Pollution-derived magnetite nanoparticles as a possible risk factor for Alzheimer's disease

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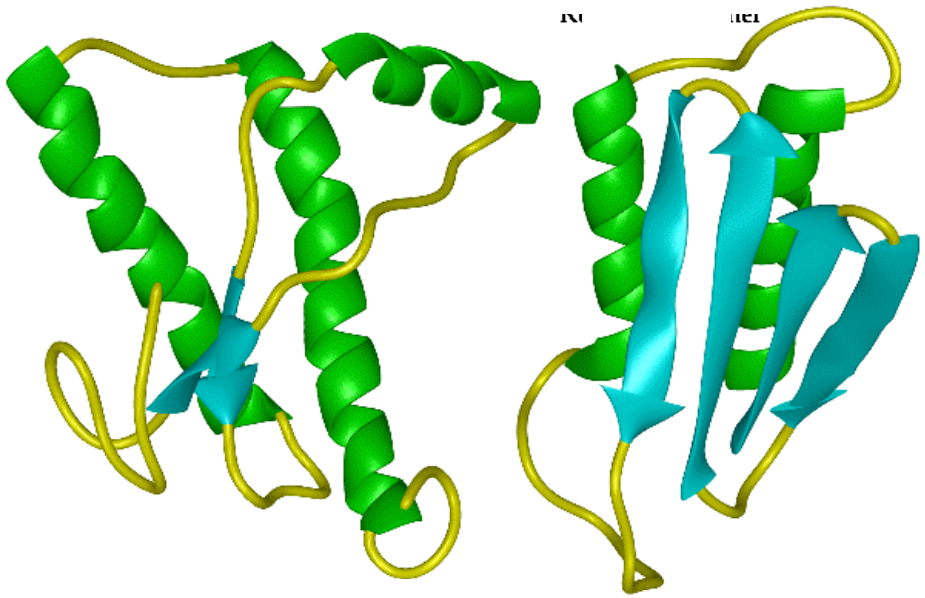
Protein aggregation in neurodegenerative diseases

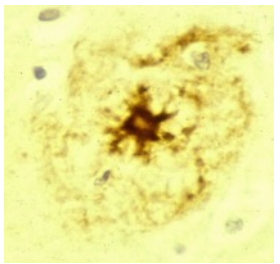
PrP^C
43% α-helix
3% β-sheet

PrP^{Sc}
34% α-helix
43% β-sheet

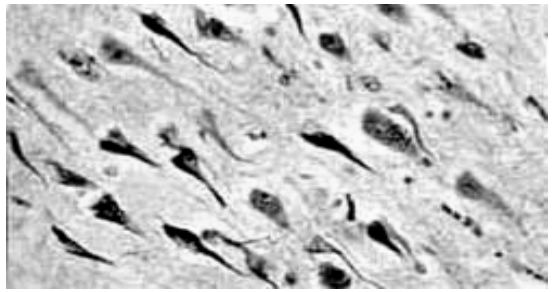


Protein misfolding is the cause of **prion disease**

Alzheimer's disease



Senile plaque (Aβ)



Neurofibrillary tangles (tau)

Protein	Disease
β-amyloid (APP)	Alzheimer's disease
α-synuclein	Parkinson's disease
tau	FTDP-17
prion protein	prion disease
huntingtin	Huntington's disease
TDP-43/SOD-1/FUS/C9orf72	MND (ALS) – FTD

Mutations in the gene encoding each aggregating protein gives rise to an inherited ND disease

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Role of metals in neurodegenerative disease

- Evidence for extensive oxidative damage to the brain or CNS in diseases such as Alzheimer's, Parkinson's and motor neuron disease (MND/ALS)
- This appears to occur very early on in the course of AD
- The brain uses large amounts of oxygen and is particularly vulnerable to damage by ROS because of low levels of protection by anti-oxidants
- The key lesions in these diseases (e.g. plaques and tangles in AD) are sites of redox-active metal ion accumulation
- Many of the key proteins (e.g. Aβ, α-synuclein, PrP) have high-affinity metal binding properties

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Huang *et al.* (1999) *Biochemistry* 38, 7609

The Aβ Peptide of Alzheimer's Disease Directly Produces Hydrogen Peroxide through Metal Ion Reduction[†]

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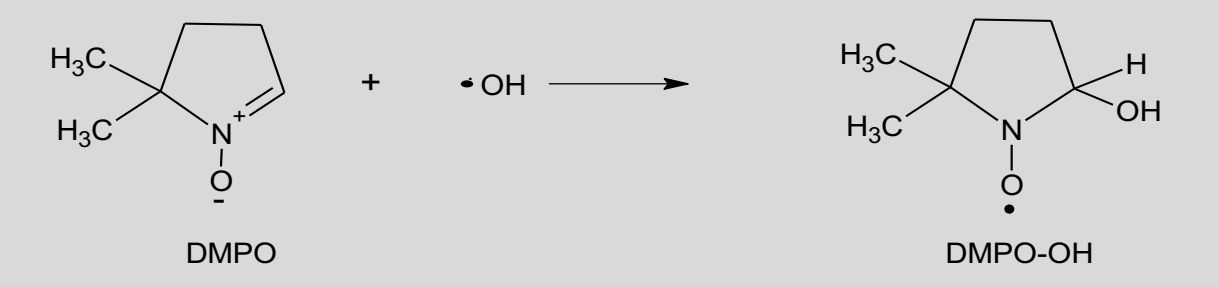
ABSTRACT: Oxidative stress markers characterize the neuropathology both of Alzheimer's disease and of amyloid-bearing transgenic mice. The neurotoxicity of amyloid Aβ peptides has been linked to peroxide generation in cell cultures by an unknown mechanism. We now show that human Aβ directly produces hydrogen peroxide (H_2O_2) by a mechanism that involves the reduction of metal ions, Fe(III) or Cu(II), setting up conditions for Fenton-type chemistry. Spectrophotometric experiments establish that the Aβ peptide reduces Fe(III) and Cu(II) to Fe(II) and Cu(I), respectively. Spectrochemical techniques are used to show that molecular oxygen is then trapped by Aβ and reduced to H_2O_2 in a reaction that is driven by substoichiometric amounts of Fe(II) or Cu(I). In the presence of Cu(II) or Fe(III), Aβ produces a positive thiobarbituric-reactive substance (TBARS) assay, compatible with the generation of the hydroxyl radical (OH·). The amounts of both reduced metal and TBARS reactivity are greatest when generated by Aβ1−42 ≫ Aβ1−40 > rat Aβ1−40, a chemical relationship that correlates with the participation of the native peptides in amyloid pathology. These findings indicate that the accumulation of Aβ could be a direct source of oxidative stress in Alzheimer's disease.

Detection of hydrogen peroxide by ESR spectroscopy



1. Incubate Aβ(1-40) for up to 48 h @ 37°C in PBS
2. Add Fe(II) sulphate (Fenton's reaction) and DMPO (spin trap)

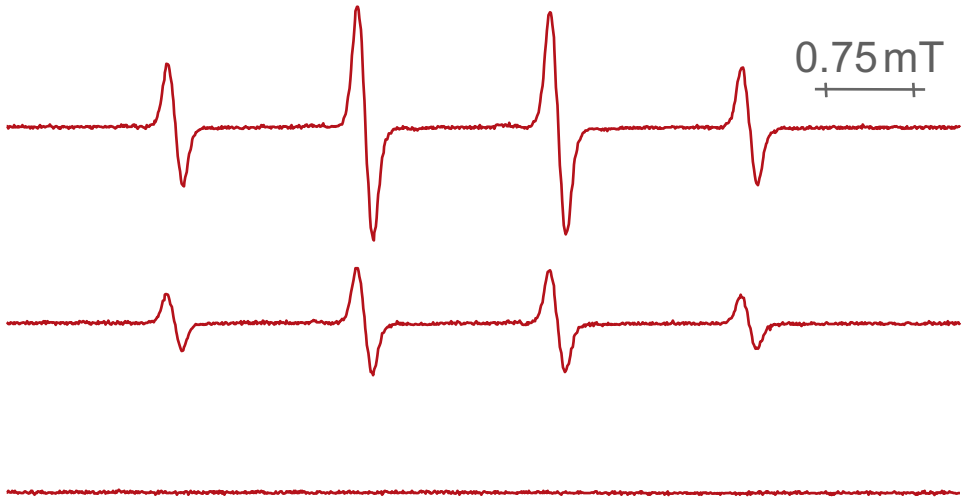
$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + \blacksquare OH + {}^{-}OH$$

