identification of teratogenic alterations in brain structure.

Historically, the prevalence of FASD in the Cape region of South Africa was much higher than other global regions, with an estimate between 13.6 to 20.9% in a high-risk community sample of South African children in the first grade (May et al., 2013), compared to conservative estimates of 1–5% in the United States (May et al., 2018). This historical high prevalence reflects the history of South Africa, where there was systematic oppression of black farmworkers, including provision of alcohol in lieu of wages. Ongoing contributing factors to this risk may include prevailing socioeconomic disadvantages, psychological distress and depression, exposure to traumatic stressors, and intimate partner violence (Tomlinson et al., 2014; Stein et al., 2015; Koen et al., 2016; Donald et al., 2018).

A longitudinal community birth cohort, the Prenatal Alcohol, Sudden Infant Death Syndrome and Stillbirth (PASS) Network in this region, allows prospective investigation of the impact of a range of prenatal alcohol and potential co-occurring tobacco exposures. Working with PASS, the present study assessed cortical and subcortical brain region sizes among 6 year olds whose parent participating in prospective PAE and PTE data collection with PASS birth cohort; and subsequent associations with: 1) PAE, PTE, and their interaction, and 2) patterns of exposure (QFT). No other brain imaging study has examined the relationship between PAE, PTE and brain volumes children all aged 6. However, based on extant literature contrasting cortical volumes in FASD and neurotypical children (Nuñez et al., 2011), and in cross-sectional subcortical effects of PAE in older children versus neurotypical (Inkelis et al., 2020) it is hypothesised that significant interactions will occur in frontal and basal ganglia regions early enough in development that it is detectable by age 6.

Materials and methods

Study design and participants.

This research was a small sub-study embedded in the Safe Passage Study within the PASS Network, Western Cape, South Africa, designed to serve as a pilot for a larger neuroimaging study. This was a unique international community-based prospective birth cohort study investigating the role of PAE in the risk for sudden infant death syndrome (SIDS), stillbirth, Fetal Alcohol Syndrome (FAS) and FASD, funded by the National Institute of Child Health and Human Development (NICHD), the National Institute on Alcohol Abuse and Alcoholism (NIAAA), and the National Institute on Deafness and Other Communication Disorders (NIDCD).

As part of the Safe Passage Study of the PASS Network at Stellenbosch University, South Africa, N=7,060 pregnant people were recruited from Bishop Lavis and Belhar residential areas between August 2007 and January 2015. These sites were selected on account of a historical reputation for having a high prevalence of PAE and SIDS and the need to include populations where the marked ethnic and socioeconomic disparities in SIDS remains understudied. Recruitment of pregnant women for the Safe Passage Study occurred between 6 weeks of gestation up to the day admitted for delivery. Methods and timelines are described in full elsewhere (Dukes et al., 2014).

The Safe Passage Study used a modified Timeline Follow-Back (TLFB) (Dukes et al., 2017), a validated method to capture alcohol exposure with a high level of detail, at the time around conception until the last day that the pregnant person reported drinking. A validation study done by Himes and colleagues (2015) using a subset of Safe Passage Study pregnant participants, indicated strong concordance between parental reports using this approach and meconium biomarkers of alcohol exposure. The modified TLFB was administered at the recruitment interview (approximately 6 weeks GA), again at three different prenatal visits (20–24 weeks, 28–32 weeks, and 34+ weeks), and at one-month post-delivery. Exposure information was collected at the recruitment interview to capture substance exposure at the time around conception (15 days before and after the last menstrual period) and for 30 days prior to the participant's last reported drinking day. In subsequent interviews, if the participant reported consumption since their previous visit, the reference period consisted of the 30 days prior to the last drinking day. Peri-conception (2 weeks prior and 2 weeks following the last menstrual period) alcohol intake information was also collected. Detailed information was obtained to standardize and calculate the total grams of alcohol consumed on each drinking day or episode and detailed information regarding the type(s) of alcoholic beverage consumed was collected: whether the drink was frozen or included ice; number, and size of containers; the number of persons sharing; and interval of ingestion were collected for each drinking day.

For the purposes of this pilot study, 80 mothers with varying levels and timing of exposure in pregnancy and who had initially indicated that they would be willing to participate in future studies, were approached when their children (born as participants in the Safe Passage Study) were approximately 6 years old and invited to participate in the pilot study. All potential participants would necessarily have fulfilled the inclusion criteria of the Safe Passage Study, and these include:

1. Pregnant parent was able to give consent;
2. Pregnant parent was at least 16 years of age at the time of consent;
3. Pregnant parent and child can speak Afrikaans and/or English. This criterion was not considered to unfairly exclude many research candidates: Afrikaans and English are the main spoken languages in the study region; thus, this criterion was included purely for practical reasons.

Additional exclusion criteria for children participating in the present study were:

1. History of traumatic brain injury with loss of consciousness exceeding 10 minutes;
2. Presence of a major medical or central nervous system disorder;
3. Prenatal exposure to drugs (aside from tobacco and alcohol);
4. Implant (e.g., metal shunt) or medical condition that posed a risk during scanning.

Of the 80 randomly selected participants invited, 72 birthing parents agreed to participate. From the 72 parent participants, 51 of their children completed the scan.

Calculating quantity, frequency, and timing of PAE and PTE.

The quantity of PAE was measured using the total amount of standard drinks consumed during pregnancy and the average number of drinks per drinking day. The *frequency* of PAE was measured using the total number of days with binge consumption (more than 4 drinks per sitting). *Timing* of PAE identified the total amount of drinks consumed per trimester.

The quantity of PTE was measured using the total amount of cigarettes smoked throughout pregnancy. *The timing* of PTE was measured using the total amount of cigarettes smoked per trimester. There was no measure of frequency of PTE.

Image acquisition.

Scanning occurred at the Cape Universities Brain Imaging Center (CUBIC) located at Tygerberg Hospital, Cape Town. Whole-brain T1-weighted images were acquired for each participant using a 3-Tesla Siemens Allegra scanner. Scan parameters were as follows: repetition time (TR) = 2530 ms; echo time (TE) = 6.53 ms; flip angle = 7°; field of view = 224×168 mm^2; voxel = 1×1×1 mm. Children were familiarized with the MRI environment during a session in a mock scanner on the same day prior to the actual scanning session.

Image processing.

