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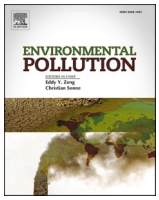

# Air pollution, white matter microstructure, and brain volumes: Periods of susceptibility from pregnancy to preadolescence☆

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ABSTRACT

Air pollution exposure during early-life is associated with altered brain development, but the precise periods of susceptibility are unknown. We aimed to investigate whether there are periods of susceptibility of air pollution between conception and preadolescence in relation to white matter microstructure and brain volumes at 9–12 years old. We used data of 3515 children from the Generation R Study, a population-based birth cohort from Rotterdam, the Netherlands (2002–2006). We estimated daily levels of nitrogen dioxide (NO2), and particulate matter (PM2.5 and PM2.5absorbance) at participants' homes during pregnancy and childhood using land-use regression models. Diffusion tensor and structural brain images were obtained when children were 9–12 years of age, and we calculated fractional anisotropy and mean diffusivity, and several brain structure volumes. We performed distributed lag non-linear modeling adjusting for socioeconomic and lifestyle characteristics. We observed specific periods of susceptibility to all air pollutants from conception to age 5 years in association with lower fractional anisotropy and higher mean diffusivity that survived correction for multiple testing (e.g., −0.85 fractional anisotropy (95%CI -1.43; −0.27) per 5 μg/m$^3$ increase in PM2.5 between conception and 4 years of age). We also observed certain periods of susceptibility to some air pollutants in relation to global brain and some subcortical brain volumes, but only the association between PM2.5 and putamen survived correction for multiple testing (172 mm$^3$ (95%CI 57; 286) per 5 μg/m$^3$ increase in PM2.5 between 4 months and 1.8 year of age). This study suggested that conception, pregnancy, infancy, toddlerhood, and early childhood seem to be susceptible periods to air pollution exposure for the development of white matter microstructure and the putamen volume. Longitudinal studies with repeated brain outcome measurements are needed for understanding the trajectories and the long-term effects of exposure to air pollution.

## 1. Introduction

There is growing evidence of the potential harmful effects of air pollution on brain development (Block et al., 2012). Due to the immaturity of detoxification mechanisms in fetuses and infants, the brain is considered particularly susceptible to external stressors during pregnancy and the first years of life, and exposures to air pollution during these developmental periods could lead to permanent alterations

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in the brain (Block et al., 2012; Grandjean and Landrigan, 2014). A large number of studies suggest that higher exposure to air pollution during early-life is associated with lower cognitive abilities, and alterations in white matter microstructure and brain morphology (Lopuszanska and Samardakiewicz, 2020). Most studies focused on prenatal exposures, however, the brain is still developing until adulthood (DeBello and Knudsen, 2004; Giedd et al., 1999; Shaw et al., 2008), and may also be vulnerable to air pollution during childhood and adulthood (Rice and Barone, 2000; Stiles and Jernigan, 2010).

To date, previous studies have investigated averaged exposure to air pollution over periods of time, and have not assessed the possible association of cumulative exposure. Thus, they did not take into account the variability of the exposure across time, and are prone to misclassification (Loomis and Kromhout, 2004). Moreover, studies focusing on a given periods of time (e.g., childhood period) without considering the other periods of exposures (e.g., pregnancy period) may overestimate the health effect of the exposure during that period. In addition, average exposure estimates might not be detailed enough to identify which periods of life are susceptible to air pollution, considering the numerous, timing-specific developmental phases of the brain (Casey et al., 2008; Stiles and Jernigan, 2010). This approach can produce biased estimates, in particular when the true critical windows do not match with the bound of the arbitrary periods of time, and mutual adjustment on the other periods of exposure may not be enough to eliminate bias (Wilson et al., 2017).

Distributed lag non-linear model (DLNM) can address this limitation and help identify the periods of susceptibility to air pollution in a data-driven way (Gasparrini et al., 2010). It also permits considering an entire period of exposure (e.g., from conception to preadolescence) at a fine temporal scale, taking into account the correlation within time series. Few studies have applied DLNM to study the association between early-life exposure to air pollution and cognitive function or behavior problems in children (Chiu et al., 2016; Raz et al., 2018; Rivas et al., 2019; Wang et al., 2021), but to our knowledge, none has applied DLNM to study brain development. As magnetic resonance imaging (MRI) data provides direct insights into brain structures, DLNM could potentially allow identification of pathways underlying any time-specific associations between exposure to air pollution and neurodevelopment (Guxens et al., 2018).

We built on previous studies in the same population, in which we have previously identified associations between averaged prenatal and childhood exposure to air pollution and brain structure (Lubczyńska et al., 2020a, 2020b). We now reanalyzed the data using air pollution estimates with a finer temporal resolution and applying DLNM to identify the potential periods of susceptibility to air pollution between conception and age 8.5 years in relation to white matter microstructure and brain volumes. We hypothesized that the risk function between exposure to air pollution and brain outcomes is not constant over time, thus specific periods of susceptibility may be present.

Therefore, we aimed to identify periods of susceptibility from conception to age 8.5 years to the exposure to three ubiquitous pollutants in relation to white matter microstructure and brain volumes in preadolescents aged 9–12 years. We hypothesized that exposure to air pollution during pregnancy and childhood was associated with lower fractional anisotropy and higher mean diffusivity. We postulated that exposure to air pollution during pregnancy and childhood was associated with smaller volume of the cortical gray matter, the cerebral white matter, the corpus callosum, the cerebellum, the thalamus, the pallidum, and the hippocampus. Also, we posited that air pollution exposure during pregnancy and childhood was associated with larger caudate, putamen, amygdala, and nucleus accumbens volumes.

## 2. Methods

### 2.1. Study population

This study used data from the Generation R Study, a population-based birth cohort set up in the area of Rotterdam, the Netherlands (Kooijman et al., 2016). A total of 8879 women were enrolled during pregnancy, and additionally 899 women were recruited shortly after the delivery between April 2002 and January 2006. We included only singleton deliveries in our study, resulting in 9610 mother-child pairs. When the children were between 9 and 12 years of age, we invited them to participate in an MRI session (n = 8548) (White et al., 2018). In total, 3839 attended the MRI visit. From this group, we excluded those with poor brain MRI data quality. This resulted in a final study population of 3515 participants. Parents provided written informed consent for themselves and their children. The Medical Ethics Committee of the Erasmus Medical Center in Rotterdam, the Netherlands, granted ethical approval for the study.

