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# Air pollution, cognitive deficits and brain abnormalities: A pilot study with children and dogs

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ABSTRACT

Exposure to air pollution is associated with neuroinflammation in healthy children and dogs in Mexico City. Comparative studies were carried out in healthy children and young dogs similarly exposed to ambient pollution in Mexico City. Children from Mexico City (n: 55) and a low polluted city (n:18) underwent psychometric testing and brain magnetic resonance imaging MRI. Seven healthy young dogs with similar exposure to Mexico City air pollution had brain MRI, measurement of mRNA abundance of two inflammatory genes cyclooxygenase-2, and interleukin 1 β in target brain areas, and histopathological evaluation of brain tissue. Children with no known risk factors for neurological or cognitive disorders residing in a polluted urban environment exhibited significant deficits in a combination of fluid and crystallized cognition tasks. Fifty-six percent of Mexico City children tested showed prefrontal white matter hyperintense lesions and similar lesions were observed in dogs (57%). Exposed dogs had frontal lesions with vascular subcortical pathology associated with neuroinflammation, enlarged Virchow–Robin spaces, gliosis, and ultrafine particulate matter deposition. Based on the MRI findings, the prefrontal cortex was a target anatomical region in Mexico City children and its damage could have contributed to their cognitive dysfunction. The present work presents a groundbreaking, interdisciplinary methodology for addressing relationships between environmental pollution, structural brain alterations by MRI, and cognitive deficits/delays in healthy children.



## 1. Introduction

There is mounting evidence that exposure to air pollution can cause stroke-related sickness and death (Hong et al., 2002; Maheswaran et al., 2006), as well as brain damage and neurodegeneration (Calderón-Garcidueñas et al., 2002, 2003b, 2004, 2007a; Dorado-Martinez, Paredes-Carbajal, Mascher, Borgonio-Perez, & Rivas-Arancibia, 2001; Peters et al., 2006). Children are a population at risk for these latter effects since childhood and

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adolescence are crucial periods of brain development associated with dynamic behavioral, cognitive and emotional changes. There is an important knowledge gap regarding the impact that chronic exposure to air pollution has on cognitive performance, neuroinflammation, and neurodegeneration in healthy children with no other risk factors for neurological diseases.

Urban dwelling children and their companion canine pets are similarly exposed to ambient environmental factors that may have pathophysiological consequences. We have previously demonstrated that health effects from air pollution in dogs mimic similar effects in humans (Calderón-Garcidueñas et al., 2002, 2003a, 2003b, 2007a, 2007b) underscoring the utility of comparative studies. Studies from our laboratory have shown that long-term exposure to severe air pollution causes neuroinflammation, and an accumulation of the 42 amino acid-isoform of beta amyloid in adult residents of megacities (average age 54.7 ± 4.8 year) (Calderón-Garcidueñas et al., 2004), and that healthy dogs younger than 1 year exhibit neuroinflammation along with disruption of the blood–brain-barrier (BBB), and accumulation of beta amyloid 42 (Calderón-Garcidueñas et al., 2002). More recently, we have shown in highly exposed children and young adults average age 25.1 ± 1.5 years, a significant upregulation of cyclooxygenase 2 (COX2), interleukin 1β (IL1β) and CD14 in olfactory bulb, frontal cortex, substantia nigrae and vagus nerves, disruption of the BBB, endothelial activation, oxidative stress, and inflammatory cell trafficking (Calderón-Garcidueñas et al., 2008).

Children living in Mexico City (MC) exhibit evidence of chronic inflammation of the upper and lower respiratory tracts, alterations in circulating inflammatory mediators, and breakdown of the nasal respiratory epithelial barrier (Calderón-Garcidueñas et al., 2001, 2003a, 2007a). These children also have elevated levels of plasma endothelin-1 (Calderón-Garcidueñas et al., 2003a, 2007b), a potent vasoconstrictor peptide involved in the homeostatic regulation of the cerebral microcirculation and upregulated after exposure to air pollutants including particulate matter (PM; Chauhan, Breznan, Thomson, Karthikeyan, & Vincent, 2005; McCarron et al., 2006; Thomson, Kumarathasan, & Vincent, 2006). Dogs exposed to the polluted environment in Mexico City exhibit respiratory tract chronic inflammation, early expression of neuronal nuclear NF κB and endothelial/glial inducible nitric oxide synthase, disruption of the nasal and olfactory barriers and the blood–brain-barrier, accumulation of Aβ42 in neurons, and increased olfactory bulb and hippocampal apurinic/apyrimidinic sites as indicators of oxidative DNA damage (Calderón-Garcidueñas et al., 2002, 2003b, 2004). T2 weighted magnetic resonance imaging (MRI) sequences are especially sensitive to structural alterations of CNS tissue (Boretius et al., 2006; T'Hart et al., 1998). White matter inflammatory lesions are visualized as high signal intensity regions by MRI (Boretius et al., 2006; T'Hart et al., 1998).

The primary purpose of the present work was to evaluate the neuropsychological functioning and the structural brain alterations as detected by MRI of clinically healthy children with a life time residency in two significantly different urban environments, one with high concentrations of air pollutants (Mexico City), and the other (Polotitlán, Mexico State) with levels within the current USA National Ambient Air Quality Standards (NAAQS). We selected a standardized neuropsychological instrument to analyze the degree of impairment of cognitive processes such as attention, working memory and executive functions: the Wechsler Intelligence Scale for Children–Revised (WISC-R 1974). We compared performance on the WISC-R for the children living in the two cites, controlling for age differences either statistically or by age-normative scaling methods made available by the test publisher (El Manual Moderno S. A. Mexico, 1981). Given that neuroinflammation is seen in both Mexico City healthy children and dogs (Calderón-Garcidueñas et al., 2002, 2003b, 2004, 2007a, 2008), we investigated the gene expression profiling and the histopathology of the MRI-detectable lesions in healthy young dogs resident in the same area as the MC children and thus exposed to the same levels of air pollution. Two key inflammatory genes: cyclooxygenase-2 (COX2), and interleukin-1beta (IL-1β), and the LPS receptor CD14, were measured by real time polymerase chain reaction. The gene selection was based on the increasing evidence that neuroinflammatory processes contribute to the cascade of events that lead to neurodegeneration (Griffin & Mrak, 2002; Hoozemans, Veerhuis, Rozemuller, & Eikelenboom, 2006; Minghetti, 2005; Qin et al., 2007; Rothwell & Luheshi, 2000; Whitton, 2007). Given that Apolipoprotein E (APOE) plays a crucial role in the maintenance and repair of neurons, and since APOE 4 is associated with a wide variety of neuropathological/neurological processes and it is a major known genetic risk factor for Alzheimer's disease (Gozal, Capdevila, Kheirandish-Gozai, & Crabtree, 2007; Mahley, Weisgraber, & Huang, 2006; Packard et al., 2007) the cohorts were genotyped for the Apolipoprotein E alleles to determine if subjects had a known risk factor for Alzheimer's disease (ie., APOE ε 4 allele carriers). We also determined the allelic frequencies of the Asp299Gly TLR4 polymorphism to determine if subjects were capable of responding to lipopolysaccharides (one of the major organic components in Mexico City particulate matter).

In the present study we have documented MRI prefrontal lesions and cognitive deficits in children exposed to ambient air pollution in Mexico City. Comparative frontal MRI lesions were present in similarly exposed laboratory housed young Mexico City dogs and subsequent histological and molecular analysis of the dog brains identified neuroinflammatory changes in the frontal cortex and white matter tracts. This study identifies a possible link between cognitive dysfunction/structural alterations to children's brains and chronic exposure to significant concentrations of air pollutants, including particulate matter. This study was done in clinically healthy cohorts with no known risk factors for cognitive dysfunction.

