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Effect of exposure to polycyclic aromatic hydrocarbons on basal ganglia and attention-deficit hyperactivity disorder symptoms in primary school children

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) have been proposed as environmental risk factors for attention deficit hyperactivity disorder (ADHD). The effects of these pollutants on brain structures potentially involved in the pathophysiology of ADHD are unknown.

Objective: The aim of this study was to investigate the effects of PAHs on basal ganglia volumes and ADHD symptoms in school children.

Methods: We conducted an imaging study in 242 children aged 8–12 years, recruited through a set of representative schools of the city of Barcelona, Spain. Indoor and outdoor PAHs and benzo[*a*]pyrene (BPA) levels were assessed in the school environment, one year before the MRI assessment. Whole-brain volumes and basal ganglia volumes (caudate nucleus, globus pallidus, putamen) were derived from structural MRI scans using automated tissue segmentation. ADHD symptoms (ADHD/DSM-IV Scales, American Psychiatric Association 2002) were reported by teachers, and inattentiveness was evaluated with standard error of hit reaction time in the attention network computer-based test.

Results: Total PAHs and BPA were associated with caudate nucleus volume (CNV) (i.e., an interquartile range increase in BPA outdoor level (67 pg/m^3) and indoor level (76 pg/m^3) was significantly linked to a decrease in CNV (mm^3) ($\beta = -150.6$, 95% CI [−259.1, −42.1], $p = 0.007$, and $\beta = -122.4$, 95% CI [−232.9, −11.8], $p = 0.030$ respectively) independently of intracranial volume, age, sex, maternal education and socioeconomic vulnerability index at home). ADHD symptoms and inattentiveness increased in children with higher exposure to BPA, but these associations were not statistically significant.

Conclusions: Exposure to PAHs, and in particular to BPA, is associated with subclinical changes on the caudate nucleus, even below the legislated annual target levels established in the European Union. The behavioral consequences of this induced brain change were not identified in this study, but given the caudate nucleus involvement in many crucial cognitive and behavior processes, this volume reduction is concerning for the children's neurodevelopment.

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1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is among the most frequently diagnosed childhood neurodevelopmental disorders. Symptoms are characterized by an inability to focus on tasks, impulsive hyperactive behavior, or lethargic inattention, and frequently lead to functional impairment in academic, family, and social settings (Barkley, 2002). ADHD affects approximately 5–7.1% of children and adolescents worldwide (Polanczyk et al., 2007; Willcutt, 2012), and persists into adulthood in 65% of cases (Faraone et al., 2006). Although considered to be a familial disorder, ADHD heritability estimated at 60% to 80% highlights the considerable role of environmental factors in disorder susceptibility (Nigg, 2009).

Influence of air pollutants on the ADHD prevalence has been poorly investigated while there is growing evidence of their detrimental effect on the central nervous system (Block et al., 2012). Among the different air pollutants, of particular concern are the polycyclic aromatic hydrocarbons (PAHs), mainly emitted into the air from anthropogenic combustion sources. PAHs are a group of ubiquitous environmental contaminants formed during the incomplete combustion of organic material and found in diverse media including cigarette smoke, charcoal-broiled food and emissions from combustion of fossil and biomass fuels (World Health Organization, 2001). Experimental studies have indeed reported that administration of benzo[*a*]pyrene (BAP) induced spontaneous motor hyperactivity in rodents (Das et al., 2016; Grova et al., 2007; Maciel et al., 2014). Moreover, prenatal exposure to PAHs was associated with attention and ADHD behavior problems in childhood in a birth cohort (Perera et al., 2011, 2012, 2014). Owing to their lipophilicity, PAHs can cross the blood-brain barrier (Yan et al., 2010) and cause pathophysiological changes such as loss of neuronal activity and synaptic plasticity (Chepelev et al., 2015), and neuronal death (Dutta et al., 2010). However, their effects on brain structures remain unknown. To date, only one magnetic resonance imaging (MRI) study, conducted in the same birth cohort reporting the association between prenatal PAHs exposure and ADHD symptoms, has investigated the changes in surface of the brain. This study has highlighted a dose-response relationship between prenatal PAHs exposure and subsequent reductions of the white matter surface (Peterson et al., 2015). In addition to changes in white matter or prefrontal cortex, reductions in basal ganglia (BG) are reported as the most consistently brain abnormalities findings in many MRI meta-analysis in ADHD children (Ellison-Wright et al., 2008; Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007). The BG thalamocortical circuits are indeed involved in processes such as motor control, reward processing, and cognitive and attentional control that are impaired in ADHD (Ring and Serra-Mestres, 2002). Therefore, BG should also be investigated as a potential mediator in the relationship between PAHs exposure and ADHD symptoms.

The main aim of this study was to investigate the effects of PAHs exposure in indoor and outdoor school environments on white matter, gray matter and BG (putamen, caudate nucleus and globus pallidus) in children from the general population. We hypothesized that higher PAHs exposure would be associated with reduced BG volumes and increased risk of ADHD symptoms.

2. Methods

2.1. Schoolchildren

This study was developed in the context of the Brain Development and Air Pollution Ultrafine Particles in School Children (BREATHE) project (Sunyer et al., 2015). Forty schools in Barcelona (Catalonia, Spain) were selected based on modeled traffic-related nitrogen dioxide (NO_2) values (Wang et al., 2013). Low- and high-NO_2 schools were paired by socioeconomic vulnerability index and type of school (i.e., public/private). A total of 39 representative schools of the city of Barcelona and 2897 children agreed to participate in the whole survey. The participating children were in grades 2 through 4 (7–10 years of age), an appropriate school age for the investigation of attention functions (Anderson, 2002; Rueda et al., 2004).

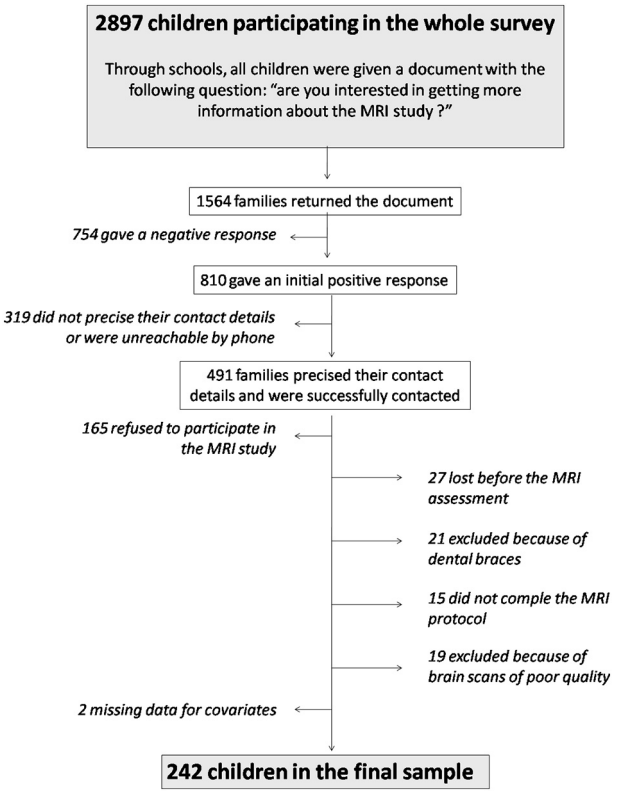

Fig. 1. Flow chart.