### 2.2. Exposure to air pollution

We estimated outdoor air pollution levels of nitrogen dioxyde (NO2), particulate matter (PM) with aerodynamic diameter <2.5 μm (PM2.5), absorbance of PM2.5 fraction (PM2.5absorbance) for each address that the participants have lived at during the period of interest, i.e. since conception until date of the MRI session, following a standardized procedure (Brunekreef, 2010). Details about the assessment are provided in Appendix Methods S1 and were published in Guxens et al. (2022) In brief, three 2-week measurements of NO2 were performed in 2009–2010 at 80 sites spread across the Netherlands and Belgium, and measurements of PM2.5 in 40 of those sites (Beelen et al., 2013; Cyrys et al., 2003; Eeftens et al., 2012b). PM2.5absorbance was measured in the PM2.5 filters (Eeftens et al., 2012b). Measurements performed at each site were averaged, resulting in one annual mean level for each pollutant per site. To adjust for temporal trends, we used data from a centrally located reference site chosen at a regional background location. A variety of geographic information systems (GIS) land use predictors was then assigned to each monitoring site and linear regression modeling was applied to determine the combination of predictors explaining the levels of the pollutants most accurately, resulting in land use regression models (Beelen et al., 2013; Eeftens et al., 2012a). Next, we assigned the GIS predictors to each address of the participants from conception until the MRI session, and applied the land use regression models to predict air pollution levels at each address. $R^2$ cross-validation were0.60 for PM2.5, 0.81 for NO2, and 0.89 for PM2.5absorbance. To capture the temporal trend and increase the temporal resolution, daily data from seven routine background monitoring sites were used to extrapolate the air pollution levels to the exact period of residency at each address, resulting in daily air pollution levels at each address the participants were living between conception until the MRI assessment (Brunekreef, 2012). For participants recruited after birth, we considered the address at birth as representative for the pregnancy period. We averaged the daily levels of each pollutant into periods of four weeks across pregnancy and across childhood, separately.

### 2.3. Magnetic resonance imaging

To familiarize the participants with the magnetic resonance environment, each child underwent a mock scanning session prior to the actual MRI session (White et al., 2013). The collection of data was performed at a single site, and all scans were performed on a 3 T General Electric scanner (GE, MR750W, Milwaukee, WI) using an 8-channel receive-only head coil. The brain MRI protocol included measurements of brain structural connectivity (i.e., white matter microstructure) using diffusion tensor imaging (DTI), and brain structural volumes using T1-weighted images. The sequence parameters, preprocessing, and assessment of the image quality are provided in Appendix Methods S2 and S3 and were published in Muetzel et al. (2018).

We processed the DTI data using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al., 2012). First, we assessed average fractional anisotropy (FA) and mean diffusivity (MD) values for twelve

commonly described white matter tracts (forceps minor and forceps major, and bilateral tracts of the cingulum bundle, corticospinal tract, inferior longitudinal fasciculus, superior longitudinal fasciculus, and uncinate) (Appendix Fig. S1). A factor analysis was run to produce a global metric of FA and a global metric MD of all twelve tracts based on the factor loadings (Muetzel et al., 2015). Global metrics are factors scores from a confirmatory factor analysis (i.e., standardized scores centered on 0 and ranging from roughly −5 to 5 for FA, and −0:5 to 0.5 for MD) and thus do not conform to the standard positive values typically seen with DTI. FA indicates the tendency for preferential water diffusion in white matter tracts, with higher values in well-organized white matter tracts. MD describes the magnitude of average water diffusion in all directions within brain tissue, with higher values generally occurring in less well-organized white matter tracts.

We processed the structural MRI data through the FreeSurfer Image Analysis Suite 6.0 (Fischl, 2012). We calculated several brain volumes, including cortical gray matter volumes, cerebral white matter volume, corpus callosum volume, and cerebellum volume. We also calculated subcortical brain volumes including the thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens. In case of bilateral volumes, we summed the volume of the left and the right hemisphere.

### 2.4. Potential confounding variables

We identified potential confounding variables based on up-to-date knowledge of the scientific literature, and on availability of data within the Generation R cohort (Guxens et al., 2018; Lubczyńska et al., 2020a, 2020b) (Appendix, Methods S4). We collected the following data by questionnaires during pregnancy: maternal and paternal countries of birth (the Netherlands, other Western, non-Western), monthly household income (<900, 900–1600, 1600–2200, >2200 euros), marital status (married, living together, no partner), maternal and paternal age at enrollment in the cohort (years), maternal and paternal educational levels (primary or lower, secondary, higher), maternal smoking and alcohol use during pregnancy (no, until pregnancy known, during pregnancy), parity (0, 1, ≥2 children), and maternal and paternal psychological distress (continuous, higher score representing higher distress) using the Brief Symptom Inventory (De Beurs, 2008). We calculated maternal and paternal body mass index (BMI) based on maternal and paternal weight and height (kg and cm, respectively) measured or self-reported in the 1st trimester of pregnancy. Maternal intelligence quotient (continuous) was assessed at child's age of 6 years with Raven's Advanced Progressive Matrices Test, set I (McKinzey et al., 2003). We collected child's sex (boy, girl) from hospital records at birth and child's age (years) at the MRI session.

### 2.5. Statistical analyses

We first imputed missing potential confounding variables among all participants that had available data for all exposures and each set of outcomes separately (N = 2954 with white matter microstructure data, and N = 3133 with brain volumes data) using chained equations. We obtained 25 datasets for each set of outcomes and used the 25th in all of the analyses mentioned hereafter. Distributions between observed and imputed datasets were similar (Appendix Table S1). Based on observed values, families of the included participants were more likely to have higher education, have higher household income, be Dutch, and be married, as compared to families of participants that were not included (Appendix Table S2). We thus corrected for potential attrition bias using inverse probability weighting (Weisskopf et al., 2015; Weuve et al., 2012) (Appendix Methods S5). In brief, we imputed missing covariates for all eligible subjects (N = 9610), and we used all the available information to predict the probability to participate in each subsample (N = 2954 with white matter microstructure data, and N = 3133 with brain volumes data). We used the inverse of those probabilities as weights, so that results would be representative for the initial population of the cohort.

