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## THE INFLUENCE OF AGE OF LEAD EXPOSURE ON ADULT GRAY MATTER VOLUME

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### Abstract

Childhood lead exposure is associated with decreased cognitive abilities and executive functioning localized within the prefrontal cortex. Several studies have observed stronger associations between blood lead measurements obtained later in life than earlier measures, but there are no imaging studies investigating the developmental trajectory of blood lead levels taken during childhood on adult gray matter volume. In this study, we recruited 157 adults ( $20.8 \pm 1.5$  years of age) from the Cincinnati Lead Study to undergo high-resolution volumetric magnetic resonance imaging. Adjusted voxel-wise regression analyses were performed for associations between adult gray matter volume loss and yearly mean blood lead levels from one to six years of age in the entire cohort and by sex. We observed significant inverse associations between gray matter volume loss and annual mean blood lead levels from three to six years of age. The extent of prefrontal gray matter associated with yearly mean blood lead levels increased with advancing age of the subjects. The inverse associations between gray matter volume loss and yearly mean blood lead measurements were more pronounced in the frontal lobes of men than women. Analysis of women yielded significantly weaker associations between yearly mean blood lead levels and gray matter volume at all ages than either men or the combined cohort of men and women together. These results suggest that blood lead concentrations obtained during later childhood demonstrate greater loss in gray matter volume than childhood mean or maximum values. The relationship between childhood blood lead levels and gray matter volume loss was predominantly observed in the frontal lobes of males. This study demonstrates that maximum blood lead levels do not fully account for gray matter changes associated with childhood lead exposure, particularly in the frontal lobes of young men.

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## Keywords

Lead; brain; gray matter; magnetic resonance imaging; voxel based morphometry; development

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## 1. Introduction

Lead is an environmental toxicant with documented effects on human cognition (Baghurst et al., 1992; Bellinger et al., 1992; Canfield et al., 2003; Dietrich et al., 1993a; Lanphear et al., 2005; Schnaas et al., 2000; Wasserman et al., 1997), behavior (Dietrich et al., 2001; Needleman et al., 1996; Stretesky and Lynch, 2004; Wright et al., 2008), and brain structure (Cecil et al., 2008; Stewart et al., 2006).

While mean and maximum childhood blood lead levels have long been regarded as the standard metrics of childhood lead exposure, several studies have noted stronger associations between neurobehavioral outcomes and blood lead levels measured in later childhood than either mean or maximum childhood blood lead levels (Bellinger et al., 1992; Chandramouli et al., 2009; Chen et al., 2007; Chen et al., 2005; Ris et al., 2004; Schnaas et al., 2000; Tong et al., 1996; Wasserman et al., 1997). In Cecil, et al. 2008, we demonstrated the adjusted association between *mean* childhood lead levels and adult gray matter volume loss using a voxel based morphometric analysis of volumetric magnetic resonance imaging (MRI) obtained from a longitudinal birth cohort. The purpose of this study was to investigate if later childhood blood levels were more strongly associated with neuroanatomical changes than mean or maximum childhood blood lead levels.

## 2. Methods

### 2.1 Participants

The Cincinnati Lead Study (CLS) is an urban, inner-city birth cohort with detailed prenatal and postnatal histories of low to moderate lead exposure and behavioral outcomes monitored over 30 years. The CLS enrolled pregnant women between 1979 and 1984 who lived in neighborhoods with historically high levels of childhood lead exposure. Women were excluded if they were known to be addicted to drugs, diabetic or had any known neurological or psychiatric disease. Infants were excluded if their birth weight was less than 1,500 g or if genetic or other serious medical issues were present at birth (Dietrich, Krafft 1987). This process netted newborns that were followed up quarterly through 5 y of age, semiannually from 5 to 6.5 y of age, again at age 10 y and between the ages of 15 and 17 y. A total of 157 CLS participants between the ages of 19 and 24 years provided informed consent and participated in this imaging study (Table 1). Some CLS participants in this imaging study did not have complete lead exposure histories; rather than try to impute the missing values these subjects were excluded from analysis of the years for which there was no lead exposure record. A summary of the cohort size and lead level by age of the participants is shown in Table 2.

