# Evidence for Chronic Aluminium Intake as the Cause of Alzheimer's Disease

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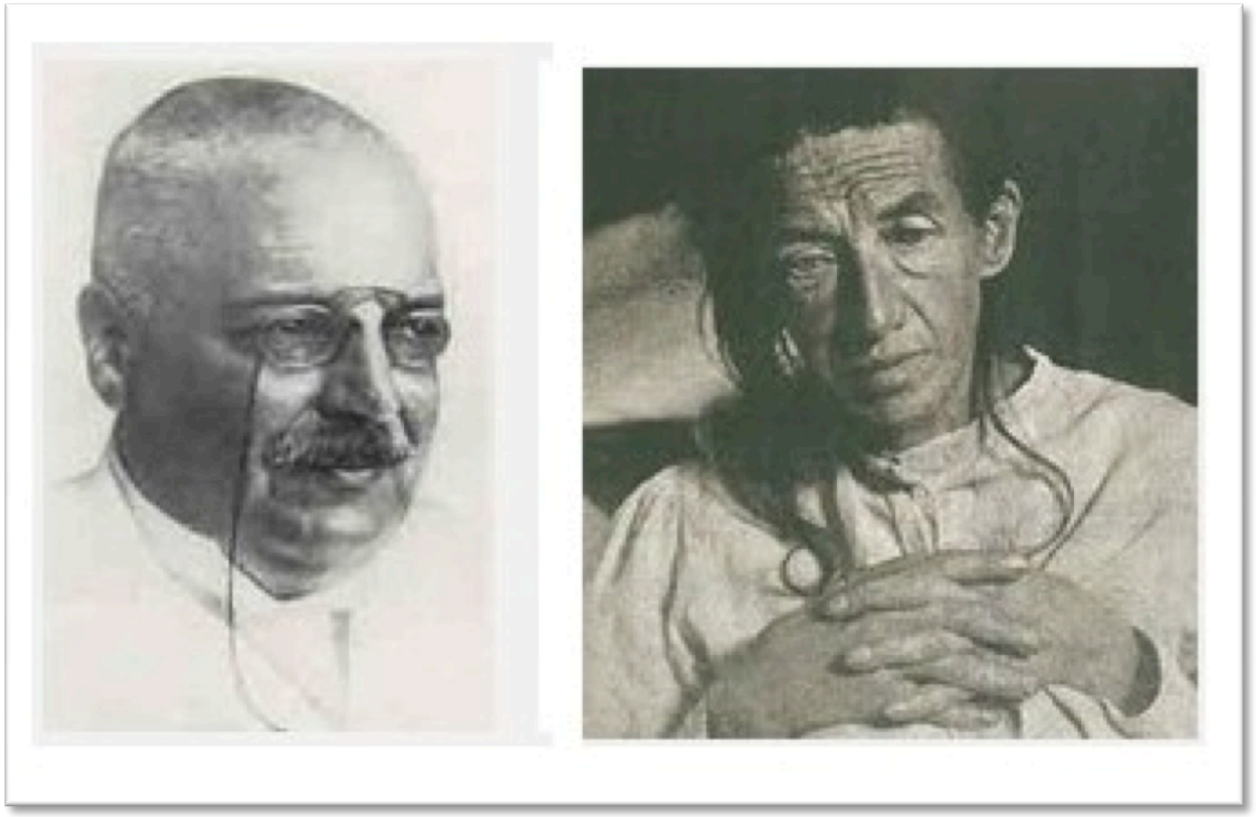
# In 1907, Dr Alois Alzheimer described, in a 51-year old woman, the disease that now bears his name



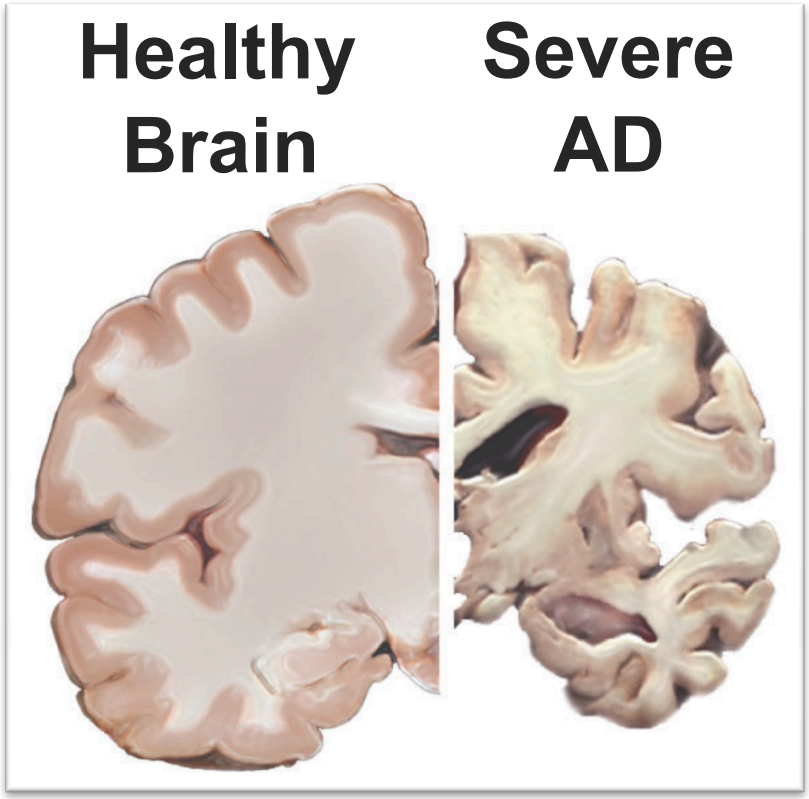
Dr Alois Alzheimer  Frau Auguste Deter

Alzheimer A. Zentralblatt Nervenheilkd Psychiatrie 30, 177-9 (1907)

## Alzheimer described this case as a new, previously unknown, disease

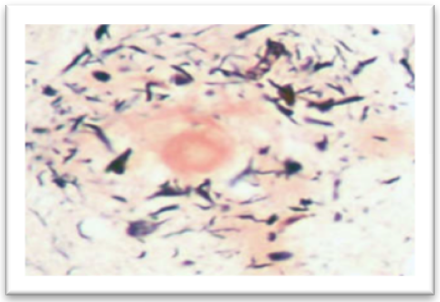
He observed the cerebral cortex of
Frau Deter's brain was greatly atrophied

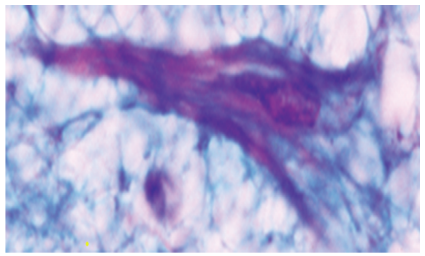
Mean brain weight
1360 g or ~3 lbs

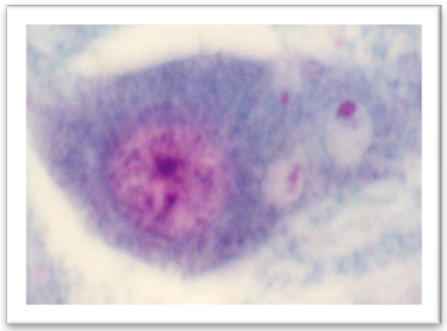



Mean brain weight
900 g or ~2 lbs

# Alzheimer studied the neuropathology of her brain







- Describing in detail the structure of miliary (i.e., β-amyloid) plaques scattered throughout her cortex
- Alzheimer realized that a chemical change occurred to produce neurofibrillary tangles (NFTs) that can ultimately kill their host cells
- Simchowicz (one of Alzheimer's students) identified granulovacuolar degeneration (GVD), the third most well-known hallmark of AD

Simchowicz T. L'Encephale 218-231 (1914)

University of New South Wales

**Alzheimer wrote in 1911, before his untimely death in 1915, "Perhaps [my observations] will encourage another, to try and solve these pressing questions with the help of new equipment and material…"**

"...vielleicht veranlassen [meine Beobachtungen] einen anderen, an der Hand eines neuen Materials die noch einer Lösung bedürftigen Fragen zu beantworten..."

Azheimer A. Z gesamte Neurologie Psychiatrie 4, 356-85 (1911)

**The conclusions from my body of work over the last 25 years, in association with the scientific literature of the past 107 years, provide the answers to Alzheimer's questions as to causality**

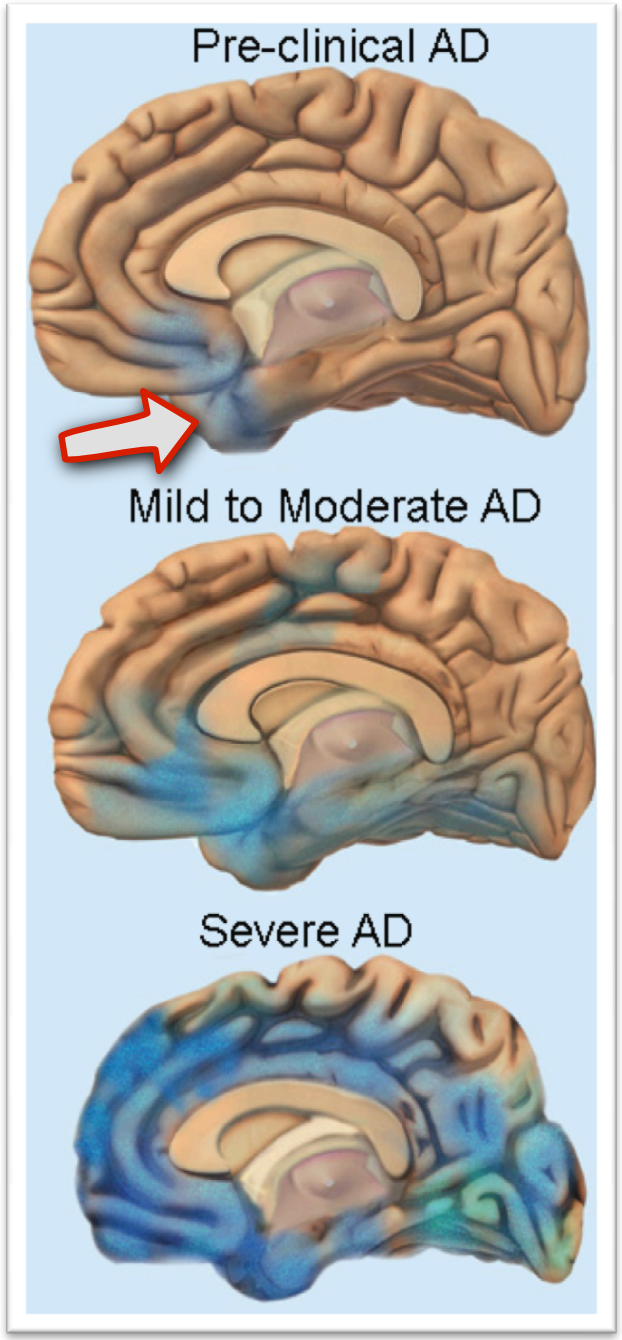
**We now know Alzheimer's disease is a disease associated with ageing in that AD develops progressively and irreversibly, over an extremely long prodromal period before becoming overt**

**Understanding the atrophic process is the key to unlocking Alzheimer's disease**

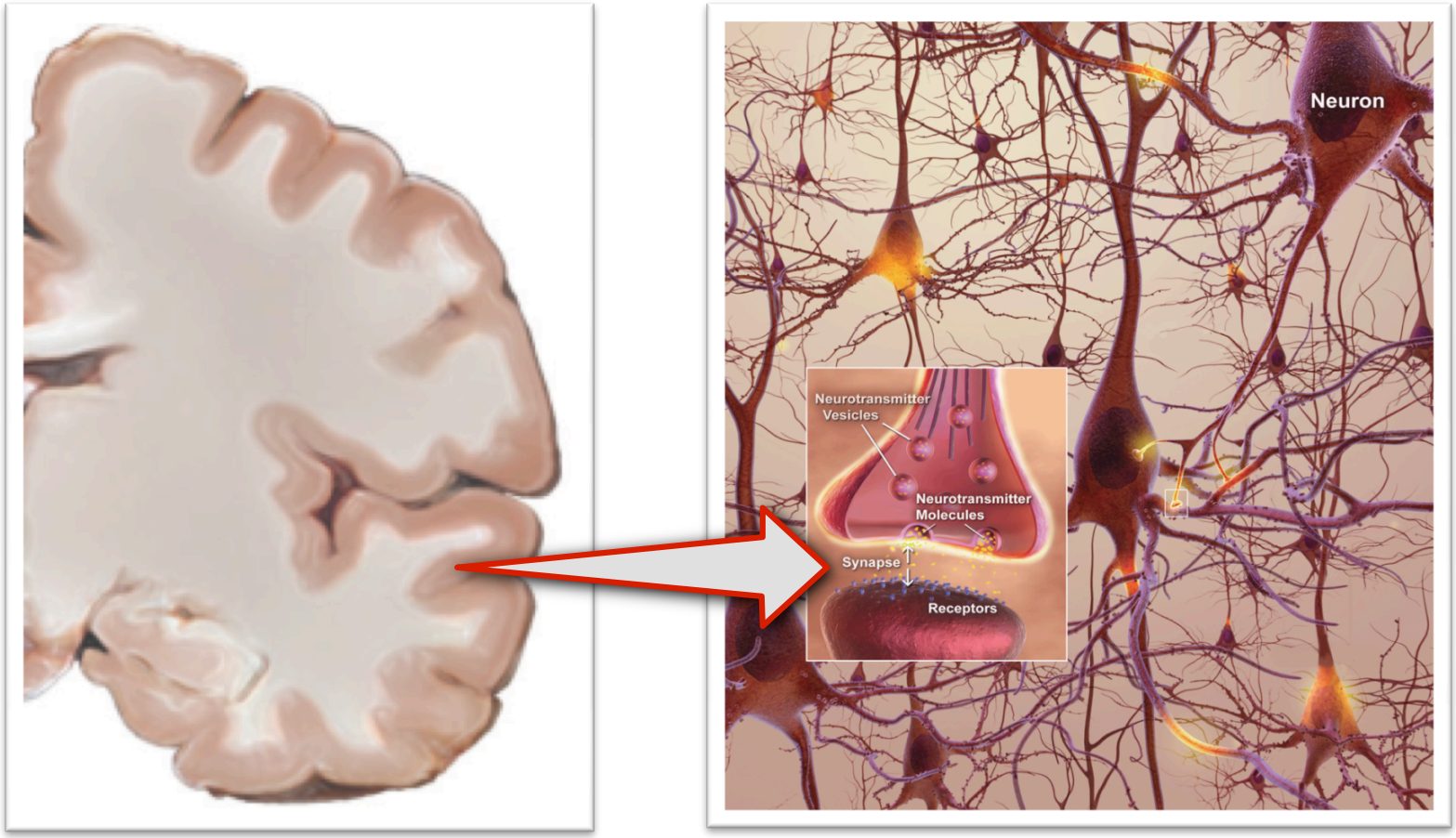
# Atrophy is the anatomical basis of AD dementia

- Serial MRIs show atrophy of the entorhinal cortex (arrow) and hippocampus (absent from this view) before AD is clinically diagnosable