FreeSurfer's v5.3 recon-all pipeline was used to perform volumetric segmentation. This pipeline involve motion correction and averaging (Reuter et al., 2010) of multiple volumetric T1 weighted images (when more than one was available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Ségonne et al., 2007), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al., 2004a; Fischl & Dale, 2000), intensity normalization (Sled et al., 1998), tessellation of the gray matter to white matter boundary, automated topology correction (Fischl et al., 2002; Ségonne et al., 2007), and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale & Sereno, 1993; Dale et al., 1999; Fischl & Dale, 2000). Once the cortical models are complete, a number of deformable procedures can be performed for further data processing and analysis including surface inflation (Fischl et al., 1999a), registration to a spherical atlas which is based on individual cortical folding patterns to match cortical

geometry across subjects (Fischl et al., 1999b), parcellation of the cerebral cortex into units with respect to gyral and sulcal structure (Fischl et al., 2004b; Desikan et al., 2006), and creation of a variety of surface based data including maps of curvature and sulcal depth. Whole brain metrics for cortical and subcortical volume Regions of Interest (ROI) were extracted using the Desikan-Kiliany atlas in tabular format. FreeSurfer's subcortical pipeline segments each voxel into one of approximately 27 ROIs. These include the following bilateral structures: cerebral white matter, cerebral cortex, lateral ventricles, inferior lateral ventricles, cerebellum white matter, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and accumbens area as well as midline and specialized labels such as: lesions, vessels, 3rd ventricle, 4thventricle, brain stem, and cerebrospinal fluid. These ROIs include the caudate of the left and right hemisphere, putamen of the left hemisphere, anterior cincular cortex and the cerebellum of the right hemisphere. The Deskian-Kiliany atlas was used to parcellate the cortical surface into 68 ROIs. Cortical volume, thickness and surface area metrics were calculated for each ROI by hemisphere.

Statistical analyses

All statistical analyses were performed in CRAN R v4.1. Linear regression was run in R with the lm() package to identify associations between structural brain metrics and prenatal exposure (PAE, PTE) as well as the PAE × PTE interaction. The regression model included age (months) and biological sex, and intracranial volume (ICV) as factors. The main effects and interactions were considered statistically significant at $p < 0.05$. The Benjamini-Hochberg false-discovery rate (FDR) correction ($q < 0.05$) was applied within each metric group (e.g., within cortical volume, cortical thickness, cortical area, subcortical volume, within each hemisphere).

Results

Demographics and patterns of PAE and PTE.

Due to missing PTE information, four children with PAE were removed from the analysis. The final sample for analysis included 30 children with PAE and 16 control children without PAE (see Table 1). There was no significant difference in sex, household income, maternal age at birth or gestational age with PAE relative to the no-PAE control group (p's>0.20). Patterns of PTE were slightly different between PAE and no-PAE control group (Table 2), where a statistical trend was observed for larger proportion of participants exposed to PTE in PAE (70%) compared to no-PAE (56%, p=0.08), larger quantity of cigarettes per day in trimester 2 in PAE (mean=4 cigs/day) compared to no-PAE (mean=2 cigs/day) groups (p=0.08). Within the PAE group, on average, the total number of alcoholic drinks consumed were highest in the first and second trimester and reduced in the third trimester. In contrast, PTE was consistent throughout pregnancy for both PAE and non-PAE groups.

Main and interactive effects of PAE and PTE on cortical ROIs.

Main effects of PAE (Figure 1) revealed larger cortical volumes (r. fusiform, l. postcentral) and larger cortical area (r. fusiform, r. pars orbitalis) compared to Control (uncorrected p's

< 0.01); however, none survived FDR correction for multiple comparisons (n=68 cortical ROIs).

Main effects of PTE (Figure 2) were primarily associated with larger brain metrics [larger cortical volume (r. lateral occipital), larger cortical area (r. temporal pole), and thicker cortices (r. medial orbital frontal, parahippocampal, pars orbitalis, pars triangularis, transverse temporal; l. cuneus, entorhinal, isthmus cingulate, lateral occipital) (uncorrected p's<0.03], with the exception of smaller right paracentral volume and area (uncorrected p's<0.05); however, none survived FDR correction for multiple comparisons (n=68 cortical ROIs).

Interactive effects of PAE and PTE (Figure 3) revealed a unique pattern of cortical alterations from what was observed for main effects of both. The interactions across 7 cortical outcomes demonstrated that the relative difference between the group means for PTE compared to no-PTE was tended to be reversed between the PAE and no-PAE conditions (e.g., larger versus smaller mean). This was observed in cortical volume (r. caudal middle frontal, l. cuneus, l. superior frontal), cortical thickness (l. interior parietal, l. cuneus, r. insula, r. temporal pole) and cortical area (r. caudal middle frontal) (uncorrected p's<0.05); however, none survived FDR correction for multiple comparisons (n=68 cortical ROIs).

Main and interactive effects of PAE and PTE on subcortical ROIs.

No associations between PAE and subcortical volumes were observed (p's>0.05) prior to FDR corrections. PTE was associated with decreased volume in the left putamen (uncorrected p = 0.03), which did not pass FDR corrections. PAE × PTE interactions were found in the anterior, central, and posterior segments of the corpus callosum (uncorrected p's < 0.03) but did not pass FDR correction, where differences between PTE and no-PTE were larger in no-PAE group compared to PAE in the central and mid posterior corpus collosum.

Associations of quantity, frequency, and timing (QFT) on cortical and subcortical ROIs.

ROIs that reached significance prior to FDR correction for either PAE main effect or the PAE × PTE interaction were further studied in relation to QFT of PAE. When characteristics of PAE patterns were examined in relation to cortical and subcortical brain outcomes, associations were observed between quantity and frequency of PAE (but not timing), and with cortical alterations only, and not subcortical. Specifically, more quantity (total drinks) and greater frequency (frequency of binge episodes) were significantly related to larger volume and larger area of the fusiform on the right hemisphere (corrected p's < 0.05). Frequency of binge episodes was also positively associated with greater surface area of the fusiform on the left hemisphere (p<0.05).

