Research Paper

Effects of prenatal alcohol exposure on neurobehavioural development and volume of rostral cingulate cortex subregions

Arash Aghamohammadi-Sereshki, PhD; Carly A. McMorris, PhD; W. Ben Gibbard, MD; Christina Tortorelli, MA, RSW; G. Bruce Pike, PhD; Catherine Lebel, PhD

Background: Maternal alcohol consumption during pregnancy can have widespread and long-lasting effects on children's cognition, behaviour, brain function and structure. The pregenual anterior cingulate cortex (ACC) and the anterior midcingulate cortex (MCC) mediate emotional and cognitive behaviours that are affected by prenatal alcohol exposure. However, the neurobehavioural development of the pregenual ACC and anterior MCC has not been examined in people with prenatal alcohol exposure. **Methods:** We recruited 30 children and adolescents with prenatal alcohol exposure and 50 age- and gender-matched unexposed controls. We acquired structural MRI data sets on a 3 T scanner. We manually delineated 2 areas of the rostral cingulate cortex — the pregenual ACC and the anterior MCC — and compared them between groups. We measured behavioural and emotional problems using the Behaviour Assessment System for Children, 2nd Edition, Parent Rating Scale, and then explored their associations with rostral cingulate cortex volumes. **Results:** Intracranial-normalized volumes of the right pregenual ACC and the right total rostral cingulate cortex were significantly smaller in individuals with prenatal alcohol exposure than in unexposed controls. The volume of the right anterior MCC had a significant positive association with scores on the Internalizing Problems scale in individuals with prenatal alcohol exposure. **Limitations:** This study was cross-sectional, and detailed information about the timing and amount of exposure was not always available. **Conclusion:** Prenatal alcohol exposure is associated with lower volumes in the right pregenual ACC. This finding may underlie some of the emotional and behavioural problems experienced by individuals with prenatal alcohol exposure.

Introduction

The first well-known reports of the dystrophic and teratogenic effects of alcohol were published in the late 1960s[1] and early 1970s.[2,3] Since then, a growing body of research has shown the widespread effects of prenatal alcohol exposure on the brain,[4,5] on mental health[6,7] and on cognition and behaviour.[7] Early studies of prenatal alcohol exposure were mainly autopsy examinations of the most severe effects of prenatal alcohol exposure — namely fetal alcohol syndrome.[2,8] However, since the 1990s, MRI studies have made it possible to investigate the deleterious effects of prenatal alcohol exposure on people with fetal alcohol spectrum disorder (FASD).[4,5] FASD is a term that includes people with a spectrum of cognitive and behavioural effects induced by prenatal alcohol exposure, regardless of facial dysmorphology.[9]

To date, most structural MRI studies of prenatal alcohol exposure have reported volume reductions in the whole brain, the total grey and white matter, and numerous subregions.[4,5] A thorough understanding of how these structural brain alterations underlie the behavioural deficits induced by prenatal alcohol exposure is critical for earlier detection of people with prenatal alcohol exposure, and for effective intervention.

The anterior cingulate cortex (ACC) and anterior midcingulate cortex (MCC) are the 2 most rostral regions of the cingulate cortex and are involved in emotional and cognitive functions,[10,11] including those impaired by prenatal alcohol exposure. Different nomenclatures are used for the cingulate cortex. In the present study, we have adopted the 4-region neurobiological model, which divides the cingulate cortex into 4 regions: the ACC, the MCC, the posterior cingulate cortex and the retrosplenial cortex.[12] The subgenual and pregenual subregions of the ACC can be distinguished

Correspondence to: C. Lebel, B4-513, Alberta Children's Hospital, 28 Oki Dr., Calgary, AB, T3B 6A8, clebel@ucalgary.ca; A. Aghamohammadi-Sereshki, 2910C, HSC Building, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB, T2N 4N1, aghamoha@ualberta.ca

Submitted Nov. 10, 2021; Revised Jan. 25, 2022; Revised Apr. 20, 2022; Accepted May 8, 2022

Cite as: *J Psychiatry Neurosci* 2022 July 26;47(4). doi:10.1503/jpn.210198

based on their connectivity profiles, cytoarchitectural and neurochemical features, and functional implications.[11,13,14] The subgenual ACC is involved in autonomic regulation and storing negative-valence memories; the pregenual ACC is involved in assessing internal emotional states and self-efficacy, value scaling of reinforcers (i.e., rewards and punishers) and cost assessment of actions.[11,15]

The MCC can be further subdivided into anterior and posterior subregions and contributes to a brain network that produces adaptive responses to environmental stimuli.[16] Shackman and colleagues[10] reviewed physiologic, anatomic and functional studies and suggested an "adaptive control hypothesis," in which the anterior MCC is a hub for integrating information about pain, negative affect and cognitive control to mediate aversively motivated behaviours. As well, Vogt[11] considered the mediation of decision-making related to reward–approach versus fear–avoidance to be the major function of the anterior MCC. In contrast to the ACC and the anterior MCC, the posterior MCC is not involved in emotional activations; it is primarily involved in reflexive skeletomotor responses to sensory stimuli.[17]

The results of structural MRI studies of the rostral cingulate cortex in people with prenatal alcohol exposure have been heterogeneous. Studies have reported volumetric reductions in the ACC;[18] volumetric increases in the ACC[19] and caudal ACC;[20] and no significant effects on the normalized rostral ACC, caudal ACC[21] or cingulate grey matter volume.[22] (Note: Some studies defined the rostral and caudal ACC as the most and second-most anterior parts of the cingulate cortex, respectively. However, it is now accepted that the second-most anterior part of the cingulate cortex should instead be labelled the anterior MCC.[17])

Most previous studies have used an automated segmentation method or have registered individual images to an average template to measure cingulate volume.[18–21] These methods minimize interindividual anatomic variations in the rostral cingulate cortex, which can obscure structural differences among individuals.[23] Furthermore, automated analyses often do not consider the superior cingulate gyrus, which originates rostrally to the main (i.e., ventral) cingulate gyrus, runs dorsally parallel to it and exists in 30% to 75% of cases.[16,24–26] The absence (i.e., single gyrus–sulcus pattern) or presence (i.e., double-parallel gyri–sulci pattern) of the superior cingulate gyrus affects the cytoarchitectural features and volume of the rostral cingulate cortex.[14,23,25,27] Therefore, the superior cingulate gyrus or paracingulate sulcus should be considered in structural analyses of the rostral cingulate cortex.[23]

The Behaviour Assessment System for Children, 2nd Edition (BASC-2),[28] is a questionnaire used to evaluate emotions and behaviours in children and adolescents. The BASC-2 Parent Rating Scale (BASC-2-PRS) is an appropriate method for the early identification of children with emotional and behavioural problems,[29] many of which are mediated by the rostral cingulate cortex, including negative affect (e.g., fear, anger, anxiety and sadness), attention, approach–avoidance behaviours and social decision-making.[10,11,15]

The first aim of the present study was to investigate volumetric differences in the pregenual ACC and anterior MCC between individuals with prenatal alcohol exposure and unexposed controls. The second aim was to explore possible associations of the volumes of the pregenual ACC and anterior MCC with behavioural, emotional and adaptive functioning in the exposed and unexposed groups separately. We did not delineate the subgenual ACC because the cingulate gyrus does not extend below the genu of the corpus callosum in all individuals.[23,30]

Methods

Participants

Through local advertisements and the Cumulative Risk Diagnostic Clinic in Calgary, Alberta, Canada, we recruited 31 children and adolescents with prenatal alcohol exposure and 54 unexposed controls to undergo behavioural assessment and MRI. We excluded 1 individual with prenatal alcohol exposure and 4 controls because of severe motion artifacts in their MRIs. Therefore, the final sample consisted of 30 individuals with prenatal alcohol exposure and 50 unexposed controls matched according to age, gender, annual household income and maternal education.