### 2.2 Imaging Analysis

We acquired whole-brain, three-dimensional, high resolution volumetric 1.5 Tesla MR data (General Electric Medical Systems, Milwaukee, WI, Signa LX EXCITE scanner operating at software platforms of 11.0 and 12.0) using a T1-weighted, axial inversion recovery preppped, fast spoiled gradient echo (3D IR FSPGR) sequence (echo time (TE) of 5 msec, repetition time (TR) of 12 msec, inversion time (TI) 300 msec, field of view (FOV) = 24 cm × 19.2 cm, 1.5-mm thick contiguous slices in a 256 × 192 × 124 matrix for a resolution of 0.94 mm \* 1 mm \* 1.5 mm) to assess global and regional changes in brain tissue (gray matter, white matter, and cerebrospinal fluid [CSF]) volume for comparison with the yearly mean of childhood blood

lead concentrations (measured in  $\mu\text{g}/\text{dL}$ ) collected between 3 and 78 months of life using voxel-based morphometry (VBM) (Ashburner and Friston, 2000). VBM requires normalizing individual structural MRI scans to a study-specific template to allow voxel-by-voxel comparisons between individuals. This approach allows for statistical analyses throughout the brain without *a priori* designation of structures of interest or manual delineation of brain structures.

### 2.3 Blood Lead Concentrations

Blood lead samples were collected and analyzed for lead by anodic stripping voltametry as been previously described in extensive detail (Dietrich et al., 1993b, Dietrich et al., 1987, and Roda et al., 1987) at the Hematology and Environmental Chemistry Laboratory in the University of Cincinnati Department of Environmental Health. This laboratory was fully accredited to carry out this work and served as a reference laboratory for several blood lead assessment programs throughout the United States. Blood lead concentrations were measured in this cohort every 3 months from birth for the first 5 y of life and every 6 months from 5 to 6.5 y. To investigate the influence of lead exposure at different ages, yearly mean blood lead levels were calculated for all individuals in this study. The mean of all blood lead levels in the preceding year was used to calculate the yearly mean blood lead level. For example, the mean of blood lead levels recorded at birth through 12 months was designated Year 1 mean blood lead level. Similarly, the mean of blood lead levels from 15 months to 24 months gave the Year 2 mean blood lead level, and so on for years 3 through 5. In year 6, blood lead levels were collected semiannually, and the mean of the 66 and 72-month blood lead levels was used to calculate the year 6 mean blood lead level. Maximum values were also noted for each individual, and associations between individual maximum lead levels and adult gray and white matter volumes were also investigated.

### 2.4 VBM Approach

Separate multiple regression models were developed to investigate associations between each yearly mean blood lead level on changes in volume for white matter, gray matter, or cerebrospinal fluid. All initial exploratory analyses were 2-tailed for positive or negative associations, representing volume gain or loss, between yearly mean lead levels and volume in all tissue classes.

Because multiple environmental and developmental factors could influence adult brain volumes, we considered several potential covariates for inclusion in the final regression models. Covariate selection was performed using a modified version of our method reported previously (Cecil et al., 2008). In summary, a simple regression analysis between blood lead level and gray matter volume was used to identify regions where mean blood lead level was associated with gray matter volume change ( $P \leq 0.001$  unadjusted, 700 voxel minimum cluster size). After performing simple regressions, possible covariates were then added individually to the otherwise simple regression model. The change in the regression coefficient (beta 1) was calculated on voxel-by-voxel basis. Covariates were retained if the addition of the potential covariate caused a change of more than 10% in beta 1 in more than 20% of the voxels where a significant association was found in the simple regression between mean childhood blood lead and volume change. This process was repeated in a stepwise fashion until no further covariates met the inclusion criteria. This method is similar to that reported previously (Cecil et al., 2008), but uses a stepwise, rather than single-step, selection of covariates.

Covariates considered included participant age at time of imaging, current marijuana use (obtained from a urine drug screening collected at time of imaging), sex, birth weight, gestational age at birth, maternal IQ (Silverstein, 1985) maternal alcohol consumption during pregnancy, maternal marijuana use during pregnancy, maternal tobacco use during pregnancy,

mean childhood Hollingshead socioeconomic status (SES) score (Cirino et al., 2002), current SES score, and HOME Inventory (using the mean Home Observation for Measurement of the Environment score measured in early childhood (Bradley and Caldwell, 1979)). Two variables—age at time of imaging and birth weight—satisfied these criteria and were included in the final multiple regression models. No significant differences were observed between males and females in the distribution of any of the potential covariates, and separate analyses for each sex were not performed.

## 2.5 Ethical Oversight

The institutional review boards of the Cincinnati Children's Hospital Medical Center and the University of Cincinnati approved the study protocol. A Certificate of Confidentiality for the study was obtained from the National Institutes of Health.