3. Immediately record ESR spectra
   - 100kHz magnetic field modulation
   - modulation amplitude 0.05 mT
   - microwave power 20 mW
   - spectrum accumulation over 25 scans

The β-amyloid peptide (Aβ) does generate H_2O_2

Characteristic 4-line (1:2:2:1) spectrum of DMPO-OH

a(N) 1.50 and *a*(H) 1.46 mT



1 mM H_2O_2

Aβ(1-40) pre-incubated at 1 mg/ml for 48 hrs

PBS control*

*Blank spectra were also seen when Aβ(1-40) was pre-incubated with DETAPAC (metal ion chelator) or with catalase. *Here, generation of H_2O_2 seems to be dependent on trace levels of metal ions present in buffers.*

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So do other protein aggregation systems

	DMPO-OH	No spectrum
Aβ peptides	Aβ(1-40) Aβ(1-42) Aβ(25-35)	Aβ(1-40)Met35Nle Aβ(40-1)
Synucleins	α-synuclein NAC(1-35) NAC(1-18)	β-synuclein γ-synuclein NAC(19-35) NAC(35-1) NAC(18-1)
Amylin	human amylin	rodent amylin

	DMPO-OH	No spectrum
PrP		full-length PrPc Doppel
PrP(106-126)	+ Cu(II)	- Cu(II) scrambled + Cu(II)
PrP(121-131)	D178N F198S E200K	wild-type
ABri	ABri (oxidised)	ABri (reduced) ABri (wild-type)

Turnbull *et al.* (2001) *FRBM*
Turnbull *et al.* (2003) *Biochemistry*
Turnbull *et al.* (2003) *Neurosci Lett*
Masad *et al.* (2007) *FRBM*
Tabner *et al.* (2006) *Free Radical Res*
Turnbull *et al.* (2003) *Neuroreport*
Tabner *et al.* (2005) *J Biol Chem*
Masad *et al.* (2011) *FRBM*

Alzheimer's
Parkinson's
Type-2 diabetes
Prion disease
familial British dementia

Natural ‘biogenic’ magnetite in the human brain

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7683–7687, August 1992
Biophysics

Magnetite biomineralization in the human brain

(iron/extremely low frequency magnetic fields)

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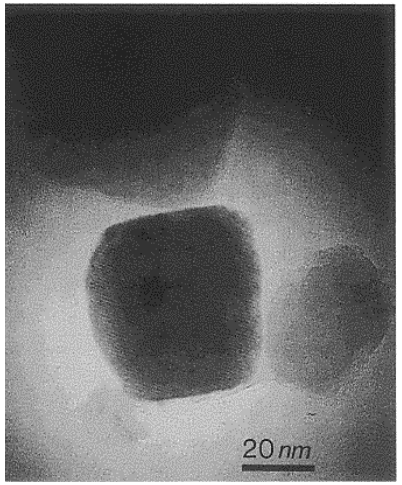
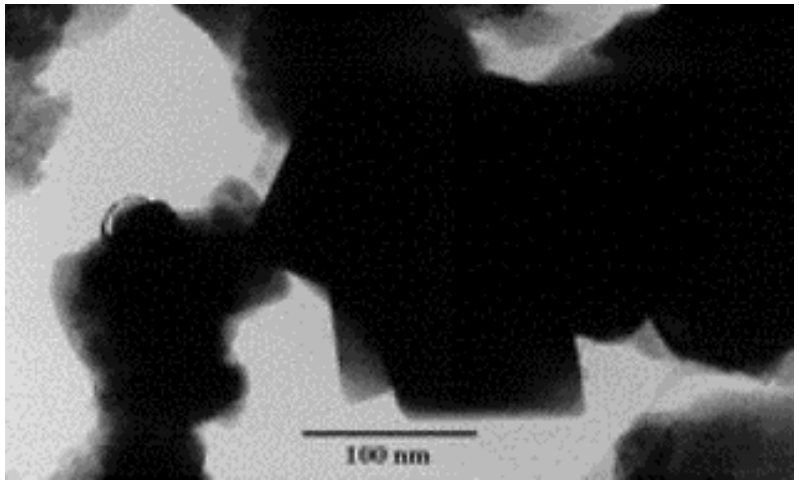
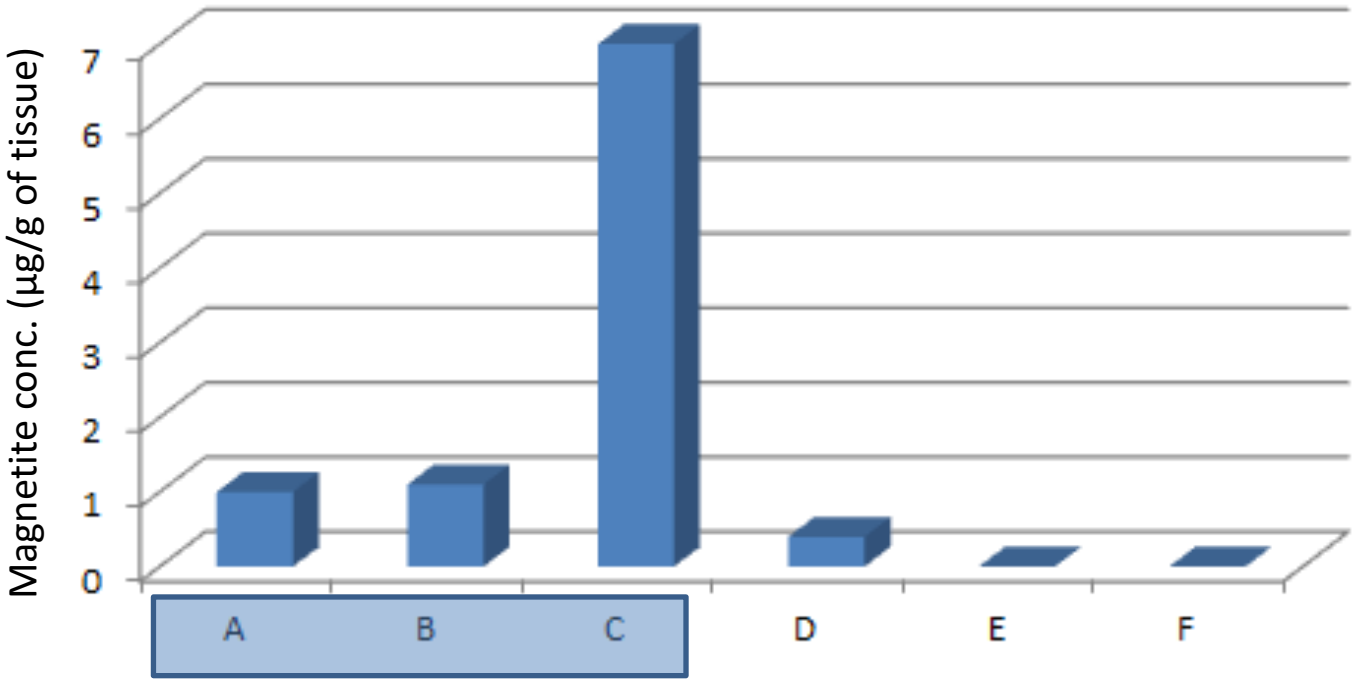




- Magnetite, a strongly magnetic mixed Fe^{2+}/Fe^{3+} iron oxide, was first identified in the human brain in 1992
- Detected by highly sensitive superconducting quantum interference device (SQUID) magnetometry and TEM analysis of brain extracts subjected to magnetic extraction
- Accumulates in the form of nano-scale euhedral crystal shapes, possibly made within the 8 nm-diameter cores of the iron storage protein, ferritin
- May play a role in safe iron storage, sense of navigation, memory?