## 2. Procedure

### 2.1. Study areas

We selected a large polluted city and a control city. Mexico City was the selected megacity with high pollution and Polotitlán was the selected control city with lower pollution levels. Mexico City represents an extreme of urban growth and environmental pollution (Bravo & Torres, 2002) covering an area of 2000 $km^2$ surrounded by a series of volcanic and discontinuous mountain ranges that restrict the natural ventilation of the topographic basin. The basin has more than 30,000 industrial facilities and 4 million vehicles with an estimated annual emission of 2.6 million tons of particulate and gaseous air pollutants (Bravo & Torres, 2002). The critical air pollutants are particulate matter (PM) and ozone ($O_3$; Bravo & Torres, 2002). The climatic conditions in Mexico City are stable throughout the seasons, thus air pollutant concentrations are relatively consistent.

Residents in Mexico City have been chronically exposed to significant concentrations of $O_3$, PM, and lipopolyssacharides-associated with PM (LPS-PM) for the last two decades (Calderón-Garcidueñas et al., 2007b). The higher 8-h $O_3$ and $PM_{2.5}$ concentrations coincide with the times children are outdoors during the school recess and physical education periods and when they play outdoors at home (Villarreal-Calderón et al., 2002). Children in Southwest Mexico City are exposed to a yearly average concentration of $PM_{2.5}$ of 25 μg/$m^3$, a value well above the annual standard of 15 μg/$m^3$. Lipopolyssacharides detected in $PM_{10}$ samples show a range of 15.3–20.6 ng/mg of $PM_{10}$, and South Mexico City PM samples show the highest endotoxin concentrations at

59 EU/mg $PM_{10}$ (Bonner et al., 1998; Osornio-Vargas et al., 2003). Significant sources of environmental endotoxins in Mexico City include open field waste areas, wastewater treatment plants, and daily outdoor deposits of more than 500 metric tons of animal and human fecal material (Estrada-García, Cerna, Thompson, & Lopez-Saucedo, 2002).

In contrast, in the control city Polotitlán all criteria pollutants ($O_3$, $PM_{10}$, $SO_2$, $NO_2$, CO and Pb) levels are below the current US standards, due to the fortunate combination of relatively few contributing emission sources from industry and cars and good ventilation conditions due to regional winds. The selection of Polotitlán as the control city was based on the low air pollution levels plus four additional factors. Polotitlán is at an altitude above sea level similar to Mexico City. The choice of Polotitlán as a control city also provided access to middle class children, and its relative proximity to Mexico City (114 km) facilitated the follow-up of these control cohorts. Previous clinical studies with the Polotitlán control cohort indicated no air pollution-related health issues (Calderón-Garcidueñas et al., 2006; Calderón-Garcidueñas et al., 2007b).

### 2.2. Study population

The Institutional Review Boards for Human Studies at the Instituto Nacional de Pediatria in Mexico City approved the study protocol. We recruited 55 children from Mexico City and 18 children from Polotitlán. Children were selected from middle class families, determined by parental occupation and income criteria. Parents and children gave written consent and oral assent to participation. Children underwent a physical by a pediatrician, drawing of blood samples, brain MRI scanning, and psychometric testing.

### 2.3. Psychometric testing

The Wechsler Intelligence Scale for Children–Revised WISC-R; (Wechsler, 1974) consists of 12 subtests (Information, Similarities, Arithmetic, Vocabulary, Comprehension, Digit Span, Picture Completion, Picture Arrangement, Block Design, Object Assembly, Coding, and Mazes). Composite IQ scores (Full Scale, Verbal, and Performance) are derived from performance on different combinations of the subtests, reflecting different content/process domains. Standard administration procedures were followed.

### 2.4. Genotyping for the Apolipoprotein E (APOE) alleles, and the Asp299Gly Toll-like receptor 4 (TLR4) polymorphism

DNA was isolated from peripheral blood as described and genotyped for the HhaI restriction site polymorphism in the APOE gene (Hixson & Vernier, 1990). Since missense mutations such as Asp299Gly are associated with a blunted response to inhaled lipopolyssacharides, we determined the allelic frequencies of the Asp299Gly TLR4 polymorphism (Garantziotis, Hollingsworth, Zaas, & Schwartz, 2008) using an allelic discrimination assay protocol according to Applied Biosystems.

### 2.5. Children's magnetic resonance imaging (MRI)

Thirty-six children had brain MRI, 23 from Mexico City (12 females; Mean age = 10.73 years, *SD* = 2.734) and 13 from Polotitlán (7 females; Mean age = 10.69 years, *SD* = 2.097). The 3D MRI for all subjects was acquired on a 1.5 Tesla 5T Signa Excite HD MR (General Electric, Milwaukee WI, USA) with an 8 Channel Brain Array. White matter lesions (WML) were defined as hyperintense focal images observed in two different sequences: T2 and T2 weighted with fluid-attenuated inversion recovery (FLAIR). White matter lesions were scored by lobe location, and number, and we obtained a quantitative measure of load by multiplying each lesion by a size-dependent constant: 0.0042 ml for 1 (<3 mm), 0.114 ml for 2 (4–10 mm), and 0.90 ml for 3(>10 mm) according to the method used by Kruit et al. (2004). The studies were coded and a pediatric radiologist and two neuroradiologists reviewed the studies independently, having access only to age and gender information in each case. The final index of number and extent of white matter lesions for each participant (Kruit score) was the result of the evaluation of the three readings.

### 2.6. Dogs' studies

The Institutional Animal Care and Use Committee at the INP approved the study protocol. We selected seven healthy 12-, 15-, and 19-month-old mongrel dogs, bred and reared at the INP animal facility located in Southwest Mexico City. To examine the mRNA in the genes of interest, COX2, IL1β and GFAP, we used fresh-frozen samples from five age-matched control dogs from our previous studies (Calderón-Garcidueñas et al., 2003b) for comparison to the samples from the Mexico City dogs. The pathology of Mexico City dogs was compared with the materials from 14 control dogs from Tlaxcala, a city located 114 km east of Mexico City at 2254 m above sea level, with low levels of air pollutants (Calderón-Garcidueñas et al., 2003b). Mexico City dogs were whelped and housed in an outdoor-indoor kennel; husbandry was in compliance with the American Association of Laboratory Animal Certification Standards. Dogs were under daily veterinary observation during their entire life, and at no time was there any evidence of overt respiratory, cardiovascular, or neurological diseases. Dogs had all applicable vaccines, and were treated with antihelmintics regularly. Mexico City dogs had a brain MRI 2–4 days prior to their euthanasia, conducted in accordance with established American Veterinary Medical Association Guidelines (Panel on Euthanasia, 2001).

We used the same scanning protocol for the dogs as for the children but only acquired axial and coronal images. The total scanning time was approximately 35 min, and dogs were anesthetized with Zoletil-Virbac 10 mg/kg IM. The resulting images were coded, a neuroradiologist reviewed the images, and the final Kruit score for each dog was the result of the evaluation of his reading.