Two individuals with prenatal alcohol exposure lived with their biological parents; the others were in foster or adoptive care. All unexposed controls lived with their biological parents. Of the 27 individuals with prenatal alcohol exposure for whom we had detailed information, 24 (89%) had prenatal exposure to other substances (tobacco, cannabis, illegal drugs or a combination of these). We verified participants' prenatal alcohol exposure by accessing child welfare, medical, police and social services records. Confirmation of prenatal alcohol exposure included reports from biological mothers, family members and close friends, or documented positive blood or urine tests during pregnancy. In the unexposed controls, we confirmed the absence of prenatal exposure to alcohol and other substances based on reports from their biological mothers. We acquired written informed consent from caregivers or guardians and assent from participants. The study was approved by the University of Calgary Health Research Ethics Board (REB17-0663). None of the participants had contraindications for MRI.

In Canada, FASD itself is a diagnosis.[9] Among individuals with prenatal alcohol exposure in the present study, 12 had been diagnosed with FASD. None of the individuals with prenatal alcohol exposure had been diagnosed with fetal alcohol syndrome. Not all participants in this study had been assessed for a diagnosis of FASD, but those who had been diagnosed with FASD were assessed according to the 2015 Canadian guideline for FASD diagnosis.[9] Fifteen individuals with prenatal alcohol exposure had co-occurring disorders, including attention-deficit/hyperactivity disorder, learning disabilities, anxiety and oppositional defiant disorder. Nineteen participants with prenatal alcohol exposure were taking medication for mental health disorders or systemic diseases. Unexposed controls had no lifetime psychiatric, neurodevelopmental or neurologic disorders reported by their caregivers.

MRI acquisition and analysis

We acquired images using a 3 T GE MR750w MRI system with a 32-channel head coil at the Alberta Children's Hospital. We acquired whole-brain T_1-weighted images using a 3-dimensional fast spoiled gradient-echo sequence (BRAVO: inversion time 600 ms, echo time 3.2 ms, repetition time 8.2 ms, acquisition time 5:38 min, native resolution 0.8 × 0.8 × 0.8 mm^3). Images were preprocessed using Computational Anatomy Toolbox (CAT12)[31] implemented in SPM12, performed in MATLAB (R2019a, MathWorks, Inc.) to correct radiofrequency inhomogeneities, perform skull-stripping and estimate intracranial volume (ICV). CAT12 quantifies image quality as a rating. Except for 5 participants with an image quality rating of B+ (87.41%–89.94%), the rest of the ratings were A– or A (90.34%–93.38%), indicating excellent quality (www.neuro.uni-jena.de/cat/index.html#About).

We analyzed a subset of these data previously using FreeSurfer.[18] In the current study, we adopted a manual method for more specific volumetric measurements and extended our analysis to the anterior MCC.

We marked the cingulate sulcus as the first prominent sulcus located dorsal and parallel to the corpus callosum in an anterior–posterior course on sagittal slices, as described previously.[24] We manually delineated 2 subregions of the rostral cingulate cortex (the pregenual ACC and the anterior MCC) using the anterior commissure and genu of the corpus callosum as landmarks, as described previously.[30] The corresponding delineated Brodmann areas (BAs) for the pregenual ACC were p24a, p24b, p24c on the ventral bank of the cingulate sulcus, and p33; BAs for the anterior MCC were a24a', a24b', a24c' on the ventral bank of the cingulate sulcus, and a33'.[14,17,25]

To control for the effects of the superior cingulate gyrus on the volumes of the pregenual ACC, anterior MCC and total rostral cingulate cortex, we marked the superior cingulate gyrus (BA32/BA32') based on the criteria of Yücel and colleagues[26] as a gyrus that originates anterior to the cingulate gyrus and extends posteriorly in a parallel direction to the cingulate gyrus, which forms a double-parallel pattern. When the superior cingulate gyrus was present, we measured the overlap between the total rostral cingulate cortex and the superior cingulate gyrus ([number of slices in which the superior cingulate gyrus was located dorsal and parallel to the rostral cingulate gyrus ÷ total number of rostral cingulate slices] × 100) and included this as a covariate in statistical analyses for all rostral cingulate regions of interest (i.e., pregenual ACC, anterior MCC and total rostral cingulate cortex; Figure 1). The overlaps were usually measured 4 mm to 5 mm lateral to the midline, where the superior cingulate gyrus could be clearly recognized and differentiated from the shallow intralimbic sulcus, if it was present.[24,27]

All manual volumetric measurements were acquired by a single rater (A.A.S.) who was blind to group assignments and all demographics, and was trained in manual delineation of the cingulate cortex. We used MNI Display for all manual delineations (www.bic.mni.mcgill.ca/software/Display/Display.html). To compensate for interindividual differences in head size, we normalized regional volumetric measurements of the rostral cingulate cortex to ICV using a proportional method: normalized volume = (individual regional volume [mm^3] ÷ individual ICV [cm^3]) × average ICV for the entire cohort (cm^3).[32] We multiplied by the group average ICV simply to adjust the scale of the numbers and make analysis easier; multiplying by this constant did not affect results.

Measurement of emotional and behavioural functioning

We measured emotional and behavioural functioning in both groups using the BASC-2 PRS,[28] typically on the same day as MRI scanning but always within 2 weeks of the scans. BASC-2 PRS is a norm-referenced multidimensional screening tool with 160 items rated by parents to study emotions and behaviours in children and adolescents aged 2–21 years. Responses are rated on a 4-point scale: never, sometimes, often and almost always. The BASC-2 PRS quantifies composites and clinical scales of composites: the Externalizing Problems scale includes hyperactivity, aggression and conduct problems; the Internalizing Problems scale includes anxiety, depression and somatization; the Behavioural Symptoms Index