Prenatal tobacco exposure

ROIs that reached significance prior to FDR correction for either PTE main effect or the PTE x PAE interaction were further studied in relation to quantity and timing of PTE (frequency data was not collected). Quantity of PTE was not associated with any cortical or subcortical brain outcomes. However, timing of PTE was associated with cortical, but not subcortical, brain outcomes. Specifically, Presence of PTE in trimester 1 was significantly

related to greater cortical thickness (r. pars orbitalis, l. lateral occipital) (corrected p's <0.05). Other findings relating to timing of PTE were found but did not survive FDR correction (thicker left entorhinal cortex with trimester 2 PTE; thinner right pars orbitalis cortex with trimester 3 PTE) (uncorrected p's<0.05).

Discussion

Several key findings emerge from this pilot study: 1) Leveraging QFT data significantly detected brain signatures of PAE and PTE at age 6 years (results passed correction for multiple comparisons after pre-screening ROIs for PAE or PTE main effects across the whole brain); 2) Examining PAE, PTE and their interaction yielded 3 distinct profiles of cortical and subcortical alterations, and 3) community-levels of PAE and PTE in a non-clinical sample of 6 year olds appears to be detectable primarily in cortical, but not as much within subcortical, brain regions. Together, these findings provide evidence towards the theory that specific combinations of prenatal substance exposures and their unique pattern of exposure result in complex profiles of teratogenic outcomes on the brain, and are not simply additive in their teratogenic potential. The use of prospective QFT data extends our understanding of how important it may be to consider the pattern of exposures and presence/absence of co-exposures when examining brain alterations following prenatal alcohol and/or tobacco exposure.

Prior to correction for multiple testing, both PAE and PTE, as well as their interaction, were associated with a range of brain alterations. PAE was only associated with cortical, and not subcortical alterations at this age range [e.g., larger cortical volume (r. fusiform, l. postcentral) and larger cortical area (r. fusiform and pars orbitalis)]. PTE was associated with both cortical and subcortical alterations, and interestingly, the brain regions did not overlap with those associated with PAE [e.g., larger cortical volume (r. lateral occipital and paracentral), altered cortical area (smaller r. paracentral and larger r. temporal pole), thicker cortices (r. medial orbital frontal, parahippocampal, pars orbitalis, pars triangularis, transverse temporal; l. cuneus, entorhinal, isthmus cingulate, lateral occipital), and decreased subcortical volume in the left putamen]. Furthermore, the profile of ROIs associated with the interactive effects of PAE with PTE exhibited a third profile that was completely distinct from PAE only alterations, had slight overlap with PTE only alterations (e.g., l. cuneus, r. temporal pole), and implicated many unique brain alterations that were not observed when examining PAE or PTE alone [e.g., altered cortical volume (r. caudal middle frontal, l. cuneus and superior frontal), altered cortical thickness (r. temporal pole and insula; l. cuneus and inferior parietal), altered cortical area (r. caudal middle frontal), and altered subcortical midline structures (3 segments of the corpus collosum]. These three profiles of brain alterations suggest that the impact of PAE on brain structure at 6 years of age is unique from PTE, and their interactive effects (PAE + PTE) are likely complex and not simply additive or synergistic in teratogenic potential. Importantly, however, these findings of PAE, PTE and their interaction on brain metrics remain preliminary, as none survived correction for multiple comparisons. It is possible results would have passed corrections for multiple comparisons with a higher-powered study (e.g., larger participant sample size, focused within clinical population known to demonstrate symptomology of fetal alcohol spectrum disorder (FASD), examined only targeted neural circuitry rather than a whole

brain analysis in present study). None the less, current statistical trends following analyses examining PAE interactions with PTE suggests this interaction deserves further study in larger samples, and further studies should also consider the higher PTE doses that naturally co-occur with PAE, compared to mono-substance use of PTE or PAE only. PAE by PTE interactions were observed within the corticospinal tract among 2 year olds participating in a research study from a neighbouring community to the one current study's participants reside in, further supporting the hypothesis that consideration of co-exposures is needed to advance our teratogenicity following often individualized patterns of prenatal exposures (Roos et al., 2021).

It is noteworthy that despite the small sample size of this pilot study, the total amount of standard drinks consumed during pregnancy and the average number of drinks per drinking day, were positively associated with cortical volume in the right fusiform gyrus. This is in contrast to other neuroimaging studies, which have consistently demonstrated reduced cortical volume in children prenatally exposed to alcohol (Rajaprakash et al., 2014; Migliorini et al., 2015). However, a study performed in neonates in an adjacent community recently found similar alterations in the fusiform following PAE (Roos et al., 2021), corroborating teratogenic impact on the fusiform by PAE in early postnatal life up to 6 years old in current study. The fusiform gyrus plays a role in high-level tasks related to visual processing, including processing of information about faces (Rangarajan et al., 2014; Weiner & Grill-Spector, 2012). Face processing is a critically important perceptual ability; identifying and interpreting facial emotions have a critical role in social functioning, and children with FASDs may have difficulty doing so (Lindinger et al., 2022). These findings, together with the observation of similar size effects for both PAE and PTE are novel and deserve to be consolidated with more extensive work.

Several limitations deserve emphasis. Firstly, as described above, work on PAE and PTE that includes a range of different QFT features will require larger sample sizes to avoid false negative findings. Secondly, we did not include as potential moderators of our findings, a range of poverty-related factors, such as maternal exposure to interpersonal violence, childhood exposure to community violence, and food insecurity (Tomlinson et al., 2014; Stein et al., 2015; Koen et al., 2016; Donald et al., 2018; Gonzalez et al., 2020; Uban et al., 2020). Thirdly, given the focus on age 6, we are unable to determine whether the findings here are true null findings, or simply underpowered. Given the dynamic trajectories of brain volumes over time, with significant increases in cortical thickness at ages 5–6 years, and then subsequent thinning when approaching pubertal maturation, it is possible that the outcomes of PAE and PTE are also likely dynamic, and alterations in brain developmental trajectories following teratogen exposures may overlap. This may result in no observable group differences at certain ages, including around age 6, as in the case in this small study. Finally, exclusion criteria based on co-use of other substances other than alcohol and tobacco may have excluded women and their developing babies that may be most impacted by teratogens, and poly-use beyond the two most common ones (alcohol, tobacco) warrants more investigation to serve this likely high-risk sub-sample.