### 2.7. Dogs' necropsy and tissue preparation

Pathology procedures were as in our earlier work (Calderón-Garcidueñas et al.,2002, 2003b, 2004). Necropsies were performed within 2–4 days after the MRI study to allow for the review of the studies conjointly by the neuroradiologists and the neuropathologist. Immunohistochemistry (IHC) antibodies included: cyclooxygenase-2 COX2 (Santa Cruz Biotechnology 1:100), glial fibrillary acidic protein GFAP (Abcam, Inc 1:500), and Zonula occludents-1 ZO-1-FITC (In Vitrogen, 1:200). A veterinarian pathologist and a human neuropathologist read sections with no access to the codes regarding the dogs' identification data. Electron microscopy was performed in selected frontal gray and white matter samples, as well as lung and heart samples, examined with a Carl Zeiss EM109T (Germany) or a JEM-1011 (Japan) microscope. For the confocal microscopy vessel diameters and tight junctions (TJ) abnormalities were assessed by two independent observers and vessels were scored as normal or abnormal on the basis of the ZO-1 staining of their TJs. Fluorescence was examined using a BioRad Radiance 2000 laser scanning confocal on an inverted Nikon TE 300 microscope with an Argon laser line, 488/514 emission.

### 2.8. Estimation of mRNA abundance by real-time RT-PCR

RT-PCR protocols were performed as described previously (Calderón-Garcidueñas et al. 2004). Relative abundances of mRNAs encoding COX2, IL1β and CD14 were estimated by quantitative

fluorogenic 5′ nuclease (TaqMan) assay of the first strand cDNAs as described in Calderón-Garcidueñas et al. (2004).

### 2.9. Statistics

Statistics were performed using Stata (StataCorp, 2005). We used several strategies to analyze the WISC-R data. Hierarchical regressions on the independent variables Age, Residency, and Gender were done first to estimate the total variance accounted for in WISC-R raw scores and to locate sub-tests showing possible effects of Residency after controlling for Age and Gender. For the seven sub-tests that did suggest a unique effect of Residency, we estimated the variance, uniquely accounted for by Residency. WISC-R raw scores were converted to age-normed "performance age" scores (the corresponding age for the raw score obtained by the subject in each scale, according to the procedure described in appendix D and using Table 21 of The Wechsler Intelligence Scale for Children–Revised. El Manual Moderno S. A. Mexico, 1981). Briefly raw scores obtained by each subject in each scale were used to find the correspondent scalar age in Table 21. Data from each child were used to compare actual chronological age vs. scalar age within each cohort. We expected these measures to show the MC children performing substantially "behind" their control cohorts (Table 2). To test the dog data, we applied a parametric Student "*t*" test or a nonparametric Wilcoxon or Mann–Whitney test procedure to compare two independent samples for the mRNA dog results in the target genes, and univariate descriptive measurements were summarized as mean values ± SEM (Table 5). Significance was assumed at $p < .05$.

## 3. Results

### 3.1. Demographic data

A total of 73 children participated, all from middle class families. Years of formal education were not different ($p > .05$) between mothers of the children from the two cities, Mean = 10.53 years, *SD* = 3.70 for the Mexico City mothers, and Mean = 9.31 years, *SD* = 3.66 for the Polotitlán mothers, respectively. Children slept in bedrooms with no carpeting/draperies and had open windows for ventilation. All households had kitchens separated from the living and sleeping areas and used gas for cooking. There were 18 children from Polotitlán (10 females; Mean age = 10.5 years, *SD* = 2.0) and 55 children from Mexico City (28 females, Mean age = 9.2 years, *SD* = 2.3), $p = .036$ Children had physical exams and their weight and height were within normal limits for their age and gender. No overweight or obese children were included.

### 3.2. Psychometric data

Age has a large effect on psychometric intelligence scores for children. Therefore, the following analyses were used to control for mean age differences between our two cohorts. Regressing WISC-R raw scores on Age, Gender, and Residency accounted for significant variance and suggested a unique Residency effect in seven of the twelve sub-tests (Object Assembly, Picture Arrangement, Digit Span, Information, Arithmetic, Mazes, and Vocabulary; the latter two are trends; Table 1). Variance partitioning (Pedhazur, 1997) indicated that Residency accounted for between 2% and 6% of the variance in these seven subtests uniquely (Table 1, column 5), after controlling for variance shared with Age and Gender. With respect to the composite IQ scores and the other five subtests for which unique effects of Residency did not approach significance, mean differences were at least in the expected direction, with control children scoring on average one to two points higher than MC children in each case.

We used WISC-R raw scores converted to age-normed "performance age" scores as a method for dealing with the confounding of age differences and city of residence. Raw scores obtained by each subject in each scale were used to find the correspondent scalar age. Data from each child was used to compare actual chronological age vs. scalar age within each cohort.

The scalar age served to locate the actual children "age of performance" and could be taken as an indicator of whether a child's cognitive development is "on track, behind, or ahead" of his or hers own chronological age. Comparing performance age to chronological age within residency groups showed significant differences in the Mexico City group for a number of WISC-R variables but none in the Polotitlán group (Table 2). These results suggest that Mexico City children, but not Polotitlán children, performed significantly "behind" their normative level of cognitive development.

### 3.3. Children's MRI data

The results of the brain MRI in 36 children are shown in Table 3. These children included 13 children from Polotitlán (7 females; Mean age = 10.69 years, *SD* = 2.097), and 23 children from Mexico City (12 females; Mean age = 10.73 years, *SD* = 2.734). Age differences were not significant between residence groups that were scanned, $p > .05$. The APOE, the TLR4 Asp299Gly polymorphism, the total number of white matter lesions (WML) and the Kruit load scores are also shown (Table 3). Hyperintense areas localized predominantly in subcortical prefrontal white matter characterized the white matter lesions. One Polotitlán child out of 13 tested (7.6%) exhibited a single white matter lesion, with APOE 3/4. In contrast, 13 of the 23 Mexico City children tested (56.5%) had white matter lesions, an outcome significantly different from chance, $X^2_{(1)} = 8.33$, $p = .003$ (test performed included 13 of 23 MC and 1 of 13 Control children with WML). There were no APOE 4 children in the Mexico City cohort, or any TLR 4 Asp299Gly carriers in the Polotitlán group. Two Mexico City children ages 14 and 17 had the TLR 4 Asp299Gly polymorphism associated with a blunted response to inhaled lipopolysaccharides. Fig. 1A–C illustrate an 8-year-old girl with persistent white matter hyperintense T2 and FLAIR lesions through 11 months of follow-up with sequential MRI. Table 4 illustrates the results of the quantification with the Kruit load scores of persistent white matter lesions in three Mexico City children that had 3–4 MRI in an 11-month period.

The results in this pilot study have suggested relationships between city of residency and cognitive deficits/delays in children, and a relationship between city of residency and white matter lesions in children.

### 3.4. Dog's MRI and gene expression data

Four of the seven Mexico City dogs (57%) exhibited white matter lesions (WML) in the frontal cortex (Fig. 2), these lesions were very similar in their characteristics, size and location to the lesions found in the children. Table 5 shows the results of the mRNA for COX2, IL1β and GFAP in samples of frontal cortex white matter in age-matched control versus Mexico City dogs. There was a significant upregulation of the inflammatory genes: COX2 and IL1β mRNA in Mexico City dogs, while GFAP showed no differences. Table 6 shows the mRNA data for the frontal white matter contrasting the values of COX2, IL1β and GFAP mRNA between the areas identified as WML by MRI, and the opposite or adjacent anatomical area with no apparent lesion by MRI. The four dogs with white matter lesions exhibited higher values of IL1β and/or COX2 in the sample corresponding to the MRI lesion. In addition, three of four dogs also showed higher mRNA for the glial acidic fibrillary protein