## 3. Results

We investigated the effects of yearly mean blood lead from 1 to 6 years of age on adult gray matter volume in a voxel-wise analysis of high resolution volumetric MR images (Table 3). We found that later ages of blood lead assessment were more strongly associated with gray matter volume loss than earlier ages of assessment, and that males were more affected than females at all ages (Figures 1A-1C). These findings were most prominent in the frontal lobes. We found the largest regions of gray matter volume loss were in males associated with mean blood lead levels measured during the fifth and sixth year of life (Figure 1B). Although maximum blood lead measures were recorded at approximately 2 years of age, the strongest associations with adult gray matter volume were observed in association with blood lead levels measured at 5 and 6 years of age (Figures 1A-1C). These results suggest that blood lead measurements obtained early in childhood, mean, or maximum blood lead levels may not fully represent the extent of lead-associated gray matter changes observed in young adults.

## 4. Discussion

While most developed countries have reduced the major sources of lead exposure in the general population, particularly from the combustion of gasoline (petrol), lead remains a threat to healthy development in children. Lead exposure in US children results primarily from the ingestion of leaded paint residues in dust and soil, although other sources such as lead in imported toys, art materials, candies, and folk medicines can still present a risk. Blood lead levels during childhood usually correspond to the intensity of normal hand-to-mouth and ambulatory behavior, and peak at approximately 2 years of age (Ris et al., 2004). Blood lead concentrations decline in older children as they grow out of their mouthing behaviors and absorption is diminished, but there is still ongoing exposure that reflects ingestion, environmental exposure, and resorption from mineral deposits in the body throughout life.

### 4.1 Maximum blood lead levels

Until only recently, it was widely believed that neurocognitive and behavioral changes in children were latent effects of peak lead exposure occurring years earlier (Bellinger et al., 1992; Pocock et al., 1994; Schwartz, 1994). Several recent studies utilizing serial blood lead measurements have observed that maximum blood lead levels were not strong predictors of all lead-associated findings (Chen et al., 2005; Tong et al., 1996; Tong et al., 1998), and that blood lead levels recorded later in childhood yielded stronger associations between cognitive (Baghurst et al., 1992; Bellinger et al., 1992; Chen et al., 2007; Chen et al., 2005; Factor-Litvak et al., 1999; Lanphear et al., 2005; Schnaas et al., 2000; Tong et al., 1996; Tong et al., 1998; Wasserman et al., 1997) and behavioral (Burns et al., 1999; Ris et al., 2004) outcomes. Comparing the effects of earlier versus later blood lead concentrations on IQ, Hornung,

Lanphear, et al. (Hornung et al., 2009) found that the greatest global cognitive deficits were observed among Cincinnati and Rochester children whose blood lead concentrations continued to rise from 2 to 6 years of age. Furthermore, adult criminal arrest rates in the Cincinnati cohort were a 3.35 times higher in subjects whose 6-year blood lead level was 50% higher than their 2-year blood lead (Hornung et al., 2009).

Our findings are consistent with the cognitive and behavioral studies by demonstrating a stronger and more widespread association for blood lead levels in years five and six of life when compared with the maximal or early childhood blood lead levels (Figures 1A & 1B). However, it is noteworthy that the maximum blood lead level did demonstrate a relationship with volume loss in the frontal lobes.

#### 4.2 Sex Differences in lead exposure outcomes

We have previously found that associations between mean childhood blood lead level and gray matter volume loss were much more widespread and significant in males than females despite comparable mean childhood blood lead levels (Cecil et al., 2008). Our previous study was the first to observe sex differences in a radiologic outcome of childhood lead exposure. Several studies, including many performed in this cohort, have observed stronger associations between lead levels in males than females by diverse neurocognitive (Bellinger et al., 1990; Cecil et al., 2008; Dietrich et al., 1987; Froehlich et al., 2007; Pocock et al., 1987; Ris et al., 2004) and behavioral (Wright et al., 2008) outcomes, though these findings are not universal (Baghurst et al., 1992; Rabinowitz et al., 1991; Tong et al., 1996). The consistency of findings of greater lead-associated neurocognitive and behavioral findings in males, and our prior work showing greater extent and significance of lead-associated gray matter volume loss in males, suggests an underlying physiologic difference in how the brains of men and women respond to childhood lead exposure.

#### 4.3 Sex differences and developmental trajectories

Men and women have brains of different sizes, and different trajectories of gray matter maturation. White matter volume increases in a closely linear fashion from birth to early adulthood (Giedd et al., 1999; Lenroot et al., 2007), reflecting consistently increasing myelination with age (Gulani et al., 2001; Hildebrand and Waxman, 1984; Partridge et al., 2004; Suzuki et al., 2003), while total gray matter volume increases in a non-linear and region-specific manner (Giedd, 2004; Giedd et al., 1999; Lenroot and Giedd, 2006; Lenroot et al., 2007; Shaw et al., 2008). Total gray matter volume peaks at approximately 11 years of age in boys and 9 years of age in girls (Lenroot et al., 2007). Frontal and parietal gray matter volumes peak at approximately 12 years of age in boys and 10 years of age in girls (Giedd et al., 1999; Lenroot et al., 2007). Peaks in gray matter volume are thought to correspond to maximum neuronal number and synaptic density, and the subsequent decline to arise from normal pruning of synapses and neurons (Huttenlocher, 1984; Low and Cheng, 2006). While the rate of volume change is comparable between boys and girls in most regions (Giedd et al., 1999; Lenroot et al., 2007), normal gray matter volume loss occurs at a faster rate in the frontal lobes of boys than girls (De Bellis et al., 2001; Giedd et al., 1999), though this finding has not been consistently replicated (Giedd, 2004; Lenroot et al., 2007).