Magnetite in brain tissue from cases of AD

Hautot *et al* (2003) Preliminary evaluation of nanoscale biogenic magnetite in Alzheimer's disease brain tissue. *Proc Biol Sci* 270, S62-64

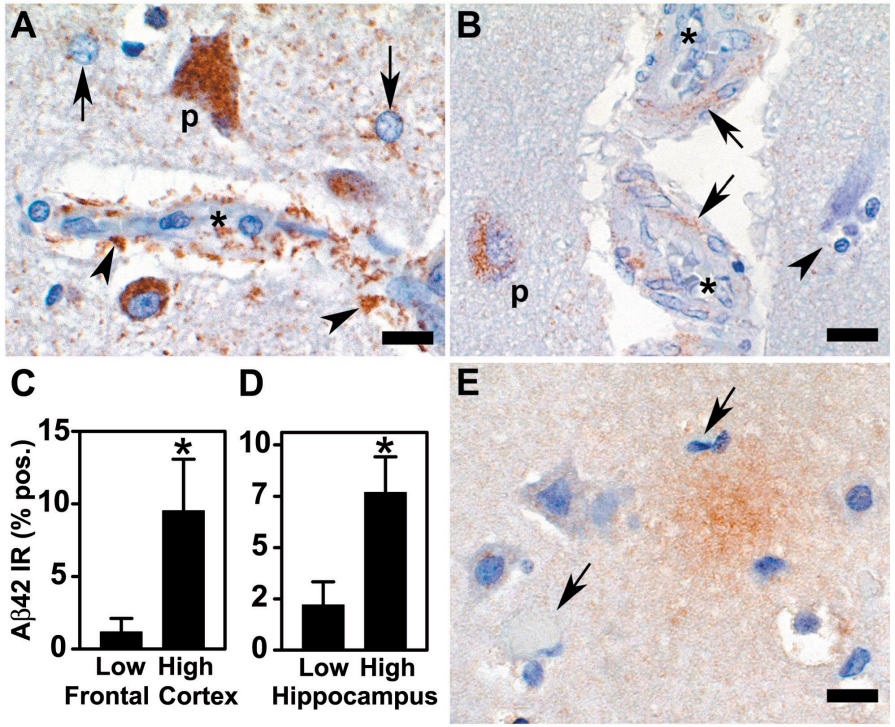


Subjects A, B, C had Alzheimer's disease - D, E, F are controls

- SQUID magnetometry indicates higher concentrations of magnetite in AD brain tissue than in controls with other diseases
- Only very small sample numbers
- Attributed to biogenic magnetite

Are air pollutants linked to early-stage Alzheimer pathology?

Calderón-Garcidueñas *et al* (2004) Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol Pathol* 32, 650-658



Aβ42 immunohistochemistry

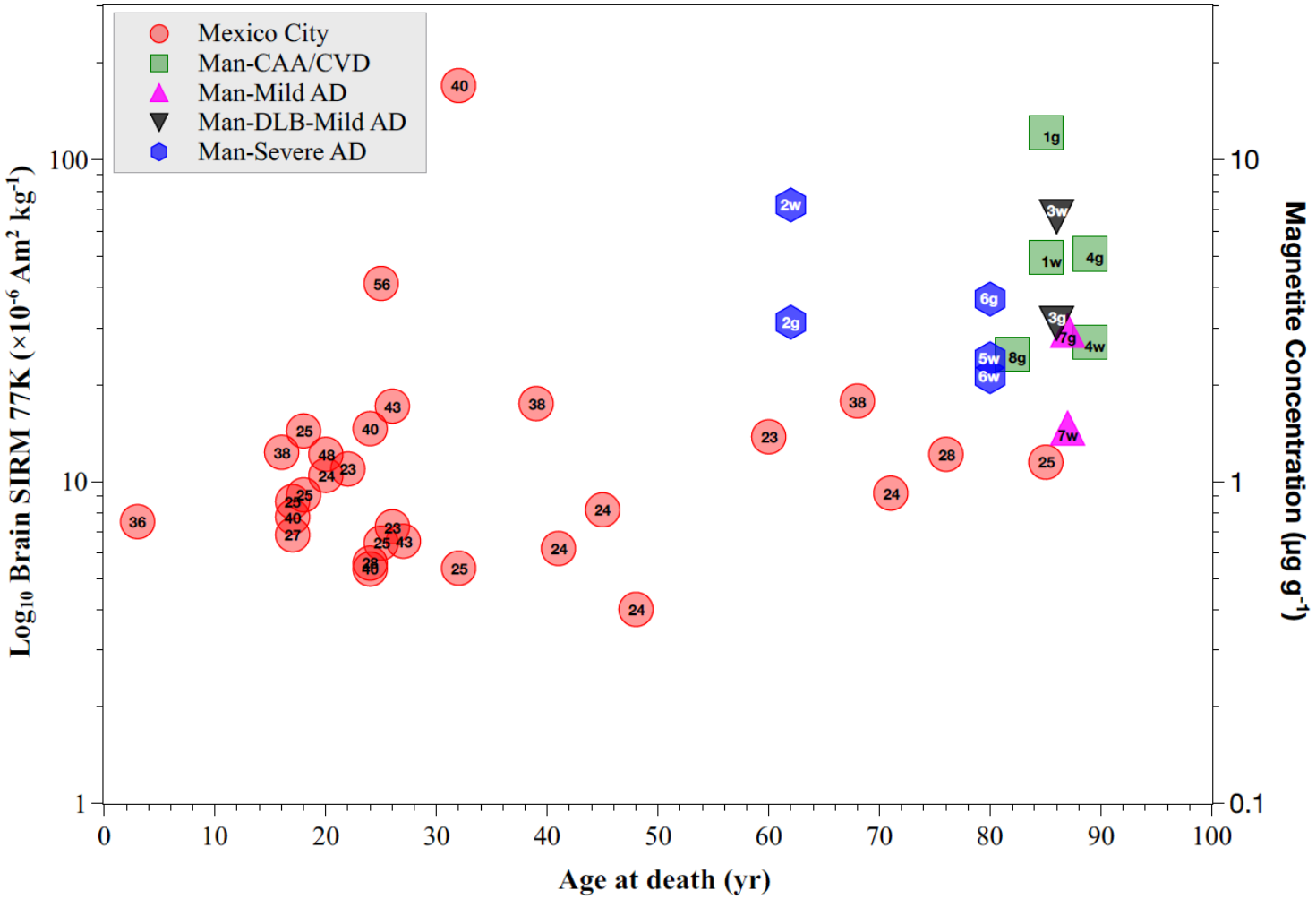
- Residents of cities with severe air pollution (e.g. Mexico City) had higher COX2 expression in frontal cortex and hippocampus and greater neuronal and astrocytic accumulation of Aβ42 than residents in low air pollution cities
- Authors suggest that exposure to severe air pollution is associated with brain inflammation and Aβ42 accumulation, two causes of neuronal dysfunction that precede the appearance of plaques and tangles

Is this connected with magnetite pollution nanoparticles?