- AD spreads from one brain region to another, showing the same neuropathology and atrophy in the newly-affected brain regions as in those initially affected, ultimately culminating in loss of function

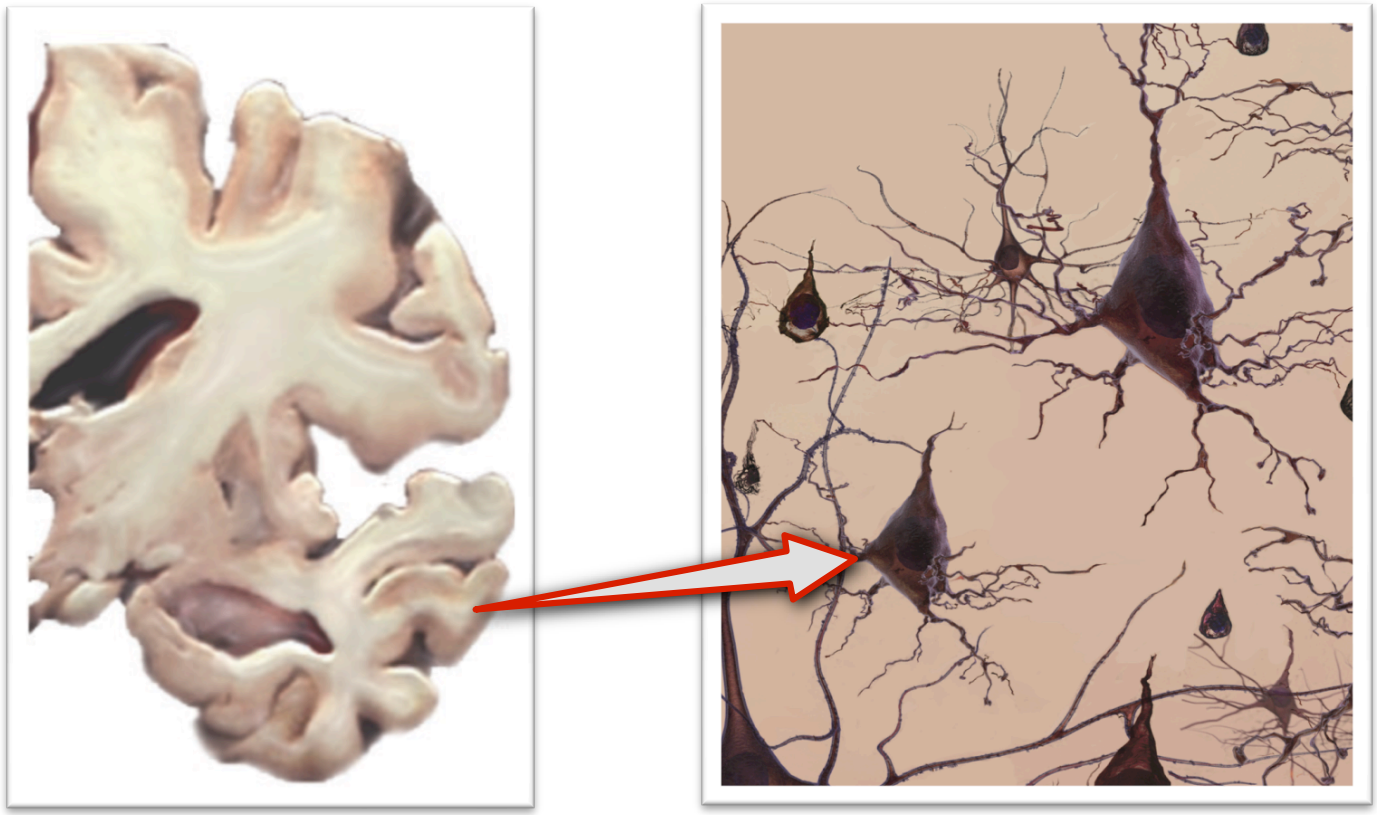


# The healthy cortex, when magnified, shows a complex network of neurons connected by synapses (arrow), being specialised junctions at the end of the neurons' long axons



**In affected brain regions of Alzheimer's disease, cell processes shrivel and retract, causing the synapses to break apart**

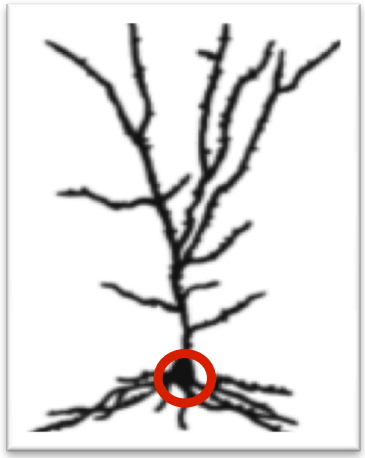
**Disconnection results, the cortex atrophies, and functions of these brain regions are lost**



Some AD researchers assume that global neuron death is responsible for atrophy of the AD brain

# Regeur's group carefully investigated this possibility and concluded otherwise

- They found a 14% volume reduction and counted 6% fewer neurons in the neocortex of severe AD cases than in controls
- Regeur's group noted many surviving neurons in severe AD neocortex exhibited dendritic dieback, accompanied by degenerating axons and synapse loss
- This led them to conclude dendritic dieback is sufficient, while neuron death is insufficient, to account for the 14% reduction in volume (atrophy) observed in the AD neocortex
- This conclusion is credible given that 95% of the neuron's volume is normally contained in its dendrites*

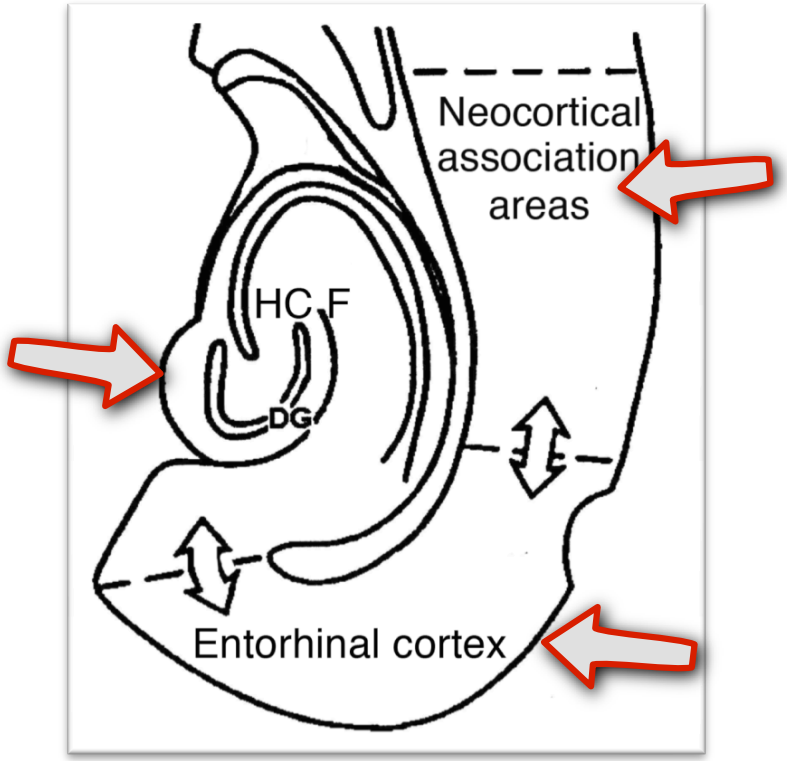


Regeur et al. Neurobiol Aging 15, 347-52 (1994)
*Buell and Coleman. Science 214, 33-41 (1979)

**Camera lucida drawings show the progressive degeneration of cell processes undergoing dieback in AD hippocampal neurons that ultimately results in disconnection from other neurons as dendrites, axons, and synapses are lost**

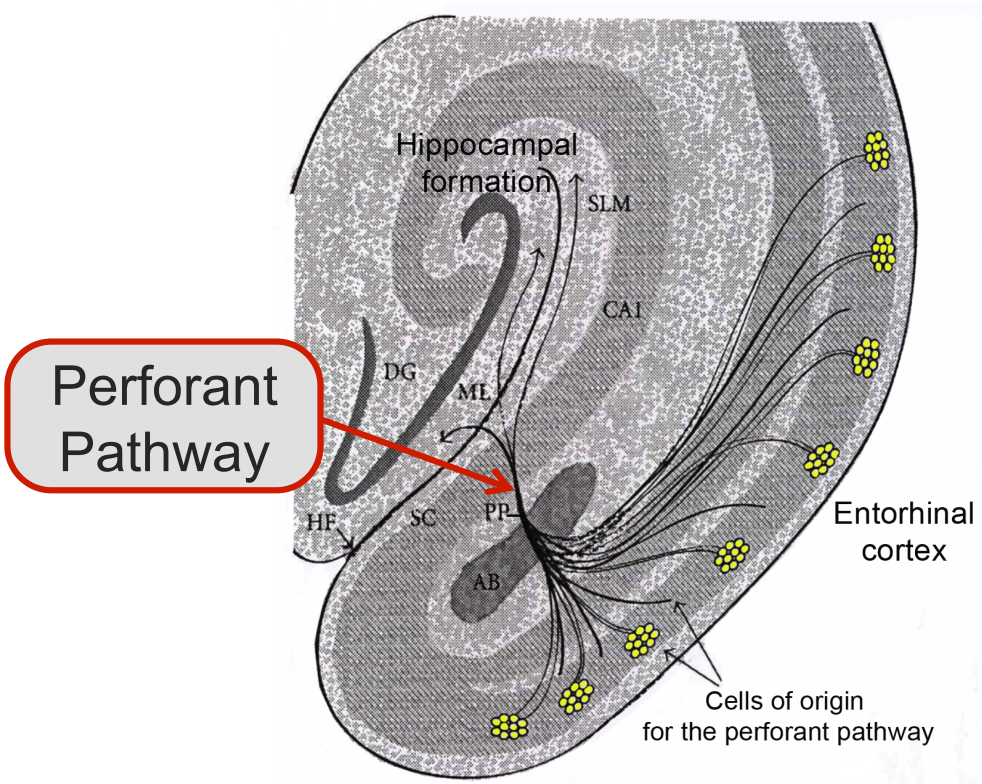
Scheibel ME. Exp Neurol 53, 420-30 (1976) with permission from Elsevier

**Certain parts of the brain are crucial for memory-processing. The hippocampal formation must communicate with the neocortex to determine whether, and where, to store new memories for future access**




The entorhinal cortex bi-directionally interconnects the hippocampal formation with the neocortex

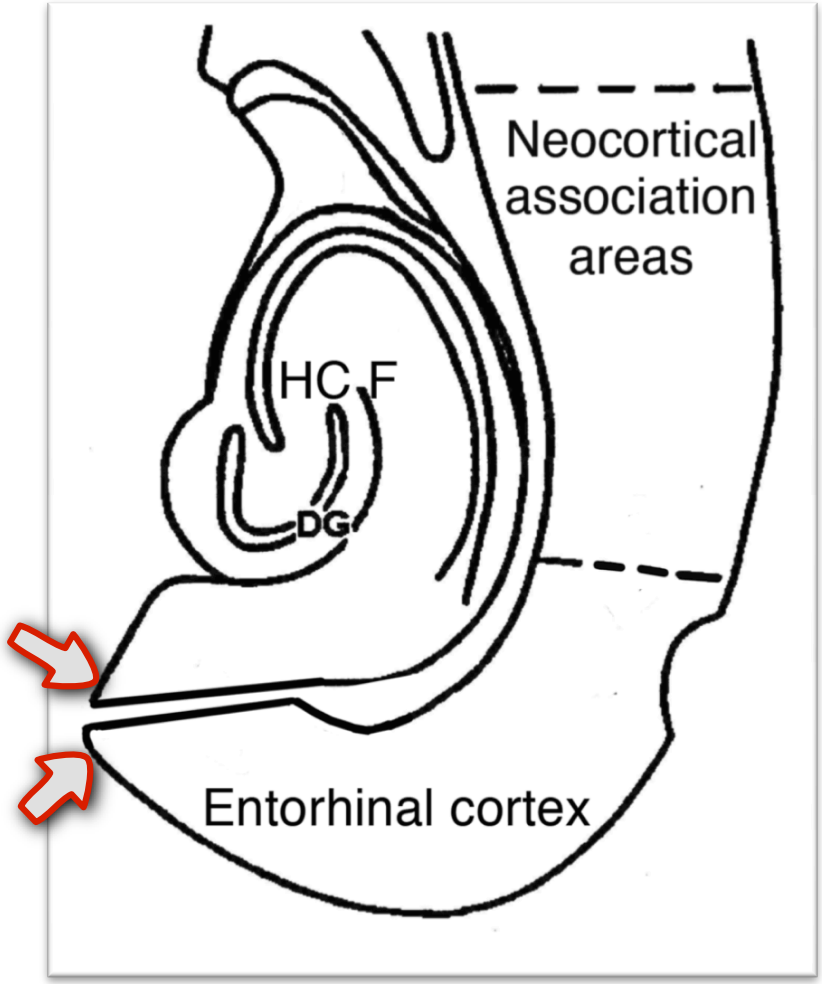
# Large cells in the entorhinal cortex (shown in yellow) receive information from the neocortex and relay that information to the hippocampus via a bundle of their axons, “the perforant pathway”




Published in Walton J. J Alzheimer’s Dis 35, 7-43 (2013)

# Van Hoesen et al. observed in relation to the agent that causes AD

- **"The precision of the pathology literally dissects the cortex, separating and isolating the hippocampal formation from the entorhinal cortex"**
- **"In early AD, confusion and inability to recall new and changing daily episodes undoubtedly relate to pathological changes in the entorhinal cortex and its associated neural systems."**




Van Hoesen GW et al., Hippocampus 1,1-8 (1991)

**The remainder of my talk describes at least 20 ways that aluminium brings about Alzheimer's disease**

No. 1

## Aluminium is a recognised neurotoxin*

- which causes a sub-acute dementia (dialysis encephalopathy syndrome)**; and

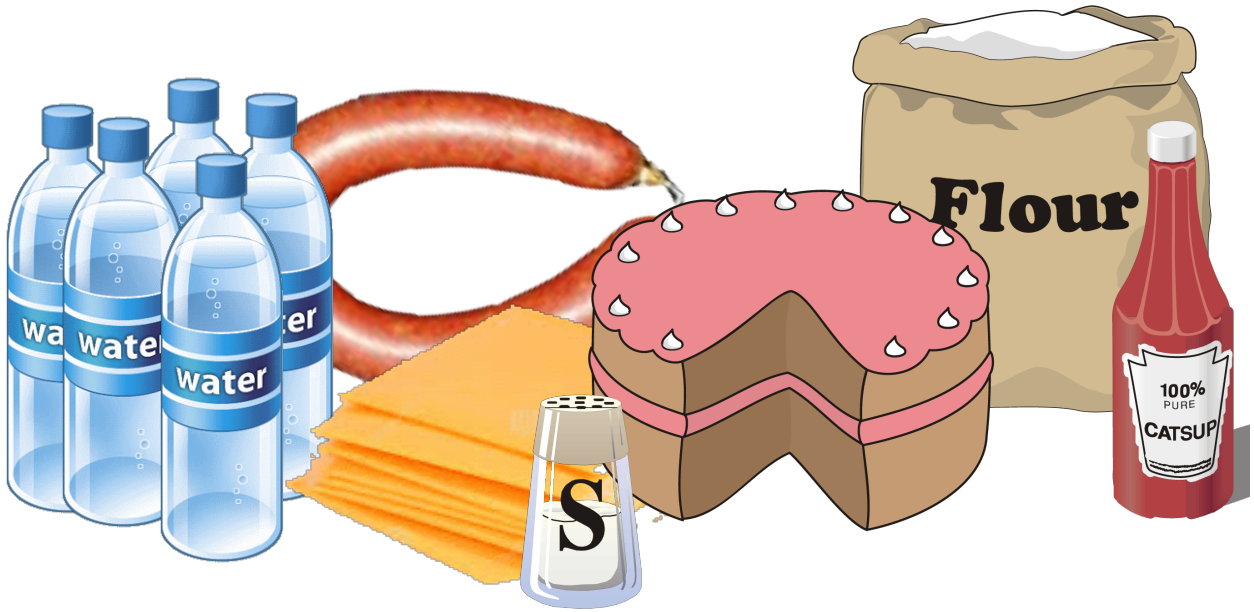
- is implicated in several chronic dementias:
  - AD
  - Early onset-AD in Down's Syndrome patients
  - Amyotrophic lateral sclerosis/Parkinsonism dementia of Guam
  - Parkinson's dementia of the Alzheimer type

