Proteins Gone Wild!

Current Research on Alzheimer's and Mad Cow diseases.

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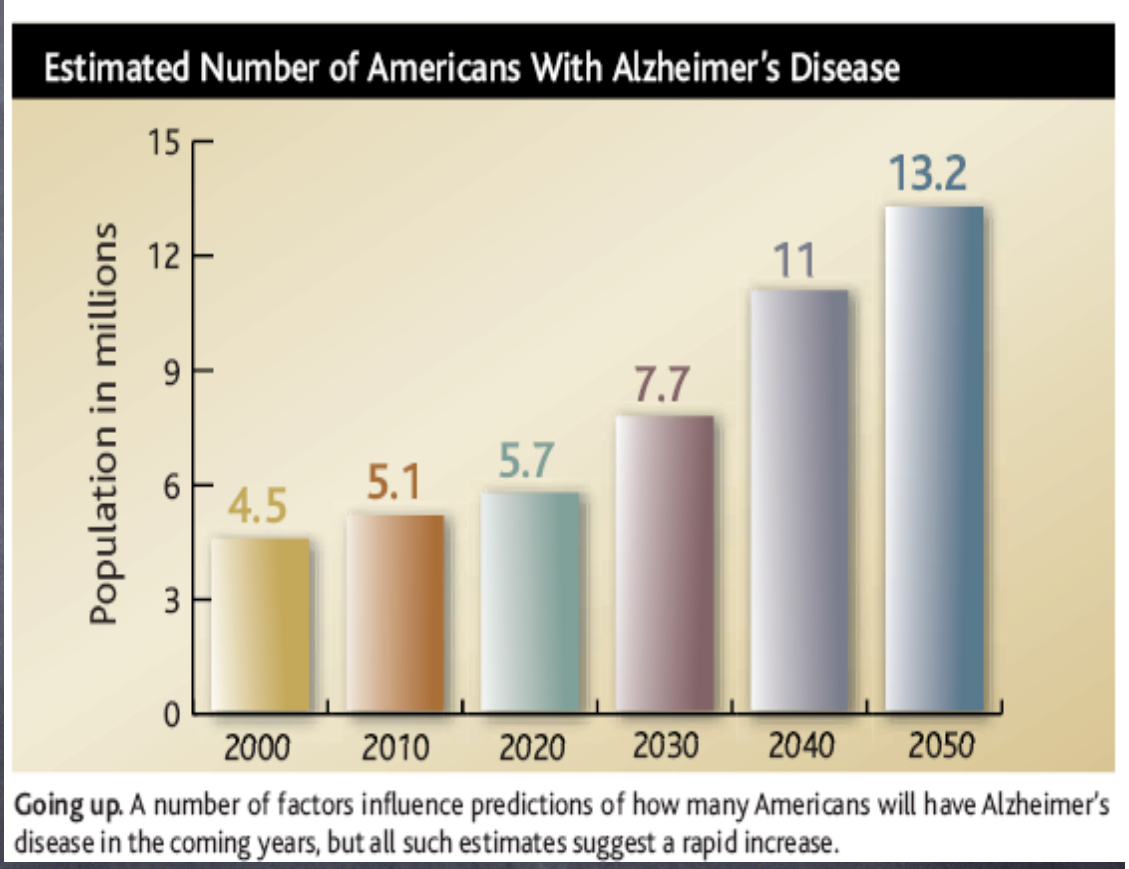
University of Wisconsin-Eau Claire

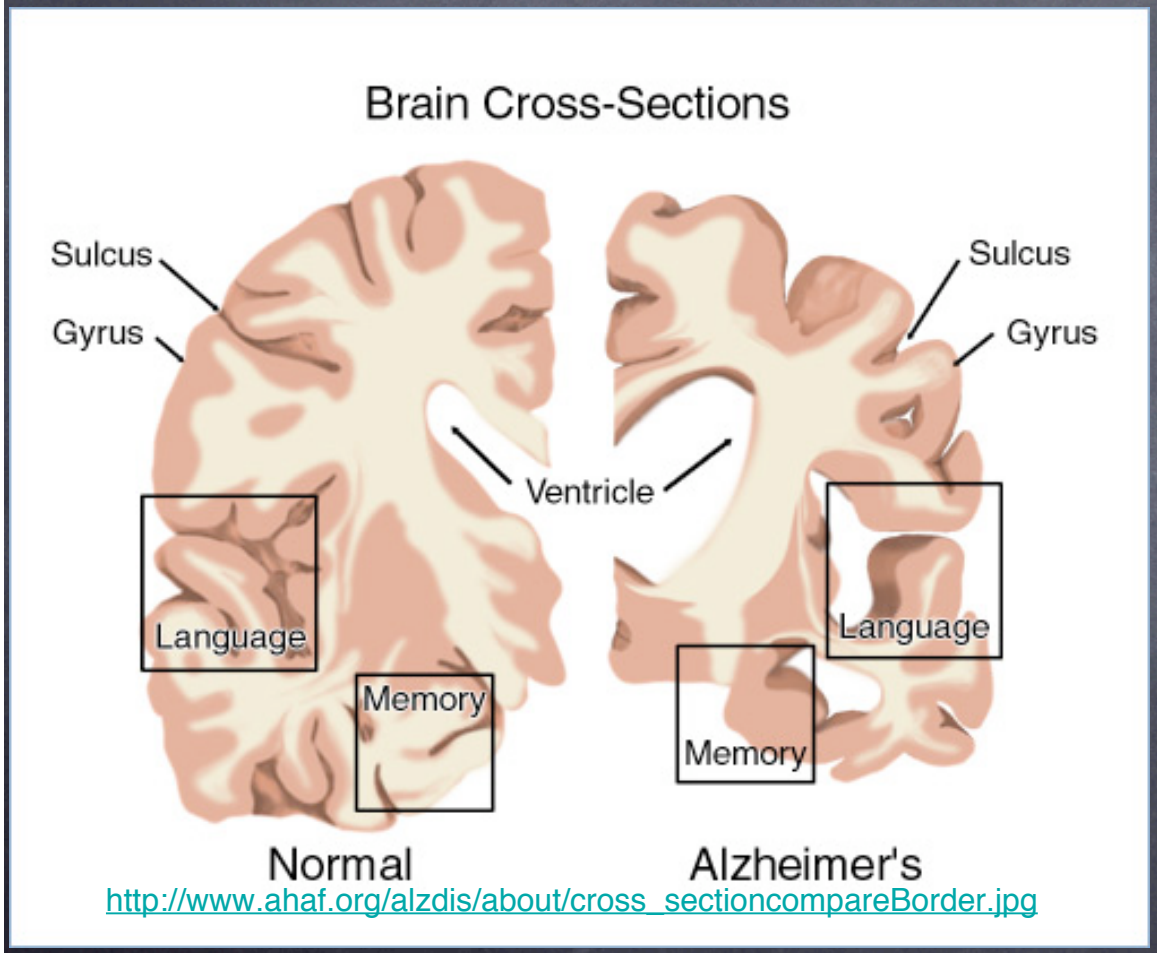
Statistics

- An estimated 4,500,000 persons in the *US alone* have Alzheimer's disease right now. The number of Americans with Alzheimer's has more than doubled since 1980. It is currently incurable. ***This is an Amyloid Disease***
- From 1995 through June 2002, a total of just 124 human cases of CJD (Human version of Mad Cow disease) were reported in the "epidemic" area, the United Kingdom, six in France, and one case each in Italy, Ireland, and the United States. It is also currently incurable. ***This is a Prion Disease***



Going up. A number of factors influence predictions of how many Americans will have Alzheimer's disease in the coming years, but all such estimates suggest a rapid increase.