The different trajectories of gray matter volume change in males and females suggest different windows of neuronal vulnerability during development. Lead has been shown to be neurotoxic to cultures of developing neurons (Basha et al., 2003; Chetty et al., 2001; Reddy and Zawia, 2000), but whether the toxicity of lead varies along the course of neuronal development is unknown. If lead-associated gray matter volume loss results from lead acting during a window of developmental neuronal vulnerability, we would expect to see roughly parallel, chronologically offset patterns of gray matter volume loss in males and females, corresponding

roughly to the parallel trajectories of developmental gray matter volume change. Instead, we observed widespread regions of lead-associated gray matter volume loss in males at several time points and an almost complete lack of findings in females. These results suggest males are intrinsically vulnerable, or females are intrinsically protected, from lead-associated gray matter volume loss. Female sex has been shown to be protective in epidemiologic studies of stroke, schizophrenia, and Parkinson's disease (Amantea et al., 2005). If the observed differences in male and female lead-associated gray matter volume loss are due to different physiology, estrogens may explain why the brains of men and women respond differently to similar lead exposures.

#### 4.4 Sex differences and estradiol

Males and females have dramatically different circulating levels of sex hormones throughout life; prepubertal estradiol is approximately 8-fold higher in girls than boys (Cutler, 1997) and 25-fold higher during puberty (Ducharme et al., 1976). Estradiol enhances cell proliferation (Tanapat et al., 1999) and neuronal density (Gould et al., 1990; Hao et al., 2006; Morrison and Hof, 2007; Pozzo-Miller et al., 1999; Tang et al., 2004) and synaptic density (Woolley et al., 1996). Multiple studies have shown that estradiol protects neurons from oxidative stress (Green and Simpkins, 2000; Kolsch et al., 2001; Schmidt et al., 2002; Teepker et al., 2003; Vedder et al., 1999; Wang et al., 2001). The mechanisms of neuroprotective actions of estradiol include activation of the mitogen activated kinase pathway, altered expression of anti-apoptotic bcl-2 genes, maintenance of calcium homeostasis via the NMDA channel, and direct antioxidative action. (Green and Simpkins, 2000; Kolsch et al., 2001; Rao and Kolsch, 2003; Schmidt et al., 2002; Teepker et al., 2003; Vedder et al., 1999; Wang et al., 2001).

In a neuronal culture model, lead-exposed neurons pretreated with estradiol showed reduced expression of the antioxidant glutathione, reduced expression of the pro-apoptotic protein caspase-3, and decreases in the number of apoptotic neurons (Chetty et al., 2007). The neuroprotective properties of estrogens (Amantea et al., 2005; Rao and Kolsch, 2003), particularly estradiol (Chetty et al., 2007), provide a potential etiology for the observed neuroanatomical differences between males and females exposed to comparable levels of lead during childhood.

### 5. Study Limitations

A single blood lead measurement reflects both recent exposure and the ongoing resorption of lead from deep physiological depots such as bone. Individual serial blood lead measurements show that individual variability decreases with age (Mushak, 1998), a finding replicated in this cohort, resulting in a stabilization of rank order of lead exposure over time (Ris et al., 2004). This stabilization of rank order should result in stronger associations between lead levels and lead-associated outcomes with age, making it difficult to determine the extent to which studies such as this one and others (Chen et al., 2007; Chen et al., 2005; Lanphear et al., 2005; McMichael et al., 1988; Song et al., 2003) reflect the decreased variability of blood lead over time.

