Prenatal Cannabis and Tobacco Exposure in Relation to Brain Morphology: A Prospective Neuroimaging Study in Young Children

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ABSTRACT
BACKGROUND: Cannabis use during pregnancy has been associated with negative behavioral outcomes and psychopathology in offspring. However, there has been little research evaluating alterations in brain structure as a result of maternal cannabis use. In this prospective study, we investigated the association between prenatal cannabis exposure and brain morphology in young children.

METHODS: We matched 96 children prenatally exposed to tobacco only (without cannabis) with 113 unexposed control subjects on the basis of age and gender and subsequently selected 54 children exposed to prenatal cannabis (mostly combined with tobacco exposure). These children (aged 6 to 8 years) were part of a population-based study in the Netherlands, the Generation R Study, and were followed from pregnancy onward. We assessed brain volumetric measures and cortical thickness in magnetic resonance imaging scans using FreeSurfer. We performed vertex-wise analyses in FreeSurfer and linear regression analyses adjusting for relevant covariates using Statistical Package for the Social Sciences.

RESULTS: Prenatal cannabis exposure was not associated with global brain volumes, such as total brain volume, gray matter volume, or white matter volume. However, prenatal cannabis exposure was associated with differences in cortical thickness: compared with nonexposed control subjects, cannabis-exposed children had thicker frontal cortices. Prenatal tobacco exposure compared with nonexposed control subjects was associated with cortical thinning, primarily in the superior frontal and superior parietal cortices.

CONCLUSIONS: Our findings suggest an association between prenatal cannabis exposure and cortical thickness in children. Further research is needed to explore the causal nature of this association.

Keywords: Brain morphology, Neuroimaging, Pediatric brain development, Population-based study, Prenatal cannabis exposure, Prenatal tobacco exposure

http://dx.doi.org/10.1016/j.biopsych.2015.08.024

Worldwide, cannabis is commonly used among pregnant women with prevalence varying from 2% to 13% (1–4). Prenatal cannabis use is an important issue, as it may have consequences on health and brain development in offspring (5,6).

The long-term consequences of prenatal cannabis exposure on neurodevelopment have been largely investigated in two longitudinal studies. In 1978, the Ottawa Prenatal Prospective Study (OPPS) collected behavioral data from offspring of a middle-class population of pregnant women who smoked cannabis (7). In 1982, the Maternal Health Practices and Child Development project investigated the long-term behavioral consequences of prenatal cannabis exposure in a low-income African-American population (8). Results from these cohorts suggest that prenatal cannabis exposure has both short- and long-term consequences. For example, prenatal cannabis has been associated with aberrant behavior in newborns (9), cognitive deficits, impairments in inhibitory control, delinquency, and increased risk of drug abuse later in life (10–15). In the Generation R Study, a population-based prospective cohort in the Netherlands, which started in 2002, our group assessed the associations between prenatal cannabis and several offspring outcomes. This recent cohort is important, because improved breeding technology has significantly increased Δ-9-tetrahydrocannabinol (THC) levels in marijuana and hashish (16). Data from the Generation R cohort have shown growth retardation and decreased blood flow in the fetus (17,18) and attention and aggression problems in childhood (19) associated with prenatal cannabis exposure.

While the above-mentioned studies suggest that prenatal cannabis exposure may be harmful for the developing fetus, there is still little information as to the extent such early cannabis exposure can have persistent effects on brain development. To our knowledge, no information is available concerning the potential consequences of prenatal cannabis exposure on brain morphological differences later in life.
In a functional magnetic resonance imaging (fMRI) study, the OPPS demonstrated that young adults prenatally exposed to cannabis have increased activation in several brain regions during a visuospatial working memory task (left inferior and middle frontal gyri, left parahippocampal gyrus, left middle occipital gyrus, and left cerebellum). During this task, decreased activation was observed in right inferior and middle frontal gyri, suggesting that prenatal marijuana alters neural function during visuospatial working memory processing (20). Additionally, during a response inhibition task, increased activation was observed in the bilateral prefrontal cortex and right premotor cortex and decreased activation was observed in the left cerebellum (21), proposing that prenatal cannabis exposure is associated with neural activation during response inhibition. However, it was unclear whether these differences in activation were the result of differences in brain morphology, as this was not assessed in these fMRI studies (20,21).

Moreover, these fMRI studies have some limitations, including a small sample size, confounding by current cannabis use, and challenges relating to the reliability of fMRI.