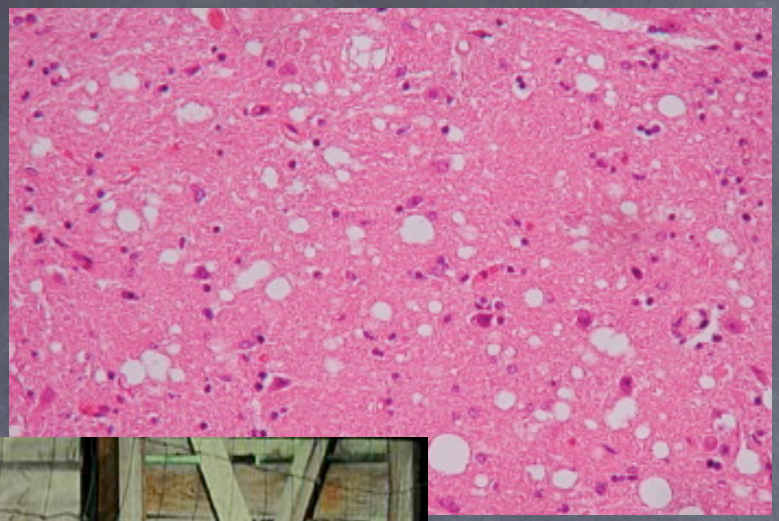
What are some Amyloid Diseases?



http://www.ahaf.org/alzdis/about/cross_sectioncompareBorder.jpg

- Alzheimer's disease
- Atrial Amyloidosis
- Hereditary Renal Amyloidosis
- Secondary Systematic Amyloidosis
- Injection-Localized Amyloidosis

What are some Prion Diseases?

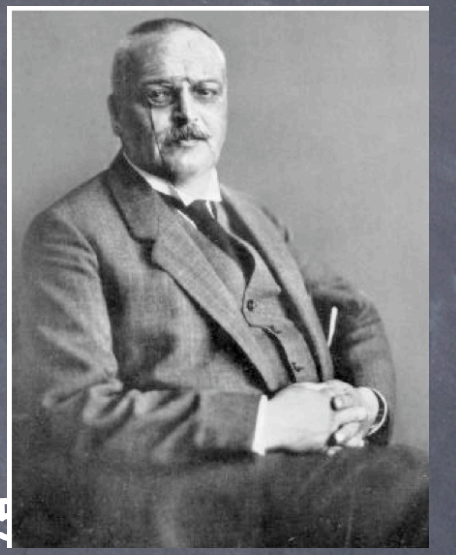


- BSE- Mad Cow Disease
- Creutzfeldt-Jakob Disease (CJD and vCJD)
- Chronic Wasting Disease (CWD)
- Scrapie
- Kuru

On to New Guinea...

- 1950's
- The Fore people were getting a strange disease
- kuru-"trembling in fear"
- traced to funeral ritual carried out by women and children-cooking and eating dead relatives
- men almost never got it
- when the rituals stopped the disease went away

On to Frankfurt....



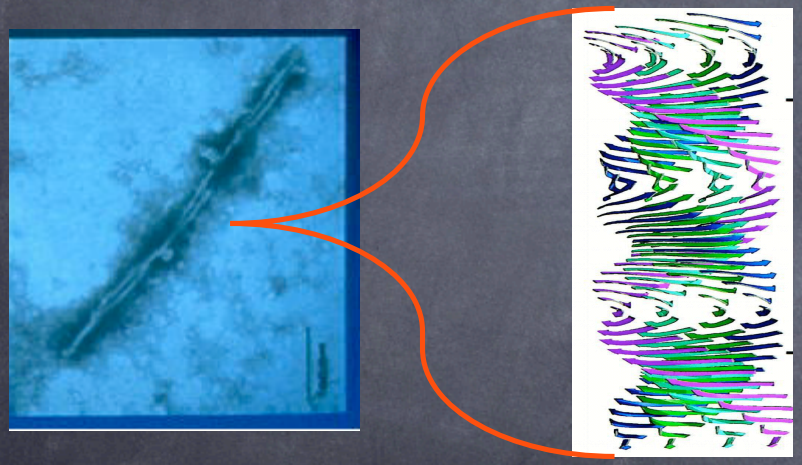
- 1907- Alois Alzheimer-German Physician
- 57-year old patient Auguste D. died after 5 years of unusual behavior and physical decline
- plaques and tangles noted in her brain-iodine staining and "starch-like" (amyloid).
- disease was considered rare at the time!!

Q: What causes Amyloid and Prion Diseases?

A: Brain proteins become permanently denatured and kill brain cells

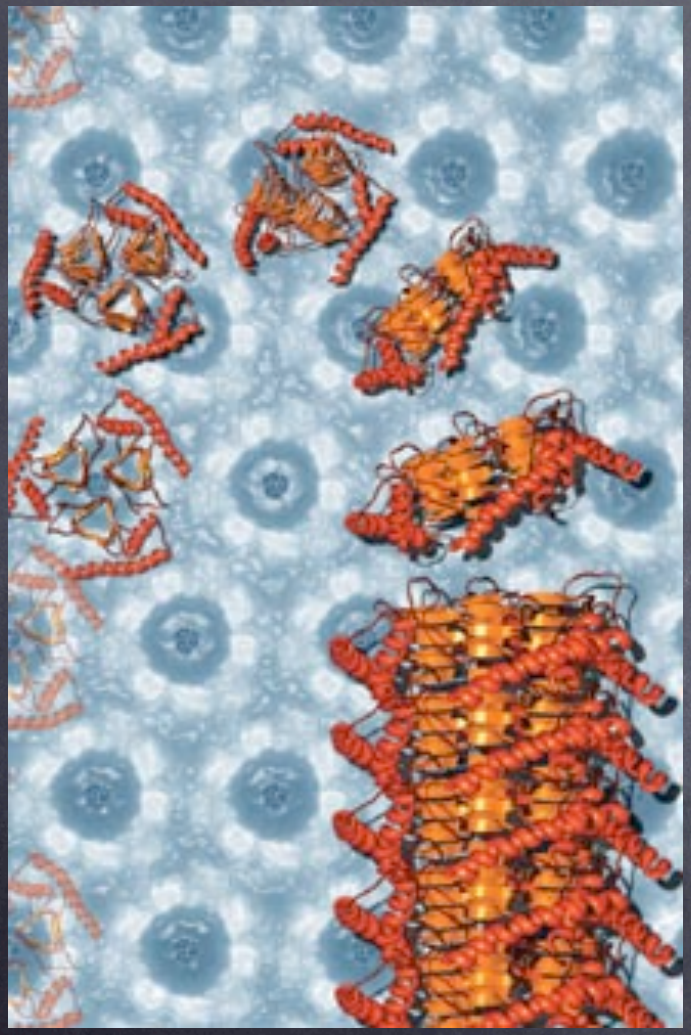
DENATURED?

These diseases involve creation of destructive fibril deposits: What is a fibril?



- Normal PrP or Amyloid Beta (ABeta 1-42) brain proteins may misfold into a flat sheet structure that coil around other sheets
- These fibrils are stable and insoluble and can't be easily "recycled" like most badly folded proteins (*see cheese!)*

How do fibrils reproduce?



- The abnormal forms can serve as seeds
- The misfolded proteins make extended fiber structures by "recruiting" other proteins
- These fibrils are stable and insoluble-in prion diseases this is what you "catch"
- Smaller parts may be the actual toxic species
- like your mother said "one bad apple..."

3D Structure of a Prion-SUP35!!

Note the
Cross beta sheet structure and predict features...

Get the original pdf article at
www.chem.uwec.edu/Chem452_F05/Prion.pdf

Nelson, R. *et al. Nature* **435,** 773-778 (2005).