Through the schools, all the participating children were given a document asking whether they were interested in further information about the MRI study. From the initial sample, 1564 families returned the document and among them, 810 gave an initial positive response. Parents of 491 children were successfully contacted. Consent to participate was finally not obtained in 165 cases, 27 children were lost before the assessment and 21 children were not eligible because of dental braces. This group was further reduced in excluding 15 children who did not complete the imaging protocol, 19 children with brain scans of poor quality, and 2 with missing data for covariates (Fig. 1). The final MRI sample included 242 children from 35 schools (49% of girls, median age at MRI of 9.7 years). The median time spent in the school before the beginning of the study was 6.5 years.

All parents or tutors signed the informed consent form approved by the Research Ethical Committee (No. 2010/41221/I) of the IMIM-Parc de Salut Mar., Barcelona, Spain and the FP7-ERC-2010-AdG Ethics Review Committee (268479-22022011).

2.2. PAHs exposure

Air pollution measurements were taken in each the 35 schools during two one-week periods separated by 6 months (sampling campaign 1: January to June 2012; sampling campaign 2: September 2012 to February 2013). Indoor air in a single classroom and outdoor air in the courtyard were measured simultaneously. Ambient particulate Matter < 2.5 μm ($PM_{2.5}$) was collected on pre-heated quartz filters (Pall, 2500Q, 15 mm) during 8-h (school time, from 09:00 to 17:00 h) using a High-Volume sampler (MCV SA, Spain). Air samples were collected at a height of around 1.3 m above floor level, which is the height at which the pupils aged 7–9 would usually inhale. A quarter of each $PM_{2.5}$ filter was used for organic analysis using organic solvent

extraction and subsequent chemical analysis by gas-chromatography coupled to mas-spectrometry detection (GC–MS) following the methodology that is described elsewhere (Alier et al., 2014; Fontal et al., 2015). PAHs included in this study were benz[*a*]anthracene (BAAN), chrysene (CHRYS), benzo[*b* + *j* + *k*]fluoranthene (BFL), benzo[*e*]pyrene (BEP), BAP, indeno[1,2,3-*c*,*d*]pyrene (IP) and benzo[*g*,*h*,*i*]perylene (BGP) which were those showing detectable levels in all samples.

Outdoor and indoor long-term school PAHs levels were obtained by averaging the two one-week measures. Given that the sampling was not performed simultaneously in all schools, to reduce temporal fluctuations when comparing the PAHs levels between the schools the data were previously seasonalized after adjusting for the mean daily BAP level measured in three urban monitoring stations of *Eixample* (429345, 4582085 UTM), *Gràcia-SantGervasi* (429323, 4583573 UTM) and *Plaça Universitat* (430275, 4582307 UTM). These three sites are considered urban road sites, exposed to traffic, and BAP is measured daily at least in one of these stations continuously during the whole study period. Seasonalized levels were obtained by multiplying the daily concentration at each school by the ratio of annual average to the same day concentration at the three fixed air quality background monitoring stations, as detailed elsewhere (Rivas et al., 2014).

Individual PAHs from the outdoor measurements showed very high correlations. In contrast, among the indoor measurements, the correlation coefficients were low between the high molecular weight PAHs (BAP, BEP, IP, BGP) and the total sum of PAHs mainly driven by the lower molecular weight PAHs (BAAN, CHRYS and BFL) (see eTables 1–3 and eFigs. 1–3 that show how these pollutants cluster). Hence, as exposure variables, we retained the total sum of PAHs (total PAHs = BAAN + CHRYS + BFL + BEP + BAP + IP + BGP), and the BAP levels (as a representative of the high molecular weight PAHs) for both indoor and outdoor measures.

A complete $PM_{2.5}$ chemical characterization (including the analysis of elemental carbon, EC) and the analysis of NO_2 levels (using passive samplers GRADKO) was also carried out in parallel at each school using the methodology reported elsewhere by Rivas et al. (2014).

2.3. MRI

2.3.1. MRI acquisition

Cerebral anatomical images were acquired one year after the PAHs measurements, between October 2012 and April 2014. A 1.5-Tesla Signa Excite system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil was used. Anatomical images were obtained using an axial T1-weighted three-dimensional fast spoiled gradient inversion recovery prepared sequence. A total of 134 contiguous slices were acquired with repetition time 11.9 msec, echo time 4.2 msec, flip angle 15°, field of view 30 cm, 256 × 256 pixel matrix, and slice thickness 1.2 mm.

2.3.2. Data preprocessing and segmentation

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Fischl et al., 2004, 2002; Ségonne et al., 2004).

Briefly, this processing includes removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Ségonne et al., 2004), automated Talairach transformation and segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2002, 2004).

In this study, we were interested in BG volumes (putamen, caudate and globus pallidus), as well as in brain parenchymal fraction (BPF), defined as the sum of gray and white matter divided by intracranial volume (ICV).

2.4. Neurobehavior assessment

ADHD symptoms (ADHD/Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Scales, American Psychiatric Association 2002) were reported by teachers. ADHD-DSM-IV consists of a list of 18 symptoms categorized under two separate symptom groups. These are inattention (nine symptoms) and hyperactivity/impulsivity (nine symptoms). Each ADHD symptom is rated on a 4-point scale (0 = never or rarely, 1 = sometimes, 2 = often, or 3 = very often). The number of ADHD cases, defined as presenting often or very often ≥ 6 inattention symptoms and/or ≥ 6 hyperactive/impulsive symptoms, was small (23 cases) and we were thus not able to consider the outcome "ADHD case" in our analyses. Therefore, we analyzed the ADHD clinical criteria as a continuous variable for the total score, the inattention and the hyperactivity subscores in order to capture and quantify the ADHD symptomatology in our MRI sample.

In addition, attention was also evaluated with the validated original child attention network test (ANT) (Rueda et al., 2004) using standard error of hit reaction time (HRT-SE) for correct responses, a measure of response speed consistency throughout the test. Inattentiveness is indicated by higher values of HRT-SE. The children were assessed with computerized tests in 4 repeat visits, every three months. Data from children with > 30% errors in any visit were excluded from the analysis (1.67%) (Rueda et al., 2004). All children had repeated inattentiveness data at least twice.

2.5. Contextual and individual covariates

Socio-demographic factors were measured using a neighborhood socio-economic vulnerability index (based on level of education, unemployment, and occupation in each census tract, the finest spatial census unit, with median area of 0.08 km^2) (Ministry of Public Works, 2012) according to both the school and home address, and maternal education (equal or less than primary, secondary or university).

Exposures at home to nitrogen dioxide (NO_2) and $PM_{2.5}$ at the time of the study and during the prenatal period were estimated at the geocoded postal address of each participant using land use regression models, details of which are explained elsewhere (Wang et al., 2013).

2.6. Statistical analyses

To determine whether brain structures might be affected by PAHs exposure, we used linear mixed effects models with the different neuroimaging variables (BPF and BG volumes) as outcomes, PAHs levels as independent variables, and schools as nested random effects due to the multilevel nature of the data.