Maher *et al* (2016) Magnetite pollution nanoparticles in the human brain. *Proc Natl Acad Sci USA* 113, 10797-107801

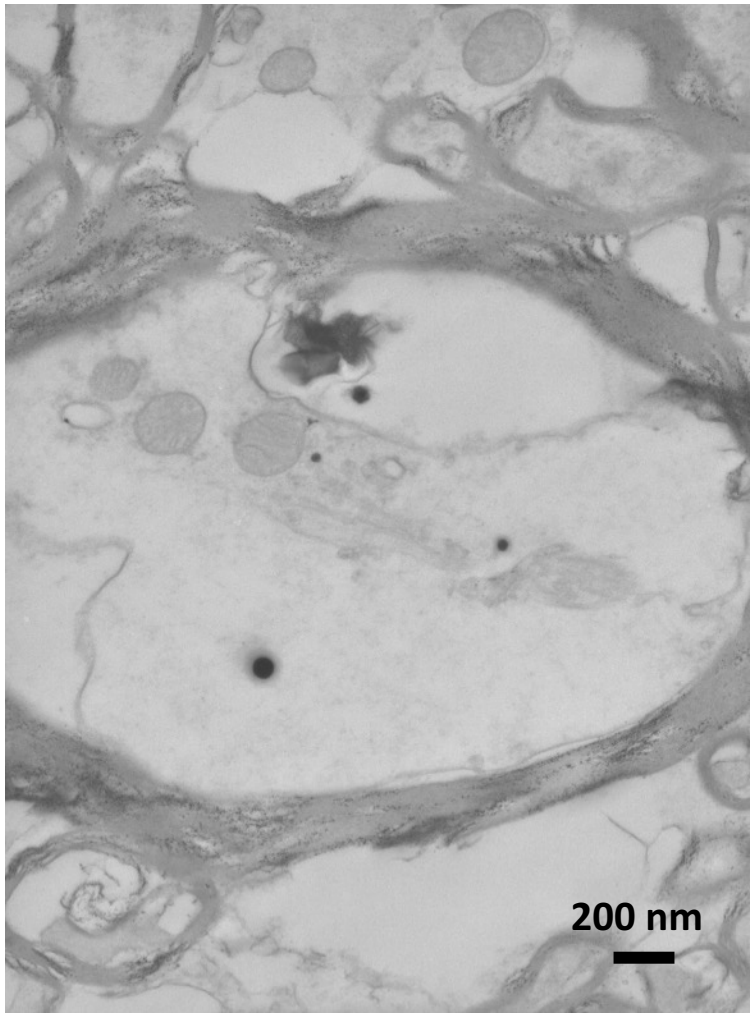
- Samples of frontal cortex were obtained from Mexico City (29 cases; ages 3 to 85) and Manchester, UK (8 cases; ages 62 to 92) – the latter included 5 cases of clinically diagnosed AD, but all had some degree of Alzheimer pathology
- To quantify brain magnetic content, a cryogenic SQUID magnetometer was used to measure the saturation magnetic remanence (SIRM) of freeze-dried tissue samples
- Magnetic brain particles *in situ* were examined by transmission electron microscopy (TEM), electron energy loss spectroscopy (EELS) and energy dispersive x ray analysis (EDXA) of ultrathin tissue sections
- Particles were also extracted magnetically from brain homogenates, prepared by digestion with the proteolytic enzyme papain
- Every analytical step was designed and monitored to minimise magnetic contamination

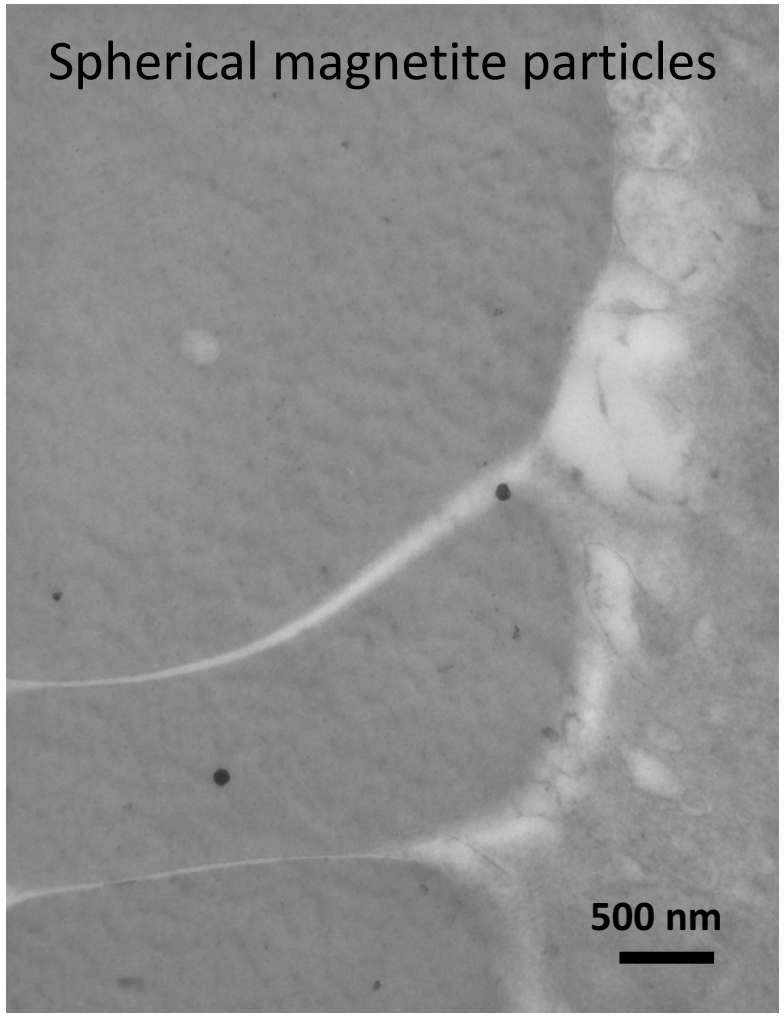
SIRM results and estimated magnetite concentration

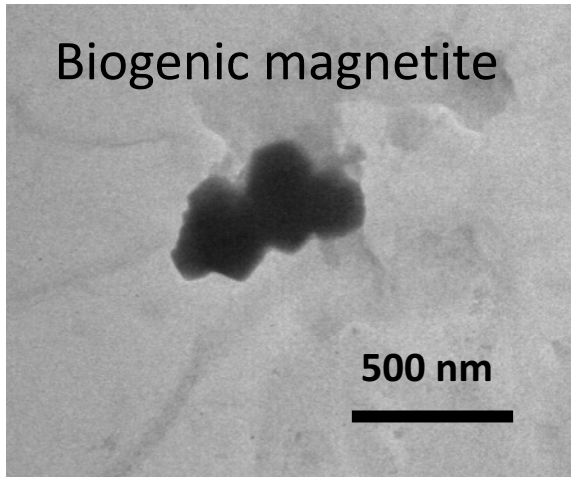


- No brains were analysed from areas of low pollution (Mexico), or without Alzheimer pathology (Manchester), and so correlation with pollution levels or disease is not possible
- However, many of the highly magnetic samples were from young (< 40 years at death) Mexico City residents, and all Manchester cases (> 60 years at death)

TEM images of brain sections







- Biogenic brain magnetite is seen in some images
- However, the majority of brain magnetite particles are spherical with diameters ranging from 5 to 150 nm