The current structural MRI study is focused on preadolescent children aged 6 to 8 years, an age range before the risk period of cannabis use in children. Pregnant mothers who smoke cannabis also tend to smoke tobacco. To disentangle the associations of smoking cannabis and tobacco with brain morphology, it is important to take into account tobacco smoking during pregnancy. Prenatal tobacco exposure has been associated with thinner orbitofrontal, middle frontal, and parahippocampal cortices, particularly in girls (22,23). Previously, we demonstrated that prenatal tobacco smoking was associated with thinner cortices in children aged 6 to 8 years (24). We used this study as a starting point to investigate the association of prenatal exposure to cannabis with brain morphology in 6- to 8-year-old children. Our hypothesis is that prenatal cannabis exposure will be associated with global morphological differences in the offspring brain, similar or even increased as compared with the association between prenatal tobacco exposure and brain morphology.

**METHODS AND MATERIALS**

**Design and Setting**

Subjects were recruited from an ongoing population-based prospective cohort, the Generation R Study. The study design has been described previously (25) and was approved by the Medical Ethics Committee (MEC) of the Erasmus Medical Centre. Written informed consent was obtained from all participants and the MEC requested to preinform the participants about the purpose of this MRI study (MEC 2008-140). In September 2009, 6- to 8-year-old children from the Generation R Study were invited to participate in an MRI component (26). Approximately 20% of the families declined to participate in this component. Exclusion criteria were having a significant motor or sensory disorder, moderate to severe head trauma with loss of consciousness, neurological disorders (including seizure disorder, neuromotor disorder, or a history of brain tumors), claustrophobia, and contraindications to MRI (e.g., having a pacemaker).

We selected all children exposed prenatally to cannabis (mostly combined with tobacco) with structural MRI data (n = 54) and children prenatally exposed to only tobacco (without cannabis) (n = 97). The unexposed control subjects (n = 113) were matched based on age and gender using a fuzzy matching procedure. This procedure randomly searches for a case-control match that falls within the set of defined criteria: an exact match for gender and a fuzzy match for age with a difference of 4 months (smaller age differences did not yield a match for each exposed child). We used this approach in our previous study on prenatal tobacco exposure and brain morphology (24); the same subjects were used in the current study.

**Prenatal Cannabis Exposure**

To optimize the cannabis exposure assessment, we used two sources of information: 1) maternal self-report of cannabis use with a questionnaire; and 2) maternal THC levels from urine. Self-reports on cannabis use during pregnancy were obtained once. In the first trimester, mothers indicated whether they used cannabis before or during pregnancy and whether they were still using cannabis, as has been described previously (4). Information about the product used and frequency of cannabis use (daily, weekly, monthly) was also available. Urine samples were collected in early, mid, and late pregnancy, and the first available sample was used for urinalysis. Urine samples were available in a subset of the cohort (4) and were tested for 11-nor-Δ9-THC-9-COOH using the DRI Cannabinoid Assay (Microgenics Corporation, Fremont, California) with a cutoff value of 50 μg/L as recommended by the manufacturer and the Substance Abuse and Mental Health Security Agency. The agreement between self-reported cannabis use and THC levels using Yule’s Y was .77, indicating substantial agreement (4). Self-reported cannabis use prevalence before and during pregnancy corresponded with the prevalence of cannabis use among Dutch women aged between 15 and 64 years in the same period (27).

**Prenatal Tobacco Exposure**

Information about maternal smoking was prospectively obtained by postal questionnaires in each trimester (28). Maternal smoking and cannabis use were both assessed in the first questionnaire; in the second and third questionnaires, information on maternal smoking was assessed. Maternal smoking at enrollment was assessed in the first questionnaire by asking whether the mother smoked during pregnancy. In the second and third questionnaires (mid and late pregnancy), mothers were asked whether they had smoked in the last 2 months. Maternal smoking was categorized on the basis of all three questionnaires into no smoking during pregnancy, until pregnancy was known, and continued during pregnancy. The group that used tobacco until the pregnancy was known was excluded from the current study. The number of cigarettes smoked was compiled into three categories: less than 5 cigarettes per day, between 5 and 9 cigarettes per day, and more than 9 cigarettes/day (only eight pregnant mothers smoked more than 20 cigarettes per day in one of the trimesters).
**Magnetic Resonance Imaging**

