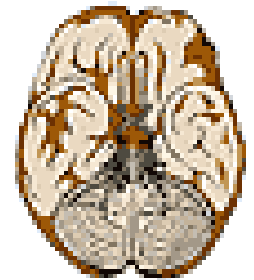


Particulate matter and the Central Nervous System.

Brain inflammation and neurodegeneration in exposed children, adolescents, and young adults

Lilian Calderón-Garcidueñas MD PhD



We have preliminary data to support the hypothesis that chronic exposure to air pollutants is associated with brain inflammation, and neuronal, endothelial and smooth muscle accumulation of the 42-amino acid form of β -amyloid ($A\beta_{42}$) in cognitively intact adults (average age 58.1 ± 13.8 years) (Tox Path 2004)

Brain inflammation and $A\beta_{42}$ accumulation precede the appearance of neuritic plaques and neurofibrillary tangles, hallmarks of **Alzheimer's disease. The accumulation of $A\beta_{42}$ is noteworthy in this environmental setting because this form of $A\beta$ is far more prone to oligomerization and fibril formation than the more abundant $A\beta_{40}$ isoform. Chronic inflammatory processes play an important role in the pathogenesis of AD.**



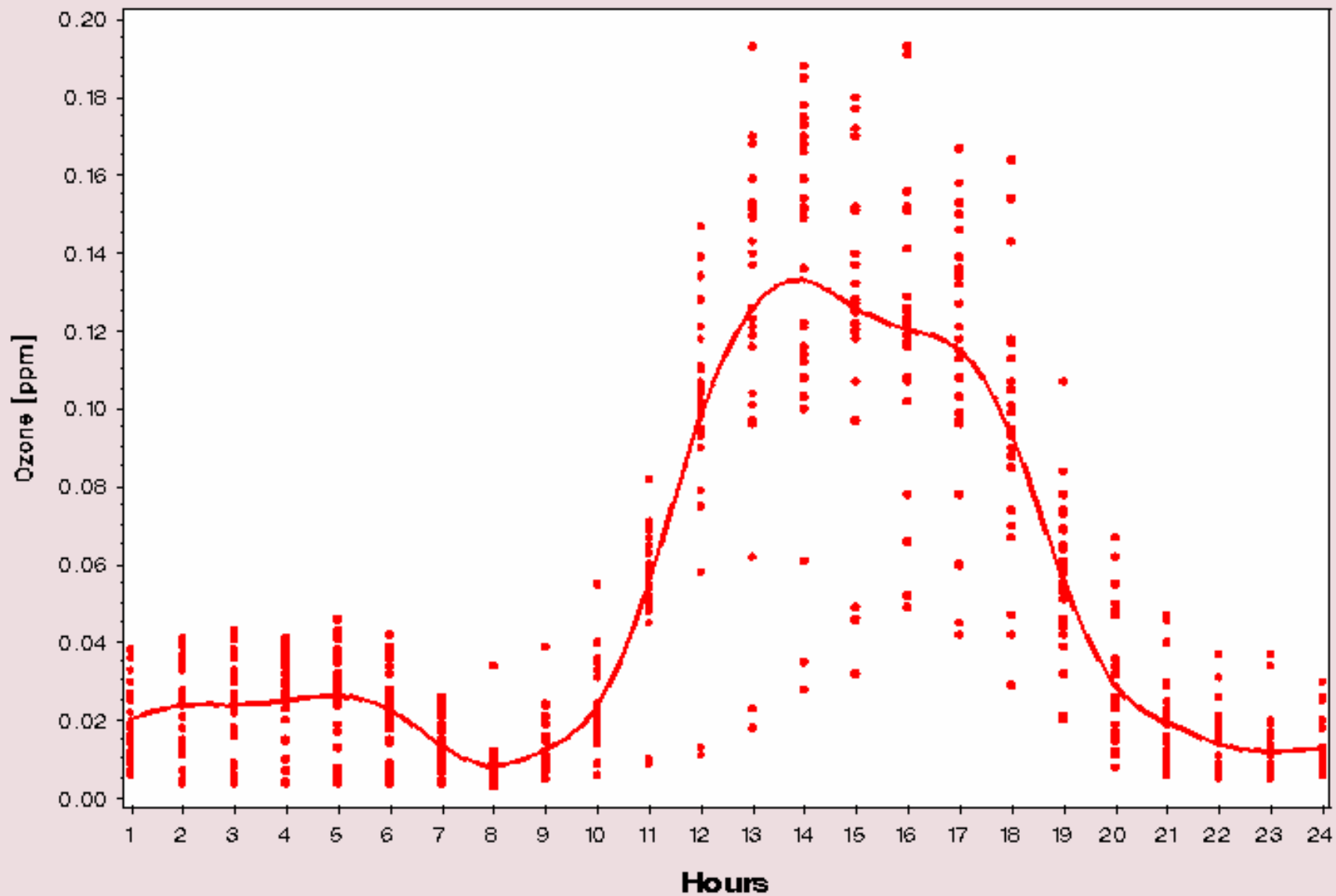






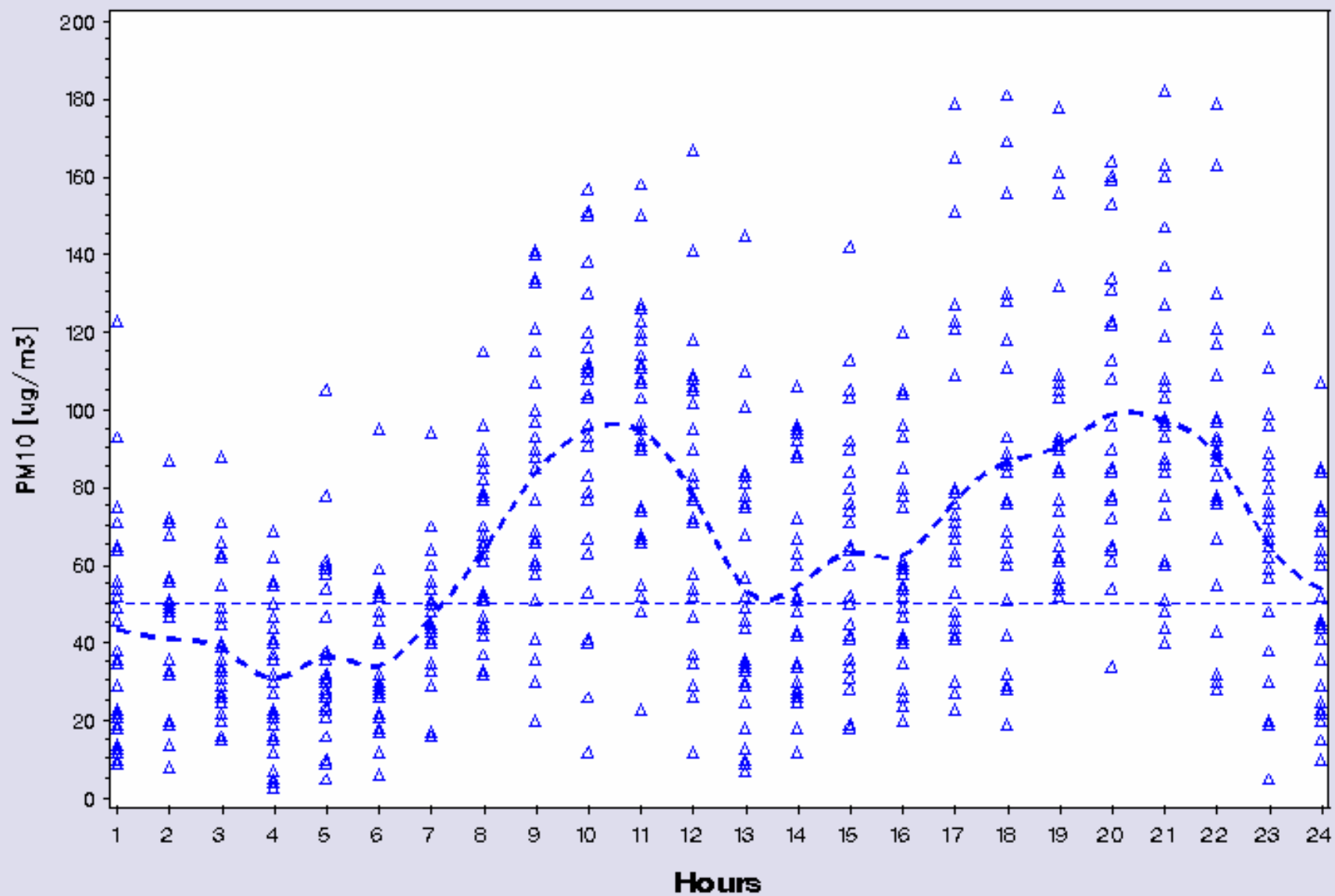
Mexico City 1999

month= February

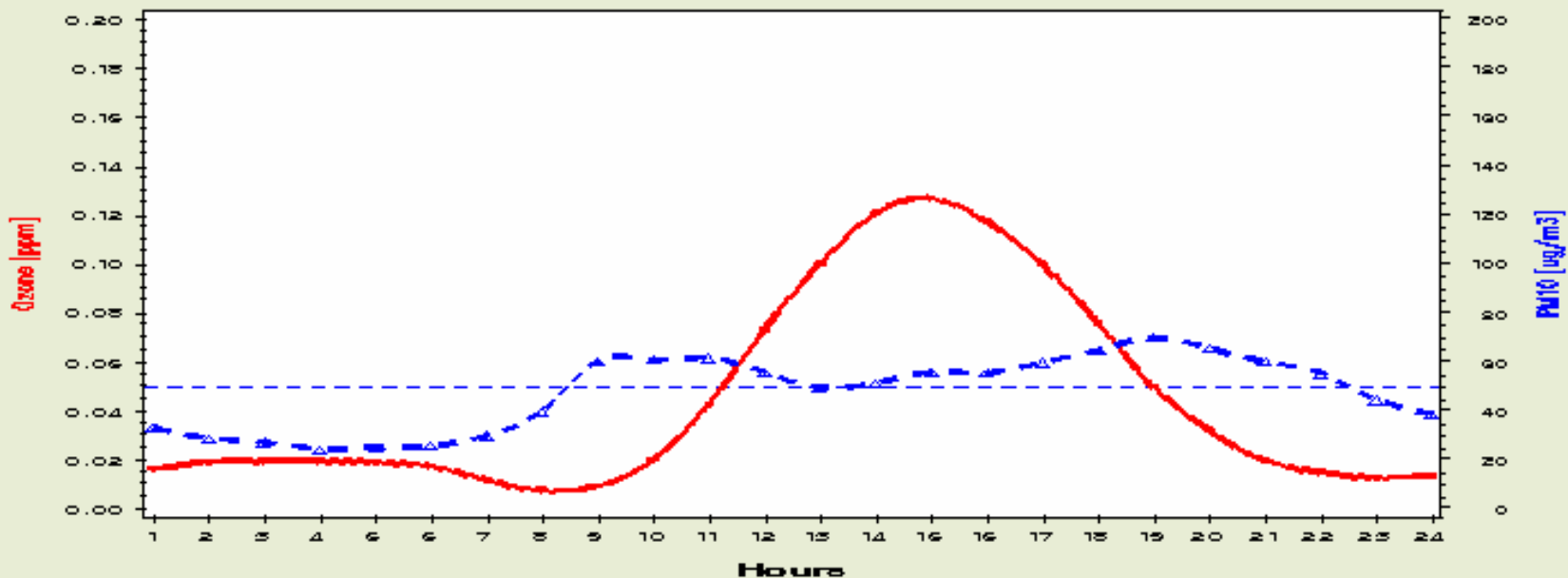


Mexico City 1999

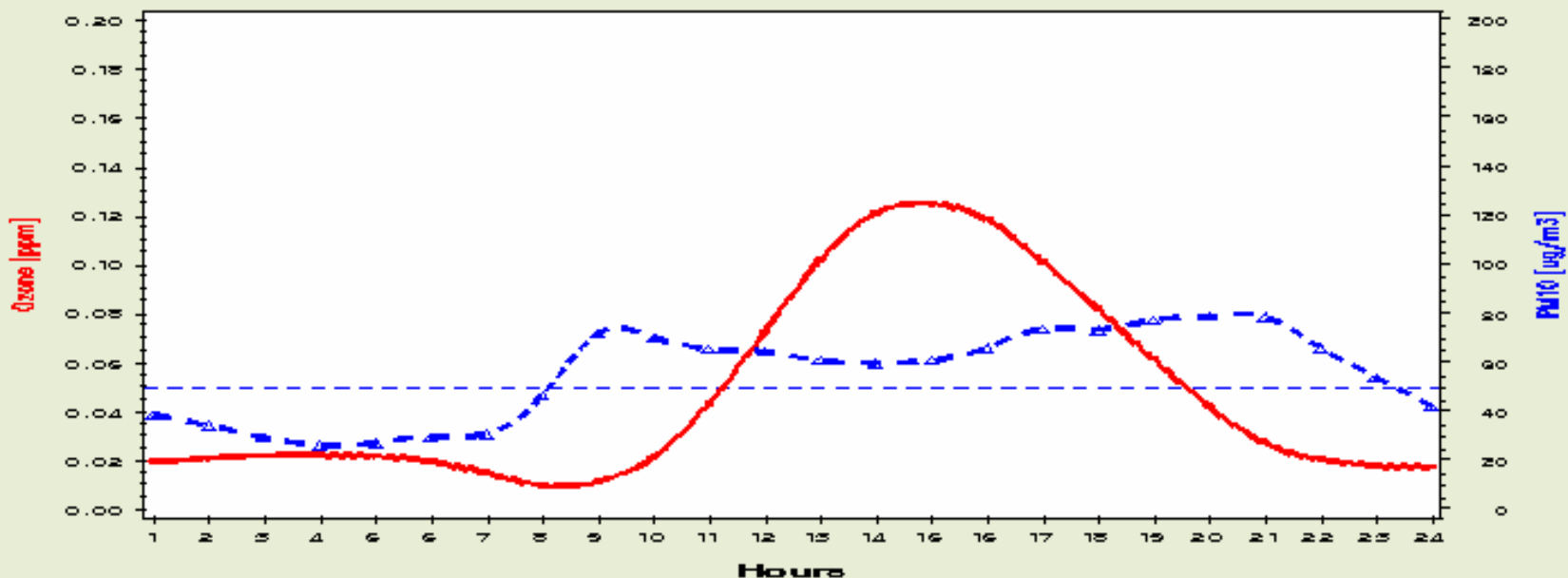
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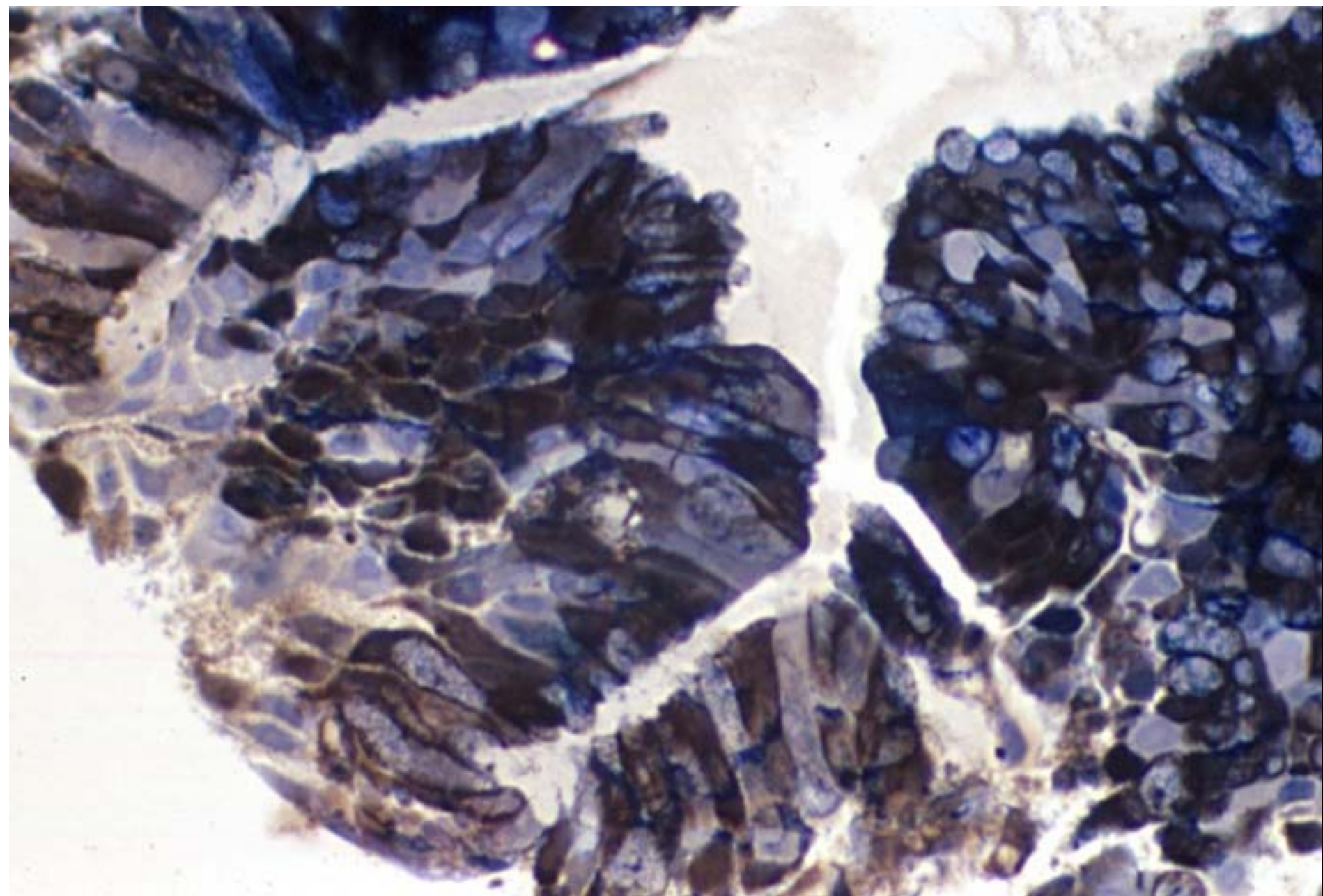


year= 1999



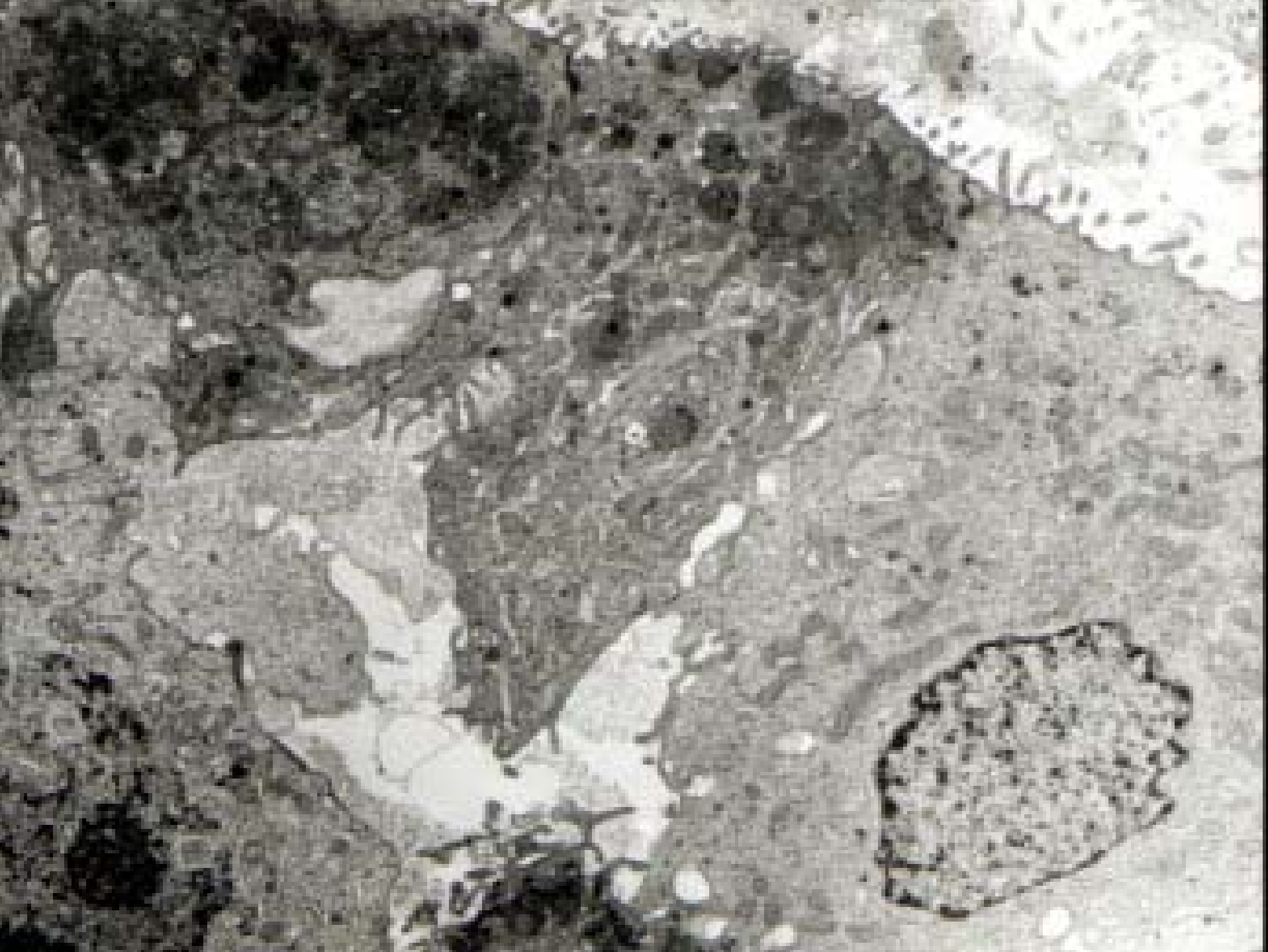
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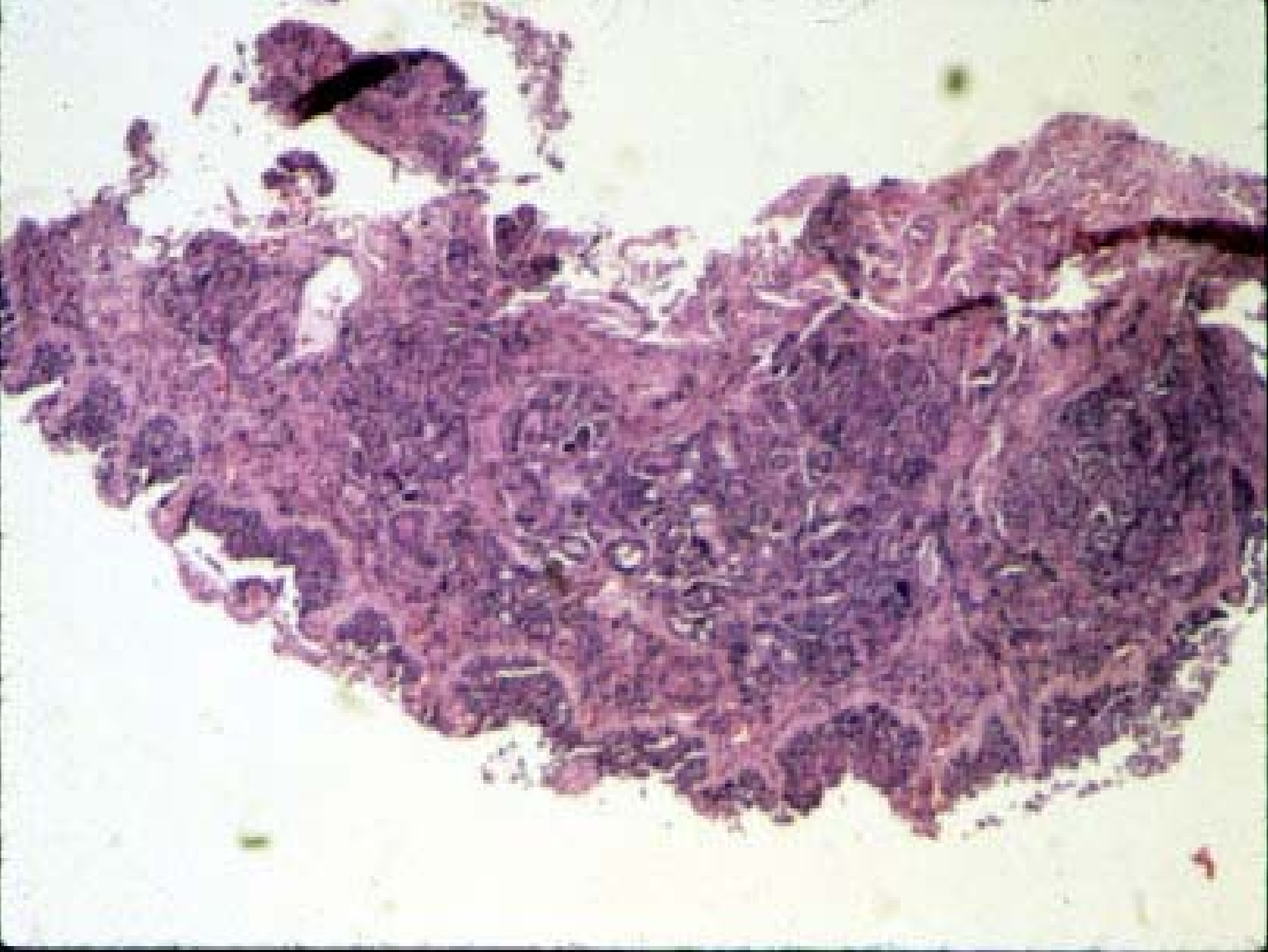