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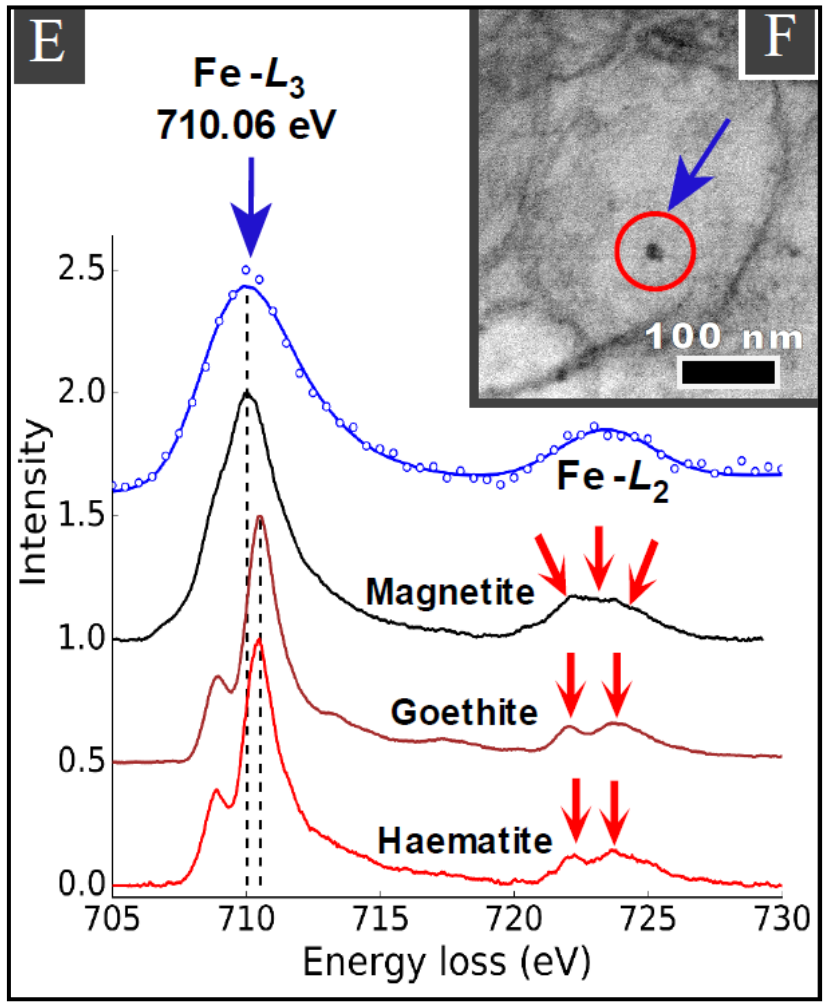
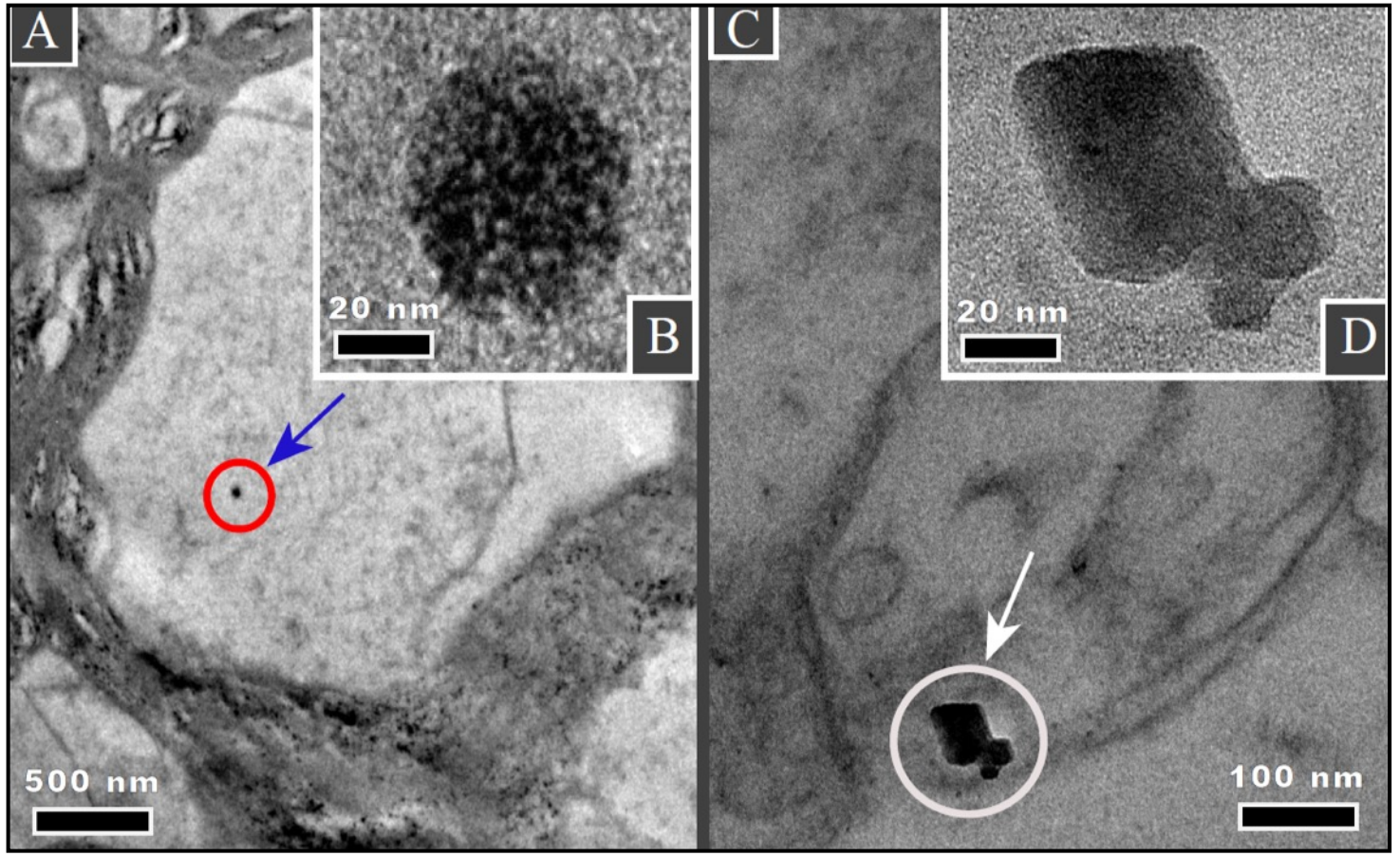
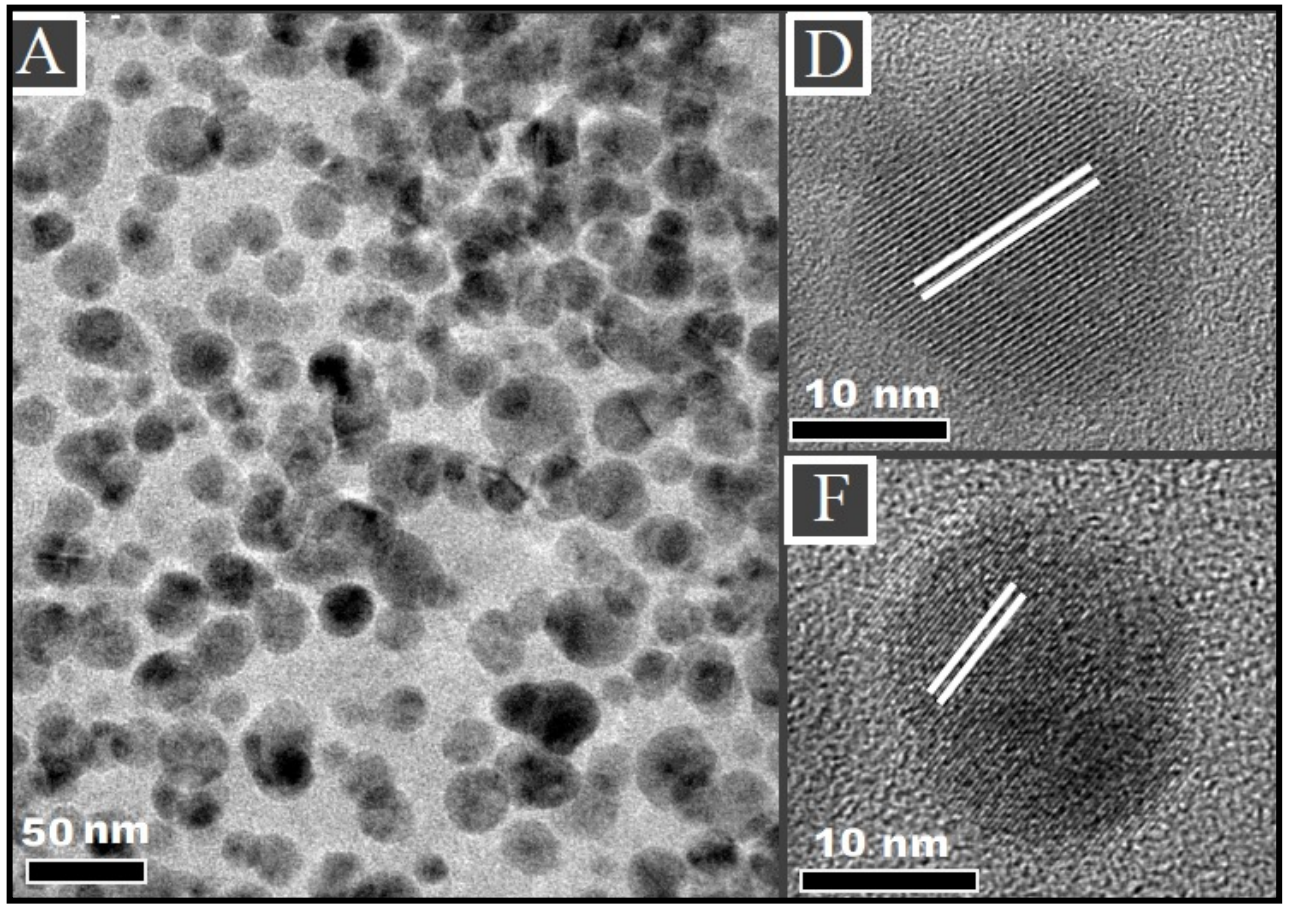
EELS identifies these structures as magnetite



