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

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Introduction

The first well-known reports of the dystrophic and teratogenic effects of alcohol were published in the late 1960s and early 1970s. Since then, a growing body of research has shown the widespread effects of prenatal alcohol exposure on the brain, on mental health and on cognition and behaviour. Early studies of prenatal alcohol exposure were mainly post-mortem examinations of the most severe effects of prenatal alcohol exposure — namely fetal alcohol syndrome. 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). FASD is a term that includes people with a spectrum of cognitive and behavioural effects induced by prenatal alcohol exposure, regardless of facial dysmorphology. 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.

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.
Prenatal alcohol exposure and the structure of the rostral cingulate cortex

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.

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 ascent 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. 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. 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.

Participants

The Behaviour Assessment System for Children, 2nd Edition (BASC-2), 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, 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.
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 $0.8 \times 0.8 \times 0.8$ mm$^3$). Images were preprocessed using Computational Anatomy Toolbox (CAT12) 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 colleagues26 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

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**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: $V_{adj-nor} = V_{nor} - B (SCG_{nor} - SCG_{mean})$, where $V_{adj-nor}$ is the adjusted ICV-normalized cingulate volumes to superior cingulate gyrus overlap in $i$th individual; $V_{nor}$ 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; $SCG_{nor}$ 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 $V_{adj-nor}$, 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.

**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).
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, larger ACC and larger left caudal ACC 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.

Most studies used FreeSurfer, harnessing the Desikan–Killiany atlas to label the cingulate cortex. 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. 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

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**Table 1: Demographic characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unexposed controls $n = 50$</th>
<th>Individuals with prenatal alcohol exposure $n = 30$</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>$9.95 \pm 2.32$</td>
<td>$10.09 \pm 2.38$</td>
<td>0.79</td>
</tr>
<tr>
<td>Boy/girl, $n$</td>
<td>22/28</td>
<td>12/18</td>
<td>0.73</td>
</tr>
<tr>
<td>Intracranial volume, cm$^3$</td>
<td>$1543.66 \pm 179.01$</td>
<td>$1489.57 \pm 114.32$</td>
<td>0.10†</td>
</tr>
<tr>
<td>Annual household income, $</td>
<td>$118055.56 \pm 47197.08$</td>
<td>$109000.00 \pm 38200.52$</td>
<td>0.45</td>
</tr>
<tr>
<td>Maternal education, yr</td>
<td>$15.04 \pm 1.46$</td>
<td>$15.20 \pm 2.48$</td>
<td>0.78†</td>
</tr>
</tbody>
</table>

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

**Table 2: BASC-2 PRS $T$ scores**

<table>
<thead>
<tr>
<th>Composite scale</th>
<th>Unexposed controls $n = 47$</th>
<th>Individuals with prenatal alcohol exposure $n = 29$</th>
<th>$q$ value*</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean rank</td>
<td>Sum of ranks</td>
<td>Mean rank</td>
<td>Sum of ranks</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>Externalizing Problems</td>
<td>29.95</td>
<td>1407.50</td>
<td>52.36</td>
<td>1518.50</td>
</tr>
<tr>
<td>Internalizing Problems</td>
<td>36.00</td>
<td>1692.00</td>
<td>42.55</td>
<td>1234.00</td>
</tr>
<tr>
<td>Behavioural Symptoms Index</td>
<td>28.66</td>
<td>1347.00</td>
<td>54.45</td>
<td>1579.00</td>
</tr>
<tr>
<td>Adaptive Skills</td>
<td>50.67</td>
<td>23891.50</td>
<td>18.78</td>
<td>544.50</td>
</tr>
</tbody>
</table>

*For Benjamini–Hochberg false discovery rate correction, we assumed 4 tests for composite scales.