In order to account for the overdispersion of ADHD scores and subscores (eFigs. 4–6), we fit negative binomial mixed regression models to test for associations between PAHs exposure and ADHD scores and subscores (semi-quantitative outcomes) with schools as random effects.

We then examined the relationship between PAHs exposure and inattentiveness using linear mixed effects models with the four repeated cognitive parameters as outcomes, and random effects for child and school.

Given the higher frequency of ADHD cases in boys (Davies, 2014), we also examined whether an interaction sex-PAHs exposure for both outcomes (brain structures and ADHD symptoms) was present.

Finally, we conducted negative binomial mixed models and linear mixed models with ADHD (scores and subscores) and inattentiveness as outcomes and BG volumes as independent variables to study the relationship between brain structures and neurobehavior.

All the analyses were adjusted for age, sex, ICV when needed, maternal education and home socioeconomic vulnerability index. Analyses were run using Stata 14 (StataCorp). Statistical significance was set at $p < 0.05$. As the exposures (BAP and PAHs levels), as well as

Table 1
Children's characteristics, n = 242.

Socio-demographic characteristics	
Age at baseline, yrs (median, range)	8.4 (7.1–10.3)
Age at MRI, yrs (median, range)	9.7 (8.0–12.1)
Girls (n, %)	119 (49%)
Years spent at the school[a] (median, range)	6.5 (0.9–9.7)
Home socioeconomic vulnerability index (median, range)	0.42 (0.06–0.90)
Maternal education level (n, %)	
Primary or less than primary	22 (9%)
Secondary	59 (24%)
University	161 (67%)
Brain structures (median, range)	
ICV, cm^3	1393 (1109–1752)
Brain parenchymal fraction, %	79 (65–85)
Putamen, mm^3	11,523 (8716–14,737)
Caudate nucleus, mm^3	7849 (5052–10,192)
Globus pallidus, mm^3	3331 (2327–4427)
ADHD symptomatology (median, range)[b]	
ADHD DSM-IV scale	
ADHD total score (n = 236)	4 (0–49)
Inattention score (n = 238)	2 (0–24)
Hyperactivity score (n = 237)	1 (0–26)
Inattentiveness (ANT, HRT-SE, milliseconds)[c]	
Baseline (n = 217)	248 (95–481)
Visit 2 (n = 220)	233 (80–509)
Visit 3 (n = 224)	224 (72–490)
Visit 4 (n = 227)	216 (77–572)

Abbreviations: ADHD, Attention-Deficit Hyperactivity Disorder; ICV, intracranial volume.
[a] Before the beginning of the study.
[b] 6 teachers gave back incomplete ADHD DSM-IV questionnaires.
[c] Accuracy rate of the test is ≥ 70% (children's data with high error rates were removed from the analysis).

the outcomes (BPF, caudate nucleus, putamen and globus pallidus volumes) were correlated, all the tests performed in our study are dependent on each other. Therefore, we did not correct our results for multiple testing as this could lead to conservative results.

In sensitivity analyses, we excluded 19 participants whose parents reported to smoke at home to minimize confounding by exposure to other sources of PAHs than those at schools. As ADHD does predict volume reduction (Castellanos et al., 2002), we examined the relationships between PAHs exposure and brain structures with an additional adjustment for ADHD status, then in children with an ADHD-negative status only. ADHD medication might also have an impact on volumes of brain structures (Castellanos et al., 2002), we thus excluded the 6 children with ADHD medication (5 with methylphenidate and 1 with atomoxetine) reported by parents from all the analyses.

We also adjusted all the analyses for exposure to NO_2 and $PM_{2.5}$ at home, and noise in classroom. Finally, we investigated the relationship between prenatal exposure to NO_2 and $PM_{2.5}$ and the brain volumes.

3. Results

Table 1 describes the children's characteristics. Forty-nine percent of children were girls and age at MRI ranged between 8.0 and 12.1 years. More than 75% of the children had been attending the same school for > 5 years before the beginning of the study. The children within the MRI sample did not differ from those of the whole BREATHE sample in terms of age and sex (eTable 4). However, higher maternal education, lower socio-economic vulnerability index and exposure levels to outdoor PAHs and BAP were observed in the MRI sample when compared to the total population (eTable 4).

PAHs levels were highly variable between schools (Table 2). Total PAHs ranged from 597 to 3235 pg/m^3 for outdoor measures, and from 484 to 5220 pg/m^3 for indoor ones (details about individual indoor and outdoor concentrations are showed in eTable 5). BAP levels were similar outdoors and indoors in the different schools. The PAHs levels were strongly correlated with others markers of vehicle exhaust such as NO_2 and EC (Pearson correlation coefficients ranged from 0.55 to 0.84 and were all significant, eTables 6 and 7).

Table 2
PAHs level in the 35 schools.

PAHS level at schools, pg/m^3	Mean (SD)	Median	Range
Outdoor			
Total PAHs[a]	1458 (704)	1222	597–3235
BAP	99 (62)	86	20–304
Indoor			
Total PAHs[a]	1710 (1107)	1486	484–5220
BAP	105 (72)	96	23–425

Abbreviations: BAP, Benzo[*a*]pyrene; PAHs, Polycyclic Aromatic Hydrocarbons.
[a] Total PAHs: Sum of Benzo[*a*]pyrene, Benzo[*e*]pyrene, Indeno[1,2,3-*cd*]pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, and Benzo[*ghi*]perylene.

3.1. PAHs exposure and brain volumes

Table 3 presents the adjusted PAHs coefficients for BPF and BG volumes which revealed a negative relationship between BAP levels (outdoor and indoor) and caudate nucleus volume (CNV). An interquartile range increase in both outdoor and indoor BAP levels (67 and 76 pg/m^3 respectively) were significantly linked to a decrease in CNV (mm^3) ($\beta = -150.6$, 95% CI [−259.1, −42.1], $p = 0.007$, and $\beta = -122.4$, 95% CI [−232.9, −11.8], $p = 0.030$ respectively) independently of intracranial volume, age, sex, maternal education and socioeconomic vulnerability index at home. The effects of outdoor total PAHs on CNV were similar but to a lesser extent than for BAP outdoor.

A similar trend was reported between total PAHs indoor and CNV, but did not reach the significance threshold.

No significant associations were observed between PAHs outdoor or indoor and any of the others different neuroimaging variables (BPF, putamen and globus pallidus volumes).

No interaction between sex and PAHs was observed for brain volumes (eTable 8). Hence, CNV was smaller with increasing levels of total PAHs and BPA both in boys and girls (eTables 9 and 10).

3.2. PAHs exposure and neurobehavior

Table 4 gives the adjusted Rate Ratios (RR) for ADHD score and subscores and coefficients for inattentiveness by school air pollution exposure to PAHs.

Results suggest a trend for higher ADHD scores with an interquartile range increase in outdoor BAP and indoor BAP levels (RR = 1.18, 95%CI [0.96, 1.45], $p = 0.109$, and RR = 1.14 95%CI [0.90, 1.45], $p = 0.274$ respectively). Similar trends for detrimental associations were observed between BAP exposure and ADHD hyperactivity and inattention subscores, and inattentiveness (i.e., increase of HRT-SE values over one year).