In conclusion, even in this small sample, significant positive associations of alcohol *quantity* with cortical brain volume and surface area of the right fusiform were observed. Larger

samples and longitudinal examination will be needed to fully delineate the impact of PAE and PTE, as well as QFT characteristics on the developing brain, particularly when investigating naturally occurring patterns of PAE among the community, rather than solely focusing on very high levels of PAE found in clinical FASD studies. Given the large range of profiles of prenatal exposures that occur in the global population, research that evolves our understanding of the complexity of teratogenicity is greatly needed. This can be achieved by examining the entire range of: 1) postnatal developmental periods including neonates to young children; 2) combinations prenatal co-exposures; 3) impact of widely variable patterns of exposures among developing babies and children; and 4) ecological contexts in which the prenatal exposures occur to understand interactions with larger sociodemographic and neighborhood factors to inform future interventions.

Acknowledgments.

The authors gratefully acknowledge the contribution of the personnel and investigators of the Safe Passage Study. We would especially like to acknowledge the families for their participation in this study.

Financial support.

The PASS Research Network was supported by the National Institute on Alcohol Abuse and Alcoholism, Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Institute on Deafness and Other Communication Disorders through the Cooperative Agreement Mechanism (U01 HD055154, U01 HD045935, U01 HD055155, U01 HD045991, and U01 AA016501). This pilot study was funded by a ABMRF grant. KAU was supported by K01AA026889. Collaboration work and future research on this topic is funded with funds from the National Institute of Alcohol Abuse and Alcoholism (R01AA025653, Sowell PI). This publication was made possible in part by a grant from Carnegie Corporation of New York, supporting the author D Jonker. The statements made and views expresses are solely the responsibility of the authors.

References

Bhattacharya D, Fujihashi A, Majrashi M, Bloemer J, Bhattacharya S, Buabeid M, Escobar M, Moore T, Suppiramaniam V and Dhanasekaran M (2020). Concurrent nicotine exposure to prenatal alcohol consumption alters the hippocampal and cortical neurotoxicity. Heliyon 6(1).

Cook JL, Green CR, Lilley CM, Anderson SM, Baldwin ME, Chudley AE, Conry JL, LeBlanc N, Loock CA, Lutke J and Mallon BF (2016). Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. Canadian Medical Association Journal 188(3),191–197. [PubMed: 26668194]

Dale AM and Sereno MI (1993). Improved localizadon of cortical activity by combining EEG and MEG with MRI cortical surface reconstruction: a linear approach. Journal of cognitive neuroscience 5(2), 162–176. [PubMed: 23972151]

Dale AM, Fischl B and Sereno MI (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. Neuroimage 9(2), 179–194. [PubMed: 9931268]

Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT and Albert MS (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31(3), 968–980. [PubMed: 16530430]

Donald KA, Hoogenhout M, du Plooy CP, Wedderburn CJ, Nhapi RT, Barnett W, Hoffman N, Malcolm-Smith S, Zar HJ and Stein DJ (2018). Drakenstein Child Health Study (DCHS): investigating determinants of early child development and cognition. BMJ paediatrics open 2(1).

Dukes KA, Burd L, Elliott AJ, Fifer WP, Folkerth RD, Hankins GD, Hereld D, Hoffman HJ, Myers MM, Odendaal HJ and Signore C (2014). The Safe Passage Study: Design, Methods, Recruitment, and Follow-Up Approach. Paediatric and Perinatal Epidemiology 28(5), 455–465. [PubMed: 25131605]

Dukes K, Tripp T, Petersen J, Robinson F, Odendaal H, Elliott A, Willinger M, Hereld D, Raffo C, Kinney HC and Groenewald C (2017). A modified Timeline Followback assessment to capture alcohol exposure in pregnant women: Application in the Safe Passage Study. Alcohol 62, 17–27. [PubMed: 28755748]

El Marroun H, Schmidt MN, Franken IH, Jaddoe VW, Hofman A, Van Der Lugt A, Verhulst FC, Tiemeier H and White T (2014). Prenatal tobacco exposure and brain morphology: a prospective study in young children. Neuropsychopharmacology 39(4), 792–800. [PubMed: 24096296]

El Marroun H, Tiemeier H, Franken IH, Jaddoe VW, van der Lugt A, Verhulst FC, Lahey BB and White T (2016). Prenatal cannabis and tobacco exposure in relation to brain morphology: a prospective neuroimaging study in young children. Biological Psychiatry 79(12), 971–979. [PubMed: 26422004]

Fischl B and Dale AM (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proceedings of the National Academy of Sciences 97(20), 11050–11055.

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, Van Der Kouwe A, Killiany R, Kennedy D, Klaveness S and Montillo A (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33(3), 341–355. [PubMed: 11832223]

Fischl B, Salat DH, van der Kouwe AJ and Makris N, Ségonne F, Quinn BT, Dale AM (2004a). Sequence-independent segmentation of magnetic resonance images. NeuroImage 23, S69–S84. [PubMed: 15501102]

Fischl B, Sereno M, Tootell R and Dale A (1999a). High-resolution intersubject averaging and a coordinate system for the cortical surface. Human Brain Mapping 8(4), 272–284. [PubMed: 10619420]

Fischl B, Sereno MI and Dale AM (1999b). Cortical surface-based analysis: II: inflation, flattening, and a surface-based coordinate system. Neuroimage 9(2), 195–207. [PubMed: 9931269]

Fischl B, Van Der Kouwe A, Destrieux C, Halgren E, Ségonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D and Caviness V (2004b). Automatisk parcellering af den menneskelige hjernebark. Cerebral Cortex (New York, NY), 14(1), 11–22.

Gonzalez MR, Palmer CE, Uban KA, Jernigan TL, Thompson WK and Sowell ER (2020). Positive economic, Psychosocial, and Physiological Ecologies predict brain structure and Cognitive performance in 9–10-year-old children. Frontiers in human neuroscience, p.436.