**Table 3: Intrarater reliability**

<table>
<thead>
<tr>
<th>Region of interest*</th>
<th>ICC (95% CI)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregenual ACC</td>
<td>0.993 (0.977–0.998)</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Anterior MCC</td>
<td>0.995 (0.982–0.998)</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Rostral cingulate cortex</td>
<td>0.993 (0.977–0.998)</td>
<td>$&lt; 0.001$</td>
</tr>
</tbody>
</table>

ACC = anterior cingulate cortex; CI = confidence interval; ICC = intraclass correlation coefficient; MCC = midcingulate cortex.
*Structures were traced twice in 6 children and adolescents ($n = 12$).
present in 30%–75% of cases and more often is in the left hemisphere,\textsuperscript{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.\textsuperscript{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.\textsuperscript{12,25} Therefore, the superior cingulate gyrus should be regarded as a confounder in the structural analysis of the rostral cingulate cortex.\textsuperscript{23}

Sowell and colleagues\textsuperscript{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;\textsuperscript{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.\textsuperscript{27} Bjorkquist and colleagues\textsuperscript{22} manually delineated the cingulate gyrus; however, it is unclear whether the superior cingulate gyrus was included in their delineation method. \textsuperscript{27}

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

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Normalized volumes, mm$^3$</th>
<th>Estimated normalized volumes adjusted for superior cingulate gyrus overlap, mm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexposed controls n = 50</td>
<td>Individuals with prenatal alcohol exposure n = 30</td>
</tr>
<tr>
<td>Right pregenual ACC</td>
<td>1642.99 ± 760.05</td>
<td>1488.72 ± 760.80</td>
</tr>
<tr>
<td>Right anterior MCC</td>
<td>2152.45 ± 601.03</td>
<td>2188.95 ± 402.93</td>
</tr>
<tr>
<td>Right rostral cingulate</td>
<td>3795.43 ± 1152.16</td>
<td>3677.67 ± 916.96</td>
</tr>
<tr>
<td>Left pregenual ACC</td>
<td>1310.12 ± 651.58</td>
<td>NA</td>
</tr>
<tr>
<td>Left anterior MCC</td>
<td>1851.89 ± 569.66</td>
<td>1851.33 ± 512.14</td>
</tr>
<tr>
<td>Left rostral cingulate</td>
<td>3162.01 ± 1096.26</td>
<td>3271.88 ± 1138.74</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Unexposed controls n = 50</th>
<th>Individuals with prenatal alcohol exposure n = 30</th>
<th>$F_{1,75}$</th>
<th>$p$ value</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right pregenual ACC</td>
<td>1642.99 ± 760.05</td>
<td>1488.72 ± 760.80</td>
<td>1378.93 ± 89.43</td>
<td>8.528</td>
<td>0.019†</td>
</tr>
<tr>
<td>Right anterior MCC</td>
<td>2152.45 ± 601.03</td>
<td>2188.95 ± 402.93</td>
<td>2146.69 ± 88.04</td>
<td>0.126</td>
<td>0.72</td>
</tr>
<tr>
<td>Right rostral cingulate</td>
<td>3795.43 ± 1152.16</td>
<td>3677.67 ± 916.96</td>
<td>3534.62 ± 120.50</td>
<td>5.890</td>
<td>0.036†</td>
</tr>
<tr>
<td>Left pregenual ACC</td>
<td>1310.12 ± 651.58</td>
<td>NA</td>
<td>NA</td>
<td>0.131</td>
<td>0.72†</td>
</tr>
<tr>
<td>Left anterior MCC</td>
<td>1851.89 ± 569.66</td>
<td>1851.33 ± 512.14</td>
<td>1771.55 ± 84.03</td>
<td>1.494</td>
<td>0.23</td>
</tr>
<tr>
<td>Left rostral cingulate</td>
<td>3162.01 ± 1096.26</td>
<td>3271.88 ± 1138.74</td>
<td>3081.14 ± 133.31</td>
<td>1.363</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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 because of violation of an ANCOVA assumption (i.e., residuals were not normally distributed).

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

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

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.
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 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.
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 mm$^3$; 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. 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, 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 and in the ACC in major depressive disorder. As well, Kano and colleagues and Perez and colleagues 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. Moreover, Jarcho and colleagues 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 set), and along with the pregenual ACC, it is affected by attention-deficit/hyperactivity disorder.

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, 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. Smaller right rostral cingulate volumes, mainly in BA24, have been reported in people with autism spectrum disorder, 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.

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.

**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 anatomical 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. These can confound results, although findings suggest that prenatal alcohol exposure may be the dominant factor.

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.

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