Children were familiarized with the MRI environment during a mock scanning session. All images were acquired using the same sequence on the same scanner, located at the radiology department in the Erasmus Medical Centre. Images were acquired on a 3 Tesla scanner (750 Discovery, GE Healthcare, Milwaukee, Wisconsin) using an eight-channel head coil. A high-resolution T1 inversion recovery fast spoiled gradient recalled sequence was acquired with the following parameters: echo time = 4.24 milliseconds, inversion time = 350 milliseconds, repetition time = 10.26 milliseconds, number of excitations = 1, flip angle = 16°, and .9 mm³ isotropic resolution. At the scanner, structural images were rated for quality. If the T1 scan was evaluated as poor, the sequence was repeated. During scanning, researchers were blind to the child’s exposure status.

**Cortical and Volumetric Measures**

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.1.0 (http://surfer.nmr.mgh.harvard.edu; Martinos Center for Biomedical Imaging, Charlestown, Massachusetts). FreeSurfer computes these measures in an automated approach, and technical procedures were described extensively (29). Cortical thickness measurements were validated against histological analysis (30) and manual measurement (31,32). FreeSurfer morphometry has demonstrated good test-retest reliability across scanner manufacturers and field strengths (33,34). Numerous studies using FreeSurfer in typical and atypical developing school-aged children are available (35–38). All FreeSurfer output was visually inspected for quality control.

**Additional Measures**

Maternal characteristics were maternal age at intake, ethnicity, educational level, household income, marital status, and drinking habits. Child characteristics were age at MRI assessment, gender, head circumference and nonverbal IQ at age 6 years, gestational age at birth, and birth weight. Maternal ethnicity was defined according to the classification of Statistics Netherlands. Maternal educational level was categorized into primary (no or primary education), secondary (lower and intermediate vocational training), and higher (higher vocational education and university) education. Information about household income and marital status was collected in the first questionnaire. Information on maternal drinking habits, like information on maternal smoking habits, was collected using questionnaires in each trimester. Nonverbal IQ was assessed at the age of 6 years with two subtests of a revised Dutch intelligence test, the Snijders-Oomen Niet-verbale intelligentie Test-Revisie (SON-R 2.5-7): Mosaics and Categories (39), and at this visit at 6 years of age, head circumference was measured by a research assistant. Child emotional and behavioral problems were assessed at the age of 5 years (40). Gestational age and weight at birth were extracted from medical records. A NEPSY-II was assessed at the time of the MRI assessment; analyses with the NEPSY-II as an outcome are presented in Supplement 1.

**Statistical Analyses**

**Demographic Measures and Volumetric Measures.** Differences in demographics and cognitive and clinical variables between the cannabis-exposed, tobacco-exposed (continued smoking), and nonexposed groups were determined using Chi-square tests for categorical data and t tests for continuous variables. Volumetric measures used included total brain volume, cortical volume, cortical gray matter volume, cortical white matter volume, and ventricular volume. These tests were performed using linear regression analyses. In all models, subjects were matched on age and gender (model I), and we adjusted for maternal education, ethnicity, and prenatal alcohol use (model II) and birth weight (model III). Covariates were selected based on prior literature or if the covariate changed the effect estimate (B) by 5% or more. In the current study, we did not further stratify the analyses for frequency of cannabis use or type of cannabis product due to small sample sizes of the specific groups.

**Vertex-wise Group Analyses.** We performed vertex-wise analyses to assess regional differences. Differences in cortical thickness smoothed with a 10 mm full-width at half maximum Gaussian kernel for both hemispheres between cannabis-exposed, tobacco-exposed, and unexposed subjects were analyzed using analysis within FreeSurfer with the built-in general linear model module QDEC. This procedure allows for generation of statistical parametric maps with an uncorrected threshold of p < .01 for initial vertex-wise comparison. Thereafter, a cluster-wise correction for multiple comparisons was performed using the built-in simulation procedure with 5000 iterations and a cluster-wise threshold of p < .05, which controls for false positive clusters (41). Clusters that were significantly associated with prenatal cannabis or tobacco exposure were extracted and linear regression analyses were performed. In all models, subjects were matched on age and gender (model I), and we adjusted for maternal education, ethnicity, household income, marital status, prenatal alcohol use, and maternal psychopathology (model II) and birth weight to check whether the findings were mediated by birth weight (model III). Further adjustment for child emotional and behavioral problems was also performed.