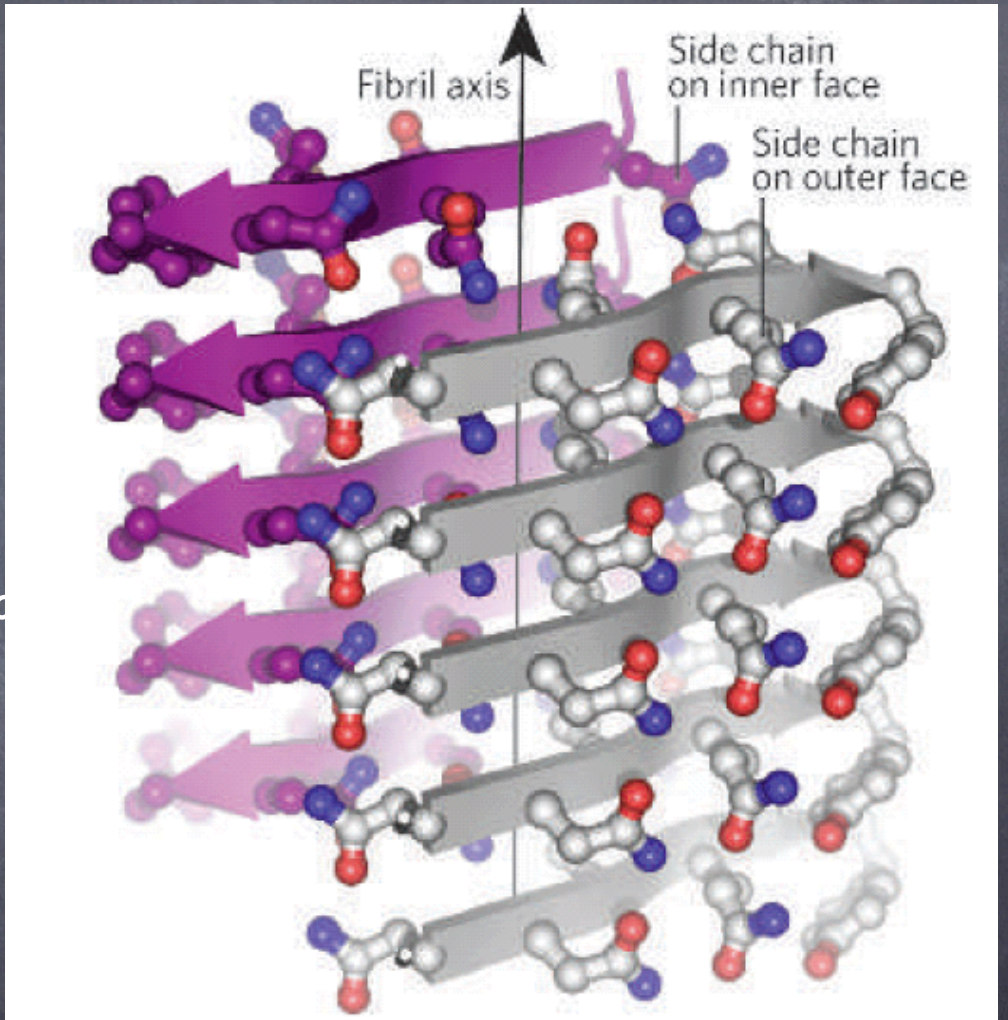
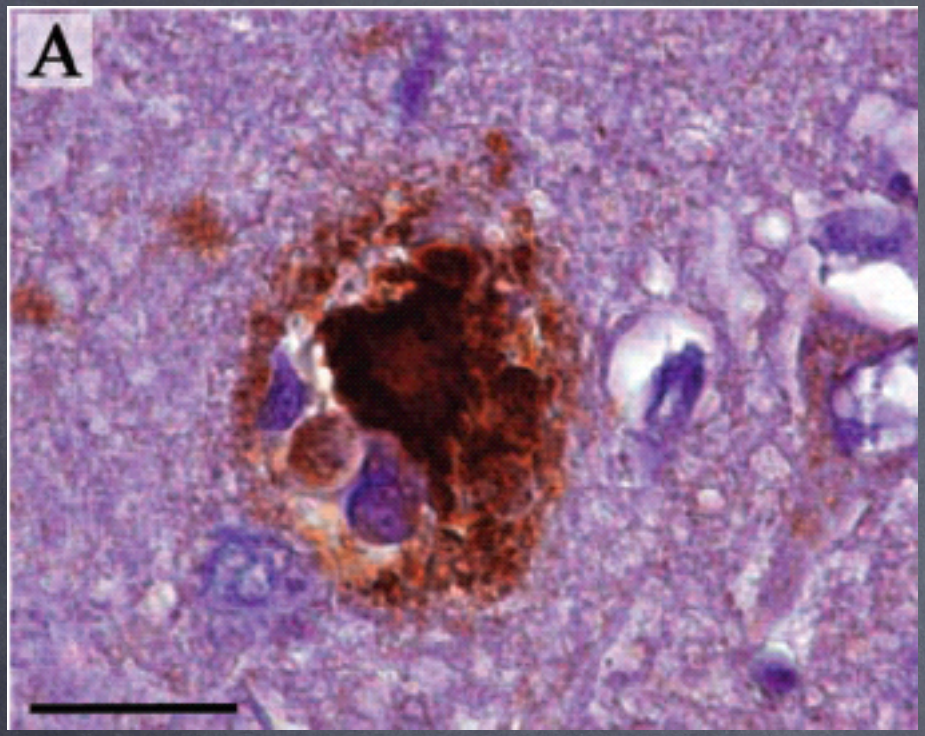
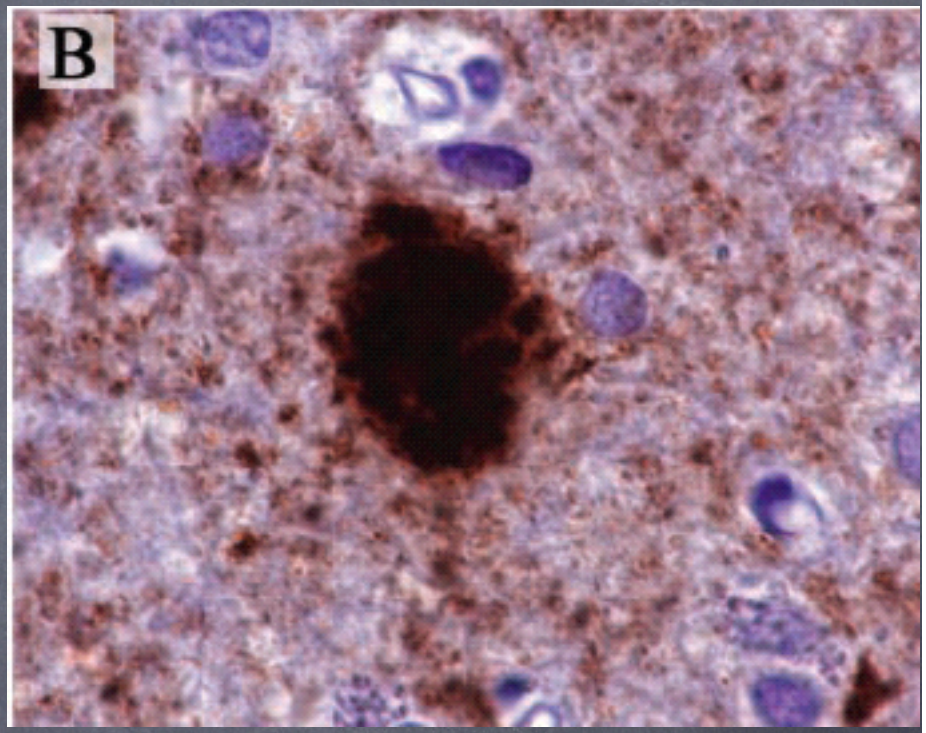


Figure 1 | Structure of the seven-residue peptide from the yeast prion, Sup35. The figure shows

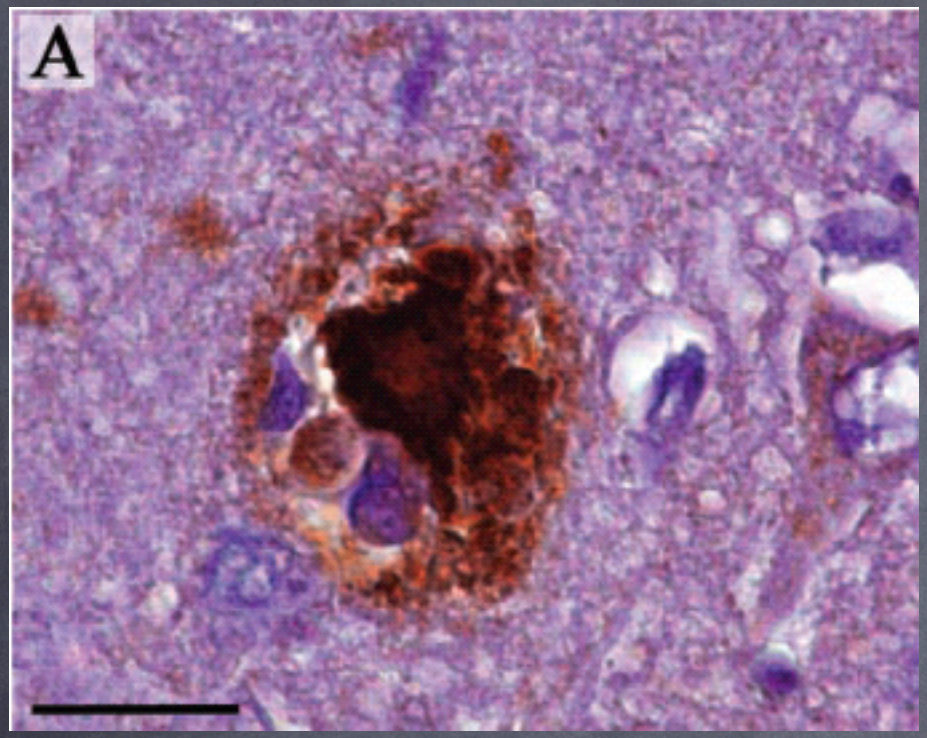
Amyloids and Prions are alike...