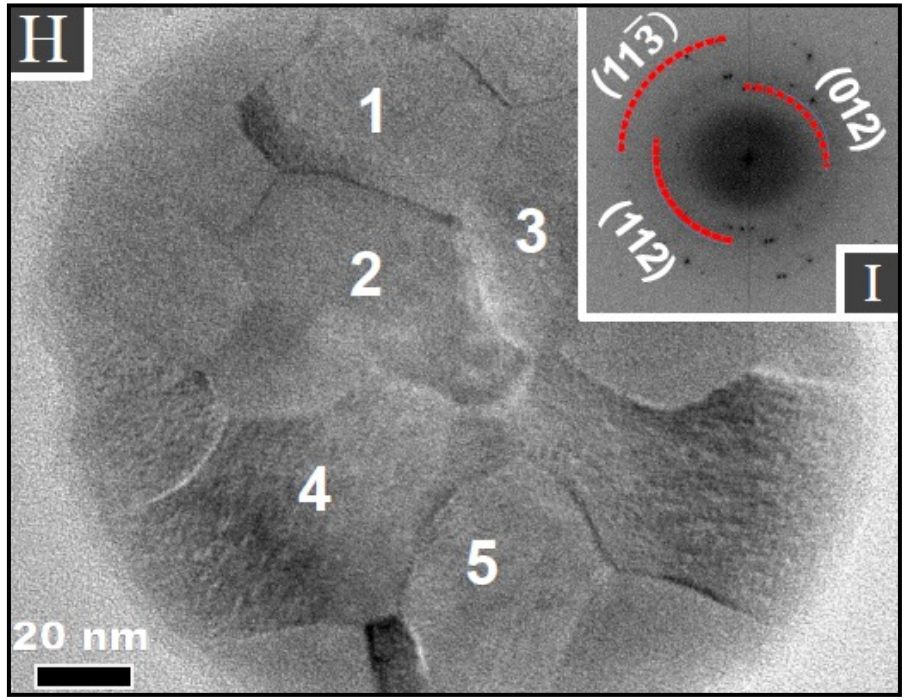


Magnetite extracted from brain



- Many of the extracted particles display rounded morphologies
- Indexing of the lattice fringes of these particles is consistent with magnetite crystal structure, with some oxidation to maghemite
- Size distribution is broad, with a median (longest) diameter of 18 nm and maximum diameter of ~150 nm
- These dimensions greatly exceed those of nanoparticles formed within the 8-nm diameter of ferritin cores

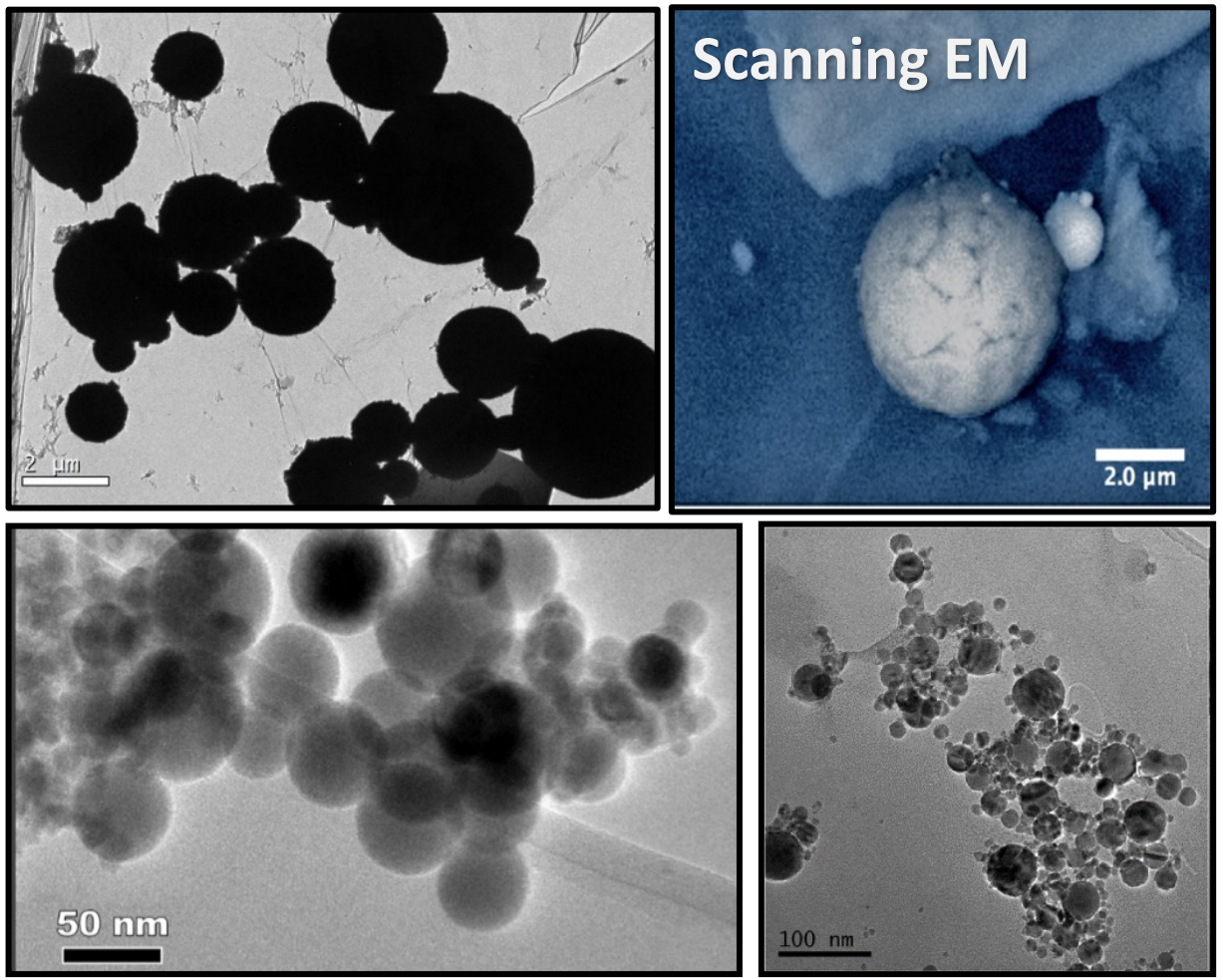
Magnetite extracted from brain



Large (~150-nm) spherical particle with fused, interlocking magnetite/maghemite surface crystallites