* Simonsen et al., Scand J Work Environ Health 20, 1-12 (1994)
** Alfrey AC et al. New Engl J Med 294, 184-8 (1976)

No. 2

## Aluminium has a GRAS rating -- (Generally Regarded as Safe)

- This encourages aluminium's widespread usage in processed foods and alum-treated drinking water that many people routinely ingest, and in other bioavailable products that can increase the brain's aluminium burden



No. 2

# Plants and animals can deal with aluminium within the geological environment

Plants (below) secrete mucilage that coats their roots and inhibits aluminium uptake

**Animals (at right) secrete mucus (arrows) that lines their intestine and reduces the absorption of solubilised aluminium and other toxins fortuitously ingested**

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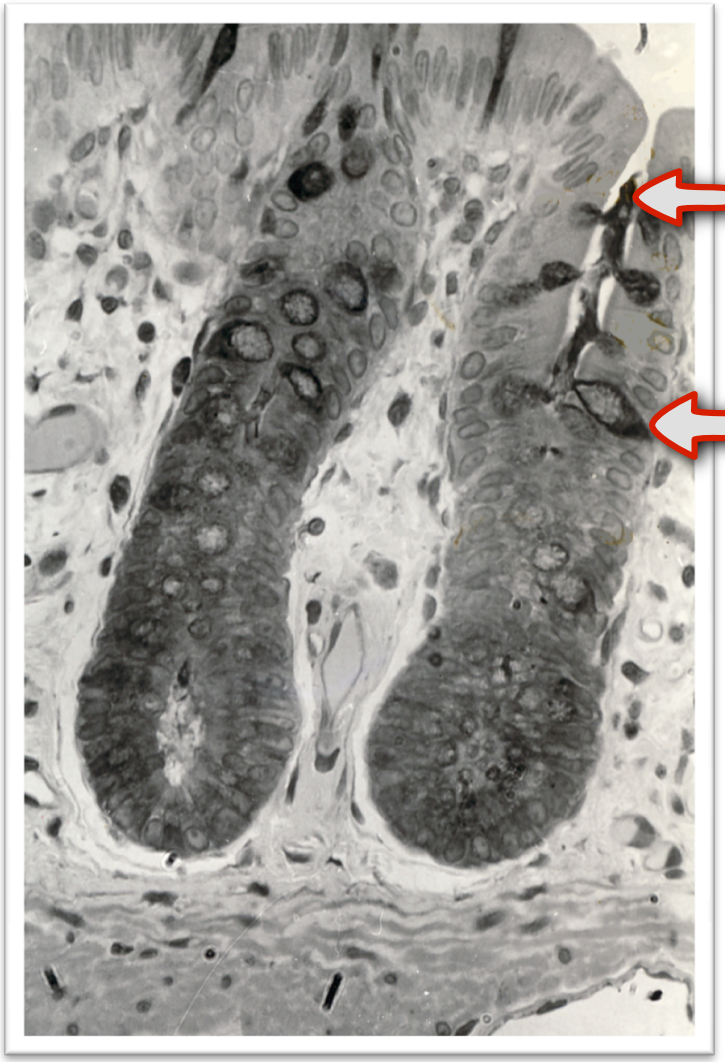


No. 3

**Observations that humans and other animals absorb low levels of aluminium led to the incorrect assumption that aluminium is safe for human ingestion**

However, man-made activities have overwhelmed evolutionary barriers to toxic levels of aluminium uptake

No. 4

## Measurable amounts of $^{26}Al$ can enter the brain from drinking the equivalent of one glass of alum-treated water

- The City of Sydney was preparing its bid for the year 2000 Olympic Games from about 1989 onwards
- The city needed to demonstrate that it could supply drinking water to comply with WHO guidelines or better
- To do so, four massive water treatment plants were needed

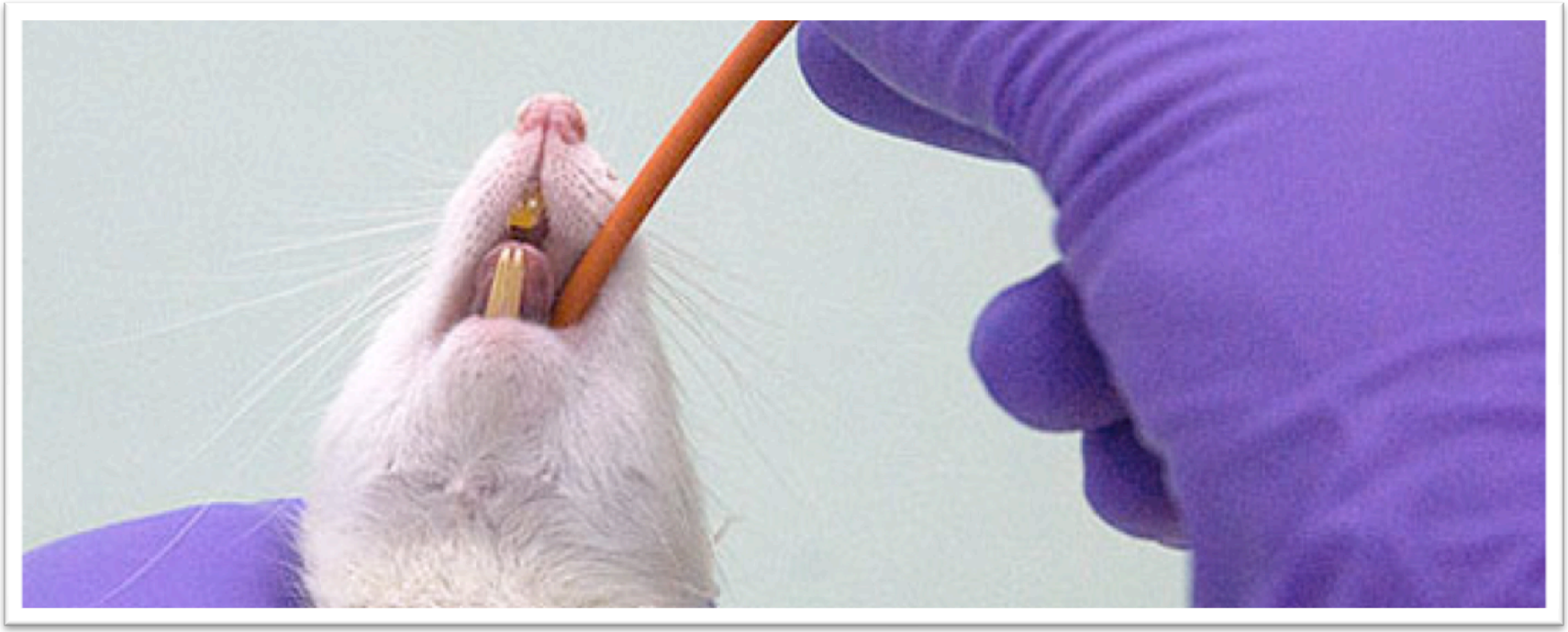
No. 4

# The question was whether to specify alum as a coagulant or ferric chloride

- In view of the Camelford incident in 1988, and the suggested environmental link between aluminium and AD, Sydney Water commissioned us to determine whether residual aluminium coagulant in drinking water could enter the brain

No. 4

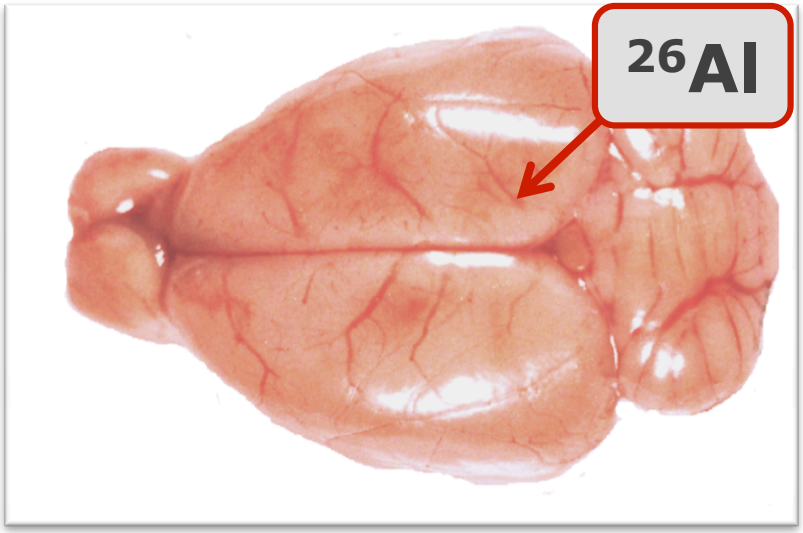
**We gave 8/10 mature outbred male Wistar rats an amount of radioactive aluminium ($^{26}$Al) equivalent to the aluminium contained in a single glass of alum-treated drinking water.** Two controls were given water without $^{26}$Al



No. 4

# Accelerator mass spectrometry detected $^{26}Al$ in (blinded) samples from 6/8 rat brains removed 2 weeks after oral $^{26}Al$ exposure

$^{26}Al$ is a synthetic aluminium isotope so this study unambiguously demonstrates aluminium uptake by the brain after $^{26}Al$ oral exposure, even at trace levels




Walton JR et al. Neurotoxicology 16, 187-90 (1995)
Jouhanneau P et al. Clin Chem 43,1023-1028 (1997)
Zafar TA et al. Proc Soc Exp Biol Med 216, 81-85 (1997)

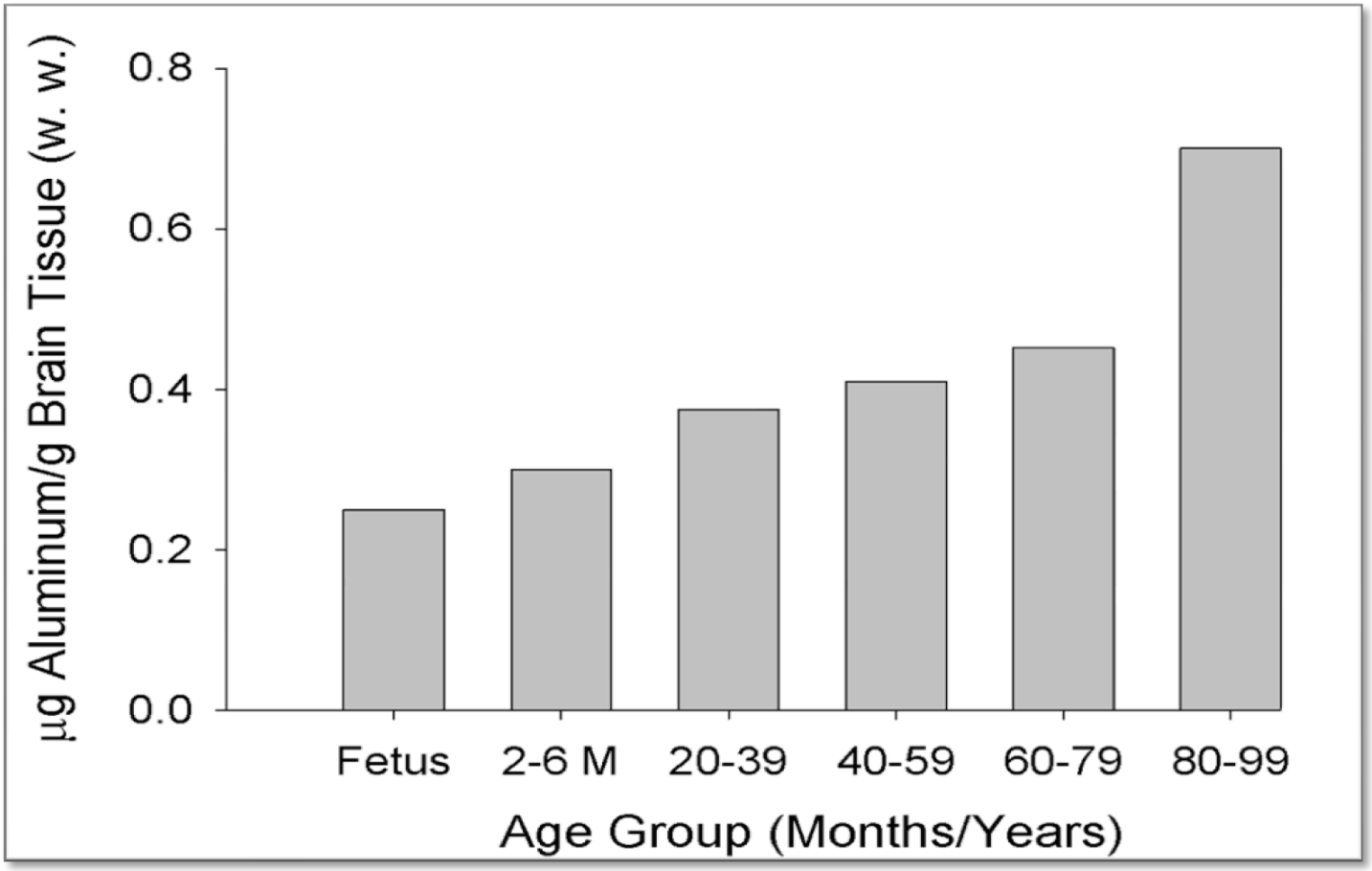
No. 4

## This example of a risk assessment and decision led Sydney Water to:

- exercise prudent avoidance of the risk
- utilise ferric iron salts rather than alum (aluminium sulphate) in the coagulation process for providing clean and safe drinking water.
- The new drinking water treatment plants were commissioned in 1996