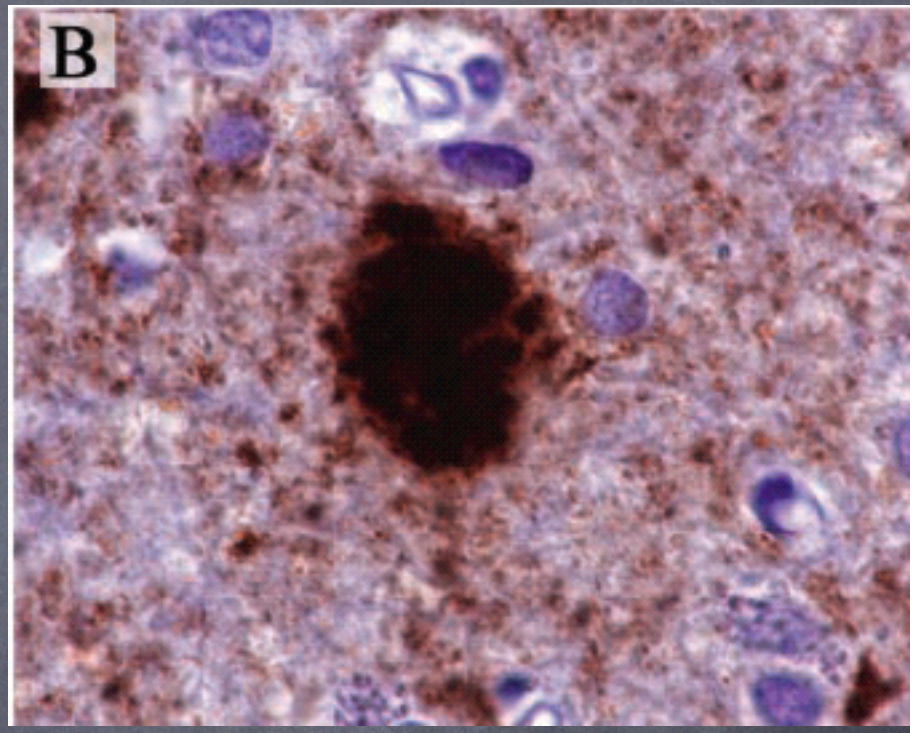




- Both AD and BSE agents show similar reproduction of fibrils in the test tube.
- Both agents cause similar symptoms (brain inflammation) and adverse affects.

BUT, Amyloids and Prions are different too

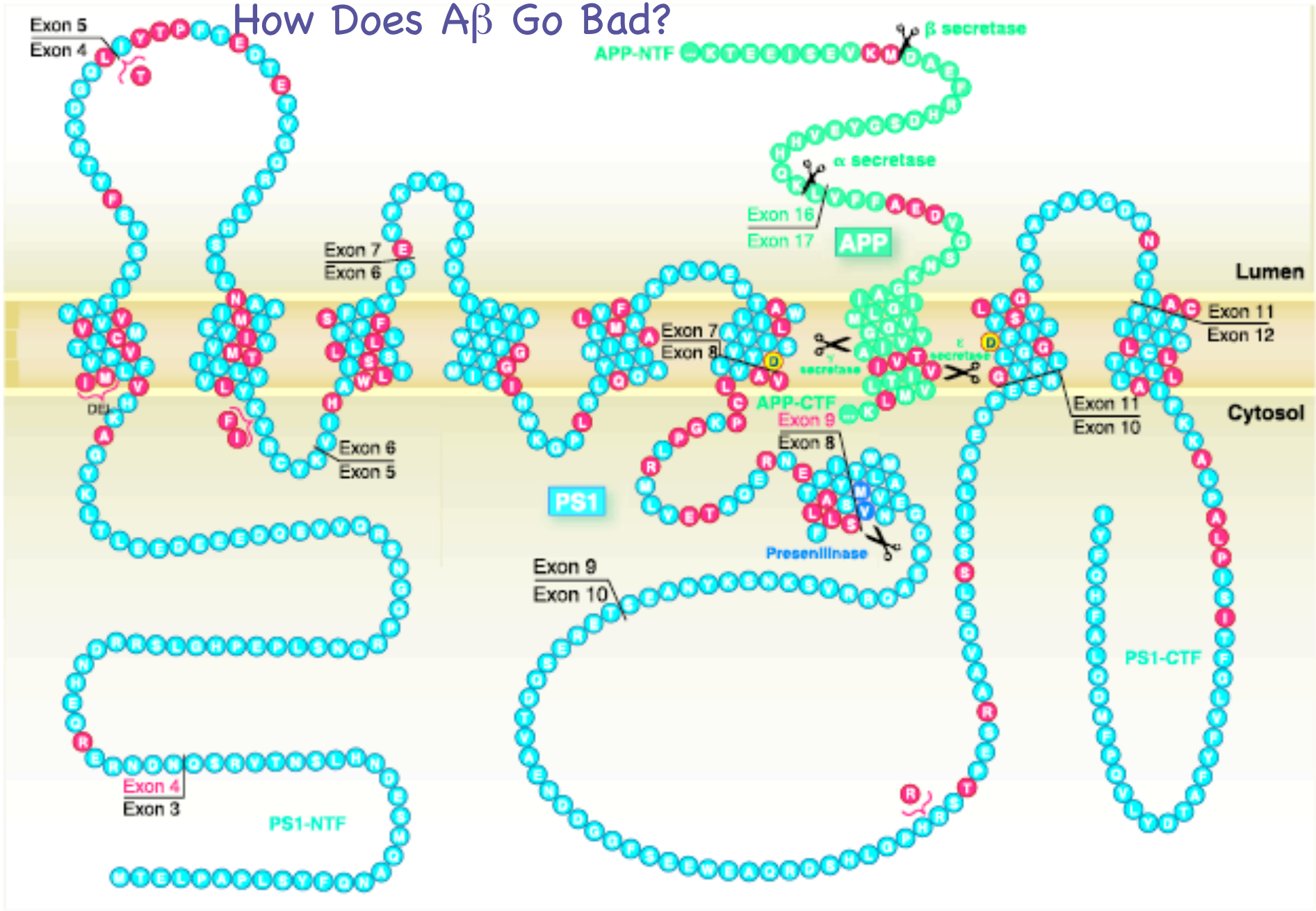




- Prions are protein-only non-living infectious agents
- Prions are known to be infectious in their spreading to different hosts, e.g. CWD, BSE, kuru, etc...
- Amyloid diseases - not *thought* to be infectious agents.