**Table 1**
Variance predicted by Age, Gender and Residency, for WISC-R sub-tests suggesting an effect of Residency, N = 73

| WISC-R subtest | Variance explained ($R^2$) | $F$ | $p$ | Variance explained uniquely by Residency | $t$ | $p$ |
|---|---|---|---|---|---|---|
| Object Assembly | .433 | 17.591 | <.001 | .033 | 2.028 | .046 |
| Picture Arrangement | .306 | 10.143 | <.001 | .038 | 1.945 | .056 |
| Digit Span | .288 | 9.325 | <.001 | .065 | 2.529 | .014 |
| Information | .657 | 43.981 | <.001 | .029 | 2.396 | .019 |
| Arithmetic | .522 | 25.139 | <.001 | .033 | 2.196 | .031 |
| Mazes | .544 | 27.384 | <.001 | .020 | 1.733 | .088 |
| Vocabulary | .549 | 27.992 | <.001 | .019 | 1.682 | .097 |

**Table 2**
Performance age compared to chronological age within each cohort for WISC-R

| | Mexico City ($N$ = 55) | | | Polotitlán ($N$ = 18) | | |
|---|---|---|---|---|---|---|
| | Mean scale age (SEM) | $t$ | $p$ | Mean scale age (SEM) | $t$ | $p$ |
| Chronological age | 9.20 (0.30) | NA | NA | 10.50 (0.53) | NA | NA |
| Information | 8.14 (0.30) | −2.3507 | 0.0190 | 9.94 (0.72) | −0.5685 | ns |
| Similarities | 8.02 (0.32) | −2.60252 | 0.0094 | 8.97 (0.73) | −1.53675 | ns |
| Arithmetic | 8.81 (0.27) | −0.86025 | ns | 10.51 (0.71) | 0.008872 | ns |
| Vocabulary | 7.86 (0.29) | −2.95074 | 0.0033 | 9.29 (0.76) | −1.21902 | ns |
| Comprehension | 8.07 (0.24) | −2.4985 | 0.0127 | 9.04 (0.45) | −1.46583 | ns |
| Digit Span | 7.11 (0.18) | −4.60666 | <.0001 | 8.53 (0.65) | −1.98346 | ns |
| Picture completion | 8.67 (0.35) | −1.1675 | ns | 9.65 (0.66) | −0.86389 | ns |
| Picture arrangement | 8.88 (0.31) | −0.71647 | ns | 11.29 (0.88) | 0.790057 | ns |
| Block design | 9.27 (0.36) | 0.154673 | ns | 10.53 (0.71) | 0.027299 | ns |
| Object assembly | 8.11 (0.32) | −2.41858 | 0.0158 | 9.93 (0.73) | −0.5819 | ns |
| Coding | 7.48 (0.31) | −3.79778 | 0.0002 | 8.79 (0.77) | −1.72213 | ns |
| Mazes | 9.57 (0.49) | 0.82379 | ns | 11.16 (0.79) | 0.661069 | ns |
| Verbal IQ | 7.79 (0.23) | −3.1259 | 0.0018 | 9.12 (0.60) | −1.5282 | ns |
| Performance IQ | 8.46 (0.29) | −1.3828 | ns | 10.00 (0.59) | −0.9143 | ns |
| Full Scale IQ | 8.14 (0.25) | −2.2544 | 0.0024 | 9.59 (0.57) | −0.9737 | ns |

consisting with the presence of reactive astrocytosis in the white matter lesions. The dog with the higher value of COX2 in frontal white matter had a white matter lesion by MRI.

### 3.5. Dogs' pathological data

Mexico City dogs' nasal respiratory epithelium displayed patchy replacement of the mucociliary epithelium by squamous metaplasia. Lungs displayed bronchiolar epithelial and mild smooth muscle hyperplasia. Chronic mononuclear cell infiltrates, and scattered mast cells along with macrophages filled with particulate matter were surrounding vascular structures and occupying alveolar spaces. Peribronchial and periaortic lymph nodes contained PM in macrophage-like cells. Control dogs findings were unremarkable. Gross examination of the brain revealed no abnormalities either in control or Mexico City dogs. Mild reactive astrocytosis was observed in all olfactory bulb layers from Mexico City dogs. Intense search by light microscopy for particulate matter in the olfactory bulbs yielded negative results. Control dogs showed unremarkable olfactory bulbs. Frontal sections in Mexico City dogs revealed widespread vascular pathology characterized by hypercellular arterioles surrounded by pyknotic nuclei and vacuolated macrophage-like perivascular cells, and enlarged Virchow–Robin spaces (Fig. 3A and B). Cortical and white matter vessels exhibited fibrin thrombi (insert in Fig. 3B), and significant reductions in their lumen similar to those seen in the olfactory bulb. Frontal white matter arterioles exhibited hyperplastic endothelial cells with elongated fronds that protruded into the lumen (Fig. 3C). The endothelial fronds reduced the vessel lumen.

Electron micrographs of frontal white matter vessels showed perivascular macrophage-like cells, with abundant lipid vacuoles in intimate contact with the vessel walls (Fig. 3D). Arteriolar luminal red blood cells exhibited ultrafine particle-like material also seen in the adjacent endothelial basement membranes (Fig. 3E). Astrocytes with a small amount of eosinophilic cytoplasm were seen around blood vessels and neurons in the frontal cortex. Astrocytic GFAP+ processes completely surrounded neuronal bodies (Fig. 4A). Reactive GFAP+ astrocytes were focally prominent in subpial, subcortical (Fig. 4B), perivascular (Fig. 4C), and deep white matter of all Mexico City dogs. This observation was also prominent in areas identified by MRI as hyperintense lesions (Fig. 4D). Significant loosening of the neuropil with leaky blood vessels, extravasation of red blood cells, along with strongly positive GFAP reactivity and enlarged Virchow–Robin spaces were the main histological features of the hyperintense lesions identified by MRI (Fig. 4D). In contrast, the same level opposite white matter exhibited less gliosis and no leaky blood vessels (Fig. 4E). Confocal microscopy for tight junctional abnormalities ZO-1: the examined vessels were less than 100 μm in diameter, and all Mexico City dogs exhibited on average 10.8% (±1.9%) of vessels with discontinuous or punctuate staining in frontal cortex, while only rarely similar abnormal staining pattern was seen in two of the control dogs (not shown).

## 4. Discussion

Based on comparative studies of healthy children and young dogs, the present investigation has identified potential relationships among exposure to Mexico City air pollution, brain structural alterations, and delays in cognitive development. Mexico City clinically healthy children with no known risk factors for neurological or cognitive impairment showed significant cognitive deficits in areas of fluid cognition, memory, and executive functions when compared to socio-economically matched children residing in a low pollution environment. Generally, Mexico City children performed at lower levels than Polotitlán children on tests of psychometric intelligence, even after accounting for age differences between groups.