Our study benefits from a large sample size and detailed histories of lead exposures and perinatal, environmental and sociohereditary influences on development. This study is limited by relatively high mean blood lead concentrations at enrollment and limited generalizability due to the primarily African-American, urban, and impoverished demographics of this cohort (Chen et al., 2007; Ris et al., 2004; Wright et al., 2008). This study utilized a cross-sectional sample of volumetric magnetic resonance images obtained during adulthood as outcome measures. While this outcome measure allows for separation of concurrent from earlier developmental effects of lead exposure, a longitudinal volumetric MRI study of lead exposure during childhood and adolescence would be helpful in delineating effects of lead exposure at

different ages on developing brain structures. At the time this study was designed, such an experiment was not technically feasible. While these results reflect altered neuroanatomy associated with lead exposure, the functional consequences of gray matter volume loss, or gain, are often difficult to interpret (Giedd, 2004; Gogtay et al., 2004; Lenroot and Giedd, 2006; Shaw et al., 2008). This problem is compounded when attempting to explain differences in cognitive outcome measures, such as IQ, which utilize widespread, non-distinct brain regions (Haier et al., 2004). More studies are necessary to fully understand the connections between neurocognitive and behavioral outcomes and the neuroanatomic findings described herein.

## Abbreviations

MR	magnetic resonance
MRI	magnetic resonance imaging
VBM	voxel based morphometry
GM	gray matter
WM	white matter