The analyses were performed in Statistical Package for Social Sciences v.20.0 (IBM Corporation, Armonk, New York). False discovery rate corrected p values were calculated using the R Statistical Software v.2.12.0 (R Core Team, Vienna, Austria).

**RESULTS**

**Descriptive Information**

Demographic characteristics are reported in Table 1. Three groups were defined: the nonexposed control subjects (n = 113), children of whom the mother continued to smoke during pregnancy (n = 96, multiple trimester exposure), and children of whom the mother used cannabis at any time during pregnancy (n = 54, prenatal cannabis exposure). Compared with nonexposed control subjects, women who used cannabis during pregnancy were younger (28.0 ± 5.7 versus
30.5 ± 4.5 years, \( p = .005 \), more often had only secondary education and thus were lower educated (67.3\% versus 46.0\%, \( p = .01 \)), and more often drank alcohol during pregnancy (16.3\% nondrinking versus 41.6\%, \( p = .003 \)). Children exposed to cannabis were more likely to have a lower birth weight birth (3203 ± 604 versus 3475 ± 520 grams, \( p = .003 \)) than the children of control subjects. Importantly, birth weight in this smaller MRI subsample was very similar to birth weight in the larger sample of cannabis-exposed children (3206 ± 535 grams, \( n = 173 \)) (17). No differences between the cannabis-exposed group and unexposed control subjects were observed on other variables, including ethnicity, nonverbal IQ, and gestational age at birth (Table 1).

Children exposed to tobacco were more likely to have a lower weight at birth (3194 ± 536 versus 3475 ± 520 grams, \( p < .001 \)) and their mothers were more likely to have only secondary education (66.6\% versus 46.0\%, \( p = .005 \)) than control subjects. No differences between the tobacco-exposed and the control subjects were found on other demographic variables including maternal age, ethnicity, drinking habits, nonverbal IQ, and gestational age at birth (Table 1).

### Table 1. Descriptive Statistics of Whole Study Population

<table>
<thead>
<tr>
<th></th>
<th>Nonexposed Control Subjects</th>
<th>Prenatal Tobacco Exposure (Multiple Trimesters)</th>
<th>Prenatal Cannabis Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 113 )</td>
<td>( n = 96 )</td>
<td>( n = 54 )</td>
</tr>
<tr>
<td>Maternal Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age at intake</td>
<td>30.5 ± 4.5</td>
<td>29.3 ± 5.8</td>
<td>28.0 ± 5.7*</td>
</tr>
<tr>
<td>Educational level (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>8.0</td>
<td>10.4*</td>
<td>13.5*</td>
</tr>
<tr>
<td>Secondary</td>
<td>46.0</td>
<td>66.6</td>
<td>67.3</td>
</tr>
<tr>
<td>Higher</td>
<td>46.0</td>
<td>23.0</td>
<td>19.2</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>67.3</td>
<td>53.1</td>
<td>51.9</td>
</tr>
<tr>
<td>Non-Dutch Western</td>
<td>5.3</td>
<td>8.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Non-Dutch Non-Western</td>
<td>27.4</td>
<td>38.6</td>
<td>38.9</td>
</tr>
<tr>
<td>Marital status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>58.3</td>
<td>31.9*</td>
<td>7.4*</td>
</tr>
<tr>
<td>Living together</td>
<td>29.6</td>
<td>37.4</td>
<td>48.1</td>
</tr>
<tr>
<td>No partner</td>
<td>12.0</td>
<td>30.8</td>
<td>44.4</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below modal €1200</td>
<td>12.1</td>
<td>32.2*</td>
<td>31.7*</td>
</tr>
<tr>
<td>Modal €1200 to 2000</td>
<td>24.3</td>
<td>24.4</td>
<td>26.8</td>
</tr>
<tr>
<td>Above modal €2000</td>
<td>63.6</td>
<td>43.3</td>
<td>41.5</td>
</tr>
<tr>
<td>Drinking habits (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never drank in pregnancy</td>
<td>41.6</td>
<td>30.2</td>
<td>16.3*</td>
</tr>
<tr>
<td>Drank until pregnancy was known</td>
<td>12.4</td>
<td>12.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Continued to drink in pregnancy</td>
<td>46.0</td>
<td>57.3</td>
<td>57.1</td>
</tr>
<tr>
<td>Smoking habits (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked in pregnancy</td>
<td>100.0</td>
<td>–</td>
<td>14.8</td>
</tr>
<tr>
<td>Less than 5 cigarettes/day</td>
<td>–</td>
<td>29.2</td>
<td>16.7</td>
</tr>
<tr>
<td>Between 5 to 9 cigarettes/day</td>
<td>–</td>
<td>17.7</td>
<td>31.5</td>
</tr>
<tr>
<td>More than 9 cigarettes/day</td>
<td>–</td>
<td>53.1</td>
<td>37.0</td>
</tr>
<tr>
<td>Child Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at MRI (months)</td>
<td>87.2 ± 9.2</td>
<td>86.2 ± 8.9</td>
<td>87.2 ± 9.6</td>
</tr>
<tr>
<td>Gender (% boys)</td>
<td>58.4</td>
<td>58.3</td>
<td>64.8</td>
</tr>
<tr>
<td>Nonverbal IQ at age 6 years</td>
<td>101.0 ± 12.8</td>
<td>99.6 ± 13.5</td>
<td>99.6 ± 12.8</td>
</tr>
<tr>
<td>Head circumference at age 6 years (cm)</td>
<td>51.4 ± 1.4</td>
<td>51.1 ± 1.5</td>
<td>51.6 ± 1.5</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>40.0 ± 1.6</td>
<td>39.7 ± 1.9</td>
<td>39.6 ± 1.6</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3475 ± 520</td>
<td>3194 ± 536*</td>
<td>3203 ± 604*</td>
</tr>
</tbody>
</table>