**Table 3**
White matter lesions in control and Mexico City children MRI in four image acquisition sequences

| Cohorts | Age/gender | ApoE | TLR4 | WML/total | Kruit load score* |
|---|---|---|---|---|---|
| Control | 12F | 3/3 | AA | 0 | 0 |
| Control | 12F | 3/3 | AA | 0 | 0 |
| Control | 10M | 3/3 | AA | 0 | 0 |
| Control | 9F | 3/3 | AA | 0 | 0 |
| Control | 9M | 3/4 | AA | 1 | 0.0042 |
| Control | 10F | 4/4 | AA | 0 | 0 |
| Control | 8M | 3/3 | AA | 0 | 0 |
| Control | 10F | 3/3 | AA | 0 | 0 |
| Control | 16M | 3/3 | AA | 0 | 0 |
| Control | 10F | 3/3 | AA | 0 | 0 |
| Control | 10M | 3/3 | AA | 0 | 0 |
| Control | 10F | 3/3 | AA | 0 | 0 |
| Control | 13M | 3/3 | AA | 0 | 0 |
| MC | 14F | 3/3 | GG | 0 | 0 |
| MC | 17M | 3/3 | GG | 3 | 0.0126 |
| MC | 18M | 3/3 | AA | 1 | 0.0042 |
| MC | 11M | 3/3 | AA | 1 | 0.0042 |
| MC | 9F | 3/3 | AA | 7 | 0.0294 |
| MC | 10F | 3/3 | AA | 3 | 0.0126 |
| MC | 8F | 3/3 | AA | 9 | 0.0378 |
| MC | 11M | 3/3 | AA | 1 | 0.0042 |
| MC | 12F | 3/3 | AA | 0 | 0 |
| MC | 10M | 3/3 | AA | 0 | 0 |
| MC | 12F | 3/3 | AA | 0 | 0 |
| MC | 10F | 3/3 | AA | 0 | 0 |
| MC | 8M | 3/3 | AA | 0 | 0 |
| MC | 11F | 3/3 | AA | 0 | 0 |
| MC | 12F | 3/3 | AA | 1 | 0.0042 |
| MC | 10F | 3/3 | AA | 0 | 0 |
| MC | 10M | 3/3 | AA | 9 | 0.0378 |
| MC | 8M | 2/3 | AA | 2 | 0.0084 |
| MC | 7F | 3/3 | AA | 1 | 0.0042 |
| MC | 11M | 3/3 | AA | 0 | 0 |
| MC | 7F | 3/3 | AA | 3 | 0.0126 |
| MC | 11M | 3/3 | AA | 1 | 0.0042 |
| MC | 10M | 3/3 | AA | 0 | 0 |

* Kruit et al. (2004) score: the white matter lesions (WML) were defined as areas with high signal intensities on T2 weighted MRI; the count of WML was combined to get a quantitative measure of load by multiplying each lesion by a size-dependent constant: 0.0042 ml for 1 (<3 mm), 0.114 ml for 2 (4–10 mm), and 0.90 ml for 3 (>10 mm).

**Table 4**
White matter lesions in three Mexico City children with repeated MRIs

| MRI date | Age/gender | White matter lesions/total | Kruit score* |
|---|---|---|---|
| 6/13/2006 | 11M | 1 | 0.0042 |
| 11/24/2006 | 11M | 1 | 0.0042 |
| 2/13/2007 | 11M | 1 | 0.0042 |
| 3/23/2006 | 7F | 2 | 0.0084 |
| 6/13/2006* | 7F | 2 | 0.0084 |
| 11/24/2006* | 8F | 2 | 0.0084 |
| 2/13/2007* | 8F | 3 | 0.0126 |
| 6/13/2006 | 11M | 0 | 0 |
| 11/24/2006 | 11M | 1 | 0.0042 |
| 2/13/2007 | 11M | 1 | 0.0042 |

* MRI shown in Fig. 3A–C.

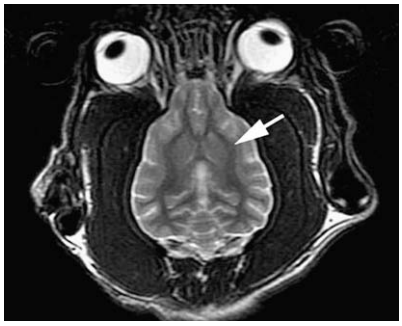

**Fig. 2.** Brain MRI in a 12-month-old healthy Mexico City dog (#7). T2 view to show the hyperintense white matter lesion (WML) in the left prefrontal region. The lesion is small, well defined, sharply demarcated (arrow), and could be seen in two different sequences: T2 and T2 FLAIR.

Results suggested that city of residence uniquely accounted for between 2–6% of the variance in about half of the tests in a standardized cognitive battery for children, and children living in the more highly polluted city performed "behind" their appropriate developmental level. Mexico City children (56.5%) that were scanned in MRI exhibited hyperintense white matter prefrontal lesions in their brain, compared to 7.6% of Polotitlán children who were scanned. The white matter lesions were persistent over time in the three children we followed up for 11 months. The presence of neuroinflammation and vascular lesions involving subcortical areas in similarly exposed healthy Mexico City 12- to 19-month-old dogs, but not in controls, provides converging evidence that exposure to air pollution is a key factor to the prevalence of brain damage in these children. Mexico City dogs exhibit MRI lesions

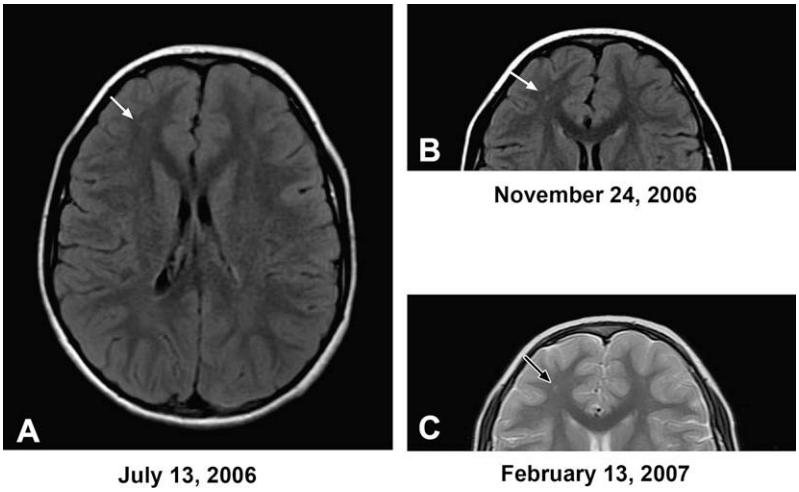


**Fig. 1.** (A–C) Corresponds to an 8.02-year-old Mexico City girl with persistent white matter hyperintense T2 and FLAIR lesions through 11 months of follow-up with sequential MRIs (July 13 and November 24, 2006, and February 13, 2007). The lesions are punctate, well defined, and persistent (arrows). This child has an IQ Full Scale of 113, verbal 56 and performance 57. However, the WISC-R subtests revealed performance behind her chronological age for Object Assembly 6.16 years, and Similarities 7.08 years.

**Table 5**
COX2, IL1β and GFAP mRNA in frontal cortex white matter in age-matched dogs from Mexico City and control city

| GROUPS | COX2/GAPDH* | IL1β/GAPDH* | GFAP/GAPDH* |
|---|---|---|---|
| CONTROL n:5 | 181.7 ± 44.75 | 746.2 ± 613.8 | 295503 ± 116145 |
| MEXICO CITY n:7 | 41764 ± 17152 | 4050 ± 1343 | 485135 ± 104243 |
| | $p = .03$ | $p = .01$ | $p = .34$ |

* Molecules per femtomol GAPDH rRNA.