Normal nasal epithelium EM

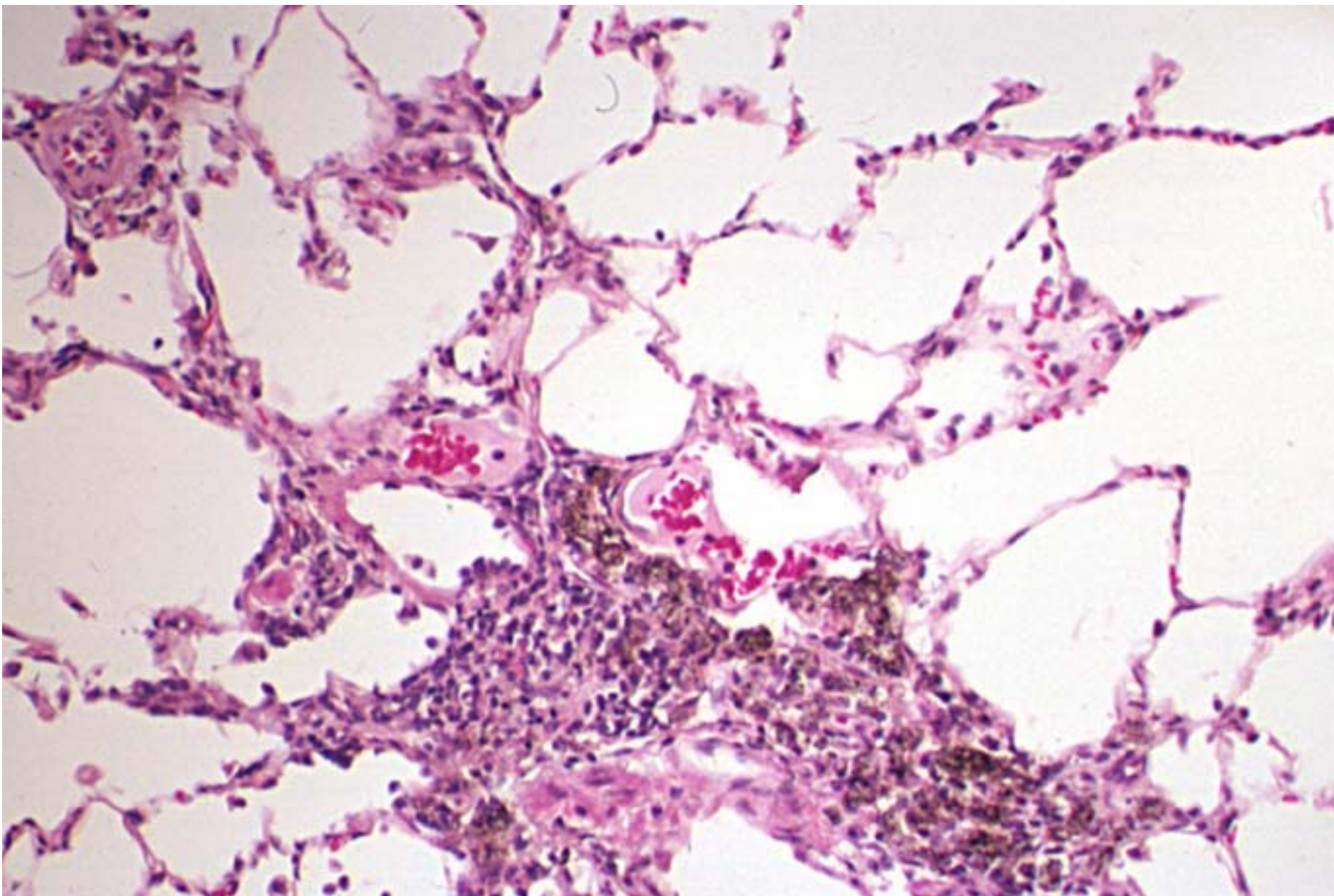


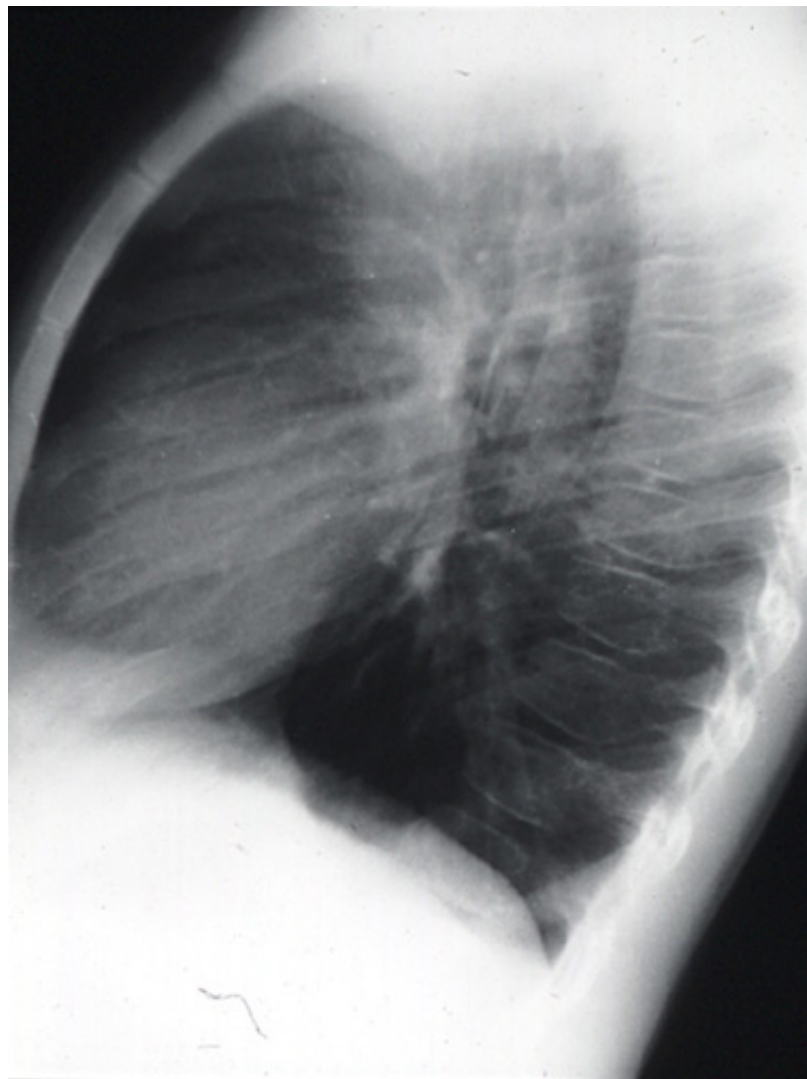




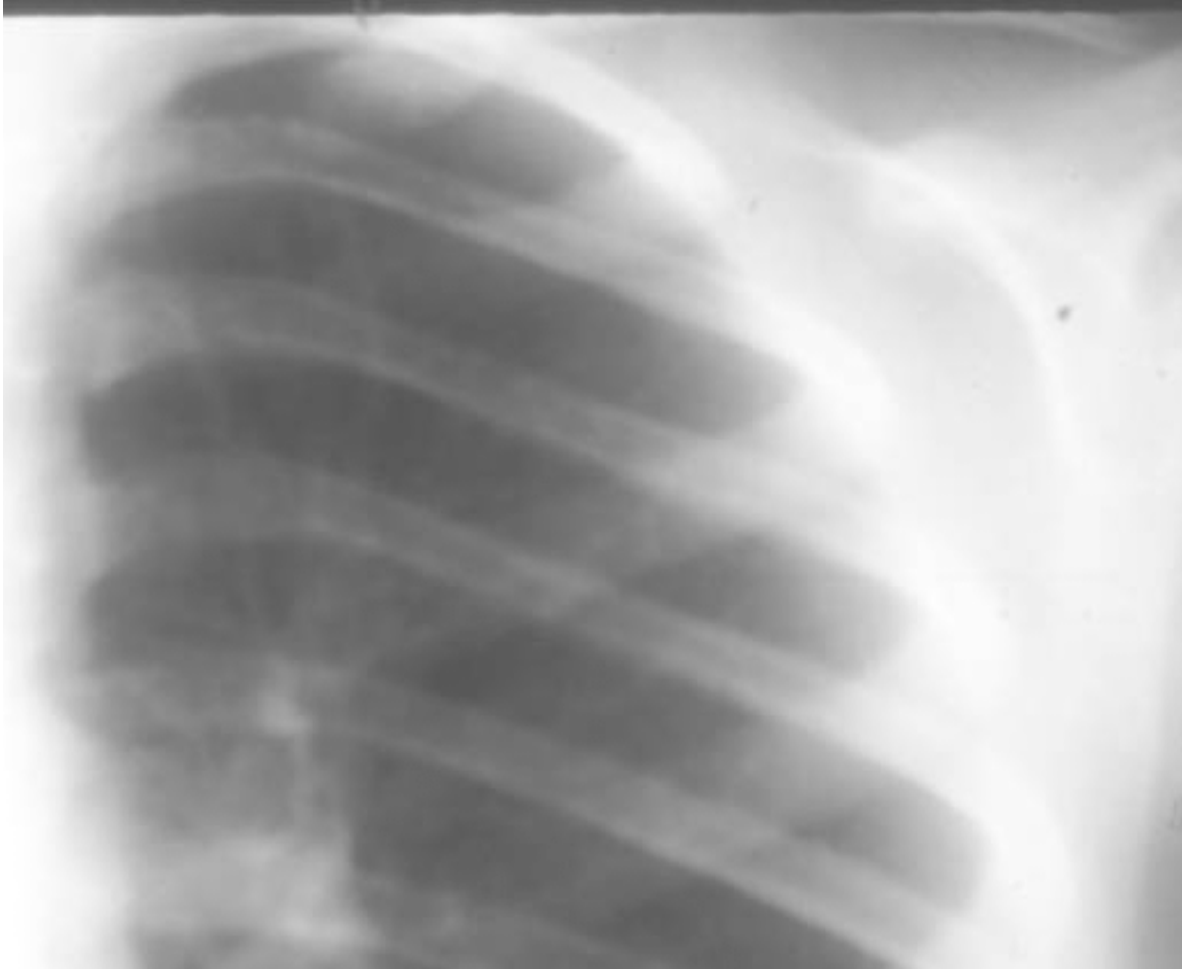
Nasal respiratory and olfactory breakdown

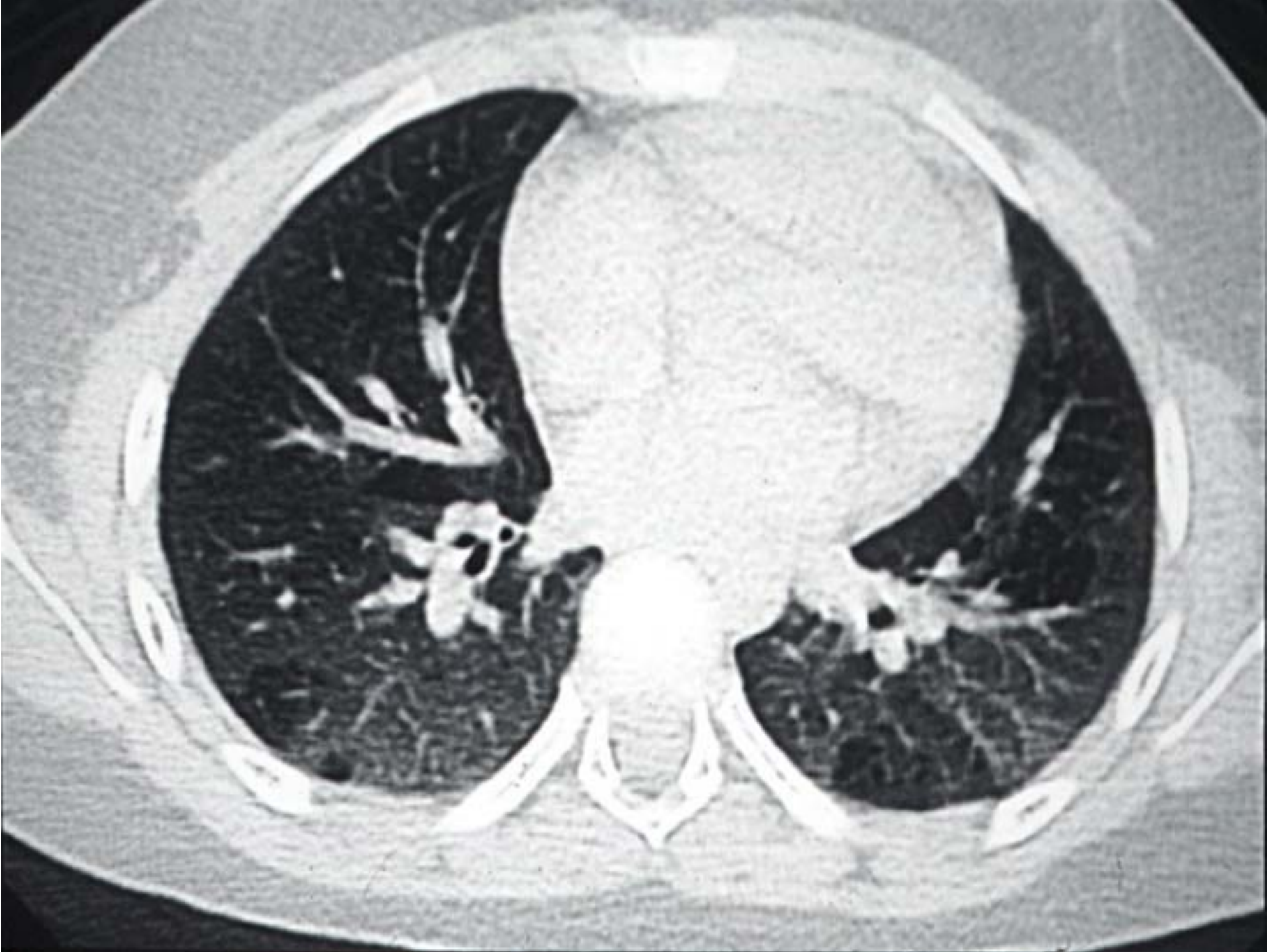
- **14% of the inspired air goes through the olfactory region**
- **The nose is a prime portal of entry of pollutants to the brain**
- **Direct contact between the environment and the olfactory bulb (OB), the first synaptic relay from the olfactory system**