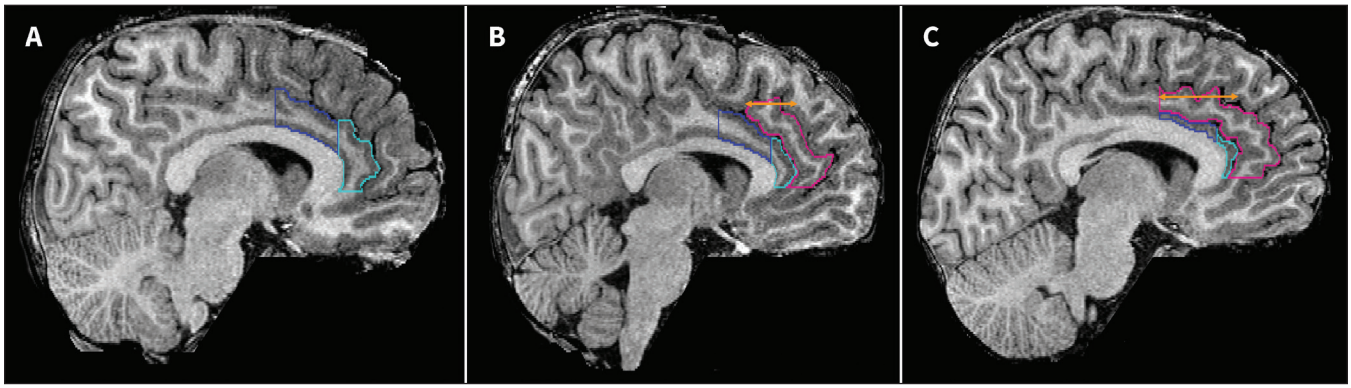

Figure 1: Delineation of the rostral cingulate cortical subregions (sagittal views). (A) The single gyrus pattern of the cingulate gyrus. (B and C) Double-parallel gyri patterns of the cingulate gyrus. The pregenual anterior cingulate cortex is outlined in cyan, the anterior midcingulate cortex is outlined in blue and the superior cingulate gyrus is outlined in pink. The orange arrows represent overlap between the superior cingulate gyrus and the total rostral cingulate cortex, used as a covariate in analyses.

includes atypicality, withdrawal and attention problems; and the Adaptive Skills scale includes adaptability, social skills, leadership, activities of daily living and functional communication.

The total raw score for each scale is transformed to a normalized *T* score based on a mean of 50 and a standard deviation of 10. Higher *T* scores represent more problematic emotions and behaviours for all composites except Adaptive Skills, in which lower *T* scores indicate deficits. We analyzed *T* scores of composites because of their high reliability compared to clinical scales.

Statistical analysis

We carried out all analyses in SPSS Statistics 28.0 (IBM). We used independent-samples *t* tests to compare age, gender, annual household income, maternal education and ICV between individuals with prenatal alcohol exposure and unexposed controls. We compared BASC-2 PRS composite *T* scores between individuals with prenatal alcohol exposure and unexposed controls using the Mann–Whitney *U* test because the Shapiro–Wilk test indicated that composite *T* scores were not normally distributed in either group.

We evaluated the intrarater reliability of the manual tracing using intraclass correlation coefficients for the volumes of the total rostral cingulate cortex, pregenual ACC and anterior MCC. We traced structures in both hemispheres twice, with intervals of 1–2 weeks, in 6 children and adolescents.

We used a series of analyses of covariance to compare volumes of the rostral cingulate cortex between individuals with and without prenatal alcohol exposure. Analyses of covariance included volume as the dependent variable, group and gender as factors, and superior cingulate gyrus overlap and age as covariates. We also tested the group × gender interaction but removed it from the model because it was not significant.

We tested correlations between rostral cingulate volumes and BASC-2 PRS scores using multivariable linear regression with a stepwise method for variable selection (with $p < 0.05$) separately in individuals with prenatal alcohol exposure and unexposed controls. To do so, we first adjusted ICV-normalized cingulate volumes to superior cingulate gyrus overlap separately in each group using the residual method[32] $V_{(adj\text{-}Nor)ith} = V_{(Nor)ith} - B\,(SCG_{ith} - SCG_{mean})$, where $V_{(adj\text{-}Nor)ith}$ is the adjusted ICV-normalized cingulate volumes to superior cingulate gyrus overlap in *i*th individual; $V_{(Nor)ith}$ is the ICV-normalized cingulate volumes in *i*th individual; *B* is the slope of the linear relationship between the superior cingulate gyrus overlap and the ICV-normalized volume of the total rostral cingulate cortex; SCG_{ith} is the overlap measure between the superior cingulate gyrus and the total rostral cingulate cortex in *i*th individual; and SCG_{mean} is the mean superior cingulate gyrus overlap measured for each group. We used $V_{(adj\text{-}Nor)ith}$, age and gender as independent variables and BASC-2 PRS composite *T* scores as dependent variables. *T* scores with more than 3 interquartile values were excluded as outliers from the regression analyses.

We used Benjamini–Hochberg false discovery rate (FDR) correction to correct for multiple comparisons for analysis of covariance (2 comparisons for total rostral cingulate cortex volume and 4 comparisons for subregion volumes) and BASC-2 PRS analyses (4 tests for Mann–Whitney *U* test, 8 tests for total rostral cingulate cortex volume and 16 tests for subregion volumes for regression analyses); *q* values are reported in the results and the tables. We used the Levene test to check homogeneity of variance, and set significance at $q < 0.05$ (2-tailed test) for all analyses.

Results

Demographic characteristics and emotional and behavioural functioning

We found no significant differences between individuals with prenatal alcohol exposure and unexposed controls in terms of age, gender, ICV, annual household income or maternal education (Table 1).

Individuals with prenatal alcohol exposure had significantly higher scores on the Externalizing Problems scale and Behavioural Symptoms Index, and significantly lower scores on the Adaptive Skills scale than unexposed controls on all measures, indicating more emotional and behavioural problems (all $q < 0.001$; Table 2). Scores on the Internalizing Problems scale did not differ significantly between groups.

Intraclass correlation coefficient analyses

Based on a mean-rating ($k = 2$), absolute-agreement, 2-way mixed-effects model, intrarater intraclass correlation coefficients for the pregenual ACC, anterior MCC and rostral cingulate cortex were greater than 0.99 (Table 3), indicating excellent reliability.[33]

Rostral cingulate cortex volumetric analyses

The volumes of the right total rostral cingulate cortex and the right pregenual ACC were significantly smaller in individuals with prenatal alcohol exposure than in unexposed controls (both $q < 0.05$). We found no other significant volume differences between groups (all $p > 0.22$; Table 4). We found no significant effects of group × gender on the volumes of the pregenual ACC, anterior MCC or total rostral cingulate cortex (all $p > 0.14$).

Behavioural assessment and rostral cingulate cortex volumes

In unexposed controls, right pregenual ACC volume was negatively associated with the Externalizing Problems scale, Internalizing Problems scale and Behavioural Symptoms Index (all $p < 0.05$). We also found a negative association between the volume of the right total rostral cingulate cortex and the Behavioural Symptoms Index. However, none of these associations was significant after FDR correction (all $q > 0.06$; Table 5 and Figure 2).

Table 1: Demographic characteristics*

Characteristic	Unexposed controls $n = 50$	Individuals with prenatal alcohol exposure $n = 30$	p value
Age, yr	9.95 ± 2.32	10.09 ± 2.38	0.79
Boy/girl, *n*	22/28	12/18	0.73
Intracranial volume, cm^3	1543.66 ± 179.01	1489.57 ± 114.32	0.10†
Annual household income, $	118 055.56 ± 47 197.08	109 000.00 ± 38 200.52	0.45
Maternal education, yr	15.04 ± 1.46	15.20 ± 2.48	0.78†

*Unless otherwise, indicated, values are mean ± standard deviation.
†Adjusted for violation of the homogeneity of variance.