**Table 6**
COX2, IL1β and GFAP mRNA in frontal cortex with white matter lesions by brain MRI's vs. normal white matter on MRI's in Mexico City dogs

| ID# DOG and Anatomical area Sampled | COX2/GAPDH* | IL1β/GAPDH* | GFAP/GAPDH* | WML by MRI | Differences between WML and/or normal white matter by MRI |
|---|---|---|---|---|---|
| 1 FL2 | 101.3 | 280.8 | 53052 | No | |
| 1 FR2 | 91.3 | 502.4 | 380598 | Yes | IL1β 1.78-fold GFAP 7.1-fold |
| 2 FL6 | 229.6 | 1217.0 | 168376 | Yes | COX2 2.1-fold IL1β 2.24-fold |
| 2 FR6 | 107.2 | 542.9 | 217355 | No | |
| 3 FL2 | 143.8 | 887.3 | 447983 | No | |
| 3 FR2 | 122.0 | 596.6 | 430648 | No | COX2 1.17-fold IL1 β 1.48-fold GFAP 1.0-fold |
| 4 FL1 | 133 | 9611 | 312843 | No | |
| 4 FL2 | 155 | 15927 | 1407132 | Yes | IL1β 1.9-fold GFAP 2.86-fold |
| 4 FL3 | 187 | 8351 | 491373 | No | |
| 4 FR3 | 36929 | 15900 | 1490285 | Yes | COX2 197-fold IL1β 1.9-fold GFAP 3.0-fold |
| 5 FR2 | 138.2 | 1303.4 | 291530 | No | |
| 5 FL2 | 72347 | 2122.7 | 531086 | No | COX2 523.4-fold IL 1 β 1.62-fold GFAP 1.82-fold |
| 6 FL2 | 54.5 | 1063.6 | 31832 | No | |
| 6 FR2 | 274.3 | 3213.5 | 276814 | No | COX2 5.03-fold IL 1 β 3.02-fold GFAP 8.69-fold |
| 7 FL | 13014.5 | 1926 | 512403 | Yes | COX2 11.7-fold IL1β 1.42-fold |
| 7 FR | 1105 | 1352 | 718854 | No | |

* Molecules per femtomol GAPDH rRNA.

similar to the ones described for children. Moreover, Mexico City dogs exhibited frontal white matter upregulation of two important inflammatory genes: COX2 and IL1β, compared to age-matched control dogs from a low polluted area. There was a significant upregulation of COX2, IL1β, and GFAP in individual dog's white matter lesions identified by MRI. The neuroinflammation seen in Mexico City dogs had been previously described by our laboratory both in dogs, and in healthy humans in Mexico City (Calderón-Garcidueñas et al., 2002, 2003b, 2004, 2007a). IL1β is the most important molecule capable of modulating cerebral functions during systemic and localized inflammation (Ferrari et al., 2006; Griffin & Mrak, 2002; Rothwell & Luheshi, 2000), while early and sustained production of COX2 results in the production of free radicals in the process of converting arachidonic acid to precursors of vasoactive prostaglandins (Choi, Langenbach, & Bosetti, 2006; Minghetti, 2005). Thus, overexpression of COX2 is both a marker and an effector of neural damage (Strauss, 2007), and the early upregulation of COX2 observed in Mexico City young healthy dogs may play a role in free-radical mediated cellular damage, alters cellular metabolism and produces vascular dysfunction, all crucial in neurodegenerative processes (Strauss, 2007).

We propose that a sustained state of brain inflammation with widespread diffuse vascular pathology could be interfering with subcortical pathways that link the prefrontal/frontal cortex with areas crucial for cognition, including functions such as working memory. Working memory is a multicomponent system responsible for active maintenance of information in the face of ongoing processing and/or distraction (Conway, Kane, & Engle, 2003). Working memory has been shown to be important for higher-order cognitive activities such as reading (Turner & Engle, 1989), and abstract reasoning or general fluid intelligence (Engle, Tuholski, Laughlin, & Conway, 1999). The neurological substrates of working memory are primarily identified with the prefrontal cortex, which holds a key status in cognitive neuroscience approaches to complex, goal-directed human behavior (Kane & Engle, 2002). Fluid cognition, supported by working memory, is distinguished from crystallized cognition, supported by previously acquired knowledge available in long-term memory. However, a directional relationship between the two has often been considered plausible. Indeed, fluid skills show a determining influence on crystallized achievements in early and middle childhood periods (Blair, 2006). Thus, finding an association between deficits in fluid cognition with exposure to air pollution is important because such deficits may result in problems later, e.g., in the self-regulation of cognition, emotion, and socially appropriate behaviour in affected children.

The prefrontal cortex seems to be a target anatomical region in pollution-exposed children and is notable for widespread connectivity with the organs in the cortico-limbic circuit, including the anterior cingulate cortex, the amygdalae, and the hippocampal formation (Carmichael & Price, 1995; Carmichael & Price, 1996; Kondo, Saleem, & Price, 2005; Markham, Morris, & Juraska, 2007). MRI changes in the prefrontal cortex are dramatic in the adolescent period (Sowell, Thompson, Tessner, & Toga, 2001; Sowell et al., 2004). Volumetric changes can be correlated with intelligence test and cognitive performance, most evident on tasks that rely heavily on the prefrontal cortex (Casey, Giedd, & Thomas, 2000; Shaw et al., 2006; Sowell et al., 2001; Sowell et al., 2004). In the period between adolescence and adulthood, rats exhibit a loss of neurons in the prefrontal cortex that make this crucial period particularly susceptible to the influence of environmental factors (Markham et al., 2007). Children with severe attention-deficit/hyperactivity disorders have "fixed" thinning of the left medial prefrontal cortex, which may compromise the anterior attentional network (Shaw et al., 2006). Adults with damage to the dorsolateral prefrontal cortex perform poorly on fluid cognitive tasks but exhibit measured general intelligence within the normal range (Duncan, Burgess, & Emslie, 1995).

There are two crucial findings in the young dogs' neuropathology that could be important for understanding the children's findings both cognitive and structural (white matter lesions in their brains). First, there is neuroinflammation in the brains of the exposed dogs, as shown by (1) the significant upregulation of inflammatory genes in the frontal white matter of Mexico City versus

control dogs, (2) the significant upregulation of COX2, IL1β, and GFAP in dogs' white matter lesions (3) the disruption of the blood–brain-barrier, and (4) the presence of ultrafine particulate matter in frontal vessels suggesting that ultrafine particles are capable of reaching the frontal lobe, and could be contributing to the neuroinflammatory process (Elder & Oberdorster, 2006; Peters et al., 2006; Rundell, Hoffman, Caviston, Bulbulian, & Hollenbach, 2007). Secondly, there are diffuse and prevalent vascular changes in subcortical areas in Mexico City dogs (equivalent to a preteen human), absent in control dogs (Calderón-Garcidueñas et al.,