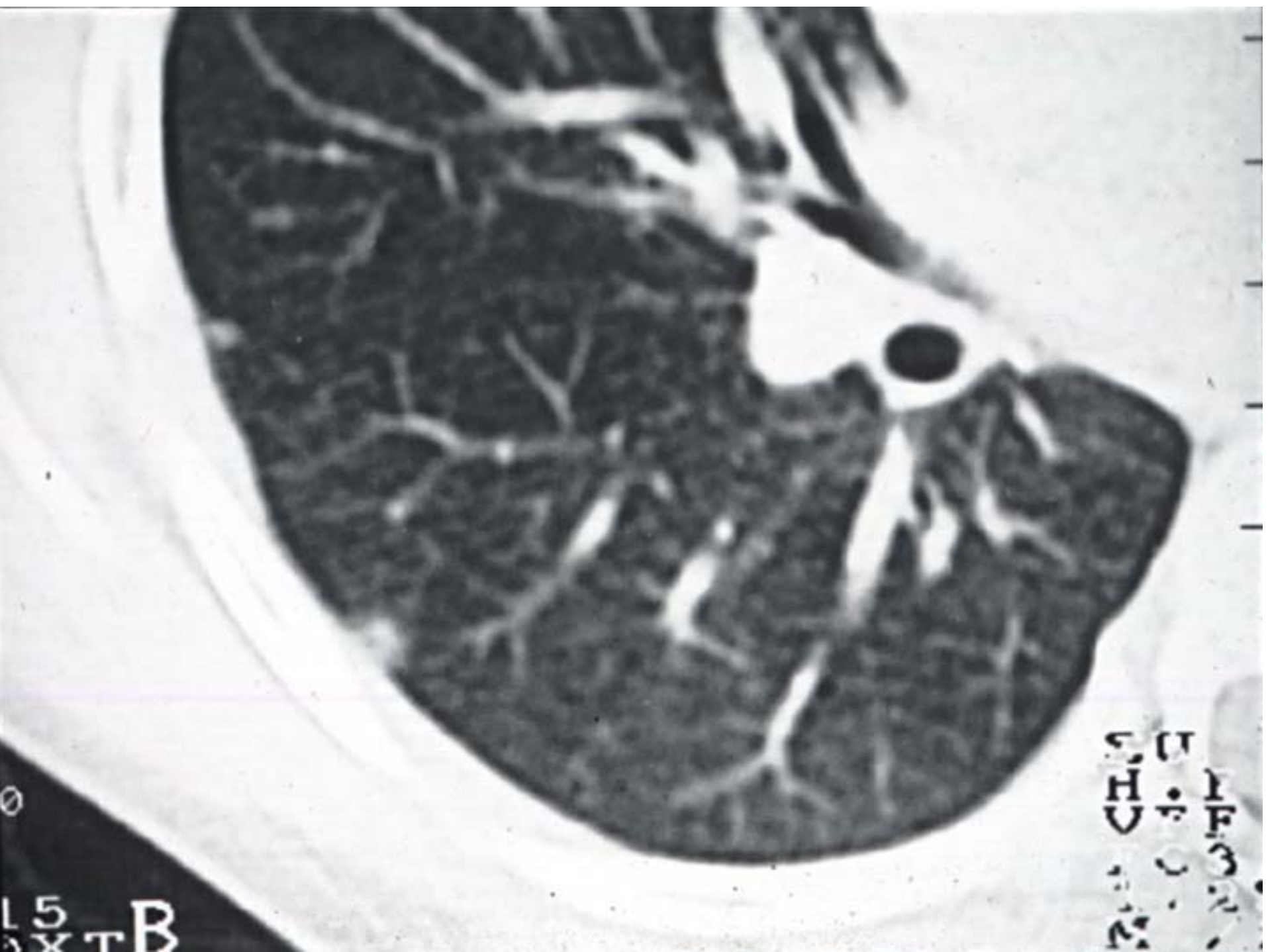




Results: Interstitial markings

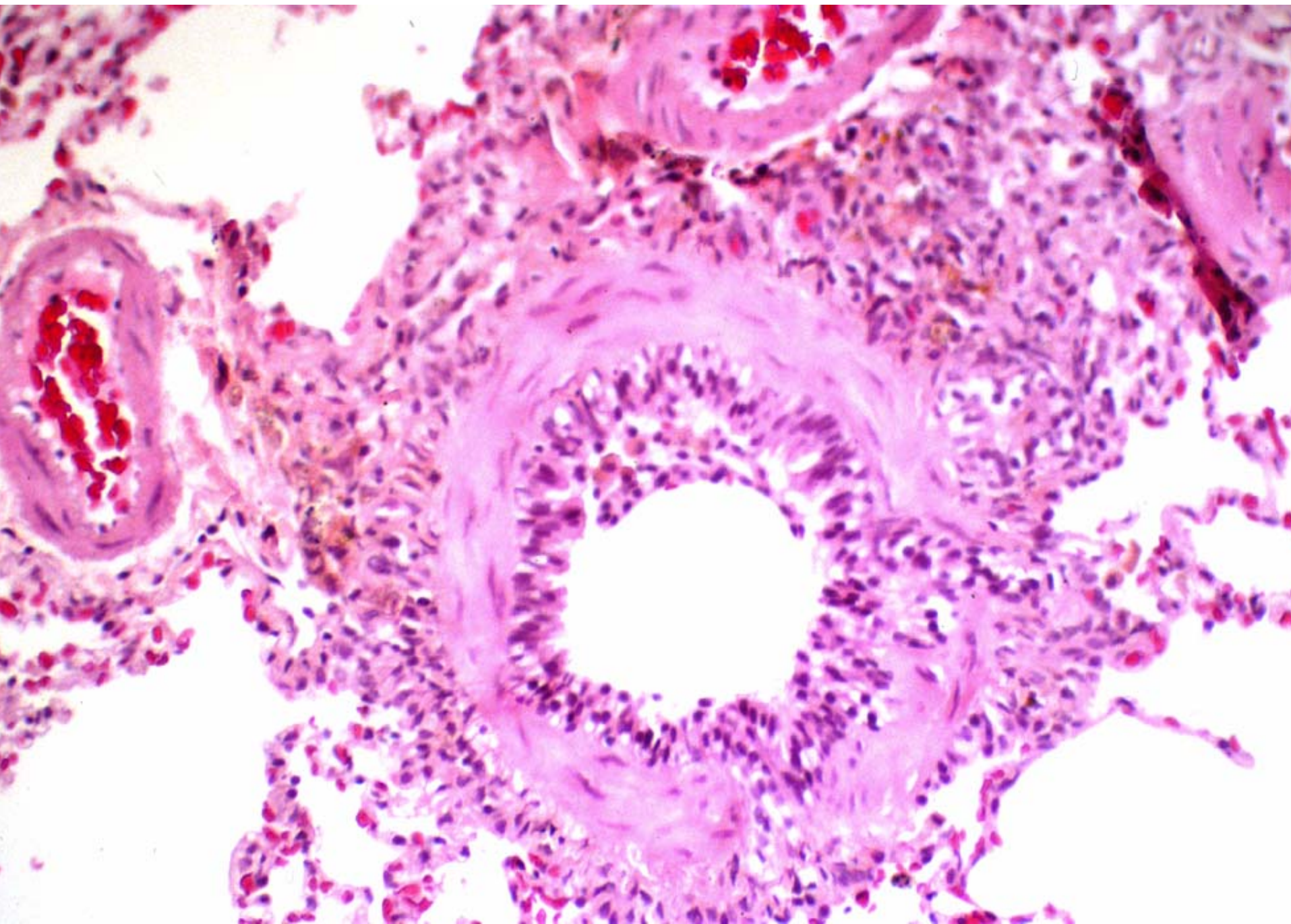




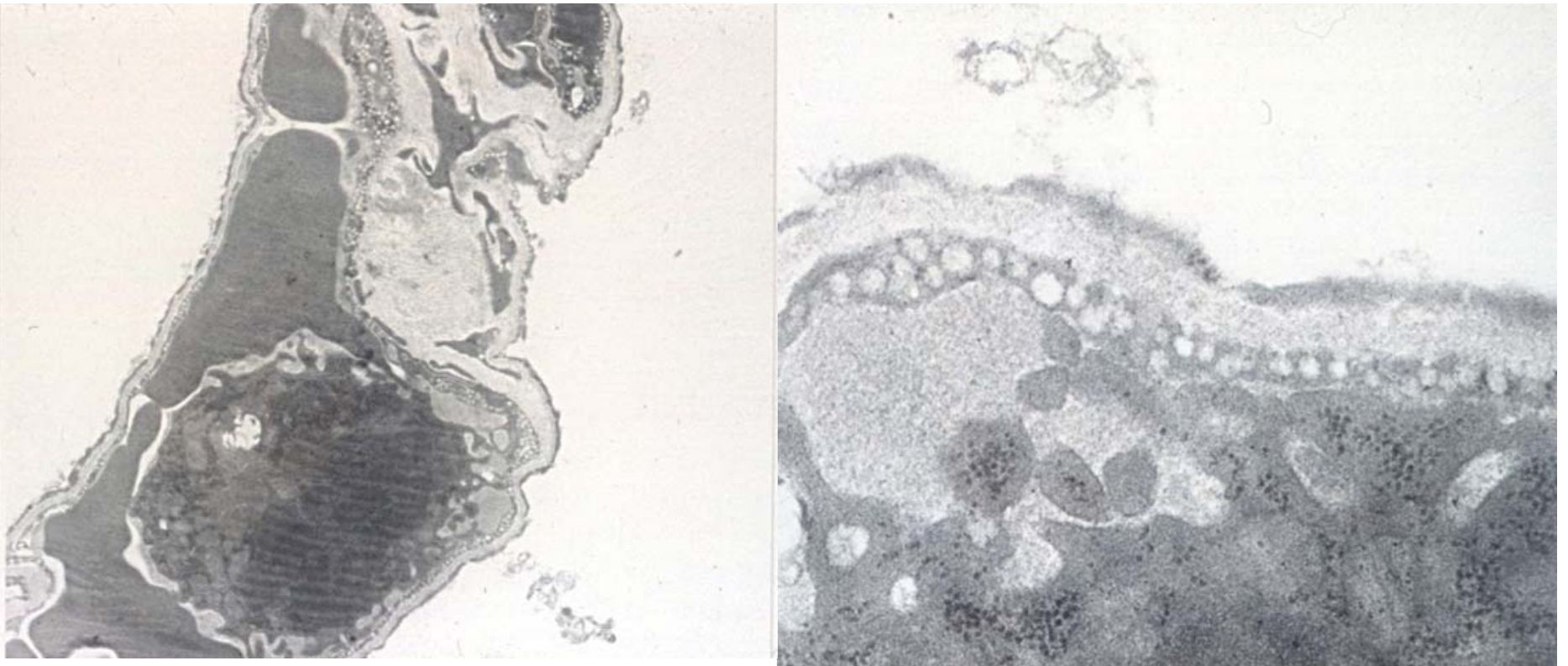


L5
XTB

CHU
10.1.9



Direct PM to the brain: intravascular macrophage-like cells



Serum Cytokines (mean \pm SD)

Cytokine	Control	SWMMC
TNF α	9.7 \pm 5	8.8 \pm 7.4
ET-1\checkmark	1.2 \pm 0.06	2.4 \pm 0.1
IL6*	2.1 \pm 0.98	4 \pm 3.7
IL8**	18.8 \pm 8.2	15.8 \pm 3.2
IL10***	4.6 \pm 2.9	19.9 \pm 3.9

* p 0.03, **p 0.01, ***p 0.0002 \checkmark 0.001

Alzheimer' Disease

- Over 7% of people >65y has AD McDowell 2001
- The proportion of new onset cases >85 will increase from 40% in 1995 to 62% in 2050
- Baby boomers (born between 1946 and 1964) will be affected substantially
- AD will affect 13.2 million USA citizens by 2050

Current hypothesis for Alzheimer's Disease

The Amyloid Hypothesis Dennis Selkoe

- **Autosomal Dominant forms**
- **Missense mutations APP, presenilin1 or 2 genes**
- **Increased A β 42 production throughout life**
- **1. Accumulation and oligomerization of A β 42 in limbic and association cortices**
- **3. Gradual deposition of A β 42 oligomers as diffuse plaques**
- **6. Altered kinase phosphatase activities =tangles**
- **7. Widespread neuronal and synaptic dysfunction**
- **Sporadic Forms**
- **Failure of A β clearance mechanisms (ie APOE4)**
- **Gradually rising A β 42 in the brain**
- **2. Subtle effects of A β 42 oligomers on synaptic efficacy**
- **4. Microglial and astrocytic activation**
- **5. Altered neuronal ionic homeostasis, oxidative injury**
- **8. DEMENTIA**

Are particles a risk factor for Alzheimer's ?