No significant relationships were observed between total PAHs and neurobehavior outcomes.

No interaction between sex and PAHs was observed for ADHD symptoms (eTable 11). Both boys and girls had higher ADHD symptoms with increasing levels of BPA, although none of the associations were statistically significant (eTables 12 and 13).

3.3. BG volumes and ADHD

A 200 mm^3 decrease in putamen and caudate nucleus were associated with significant higher inattention subscore (5% increase) and ADHD score (4% increase for putamen only) independently of age and sex (Table 5). However, these relationships were no longer significant when adjusted for ICV.

Table 3
Difference (95%CI) in brain parenchymal fraction and basal ganglia volumes by school air pollution exposure to PAHs (interquartile range increase) in 242 children from 35 schools.

	BPF, %		Putamen, mm^3		Caudate nucleus, mm^3		Globus pallidus, mm^3	
	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p	β (95%CI)	p
Outdoor								
Total PAHs[a]	0.3 (−0.2, 0.7)	0.240	26.4 (−128.3, 181.2)	0.738	−132.9 (−245.0, −20.8)	0.020	4.5 (−46.1, 55.2)	0.861
BAP	0.0 (−0.4, 0.5)	0.929	13.0 (−139.2, 165.2)	0.867	−150.6 (−259.1, −42.1)	0.007	−16.9 (−64.9, 31.0)	0.488
Indoor								
Total PAHs[a]	0.2 (−0.03, 0.3)	0.098	11.8 (−53.3, 76.8)	0.723	−30.5 (−82.5, 21.6)	0.252	6.0 (−15.6, 27.5)	0.588
BAP	0.2 (−0.2, 0.6)	0.377	−29.7 (−182.8, 123.4)	0.704	−122.4 (−232.9, −11.8)	0.030	14.4 (−34.3, 63.0)	0.563

Difference (95% CI) in brain structures adjusted for age at MRI, sex, intracranial volume, maternal education and residential neighborhood socioeconomic status; school as nested random effects.
Abbreviations: BAP, Benzo[*a*]pyrene; BPF, Brain Parenchymal Fraction; PAHs, Polycyclic Aromatic Hydrocarbons.
[a] Total PAHs: Sum of Benzo[*a*]pyrene, Benzo[*e*]pyrene, Indeno[1,2,3-*cd*]pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, and Benzo[*ghi*]perylene.

3.4. Sensitivity analyses

Excluding participants whose parents reported to smoke at home or participants with ADHD medication, as well as adjustment for ADHD status, NO_2 and $PM_{2.5}$ exposures at home or noise in classroom did not change the results (data not shown). Furthermore, the results in ADHD-negative children were similar to those observed in the whole MRI sample (data not shown).We did not observe any statistically significant association between the prenatal exposures to NO2 and $PM_{2.5}$ and the brain volumes, although the CNV was slightly smaller with higher prenatal exposures (eTable 14).

4. Discussion

In this study, we examined the relationship between PAHs exposure and BG volumes and ADHD symptoms. In our sample, we found that exposure to BAP may induce structural changes in the caudate nucleus in children from the general population. An increase of approximately 70 pg/m^3 in the concentration of indoor or outdoor BAP in the school environment was indeed significantly associated with a reduction corresponding to almost 2% of the mean CNV in our population of children aged 8 to 12 years. This reduction appeared to be subclinical given that it was not significantly associated with ADHD symptoms. We also observed trends for associations between BAP exposure and higher ADHD scores and inattentiveness, but these relationships were not statistically significant.

Neurotoxicity of PAHs, most widely studied via BAP, is likely to occur through different mechanisms. First, by binding to aryl hydrocarbon receptor, one of the most well characterized transcription factors, BAP may alter gene expression of the *N*-methyl-D-aspartate receptor (Chepelev et al., 2015), a glutamate receptor and ion channel protein in nerve cells involved in synaptic plasticity, memory function (Li and Tsien, 2009) and cell death pathways (Vanhoutte and Bading, 2003). Furthermore, BAP may be a source of oxidative stress (Dutta et al., 2010; Saunders et al., 2006) which induces alteration in gene expression, impairs cellular signaling, disrupts membrane integrity, alters neurotransmission and finally causes neuronal death (Cardozo-Pelaez et al., 1999; LeBel and Bondy, 1991). Oxidative stress can affect the entire brain. Nevertheless, our findings are in agreement with in vitro observations suggesting that striatum (caudate and putamen nuclei) is more vulnerable to oxidative damage than the others brain areas (Cardozo-Pelaez et al., 1999; Rivas-Arancibia et al., 2003). Increased levels in lipid peroxidation, a major consequence of oxidative stress, were indeed observed specifically in rodents striatum after exposure to BAP or ozone (Rivas-Arancibia et al., 2000; Saunders et al., 2006). Actually, the activity of cortico-striatal circuits is modulated by dopamine, a neurotransmitter prone to oxidation at its electron-rich catechol moiety, and which metabolism itself serves as a major source of intracellular reactive oxygen species (Meiser et al., 2013). Hence, oxidative stress appears to be preferably established in this part of the brain, certainly because of a depletion in the local antioxidant systems occurring in case of any additional oxidative challenge (Dorado-Martínez et al., 2001).

In animal studies, deleterious effects on striatum were observed for acute BAP exposures (Das et al., 2016; Saunders et al., 2006). In our study, the BAP levels encountered by the children in their school environment are obviously lower than those used in laboratory, but also far below the legislated annual target levels of 1000 pg/m^3 for BAP established in the European Union (European Council Directive 2004/107/EC, http://eur-lex.europa.eu/eli/dir/2004/107/oj). The BAP concentrations that we observed in our study are in the range of those observed in Los Angeles in the United States (US) during the summer

Table 4
Attention deficit hyperactivity disorder symptoms by air pollution exposure to PAHs (interquartile range increase) in the 35 schools.

	ADHD score[a]		ADHD subscores[a]				Inattentiveness[b]	
	RR (95%CI)	p	Inattention score		Hyperactivity score		12 months change	
			RR (95%CI)	p	RR (95%CI)	p	β(95%CI)	p
Outdoor								
Total PAHs[c]	1.02 (0.81, 1.29)	0.865	1.03 (0.82, 1.30)	0.794	1.03 (0.79, 1.35)	0.820	6.4 (−2.9, 15.8)	0.179
BAP	1.18 (0.96, 1.45)	0.109	1.20 (0.98, 1.46)	0.079	1.17 (0.92, 1.47)	0.193	3.9 (−5.9, 13.7)	0.434
Indoor								
Total PAHs[c]	0.94 (0.85, 1.04)	0.242	0.93 (0.84, 1.03)	0.165	0.95 (0.85, 1.07)	0.435	0.3 (−3.5, 4.1)	0.879
BAP	1.14 (0.90, 1.45)	0.274	1.13 (0.89, 1.44)	0.328	1.17 (0.89, 1.54)	0.269	−2.9 (−13.2, 7.4)	0.584

Abbreviations: ADHD, attention deficit hyperactivity disorder; BAP, Benzo[*a*]pyrene; PAHs, Polycyclic Aromatic Hydrocarbons; RR, Rate Ratio.
[a] Negative binomial mixed regression models adjusted for age, sex, maternal education and residential neighborhood socioeconomic status; school as nested random effects.
[b] Mixed regression models adjusted for age, sex, maternal education and residential neighborhood socioeconomic status; school and individual as nested random effects.
[c] Total PAHs: Sum of Benzo[*a*]pyrene, Benzo[*e*]pyrene, Indeno[1,2,3-*cd*]pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, and Benzo[*ghi*]perylene.