All continuous variables are presented as mean ± standard deviation; all categorical variables are presented as percentages. \( t \) tests were used for continuous variables and Chi-square tests for categorical variables with the unexposed group as the reference. Data from the tobacco-exposed group and nonexposed control subjects have been published previously (24).

MRI, magnetic resonance imaging.

* \( p < .01 \).

** \( p < .05 \).
Tobacco Smoking in Cannabis Users

Table 2 demonstrates that prenatal cannabis use commonly co-occurs with smoking during pregnancy. Of all cannabis users, only 14.8% did not smoke tobacco during pregnancy, while 74.1% continued smoking during pregnancy. Of the pregnant cannabis users that continued smoking cigarettes during pregnancy, only 20% smoked more than nine cigarettes per day (Table 2). In comparison, among the women who did not use cannabis but did continue smoking during pregnancy, the proportion of women who smoked more than nine cigarettes per day was 53.1% (Table 1).

Global Brain Volumes

Prenatal cannabis use was not associated with differences in total brain volume, cortical gray matter volume, cortical white matter volume, and ventricular volume (Supplement 1). The results (corrected for covariates) also showed that prenatal tobacco smoking (continued use in multiple trimesters) was associated with decreased brain volume, cortical gray matter volume, and cortical white matter volume (Supplement 1). These findings are similar to our previous results on prenatal tobacco exposure and brain morphology (17). Prenatal cannabis use was associated with increased scores of the language domain (Supplement 1).

Vertex-wise Group Analyses

Compared with nonexposed control subjects, cannabis-exposed children had thicker frontal cortices (Figure 1). Specifically, cannabis-exposed children had a thicker superior frontal area of the left hemisphere (cluster size 2268.3 mm², cluster-wise p value < .001) and a thicker frontal pole of the right hemisphere (cluster size 1874.3 mm², cluster-wise p value .003).

Compared with nonexposed control subjects, prenatal tobacco exposure was associated with thinner cortices in the left and right hemisphere, namely the superior parietal (cluster size 2135.4 mm², cluster-wise p value < .001) and superior frontal region (cluster size 5438.8 mm², cluster-wise p value < .001) of the left hemisphere and the caudal middle frontal region of the right hemisphere (cluster size 2010.7 mm², cluster-wise p value < .001). When comparing the cannabis-exposed children with the tobacco-exposed children, the differences in the cortical areas were even more pronounced (Figure 1).

The associations between prenatal cannabis exposure and the thicker cortices remained significant after additionally correcting for covariates including maternal education, household income, marital status, ethnicity, alcohol use, maternal psychopathology, and child’s IQ and birth weight (Table 3). Likewise, the associations between prenatal tobacco exposure and thinner cortices remained statistically significant after taking into account these covariates (Table 3). Moreover, further adjustment for child emotional and behavioral problems did not change the associations.