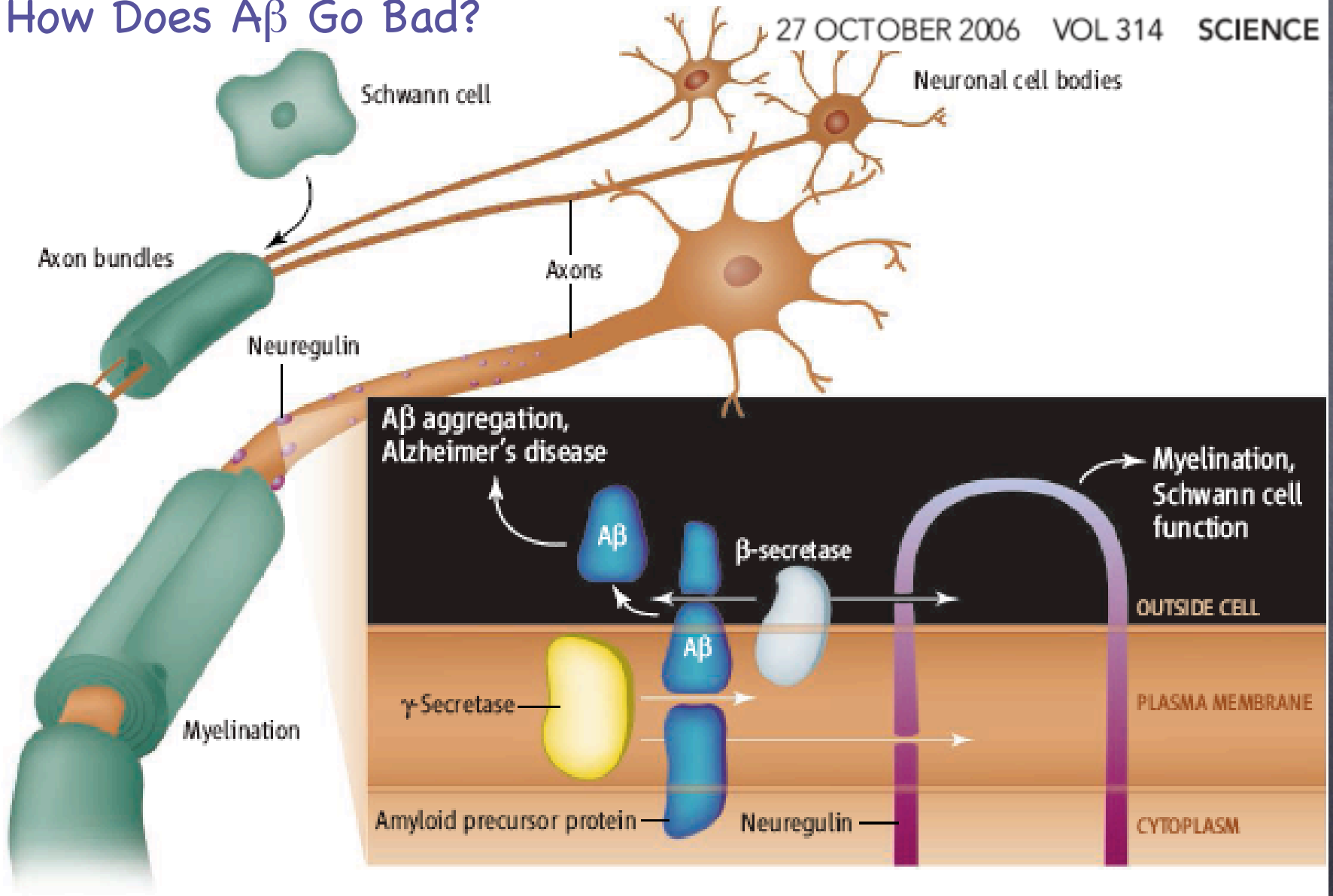
APP

How Does Aβ Go Bad?

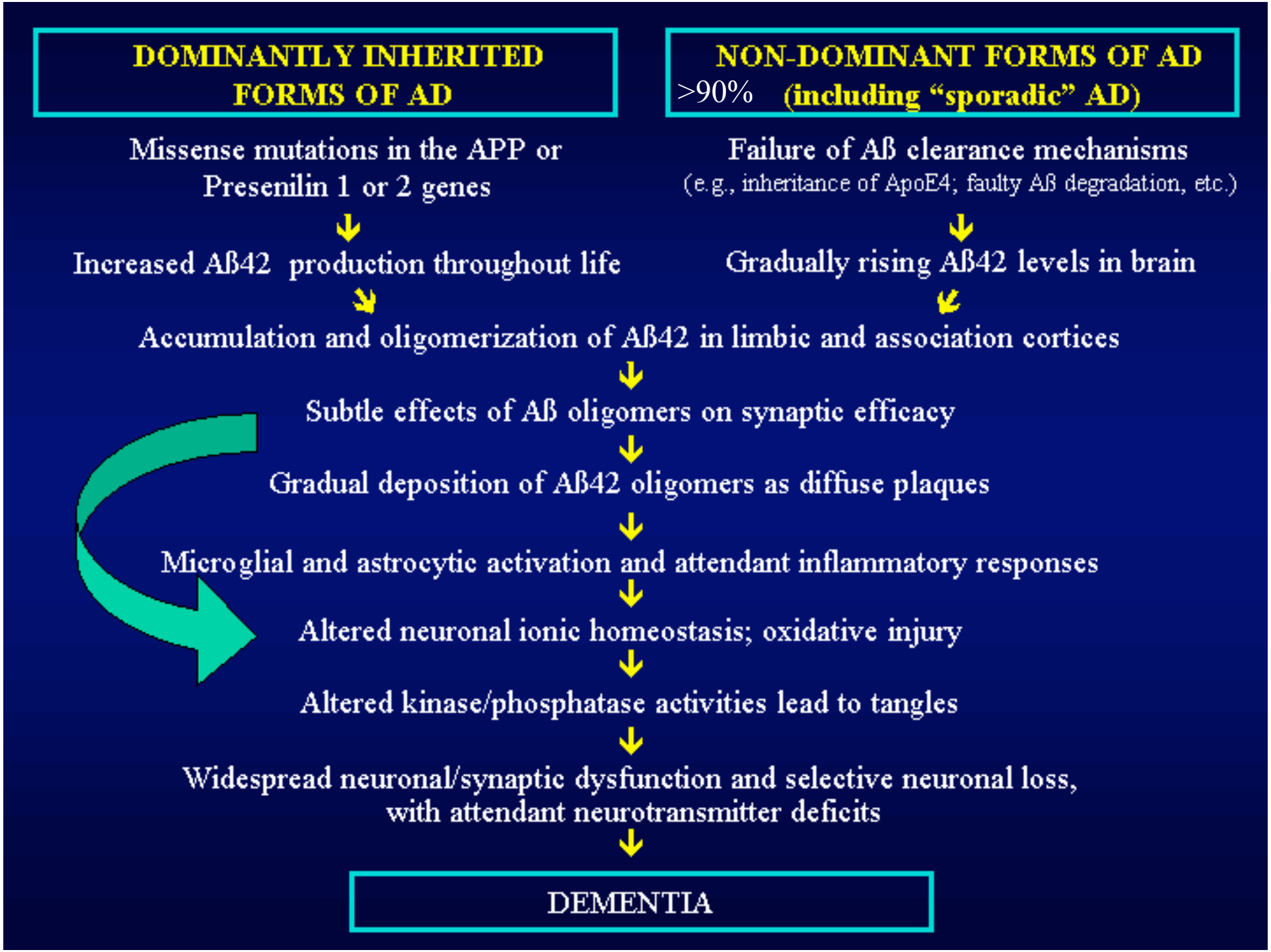


How Does Aβ Go Bad?

27 OCTOBER 2006 VOL 314 SCIENCE



Myelination in jeopardy? Just like amyloid precursor protein, type III neuregulin 1 is also cleaved by β-secretase. Proteolytic cleavage of neuregulin 1 by β-secretase is critical for peripheral nerve myelination by Schwann cells. Drugs that target β-secretase could affect peripheral nerve development and function.


DOMINANTLY INHERITED FORMS OF AD
NON-DOMINANT FORMS OF AD
>90% (including "sporadic" AD)
Missense mutations in the APP or Presenilin 1 or 2 genes
Failure of Aß clearance mechanisms
(e.g., inheritance of ApoE4; faulty Aß degradation, etc.)
Increased Aß42 production throughout life
Gradually rising Aß42 levels in brain
Accumulation and oligomerization of Aß42 in limbic and association cortices
Subtle effects of Aß oligomers on synaptic efficacy
Gradual deposition of Aß42 oligomers as diffuse plaques
Microglial and astrocytic activation and attendant inflammatory responses
Altered neuronal ionic homeostasis; oxidative injury
Altered kinase/phosphatase activities lead to tangles
Widespread neuronal/synaptic dysfunction and selective neuronal loss, with attendant neurotransmitter deficits
DEMENTIA

Possible approaches to treating Amyloid and Prion Diseases.

- Physical blockage or aggregation inhibition of fibril growth (by small molecules).
- Damage Control of existing fibrils(?) (NSAIDs -- Non-steroidal Anti-Inflammatory Drugs, Statins). Strong epidemiological evidence. May prevent initial deposit as enzyme inhibitor (ibuprofen).
- Effecting an immune response-Vaccine or antiserum
- Alter production/clearance of protein (enzyme inhibitors for secretases -ibuprofen?)
- Symptomatic (anticholinergics)
- Use it or lose it!

Possible approaches to treating Amyloid and Prion Diseases. Currently approved for AD

Table 1. Food and Drug Administration–approved treatments for AD.

Drug	Approved for
Cholinesterase inhibitors	
Donepezil (Aricept)	Mild to moderate AD
Galantamine	Mild to moderate AD
Rivastigmine	Mild to moderate AD
Tacrine	Mild to moderate AD
NMDA receptor antagonist	
Memantine	Moderate to severe AD

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Possible approaches to treating Amyloid and Prion Diseases.