Table 5
Attention deficit hyperactivity disorder symptoms by basal ganglia volumes (200 mm^3 decrease) in the 35 schools.

	ADHD score[a]		ADHD subscores[a]				Inattentiveness[b]	
	Rate ratio (95%CI)	p	Inattention score		Hyperactivity score		12 months change	
			Rate ratio (95%CI)	p	Rate ratio (95%CI)	p	β(95%CI)	p
Model 1[c]								
Putamen	1.04 (1.01, 1.07)	0.018	1.05 (1.01, 1.08)	0.008	1.03 (0.99, 1.07)	0.104	0.36 (− 1.00, 1.72)	0.601
Caudate	1.03 (0.99, 1.07)	0.172	1.05 (1.01, 1.10)	0.026	1.00 (0.95, 1.05)	0.907	− 0.71 (− 2.51, 1.10)	0.442
Globus pallidus	1.06 (0.96, 1.17)	0.244	1.09 (0.98, 1.21)	0.097	1.02 (0.91, 1.16)	0.701	0.29 (− 4.11, 4.69)	0.898
Model 2[d]								
Putamen	1.03 (1.00, 1.06)	0.071	1.03 (1.00, 1.07)	0.054	1.03 (0.99, 1.07)	0.136	0.36 (− 1.00, 1.72)	0.607
Caudate	1.01 (0.96,1.06)	0.739	1.02 (0.98, 1.07)	0.343	0.98 (0.93, 1.04)	0.579	− 0.71 (− 2.51, 1.10)	0.443
Globus pallidus	1.02 (0.92, 1.14)	0.701	1.04 (0.93, 1.16)	0.461	1.01 (0.88, 1.15)	0.905	0.38 (− 4.02, 4.79)	0.864

Abbreviations: ADHD, attention deficit hyperactivity disorder.
[a] Negative binomial mixed regression models; school as nested random effects.
[b] Mixed regression models; school and individual as nested random effects.
[c] Model 1: adjusted for age and sex.
[d] Model 2: adjusted for age, sex and intracranial volume.

2006 (Ning et al., 2007). However, many other studies report higher annual average BAP levels worldwide: between 400 and 500 pg/m^3 in urban areas in Greece (Manoli et al., 2016) and in Germany (Pietrogrande et al., 2011), between 500 and 1000 pg/m^3 in Croatia (Godec et al., 2016) and in Texas (US) (Maypole-Keenan et al., 2016), or even > 1000 pg/m^3 in Northeast Italy (Masiol et al., 2013, 2012) and Czech Republic (Křůmal et al., 2013).

Our PAHs assessments actually reflect the dose at which children are chronically exposed. Indeed, > 75% of the children had been attending the same school for > 5 years before the beginning of the study, and annual BAP levels have been quite constant for this period as shown by the monitoring stations in the City of Barcelona (mean (SD) inter-annual variation between 2008 and 2013 in six different areas in Barcelona was 17 (14) pg/m^3, http://qualitatdelaire.cat/contaminant/cerca/14/1/4.html). Consequently, the BAP impact that we observed on the CNV is probably rather attributable to a cumulative effect over a long period of time. The magnitude of the effect that we observed, a 2% decrease of the mean CNV for a 70 pg/m^3 BAP increase, might be of concern since, compared with healthy controls, the mean CNV is only 7 to 8% smaller in children with ADHD (Frodl and Skokauskas, 2012). Furthermore, in those children, the CNV correlates with ADHD severity (Castellanos et al., 2002) and with attentional performance on response inhibition tasks (Casey et al., 1997). In our sample, a 200 mm^3 decrease in the CNV was associated with a 5% increase inattention ADHD subscore. Nevertheless, this relationship was no longer significant when adjusted for ICV. The investigation of the association between attentional performance and MRI-based anatomical measures of volume are actually very rude, particularly in healthy populations. Functional MRI might be of interest to more directly assess structure-function relation in this case.

The reasons why the BAP action was only detected in the caudate nucleus are unclear. In previous work and with others neuroimaging methods, we also found that the detrimental effect of airborne copper was circumscribed to the caudate nucleus in our population (Pujol et al., 2016a). The caudate nucleus is among the few structures displaying a striking developmental variation across childhood and adolescence. While the pre-pubertal period is characterized by greater synaptogenesis, adolescence is the seat of normative pruning (Giedd and Rapoport, 2010). These intense modifications on such a short period probably make this structure particularly vulnerable in the age range (8–12 years) of our study. The absence of associations between prenatal exposure to NO_2 and $PM_{2.5}$ and the CNV in our sample also supports this hypothesis.

We however did not observe any changes in brain BPF, parameter that we used to examine fluctuations in total gray and white matter volumes, while Peterson et al. (2015) found an association between prenatal exposure to PAHs and substantial reductions in white matter surface. However, the differences in the neuroimaging methods and markers retained to evaluate the brain changes (volumes versus surface morphologies) make comparison between the two studies difficult. In addition, the children's mothers were coming from a minority population with high poverty and very high levels of PAHs in the Peterson's study. Oxidative damages might be extended to a larger range of brain structures with greater exposures.

We did not either observed any significant relationships between PAHs exposure and ADHD symptoms. Nevertheless, the trends we observed between ADHD symptoms and BAP parallel the effects of these compounds on the CNV, suggesting that the levels encountered in our study are probably too low to detect their clinical effect. In the absence of such an association, we were unable to investigate whether the reduction in CNV might mediate the effect of PAHs on ADHD symptoms. For the same reason, Peterson et al. (2015) could not reach a clear conclusion about the potential mediator role of the white matter reduction surface in the relationship between prenatal exposure to PAHs and ADHD behavior problems in their population. Other studies with larger MRI sample, with wilder range of exposure levels at different time windows are still needed to understand the impact of PAHs-related brain changes on neurodevelopment.

The strong correlations among the individual PAHs from the outdoor measurements indicate that these distributions were essentially generated by a common source, i.e. the traffic which is the dominating source of PAHs emission in the studied urban area (Alier et al., 2013; Reche et al., 2012; van Drooge et al., 2012). Conversely, the weaker correlation coefficients observed for the indoor measurements could be due to the partitioning of the more volatile PAHs (such as BAAN and CHRYS) from the gas phase towards the particulate phase because of the high indoor organic carbon concentrations (Lohmann and Lammel, 2004). Hence, the indoor level of total PAHs partly relies on the outdoor PAHs emissions and partly on the indoor circumstances in the different schools as well as children activities (Amato et al., 2014; Rivas et al., 2014). However, higher molecular weight PAHs, such as BAP, are exclusively confined to the particles phase and more related to the outdoor traffic emissions (Alier et al., 2013; Lohmann and Lammel, 2004). Furthermore, outdoor and indoor BAP showed consistent associations with reduction of CNV and seem more likely to be involved in the detrimental effects observed on the caudate nucleus.