No. 5

# Aluminium slowly accumulates in the cerebral cortex with increasing age, accounting for AD's long prodromal phase



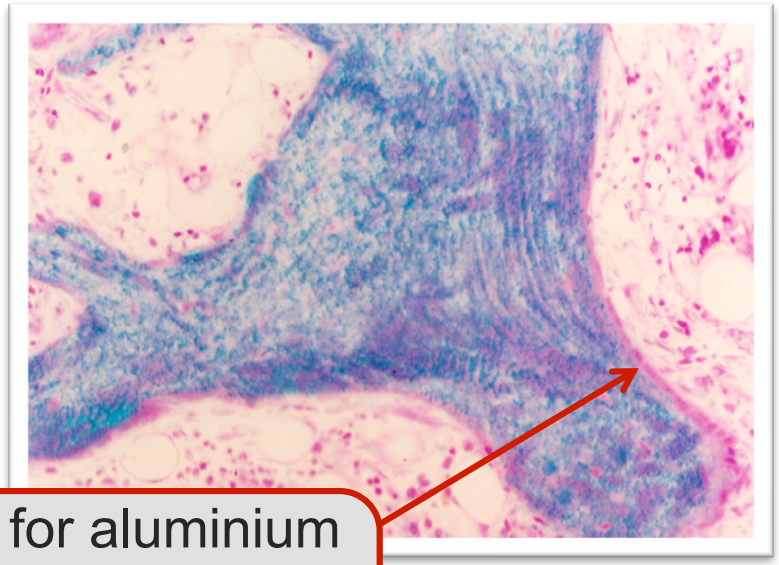

Markesbery WR et al., Neurobiol Aging 5, 19-28 (1984) with permission from Elsevier

No. 6

# Several age-related disorders elevate the risk for aluminium accumulation in older brains

- Digestive disorders in old age may result in the use of aluminium-based antacids


- Bone is a major aluminium storage tissue throughout life.
- During osteoporosis, bone matrix breaks down and releases aluminium into the bloodstream, making it available for brain aluminium uptake



Walton stain for aluminium showing Bone Al

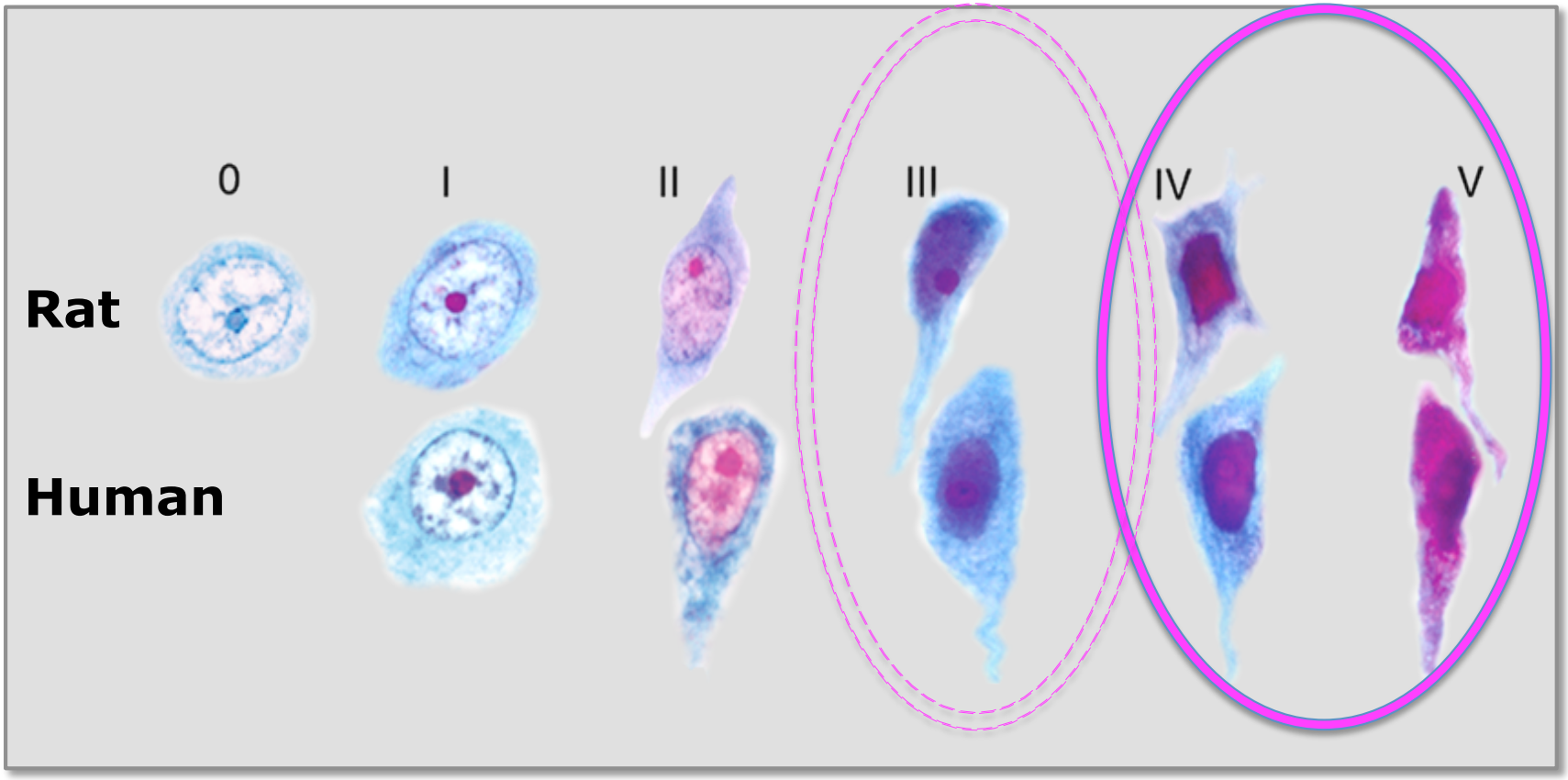
# No. 6

- After age 30, humans lose approximately 7% of kidney function each decade.
  By age 60, kidney function may be reduced by 25%, hindering renal excretion of plasma aluminium


- Older people are urged to have annual flu vaccines which contain aluminium as the adjuvant. Injections furnish ~1000x more aluminium than oral ingestion as injections bypass the gastrointestinal barrier

No. 7

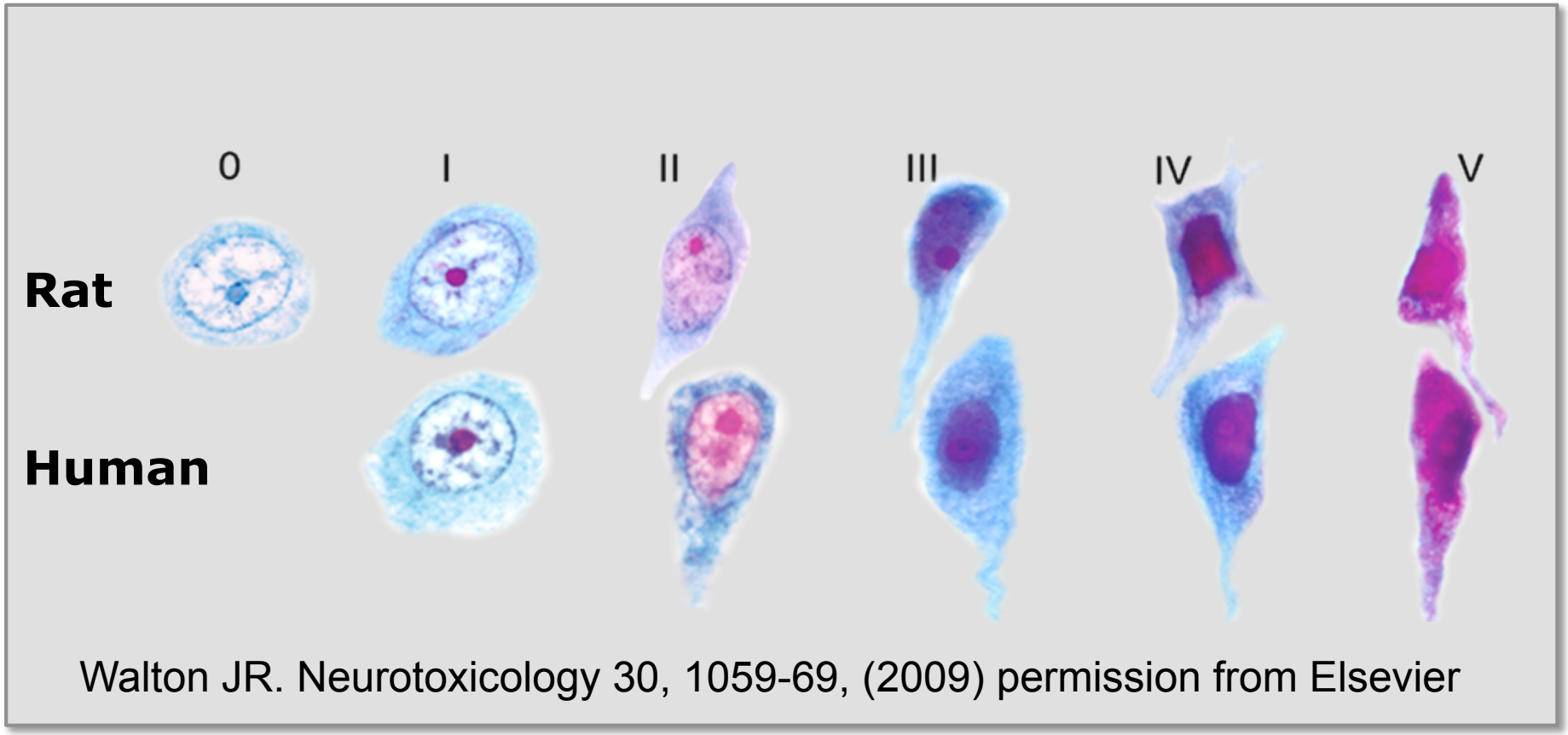
# Aluminium progressively accumulates in neurons to toxic levels (stages IV and V)




Neurons processed with the Walton stain for aluminium appear pink, purple or bright magenta (fuchsia). The staining protocol is described in Walton JR, Biotech Histochem 79,169-76 (2004)

No. 7

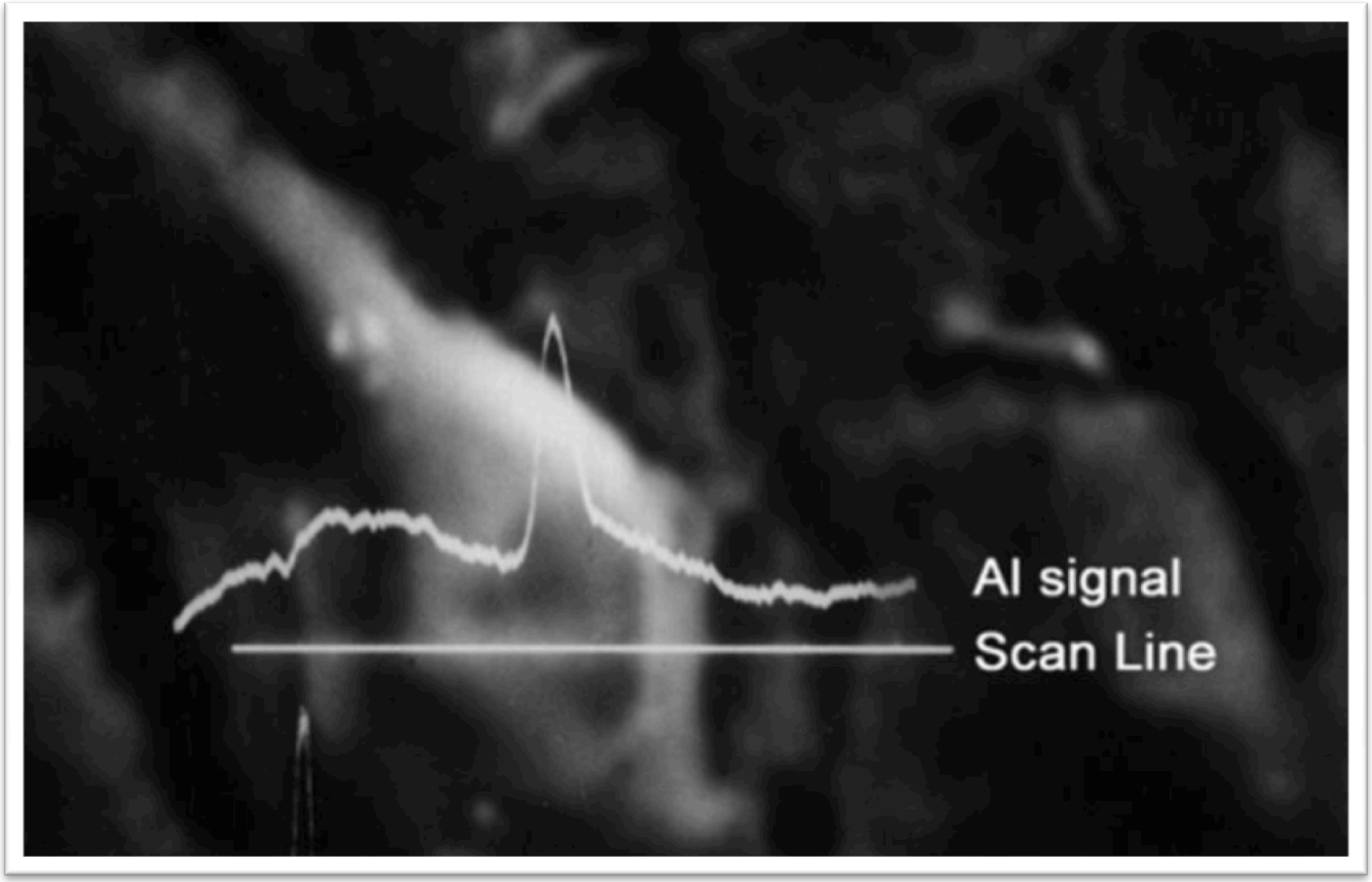
# Whether or not one develops AD depends on (1) the rate of aluminium absorption and (2) the proportion of neurons that have accumulated aluminium to toxic levels in memory-processing regions of the brain




Walton JR. Neurotoxicology 30, 1059-69, (2009) permission from Elsevier

No. 7

# Aluminium accumulation in the nucleus of neurons in AD brains



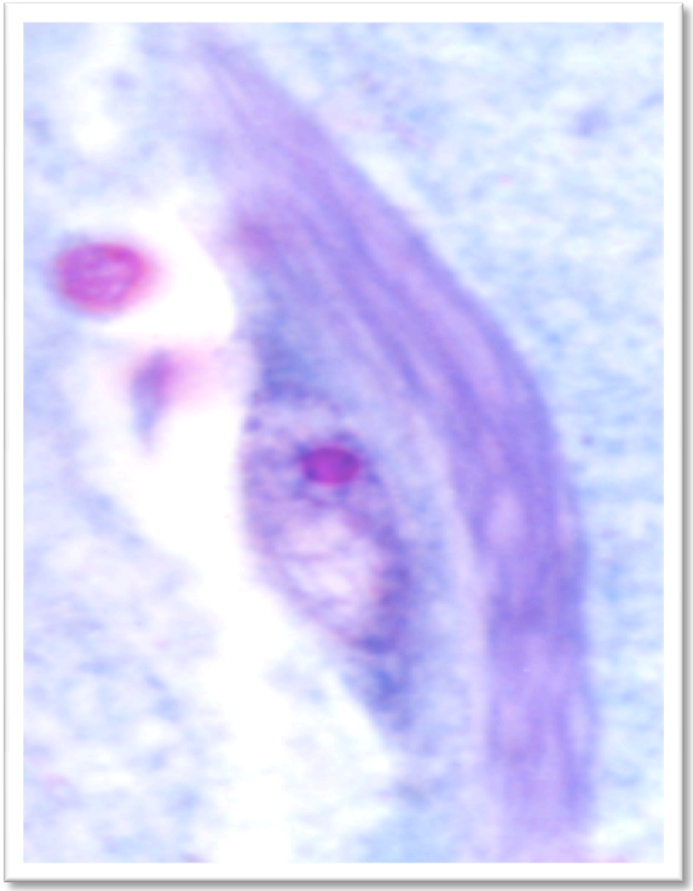

Electron micrograph (with X-ray analysis) kindly provided by Daniel Perl, published in Science 208, 297-9 *(*1980) with permission from the AAAS.