We used DLNM to estimate the exposure-response relationship between each air pollutant and each white matter microstructure and brain volume outcome, while simultaneously capturing the change of this exposure-response relationship along the lags (i.e., time periods) (Gasparrini et al., 2010). All models were adjusted for the potential confounding variables described in the section above. To account for potential temporal trends, we additionally adjusted for the year (2001–2005) and month of conception (January–December). We further adjusted corpus callosum, cerebellum, and subcortical volumes for intracranial volume to ascertain relativity to the head size. We did not adjust cortical gray matter, and cerebral white matter volumes for intracranial volume due to their high correlation (>0.8).

DLNM are based on a cross-basis, a dimensional space of two functions that define the exposure-response relationship and the lag-response relationship, respectively (Gasparrini, 2014). First, we visually inspected the relationship of each averaged exposure at each trimester of pregnancy and year of childhood with the outcome. We observed that all relationships were linear, and selected a linear regression shape for the exposure-response relationship. We also checked that the rest of assumptions of linear regression models were met (i.e. normality of the residuals, homoscedasticity, no collinearity between covariates). Next, we selected a natural cubic B-spline with an intercept for the lag-response relationship, assuming the association between exposure and outcome varies smoothly across lags (Gasparrini et al., 2010). Each lag corresponded to a four-week period.

As we considered exposures starting at conception having the same time length for all children, we only included children born after 32 weeks of gestation (N = 2911 with white matter microstructure data, and N = 3089 with brain volumes data) in the main analysis. This resulted in a pregnancy period of 9 four-week lags. We decided not to exclude preterm births, because prematurity may be a mediator in the pathway between air pollution and brain outcomes and their exclusion could lead to an incorrect specification of the estimates due to collider bias. For participants that were born between week 33 and 36, we averaged their available exposures between those weeks and considered this as representative of the 9th four-week lag. For participants that were born after week 36, we did not consider the exposure periods after week 36 in the main analysis. The childhood period started at birth and in the main analysis it was truncated for all participants at the age of the youngest child undergoing the MRI session (8.5 years), corresponding to 111 four-week lags. Because there is no *a priori* knowledge regarding the number and position of knots in the cross-basis matrix, we ran adjusted linear regressions between each exposure at each lag separately with each outcome. We plotted the beta coefficients and their 95% confidence intervals (CI) across time. Three researchers (ACB, MvdD, MG) visually inspected each plot independently and decided whether no knot, one knot, or several knots needed to be placed and in which lag, based on the number of changes in their slopes and the parsimony principle. Disagreements on knot placement between the 3 researchers were resolved by discussion.

First, we assessed whether there were periods of susceptibility to each air pollutant between conception and 8.5 years in relation to each global brain white matter microstructure (i.e., global brain FA, global brain MD), global brain volume (i.e., cortical gray matter, cerebral white matter, corpus callosum, cerebellum), and subcortical brain volumes (i.e., thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens) separately. A period of susceptibility was first identified by a statistically significant association between air pollution exposure and the outcome in a specific lag (p-value<0.05). Then, we corrected the p-values for multiple testing considering three exposures, two indicators of white matter microstructure, and eleven indicators of brain volumes. To confirm independence among the outcomes and the exposures, we extracted eigenvalues from individual-level matrix of phenotype data using the *meff* function from the *poolr*

package in R to confirm the effective number of tests, which was estimated at two (2 effective exposures* 1 effective outcome) for the white matter microstructure outcomes and fourteen (2 effective exposures* 7 effective outcomes) for the brain volume outcomes, using the approach recommended by Galwey (2009). The new p-values were 0.05/2 = 0.025 for white matter microstructure outcomes, and 0.05/14 = 0.004 for brain volume outcomes. We estimated the association of each period of susceptibility by combining the estimates of the consecutive statistically significant lags that survived correction for multiple testing. For the confidence intervals, we considered the covariance matrix along consecutive statistically significant lags that survived correction for multiple testing.

We conducted several sensitivity analyses for investigating the influence of our methodological choices: i) we repeated the selection of the lag-response relationship (i.e., number and position of the knots) to minimize the Bayesian Information Criterion (BIC), in which the lowest BIC was a linear relationship (i.e., without a knot) in all models; ii) to investigate the influence of moderate to late premature birth, and the influence of exposure in late pregnancy, we only included children born after week 36 (N = 2797 with white matter microstructure data and N = 2973 with brain volumes data) and added a 10th four-week lag during pregnancy corresponding to the pregnancy period between week 36 and 40,; iii) to investigate a possible selection bias we included very preterm and moderate to late preterm births and truncated the pregnancy period to week 28, resulting in a pregnancy period of 7 four-week lags (N = 2954 with white matter microstructure data and N = 3133 with brain volumes data); iv) to investigate the influence of exposure at a later postnatal period we included 124 lags during childhood, corresponding to children that attended the MRI session after 9.5 years (N = 2815 with white matter microstructure data and N = 3002 with brain volumes data); v) we estimated separately the associations between each air pollutant and the left and right brain volumes (i.e., cortical gray matter, cerebral white matter, cerebellum, thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens) to investigate a potential hemisphere-specific effect; and vi) we stratifyed the models by sex to investigate a potential sex-specific effect. Finally, if we identified a window of susceptibility to air pollution with global brain FA or global brain MD, we followed-up the analyses by estimating the associations of each air pollutant and the twelve individual white matter tracts (i.e., forceps minor, forceps major, and bilateral tracts of the cingulum bundle, corticospinal tract, inferior longitudinal fasciculus, superior longitudinal fasciculus, and uncinate fasciculus). Sensitivity and follow-up analyses were not corrected for multiple testing (i.e. p-value<0.05). All analyses were carried out with R version 4.0.3 (R Core Team, 2020), the DLNM analyses were performed using the R package *dlnm*.