Table 2: BASC-2 PRS *T* scores

	Unexposed controls $n = 47$		Individuals with prenatal alcohol exposure $n = 29$			
Composite scale	Mean rank	Sum of ranks	Mean rank	Sum of ranks	q value*	η^2
Externalizing Problems	29.95	1407.50	52.36	1518.50	< 0.001	0.24
Internalizing Problems	36.00	1692.00	42.55	1234.00	0.21	0.02
Behavioural Symptoms Index	28.66	1347.00	54.45	1579.00	< 0.001	0.32
Adaptive Skills	50.67	23 891.50	18.78	544.50	< 0.001	0.49

BASC-2-PRS = Behaviour Assessment System for Children, 2nd edition, Parent Rating Scale.
*For Benjamini–Hochberg false discovery rate correction, we assumed 4 tests for composite scales.

Table 3: Intrarater reliability

Region of interest*	ICC (95% CI)	p value
Pregenual ACC	0.993 (0.977–0.998)	< 0.001
Anterior MCC	0.995 (0.982–0.998)	< 0.001
Rostral cingulate cortex	0.993 (0.977–0.998)	< 0.001

ACC = anterior cingulate cortex; CI = confidence interval; ICC = intraclass correlation coefficient; MCC = midcingulate cortex.
*Structures were traced twice in 6 children and adolescents ($n = 12$).

In individuals with prenatal alcohol exposure, right anterior MCC volume showed a significant positive association with the Internalizing Problems scale. Moreover, we found a positive correlation between the volume of the right total rostral cingulate cortex and the Internalizing Problems scale, but this finding was not significant after correction for multiple comparisons. The left and right anterior MCC were positively and negatively associated with the Behavioural Symptoms Index and the Adaptive Skills scale, respectively (all $p < 0.05$). However, these associations were not statistically significant after FDR correction (Table 5 and Figure 3).

Age and gender were not significantly associated with BASC-2-PRS scores in either group. We performed a secondary analysis comparing BASC-2-PRS scores, rostral cingulate cortex volumes and their associations between individuals with prenatal alcohol exposure and a diagnosis of FASD or with no FASD. We found no significant differences in BASC-2-PRS score, rostral cingulate cortex volumes or their associations between these 2 groups.

Discussion

We found significantly smaller right pregenual ACC volume, as well as more behavioural and emotional problems, in individuals with prenatal alcohol exposure versus unexposed controls. We also found different associations between rostral cingulate cortex volumes and behavioural and emotional problems in individuals with prenatal alcohol exposure versus unexposed controls, suggesting a deviation from the normal behavioural development of the rostral cingulate cortex in individuals with prenatal alcohol exposure.

Previous researchers have reported mixed findings related to volumetric changes in the rostral cingulate cortex, including smaller ACC in an overlapping data set,[18] larger ACC[19] and larger left caudal ACC[20] in individuals with prenatal alcohol exposure than in unexposed controls. However, other studies have found no significant volume differences in cranial or brain-normalized volumes of cingulate grey matter or rostral and caudal ACC between individuals with prenatal alcohol exposure and unexposed controls.[21,22]

Most studies[18,20,21] used FreeSurfer, harnessing the Desikan–Killiany atlas to label the cingulate cortex.[35] A neuroanatomical limitation of this method is that it does not consider the superior cingulate gyrus or paracingulate sulcus in the structural analysis of the rostral cingulate cortex. Recently, a modified successor of the Desikan–Killiany atlas was published in which the superior cingulate gyrus was included as a part of the cingulate gyrus when the double-parallel pattern was formed.[36] However, this method is not optimal for use in volumetric analysis of the rostral cingulate gyrus for several reasons. First, the superior cingulate gyrus is

Table 4: ANCOVA and descriptive statistics for rostral cingulate cortex volumes

	Normalized volumes, mm³*		Estimated normalized volumes adjusted for superior cingulate gyrus overlap, mm³*				
Region of interest	Unexposed controls $n = 50$	Individuals with prenatal alcohol exposure $n = 30$	Unexposed controls $n = 50$	Individuals with prenatal alcohol exposure $n = 30$	$F_{1,75}$	p value	η_p^2
Right pregenual ACC	1642.99 ± 760.05	1488.72 ± 760.80	1717.25 ± 68.77	1387.93 ± 89.43	8.528	0.019†	0.102
Right anterior MCC	2152.45 ± 601.03	2188.95 ± 402.93	2186.14 ± 67.7	2146.69 ± 88.04	0.126	0.72	0.002
Right rostral cingulate cortex	3795.43 ± 1152.16	3677.67 ± 916.96	3903.39 ± 92.66	3534.62 ± 120.50	5.890	0.036†	0.073
Left pregenual ACC	1310.12 ± 651.58	1420.55 ± 802.53	NA	NA	0.131	0.72‡	0.010
Left anterior MCC	1851.89 ± 569.66	1851.33 ± 512.14	1901.34 ± 64.0	1771.55 ± 84.03	1.494	0.23	0.020
Left rostral cingulate cortex	3162.01 ± 1096.26	3271.88 ± 1138.74	3277.63 ± 102.36	3081.14 ± 133.31	1.363	0.25	0.018

ACC = anterior cingulate cortex; ANCOVA = analysis of covariance; FDR = false discovery rate; MCC = midcingulate cortex; NA = not applicable.
*Values are mean ± standard deviation.
†Represent a q value (i.e., a Benjamini–Hochberg FDR-corrected p value). For Benjamini–Hochberg FDR correction, we assumed 2 tests for total rostral cingulate cortex volume and 4 tests for subregion volumes.
‡Adjusted for rank analysis of covariance[34] because of violation of an ANCOVA assumption (i.e., residuals were not normally distributed).

Table 5: Regression models of the effects of the rostral cingulate cortex regions of interest on BASC-2 PRS *T* scores

Group	Composite scales	Rostral cingulate cortex regions of interest*
Unexposed controls	Externalizing Problems ($n = 47$)	Right pregenual ACC ($\beta = -0.317$, $p = 0.030$)
	Internalizing Problems ($n = 47$)	Right pregenual ACC ($\beta = -0.369$, $p = 0.011$, $q = 0.09$)
	Behavioural Symptoms Index ($n = 47$)	Right pregenual ACC ($\beta = -0.409$, $p = 0.004$, $q = 0.06$)
		Right total rostral cingulate cortex ($\beta = -0.295$, $p = 0.044$)
	Adaptive Skills ($n = 47$)	NS
Individuals with prenatal alcohol exposure	Externalizing Problems ($n = 28$)	NS
	Internalizing Problems ($n = 29$)	Right anterior MCC ($\beta = 0.536$, $p = 0.003$, $q = 0.048$)
		Right total rostral cingulate cortex ($\beta = 0.474$, $p = 0.009$, $q = 0.075$)
	Behavioural Symptoms Index ($n = 29$)	Right anterior MCC ($\beta = 0.429$, $p = 0.020$)
		Left anterior MCC ($\beta = 0.385$, $p = 0.038$)
	Adaptive Skills ($n = 29$)	Right anterior MCC ($\beta = -0.374$, $p = 0.046$)
		Left anterior MCC ($\beta = -0.369$, $p = 0.049$)

ACC = anterior cingulate cortex; BASC-2-PRS = Behaviour Assessment System for Children, 2nd edition, Parent Rating Scale; FDR = false discovery rate; MCC = midcingulate cortex; NS = not significant.
*Benjamini–Hochberg FDR-corrected p values are shown as q values. For Benjamini–Hochberg FDR correction, we assumed 8 tests for the associations between the BASC-2 PRS and total rostral cingulate cortex volume and 16 tests for the associations between the BASC-2 PRS and subregion volumes.