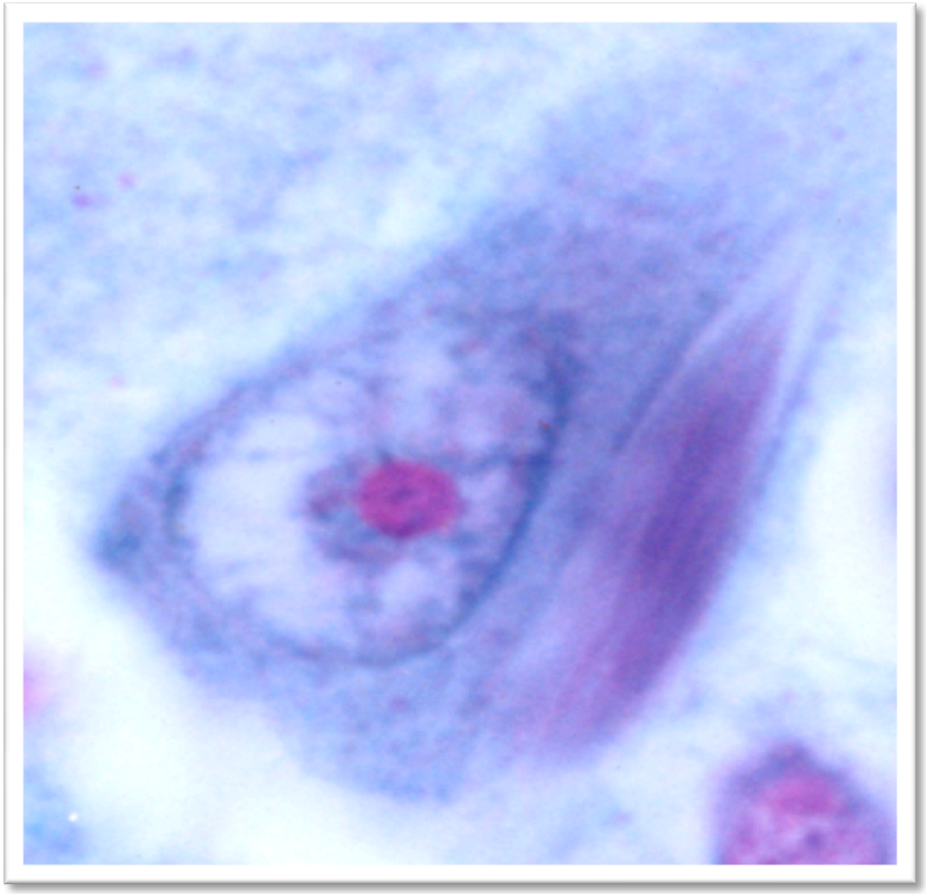


JR Walton Copyright 2014

No. 7

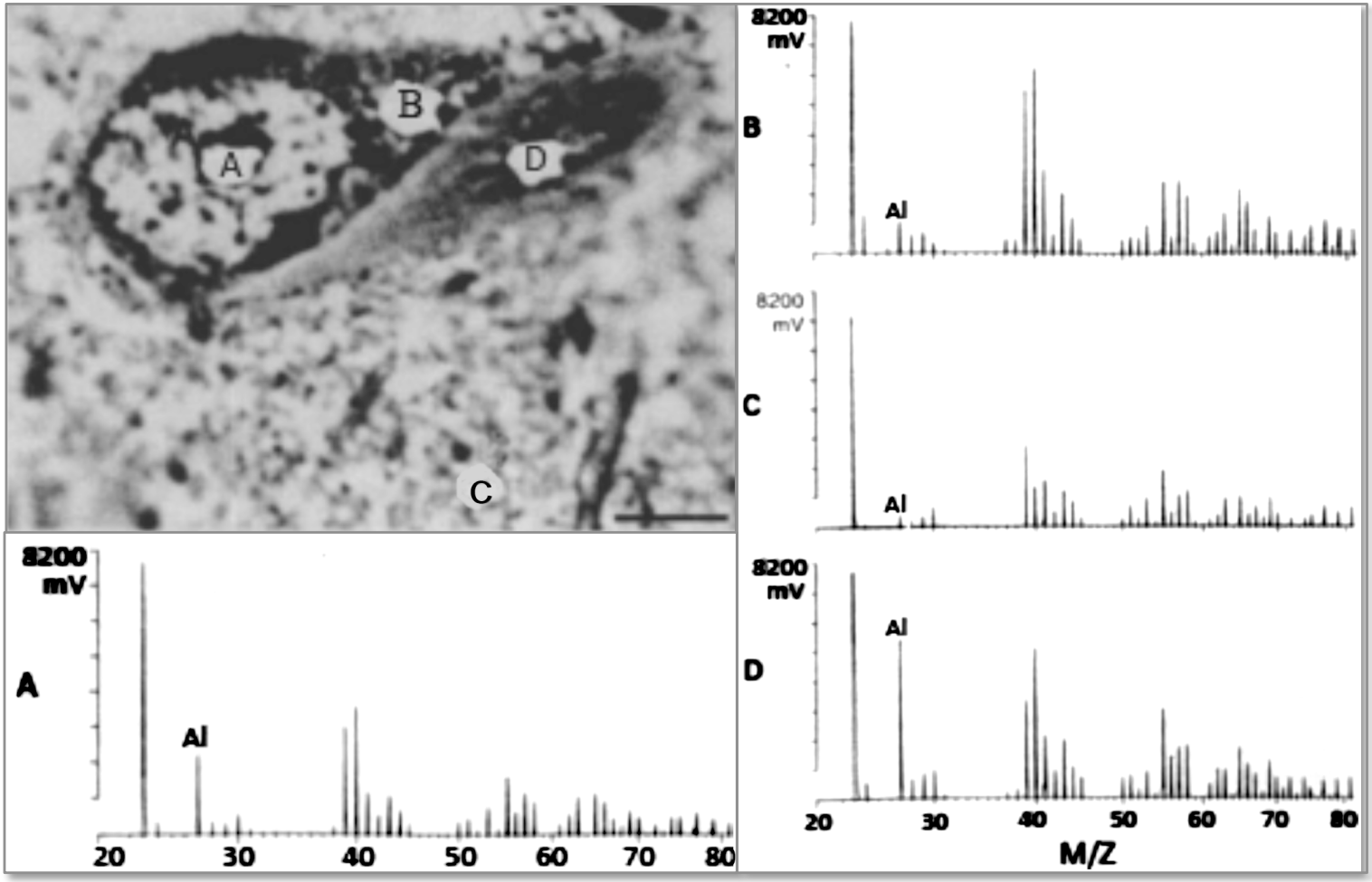
**Intracellular NFTs are a cell response, evolved in human neurons, that slows aluminium uptake into the nucleus by sequestering and storing it in large amounts**





No. 7

# The concentration of aluminium in NFTs is 3x higher than in the nucleolus

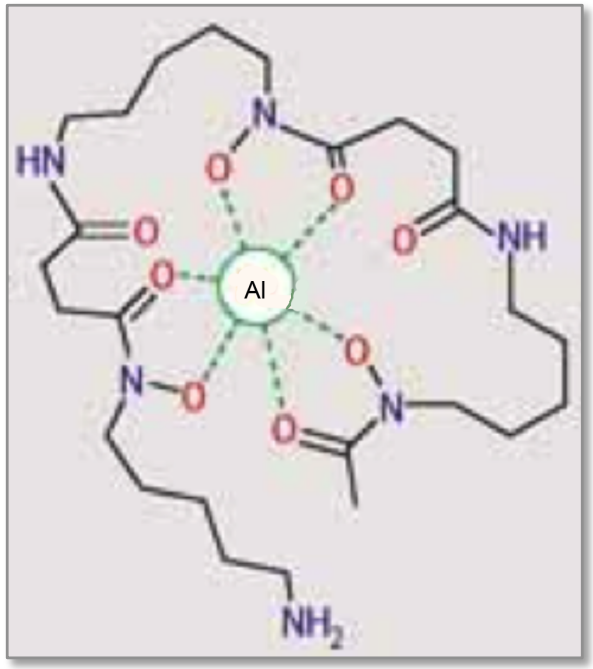



Laser Microprobe Mass Analysis (LAMMA) Good PF, Perl DP. Ann Neurol 31, 86-292 (1992) with permission from Wiley

No. 8

# Aluminium chelation by desferrioxamine (DFO), which removes aluminium from the brain, is the only treatment, to date, shown to slow the rate of AD deterioration

- Videotaping showed AD patients receiving DFO took twice as long for their activities of daily living to deteriorate compared to AD patients in a placebo group




McLachlan et al., Lancet 337, 1304-8 (1991)

McLachlan et al., Ther Drug Monit 15, 602-7 (1993)

No. 9

# Nine of thirteen epidemiological studies* reported significant relationships between aluminium levels in the drinking water supply and risk for AD

- Most positive studies recommended drinking water with less than 100 µg aluminium/L, or less than 100 µg aluminium/day provided in the form of water
- One “no effect” study was disqualified because subjects were exposed to “high Al” (alum-treated) drinking water <1 year
- Three negative studies were disqualified due to inappropriate subjects: one with cognitively-impaired subjects of all types (instead of all AD) and two based on early-onset AD patients
- One positive study was also disqualified due to early-onset AD patients. Hence, 8 studies remained; all were positive

*Flaten TP. Brain Res Bull 55, 187-96 (2001)

No. 9

# In principle, the highest level of proof that aluminium causes AD would be a prospective interventional human study

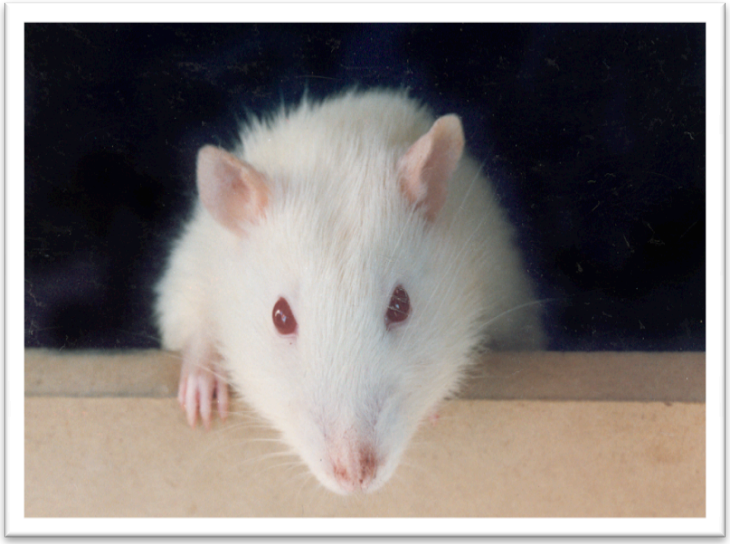
- where subjects are randomly assigned to cohorts that ingest total dietary aluminium at three levels in the range for total dietary aluminium that humans ingest (low, intermediate and the high end)
  - from their food and beverages, including drinking water
  - over a significant proportion of their life span to discover the outcomes of these treatments

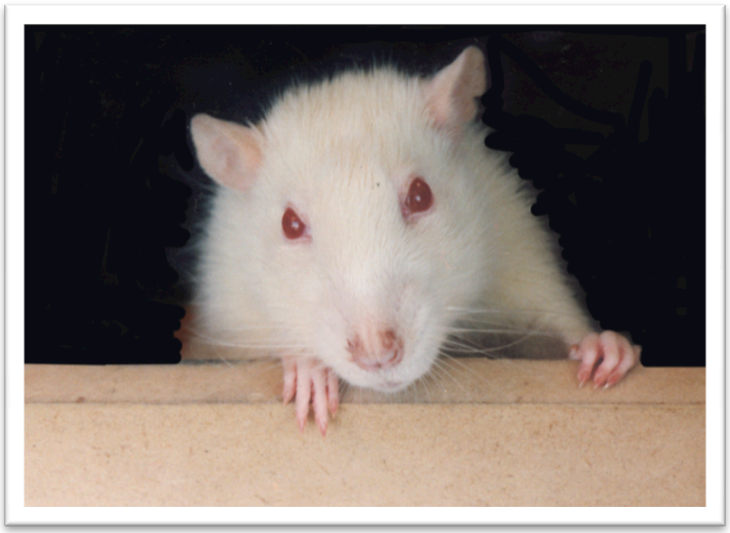
- However, it would be clearly unethical as well as impractical to perform a study like this in humans

No. 10

# Chronic Al intake produces AD-equivalent dementia in aged rats

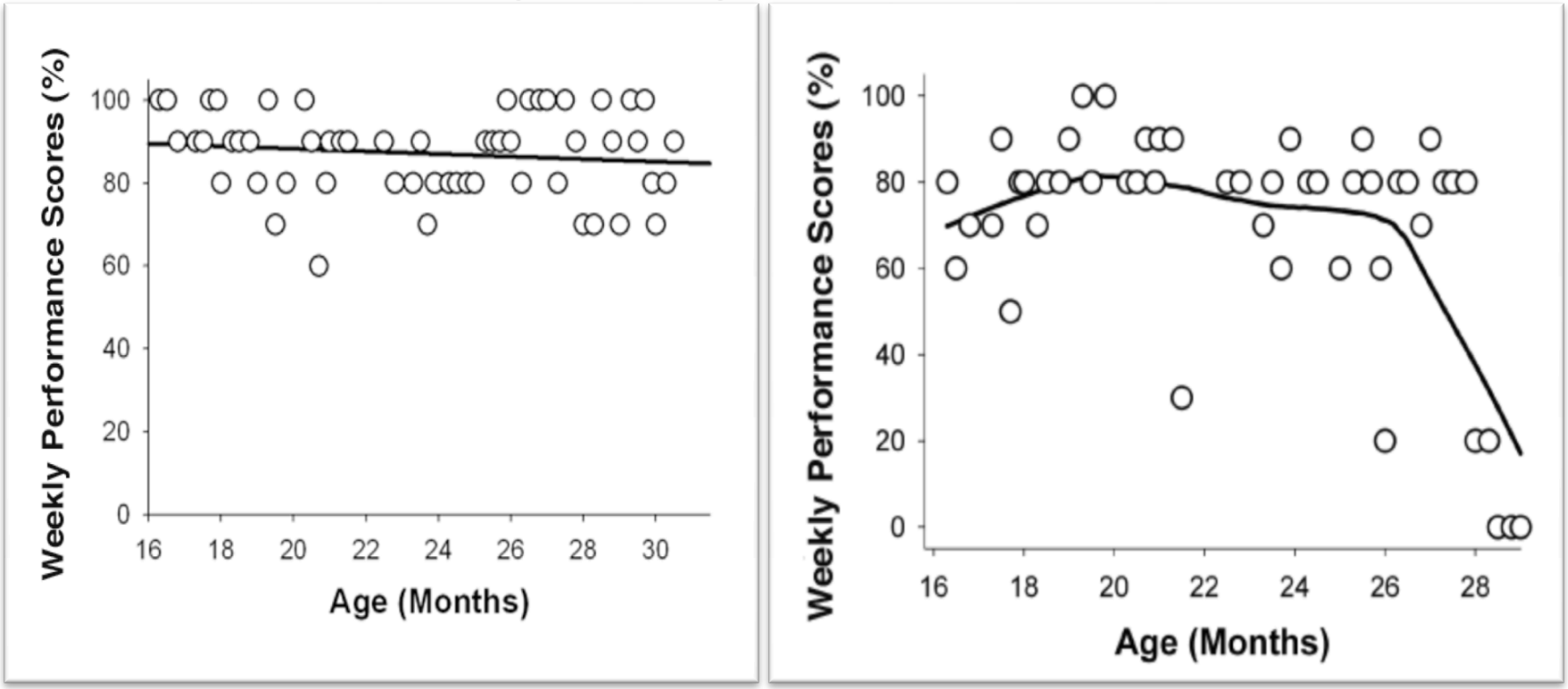
- In lieu of a comparable human study, three groups of rats were given total aluminium in their feed and water, throughout their middle age and old age, at three levels equivalent to total dietary aluminium levels routinely consumed by humans
- The three cohorts showed a dose-response effect with most (80%) rats, that ingested aluminium at the high end of the human total dietary Al range, developing Alzheimer's disease-equivalent dementia (ADED) in old age