## 3. Results

### 3.1. Study population

At enrollment, mothers were 31 years of age on average, 52% had a high education, and 58% were Dutch (Table 1). Mean air pollution exposure concentrations decreased for all pollutants between conception and age 8.5 years. For example, NO2 concentrations were on average 39.5 μg/m$^3$ during pregnancy and 28.6 μg/m$^3$ between ages 8 and 9 years (Table 2 and Appendix Table S3 and Fig. S2). Global brain FA was −0.027 on average, and global brain MD was −0.002, with an inverse correlation of 0.61 between global brain FA and MD (Appendix Table S4). Cortical gray matter volume was 582,106 mm$^3$ on average and its correlation with other brain volumes varied from 0.29 with corpus callosum volume to 0.81 with cerebral white matter volume (Appendix Table S5 and Fig. S3).

**Table 1**
Characteristics of the study population.

| Variable | Study population (N = 3515) |
|---|---|
| Maternal country of birth | |
| The Netherlands | 57.6% |
| other Western | 8.4% |
| non-Western | 34.0% |
| Paternal country of birth | |
| The Netherlands | 68.1% |
| other Western | 6.0% |
| non-Western | 25.9% |
| Monthly household income at enrollment | |
| <900€ | 7.7% |
| 900–1600€ | 14.3% |
| 1600–2200€ | 14.5% |
| >2200€ | 63.5% |
| Family status at enrollment | |
| married | 50.3% |
| living together | 38.7% |
| no partner | 11.0% |
| Maternal age at enrollment (years) | 31.1 ± 4.9 |
| Paternal age at enrollment (years) | 33.5 ± 5.4 |
| Maternal educational level | |
| primary or lower | 6.7% |
| secondary | 41.0% |
| higher | 52.3% |
| Paternal educational level | |
| primary or lower | 5.1% |
| secondary | 38.9% |
| higher | 56.0% |
| Maternal pre-pregnancy BMI (kg/m$^2$) | 23.4 ± 4.1 |
| Paternal BMI (kg/m$^2$) | 25.3 ± 3.3 |
| Maternal height (cm) | 167.9 ± 7.4 |
| Paternal height (cm) | 182.4 ± 7.7 |
| Maternal psychological distress during pregnancy | 0.3 ± 0.3 |
| Paternal psychological distress during pregnancy | 0.1 ± 0.2 |
| Maternal IQ score | 97.6 ± 14.8 |
| Maternal smoking during pregnancy | |
| never | 77.5% |
| until pregnancy known | 9.0% |
| during pregnancy | 13.5% |
| Maternal alcohol use during pregnancy | |
| never | 42.2% |
| until pregnancy known | 14.5% |
| during pregnancy | 43.3% |
| Maternal parity | |
| no child | 58.0% |
| 1 child | 30.0% |
| ≥2 children | 12.0% |
| Child's sex | |
| Boy | 49.6% |
| Girl | 50.4% |
| Child's age at MRI session (years) | 10.1 ± 0.6 |

Values are mean ± standard deviation for continuous variables and percentage for categorical variables. Distribution is displayed over non-imputed values.

### 3.2. Periods of susceptibility to air pollution of white matter microstructure

We observed a window of susceptibility to all air pollutants for each white matter microstructure outcome (Fig. 1, Table S3, and Table S6). After correction for multiple testing, we found that higher levels of NO2 between 3.6 and 4.8 years of age were associated with lower global brain FA (−0.02 (95%CI -0.04; −0.00) per 10 μg/m$^3$ increase in NO2) (Fig. 1 and Table 3). Higher levels of PM2.5 from conception to 4 years of age were associated with lower global brain FA (−0.85 (95%CI -1.43; −0.27) per 5 μg/m$^3$ increase in PM2.5). Higher levels of PM2.5absorbance during the first 5 years of age were associated with lower global brain FA (−0.18 (95%CI -0.31; −0.05) per $10^{-5}$ m$^{-1}$ increase in PM2.5absorbance). We observed similar periods of susceptibility for global brain MD, with higher levels of all air pollutants associated with higher global brain MD (Fig. 1, Table 3, and Table S6).

**Table 2**
Characteristics of the levels of air pollutants (Generation R, the Netherlands, 2002–2006) (N = 3515).

| | Pregnancy | Birth-1 year | 1–2 years | 2–3 years | 3–4 years | 4–5 years | 5–6 years | 6–7 years | 7–8 years | 8–9 years |
|---|---|---|---|---|---|---|---|---|---|---|
| NO2 (in μg/m$^3$) | 39.5 ± 6.4 | 37.5 ± 5.4 | 34.9 ± 5.3 | 33.2 ± 5.6 | 33.3 ± 6.7 | 32.7 ± 7.0 | 32.2 ± 7.1 | 30.3 ± 6.7 | 29.5 ± 6.5 | 28.6 ± 6.3 |
| PM2.5 (in μg/m$^3$) | 19.8 ± 2.4 | 18.3 ± 1.4 | 17.5 ± 0.7 | 16.9 ± 1.0 | 16.5 ± 0.9 | 16.2 ± 0.7 | 16.7 ± 1.0 | 16.6 ± 1.1 | 15.8 ± 1.7 | 14.1 ± 1.4 |
| PM2.5 abs (in $10^{-5}$/m) | 1.7 ± 0.4 | 1.7 ± 0.3 | 1.6 ± 0.3 | 1.6 ± 0.3 | 1.6 ± 0.3 | 1.6 ± 0.3 | 1.5 ± 0.3 | 1.5 ± 0.3 | 1.4 ± 0.3 | 1.3 ± 0.3 |

Values are mean ± standard deviation.
NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.