To better understand the observed neurodevelopmental differences between the cannabis-exposed children and the tobacco-exposed children, we compared the subset of children that were prenatally exposed to both cannabis and continued maternal smoking (Table 2, n = 40) with children exposed only to continued maternal smoking (n = 96). Figure 2 shows that the cannabis-exposed children who were also exposed to continued tobacco use during pregnancy

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**Table 2. Maternal Cannabis Use During Pregnancy and Cigarette Smoking Behavior**

<table>
<thead>
<tr>
<th>Cannabis Use During Pregnancy</th>
<th>n = 54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette Smoking Behavior</td>
<td></td>
</tr>
<tr>
<td>No smoking</td>
<td>14.8 (8)</td>
</tr>
<tr>
<td>Quit smoking when pregnancy was known</td>
<td>11.1 (6)</td>
</tr>
<tr>
<td>Continued smoking (multiple trimesters)</td>
<td>74.1 (40)</td>
</tr>
<tr>
<td>Less than 5 cigarettes/day</td>
<td>40.0 (16)</td>
</tr>
<tr>
<td>Between 5 to 9 cigarettes/day</td>
<td>40.0 (16)</td>
</tr>
<tr>
<td>More than 9 cigarettes/day</td>
<td>20.0 (8)</td>
</tr>
</tbody>
</table>

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**Figure 1.** Vertex-wise analysis: The association between prenatal cannabis and/or tobacco exposure and cortical thickness.
Table 3. Prenatal Cannabis and Tobacco Exposure in Relation to Cortical Thickness in Young Children

<table>
<thead>
<tr>
<th>Cortical Thickness</th>
<th>Model I B (95% CI)</th>
<th>Model I p Value</th>
<th>Model II B (95% CI)</th>
<th>Model II p Value</th>
<th>Model III B (95% CI)</th>
<th>Model III p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior frontal area (LH)</td>
<td>.18 (.07 to .28)</td>
<td>.001</td>
<td>.15 (.04 to .26)</td>
<td>.008</td>
<td>.13 (.02 to .24)</td>
<td>.02</td>
</tr>
<tr>
<td>Frontal pole (RH)</td>
<td>.20 (.09 to .30)</td>
<td>&lt;.001</td>
<td>.17 (.06 to .28)</td>
<td>.004</td>
<td>.15 (.04 to .26)</td>
<td>.01</td>
</tr>
<tr>
<td>Superior parietal (LH)</td>
<td>-.17 (-.22 to -.11)</td>
<td>&lt;.001</td>
<td>-.16 (-.21 to -.10)</td>
<td>&lt;.001</td>
<td>-.16 (-.22 to -.10)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Superior frontal (LH)</td>
<td>-.17 (-.24 to -.10)</td>
<td>&lt;.001</td>
<td>-.14 (-.21 to -.07)</td>
<td>&lt;.001</td>
<td>-.15 (-.23 to -.08)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Caudal middle frontal (RH)</td>
<td>-.17 (-.24 to -.10)</td>
<td>&lt;.001</td>
<td>-.14 (-.21 to -.07)</td>
<td>&lt;.001</td>
<td>-.15 (-.22 to -.07)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Prenatal Cannabis Exposure Versus Nonexposed Control Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus (LH)</td>
<td>.21 (.13 to .28)</td>
<td>&lt;.001</td>
<td>.20 (.13 to .28)</td>
<td>&lt;.001</td>
<td>.20 (.12 to .28)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Caudal middle frontal (LH)</td>
<td>.18 (.11 to .25)</td>
<td>&lt;.001</td>
<td>.17 (.09 to .24)</td>
<td>&lt;.001</td>
<td>.17 (.09 to .24)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lateral occipital (LH)</td>
<td>.21 (.13 to .29)</td>
<td>&lt;.001</td>
<td>.20 (.13 to .28)</td>
<td>.001</td>
<td>.22 (.13 to .30)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Precentral (RH)</td>
<td>.17 (.09 to .25)</td>
<td>&lt;.001</td>
<td>.15 (.08 to .23)</td>
<td>&lt;.001</td>
<td>.15 (.07 to .23)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pars opercularis (RH)</td>
<td>.17 (.08 to .25)</td>
<td>&lt;.001</td>
<td>.17 (.08 to .25)</td>
<td>&lt;.001</td>
<td>.16 (.07 to .25)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Model I, matched on age and gender; Model II, Model I + adjustment for maternal ethnicity, education, household income, marital status, alcohol use, maternal psychopathology, and child’s IQ; Model III, Model II + adjustment for birth weight.

CI, confidence interval; LH, left hemisphere; RH, right hemisphere.