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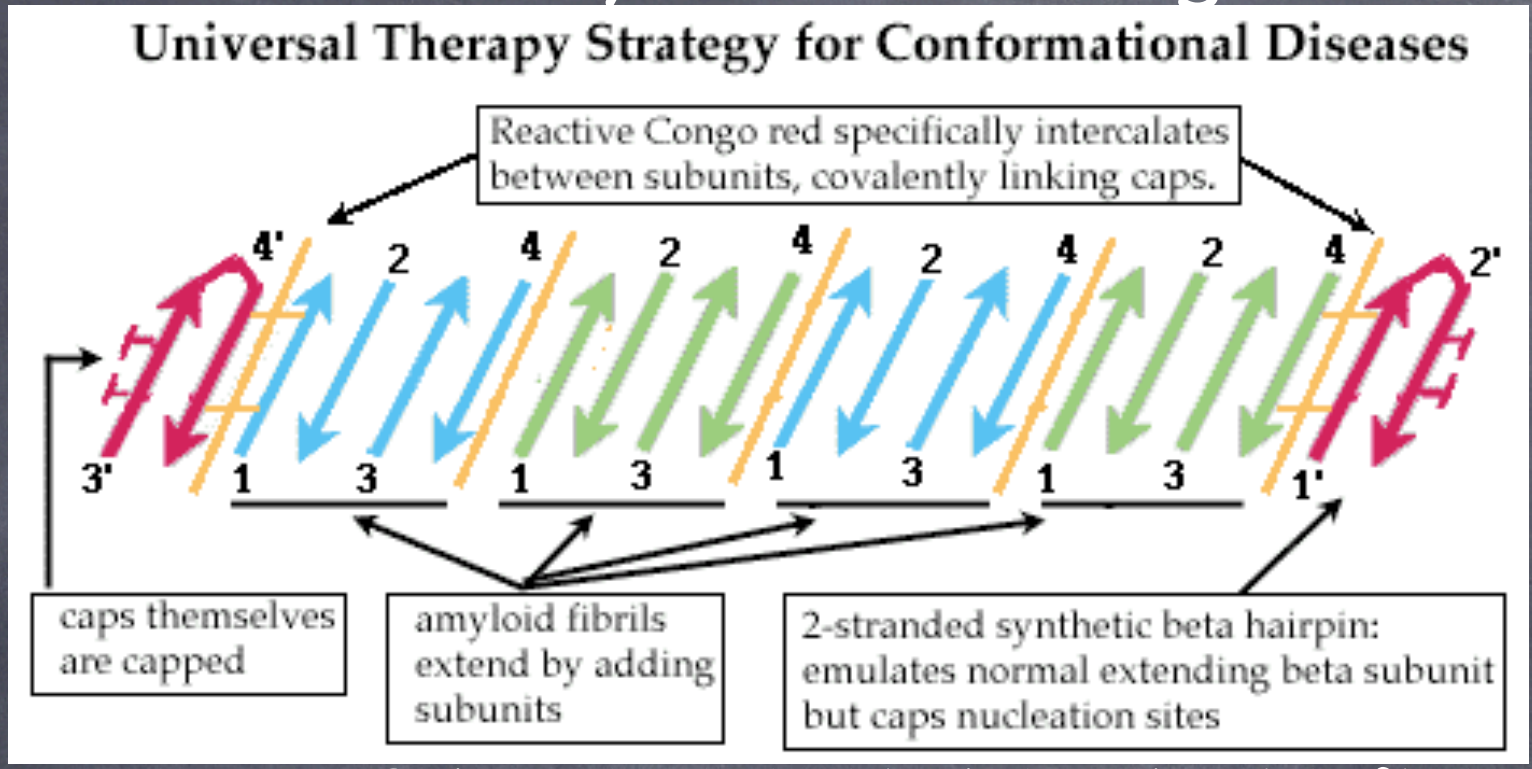
Table 2. Selected treatments in clinical trials for AD. For more information on these and other trials, see (*15*, *89*, *90*).

Treatment strategies
Phase III
Aβ aggregation inhibitors
Antioxidants
γ-Secretase modulators
NGF mimics
PPARγ agonists
HMG-CoA reductase inhibitors (statins)
Phase II
Ampakines
Calcium channel blockers
GABA receptor antagonists
γ-Secretase inhibitors
Glycogen synthase kinase inhibitors
Intravenous immunoglobulin
Muscarinic receptor agonists
New cholinesterase inhibitors
Nicotinic receptor modulators
Passive Aβ immunization
Phosphodiesterase inhibitors
Serotonin receptor antagonists
Phase I
Active Aβ immunization
NGF gene therapy

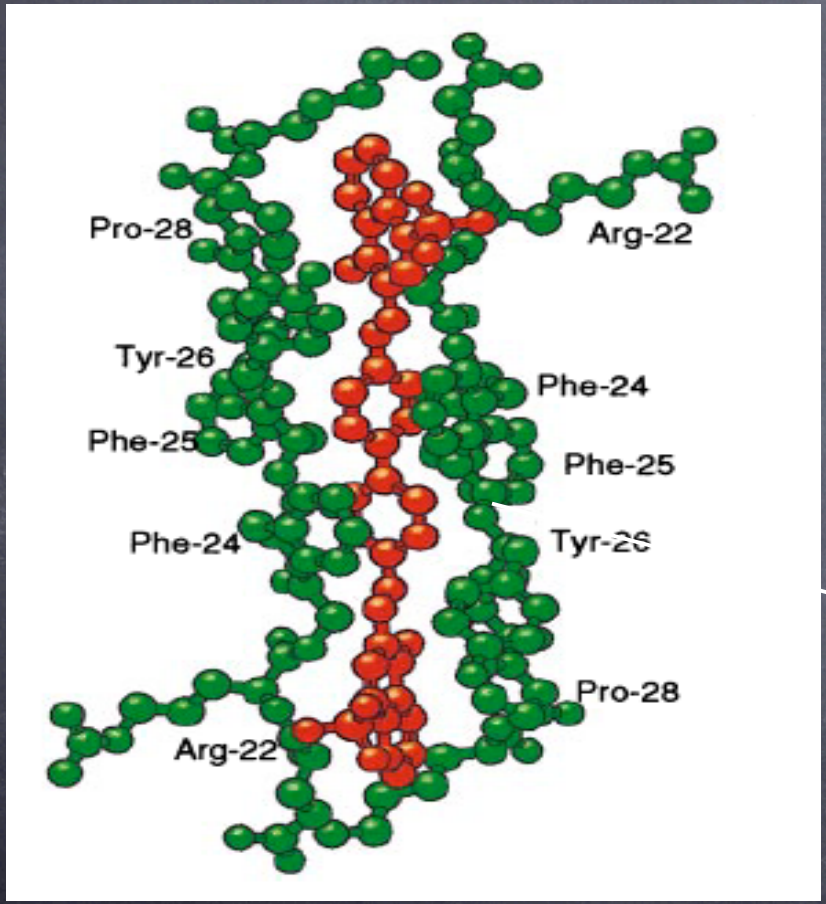
How Would Physical Blockage work?

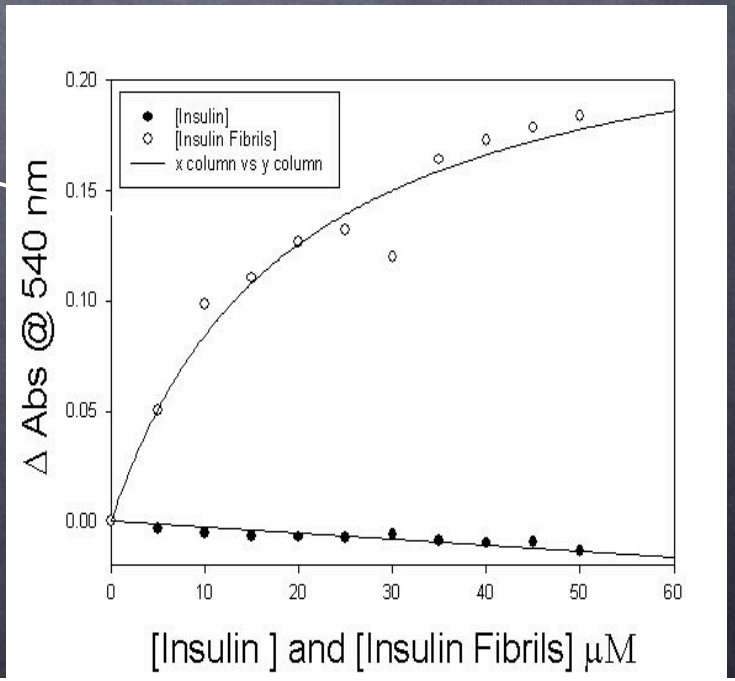


- For example, Congo Red: a molecule that binds to and inhibits fibril growth.
- Congo Red is an azo dye that was used as early as 1922 (by injection!) to diagnose amyloid diseases
- Some limited success has been seen in models using small blocking molecules (Alzhemed for AD, in Phase III).
- We have shown that Amphotericin B, and antifungal (an anti-prion?) drug can stop fibril growth in AD model systems.