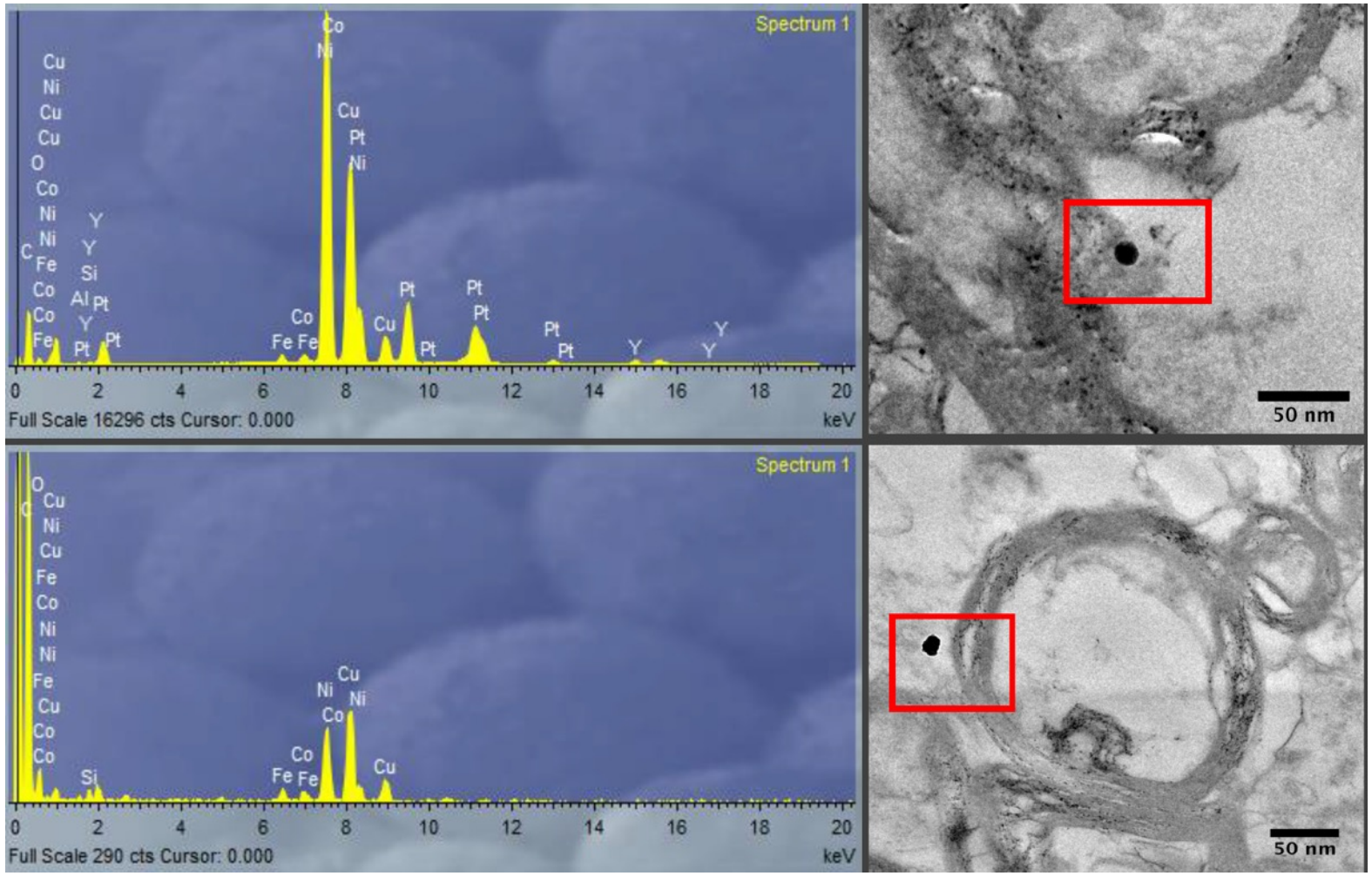
- These rounded magnetite nanoparticles, with distinctive surface textures, have not been identified previously in brain tissue sections
- Difficult to reconcile with low-temperature growth
- They bear compelling resemblance to magnetite ‘nanospheres’ found in airborne, combustion-derived, particulate matter (PM) pollution
- Their rounded shapes and fusing of interlocking, surface crystallites, reflect high-temperature formation and crystallization upon cooling

Magnetite ‘nanospheres’ from airborne pollution



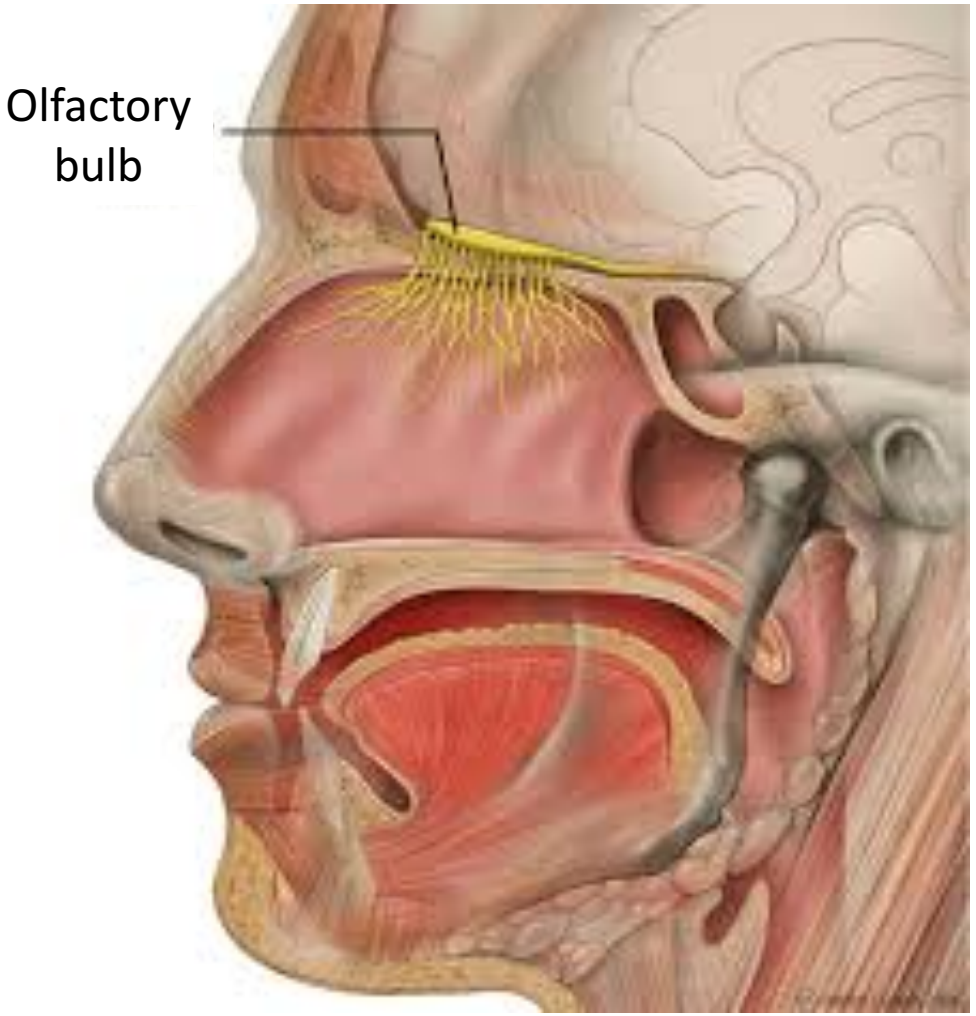
- Magnetite arises from combustion of iron-containing organic matter
- Combustion-derived, iron-rich droplets condense and cool on airborne release
- Often found with other metal particles, ranging in size from <5 nm to >100 nm
- Vehicles are a major source due to fuel combustion, iron wear from engine, and frictional heating of brake pads
- Larger magnetite spherules (>10 µm) are associated with industry/power stations

EDXA of brain sections identifies other metal nanoparticles



- The presence in brain of other metal nanoparticles containing Pt, Ni, and Co is identified by EELS and EDXA
- These cannot have a biogenic origin
- Pt release, for example, is associated with catalytic converters of vehicles

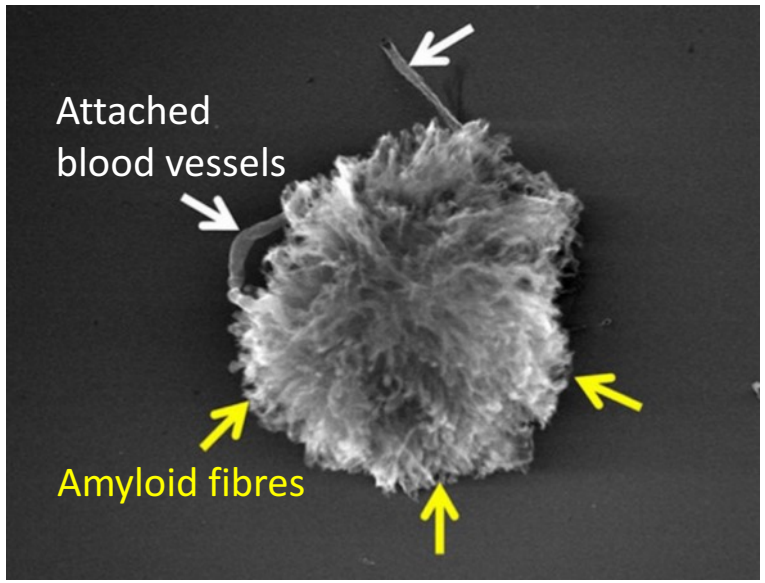
How could magnetite nanoparticles get into the brain?