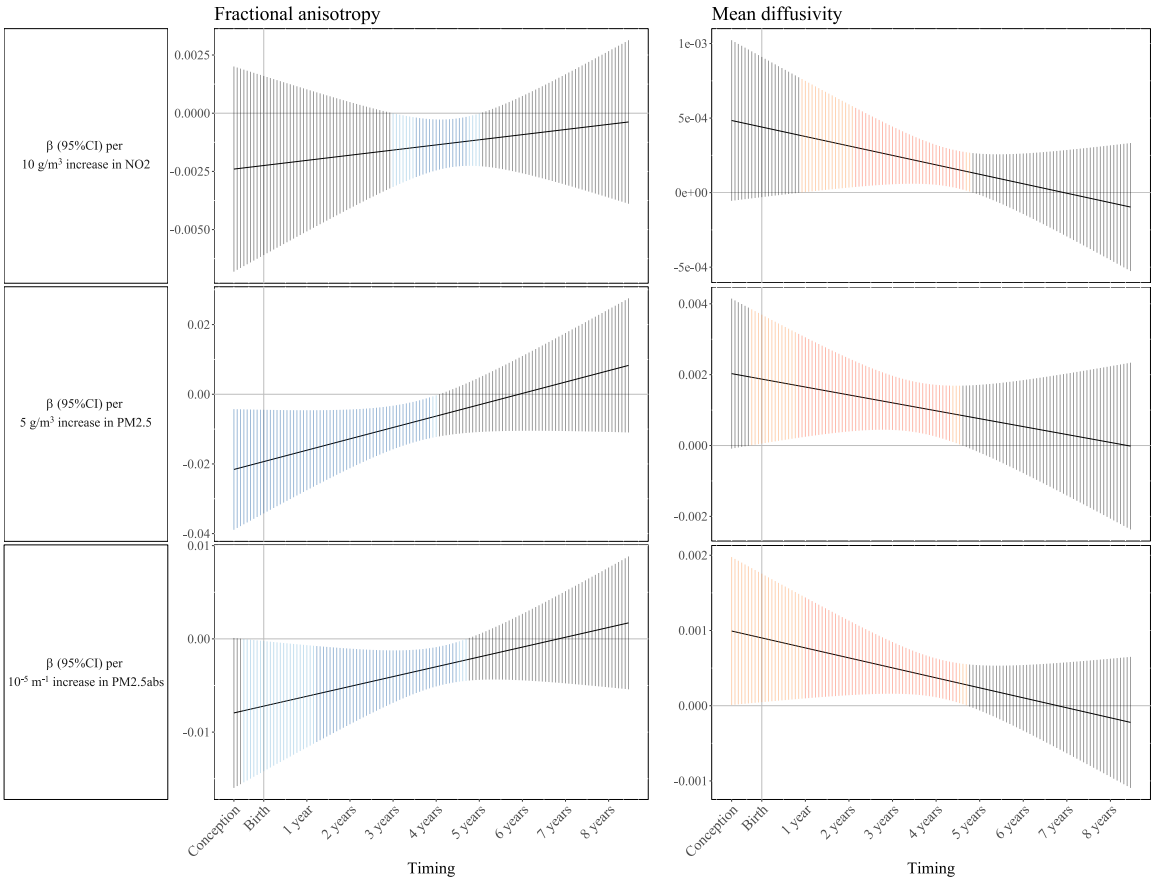


**Fig. 1.** Adjusted associations between exposure to air pollutants from conception to age 8.5 years and global fractional anisotropy and mean diffusivity at 9–12 years of age (N = 2911).
Adjusted for maternal and paternal educational levels, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking and alcohol use during pregnancy, parity, marital status, maternal and paternal psychological distress, maternal and paternal BMI, maternal intelligence quotient, child's sex and age at the MRI session, year and month of conception.
NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.
Blackline represents the beta estimate of the association between the exposure at each specific lag and the outcome. Vertical gray, blue, and orange lines represent 95% CI and indicate no divergence from the null, significant divergence from negative association, and significant divergence from positive association, respectively. Darker blue and orange colors indicate associations after correction for multiple testing (p-value < 0.025). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 3.3. Periods of susceptibility to air pollution of brain volumes

We observed some periods of susceptibility for cortical gray matter, cerebral white matter, corpus callosum, cerebellum, hippocampus, amygdala, and nucleus accumbens volumes, but these associations did not survive correction for multiple testing (Table 4, Fig. 2, Fig. 3, Table S7, and Table S8). When correcting for multiple testing, only the association between PM2.5 and putamen volume survived (172 mm$^3$ (95%CI 57; 286) per 5 μg/m$^3$ increase in PM2.5 between 4 months and 1.8 year of age) (Fig. 3). We found no period of susceptibility of air pollution in relation to thalamus, caudate, and pallidum volumes (Appendix Fig. S4)

### 3.4. Sensitivity and follow-up analysis

When we used a linear association between each air pollutant and each brain volume outcome across time, the associations remained similar (Appendix Tables S9–S10). All results were similar when adding a 10th four-week lag during the prenatal period and excluding moderate to late preterms, when truncating the prenatal period to week 28 of pregnancy, and when extending the childhood period up to 9.5 years (Appendix Tables S11–S19).

We found no evidence of differences by hemisphere for any of the bilateral brain volume (data not shown). We found no evidence either of sex-specific effect differences for any association between any air pollutant and any brain outcome (data not shown).

**Table 3**
Adjusted associations between exposure to air pollutants from conception to age 8.5 years and global brain fractional anisotropy and mean diffusivity at 9–12 years of age (N = 2911).

| Air pollutants | Fractional anisotropy | | Mean diffusivity | |
|---|---|---|---|---|
| | Lags | Estimate (95%CI) * | Lags | Estimate (95%CI) * |
| NO2 (Δ 10 μg/m$^3$) | 3.6 years–4.8 years | −0.02 (−0.04; −0.00) | 2.2 years–4.7 years | 0.01 (0.00; 0.01) |
| PM2.5 (Δ 5 μg/m$^3$) | Conception – 3.9 years | −0.85 (−1.43; −0.27) | 9 months - 4.4 years | 0.06 (0.02; 0.10) |
| PM2.5abs (Δ $10^{-5}$ m$^{-1}$) | 1.3 year - 4.5 years | −0.18 (−0.31; −0.05) | 1.1 year - 4.8 years | 0.02 (0.00; 0.04) |

Estimates and 95% CI from distributed lag non-linear model, adjusted for maternal and paternal educational levels, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking and alcohol use during pregnancy, parity, marital status, maternal and paternal psychological distress, maternal and paternal body mass index, maternal intelligence quotient, child's sex and age at the magnetic resonance imaging session, year and month of conception.
* Associations after correction for multiple testing ($p < 0.025$).
CI, confidence intervals; NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.