present in 30%–75% of cases and more often is in the left hemisphere,[16,24–26] which results in substantial interindividual or hemispheric variation. Second, the volume of the superior cingulate gyrus and the volume of the ventral rostral cingulate gyrus in the left ($r = -0.48$) and right ($r = -0.42$) hemispheres are negatively associated.[27] Finally, the superior cingulate gyrus (BA32/32') is considered the "cingulofrontal transition area" — the transition area between the cingulate gyrus and neighbouring frontal structures, with cytoarchitectonic characteristics of these structures.[12,25] Therefore, the superior cingulate gyrus should be regarded as a confounder in the structural analysis of the rostral cingulate cortex.[23]

Sowell and colleagues[19] used tensor-based morphometry, in which individual images were registered to a common anatomic template, to study structural differences between groups. In general, tensor-based morphometry and other automated methods (e.g., voxel-based morphometry) that normalize images to a common template have limitations for brain structural analysis: first, registration to a standard template minimizes interindividual variations of the brain structures;[23] second, a precise match to the common space might not be acquired for brain images with specific properties (e.g., superior cingulate gyrus) that are not present in the common space; and third, results are affected by the warping method harnessed for registration.[37] Bjorkquist and colleagues[22] manually delineated the cingulate gyrus; however, it is unclear whether the superior cingulate gyrus was included in their analysis. Furthermore, prenatal alcohol exposure is a heterogeneous condition, and differences between studies in terms of the dose, amount and gestational time of alcohol exposure may lead to differential observations for brain structure.[5,7] It is difficult to compare our findings with previous studies because of disparate methods and heterogeneous samples.

Individuals with prenatal alcohol exposure in the present study had significantly higher scores on the Externalizing

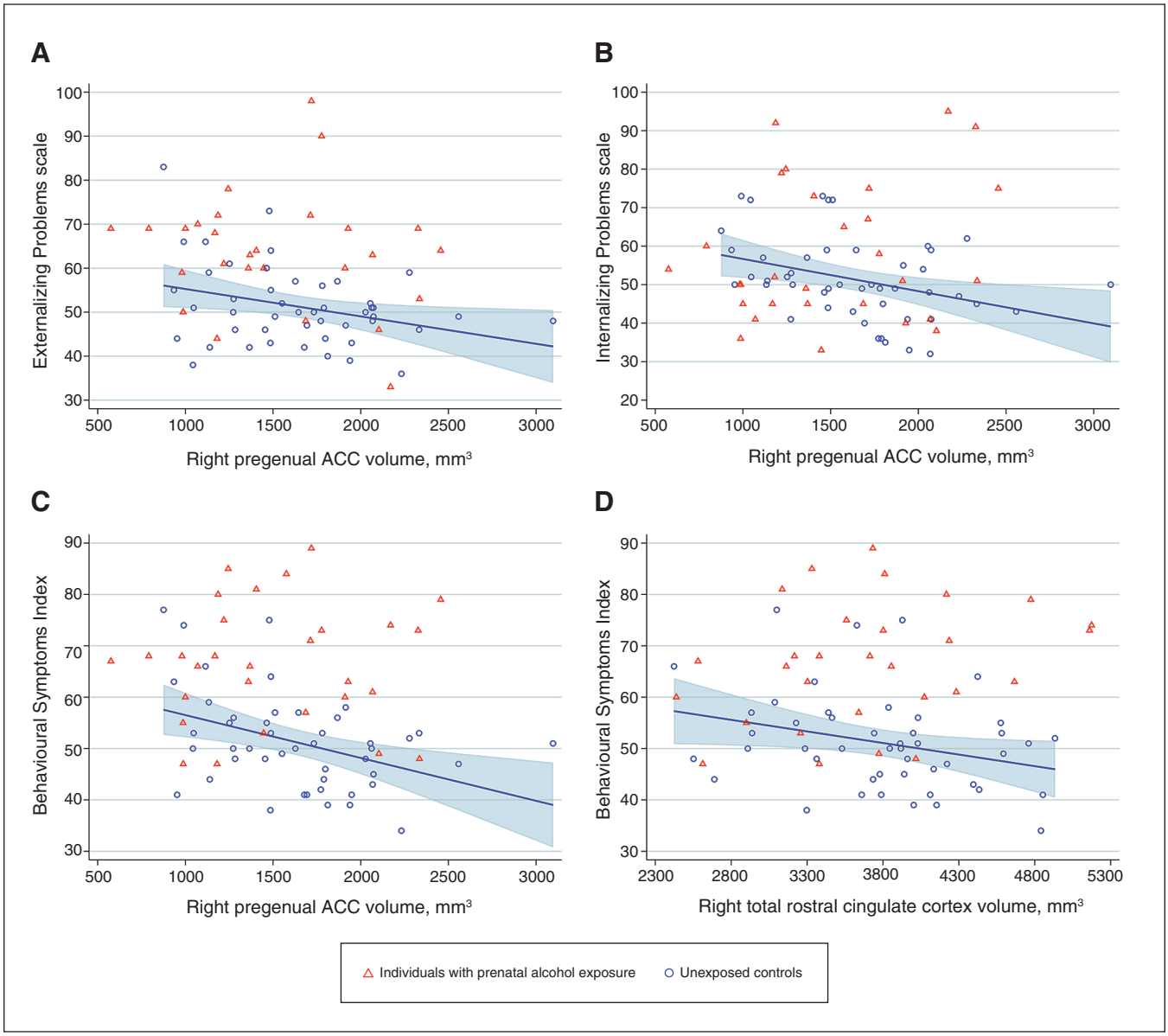

Figure 2: Regression plots showing the relationship between *T* scores on the BASC-2-PRS and rostral cingulate cortex volumes. (A) Externalizing Problems scale and volume of the right pregenual ACC. (B) Internalizing Problems scale and volume of the right pregenual ACC. (C) Behavioural Symptoms Index and volume of the right pregenual ACC. (D) Behavioural Symptoms Index and volume of the right total rostral cingulate cortex. Regression lines and confidence intervals demonstrate associations observed in unexposed controls. All volumes are in mm^3; they have been normalized to intracranial volume and adjusted for the superior cingulate gyrus. ACC = anterior cingulate cortex; BASC-2-PRS = Behaviour Assessment System for Children, 2nd edition, Parent Rating Scale.

Problems scale and Behavioural Symptoms Index and significantly lower scores on the Adaptive Skills scale than unexposed controls. These findings are in agreement with those of previous studies, which reported more externalizing and attention problems and fewer adaptive skills in individuals with prenatal alcohol exposure.[7,20] However, in contrast to previous studies that reported higher internalizing symptoms in children with prenatal alcohol exposure,[20,38] we did not find a significant difference between exposed and unexposed individuals on the Internalizing Problems scale. Externalizing problems tend to be easier to measure via caregiver report because they are directed outward to the environment; internalizing problems may be less apparent.[39] Self-reports in future studies will help further clarify whether internalizing symptoms are consistently affected in children with prenatal alcohol exposure.