Himes SK, Dukes KA, Tripp T, Petersen JM, Raffo C, Burd L, Odendaal H, Elliott AJ, Hereld D, Signore C and Willinger M (2015). Clinical sensitivity and specificity of meconium fatty acid ethyl ester, ethyl glucuronide, and ethyl sulfate for detecting maternal drinking during pregnancy. Clinical chemistry 61(3), 523–532. [PubMed: 25595440]

Inkelis SM, Moore EM, Bischoff-Grethe A and Riley EP (2020). Neurodevelopment in adolescents and adults with fetal alcohol spectrum disorders (FASD): A magnetic resonance region of interest analysis. Brain Research, 1732, 146654. [PubMed: 31930998]

Koen N, Brittain K, Donald KA, Barnett W, Koopowitz S, Maré K, Zar HJ and Stein DJ (2016). Psychological trauma and posttraumatic stress disorder: risk factors and associations with birth outcomes in the Drakenstein Child Health Study. European journal of psychotraumatology 7(1), p.28720. [PubMed: 26886489]

Lange S, Probst C, Gmel G, Rehm J, Burd L and Popova S (2017). Global prevalence of fetal alcohol spectrum disorder among children and youth: a systematic review and meta-analysis. JAMA pediatrics 171(10), 948–956. [PubMed: 28828483]

May PA, Blankenship J, Marais AS, Gossage JP, Kalberg WO, Barnard R, De Vries M, Robinson LK, Adnams CM, Buckley D and Manning M (2013). Approaching the Prevalence of the Full Spectrum of Fetal Alcohol Spectrum Disorders in a South African Population-Based Study. Alcoholism: Clinical and experimental research, 37(5), 818–830. [PubMed: 23241076]

May PA, Chambers CD, Kalberg WO, Zellner J, Feldman H, Buckley D, Kopald D, Hasken JM, Xu R, Honerkamp-Smith G and Taras H (2018). Prevalence of fetal alcohol spectrum disorders in 4 US communities. Jama 319(5), pp.474–482. [PubMed: 29411031]

Migliorini R, Moore E, Glass L, Infante M, Tapert S, Jones K, Mattson S and Riley E (2015). Anterior cingulate cortex surface area relates to behavioral inhibition in adolescents with and without heavy prenatal alcohol exposure. Behavioural Brain Research, 292, 26–35. [PubMed: 26025509]

McLachlan K, Paolozza A, Kully-Martens K, Portales-Casamar E, Pavlidis P, Andrew G, Hanlon-Dearman A, Loock C, McFarlane A, Nikkel SM and Pei J (2017). Unpacking the heterogeneity of cognitive functioning in children and adolescents with fetal alcohol spectrum disorder: determining the role of moderators and strengths. Advances in Neurodevelopmental Disorders 1(4), 271–282.

Nunez CC, Roussotte F and Sowell ER (2011). Focus on: structural and functional brain abnormalities in fetal alcohol spectrum disorders. Alcohol Research Health 34(1), 121–131. [PubMed: 23580049]

Odendaal H, Kruger M and Botha HM (2020). Dangers of smoking cigarettes and drinking alcohol during pregnancy. SAMJ: South African Medical Journal, 110(11), 1066–1067. [PubMed: 33403977]

Popova S, Lange S, Probst C, Gmel G and Rehm J (2017). Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. The Lancet Global Health 5(3), e290–e299. [PubMed: 28089487]

Popova S, Lange S, Shield K, Mihic A, Chudley AE, Mukherjee RA, Bekmuradov D and Rehm J (2016). Comorbidity of fetal alcohol spectrum disorder: a systematic review and meta-analysis. The Lancet 387(10022), 978–987.

Rajaprakash M, Chakravarty M, Lerch J and Rovet J (2013). Cortical morphology in children with alcohol-related neurodevelopmental disorder. Brain and Behavior, 4(1), 41–50. [PubMed: 24653953]

Rangarajan V, Hermes D, Foster B, Weiner K, Jacques C, Grill-Spector K. and Parvizi J, (2014). Electrical Stimulation of the Left and Right Human Fusiform Gyrus Causes Different Effects in Conscious Face Perception. Journal of Neuroscience 34(38),12828–12836. [PubMed: 25232118]

Reuter M, Rosas HD and Fischl B (2010). Highly accurate inverse consistent registration: a robust approach. Neuroimage 53(4), 1181–1196. [PubMed: 20637289]

Roozen S, Peters GJY, Kok G, Townend D, Nijhuis J and Curfs L (2016). Worldwide prevalence of fetal alcohol spectrum disorders: A systematic literature review including meta-analysis. Alcoholism: Clinical and Experimental Research 40(1), 18–32. [PubMed: 26727519]

Ségonne F, Pacheco J and Fischl B (2007). Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. IEEE transactions on medical imaging, 26(4), 518–529. [PubMed: 17427739]

Sled JG, Zijdenbos AP and Evans AC (1998). A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE transactions on medical imaging 17(1), 87–97. [PubMed: 9617910]

Stein DJ, Koen N, Donald KA, Adnams CM, Koopowitz S, Lund C, Marais A, Myers B, Roos A, Sorsdahl K and Stern M (2015). Investigating the psychosocial determinants of child health in Africa: The Drakenstein Child Health Study. Journal of neuroscience methods 252, 27–35. [PubMed: 25797842]

Sulik KK (1984). Critical periods for alcohol teratogenesis in mice, with special reference to the gastrulation stage of embryogenesis. In Ciba Foundation Symposium (105), 124–141. [PubMed: 6563984]

Sulik KK, Johnston MC, Daft PA, Russell WE, Dehart DB, Opitz JM and Reynolds JF (1986). Fetal alcohol syndrome and DiGeorge anomaly. Critical ethanol exposure periods for craniofacial malformations as illustrated in an animal model. American Journal of Medical Genetics 25(S2), 97–112.

FreeSurfer.(https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferMethodsCitation). [Accessed 1 June 2022].

Tomlinson M, O'Connor MJ, Le Roux IM, Stewart J, Mbewu N, Harwood J and Rotheram-Borus MJ (2014). Multiple risk factors during pregnancy in South Africa: the need for a horizontal approach to perinatal care. Prevention Science 15(3), 277–282. [PubMed: 23475562]

Uban KA, Kan E, Wozniak JR, Mattson SN, Coles CD and Sowell ER (2020). The relationship between socioeconomic status and brain volume in children and adolescents with prenatal alcohol exposure. Frontiers in Human Neuroscience 14, 85. [PubMed: 32322193]

Weiner K and Grill-Spector K (2012). The improbable simplicity of the fusiform face area. Trends in Cognitive Sciences, 16(5), pp.251–254. [PubMed: 22481071]

Wiebe SA, Clark CA, De Jong DM, Chevalier N, Espy KA and Wakschlag L (2015). Prenatal tobacco exposure and self-regulation in early childhood: Implications for developmental psychopathology. Development and Psychopathology, 27(2), 397–409. [PubMed: 25997761]

Significant outcomes

- Leveraging data that reflects *patterns* of prenatal alcohol (quantity, frequency) and tobacco (timing) exposure data strengthened ability to statistically detect brain signatures of PAE and PTE in a small cohort of 6 year olds.
- Examining PAE, PTE and their interaction yielded 3 distinct profiles of cortical and subcortical alterations, suggesting teratogenicity of co-exposures is not as simple as being additive or synergistic, and rather is highly complex.
- Community-levels of PAE and PTE in a non-clinical sample of 6-year-olds is detectable throughout the brain.