**Table 4**
Adjusted associations between exposure to air pollutants from conception to age 8.5 years and subcortical brain volumes at 9–12 years of age (N = 3089).

| Air pollutants | Putamen volume | |
|---|---|---|
| | Lags | Estimate (95%CI) * |
| NO2 (Δ 10 μg/m$^3$) | – | – |
| PM2.5 (Δ 5 μg/m$^3$) | 4 months–1.8 year | 172 (57; 286) |
| PM2.5abs (Δ $10^{-5}$ m$^{-1}$) | – | – |

Estimates and 95% CI from distributed lag non-linear model, adjusted for maternal and paternal educational levels, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking and alcohol use during pregnancy, parity, marital status, maternal and paternal psychological distress, maternal and paternal body mass index, maternal intelligence quotient, child's sex and age at the magnetic resonance imaging session, year and month of conception, and intracranial volume.
* Associations after correction for multiple testing ($p < 0.004$).
CI, confidence intervals; NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.

Investigating the associations between each air pollutant and the individual white matter tracts, we observed periods of susceptibility to air pollution exposure between conception and 6 years of age in association with lower FA and higher MD in the forceps minor, the left and right inferior longitudinal fasciculus, the left and right superior longitudinal fasciculus, and the left and right uncinate fasciculus tracts (Appendix, Tables S21, S24-S26). We found additional periods of susceptibility to air pollution exposure between 1.5 and 4 years of age in association with lower FA in the forceps major tract, to air pollution exposure from conception to 4 years of age in association with lower FA in the left and the right corticospinal tract, and to air pollution exposure between 3 and 6 years of age in association with higher MD in the left and right cingulum bundle tract (Appendix, Tables S20, S22-S23).

## 4. Discussion

This study is one of the first to investigate the association of exposure to air pollution, estimated from conception to age 8.5 years on a monthly basis, with white matter microstructure and brain volumes. We observed specific periods of susceptibility from conception to age 5 years to air pollution for white matter microstructure. A period of susceptibility for putamen was also suggested, in the first 2 years of age.

We have identified a period of susceptibility in relation to white matter microstructure to NO2 between 2 and 5 years of age, to PM2.5 from conception until 5 years of age, and to PM2.5absorbance between 1 and 5 years of age. White matter tracts emerge in the fetal brain between 13 and 18 weeks of gestation, and all major white matter tracts are present by the end of gestation. Myelination begins at approximately 20 weeks of gestation, advances rapidly throughout the first 5 years of life, and progresses until adulthood (Lebel and Deoni, 2018; White et al., 2008; Yakovlev and Lecours, 1967). Previous work has linked air pollution to neuroinflammatory mechanisms and microglial activation, suggesting that air pollution exposure may interfere with neurogenic/gliogenic events and myelination processes (Block et al., 2012; Boda et al., 2020; Calderón-Garcidueñas et al., 2012). Pujol et al. (2016) did not observe any cross-sectional association between exposure to elemental carbon or NO2 at school and white matter microstructure in 8–12-year-old Spanish children. In 9-10-year-old children from the United States, Burnor et al. (2021) observed that residential PM2.5 exposure at the time of the MRI assessment was associated with higher restricted isotropic diffusion, a marker of intracellular white matter microarchitecture, and lower MD. In the present study, we found no associations between exposure to air pollution at 8–9 years of age and white matter microstructure outcomes. Our results suggest a susceptibility to air pollutants of white matter microstructure, with less well-organized white matter tracts. This susceptibility seems to occur in particular during the period of rapid myelination of the brain. Due to the plasticity of the white matter, repeated measurements of the white matter microstructure would be necessary to investigate the potential longitudinal effects of air pollution.

Regarding brain volumes outcomes, Cserbik et al. (2020) reported that residential PM2.5 exposure at the time of the MRI assessment was associated with larger right thalamic, right pallidum, and left nucleus accumbens volumes, and with smaller left putamen and left pallidum volumes in 9–10-year-old children from the United States. In our study, we did not observe a hemisphere-specific effect on air pollution on brain volumes, and we found that PM2.5 exposure in the first years of life was only associated with larger putamen volume. In a vertex-wise analysis, Beckwith et al. (2020) observed that higher residential elemental carbon levels in the first year of life were related to smaller regional gray matter volumes in the left pre- and post-central gyri, in the inferior parietal lobe, and in the cerebellum in 12-year-old children from the United States. In our study, we did not find windows of susceptibility to air pollution for the cortical gray matter and the cerebellum, although we used a different approach than this previous study that did not enable us to investigate region-specific associations. Mortamais et al. (2019) reported that higher levels of residential PM2.5 exposure during the 3rd trimester of pregnancy were associated with smaller corpus callosum volume in 8–12-year-old Spanish children, but this association did not survive correction for multiple testing. In the present study, we found null associations between PM2.5 levels and corpus callosum volume. We found some periods of susceptibility for cortical gray matter and cerebral white matter volume, and corpus callosum, cerebellum, hippocampus, amygdala, and nucleus accumbens volumes, but these associations disappeared after correction for multiple testing. We cannot rule out the possibility that we lacked statistical power to detect the windows of susceptibility to air pollution for brain volumes, if any. Moreover, the interpretation of the effect of growth patterns is limited by the design of our study, because the single MRI measurement preventing us to draw robust conclusions on potential long-term effects of