Scores on the Externalizing Problems scale were negatively associated with right pregenual ACC volume in unexposed controls, but not in individuals with prenatal alcohol exposure. This finding is consistent with a meta-analysis that found a significant reduction in both the

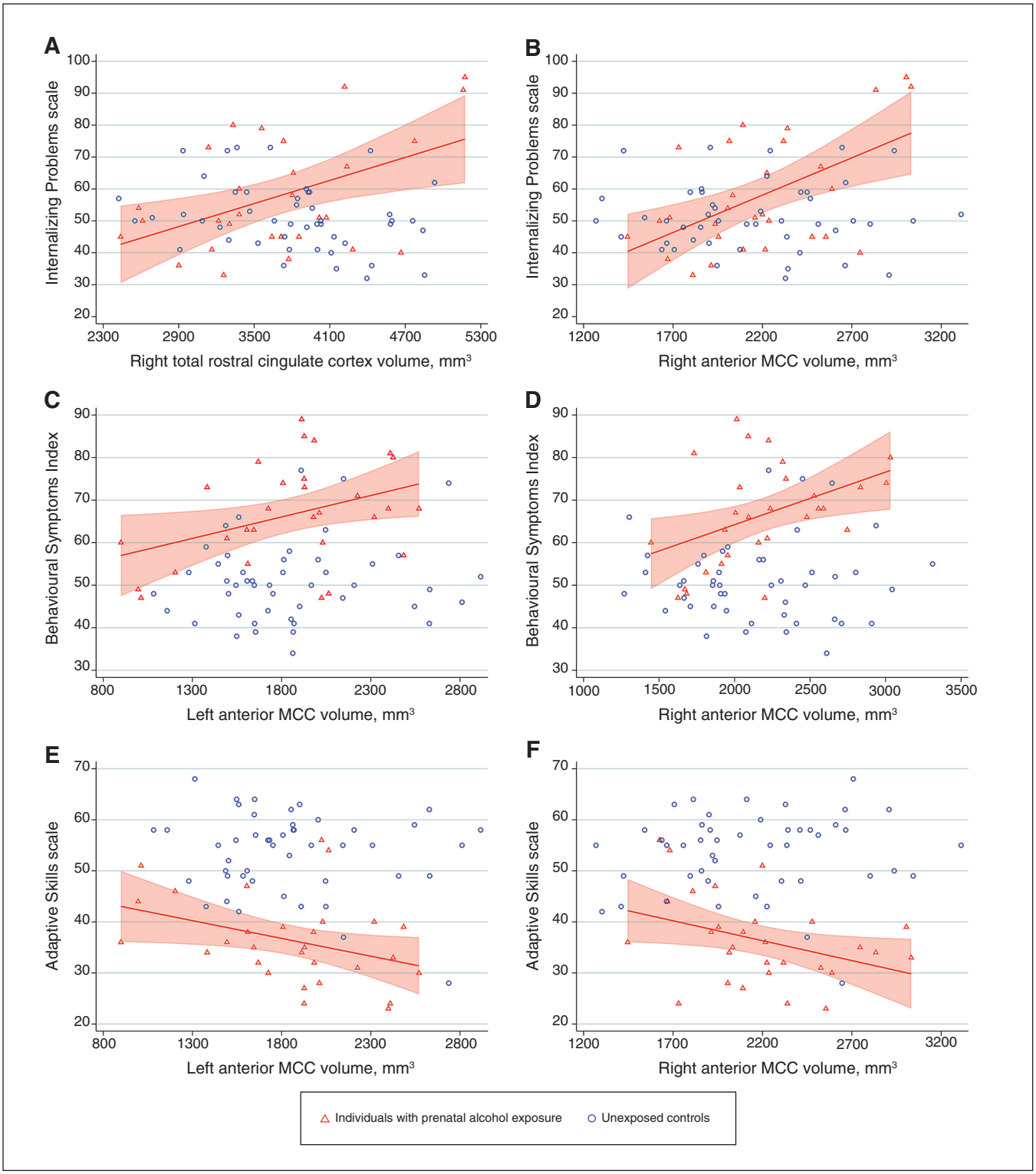

Figure 3: Regression plots showing the relationship between *T* scores of the BASC-2 PRS and rostral cingulate cortex volumes. (A) Internalizing Problems scale and right total rostral cingulate cortex. (B) Internalizing Problems scale and right anterior MCC. (C) Behavioural Symptoms Index and left anterior MCC. (D) Behavioural Symptoms Index and right anterior MCC. (E) Adaptive Skills scale and left anterior MCC. (F) Adaptive Skills scale and right anterior MCC. Regression lines and their confidence intervals demonstrate associations observed in individuals with prenatal alcohol exposure. All volumes are in mm³; they have been normalized to intracranial volume and adjusted for the superior cingulate gyrus. BASC-2-PRS = Behaviour Assessment System for Children, 2nd edition, Parent Rating Scale; MCC = midcingulate cortex.

activation and volume of the ACC (BA24/32) in the right hemisphere in individuals with antisocial behaviour, including aggression, psychopathy and conduct problems.[40] Furthermore, a consistent reduction was reported in the activity of the pregenual ACC and anterior MCC in adolescents with disruptive behaviour disorder (i.e., conduct disorder and oppositional defiant disorder) compared to typically developing adolescents across hot and cool executive functions, emotion processing and empathic pain in a meta-analysis of functional MRI task contrast studies,[41] indicating the involvement of these structures in externalizing behaviours.

In contrast to the lack of significant differences on the Internalizing Problems scale between individuals with prenatal alcohol exposure and unexposed controls, we did find differing associations between the Internalizing Problems scale and rostral cingulate cortex volumes. The right pregenual ACC had a negative association with the Internalizing Problems scale in unexposed controls, and the right anterior MCC had a significant positive association with the Internalizing Problems scale in individuals with prenatal alcohol exposure. We also found a positive correlation between the Internalizing Problems scale and the total volume of the right rostral cingulate cortex in individuals with prenatal alcohol exposure. In line with our finding in unexposed controls, meta-analyses have demonstrated volumetric reductions in the right pregenual ACC in anxiety disorders[42] and in the ACC in major depressive disorder.[43] As well, Kano and colleagues[44] and Perez and colleagues[45] reviewed abnormalities of brain structures, including the pregenual ACC and anterior MCC, that might be involved in dysfunctional interactions between brain and body in somatization. These studies suggest that internalizing symptoms affect the rostral cingulate cortex.

We found negative associations between the Behavioural Symptoms Index and the volumes of the right pregenual ACC and total rostral cingulate in unexposed controls. In contrast, the Behavioural Symptoms Index was positively associated with the volumes of the anterior MCC in both hemispheres in individuals with prenatal alcohol exposure. The involvement of the pregenual ACC in social decision-making and enhanced activation of the anterior MCC in social exclusion have been reported.[15,46] Moreover, Jarcho and colleagues[47] showed that preadolescents with high social reticence (i.e., shy and anxiously avoidant behaviour) in early childhood had higher anterior MCC activation when waiting for an unpredictable social appraisal. The anterior MCC is also involved in goal maintenance (e.g., attentional sets), and along with the pregenual ACC, it is affected by attention-deficit/hyperactivity disorder.[10,48]

Together, our findings and the above-mentioned studies support the involvement of the rostral cingulate cortex in behavioural problems. Emotion- and behaviour-related associations with the rostral cingulate cortex suggest a deviation from normal neurobehavioural development in individuals with prenatal alcohol exposure. Our findings suggest that the right pregenual ACC in unexposed controls is the major region of the rostral cingulate cortex to mediate BASC-2-PRS–relevant emotional and cognitive behaviours. In contrast, a smaller right pregenual ACC mainly induced by prenatal alcohol exposure might cause the anterior MCC to abnormally process BASC-2-PRS–relevant emotional and cognitive behaviours.