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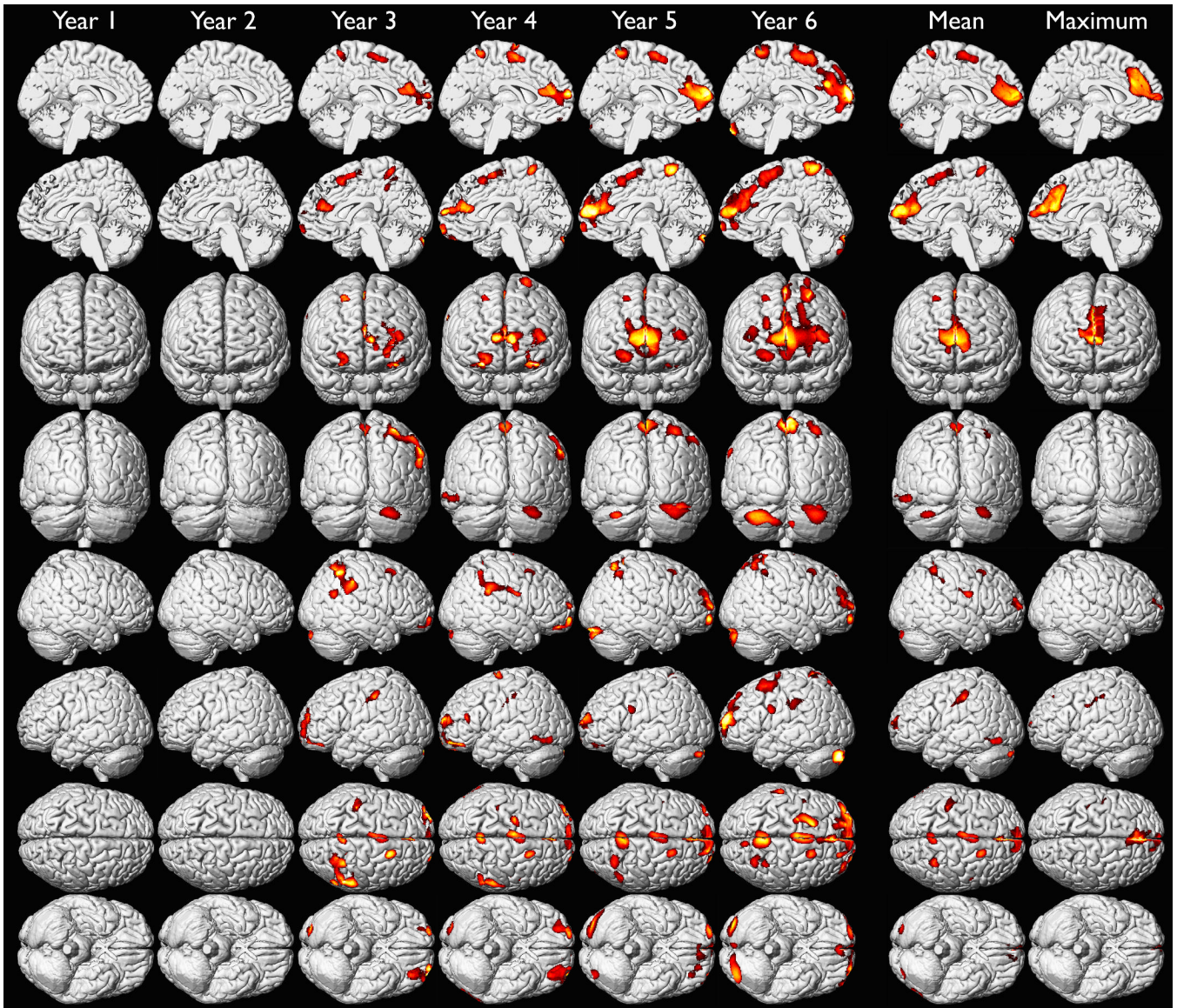
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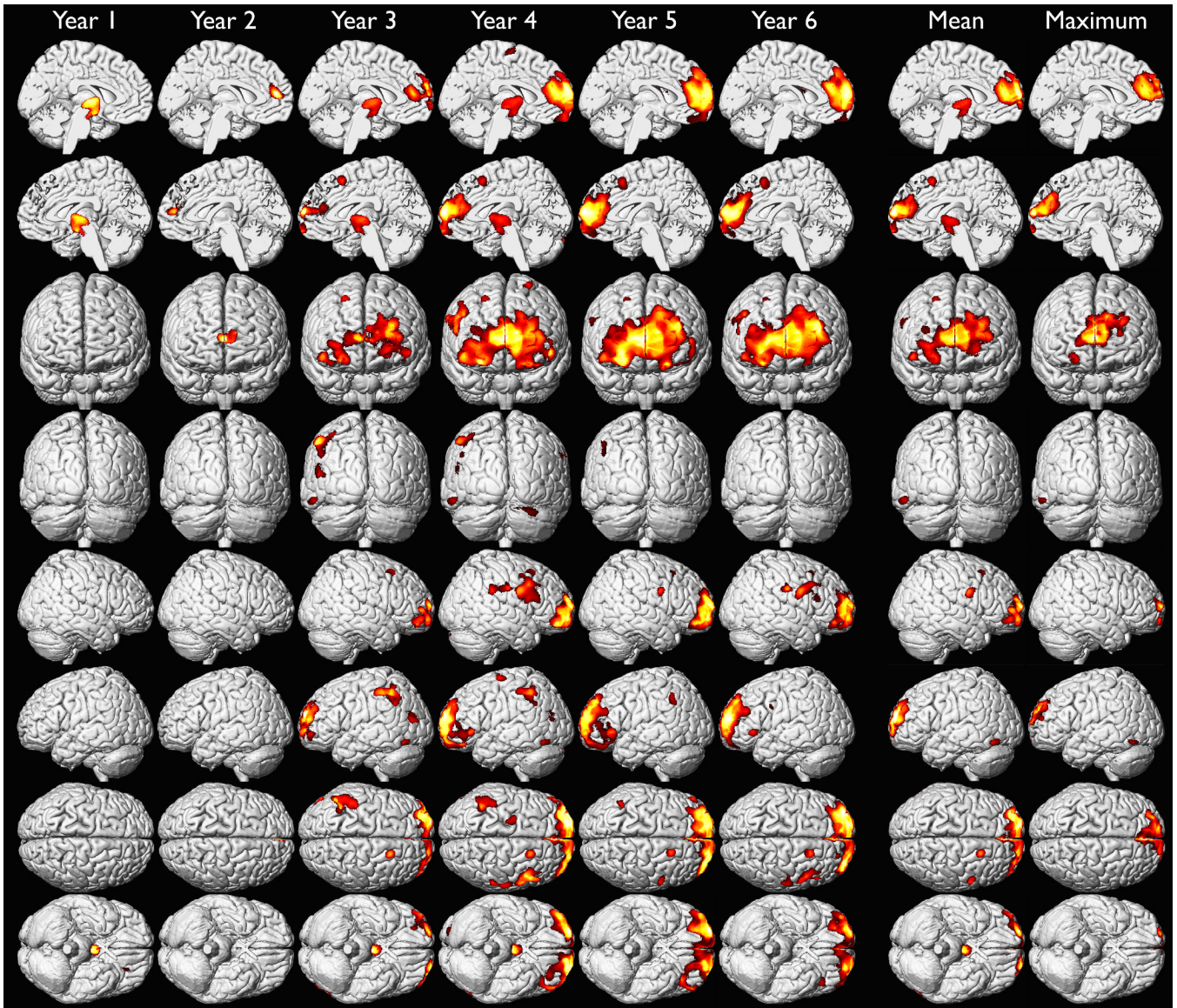
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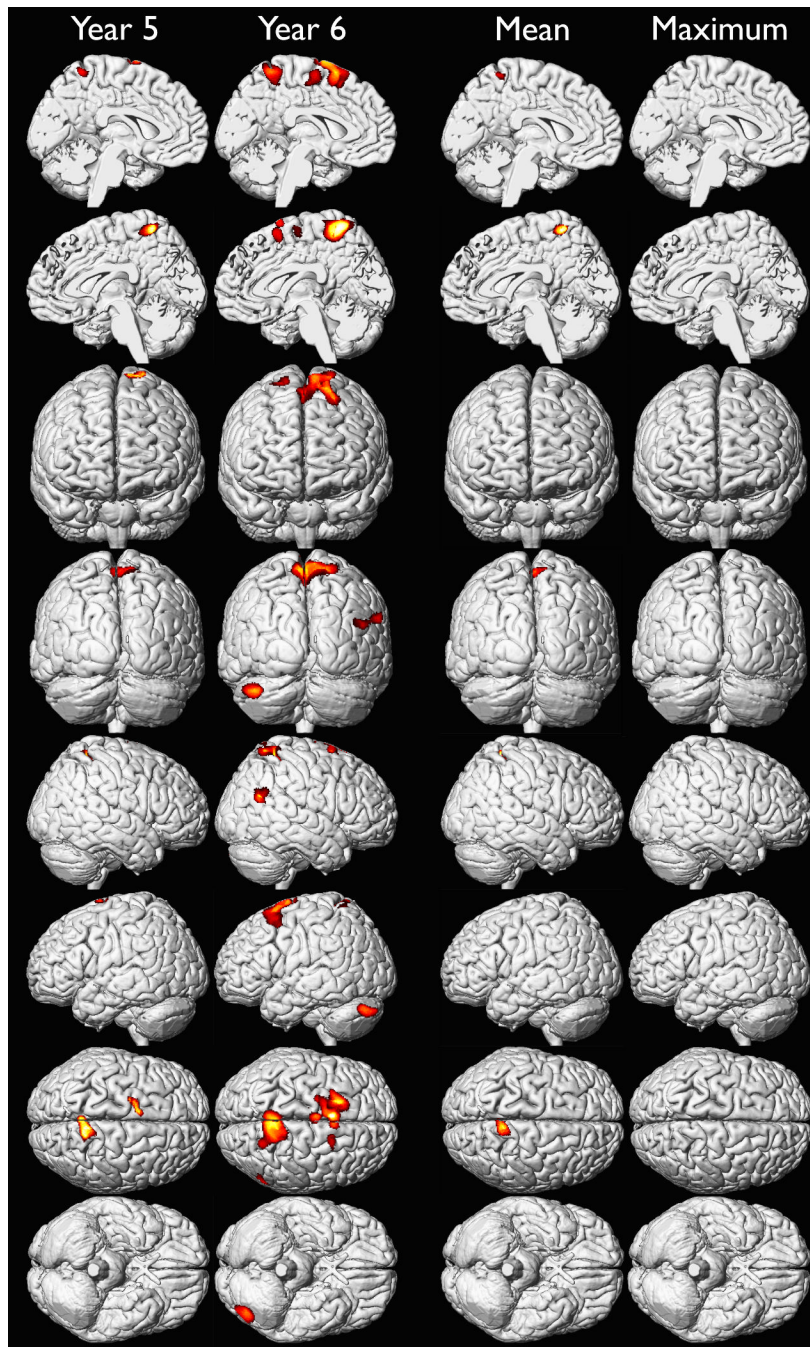
**Figure 1A.**