A number of limitations in this study must be considered. Firstly, our MRI study participants, because of their high socio-economic levels and low PAHs exposure levels, might not be representative of the general population. Secondly, our PAHs exposure assessment in school

children does not permit us to consider to what extent anterior PAHs exposure during the critical prenatal period might have influenced the BG volumes. Then, we did not have personal monitoring nor biomarkers, thus our PAHs measurements only provide indirect estimates of the real exposure to PAHs. However, given the long period of time spent in the school by the children, as well as the stability of the BAP levels in Barcelona, our exposure assessment is likely to well reflect the exposure throughout childhood. Indeed, exposure assessment at school appears to be particularly relevant. We previously showed that exposure to traffic-related air pollution at school was significantly higher than at home and did not change by commuting mode (Sunyer et al., 2015). This higher exposure level at school could be attributed to peaks of pollution occurring during school time, and higher inhaled dose during school time due to exercise and physical activity at schools.

Another potential limitation is that air pollution is actually a mixture of numerous environmental contaminants and the effects observed here might be not only attributable to BAP. Indeed, the PAHs levels are strongly correlated with others markers of vehicle exhaust such as NO_2 and EC. However, the investigation of the potential impact of NO_2 and EC revealed no significant link between the CNV and both outdoor and indoor EC levels, as well as inconsistent associations between the CNV and the outdoor and indoor NO_2 levels (eTable 15). Furthermore, using voxel-based morphometry analyses, we recently reported no associations between combined measurements of EC and NO_2 and brain anatomy in our population (Pujol et al., 2016b), which supports our observation that PAHs might have a specific neurotoxic effect on BG.

5. Conclusions

This study provides evidence that chronic exposure to PAHs during the pre-adolescent school-age years is associated with subclinical changes on the caudate nucleus, even for levels below the legislative annual target level established in the European Union. These findings add to a significant body of studies underlying the urgent necessity to reduce anthropogenic emissions of air pollutants and may suggest the need to re-evaluate the legislative annual target levels. The behavioral consequences of this induced brain change were not identified in this study, but given the caudate nucleus involvement in many crucial cognitive and behavior processes, this volume reduction is of health concern for the children's neurodevelopment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2017.04.011.

References

Alier, M., van Drooge, B.L., Dall'Osto, M., Querol, X., Grimalt, J.O., Tauler, R., 2013. Source apportionment of submicron organic aerosol at an urban background and a road site in Barcelona (Spain) during SAPUSS. Atmos. Chem. Phys. 13, 10353–10371. http://dx.doi.org/10.5194/acp-13-10353-2013.

Alier, M., Osto, M.D., Lin, Y.-H., Surratt, J.D., Tauler, R., Grimalt, J.O., van Drooge, B.L., 2014. On the origin of water-soluble organic tracer compounds in fine aerosols in two cities: the case of Los Angeles and Barcelona. Environ. Sci. Pollut. Res. Int. 21, 11649–11660. http://dx.doi.org/10.1007/s11356-013-2460-9.

Amato, F., Rivas, I., Viana, M., Moreno, T., Bouso, L., Reche, C., Àlvarez-Pedrerol, M., Alastuey, A., Sunyer, J., Querol, X., 2014. Sources of indoor and outdoor $PM_{2.5}$ concentrations in primary schools. Sci. Total Environ. 490, 757–765. http://dx.doi.org/10.1016/j.scitotenv.2014.05.051.

Anderson, P., 2002. Assessment and development of executive function (EF) during childhood. Child Neuropsychol. J. Norm. Abnorm. Dev. Child. Adolesc. 8, 71–82. http://dx.doi.org/10.1076/chin.8.2.71.8724.

Barkley, R.A., 2002. Major life activity and health outcomes associated with attention-deficit/hyperactivity disorder. J. Clin. Psychiatry 63 (Suppl. 12), 10–15.

Block, M.L., Elder, A., Auten, R.L., Bilbo, S.D., Chen, H., Chen, J.-C., Cory-Slechta, D.A., Costa, D., Diaz-Sanchez, D., Dorman, D.C., Gold, D.R., Gray, K., Jeng, H.A., Kaufman, J.D., Kleinman, M.T., Kirshner, A., Lawler, C., Miller, D.S., Nadadur, S.S., Ritz, B., Semmens, E.O., Tonelli, L.H., Veronesi, B., Wright, R.O., Wright, R.J., 2012. The outdoor air pollution and brain health workshop. Neurotoxicology 33, 972–984. http://dx.doi.org/10.1016/j.neuro.2012.08.014.

Cardozo-Pelaez, F., Song, S., Parthasarathy, A., Hazzi, C., Naidu, K., Sanchez-Ramos, J., 1999. Oxidative DNA damage in the aging mouse brain. Mov. Disord. Off. J. Mov. Disord. Soc. 14, 972–980.

Casey, B.J., Castellanos, F.X., Giedd, J.N., Marsh, W.L., Hamburger, S.D., Schubert, A.B., Vauss, Y.C., Vaituzis, A.C., Dickstein, D.P., Sarfatti, S.E., Rapoport, J.L., 1997. Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. J. Am. Acad. Child Adolesc. Psychiatry 36, 374–383. http://dx.doi.org/10.1097/00004583-199703000-00016.

Castellanos, F.X., Lee, P.P., Sharp, W., Jeffries, N.O., Greenstein, D.K., Clasen, L.S., Blumenthal, J.D., James, R.S., Ebens, C.L., Walter, J.M., Zijdenbos, A., Evans, A.C., Giedd, J.N., Rapoport, J.L., 2002. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. JAMA 288, 1740–1748.

Chepelev, N.L., Moffat, I.D., Bowers, W.J., Yauk, C.L., 2015. Neurotoxicity may be an overlooked consequence of benzo[a]pyrene exposure that is relevant to human health risk assessment. Mutat. Res. Rev. Mutat. Res. 764, 64–89. http://dx.doi.org/10.1016/j.mrrev.2015.03.001.

Das, S.K., Patel, B., Patri, M., 2016. Neurotoxic effect of benzo[a]pyrene and its possible association with 6-hydroxydopamine induced neurobehavioral changes during early adolescence period in rats. J. Toxicol. 2016, 8606410. http://dx.doi.org/10.1155/2016/8606410.

Davies, W., 2014. Sex differences in attention deficit hyperactivity disorder: candidate genetic and endocrine mechanisms. Front. Neuroendocrinol. 35, 331–346. http://dx.doi.org/10.1016/j.yfrne.2014.03.003.

Dorado-Martínez, C., Paredes-Carbajal, C., Mascher, D., Borgonio-Pérez, G., Rivas-Arancibia, S., 2001. Effects of different ozone doses on memory, motor activity and lipid peroxidation levels, in rats. Int. J. Neurosci. 108, 149–161.

van Drooge, B.L., Crusack, M., Reche, C., Mohr, C., Alastuey, A., Querol, X., Prevot, A., Day, D.A., Jimenez, J.L., Grimalt, J.O., 2012. Molecular marker characterization of the organic composition of submicron aerosols from Mediterranean urban and rural environments under contrasting meteorological conditions. Atmos. Environ. 61, 482–489. http://dx.doi.org/10.1016/j.atmosenv.2012.07.039.