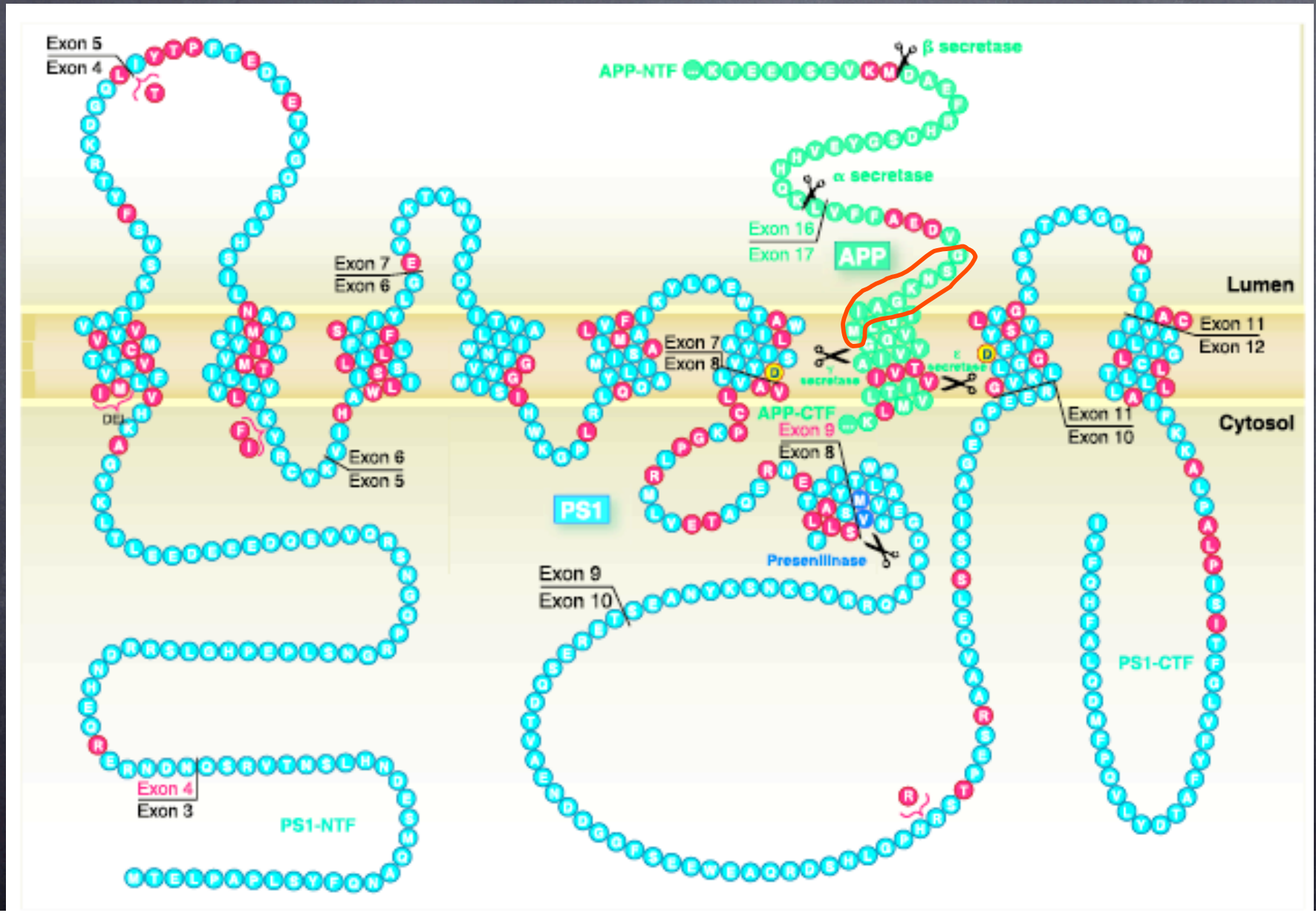
AD model system #1: Characterizing fibrils

- Congo Red binds to fibrils very specifically.
- Absorbance at 540 nm can be used to quantitate [fibril] formation
- The Insulin Fibril system is a proven amyloid model system.





AD model system #2: AD APP-fragment #25-35= GSNKGAIIGLM



NSAIDS (aspirin-like substances)

1: N Engl J Med. 2001 Nov 22;345(21):1515-21.

Comment in:

- N Engl J Med. 2001 Nov 22;345(21):1567-8.
- N Engl J Med. 2002 Apr 11;346(15):1171-3.
- N Engl J Med. 2002 Apr 11;346(15):1171-3.
- N Engl J Med. 2002 Apr 11;346(15):1171-3.
- N Engl J Med. 2002 Apr 11;346(15):1171-3.

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Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease.

in t' Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MM, Stricker BH.

Department of Epidemiology and Biostatistics, Eramus Medical Center, Rotterdam, The Netherlands.

BACKGROUND: Previous studies have suggested that the use of nonsteroidal antiinflammatory drugs (NSAIDs) may help to prevent Alzheimer's disease. The results, however, are inconsistent. METHODS: We studied the association between the use of NSAIDs and Alzheimer's disease and vascular dementia in a prospective, population-based cohort study of 6989 subjects 55 years of age or older who were free of dementia at base line, in 1991. To detect new cases of dementia, follow-up screening was performed in 1993 and 1994 and again in 1997 through 1999. The risk of Alzheimer's disease was estimated in relation to the use of NSAIDs as documented in pharmacy records. We defined four mutually exclusive categories of use: nonuse, short-term use (1 month or less of cumulative use), intermediate-term use (more than 1 but less than 24 months of cumulative use), and long-term use (24 months or more of cumulative use). Adjustments were made by Cox regression analysis for age, sex, education, smoking status, and the use or nonuse of salicylates, histamine Hz-receptor antagonists, antihypertensive agents, and hypoglycemic agents. RESULTS: During an average follow-up period of 6.8 years, dementia developed in 394 subjects, of whom 293 had Alzheimer's disease, 56 vascular dementia, and 45 other types of dementia. The relative risk of Alzheimer's disease was 0.95 (95 percent confidence interval, 0.70 to 1.29) in subjects with short-term use of NSAIDs, 0.83 (95 percent confidence interval, 0.62 to 1.11) in those with intermediate-term use, and 0.20 (95 percent confidence interval, 0.05 to 0.83) in those with long-term use. The risk did not vary according to age. The use of NSAIDs was not associated with a reduction in the risk of vascular dementia. CONCLUSIONS: The long-term use of NSAIDs may protect against Alzheimer's disease but not against vascular dementia.

PMID: 11794217 [PubMed - indexed for MEDLINE]

Vaccines?

1: Curr Alzheimer Res. 2004 Aug;1(3):149-63.

Related Articles, Links

Alzheimer's disease and immunotherapy.

Solomon B.

Department of Molecular Microbiology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv, P.O. Box 69978, Israel. beka@post.tau.ac.il

Site-directed antibodies which modulate conformation of beta-amyloid peptide became the theoretical basis of the immunological approach for treatment of Alzheimer's disease (AD). Indeed, antibodies towards the EFRH sequence, located between amino acids 3-6 of the N-terminal region of Alzheimer's AbetaP, found to be a key position in protein conformation modulation, suppress formation of beta-amyloid and dissolve already formed fibrillar amyloid. The performance of anti-beta-amyloid antibodies in transgenic mice models of AD showed they are delivered to the central nervous system (CNS), preventing and dissolving beta-amyloid plaques. Moreover, these antibodies protected the mice from learning and age-related memory deficits. Naturally occurring anti-AbetaP antibodies have been found in human CSF and in the plasma of healthy individuals, but were significantly lower in AD patients, suggesting that AD may be an immunodeficient disorder. Active and/or passive immunization against beta-amyloid peptide has been proposed as a method for preventing and/or treating Alzheimer's disease. Experimental active immunization with Abeta 1-42 in humans was stopped in phase II clinical trials due to unexpected neuroinflammatory manifestations. Antibodies generated with this first-generation vaccine might not have the desired therapeutic properties to target the "correct" mechanism, however, new clinical approaches are now under consideration. Immunotherapy represents fascinating ways to test the amyloid hypothesis and offers genuine opportunities for AD treatment, but requires careful antigen and antibody selection to maximize efficacy and minimize adverse events.