*All models survived a false discovery rate correction for multiple testing.

The subset of \( n = 40 \) and the children exposed only to tobacco differed significantly in clusters in the caudal middle frontal region of the left hemisphere (cluster size 6674.7 mm\(^2\), cluster-wise \( p \) value < .001) and the precentral region of the right hemisphere (cluster size 5895.3 mm\(^2\), cluster-wise \( p \) value < .001).

DISCUSSION

The aim of this study was to investigate the association between prenatal cannabis exposure and brain morphology in childhood. The hypothesis was that prenatal cannabis exposure would be associated with global morphological differences in the offspring brain, similar or even greater in magnitude than the association between prenatal tobacco exposure and brain morphology. Our hypothesis was only partially confirmed. Compared with nonexposed children, we observed smaller global brain volumes in tobacco-exposed children, whereas this was not found in cannabis-exposed children. Additionally, compared with non-exposed control subjects, we observed thinner cortices throughout the brain in tobacco-exposed children, whereas in cannabis-exposed children, we observed thicker cortices but only in the frontal regions in both hemispheres.

In the first years of life, the association between prenatal cannabis exposure and child neurodevelopmental function is inconsistent. In the OPPS study, no association was observed between prenatal cannabis exposure and infant development at 1 year of age (42), whereas the Maternal Health Practices and Child Development study demonstrated an association between prenatal cannabis exposure and a decrease in mental scores on the Bayley Scales of Infant Development at 9 months of age, which disappeared at 18 months (43). We previously showed that prenatal cannabis exposure was associated with increased aggression and attention problems in offspring, particularly in girls (19). As the children become older, this association may become more evident. Indeed, in preschoolers, prenatal cannabis exposure was associated with lower scores on language, memory, and abstract/visual reasoning domains (11,44). Furthermore, in school-aged children, prenatal cannabis exposure was related to attention problems (45,46). In children aged between 9 and 12 years, prenatal cannabis exposure was associated with decreased performance in executive functions (47,48), and these deficits were observed in 13- to 16-year-olds (7) and 18- to 22-year-olds as well (20). These findings have led to the hypothesis that prenatal cannabis use may have selective deleterious consequences on developing executive functions, i.e., certain higher cognitive abilities that cannot be assessed with global tests.

More specifically, our findings are in line with the fMRI study that showed that young adults exposed to cannabis in utero had increased activation in the prefrontal cortices (20,21). However, this fMRI study also showed activation differences in other regions, such as the cerebellum and occipital gyrus. It may be possible that the activation differences in other brain regions are the result of current cannabis use, as these young adults smoked cannabis themselves.

A possible interpretation for our finding of a thicker prefrontal cortex in cannabis-exposed children is altered neurodevelopmental maturation. The prefrontal cortex is the most rostral portion of the neocortex and is involved in cognitive functions. It is one of the higher-order cortical regions to undergo later maturation as compared with regions that are associated with lower-order cortices such as the somatosensory and the visual cortices (49,50). The prefrontal cortex supports functions such as the ability to suppress responses and thoughts, attention, higher-order motor control, and working memory (51). Deficits in these cognitive functions were implicated in children prenatally exposed to cannabis (48,52–54). Moreover, cannabinoid type 1 receptors are widely distributed in the brain (55,56) and the distribution of cannabinoid receptors seems to be more widespread in the fetal and neonatal brain than in the adult brain (57,58). Exogenous and endogenous cannabinoids may play a role in cannabinoid type
Cannabinoids play an important role in synaptic pruning (60), and synaptogenesis (59). Additionally, later in life, endogenous 1 receptor-mediated neurodevelopmental processes, including neuronal proliferation, migration, differentiation, survival, and synaptogenesis (59). Additionally, later in life, endogenous cannabinoids play an important role in synaptic pruning (60), and prenatal exposure to cannabis may interfere with the endocannabinoid system and thereby inhibit synaptic pruning, particularly in the receptor-enriched area such as the frontal cortex (61). Thus, it may be possible that the frontal cortex in cannabis-exposed children undergoes altered neurodevelopmental maturation (i.e., having differences in cortical trajectories) as compared with nonexposed control subjects. However, to investigate this potential explanation, repeated assessments of brain morphology over a longer follow-up period are necessary.