Gray matter volume loss associated with adjusted yearly mean, childhood mean and maximum blood lead levels in the whole CLS cohort. Hotter colors indicate greater strength of association between blood lead level (annual, mean and maximum of childhood) and gray matter volume loss upon adjustment for age at imaging and birth weight. Significance thresholds were set at uncorrected  $p < 0.001$  and 700 voxel minimum contiguous cluster size.



**Figure 1B.**

Gray matter volume loss associated with adjusted yearly mean, childhood mean and maximum blood lead levels in the males of CLS cohort. Hotter colors indicate greater strength of association between blood lead level (annual, mean and maximum of childhood) and gray matter volume loss in males upon adjustment for age at time of imaging and birth weight. Significance thresholds were set at uncorrected  $p < 0.001$  and 700 voxel minimum contiguous cluster size.



**Figure 1C.**

Gray matter volume loss associated with adjusted yearly mean, childhood mean and maximum blood lead levels in the females of CLS cohort. Hotter colors indicate greater strength of association between blood lead level (Year 5, Year 6, mean and maximum of childhood) and gray matter volume loss in females upon adjustment for age at time of imaging and birth weight. Significance thresholds were set at uncorrected  $p < 0.001$  and 700 voxel minimum contiguous cluster size. No significant findings for years 1-4.