No. 10

# Rat T-maze performance scores of a low-aluminium control (left) averages 90% to the end of his life and a high-aluminium rat (right) shows decline in his scores during old age




Walton JR. Neurotoxicology 30, 182-93 (2009)

# What is the mechanism by which aluminium causes AD?

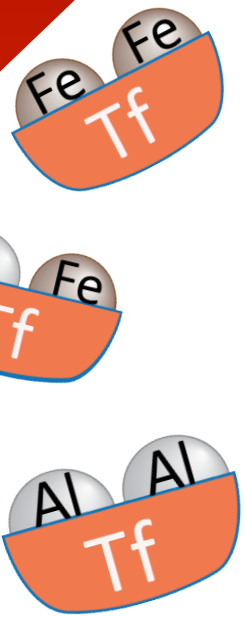
No. 11

# Aluminium's neurotoxicity is due to its inherent physical properties

- The aluminium ion is slightly smaller than iron and magnesium ions, meaning aluminium can readily substitute for these essential metals in cell transport and regulatory proteins

| Ion (Coordination number) | Ionic Radii (Å) | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 |
| $Al^{3+}$ | 0.39 | 0.48 | 0.54 | | | |
| $Fe^{3+}$ | 0.49 | 0.58 | 0.65 | | | |
| $Mg^{2+}$ | 0.57 | 0.66 | 0.72 | | 0.89 | |
| $Zn^{2+}$ | 0.60 | 0.68 | 0.74 | | 0.90 | |
| $Ca^{2+}$ | | | 1.00 | 1.06 | 1.12 | 1.18 |

No. 11




# Up to two iron atoms (Fe) can be transported by each transferrin protein (Tf) in blood as Fe Fe-Tf

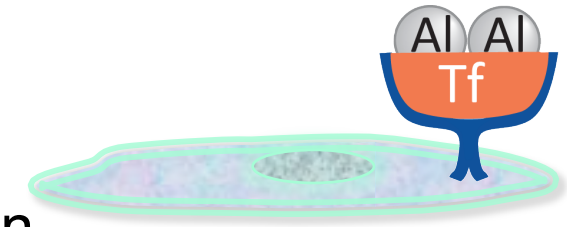
- Normally, iron occupies only 30% of the available binding sites in Tf
- Up to 90% of aluminium (Al), circulating in plasma, binds to the vacant iron-binding sites in Tf,* resulting in Fe Al-Tf or Al Al-Tf
- This allows efficient transport of aluminium throughout the circulatory system and protects aluminium from being excreted by the kidneys

*Shirley DG, Lote CJ. Nephron Physiol 101, 99-103 (2005)
**Morris CM et al. J Neurol Sci 94, 295-306 (1984)

# No. 12

## Al-Tf in the circulatory system allows aluminium to preferentially accumulate in the same neurons that are most damaged in AD, via Tf receptors ( )

- First, the blood-borne Al-Tf docks on Tf receptors located on brain capillary cells*



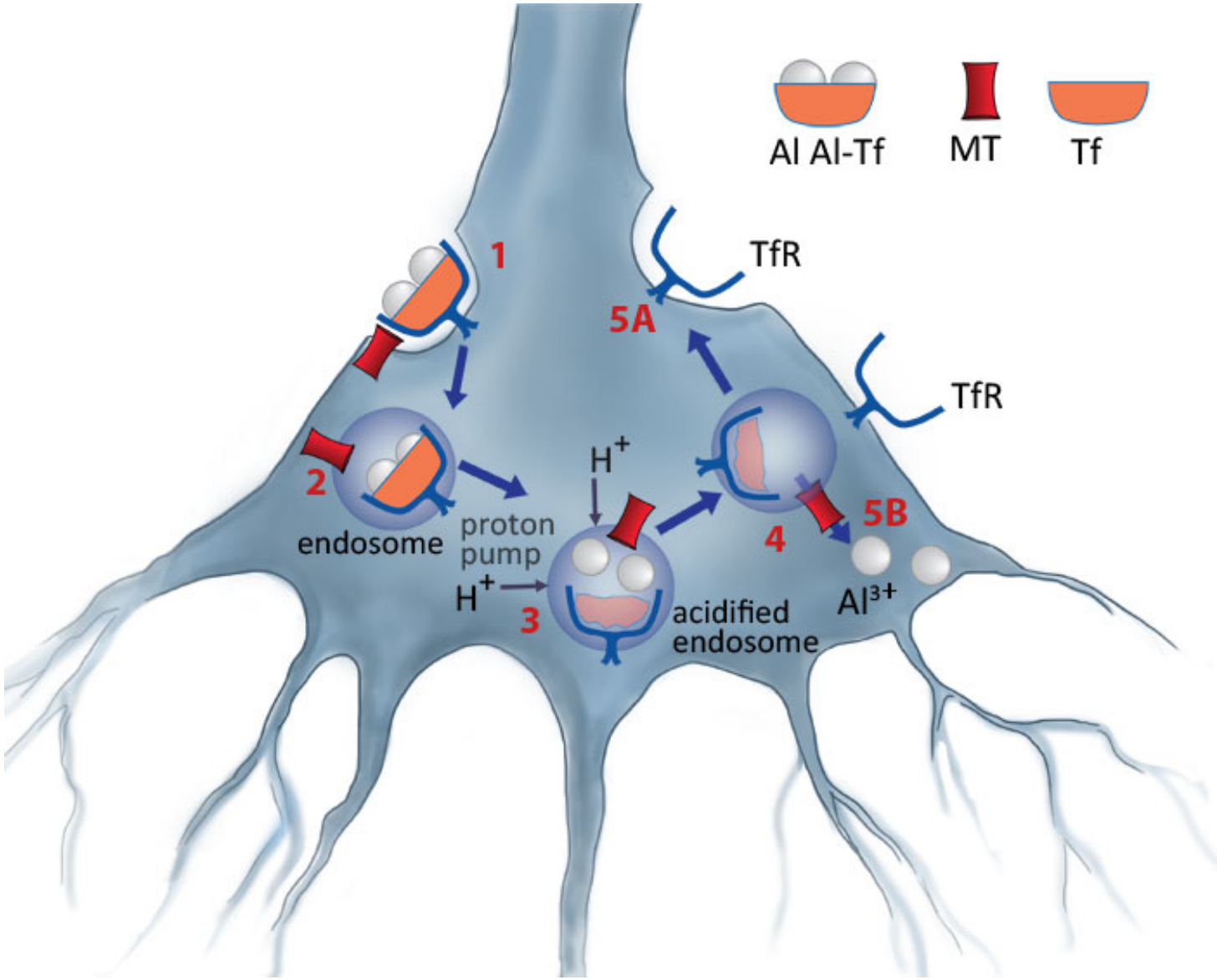

- Such docking allows aluminium to cross the blood-brain barrier and enter the brain
- Then, Al-Tf binds to Tf receptors on the surface of large neurons with high iron and energy demands** such as the entorhinal cortical cells of origin for the perforant pathway and hippocampal CA1 cells
- This allows aluminium entry and accumulation in AD-vulnerable neurons

*Jefferies WA et al., Nature 312, 162-3 (1984)

**Roskams AJ, Connor JR. Proc Nat Acad Sci 87, 9024-7 (1990)

No. 12

# Tf receptors (1) allow aluminium to enter and accumulate in the cell



No. 13

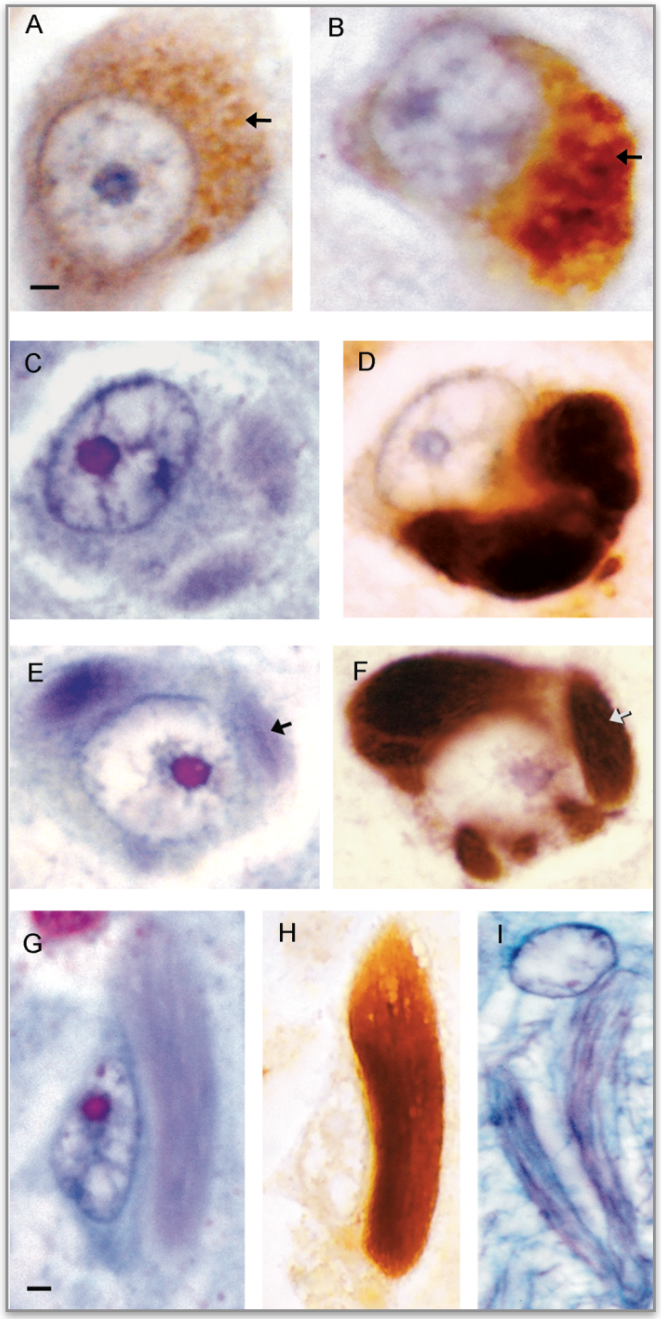
# Aluminium substitution for iron in iron regulatory protein 2 disregulates iron metabolism in neurons* **

- causing neurons to behave as though permanently iron-deficient so the neurons continue to synthesize transferrin receptors and import even more aluminium and iron
  - Aluminium-induced iron disregulation accelerates Al and Fe accumulation in neurons, thereby increasing oxidative damage

* Yamanaka K et al., FEBS Lett 462, 216-220 (1999)
** Ward RM et al., J Inorg Biochem 87, 9-14 (2001)

No. 14

# Chronic aluminium uptake into human neurons leads to the formation and growth of NFTs*



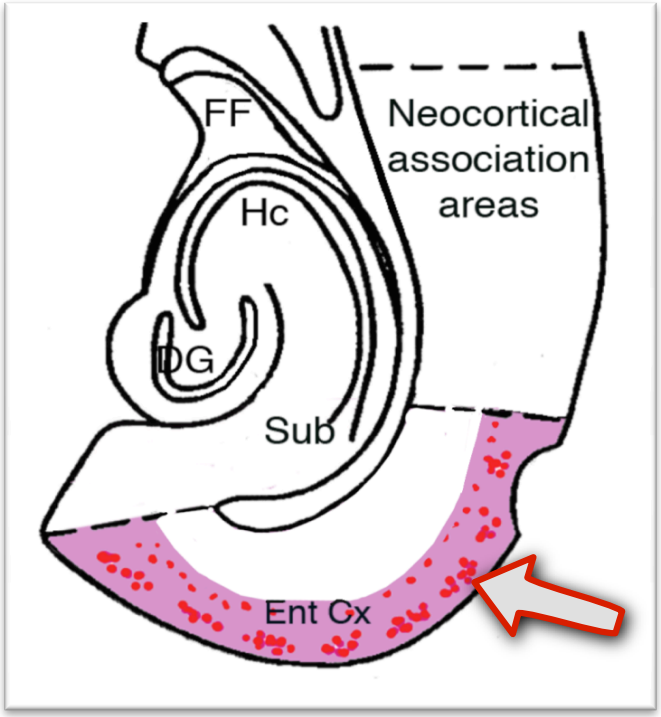

First, aluminium causes granules (arrow in A) of hyperphosphorylated tau to form which fuse (B) to form large pools of Hyp tau in the cytoplasm (D). Aluminum (purple in C,E,G,I) co-localizes with the Hyp tau, suggesting they form an aluminium/Hyp tau complex that slows aluminium uptake into the nucleus. NFT filaments begin to form in the complex (arrows in E&F) and continue to grow (G&H) producing NFTs that eventually displace the nucleus, resulting in enucleation and cell death (I).