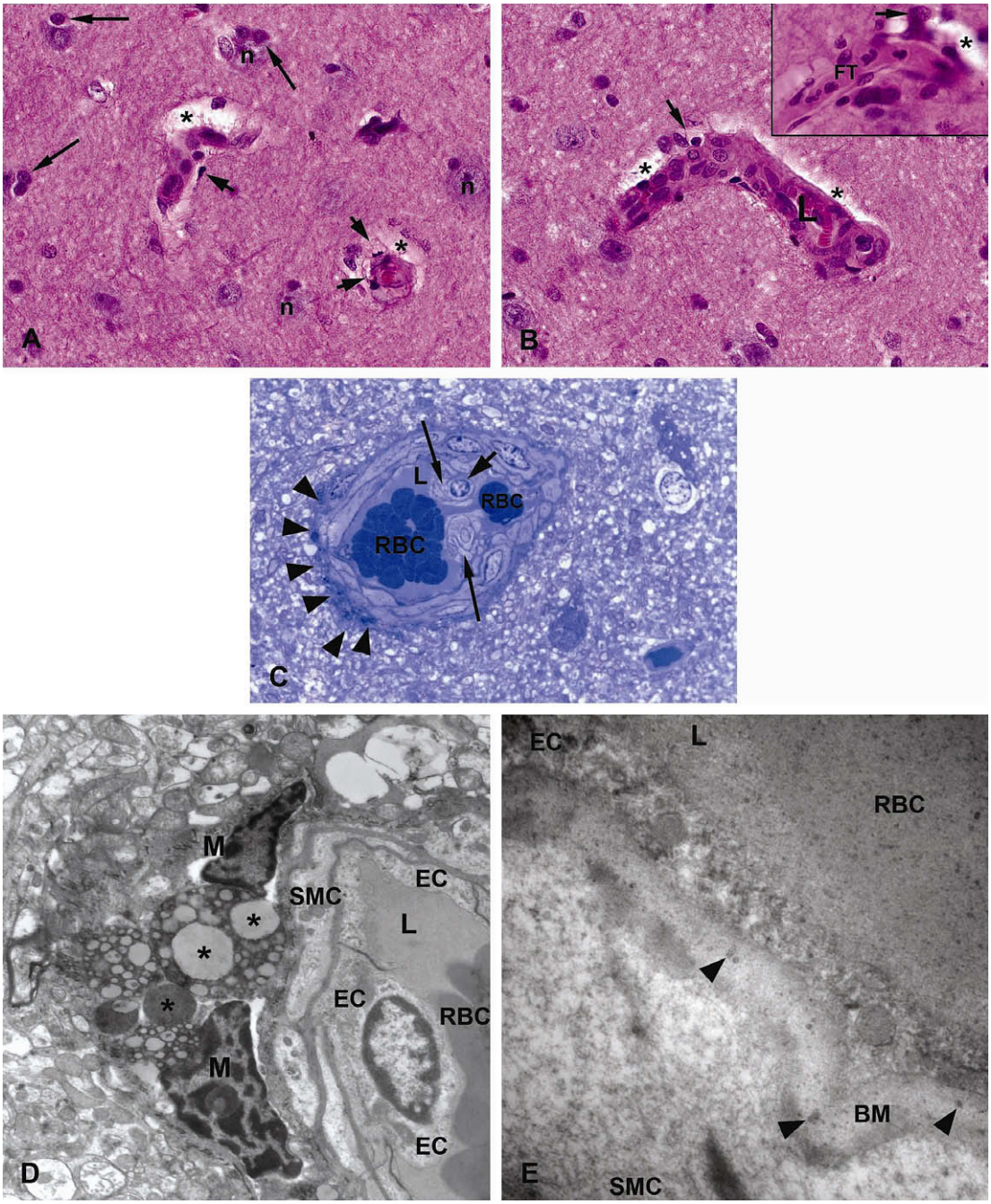


**Fig. 3.** (A) A 15-month-old dog prefrontal cortex shows small arterioles with perivascular foamy cells (short arrows) that exhibit picknotic nuclei. Virchow–Robin spaces are enlarged (∗). Perineuronal astrocytes with eosinophilic cytoplasm are also seen (long arrows). Neurons are marked n. Hematoxylin–eosin 214×. (B) Same dog as in (A) to show a gray matter frontal section with a blood vessel cut longitudinally. This vessel exhibits hypercellular walls, perivascular foamy cells (short arrow) and reduction in the lumen (L). Virchow–Robin spaces (∗) are expanded. H&E 420×. In the right upper insert we observed a small cortical frontal vessel from a 19-month-old Mexico City dog with the lumen completely occupied by a fibrin thrombus (FT) with embedded degenerating neutrophils. A reactive astrocyte (short arrow) is seen adjacent to the vessel wall surrounded by an expanded Virchow–Robin space (∗) HE 214×. (C) One micron toluidine blue frontal white matter arteriole with hyperplasic endothelial cells exhibiting elongated fronds that protruded into the lumen (long arrows). Notice the presence of a mononuclear cell (short arrow) apparently enclosed in the hyperplastic endothelial protrusions. Red blood cells (RBC) are seen in the reduced vessel lumen (L). There are several perivascular macrophage-like cells with picknotic nuclei and cytoplasmic debri occupying approximately 30% of the vessel perimeter (arrow heads). Toluidine blue 420×. (D) Frontal white matter arteriole from a 15-month-old Mexico City dog. Notice the perivascular macrophage-like cell (M) with numerous lipid vacuoles (∗) in intimate contact with the vessel wall. The endothelial cell cytoplasm is marked EC, the arteriole smooth muscle cell cytoplasm is marked SMC, and the vessel lumen occupied by RBC is marked L. Electron Micrograph 12,000×. (E) Arteriole from frontal white matter in a 19-month-old Mexico City dog. Ultrafine particulate-like material is seen in the red blood cell (RBC) in the vessel lumen (L). Similar PM material (head arrows) is seen in the basement membrane of the endothelial cell (BM). The endothelial cell cytoplasm is marked EC and the smooth muscle cell cytoplasm (SMC). Electron Micrograph 25,000×.

2003b). The neuroinflammation, the vascular pathology, the enlargement of the Virchow–Robin spaces, and the perivascular gliosis could be the morphological bases for the cognitive deficits observed in the highly exposed children.

Neuroinflammation is a harmful process, triggers disruption of the blood–brain-barrier, alters the brain homeostasis, and is a key factor in the pathogenesis of many central nervous system disorders, including chronic neurodegenerative diseases such as Alzheimer's and Parkinson's (Hoozemans et al., 2006; Minghetti, 2005; Qin et al., 2007; Whitton, 2007). The upregulation of IL1β is a significant finding in the exposed dog cohorts given that IL1β is kept at low constitutive levels in the normal brain but markedly increases after injury or an inflammatory process, thus IL1β is considered a candidate neurotoxin that contributes to the progression of neurodegenerative processes (Argaw et al., 2006; Griffin & Mrak, 2002; Rothwell & Luheshi, 2000; Vela, Molina-Holgado, Arévalo-Martín, Almazán, & Guaza, 2002; Zhang & Rivest, 2003). Cytokines play a central role in the self-propagation of neuroinflammation with IL1β having a prominent function (Minghetti, 2005).

The importance of subcortical frontal hyperintense white matter lesions has not been addressed in air pollution-exposed clinically healthy children; however it has been reported in children with sickle cell disease (Hogan, Vargha-Khadem, Saunders, Kirkham, & Baldewegm, 2006). Hogan et al. suggested that given the presence of MRI frontal white matter lesions in these children, the dorsolateral frontal cortex and the posterior medial frontal cortex could be