Calderón-Garcidueñas L, Reed W, et al

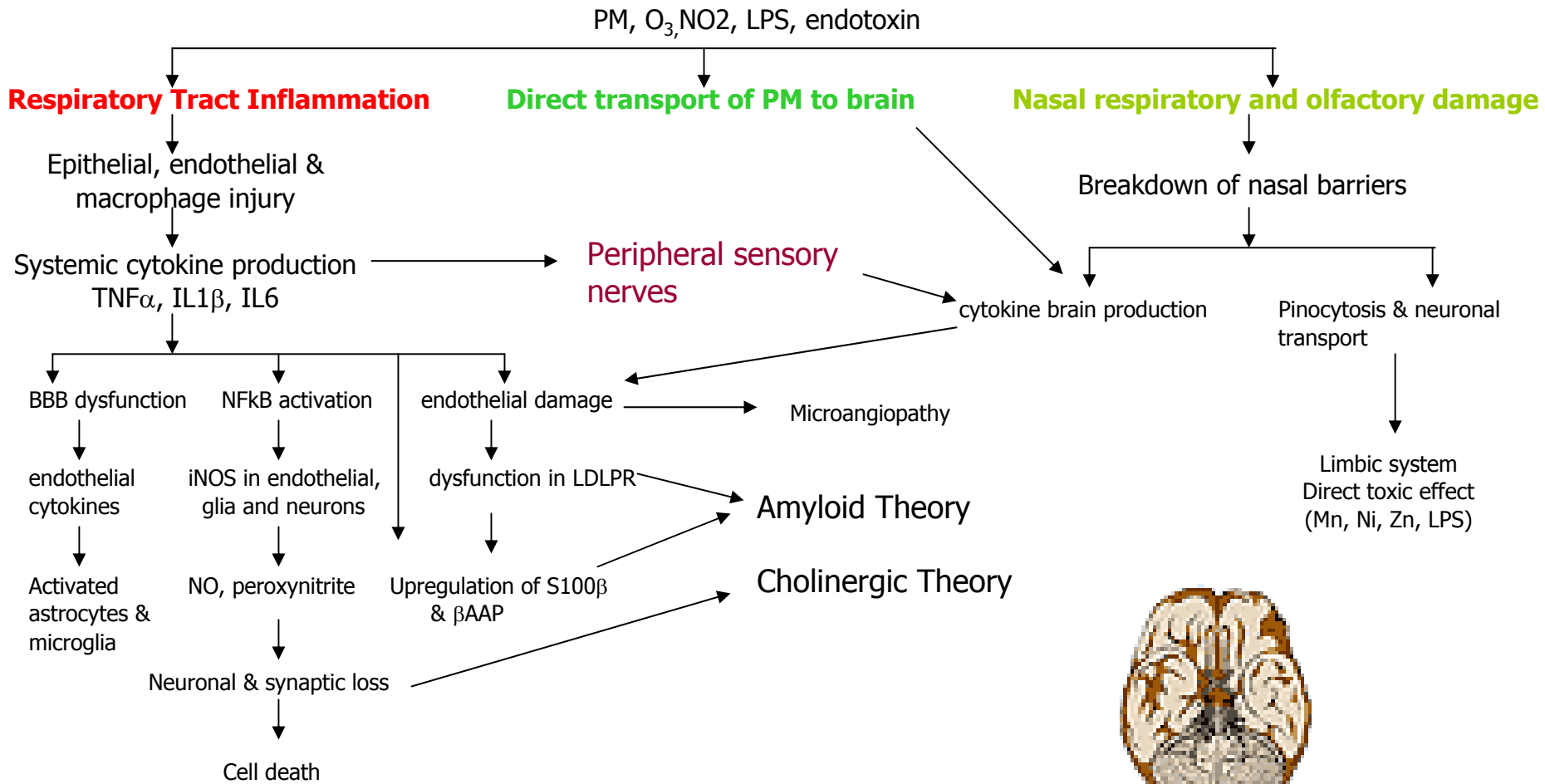
- Dogs exposed to severe air pollution exhibit chronic brain inflammation and acceleration of Alzheimer's-like pathology
- Residents of cities with severe air pollution have significantly higher COX2 expression in frontal cortex and hippocampus and greater neuronal and astrocytic accumulation of A β 42 compared to residents in low air pollution cities
- These findings suggest that exposure to severe air pollution in humans is associated with brain inflammation and A β 42 accumulation, two causes of neuronal dysfunction that precede the appearance of neuritic plaques and neurofibrillary tangles, hallmarks of Alzheimer's disease.

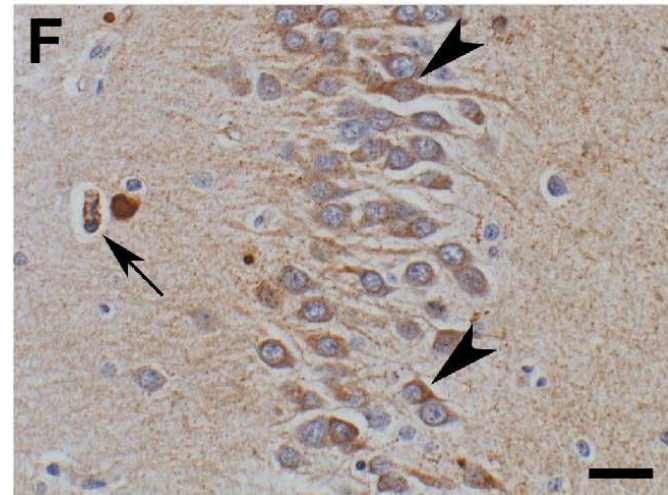
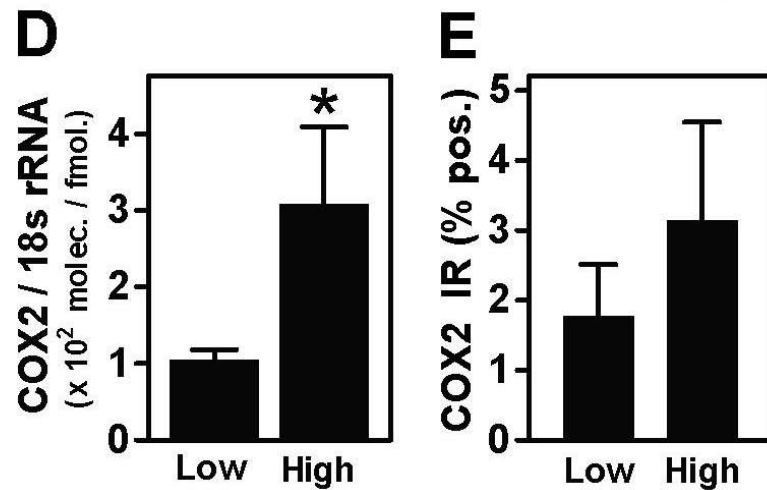
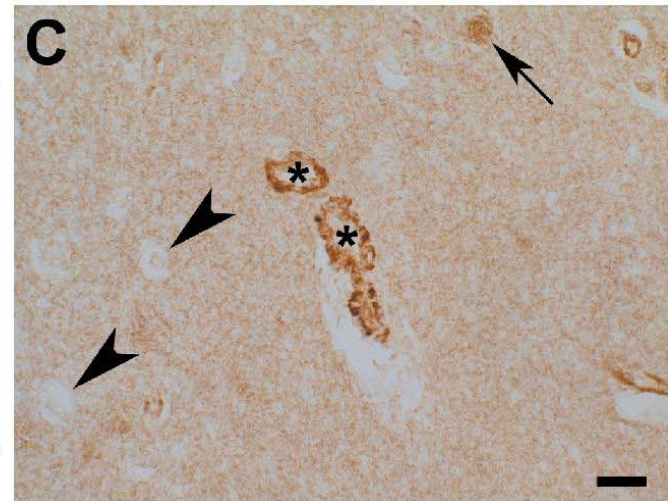
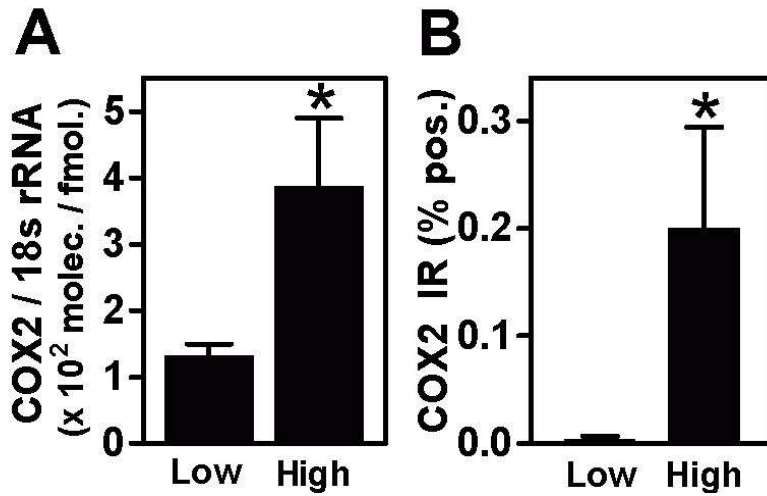
Are particles a risk factor for Alzheimer's ?

Calderón-Garcidueñas L, Reed W, et al

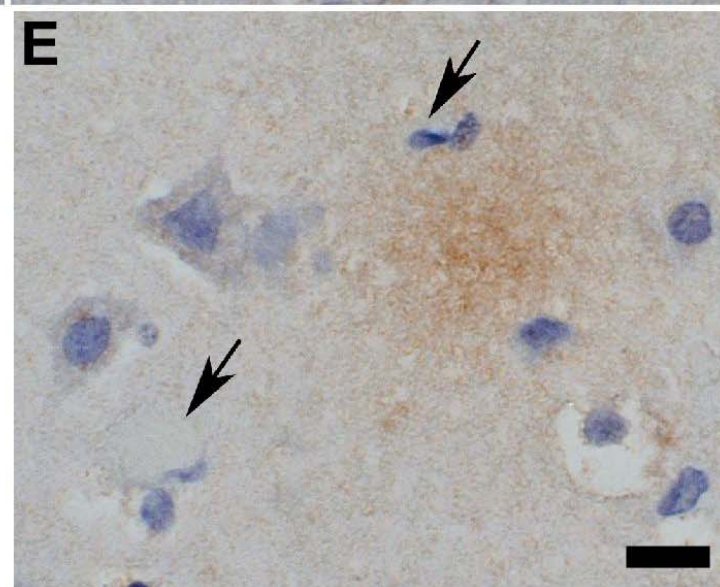
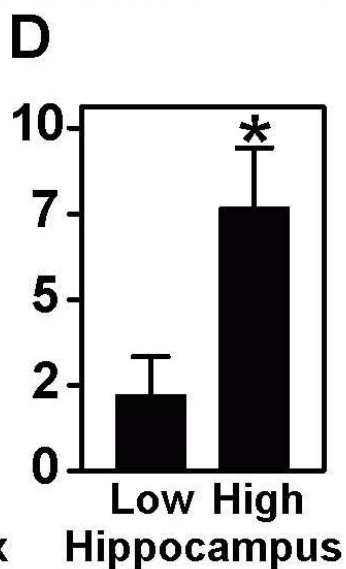
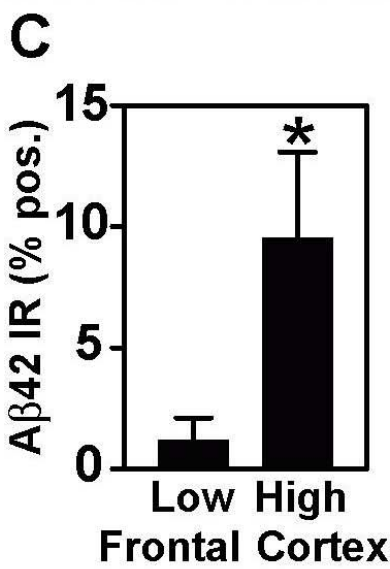
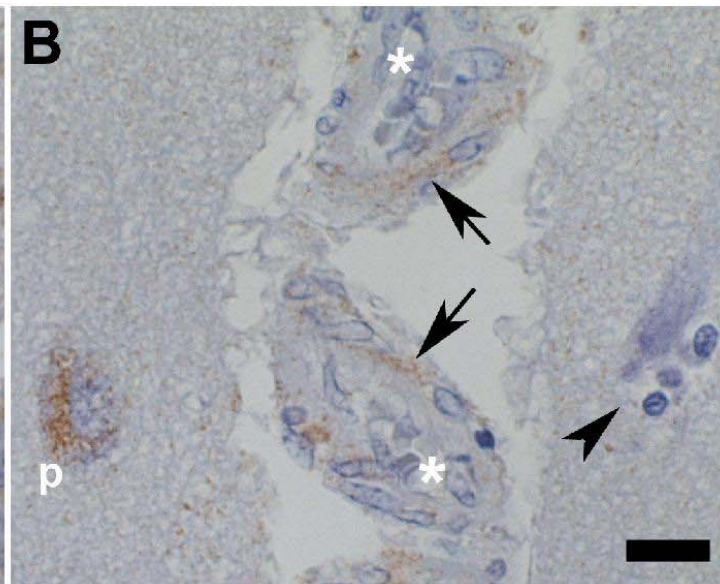
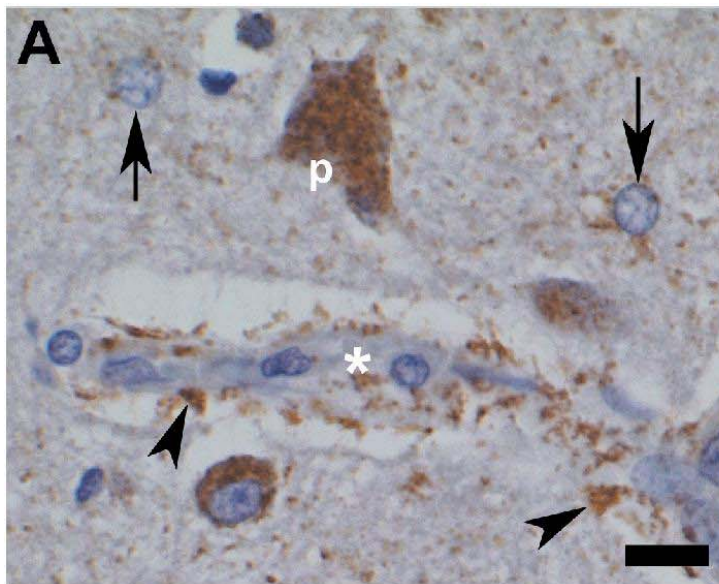
- **Pathways of brain damage by PM**
- **i. Respiratory tract inflammation and secondary systemic inflammation**
- **ii. Nasal respiratory and olfactory damage and breakdown of barriers**
- **iii. Direct transport of PM to the brain**
- **iv. Mixed cranial nerves**

Air Pollution and Brain Damage





Tox Path 2004;32:650-658 (58.1 ± 13.8y)



Tox Path 2004;32:650-658 (58.1 ± 13.8y)

New data

The current study focused on children, adolescents and young adults.