Considerations

- Research on PAE, PTE and their interaction that includes a range of different QFT features will require larger sample sizes, or targeted neural circuitries and not whole brain, to avoid false negative findings.
- Given the dynamic trajectories of brain volumes over time, with significant increases in cortical thickness at ages 5–6 years, and then subsequent thinning when approaching pubertal maturation, it is possible that the outcomes of PAE and PTE are also dynamic, and alterations in brain developmental trajectories following teratogen exposures may overlap. This may result in no observable group differences at certain ages, including around age 6, as in the case in this small study that served as a pilot for a larger study.

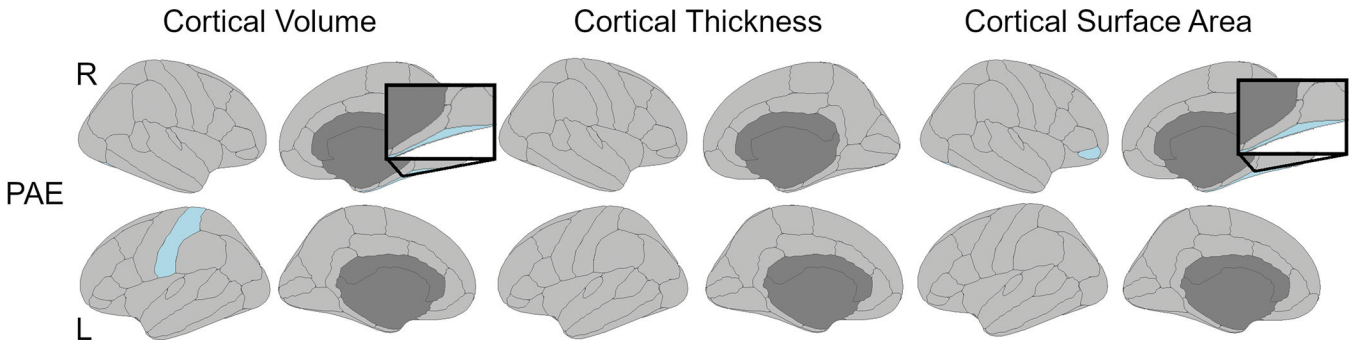

Figure 1.

Cortical brain outcomes: main effects of PAE

Relative to controls, larger sizes were observed with PAE through larger cortical brain volumes (right fusiform, left postcentral cortex) and larger cortical areas (right fusiform and pars orbitalis). No differences were observed with cortical thickness. None of these results survived FDR correction for multiple comparisons with whole-brain analysis.

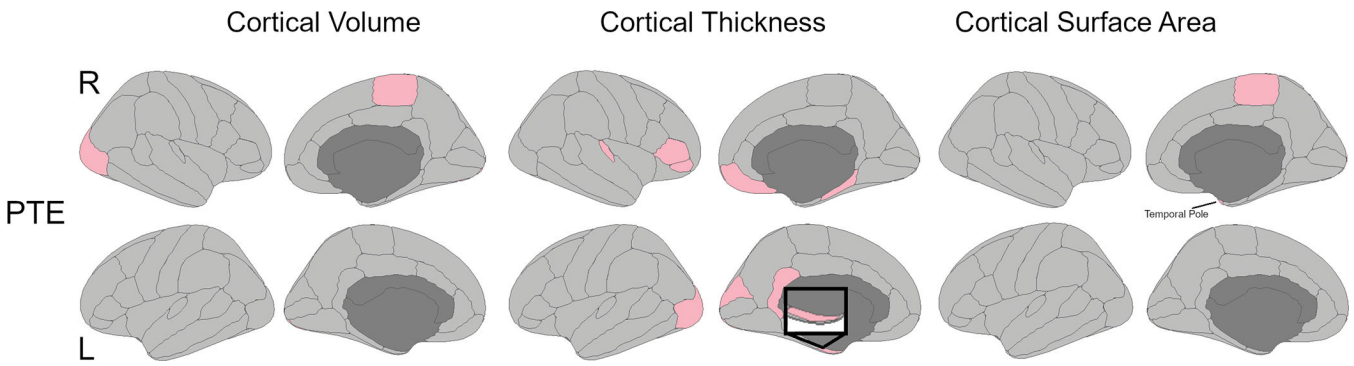

Figure 2.

Cortical outcomes: main effects of PTE

Relative to controls, smaller brain sizes were observed with PTE through smaller cortical brain volume (right lateral occipital), thinner cortices (right: medial orbital frontal, parahippocampal, pars orbitalis, pars triangularis, transverses temporal; and left: cuneus, entorhinal, isthumus cingulate, lateral occipital), and smaller cortical area (right temporal pole). Additionally, relative to controls, larger brain sizes were observed with PTE through larger cortical brain volume (right paracentral) and larger cortical area (right temporal pole). None of these results survived FDR correction for multiple comparisons with whole-brain analysis.