We found negative associations between Adaptive Skills scores and volumes of the right and left anterior MCC in individuals with prenatal alcohol exposure, but not in unexposed controls. The anterior MCC, along with other structures in the posterior medial frontal and lateral prefrontal cortices, is implicated in mediating adaptive goal-directed behaviours,[49] and it has been suggested that the rostral cingulate cortex is involved in joint attention and social-cognitive mentalizing, which enable individuals to share a common attitude with others and interpret others' beliefs or intentions.[50] Smaller right rostral cingulate volumes, mainly in BA24', have been reported in people with autism spectrum disorder,[51] suggesting the involvement of the rostral cingulate cortex in social aspects of adaptive functioning. The negative associations found here suggest that the anterior MCC also contributes to aspects of adaptive functioning in individuals with prenatal alcohol exposure, perhaps related to deficits in social communications, which are commonly associated with prenatal alcohol exposure.[7]

Most of the above-mentioned associations between the rostral cingulate cortex and behaviour occurred in the right hemisphere. These findings may reflect asymmetric processing of emotional stimuli, because previous studies have suggested that the right hemisphere dominates emotion, arousal and attention.[52,53]

Limitations

The present study was cross-sectional. In general, methods used for in vivo volumetric studies of the regions of the human rostral cingulate cortex do not exactly match the intra-anatomic boundaries of these structures; instead, different landmarks and geometrical rules are harnessed, which approximately match the location and orientation of these structures based on histological references.

As is typical of studies of individuals with prenatal alcohol exposure, it can be difficult to obtain detailed information about exposure amounts, frequency, duration and timing. We acquired extensive documentation to obtain detailed retrospective data and confirmed alcohol exposure in all cases. However, information regarding the exact amount, frequency, timing and duration of alcohol consumption during pregnancy was not always available.

Additional exposures (e.g., tobacco, cannabis, postnatal adversity) are common in individuals with prenatal alcohol exposure.[54,55] These can confound results, although findings suggest that prenatal alcohol exposure may be the dominant factor.[56]

Future studies with a larger sample size are required to clarify associations between volume and BASC-2-PRS behaviours that were not significant after FDR correction.

We used parent questionnaires to assess behaviours in this study. Although these are adequate for assessing children's behaviours and emotions at school age, a multi-informant method would have provided a more comprehensive picture of participants' behaviours and emotions and should be considered for future studies.[29]

Conclusion

This study provides in vivo evidence that prenatal alcohol exposure is associated with lower volume and impaired behavioural development of the rostral cingulate cortex. Volumetric reduction in the right pregenual ACC may partially underlie the emotional and behavioural problems associated with prenatal alcohol exposure.

Acknowledgement: The authors are thankful to all individuals who participated in this research.

Affiliations: From the Department of Radiology, Cumming School of Medicine, University of Calgary, Calgary, Alta. (Aghamohammadi-Sereshki, Pike, Lebel); the Alberta Children's Hospital Research Institute, Calgary, Alta. (McMorris, Gibbard, Lebel); the Werklund School of Education, University of Calgary, Calgary, Alta. (McMorris); the Department of Pediatrics, University of Calgary, Calgary, Alta. (Gibbard); the Department of Child Studies and Social Work, Mount Royal University, Calgary, Alta. (Tortorelli); the Department of Clinical Neurosciences, Cumming School of Medicine, University of Calgary, Alta. (Pike); the Hotchkiss Brain Institute, University of Calgary, Calgary, Alta. (Pike, Lebel).

Funding: This work was supported by a grant from the Canadian Institutes for Health Research (C. Lebel, C. McMorris, W. Gibbard, C. Tortorelli) and the Natural Sciences and Engineering Research Council Addictions and Mental Health Strategic Clinical Network Canada Research Chairs program. This work was also supported in part by funding from Brain Canada, in partnership with Health Canada, for the Canadian Open Neuroscience Platform initiative (A. Aghamohammadi-Sereshki);

Competing interests: C. Lebel's spouse is an employee of General Electric Healthcare. No other competing interests were delcared.

Contributors: G. Pike and C. Lebel designed the study. A. Aghamohammadi-Sereshki and C. McMorris acquired the data, which W. Gibbard and C. Tortorelli analyzed. A. Aghamohammadi-Sereshki wrote the article, which C. McMorris, W. Gibbard, C. Tortorelli, G. Pike and C. Lebel reviewed. All authors approved the final version to be published, agree to be accountable for all aspects of the work and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

Data statement: Neuroimaging data for the unexposed control group are publicly available here: https://doi.org/10.6084/m9.figshare.6002273.v2 (Lebel, 2018). Data on the prenatal alcohol exposure groups are available on request from Dr. Catherine Lebel.