**Table 1**  
 Characteristics of the Children and of their Mothers in the Cincinnati Lead Study (N=157) with Comparison by Sex

Characteristic	Cohort	Range	Men (N=83)	Women (N=74)	p value
Mean childhood blood lead concentration ( $\mu\text{g}/\text{dL}$ )	13.3 $\pm$ 5.9	(4.6 - 37.2)	13.6 $\pm$ 6.3	13.1 $\pm$ 5.5	0.50
Maximum blood lead concentration ( $\mu\text{g}/\text{dL}$ )	23.1 $\pm$ 11.2	(7.8 - 83.2)	23.5 $\pm$ 11.0	22.7 $\pm$ 11.6	0.64
Age at Max Pb level (months)	23.4 $\pm$ 10.8	(3 - 66)	23.6 $\pm$ 11.5	23.2 $\pm$ 10.0	0.85
Age at imaging (years)	20.8 $\pm$ 0.9	(19.7 - 24.3)	20.8 $\pm$ 0.9	20.9 $\pm$ 0.9	0.50
Gestational age (weeks)	39.4 $\pm$ 1.7	(35 - 43)	39.5 $\pm$ 1.7	39.4 $\pm$ 1.7	0.38
Birth weight (grams)	3103 $\pm$ 468	(1814 - 4260)	3137 $\pm$ 508	3075 $\pm$ 412	0.63
SES, 78 months	18.1 $\pm$ 5.1	(11 - 49)	18.9 $\pm$ 5.7	17.6 $\pm$ 4.3	0.80
IQ-FSIQ at 7 years	86.7 $\pm$ 11.9	(50 - 116)	85.2 $\pm$ 12.1	88.2 $\pm$ 11.6	0.89
Educational Level (20 yrs)	11.5 $\pm$ 1.4	(8 - 16)	11.3 $\pm$ 1.3	11.6 $\pm$ 1.6	0.78
Maternal FSIQ	75.3 $\pm$ 8.7	(55 - 100)	74.6 $\pm$ 8.1	76.0 $\pm$ 9.4	0.75
Marijuana Usage	76 positive (48%)		46 positive (55%)	30 positive (41%)	0.95
Maternal Alcohol Usage	24 yes (15%)		11 yes (13%)	13 yes (18%)	0.65
Maternal Tobacco Usage	71 yes (45%)		35 yes (42%)	36 yes (49%)	0.68
Maternal Marijuana Usage	18 yes (11%)		9 yes (11%)	9 yes (12%)	0.43

Abbreviations: SES-Hollingshead Socioeconomic Status, FSIQ-Full Scale Intelligence Quotient, p value represents difference between men and women using the student's t-test.

Table 2

## Annual Characteristics of the Cincinnati Lead Study

Cohort	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Mean Yrs 1-6
<b>Men</b>							
Number	157	157	156	156	153	149	157
Mean Pb level	10.6 ± 5.4	17.2 ± 8.5	16.3 ± 7.7	14 ± 6.8	11.8 ± 5.9	9.6 ± 5.2	13.3 ± 5.9
Pb level range	3.1 to 35	5.7 to 49.3	4.3 to 50.3	3.1 to 45.2	3.3 to 38.3	2.4 to 32.7	4.7 to 37.2
<b>Women</b>							
Number	83	83	82	82	79	77	83
Mean Pb level	10.8 ± 5.8	17.7 ± 9.2	16.4 ± 8.1	14 ± 6.9	11.8 ± 6	10.1 ± 5.4	13.5 ± 6.3
Pb level range	3.1 to 35	5.4 to 47.8	4.3 to 43.8	3.1 to 38.2	3.3 to 31.8	2.4 to 24.3	4.7 to 34.8
<b>Combined</b>							
Number	74	74	74	74	74	72	74
Mean Pb level	10.3 ± 4.8	16.7 ± 7.8	16.2 ± 7.3	14 ± 6.8	11.8 ± 5.9	9.2 ± 5.0	13.1 ± 5.5
Pb level range	3.9 to 23.5	6.4 to 49.3	4.3 to 50.3	3.9 to 45.5	3.3 to 38.8	3.5 to 32.7	4.8 to 37.2

Note to Table 2: The yearly mean blood lead levels were not significantly different between men and women, men and the combined cohort, or women and the whole cohort (data not shown.)



**Table 3**  
 Number of Voxels within Significant Gray Matter Clusters Associated with Mean Childhood Blood Lead

Year	Population	All	Frontal	Temporal	Parietal	Cerebellar
1	Group	855	0	0	855	0
	Men	5334	842	0	0	0
	Women	0	0	0	0	0
2	Group	0	0	0	0	0
	Men	5202	2502	0	0	0
	Women	0	0	0	0	0
3	Group	26304	14151	871	938	1944
	Men	34279	22788	1246	4898	1246
	Women	0	0	0	0	0
4	Group	34253	22913	0	5746	1557
	Men	86815	74836	1026	5203	872
	Women	0	0	0	0	0
5	Group	41116	29538	0	5949	5629
	Men	91692	89867	0	812	0
	Women	2992	0	0	0	0
6	Group	68028	44207	0	10978	12843
	Men	84784	78956	0	1982	0
	Women	22770	0	1791	0	2101