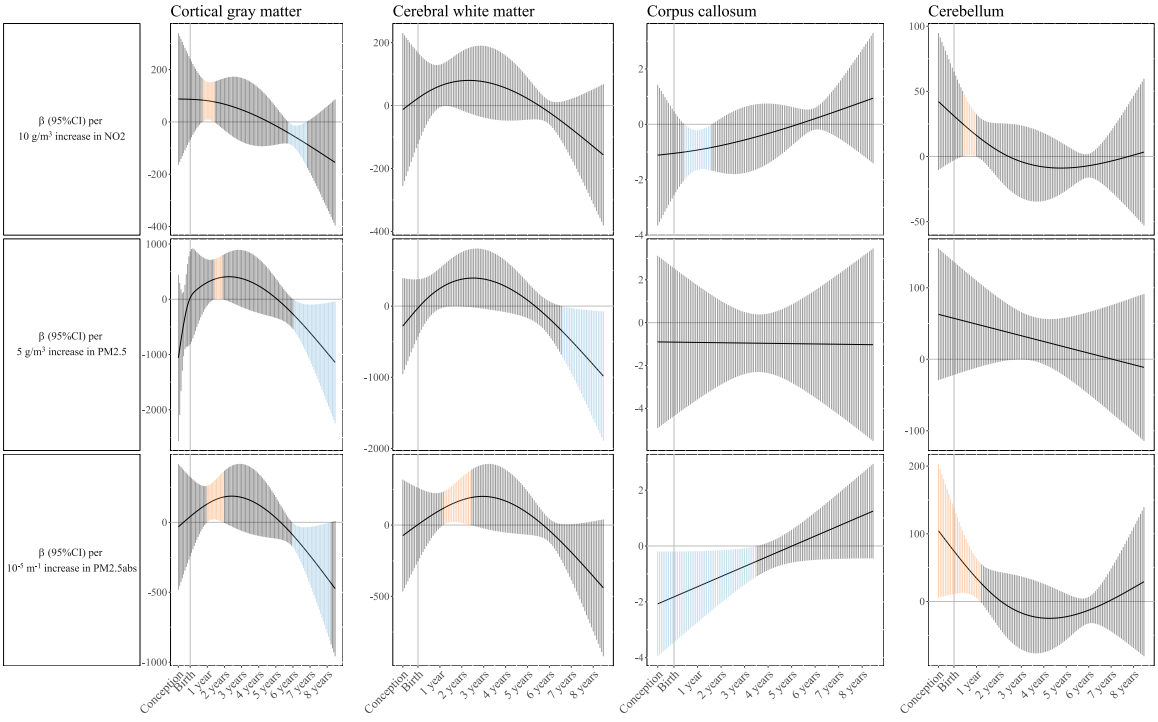


**Fig. 2.** Adjusted associations between exposure to air pollutants from conception to age 8.5 years and cortical gray matter, cerebral white matter, corpus callosum, and cerebellum volumes at 9–12 years of age (N = 3089).
Adjusted for maternal and paternal educational levels, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking and alcohol use during pregnancy, parity, marital status, maternal and paternal psychological distress, maternal and paternal BMI, maternal intelligence quotient, child's sex and age at the MRI session, year and month of conception. Corpus callosum, and cerebellum volumes were additionally adjusted for intracranial volume.
NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.
Black line represents the beta estimate of the association between the exposure at each specific lag and the outcome. Vertical gray, blue, and orange lines represent 95% CI and indicate no divergence from the null, significant divergence from negative association, and significant divergence from positive association, respectively.
. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

air pollution.

Levels of NO2 and PM2.5 reported in the present study were above the 2021 World Health Organization guidelines (10 μg/m$^3$ and 5 μg/m$^3$, respectively) but all air pollutants were below the European Union standards, suggesting that air pollution may affect brain development, at lower levels than the current standards for air quality. In Europe, the predominant source of nitrogen gases is diesel fuel (Cyrys et al., 2003). A major fraction of PM2.5 is formed by gas-to-particle conversion processes from products of long-range transport (Cyrys et al., 2003). PM2.5absorbance characterizes local soot emissions from combustion sources (e.g., diesel vehicles and residential wood combustion) (Eeftens et al., 2012b). Air pollution can impact the brain structure through oxidative stress and neuroinflammation (Costa et al., 2019). Air pollution has also been shown to activate the stress axis, including the hypothalamic-pituitary adrenal axis, and activate the release of stress hormones (Thomson, 2019). Chronic stress has been linked to alterations in various brain structures (Buss et al., 2012; Humphreys et al., 2019; Lupien et al., 2011; Teicher et al., 2004). Our findings highlight the importance of prospective longitudinal studies, with repeated measurements of the exposure during pregnancy and childhood, when investigating the effects of early-life exposure to air pollution on the brain.

Our study has several strengths, including i) its large sample size from a population-based cohort, ii) assessment of air pollution from pregnancy to 8.5 years of age in a very fine time scale, using a standardized and validated method of back-extrapolation to the period of interest, iii) multimodal imaging with two sequence of MRI to investigate both white matter microstructure and brain volumes, iv) adjustment of various socioeconomic and lifestyle variables that are known to be associated with air pollution exposure and brain development, and v) the use of an advanced statistical method, namely DLNM, to estimate the association of exposure to air pollution from pregnancy to adolescence on white matter microstructure and brain volumes with unbiased estimates, to consider each 4-week periods separately, and to identify periods of susceptibility without defining arbitrary periods (e.g., trimesters of pregnancy) *a priori*. Another advantage of DLNM is the cross-basis that allows for the simultaneous evaluation of the lag-exposure-outcome relationship, thus overcoming the problem of multiple comparisons of an averaged exposure approach with repeated measurements.