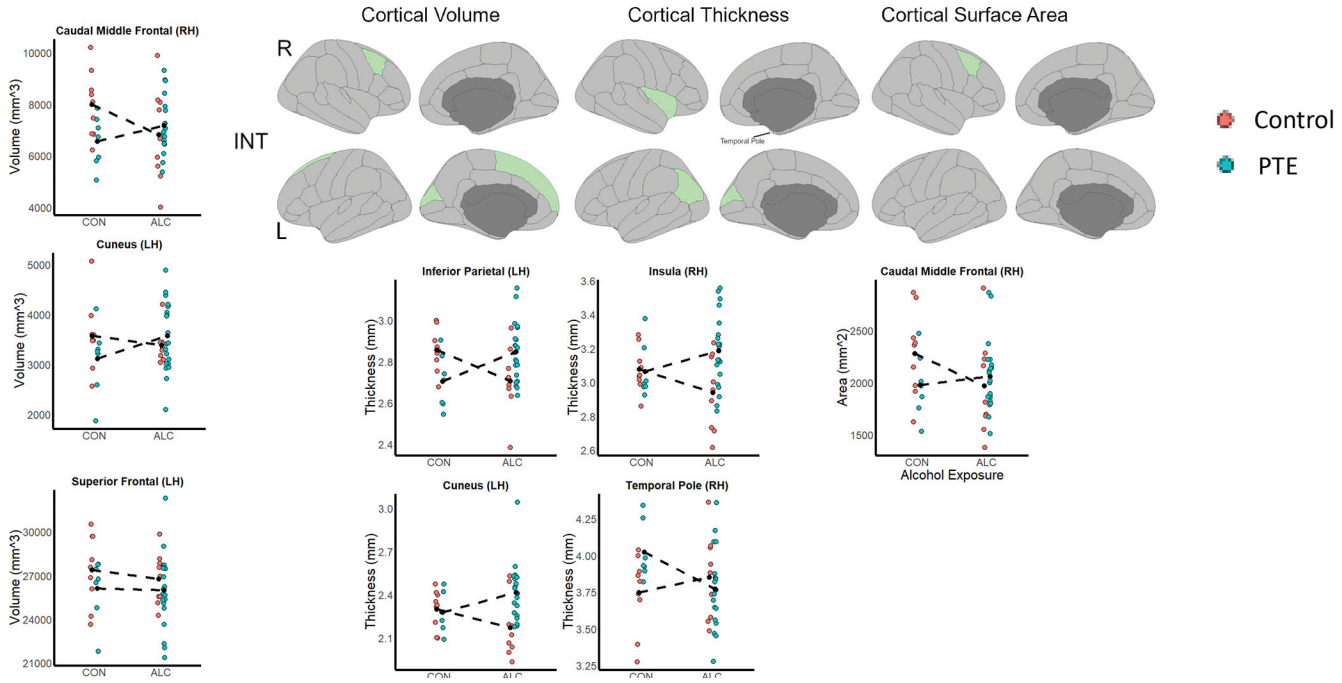

Figure 3.

Cortical outcomes: interactive effects of PAE plus PTE

Interaction effects of PAE and PTE were observed with all cortical measures, and in a ROI-specific manner. None of these results survived FDR correction for multiple comparisons with wholebrain analysis.

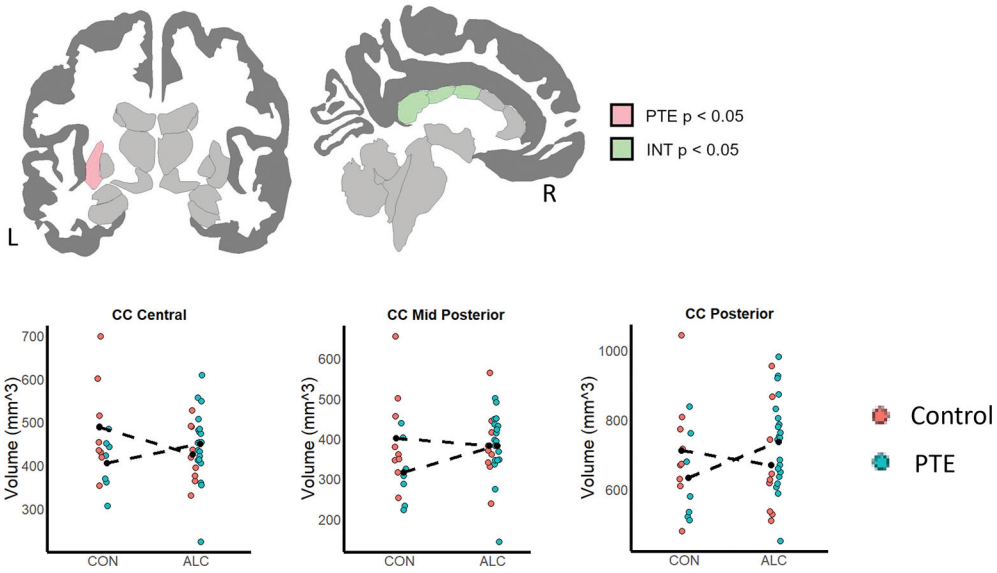

Figure 4.

Subcortical brain outcomes: main and interactive effects of PAE and PTE

PTE was associated with reduced left putamin volume. There were no main effects of PAE. Interaction effects of PAE and PTE were observed with all three corpus collosum ROIs. None of these results survived FDR correction for multiple comparisons with whole-brain analysis.

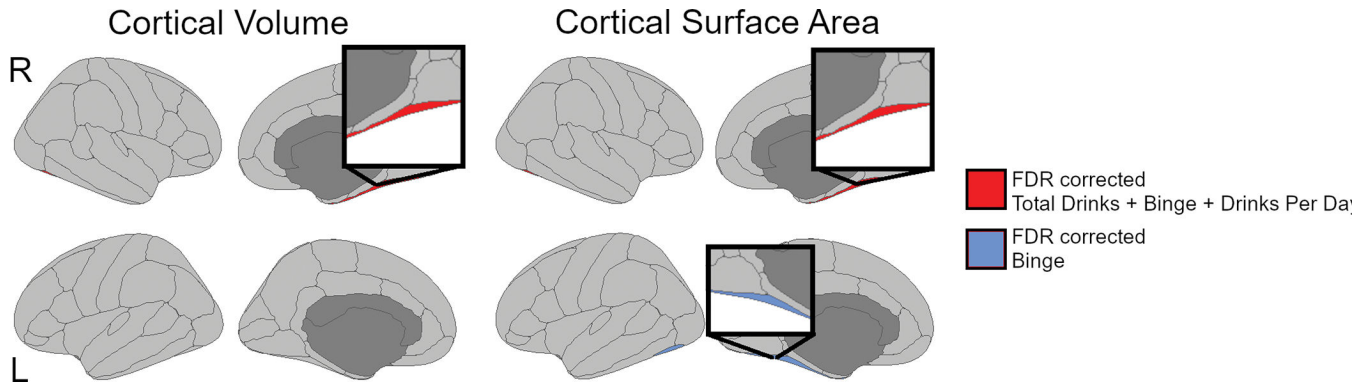

Figure 5.