Our primary question was:

Do Apo E 4 -clinically healthy and cognitively intact children, adolescents and young adults, exhibit an upregulation of inflammatory genes and accumulation of A β 42 in brain target areas?

Objectives

The objective of this work was to determine if a **younger population** chronically exposed to severe air pollution exhibits evidence of brain inflammation and A β 42 accumulation.

We studied subjects from two age- and gender-matched cohorts of clinically cognitively and neurologically intact **children, adolescents and young adults, all of whom died sudden deaths.**

The high exposure cohort (**n:28, 23.6 \pm 8.6 years**) was composed of residents of Mexico City who were chronically exposed to ozone (O₃) and particulate matter (PM) air pollution levels that regularly exceeded the U.S. air quality standards. The control cohort (**n:10, 22.6 \pm 6.5y**) included subjects from 2 cities with pollution levels that rarely exceed the standards.

We compared the expression of mRNAs encoding the inflammatory mediator genes cyclooxygenase-2 (COX2) and interleukin-1b (IL-1 β) and localized A β 42 in olfactory bulb, frontal cortex, and hippocampus.

Methods

Autopsy materials: All subjects died sudden deaths. Autopsies were performed 4.1 ± 1.3 h after death. All subjects had complete autopsies and neuropathological examinations, and were included in the immunohistochemical (IHC) and the mRNA abundance (RT-PCR) studies.

Data available for all subjects included age, gender, place of birth, place of residency, occupation, years of completed education, academic outcomes, smoking habits, clinical histories, cause of death, and time between death and autopsy.

The selected cohorts had no clinical history or pathological evidence of short or long-term inflammatory processes, administration of anti-inflammatory drugs or hormones, or events such as cerebral ischemia, head trauma or epilepsy.

Materials and Methods

- **Real-time RT-PCR:** Total RNA was extracted from frozen tissues using Trizol Reagent (Invitrogen Corp, Carlsbad CA). Random-primed first-strand cDNAs were generated and relative abundances of mRNAs encoding COX2, and IL-1 β were estimated by quantitative fluorogenic 5' nuclease (TaqMan) assay of the first strand cDNAs.
- **Histology and IHC:** Sections were taken from the olfactory bulb, superior frontal gyrus, and hippocampus, for both light and electron microscopy. Paraffin sections 8 μ m thick were cut and routinely stained with hematoxylin and eosin. IHC was performed using antibodies to glial fibrillary acidic protein (GFAP) and A β 42.
- **Data Analysis:** Statistics were performed using Stata Statistical software (College Station, TX). We applied the parametric procedure t that considers the differences among variances of the variables of interest COX2 and IL1 β . Significance was assumed at $p < 0.05$. Data are expressed as mean values \pm SEM.

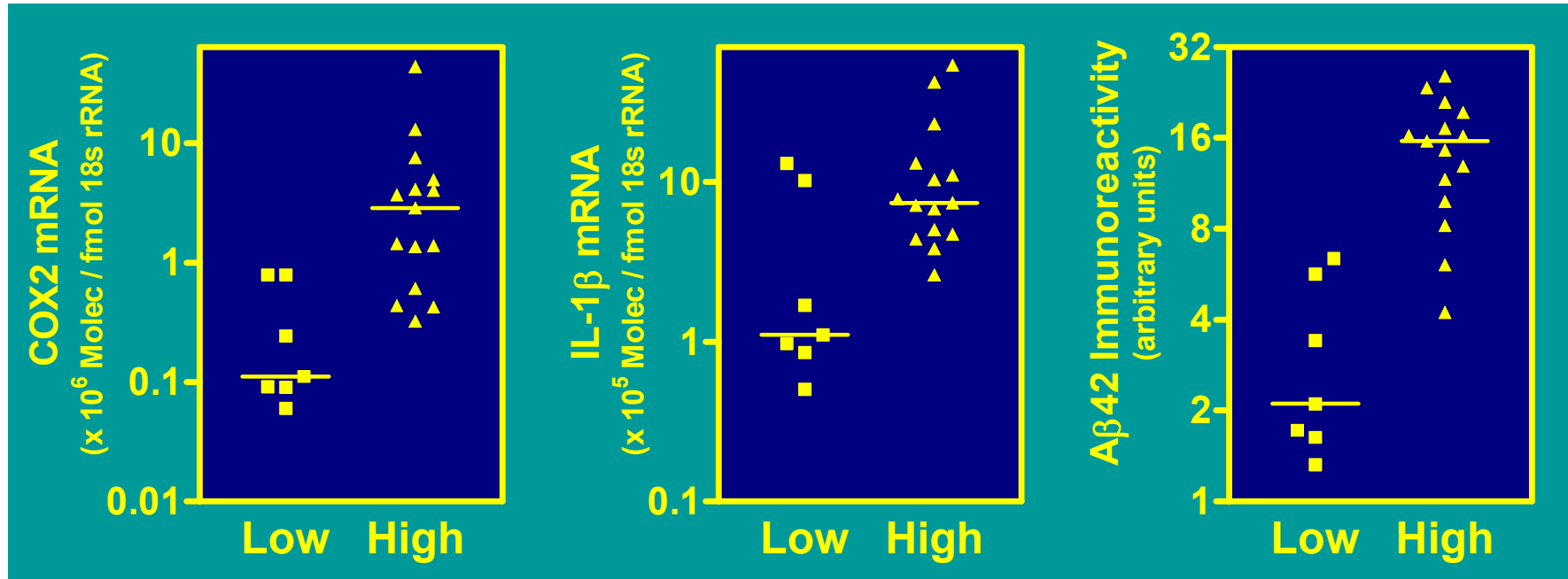
COX2 and proinflammatory cytokines

- The membrane glycoprotein inducible COX2 is regulated at transcriptional and posttranscriptional levels by pro-inflammatory agents, cytokines, growth factors, oncogenes, and tumor-promoters.
- **COX2 is an extremely potent biologically active mediator of inflammation**, involved in the conversion of arachidonic acid into prostaglandins and thromboxanes, and the synthesis of malonaldehyde, a mutagen and the production of hydrogen peroxide. COX2 is expressed in inflamed and neoplastic tissues
- Pro-inflammatory cytokines: IL1 β , IL6, IL8, IL12 and TNF-alpha
- IL1 β is a very potent pro-inflammatory cytokine playing a role in both acute and chronic neurodegenerative disorders.

Amyloid β 42

- Natural oligomers of the amyloid- β protein specifically disrupt cognitive function (Clearly et al 2005)
- A β plays a central role in the pathogenesis of neuronal dysfunction in AD
- A β is the subunit of the amyloid that is progressively deposited in neuritic plaques in the limbic and association cortices of all AD patients.
- The APP gene is on the 21q chromosome and its duplication leads to the typical AD neuropathology in middle-age Down patients.
- Inheritance of APOE4 allele increases cerebral A β burden. APOE4 is a strong genetic risk factor for AD

COX2 and IL-1 β mRNA Expression in Olfactory Bulb are Significantly Elevated in children, adolescents and young adults exposed to severe air pollution

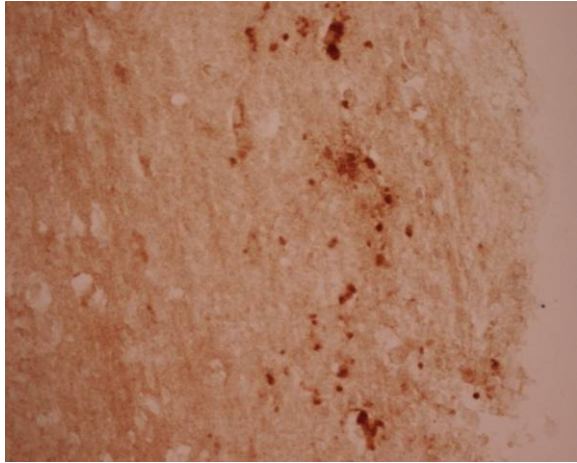


* $p < 0.002$

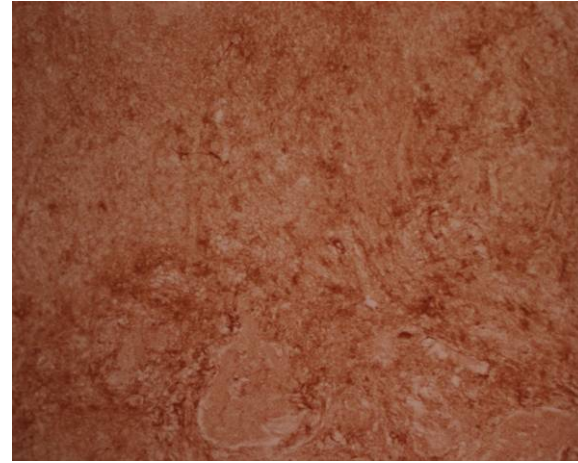
* $p < 0.024$

* $p < 0.001$

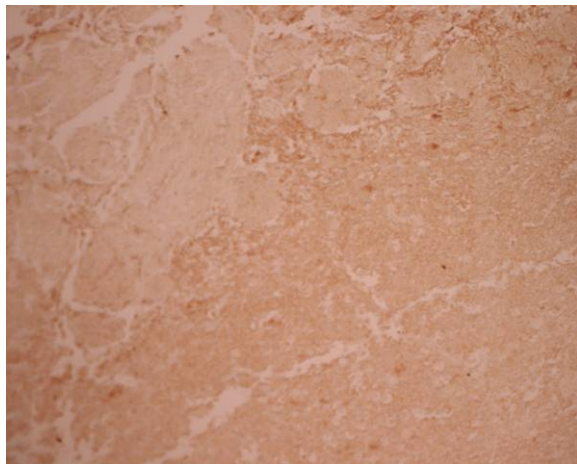
Beta amyloid 42 in olfactory bulb



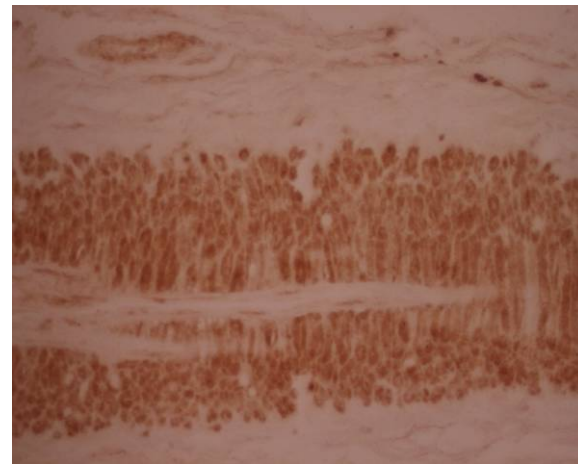
75y old male with β 42 +



42 y old male with significant deposition of β A
In the glomerular region of the OB