We also faced several limitations. First, sampling campaigns were carried out when participants were between 3.5 and 9 years of age. We used historical pollution data from routine monitoring stations to back-extrapolate the levels to the periods of interest for each child, which have been shown to remain spatially stable over time for a period up to 8 or 18 years (Eeftens et al., 2012a; Gulliver et al., 2013), but we cannot discard the introduction of misclassification error. Second, the DLNM model requires tuning parameters (i.e., shape of the relationship), without clear guidelines, and has demonstrated to be sensitive to the parameters used in the analyses (Wilson et al., 2017). We have intended to overcome this methodological limitation by i) determining the position and the number of knots on a parsimonious principle, to not overfit the model, and ii) repeating the analyses with a linear lag-response relationship to minimize the BIC. Then, because the DLNM does not permit missing values in the exposure matrix, the main analyses included pregnancy exposure until the weeks 32–36 and childhood

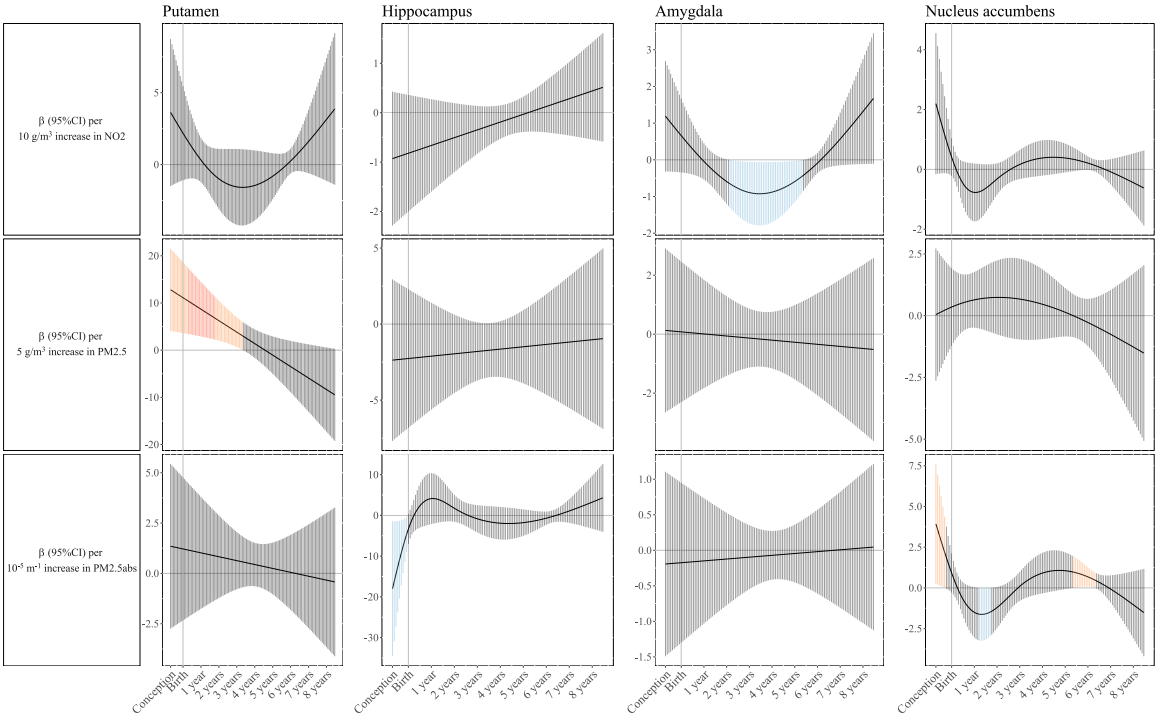


**Fig. 3.** Adjusted associations between exposure to air pollutants from conception to age 8.5 years and subcortical brain volumes at 9–12 years of age (N = 3089). Adjusted for maternal and paternal educational levels, monthly household income, maternal and paternal country of birth, maternal and paternal age at enrollment in the cohort, maternal smoking and alcohol use during pregnancy, parity, marital status, maternal and paternal psychological distress, maternal and paternal BMI, maternal intelligence quotient, child's sex and age at the MRI session, year and month of conception, and intracranial volume.
NO2, nitrogen dioxide; PM2.5, particulate matter with aerodynamic diameter <2.5 μm; PM2.5abs, absorbance of PM2.5 filters.
Blackline represents the beta estimate of the association between the exposure at each specific lag and the outcome. Vertical gray, blue, and orange lines represent 95% CI and indicate no divergence from the null, significant divergence from negative association, and significant divergence from positive association, respectively. Darker orange color indicates associations after correction for multiple testing (p-value < 0.004). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

exposure until the age of 8.5 years, in which extremely and very preterm children were excluded. However, we have conducted several sets of sensitivity analyses to evaluate the impact of the inclusion of larger periods of pregnancy and childhood exposure and a shorter period of pregnancy exposure, and we obtained similar associations, suggesting that our results were robust. Moreover, we have investigated the influence of moderate to late premature birth, and a possible selection bias on preterm children, and we found no evidence of influence of premature birth on the associations nor on the windows of susceptibility. Third, we did not perform multi-pollutant analyses and we assessed each air pollutant separately with each brain outcome. We considered NO2, PM2.5, and PM2.5absorbance as markers of traffic-related air pollution. It is difficult to disentangle the effects of each air pollutant, but our results suggest that traffic-related air pollution is associated with brain development. Fourth, participants included in this study had a higher socioeconomic status than non-participants. We used inverse probability weighting to address that limitation, but we cannot rule out a potential selection bias because we might have missed important predictors of the risk of loss-to-follow-up. Fifth, despite our extensive selection of potential confounders, we cannot discard the possibility of residual confounding. Moreover, we adjusted our models for covariates collected at one single timepoint, and we did not consider a possible time varying socioeconomic level and lifestyle environment of the participants during the period of interest of our study. Residual confounding could introduce bias and lead to incorrect estimates of the associations (Weisskopf et al., 2018). Sixth, our study is based on a single measurement of the white matter microstructure and brain volume in preadolescence. Repeated measurements across time would give insight into trajectories of brain maturation, and could help to understand the potential long-term effects of air pollution exposure on the brain.

## 5. Conclusion

Our study demonstrates that exposures to various air pollutants from conception to age 8.5 years were associated with altered white matter microstructure in preadolescents of 9–12 years of age, with specific periods of susceptibility from conception to age 5 years. The first 2 years of life were also a likely susceptible period for the association between air pollution and larger putamen volumes in preadolescence. Overall, reducing the exposure to air pollutants during pregnancy and in infancy, toddlerhood, and early childhood seems to be essential to promote a normal brain development.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120109.

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