*Reprinted from Walton JR. J Alzheimer's Dis 22, 65-72 (2010)

# No. 15

## In AD, the cells of origin for the perforant pathway of the entorhinal cortex are more severely damaged by aluminium accumulation and NFTs than neurons in any other brain region

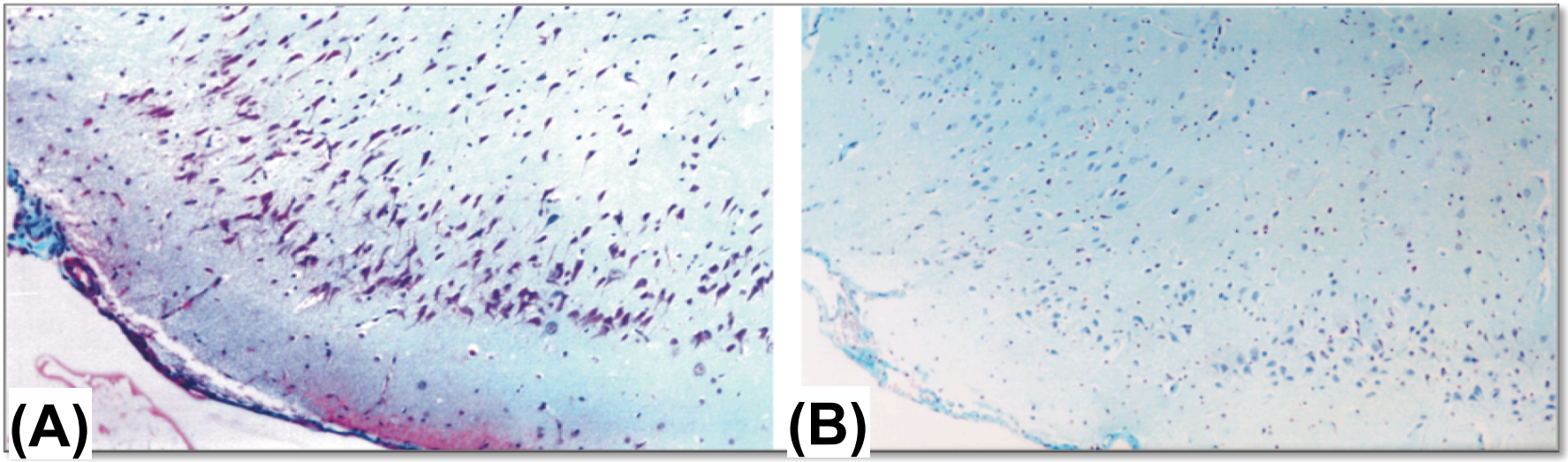
- These large cells (arrow) are, invariably, the first to develop NFTs in AD




Arnold SE et al., Cerebr Cortex 1, 106-13 (1991)
Hyman BT et al., Science 225, 1168-70 (1984)
Van Hoesen et al., Hippocampus 1,1-8 (1991)

No. 15

# Similarly, more cells of origin for the perforant pathway in the entorhinal cortex of ADED rats have toxic aluminium levels than any other brain region analysed

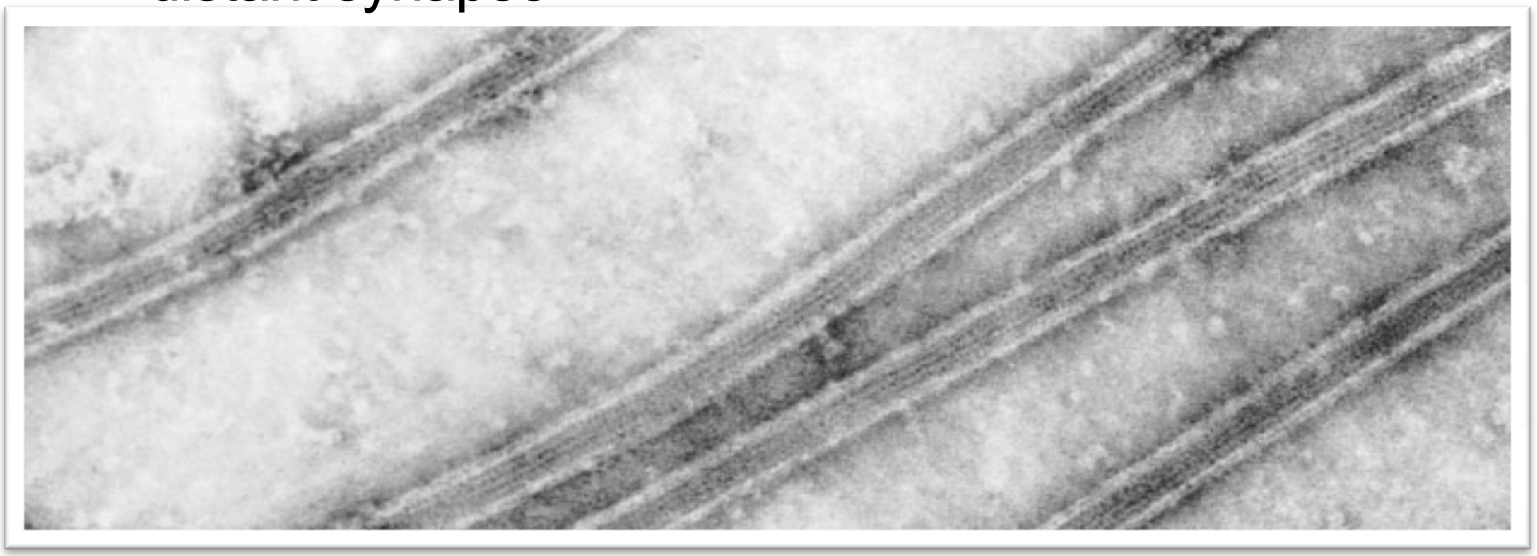



Also, significantly more cells of origin for the perforant pathway in ADED rat brains exhibit toxic aluminium levels (A) than the same cell population of low-aluminium controls (B). A major difference between A & B is cells of controls (B) have microtubules

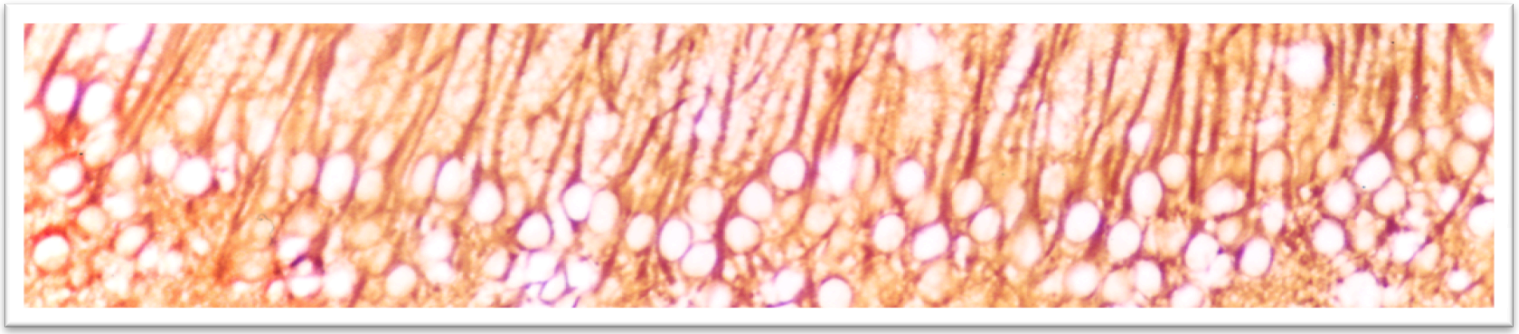
Reprinted from Walton JR, *Aging and Vulnerability to Environmental Chemicals* Weiss B [ed] (2013) pp 31-82, Royal Society for Chemistry

# No. 15

Electron microscopy of healthy cells shows microtubules which provide: (1) structural strength for the neurons' long cell processes; and (2) transport of vesicles, mitochondria and molecules between the nucleus and the cell's most distant synapse



Immunostained microtubules in healthy CA1 neurons



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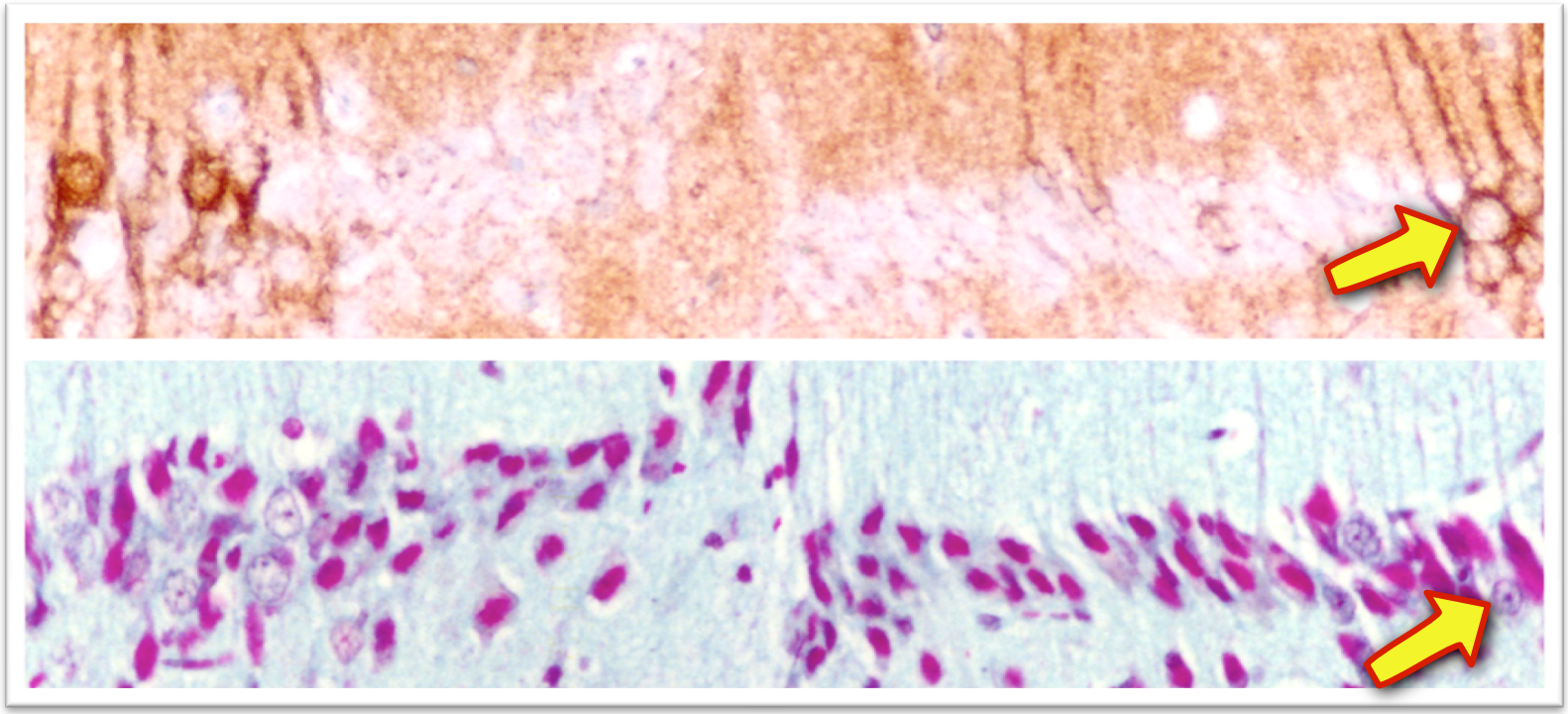
No. 16

# Toxic aluminium levels deplete neurons of their microtubules

- All rats that developed ADED had at least one lesion of microtubule-depleted aluminium-rich cells in the hippocampal CA1/subiculum zone



Stage I cells (arrows) have low aluminium accumulation with microtubules. Stage IV cells (centre) show microtubule loss

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No. 16

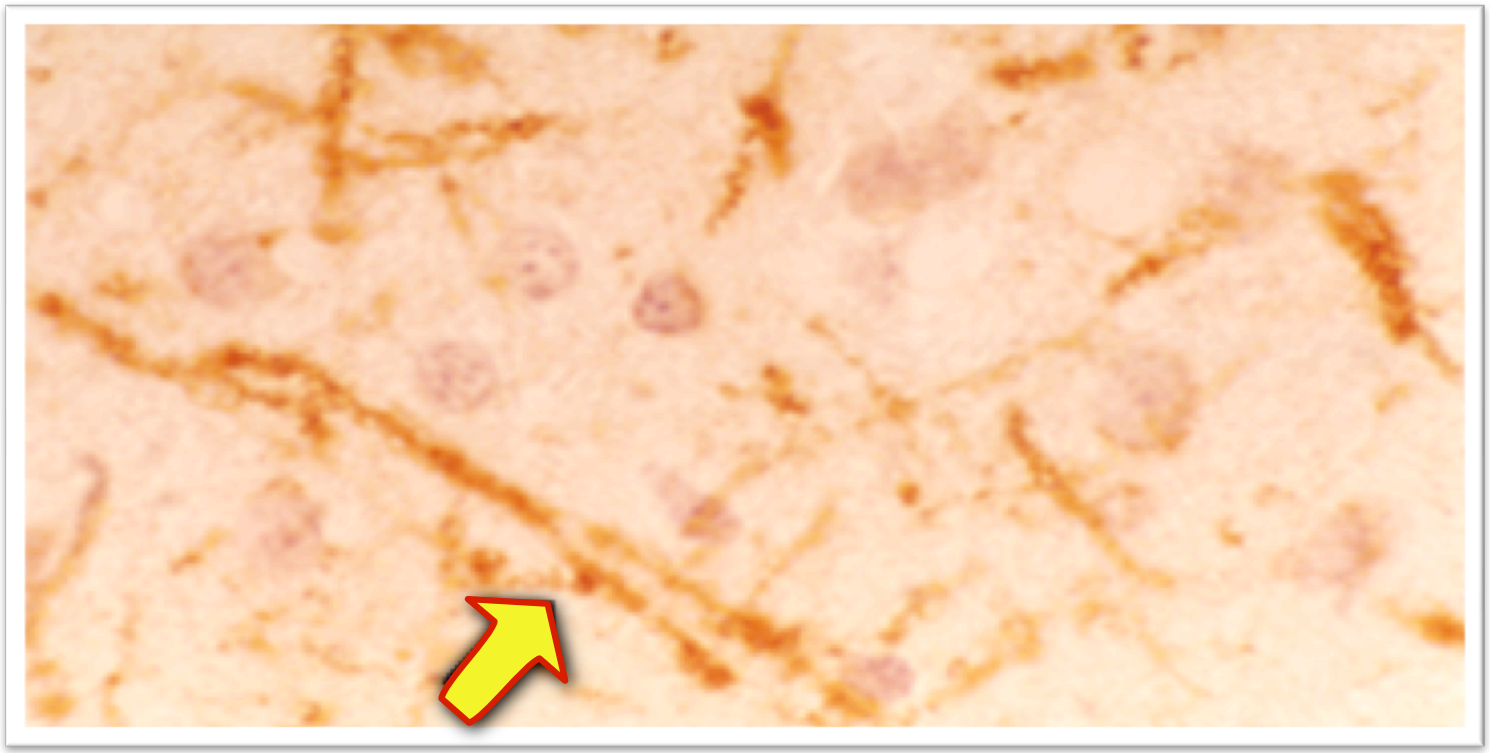
**Equivalent lesions, with NFTs, have been identified in small samples taken from AD cortex and hippocampus, analysed with graphite furnace atomic absorption spectroscopy, referred to as "aluminium hot spots"***

**Human neurons with NFTs are at toxic aluminium stages and are known to be microtubule-depleted****

*Crapper DR. Science 180, 511-513 (1973)
**Gray EG et al. Neuropathol Appl Neurobiol 13, 91-110 (1987)
**Hempen B, Brion J-P. J Neuropathol Exp Neurol 55, 964-72 (1996)

No. 17

**Microtubule depletion in neurons of humans with AD and ADED rats results in axonopathy,** shown here as dilations and constrictions in axons (arrow), reflecting transport failure