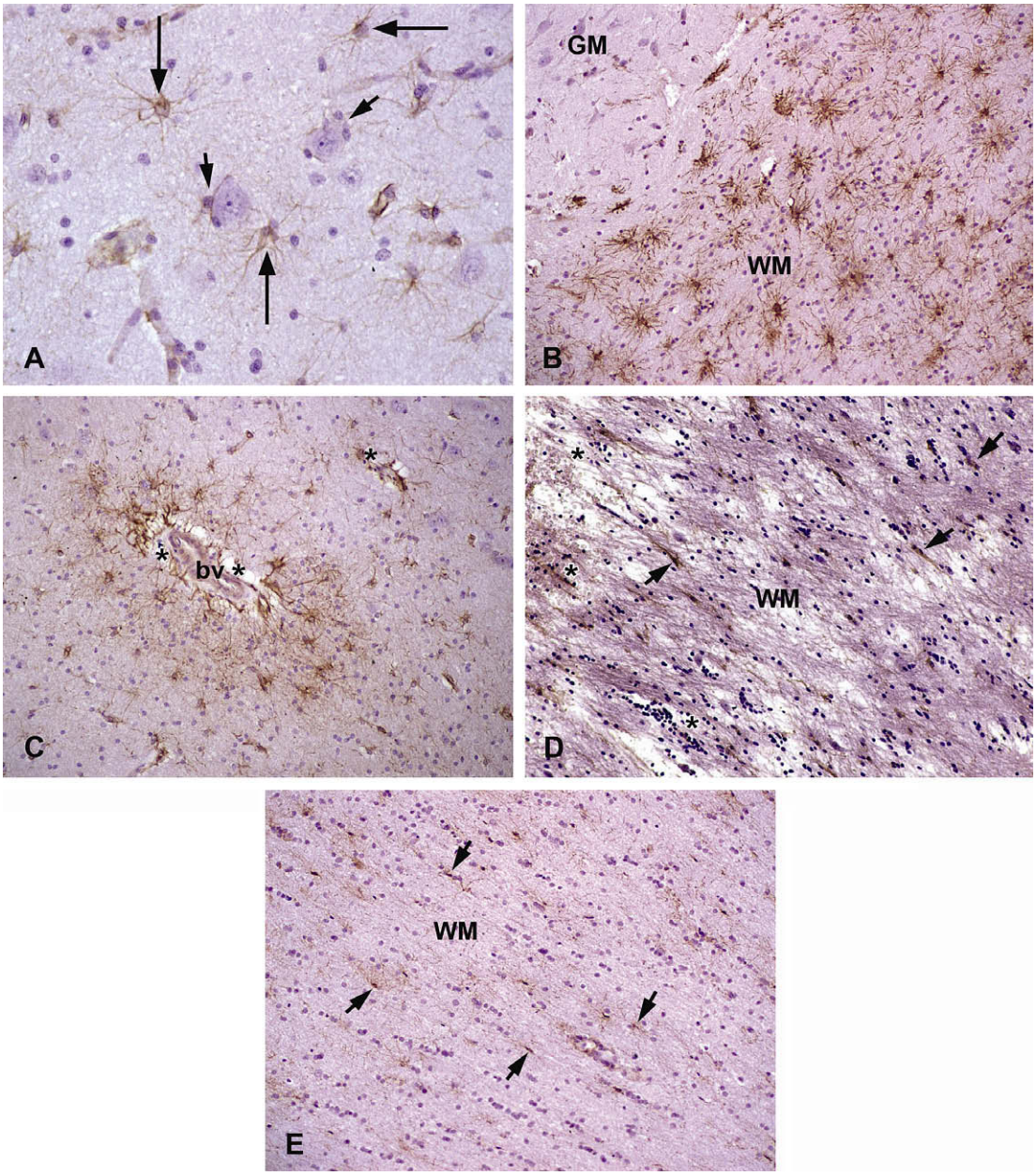


**Fig. 4.** (A) A 15-month-old Mexico City dog's frontal gray matter, immunoperoxidase for glial fibrillary acidic protein (GFAP) that labels reactive astrocytes. Notice the presence of reactive astrocytes GFAP+ surrounding neurons (short arrows), and well defined GFAP+ processes in large reactive astrocytes (long arrows). GFAP 420×. (B) A subcortical frontal sample from a 15-month-old Mexico City dog. The upper left corner is labeled gray matter (GM) and we can see a few reactive GFAP+ astrocytes, while in the area labeled white matter (WM) numerous reactive GFAP+ astrocytes are present. GFAP 214×. (C) Deep white frontal white matter in a 15-month-old Mexico City dog. Numerous reactive astrocytes GFAP+ are seen surrounding blood vessels (bv). The Virchow–Robin spaces are enlarged (∗). GFAP 214x. (D) A typical white matter lesion identified in this 15-month-old dog's brain MRI as a hyperintense area. Notice the significant loosening of the neuropil with leaky blood vessels in the white matter (WM), extravasation of RBC (∗), along with positive GFAP reactivity (short arrows). GFAP x 214. E. In contrast, this area of white matter corresponds to the opposite frontal white matter lobe. The neuropil is preserved (WM) and there is a small number of reactive GFAP+ astrocytes (short arrows). GFAP x 214

damaged, thus a disruption to event-related potential markers could indicate cortical disconnection in frontal areas. Children with sickle cell disease have a brain vasculopathy related to their abnormal hemoglobin, and thus a vascular component is the key for their white matter lesions. White matter lesions in adults and elderly people are associated with cognitive dysfunction, gait abnormalities, falls, and depression (Breteler et al., 1994; Longstreth et al., 1996; van Straaten et al., 2006). White matter lesions impair frontal lobe function (Tullberg et al., 2004), and indicate abnormalities of the subcortical fiber system (Gootjes et al., 2004). Histopathology correlates in the elderly include enlarged Virchow–Robin spaces, degeneration of axons and myelin, and gliosis (Scarpelli et al., 1994; Wahlund et al., 2001). Progressive white matter hyperintensities in older adults are related to changes in regional cerebral blood flow (Kraut, Beason-Held, Elkins, & Resnick, 2008). Vascular changes predominantly involving subcortical areas (e.g., white matter, basal ganglia, thalamus, and hippocampus) are seen in both Alzheimer's and vascular dementia patients, and in mixed dementia cases (Jellinger & Attems, 2007). The common denominator for the hyperintense white matter lesions detected by brain MRI appears to be a vascular lesion with perivascular gliosis and enlarged Virchow–Robin spaces. Based on our pilot results we suggest that the white matter lesions detected in 56.5% of Mexico City children tested represent the extreme of the vascular lesions with a breakdown of the blood–brain-barrier, and that the vascular pathology is likely of a diffuse nature as seen in the young dogs.

In summary, clinically healthy children with no known risk factors for neurological or cognitive disorders residing in a highly polluted urban environment exhibited deficits in fluid cognition, memory, and executive functions, relative to children living in a less polluted urban environment. In parallel, a number of the children living in the highly polluted city exhibited white matter hyperintense lesions in MRI, while only one child living in the less polluted city exhibited a single lesion. These white matter lesions along with the presence of more diffuse vascular pathology are likely to interrupt subcortical prefrontal cortex connections and other white matter tracts, potentially contributing to the cognitive dysfunction. Ultrafine particulate matter reaching the frontal cortex in the highly exposed dogs and in young Mexico City adults (Calderón-Garcidueñas et al., 2008) is likely contributing to the neuroinflammation.

In the USA 158 million people live in areas where ozone exceeds the 8 h standard, 29 million are exposed to $PM_{10}$ and 88 million to $PM_{2.5}$ (U.S.Environmental Protection Agency. Green Book: Particulate Matter Nonattainment Area, E2004). Urban sites with high PM contributions from vehicles and industry (Seagrave et al., 2006) are highly visible polluted sources, although indoor pollution is also very important particularly for disadvantage populations living in high-density multiunit dwellings in large urban areas (Baxter, Clougherty, Laden, & Levy, 2007). The issue of air pollution causing cognitive deficits and brain structural changes in healthy children with all their potential consequences ought to be of major public importance. Alterations in measures of fluid intelligence and cognitive control predict school performance, complex learning, ability to control attention and avoid distraction, reading and listening comprehension, reasoning, and of key importance from the social point of view: the ability to block impulsive anti-social behavior.

MRI techniques have made it possible to study structural and metabolic brain development across age groups, including children (Evans, 2006), and regional white matter alterations are related to decreases in performance on neuropsychological tests (Brickman et al., 2006). Thus, as suggested by the interdisciplinary methodology of the present investigation, it is possible to comprehensively examine brain structural changes and to determine the interrelationships between age, white matter changes, hippocampal measurements, cognitive function, and the cumulative doses of air pollutants. The work here presents a pioneering attempt to answer such urgent questions, and initial findings give encouragement that future, large-scale work in this area has great potential to give much-needed answers.

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