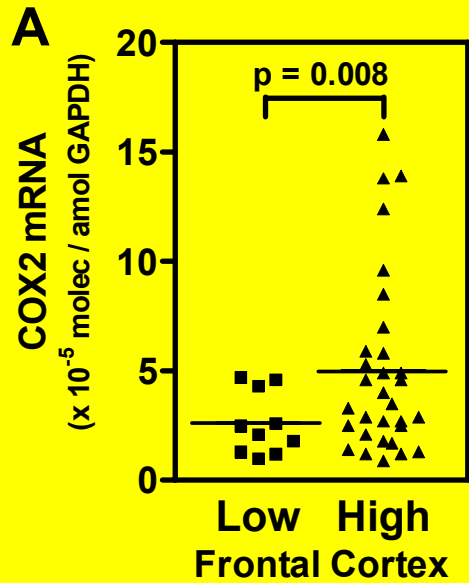


14y old male with few + β A cells in OB

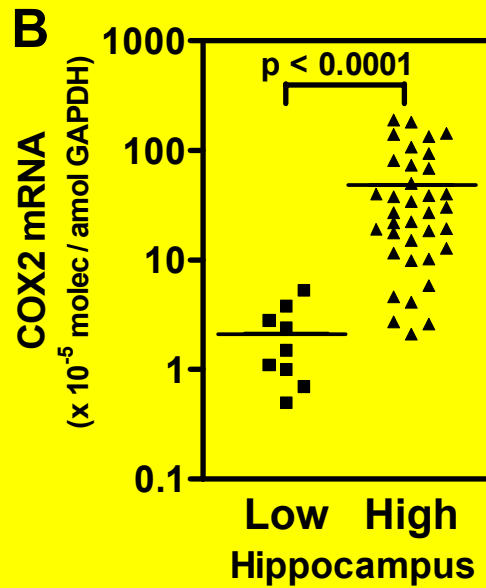


14y old male with intense β A in
smooth muscle cells, arterial OB

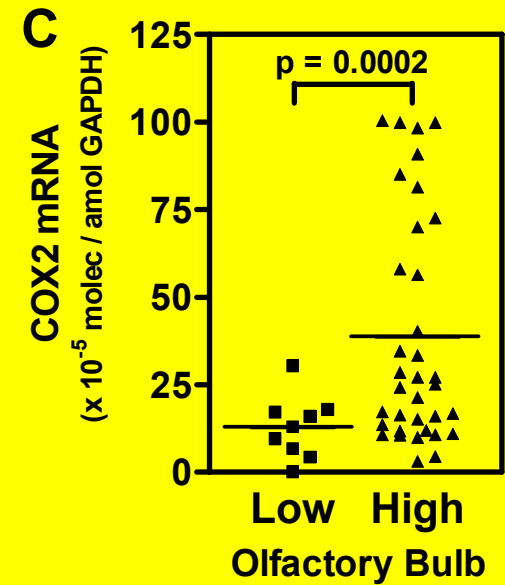
COX2 mRNA in frontal cortex, hippocampus and olfactory bulb



P=0.008

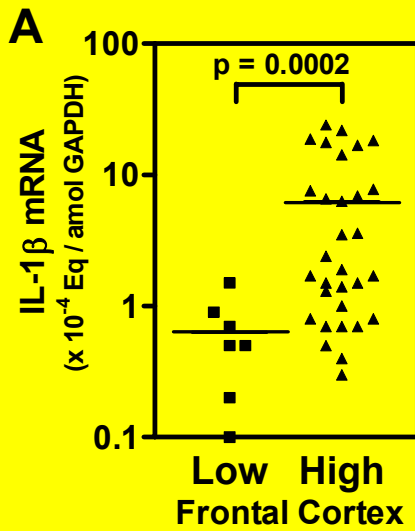


P<0.0001

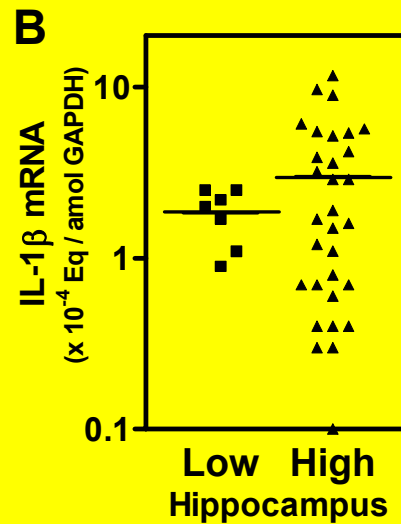


P=0.0002

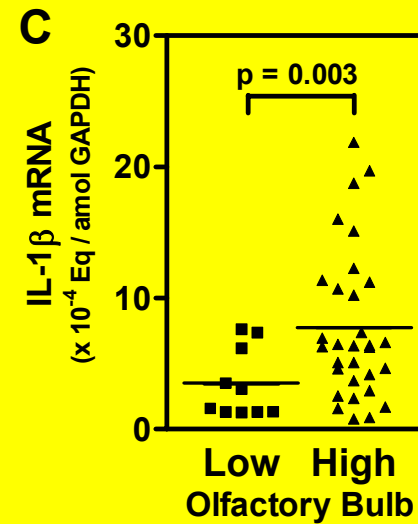
IL-1 β mRNA in frontal cortex, hippocampus and olfactory bulb



P=0.002



NS

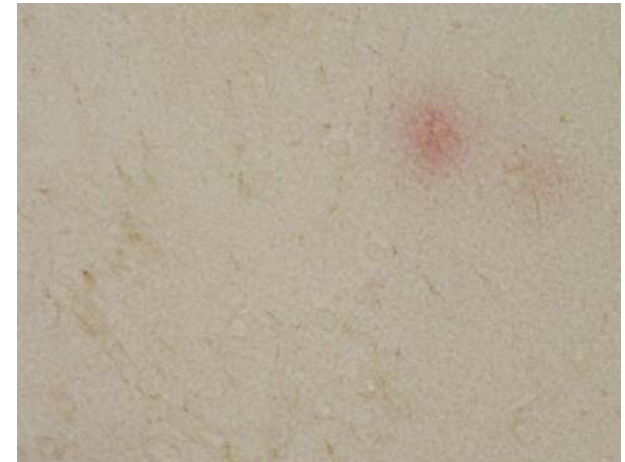
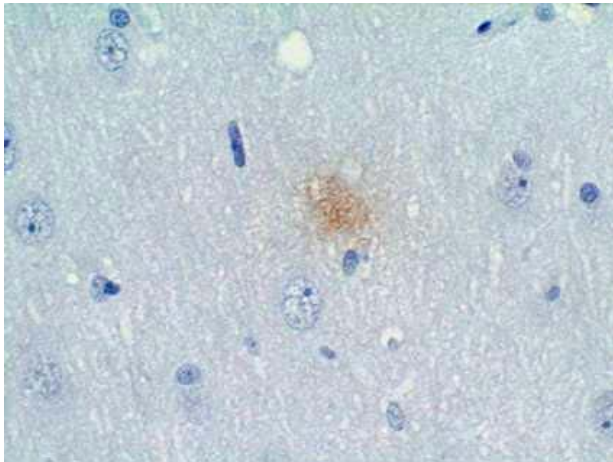
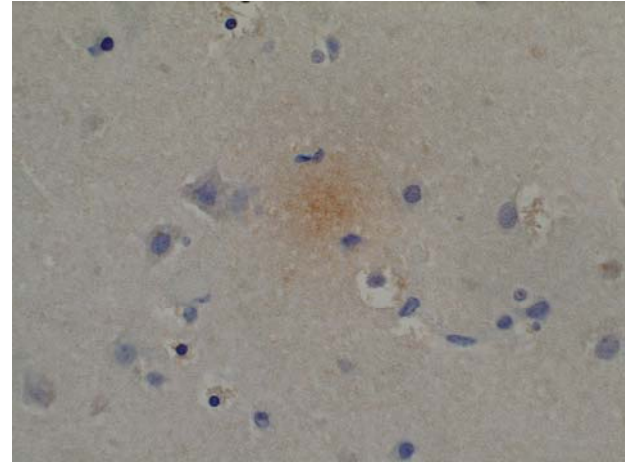
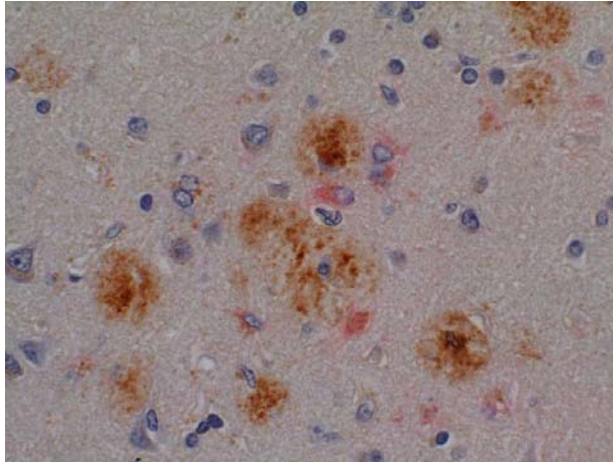


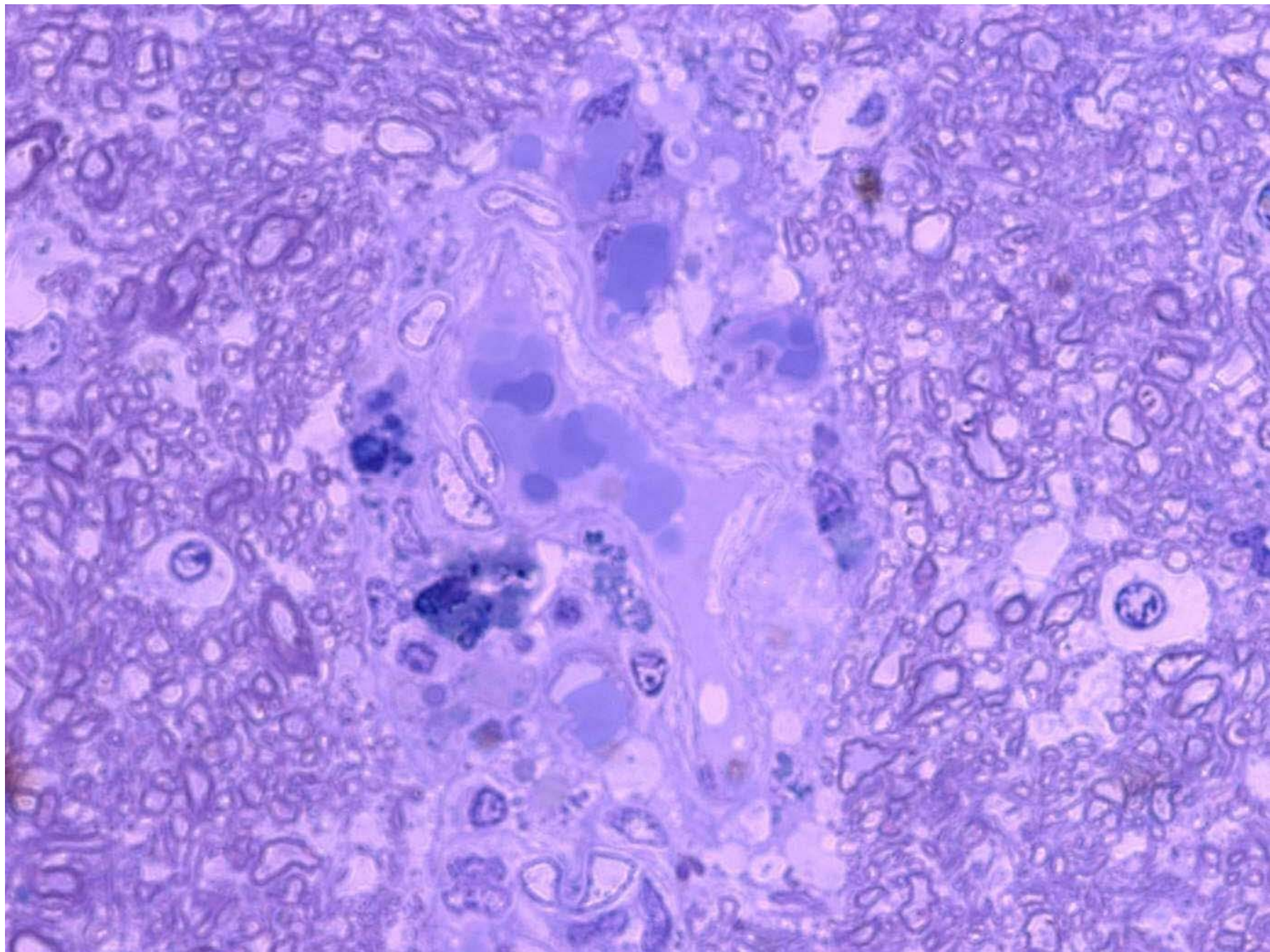
P=0.003

RT-PCR RESULTS

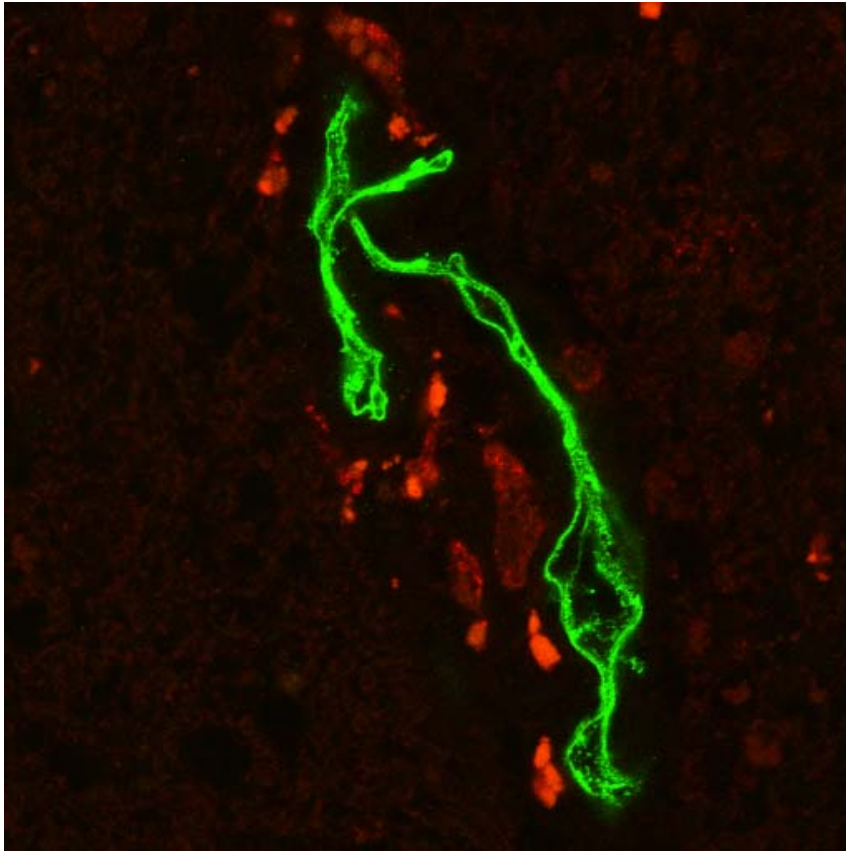
We found a significant upregulation of COX2 and IL1 β mRNA expression in olfactory bulb and frontal cortex of young subjects residing in Mexico City. The highly exposed subjects exhibited significant lung inflammation, A β 42 deposition in neurons, astrocytes and smooth muscle cells of blood vessels in the olfactory bulb and diffuse amyloid plaques in the frontal cortex.