The influence of cannabinoids on neuronal viability is complicated, as both neurotoxic and neuroprotective effects were reported [reviewed in (62)]. It has been shown that exposure to THC in neonatal rats induces cell death in the cerebral cortex (62,63). On the other hand, administration of THC to adult rats was not associated with the apoptotic pathway in the cerebral cortex (62,63). Thus, our previous finding that prenatal cannabis exposure was associated with decreased fetal head growth (17) is in line with this animal study. However, the long-term neurotoxicity of prenatal cannabis exposure was not demonstrated in the current study. Rather, we observed that the frontal cortex was thicker in children prenatally exposed to cannabis, which may also represent a neuroprotective process of prenatal cannabis exposure. In adults and adolescents, structural changes associated with cannabis use have not been consistent. While some studies reported decreases in regional brain volumes, such as the hippocampus, prefrontal cortex, and amygdala (64–67), other studies reported increases in amygdala nucleus accumbens and cerebellar volumes in cannabis users (68–70).

The strengths of this study are the prospective design, including the prospective measurement of prenatal cannabis use through questionnaires and urinalysis, the relatively large group that underwent neuroimaging, and the young age of the children. Particularly, in the current study of preadolescent children, the results were not confounded by smoking (cannabis) by the children, as has been the case in the existing studies that examined the association between prenatal cannabis exposure and brain morphology in young adulthood. Nevertheless, this study has its limitations. We assessed brain morphology at one time point. Therefore, it is impossible to infer conclusions about the trajectory of neurodevelopment in these children.

Neuroimaging assessments at multiple time points are needed to evaluate longitudinal associations. Additionally, FreeSurfer makes use of an adult atlas for brain imaging analyses, as a suitable child atlas is not available. Nonetheless, numerous studies in typical and atypical developing school-aged children have used FreeSurfer successfully (35–38). Moreover, cannabis use was only assessed once during pregnancy; self-reported information about cannabis use in the second and third trimesters was not available. Additionally, no information about maternal cannabis use in the postnatal period was available. Furthermore, the rate of participation among the cannabis-exposed children was lower than for typically developing children. In the cannabis-exposed group, 30.5% declined participation, while in the typically developing children, only 21.5% declined participation in the neuroimaging study (26). This selection may have influenced our findings. However, based on the descriptive information on birth weight, such selection bias seems not very likely. The mean birth weight in the current study is very similar to birth weight in the larger cannabis-exposed group that we investigated in the prenatal period (17). Finally, 74.1% of the cannabis-using mothers also continued using tobacco during pregnancy. This high degree of co-occurrence of cannabis and tobacco exposure makes it very difficult to conclude that the observed effects in the cannabis-exposed children are driven by cannabis exposure only.

Overall, we detected significant associations between prenatal cannabis exposure and brain morphology in young children, particularly in the frontal brain. The current study combined with the existing literature about the long-term consequences of prenatal cannabis and tobacco exposure support the importance of preventing and reducing smoking cannabis and cigarettes during pregnancy. More research is needed to explore the causal nature of this association.

**ACKNOWLEDGMENTS AND DISCLOSURES**

The Sophia Children’s Hospital Fund (SSWO-553) supported this work, which was financially awarded to Dr. Tiemeier. The study was made possible by financial support from the Erasmus Medical Centre and the Netherlands Organization for Health Research and Development (Zon MW Geestkracht Program 10.000.1003 and Zon MW TOP 40-00812-98-11021). Magnetic resonance imaging data acquisition was sponsored, in part, by the European Community’s 7th Framework Programme (FP7/2008-2013, 212652). The funding agencies had no role in the design and conduct of the study; collection, management, analyses, and interpretation of the data; and preparation, review, or approval of the manuscript and the decision to submit it for publication.

The Generation R Study is conducted by the Erasmus Medical Centre in close collaboration with the Municipal Health Service Rotterdam area, the Rotterdam Homecare Foundation, and the Stichting Trombosöedienst en Arsenlaboratorium Rijnmond, Rotterdam. We gratefully acknowledge the contribution of general practitioners, hospitals, midwives, and pharmacies in Rotterdam. Supercomputing...
computations were supported by the Netherlands Organisation for Scientific Research Physical Sciences Division (Exatec Wetenschappen) and SURFsara (Lisa compute cluster, www.surfsara.nl).

All authors report no biomedical financial interests or potential conflicts of interest.

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Received May 21, 2015; revised Jul 25, 2015; accepted Aug 25, 2015.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.08.024.

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Prenatal Cannabis Exposure and Child Brain Morphology