References

1. Lemoine P, Harousseau H, Borteyru J, et al. Les enfants des parents alcooliques: anomalies observees apropos de 127 cas. *Ouest Med* 1968;21:476-82.
2. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* 1973;302:999-1001.
3. Jones KL, Smith DW, Ulleland CN, et al. Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* 1973;1:1267-71.
4. Lebel C, Roussotte F, Sowell ER. Imaging the impact of prenatal alcohol exposure on the structure of the developing human brain. *Neuropsychol Rev* 2011;21:102-18.
5. Moore EM, Migliorini R, Infante MA, et al. Fetal alcohol spectrum disorders: recent neuroimaging findings. *Curr Dev Disord Rep* 2014;1:161-72.
6. Easey KE, Dyer ML, Timpson NJ, et al. Prenatal alcohol exposure and offspring mental health: a systematic review. *Drug Alcohol Depend* 2019;197:344-53.
7. Mattson SN, Bernes GA, Doyle LR. Fetal alcohol spectrum disorders: a review of the neurobehavioral deficits associated with prenatal alcohol exposure. *Alcohol Clin Exp Res* 2019;43:1046-62.
8. Clarren SK, Alvord EC Jr, Sumi SM, et al. Brain malformations related to prenatal exposure to ethanol. *J Pediatr* 1978;92:64-7.
9. Cook JL, Green CR, Lilley CM, et al., Canada Fetal Alcohol Spectrum Disorder Research Network. Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. *CMAJ* 2016;188:191-7.
10. Shackman AJ, Salomons TV, Slagter HA, et al. The integration of negative affect, pain and cognitive control in the cingulate cortex. *Nat Rev Neurosci* 2011;12:154-67.
11. Vogt BA. Submodalities of emotion in the context of cingulate subregions. *Cortex* 2014;59:197-202.
12. Vogt BA, Palomero-Gallagher N. Cingulate vortex. In: Mai JK, Paxinos G, editors. *The human nervous system.* 3rd ed. London: Elsevier Academic Press; 2012: 943-87.
13. Vogt BA. The cingulate cortex in neurologic diseases: history, structure, overview. *Handb Clin Neurol* 2019;166:3-21.
14. Palomero-Gallagher N, Mohlberg H, Zilles K, et al. Cytology and receptor architecture of human anterior cingulate cortex. *J Comp Neurol* 2008;508:906-26.
15. Lockwood PL, Wittmann MK. Ventral anterior cingulate cortex and social decision-making. *Neurosci Biobehav Rev* 2018;92:187-91.
16. Amiez C, Procyk E. Midcingulate somatomotor and autonomic functions. *Handb Clin Neurol* 2019;166:53-71.
17. Vogt BA. Midcingulate cortex: structure, connections, homologies, functions and diseases. *J Chem Neuroanat* 2016;74:28-46.
18. Andre QR, McMorris CA, Kar P, et al. Different brain profiles in children with prenatal alcohol exposure with or without early adverse exposures. *Hum Brain Mapp* 2020;41:4375-85.
19. Sowell ER, Leow AD, Bookheimer SY, et al. Differentiating prenatal exposure to methamphetamine and alcohol versus alcohol and not methamphetamine using tensor-based brain morphometry and discriminant analysis. *J Neurosci* 2010;30:3876-85.
20. Lees B, Mewton L, Jacobus J, et al. Association of prenatal alcohol exposure with psychological, behavioral, and neurodevelopmental outcomes in children from the Adolescent Brain Cognitive Development Study. *Am J Psychiatry* 2020;177:1060-72.
21. Chen X, Coles CD, Lynch ME, et al. Understanding specific effects of prenatal alcohol exposure on brain structure in young adults. *Hum Brain Mapp* 2012;33:1663-76.
22. Bjorkquist OA, Fryer SL, Reiss AL, et al. Cingulate gyrus morphology in children and adolescents with fetal alcohol spectrum disorders. *Psychiatry Res* 2010;181:101-7.
23. Fornito A, Whittle S, Wood SJ, et al. The influence of sulcal variability on morphometry of the human anterior cingulate and paracingulate cortex. *Neuroimage* 2006;33:843-54.
24. Paus T, Tomaiuolo F, Otaky N, et al. Human cingulate and paracingulate sulci: pattern, variability, asymmetry, and probabilistic map. *Cereb Cortex* 1996;6:207-14.
25. Vogt BA, Nimchinsky EA, Vogt LJ, et al. Human cingulate cortex: surface features, flat maps, and cytoarchitecture. *J Comp Neurol* 1995;359:490-506.
26. Yücel M, Stuart GW, Maruff P, et al. Hemispheric and gender-related differences in the gross morphology of the anterior cingulate/paracingulate cortex in normal volunteers: an MRI morphometric study. *Cereb Cortex* 2001;11:17-25.

27. Paus T, Otaky N, Caramanos Z, et al. In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences and probability maps. *J Comp Neurol* 1996;376:664-73.
28. Reynolds CR, Kamphaus RW. *The behavior assessment system for children.* 2nd ed. Bloomington (MN): Pearson; 2004.
29. Cianchetti C. Early detection of behavioral and emotional problems in school-aged children and adolescents: the parent questionnaires. *Clin Pract Epidemiol Ment Health* 2020;16:7-16.
30. Malykhin NV, Carter R, Hegadoren KM, et al. Fronto-limbic volumetric changes in major depressive disorder. *J Affect Disord* 2012;136:1104-13.
31. Gaser C, Dahnke R, Kurth K, et al. CAT — a computational anatomy toolbox for the analysis of structural MRI data. *bioRxiv* 2022 June 13. doi:10.1101/2022.06.11.495736
32. Nordenskjöld R, Malmberg F, Larsson EM, et al. Intracranial volume normalization methods: considerations when investigating gender differences in regional brain volume. *Psychiatry Res* 2015;231:227-35.
33. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 2016;15:155-63.
34. Quade D. Rank analysis of covariance. *J Am Stat Assoc* 1967;62: 1187-200.
35. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006;31:968-80.
36. Klein A, Tourville J. 101 labeled brain images and a consistent human cortical labeling protocol. *Front Neurosci* 2012;6:171.
37. Ashburner J, Friston KJ. Morphometry. In: Frackowiak RSJ, Friston KJ, Frithet CD, et al, editors. *Human brain function.* 2nd ed. Cambridge (MA): Academic Press; 2003: chapter 6.
38. Krueger AM, Roediger DJ, Mueller BA, et al. Para-limbic structural abnormalities are associated with internalizing symptoms in children with prenatal alcohol exposure. *Alcohol Clin Exp Res* 2020;44:1598-608.
39. Valla JP, Kovess V, Chan Chee C, et al. A French study of the Dominic Interactive. *Soc Psychiatry Psychiatr Epidemiol* 2002;37:441-8.
40. Yang Y, Raine A. Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: a meta-analysis. *Psychiatry Res* 2009;174:81-8.
41. Alegria AA, Radua J, Rubia K. Meta-analysis of fMRI studies of disruptive behavior disorders. *Am J Psychiatry* 2016;173:1119-30.
42. Shang J, Fu Y, Ren Z, et al. The common traits of the ACC and PFC in anxiety disorders in the DSM-5: meta-analysis of voxel-based morphometry studies. *PLoS One* 2014;9:e93432.
43. Koolschijn PC, van Haren NE, Lensvelt-Mulders GJ, et al. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp* 2009;30: 3719-35.
44. Kano M, Oudenhove LV, Dupont P, et al. Imaging brain mechanisms of functional somatic syndromes: potential as a biomarker? *Tohoku J Exp Med* 2020;250:137-52.
45. Perez DL, Barsky AJ, Vago DR, et al. A neural circuit framework for somatosensory amplification in somatoform disorders. *J Neuropsychiatry Clin Neurosci* 2015;27:e40-50.
46. Eisenberger NI, Lieberman MD, Williams KD. Does rejection hurt? An FMRI study of social exclusion. *Science* 2003;302:290-2.
47. Jarcho JM, Davis MM, Shechner T, et al. Early-childhood social reticence predicts brain function in preadolescent youths during distinct forms of peer evaluation. *Psychol Sci* 2016;27:821-35.
48. Vogt BA. Cingulate impairments in ADHD: comorbidities, connections, and treatment. *Handb Clin Neurol* 2019;166:297-314.
49. Ridderinkhof KR, Ullsperger M, Crone EA, et al. The role of the medial frontal cortex in cognitive control. *Science* 2004;306:443-7.
50. Mundy P. A review of joint attention and social-cognitive brain systems in typical development and autism spectrum disorder. *Eur J Neurosci* 2018;47:497-514.
51. Haznedar MM, Buchsbaum MS, Wei TC, et al. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *Am J Psychiatry* 2000;157:1994-2001.
52. Gainotti G. Emotions and the right hemisphere: can new data clarify old models? *Neuroscientist* 2019;25:258-70.
53. Hartikainen KM. Emotion-attention interaction in the right hemisphere. *Brain Sci* 2021;11:1006.
54. Lebel CA, McMorris CA, Kar P, et al. Characterizing adverse prenatal and postnatal experiences in children. *Birth Defects Res* 2019;111: 848-58.
55. Astley SJ. Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. *Can J Clin Pharmacol* 2010;17:e132-64.
56. Hemingway SJA, Davies JK, Jirikowic T, et al. What proportion of the brain structural and functional abnormalities observed among children with fetal alcohol spectrum disorder is explained by their prenatal alcohol exposure and their other prenatal and postnatal risks? *Adv Pediatr Res* 2020;7:41.