Beta Amyloid 1-42 Plaques

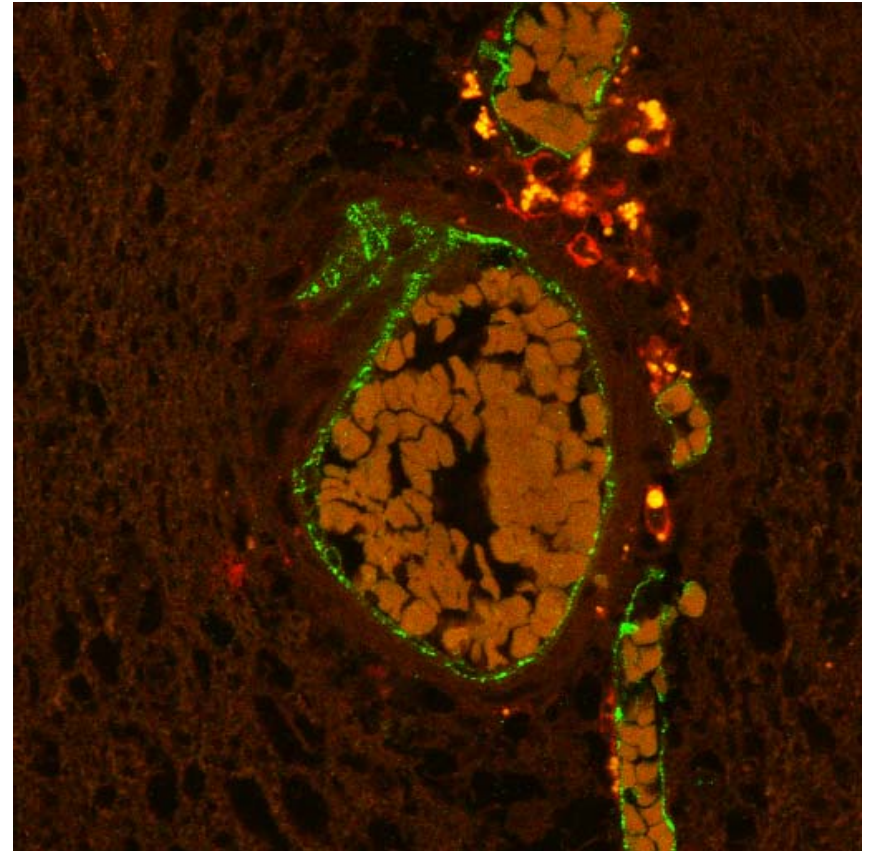




CD163 + brain perivascular macrophages

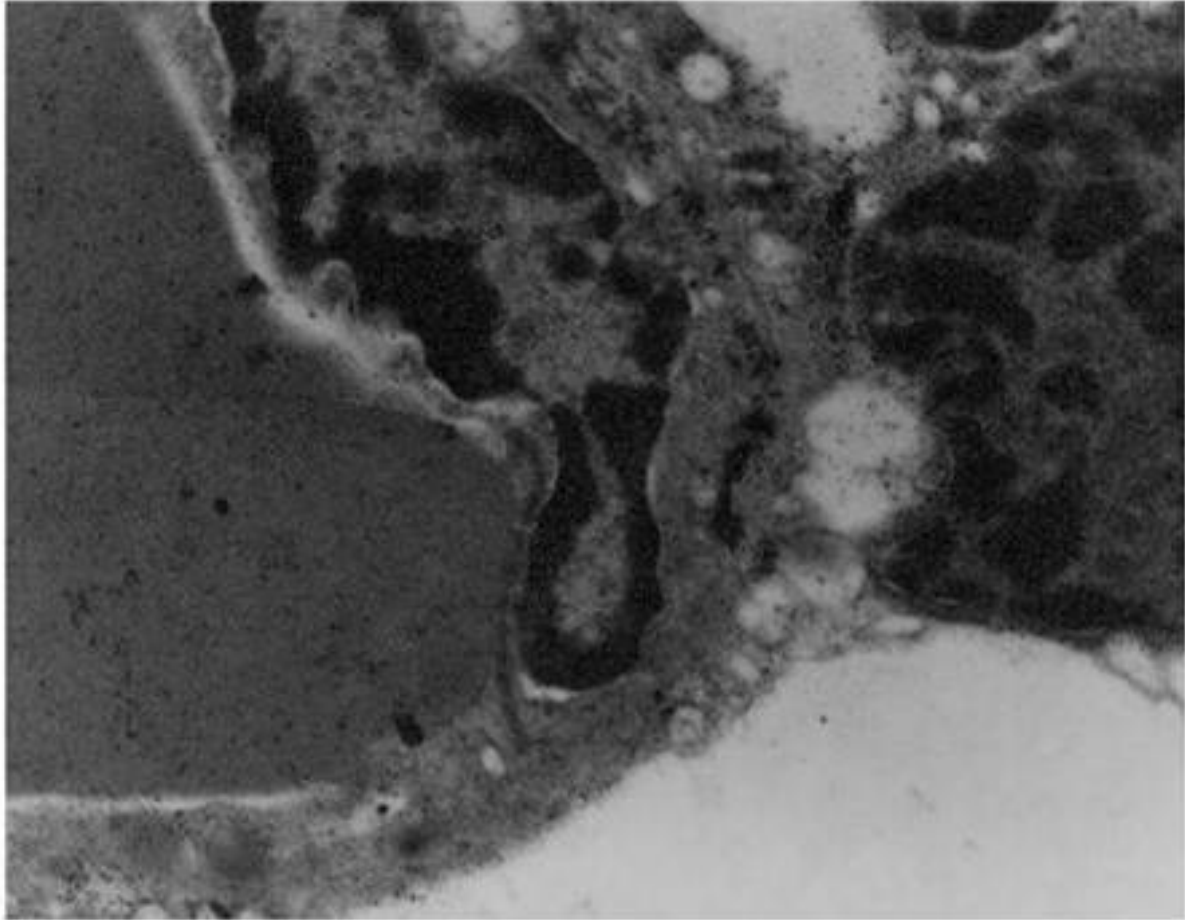


Frontal cortex 37y male



Midbrain 14y male

CORTEZA SEM-86



12 000 X

Results in children, adolescents and young adults chronically exposed to significant levels of air pollution

- Olfactory bulb, frontal cortex and hippocampal formation in exposed subjects exhibit evidence of **chronic inflammation** (COX2 and IL1 β mRNA upregulation)
- **There is trafficking of inflammatory cells into the brain parenchyma**
- There is a **breakdown of the BBB** with an increase of CD163+ perivascular macrophages. CD163 mediates removal of Hb-heptaglobin and its soluble form regulates inflammation
- **Diffuse A β 42 cortical plaques are seen in teens and young adults APO E 4 negative and with no family history of dementia. In addition, A β 42 accumulates in blood vessels in exposed teens**

Children, adolescents and young adults chronically exposed to severe air pollution exhibit evidence of brain inflammation in olfactory bulb, frontal cortex, and hippocampus. Diffuse amyloid plaques and accumulation of A β 42 are present in frontal cortex.

Early and sustained exposure to significant concentrations of air pollutants might be an early risk factor for Alzheimer's disease.

FUTURE RESEARCH

- Clinical/brain MRI studies in pediatric populations
- Comparative neuropathology in sentinel animals
- Epidemiological studies that link AD with exposure to pollutants
- Forensic studies in diverse populations: AD changes in young subjects exposed to pollutants.
- Nonsteroidal anti-inflammatory drugs (NSAIDS) in experimental animals exposed to air pollutants

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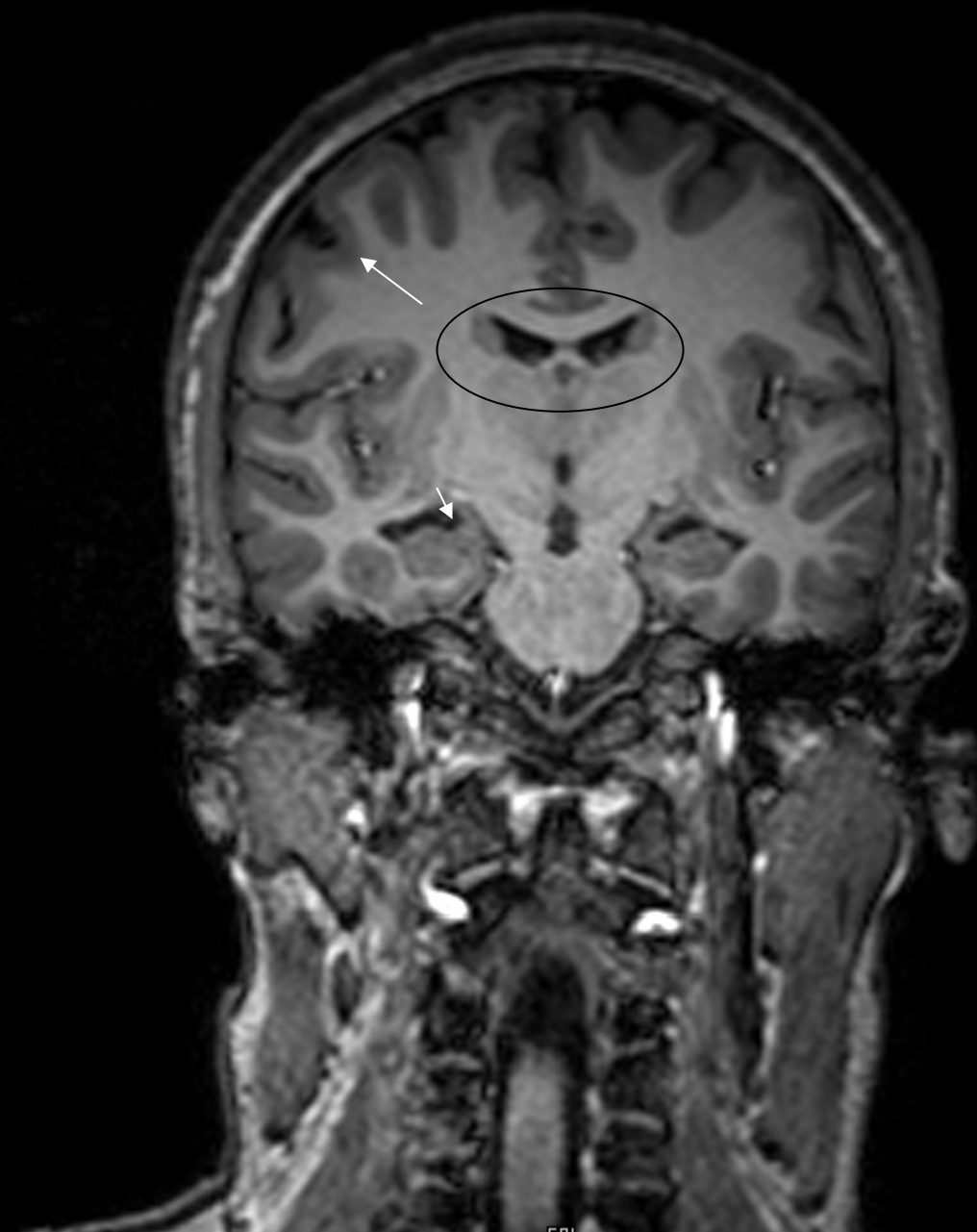
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Is chronic exposure to air pollution an early risk factor for Alzheimer's disease?
Are our children at risk?



How early? How bad?



Study: Alzheimer's Expected to Soar in U.S. Hispanic Community

Hispanics living in the United States will experience a more than **six-fold increase** in Alzheimer's disease and related dementias over the next 50 years, according to a report issued May 18, 2004 by the Alzheimer's Association. Warning that dementia is a looming but unrecognized public health crisis in Hispanic communities in the United States, the report projected that **1.3 million American Hispanics would develop Alzheimer's disease and other dementias in the first half of the 21st century, compared to fewer than 200,000 with it today.**

The report attributed the expected rise in Alzheimer's disease and other forms of dementia in this group to the expected increase in life expectancy, high rates of vascular disease and **low education** levels among Hispanics.