Dutta, K., Ghosh, D., Nazmi, A., Kumawat, K.L., Basu, A., 2010. A common carcinogen benzo[a]pyrene causes neuronal death in mouse via microglial activation. PLoS One 5, e9984. http://dx.doi.org/10.1371/journal.pone.0009984.

Ellison-Wright, I., Ellison-Wright, Z., Bullmore, E., 2008. Structural brain change in attention deficit hyperactivity disorder identified by meta-analysis. BMC Psychiatry 8, 51. http://dx.doi.org/10.1186/1471-244X-8-51.

Faraone, S.V., Biederman, J., Mick, E., 2006. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol. Med. 36, 159–165. http://dx.doi.org/10.1017/S003329170500471X.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.

Fischl, B., Salat, D.H., van der Kouwe, A.J.W., Makris, N., Ségonne, F., Quinn, B.T., Dale, A.M., 2004. Sequence-independent segmentation of magnetic resonance images. NeuroImage 23 (Suppl. 1), S69–S84. http://dx.doi.org/10.1016/j.neuroimage.2004.07.016.

Fontal, M., van Drooge, B.L., López, J.F., Fernández, P., Grimalt, J.O., 2015. Broad spectrum analysis of polar and apolar organic compounds in submicron atmospheric particles. J. Chromatogr. A 1404, 28–38. http://dx.doi.org/10.1016/j.chroma.2015.05.042.

Frodl, T., Skokauskas, N., 2012. Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. Acta Psychiatr. Scand. 125, 114–126. http://dx.doi.org/10.1111/j.1600-0447.2011.01786.x.

Giedd, J.N., Rapoport, J.L., 2010. Structural MRI of pediatric brain development: what have we learned and where are we going? Neuron 67, 728–734. http://dx.doi.org/10.1016/j.neuron.2010.08.040.

Godec, R., Jakovljević, I., Šega, K., Čačković, M., Bešlić, I., Davila, S., Pehnec, G., 2016. Carbon species in PM_{10} particle fraction at different monitoring sites. Environ. Pollut. 216, 700–710. http://dx.doi.org/10.1016/j.envpol.2016.06.034.

Grova, N., Valley, A., Turner, J.D., Morel, A., Muller, C.P., Schroeder, H., 2007. Modulation of behavior and NMDA-R1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. Neurotoxicology 28, 630–636. http://dx.doi.org/10.1016/j.neuro.2007.01.010.

Křůmal, K., Mikuška, P., Večeřa, Z., 2013. Polycyclic aromatic hydrocarbons and hopanes in PM1 aerosols in urban areas. Atmos. Environ. 67, 27–37. http://dx.doi.org/10.1016/j.atmosenv.2012.10.033.

LeBel, C.P., Bondy, S.C., 1991. Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol. Teratol. 13, 341–346.

Li, F., Tsien, J.Z., 2009. Memory and the NMDA receptors. N. Engl. J. Med. 361, 302–303. http://dx.doi.org/10.1056/NEJMcibr0902052.

Lohmann, R., Lammel, G., 2004. Adsorptive and absorptive contributions to the gas-particle partitioning of polycyclic aromatic hydrocarbons: state of knowledge and recommended parametrization for modeling. Environ. Sci. Technol. 38, 3793–3803.

Maciel, E.S., Biasibetti, R., Costa, A.P., Lunardi, P., Schunck, R.V.A., Becker, G.C., Arbo, M.D., Dallegrave, E., Gonçalves, C.A., Saldiva, P.H.N., Garcia, S.C., Leal, R.B., Leal, M.B., 2014. Subchronic oral administration of Benzo[a]pyrene impairs motor and cognitive behavior and modulates S100B levels and MAPKs in rats. Neurochem. Res. 39, 731–740. http://dx.doi.org/10.1007/s11064-014-1261-y.

Manoli, E., Kouras, A., Karagkiozidou, O., Argyropoulos, G., Voutsa, D., Samara, C., 2016. Polycyclic aromatic hydrocarbons (PAHs) at traffic and urban background sites of northern Greece: source apportionment of ambient PAH levels and PAH-induced lung cancer risk. Environ. Sci. Pollut. Res. Int. 23, 3556–3568. http://dx.doi.org/10.1007/s11356-015-5573-5.

Masiol, M., Hofer, A., Squizzato, S., Piazza, R., Rampazzo, G., Pavoni, B., 2012. Carcinogenic and mutagenic risk associated to airborne particle-phase polycyclic aromatic hydrocarbons: a source apportionment. Atmos. Environ. 60, 375–382. http://dx.doi.org/10.1016/j.atmosenv.2012.06.073.

Masiol, M., Formento, G., Pasqualetto, A., Pavoni, B., 2013. Seasonal trends and spatial variations of PM10-bounded polycyclic aromatic hydrocarbons in Veneto Region, Northeast Italy. Atmos. Environ. 79, 811–821. http://dx.doi.org/10.1016/j.atmosenv.2013.07.025.

Maypole-Keenan, C.M., Symanski, E., Stock, T.H., Waller, D.K., 2016. Association between ambient levels of polycyclic aromatic hydrocarbons and small for gestational age Hispanic infants born along the United States-Mexico border. J. Immigr. Minor. Health 18, 143–149. http://dx.doi.org/10.1007/s10903-014-9994-x.

Meiser, J., Weindl, D., Hiller, K., 2013. Complexity of dopamine metabolism. Cell Commun. Signal 11, 34. http://dx.doi.org/10.1186/1478-811X-11-34.

Ministry of Public Works, 2012. Atlas of Urban Vulnerability in Spain: Methodology and Contents.

Nakao, T., Radua, J., Rubia, K., Mataix-Cols, D., 2011. Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. Am. J. Psychiatry 168, 1154–1163. http://dx.doi.org/10.1176/appi.ajp.2011.11020281.

Nigg, J.T., 2009. What Causes ADHD?: Understanding What Goes Wrong and Why, first ed. The Guilford Press, New York.

Ning, Z., Geller, M.D., Moore, K.F., Sheesley, R., Schauer, J.J., Sioutas, C., 2007. Daily variation in chemical characteristics of urban ultrafine aerosols and inference of their sources. Environ. Sci. Technol. 41, 6000–6006.

Perera, F.P., Wang, S., Vishnevetsky, J., Zhang, B., Cole, K.J., Tang, D., Rauh, V., Phillips, D.H., 2011. Polycyclic aromatic hydrocarbons-aromatic DNA adducts in cord blood and behavior scores in New York City children. Environ. Health Perspect. 119, 1176–1181. http://dx.doi.org/10.1289/ehp.1002705.

Perera, F.P., Tang, D., Wang, S., Vishnevetsky, J., Zhang, B., Diaz, D., Camann, D., Rauh, V., 2012. Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6–7 years. Environ. Health Perspect. 120, 921–926. http://dx.doi.org/10.1289/ehp.1104315.