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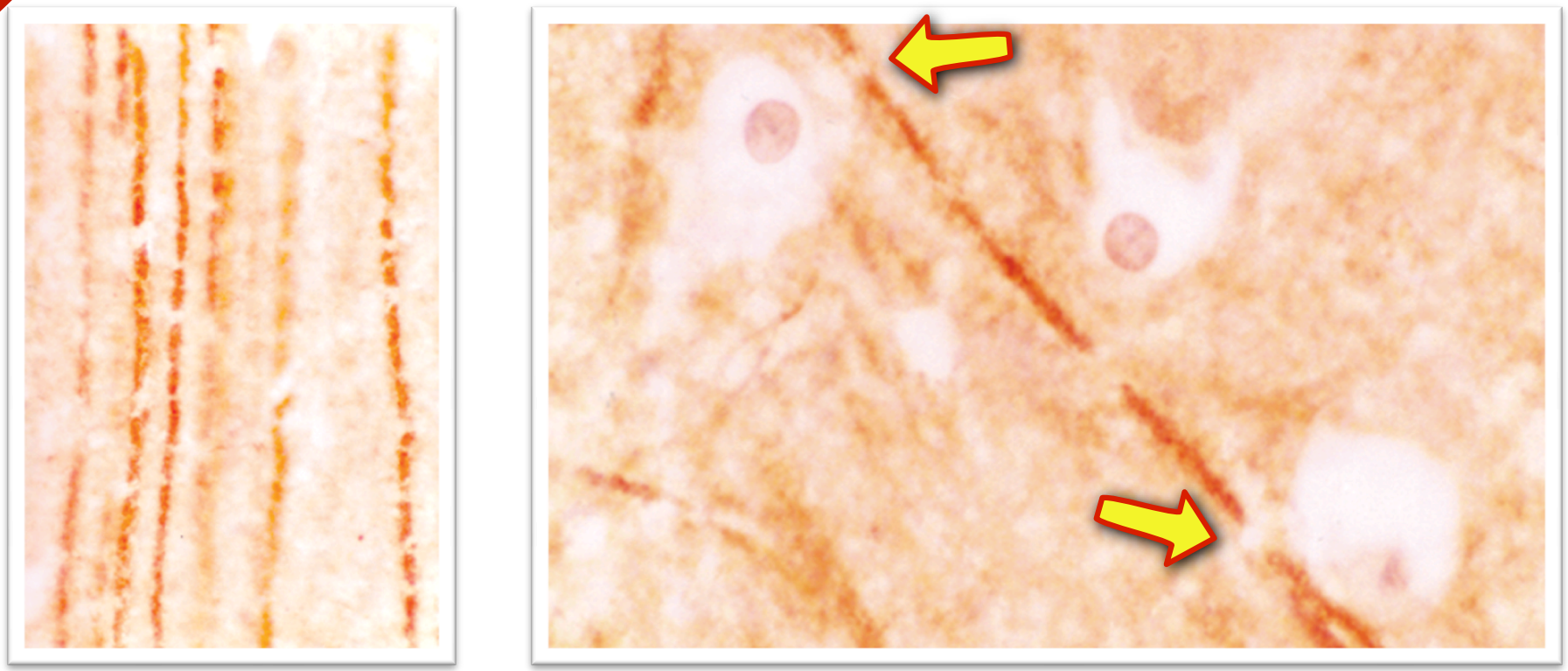
No. 18

# Dendrites segment and die back in brains of humans with AD and rats with ADED



Astrocytes (r), in close proximity to microbule-depleted dendrites, may be responsible for dendritic segmentation in preparation for their removal by brain macrophages

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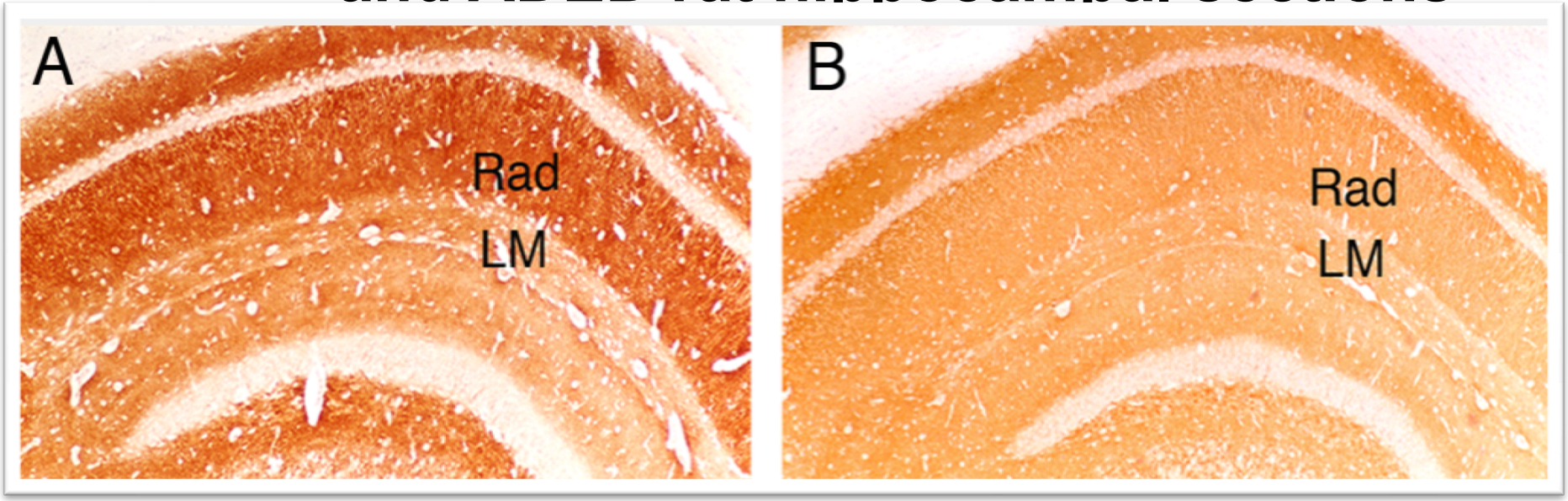
# No. 19

## Synapse breakdown and loss is evident in brains of humans with AD* and rats with ADED, as shown here in control rat and ADED rat hippocampal sections**




A. Control hippocampus: note darker synaptogyrin staining (more synapses) in the stratum radiatum (Rad) than the lacunosum moleculaire (LM). B. ADED Rad stains less for synaptogyrin.

*Terry RD et al. Ann Neurol 30, 572-580 (1991)

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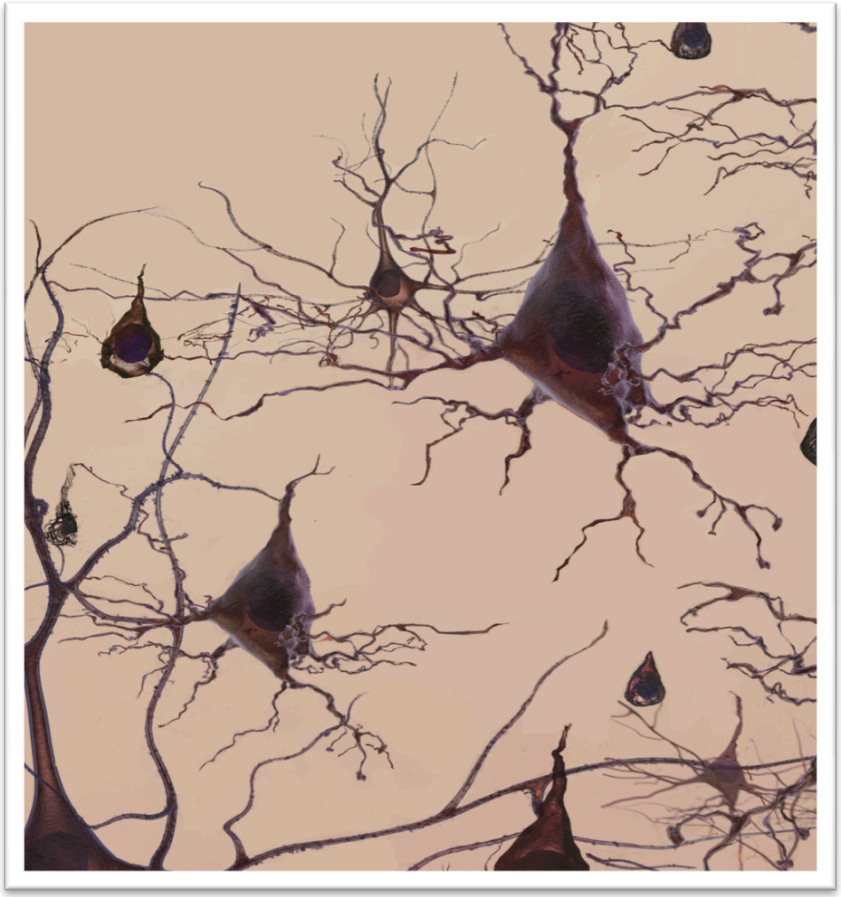
No. 19

# Aluminium-induced microtubule depletion, dendritic dieback, axonopathy and synapse break-down ultimately result in disconnection

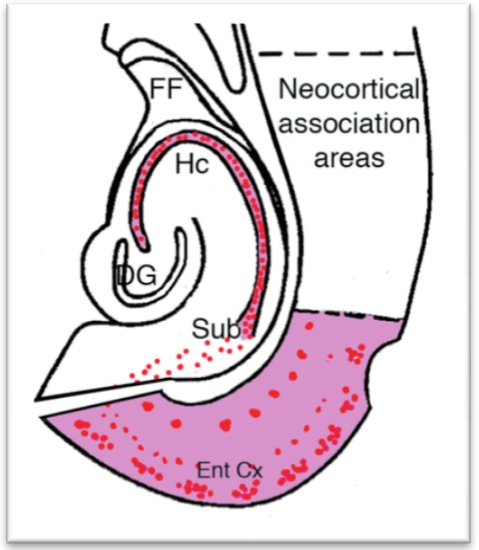
- between neurons
- between entire brain regions in the same hemisphere; and
- between the same regions in both hemispheres of the brain



Lakmache T et al. PNAS USA 95, 9042-6 (1998)

No. 19

# Aluminium targets the selective vulnerability of entorhinal and hippocampal neurons in AD and ADED, destroying their microtubules and axons of the perforant pathway

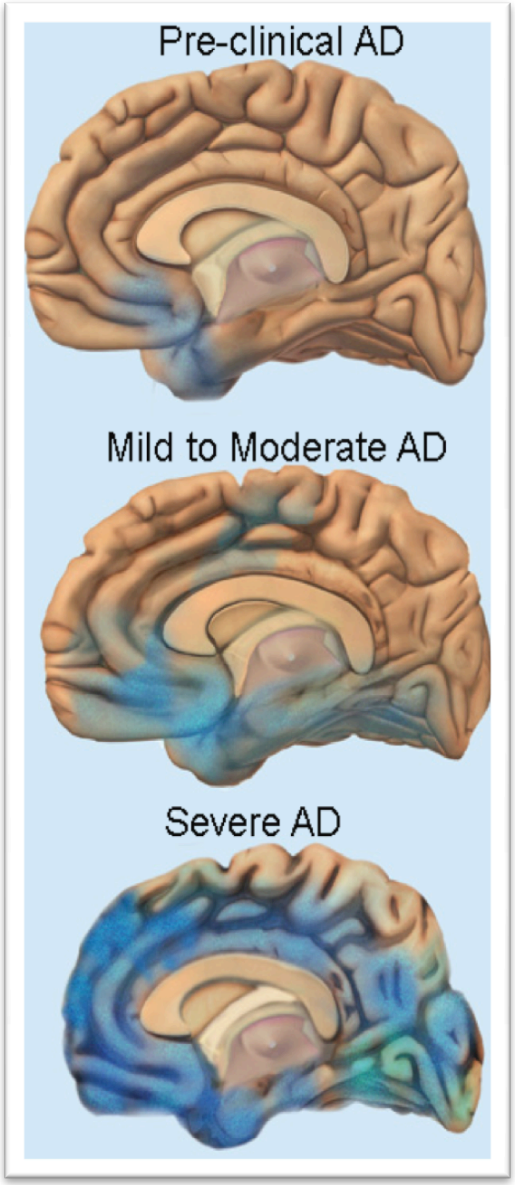



- Hence, aluminium is the agent that literally dissects the cortex, disconnecting and isolating the hippocampal formation from the neocortex in AD and ADED

- These events can account for the loss of episodic memory or memory for recent events in both species. Thus, ADED is a reliable translational animal model for AD

No. 20

# Aluminium spreads, from one brain region to another, in a hierarchical manner

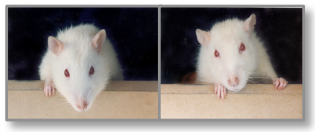



- Perl and Good demonstrated aluminium spreads between interconnected brain regions of the AD hierarchy*

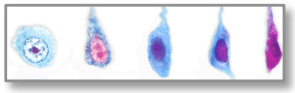
- As aluminium spreads, AD progresses. More brain regions become disconnected and lose function, resulting in: attentional deficits, inability to synthesize neurotransmitters and, eventually, inability to recognize one's self and others

* Perl DP & Good PF, Lancet I, 1028 (1987)

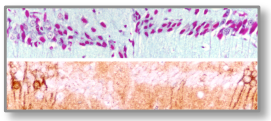
# In summary --



A translational rat model for AD has been instrumental in helping to explain the process of cortical atrophy responsible for the dementia of AD

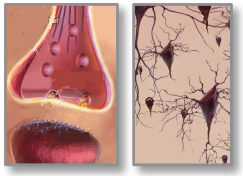


1. Aluminium (Al) from the diet and other sources chronically enters the brain more readily than exits, resulting in net aluminium accumulation. The physical resemblance of aluminium to ferric iron allows aluminium accumulation to toxic levels in AD-vulnerable brain regions, of many individuals

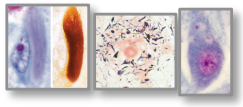


2. Toxic levels of intracellular aluminium deplete neurons of their microtubules

3. Microtubule depletion results in dieback of the dendrites and accounts for axonopathy or failure of axonal transport



4. Synapses break down as cell processes shrivel and die back, resulting in disconnection of cells and entire brain regions with loss of specific brain functions, beginning with loss of episodic memory



5. Independent of this microtubule depletion cascade, which accounts for cortical atrophy, aluminium plays critical roles in the formation and growth of neurofibrillary tangles, amyloid deposits and granulovacuolar degeneration

6. As AD severity increases, aluminium spreads in a stepwise manner to other interconnected brain regions in the AD hierarchy, repeating the aluminium-induced microtubule depletion cascade and resulting in further loss of regional functions

# Conclusion

- The evidence shows, on the balance of probabilities, that chronic intake of aluminium causes AD

# Recommendations

- Treated public drinking water, and bottled waters, be restored to naturally occurring aluminium levels, such as the parameters of the Sydney public water model (averaging <20 μg aluminium/L to a maximum of 40 μg/L); i.e. 1/10 to 1/5 of the WHO limit
- Progressively remove added aluminium from all commercially prepared foodstuffs, skin treatments and other products with potential aluminium bioavailability
- Restore human aluminium exposure to pre-industrial levels as efficiently and as soon as reasonably practicable to enable an acceptable daily intake (ADI) of up to 10 mg aluminium/day; i.e., the amount naturally contained in a fresh food diet

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