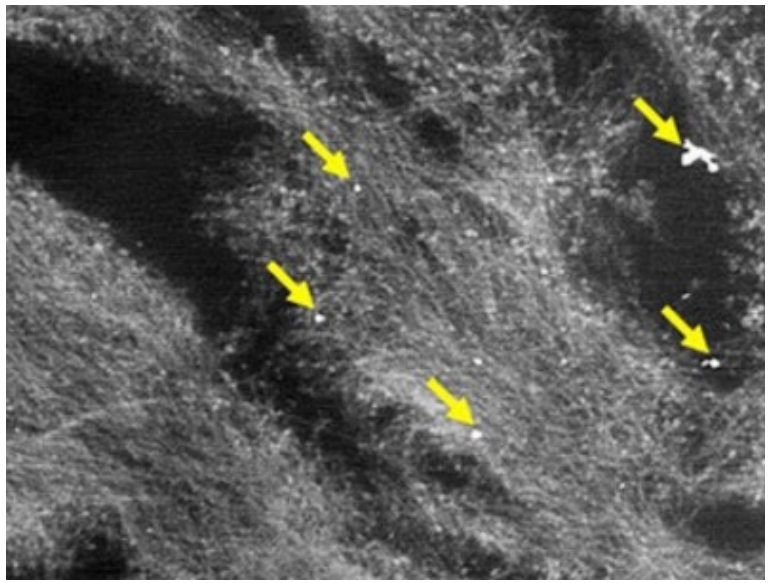
- Magnetite pollution particles <200 nm could enter the brain directly - breathed in through the nose and through the nerve cells of the olfactory bulb
- No blood-brain barrier (BBB) with this intranasal delivery mechanism
- Could then spread to other areas, including hippocampus and cerebral cortex – regions affected in AD
- Could also enter general blood circulation *via* lungs and then cross the BBB
- Spread through brain *via* extracellular fluid flow or axonal transport processes

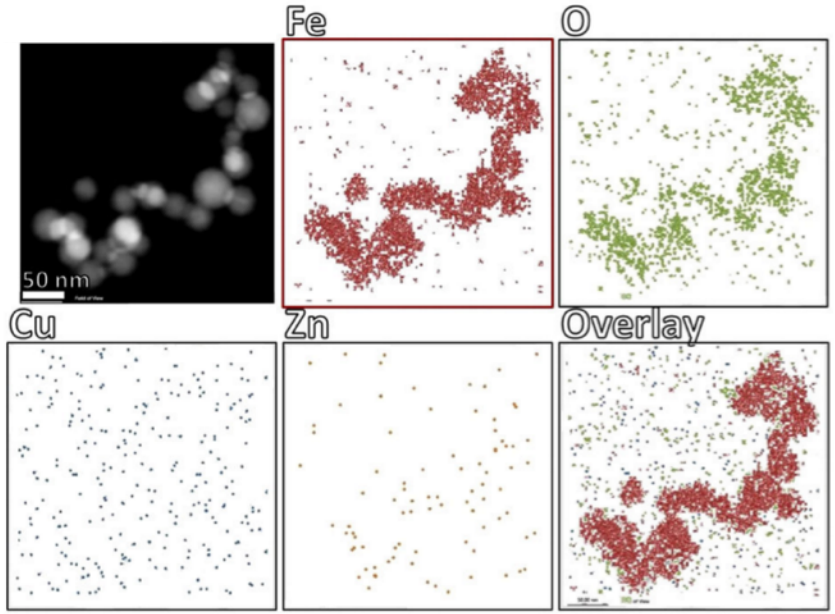
Magnetite nanoparticles are found in amyloid plaque ‘cores’

Plascencia-Villa *et al* (2016) High-resolution analytical imaging and electron holography of magnetite particles in amyloid cores of Alzheimer’s disease. *Scientific Reports* 6, doi:10.1038/srep24873



STEM image of isolated plaque ‘core’

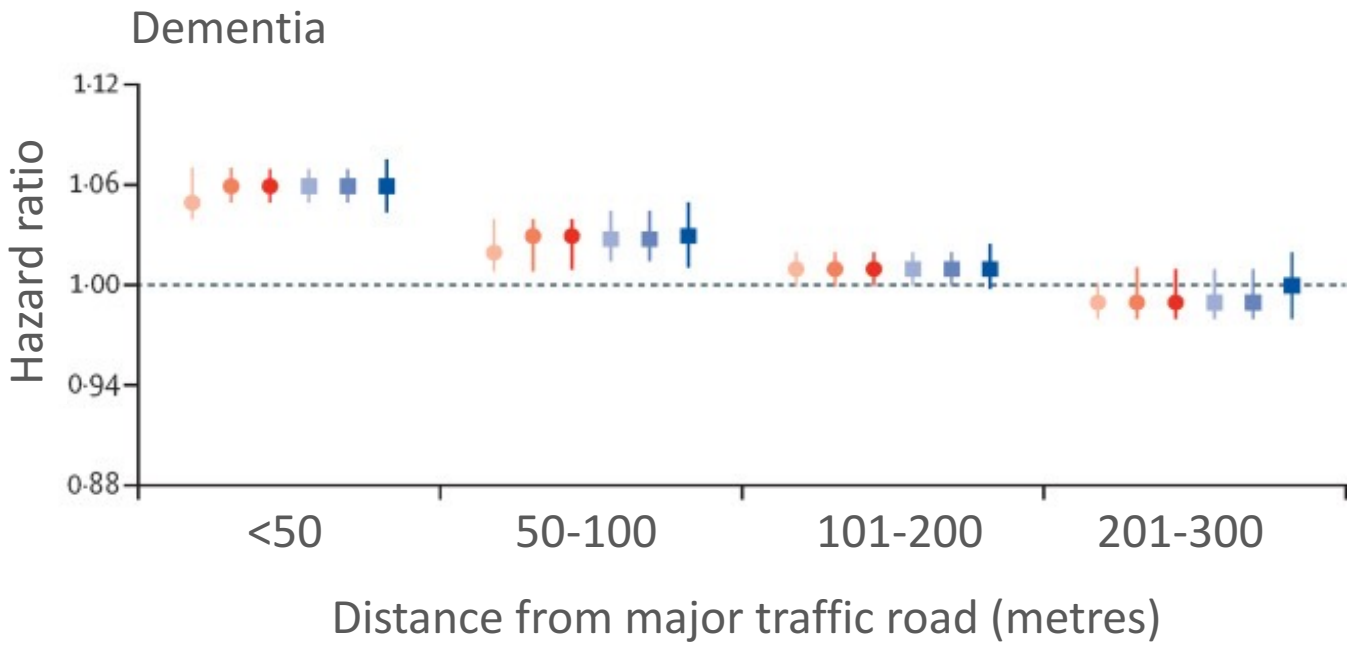




High contrast ‘spots’ seen by STEM are identified by spectroscopy as magnetite

Support from epidemiological studies

Chen *et al* (2016) Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: a population-based cohort study. *Lancet* 389, 718-726



- Study in Ontario, Canada
- The adjusted hazard ratio of incident dementia was 1·07 for people living less than 50 m from a major traffic road, *vs* further than 300 m (p = 0·0349)
- No association was found with Parkinson's disease or multiple sclerosis

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Summary

- Redox-active metal ions, ROS and oxidative damage are involved in many important diseases of the brain and CNS, including Alzheimer's disease
- Key lesions in these diseases (e.g. plaques and tangles in AD) are sites of redox-active metal ion accumulation, including magnetite in amyloid cores
- Key proteins (e.g. Aβ, α-synuclein, PrP) in association with redox-active metal ions can generate ROS, including hydrogen peroxide and hydroxyl radicals
- Magnetite nanoparticles which are remarkably similar to those present in abundance in airborne pollution have been found in the human brain
- Living close to a major road increases the risk of dementia
- Pollution-derived magnetite or other metallic nanoparticles could be involved in ROS formation and so be a major risk factor for AD
- This would have very important policy implications

Acknowledgements

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