Perera, F.P., Chang, H., Tang, D., Roen, E.L., Herbstman, J., Margolis, A., Huang, T.-J., Miller, R.L., Wang, S., Rauh, V., 2014. Early-life exposure to polycyclic aromatic hydrocarbons and ADHD behavior problems. PLoS One 9, e111670. http://dx.doi.org/10.1371/journal.pone.0111670.

Peterson, B.S., Rauh, V.A., Bansal, R., Hao, X., Toth, Z., Nati, G., Walsh, K., Miller, R.L., Arias, F., Semanek, D., Perera, F., 2015. Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on the development of brain white matter, cognition, and behavior in later childhood. JAMA Psychiatry 72, 531–540. http://dx.doi.org/10.1001/jamapsychiatry.2015.57.

Pietrogrande, M.C., Abbaszade, G., Schnelle-Kreis, J., Bacco, D., Mercuriali, M., Zimmermann, R., 2011. Seasonal variation and source estimation of organic compounds in urban aerosol of Augsburg, Germany. Environ. Pollut. 159, 1861–1868. http://dx.doi.org/10.1016/j.envpol.2011.03.023.

Polanczyk, G., de Lima, M.S., Horta, B.L., Biederman, J., Rohde, L.A., 2007. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am. J. Psychiatry 164, 942–948. http://dx.doi.org/10.1176/ajp.2007.164.6.942.

Pujol, J., Fenoll, R., Macià, D., Martínez-Vilavella, G., Alvarez-Pedrerol, M., Rivas, I., Forns, J., Deus, J., Blanco-Hinojo, L., Querol, X., Sunyer, J., 2016a. Airborne copper exposure in school environments associated with poorer motor performance and altered basal ganglia. Brain Behav. 6, e00467. http://dx.doi.org/10.1002/brb3.467.

Pujol, J., Martínez-Vilavella, G., Macià, D., Fenoll, R., Alvarez-Pedrerol, M., Rivas, I., Forns, J., Blanco-Hinojo, L., Capellades, J., Querol, X., Deus, J., Sunyer, J., 2016b. Traffic pollution exposure is associated with altered brain connectivity in school children. NeuroImage 129, 175–184. http://dx.doi.org/10.1016/j.neuroimage.2016.01.036.

Reche, C., Moreno, T., Amato, F., Viana, M., van Drooge, B.L., Chuang, H.-C., Bérubé, K., Jones, T., Alastuey, A., Querol, X., 2012. A multidisciplinary approach to characterise exposure risk and toxicological effects of PM_{10} and $PM_{2.5}$ samples in urban environments. Ecotoxicol. Environ. Saf. 78, 327–335. http://dx.doi.org/10.1016/j.ecoenv.2011.11.043.

Ring, H.A., Serra-Mestres, J., 2002. Neuropsychiatry of the basal ganglia. J. Neurol. Neurosurg. Psychiatry 72, 12–21.

Rivas, I., Viana, M., Moreno, T., Pandolfi, M., Amato, F., Reche, C., Bouso, L., Àlvarez-Pedrerol, M., Alastuey, A., Sunyer, J., Querol, X., 2014. Child exposure to indoor and outdoor air pollutants in schools in Barcelona, Spain. Environ. Int. 69, 200–212. http://dx.doi.org/10.1016/j.envint.2014.04.009.

Rivas-Arancibia, S., Dorado-Martínez, C., Borgonio-Pérez, G., Hiriart-Urdanivia, M., Verdugo-Diaz, L., Durán-Vázquez, A., Colin-Baranque, L., Avila-Costa, M.R., 2000. Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environ. Res. 82, 7–17.

Rivas-Arancibia, S., Dorado-Martínez, C., Colin-Barenque, L., Kendrick, K.M., de la Riva, C., Guevara-Guzmán, R., 2003. Effect of acute ozone exposure on locomotor behavior and striatal function. Pharmacol. Biochem. Behav. 74, 891–900.

Rueda, M.R., Fan, J., McCandliss, B.D., Halparin, J.D., Gruber, D.B., Lercari, L.P., Posner, M.I., 2004. Development of attentional networks in childhood. Neuropsychologia 42, 1029–1040. http://dx.doi.org/10.1016/j.neuropsychologia.2003.12.012.

Saunders, C.R., Das, S.K., Ramesh, A., Shockley, D.C., Mukherjee, S., 2006. Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. J. Appl. Toxicol. 26, 427–438. http://dx.doi.org/10.1002/jat.1157.

Ségonne, F., Dale, A.M., Busa, E., Glessner, M., Salat, D., Hahn, H.K., Fischl, B., 2004. A hybrid approach to the skull stripping problem in MRI. NeuroImage 22, 1060–1075. http://dx.doi.org/10.1016/j.neuroimage.2004.03.032.

Sunyer, J., Esnaola, M., Alvarez-Pedrerol, M., Forns, J., Rivas, I., López-Vicente, M., Suades-González, E., Foraster, M., Garcia-Esteban, R., Basagaña, X., Viana, M., Cirach, M., Moreno, T., Alastuey, A., Sebastian-Galles, N., Nieuwenhuijsen, M., Querol, X., 2015. Association between traffic-related air pollution in schools and cognitive development in primary school children: a prospective cohort study. PLoS Med. 12, e1001792. http://dx.doi.org/10.1371/journal.pmed.1001792.

Valera, E.M., Faraone, S.V., Murray, K.E., Seidman, L.J., 2007. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biol. Psychiatry 61, 1361–1369. http://dx.doi.org/10.1016/j.biopsych.2006.06.011.

Vanhoutte, P., Bading, H., 2003. Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. Curr. Opin. Neurobiol. 13, 366–371.

Wang, M., Beelen, R., Basagana, X., Becker, T., Cesaroni, G., de Hoogh, K., Dedele, A., Declercq, C., Dimakopoulou, K., Eeftens, M., Forastiere, F., Galassi, C., Gražulevičienė, R., Hoffmann, B., Heinrich, J., Iakovides, M., Künzli, N., Korek, M., Lindley, S., Mölter, A., Mosler, G., Madsen, C., Nieuwenhuijsen, M., Phuleria, H., Pedeli, X., Raaschou-Nielsen, O., Ranzi, A., Stephanou, E., Sugiri, D., Stempfelet, M., Tsai, M.-Y., Lanki, T., Udvardy, O., Varró, M.J., Wolf, K., Weinmayr, G., Yli-Tuomi, T., Hoek, G., Brunekreef, B., 2013. Evaluation of land use regression models for NO_2 and particulate matter in 20 European study areas: the ESCAPE project. Environ. Sci. Technol. 47, 4357–4364. http://dx.doi.org/10.1021/es305129t.

Willcutt, E.G., 2012. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. Neurother. J. Am. Soc. Exp. Neurother. 9, 490–499. http://dx.doi.org/10.1007/s13311-012-0135-8.

World Health Organization, 2001. Polycyclic aromatic hydrocarbons. In: Air Quality Guidelines for Europe, European Series, No. 91, second ed. .

Yan, T., Chengzhi, C., Haiyan, Y., Baijie, T., 2010. Distribution of benzo[a]pyrene in discrete regions of rat brain tissue using light microscopic autoradiography and gamma counting. Toxicol. Environ. Chem. 92, 1309–1317. http://dx.doi.org/10.1080/02772240903503898.