Publication Types:
- Review
- Review, Tutorial

Vaccines?

1: Expert Opin Biol Ther. 2005 Jan;5(1):97-110.

Related Articles, Links

Full text article at www.ashley-pub.com

Progress in prion vaccines and immunotherapies.

Griffin JK, Cashman NR.

University of Toronto, Centre for Research in Neurodegenerative Diseases, 6 Queen's Park Crescent West, Toronto, ON M5S3H2, Canada. jennifer.griffin@utoronto.ca.

The transmissible spongiform encephalopathies have presented a challenge to physicians and scientists attempting to develop immunologically-based treatments. Self-tolerance has been one of the major obstacles to successfully raising antibodies against the prion protein (PrP), the host-encoded protein whose misfolded form (PrPSc) is linked to the protein-only infectious agent responsible for these disorders. Recently, it has been shown that antibodies directed against the normal cellular isoform of PrP (PrPC) can reduce or eliminate PrP isoform conversion in both in vitro and in vivo model systems. Similar studies with a PrPSc-specific epitope target are in progress. There is now rational hope that this devastating group of diseases may soon be amenable to immunotherapy and immunoprophylaxis.

PMID: 15709913 [PubMed - in process]

Use it or lose it

ALZHEIMER'S DISEASE

SCIENCE VOL 307 11 MARCH 2005

Play and Exercise Protect Mouse Brain From Amyloid Buildup

As the population ages, finding ways to stave off the debilitating brain degeneration of Alzheimer's disease becomes ever more critical. New results with a mouse model of the condition now provide further support for the idea that "use it or lose it" applies as much to the mind as to the body.

A leading explanation for Alzheimer's disease blames abnormal buildup of a small protein called β amyloid, which accumulates in pathological structures called plaques in patients' brains. Now, working with mice genetically engineered to produce similar β-amyloid plaques, a research team led by Sam Sisodia of the University of Chicago, Illinois,

mally show symptoms of Alzheimer's disease; the genetically modified animals they used ordinarily develop β-amyloid plaques by about 4.5 months of age. The researchers put seven animals in standard cages and another nine in the enriched environment, where the activities of the mice were closely monitored.

After 5 months, the researchers killed both sets of mice and examined their brains. Animals kept in the enriched environment showed "a marked reduction in amyloid burden," Sisodia says. The decrease appeared to be related to exercise. "The animals that were most active as

land, and their colleagues reported that enriched environments actually increase plaque formation. The reason for the discrepancy is unclear, although the design of the 2003 experiment was different. For one, that study involved only female mice, whereas the Sisodia team used males. The Jankowsky-Borchelt group also had many more animals in their enriched cages and added young mice as they removed older ones. "To me that spells stress," says David Arendash of the University of South Florida in Tampa, who also studies the effects of enrichment on Alzheimer's mice. That stress might have overcome any beneficial effects of the enhanced environments.

Sisodia's group didn't test whether the

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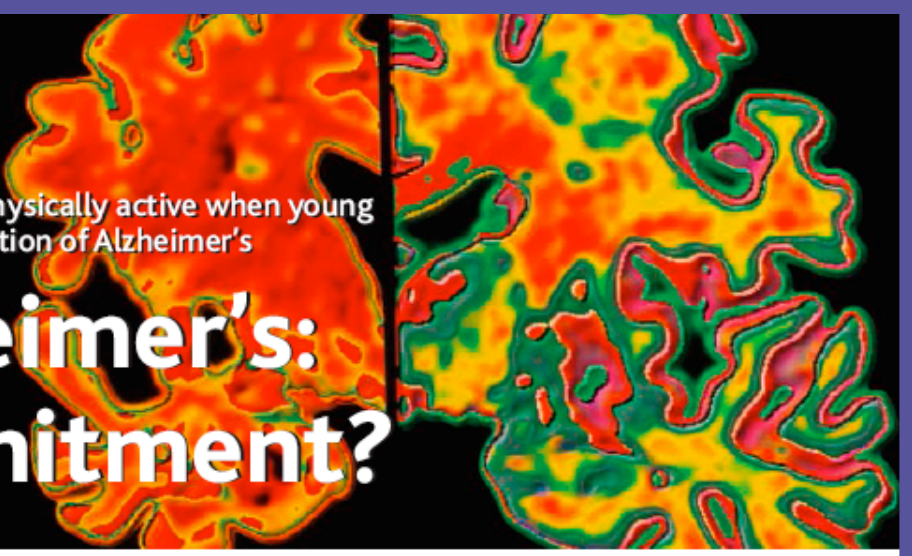
News Focus

5 AUGUST 2005 VOL 309 SCIENCE

y and physically active when young and middle-aged can help stave off the brain degeneration of Alzheimer's

Preventing Alzheimer's: A Lifelong Commitment?



Hormones?

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Last Updated: Tuesday, 25 March 2008, 12:05 GMT

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HRT 'might ward off Alzheimer's'

Hormone replacement therapy (HRT) may protect post-menopausal women against memory loss and Alzheimer's disease.

There is no cure for dementia

A study found women's memories are affected when their bodies stop producing the hormone oestrogen - as happens at the menopause.

However, London's Institute of Psychiatry found memory recovered when hormone supplies were restored - the effect achieved by HRT.

Women have a higher risk of developing Alzheimer's than men.

It is estimated that around 450,000 women in the UK have the disease.

There is a theory that oestrogen may help prevent the build up of damaging protein tangles in the brain which are thought to trigger cell death, and Alzheimer's.

> **"There may be a critical window of time around the menopause when HRT may have a beneficial effect in protecting against Alzheimer's dementia"**
>
> Dr Michael Craig
> Institute of Psychiatry

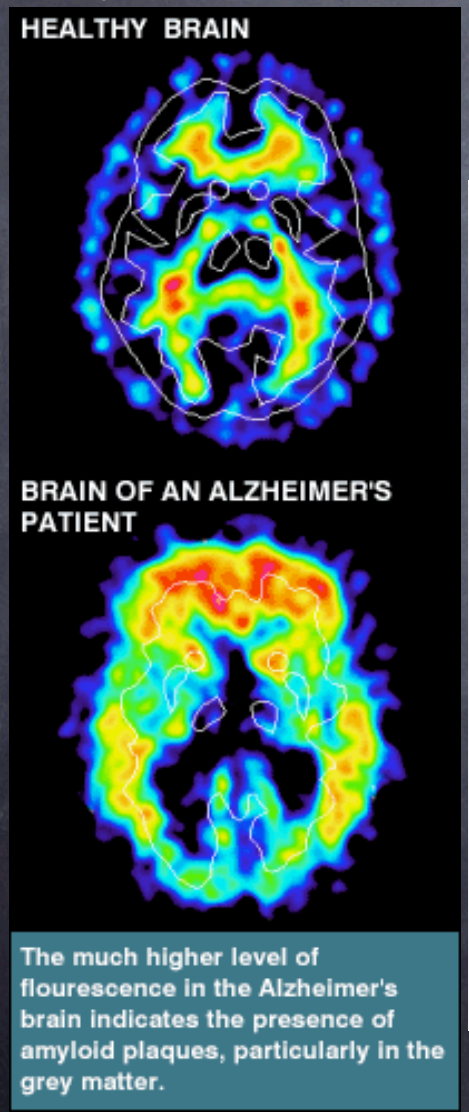
Conclusions and Prospects

- AD and BSE have a fundamental similarity- both are caused by self-propagating misfolded proteins which cause brain damage
- One promising approach is developing small molecules which delay or prevent this conversion or prevent inflammation
- In truth, vaccination (active or passive) may be the best ultimate means for a treatment
- Until then improving therapies such as statins, anti-inflammatory drugs, exercise and diet may make AD (and the very rare vCJD) a managable chronic condition like HIV disease.
- GOOD EARLY DIAGNOSTIC TEST DEVELOPMENT IS CRITICAL!!

Conclusions and Prospects

- GOOD EARLY DIAGNOSTIC TEST DEVELOPMENT IS CRITICAL!!

Thioflavin T PET SCAN



Apo E4 Diagnostic test