All brain associations with quantity and frequency of PAE

Quantity of PAE was associated with larger cortical volume (right fusiform) and larger cortical area (right fusiform, trend for left fusiform). Frequency of binge episodes was associated with larger cortical volume (right fusiform) and larger cortical area (bilateral fusiform). All results survived FDR correction for multiple comparisons (n=4 ROIs in follow-up analysis). No significant QFT associations were found for subcortical ROIs. There were no associations between timing of PAE and brain alterations.

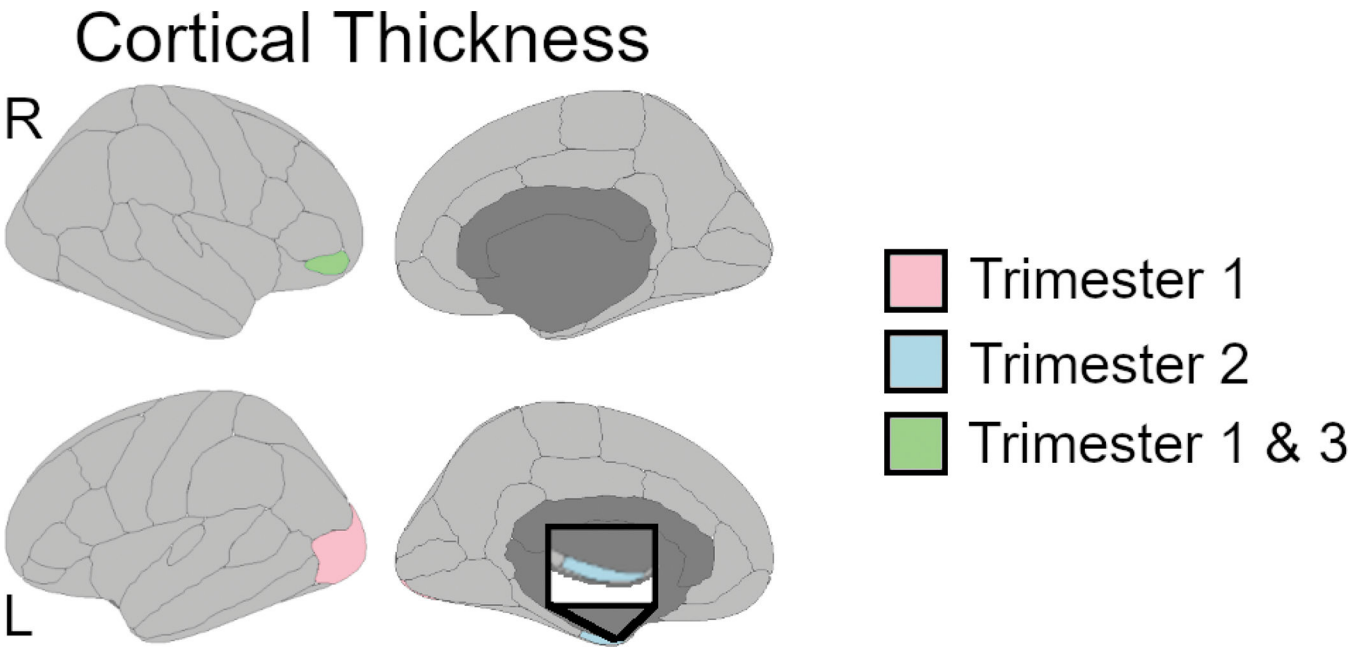

Figure 6.

All brain associations with quantity and timing of PTE

Presence of PTE in trimester 1 was associated with greater cortical thickness (trimester 1: right pars orbitalis, left lateral occipital), passing FDR correction for multiple comparisons (N = 10 ROIs for follow-up analysis). Other findings relating to timing of PTE were found but did not survive FDR correction (thicker left entorhinal cortex with trimester 2 PTE; thinner right pars orbitalis cortex with trimester 3 PTE). There were no associations between PTE quantity and subcortical volumes.

Table 1.

Descriptive characteristics of the participants

	Control (n=16)	PAE (n=30)	p value
Sex at Birth			
Female	n=8 (50%)	n=16 (53.3%)	
Male	n=8 (50%)	n=14 (46.7%)	0.82
Age at scan (range)	6.32 ± 0.28 years (5.8–6.8)	6.29 ± 0.13 years (6.0–6.7)	0.64
Monthly income	469.25 ±313.31 ZAR (71 – 1,000)	653.39 ±498.19 ZAR (71–2,000)	0.20
Maternal age at birth	26.37 ± 6.52 years (19–38)	25.16 ± 5.09 years (17– 40)	0.49
Gestational age	272 ±13 days (243–289)	272 ±13 days (237–290)	0.89

Group statistics presented as Mean ± STDEV, (range). P values of t-tests demonstrate no significant between-group (Control vs PAE) differences in demographic variables.

Note: n=5 missing data points for monthly income.

Table 2.

Details of PAE and PTE quantity, frequency, and timing.

	Control (n=16)	PAE (n=30)	p value
PAE Quantity	0.00 ± 0 total drinks (0–0)	23.09 ± 34 total drinks (0.6–142)	*0.01
	0.00 ± 0 drinks per sitting (0–0)	2 ± 2 drinks per sitting (0.2–12)	***<0.001
PAE Frequency	0.00 ± 0 binge episodes (0–0)	2 ± 2 binge episodes (0–10)	**0.003
PAE Timing			
Trimester 1	0.00 ± 0 total drinks (0–0)	10 ± 13 total drinks (0–48)	**0.005
Trimester 2	0.00 ± 0 total drinks (0–0)	10 ± 20 total drinks (0–80)	0.054
Trimester 3	0.00 ± 0 total drinks (0–0)	3 ± 7 total drinks (0–34)	*0.04
PTE	n=7 (56%)	n=21 (70%)	0.08
PTE Quantity	497 ± 694 total cigs (0–1,893)	1,020 ±1,176 total cigs (0–4,954)	0.11
PTE Timing			
Trimester 1	2 ± 3 total cigs per day (0–9)	4 ± 6 total cigs per day (0–23)	0.14
Trimester 2	1 ± 2 total cigs per day (0–7)	3 ± 3 total cigs per day (0–9)	0.08
Trimester 3	1 ± 2 total cigs per day (0–5)	3 ± 5 total cigs per day (0–25)	0.20