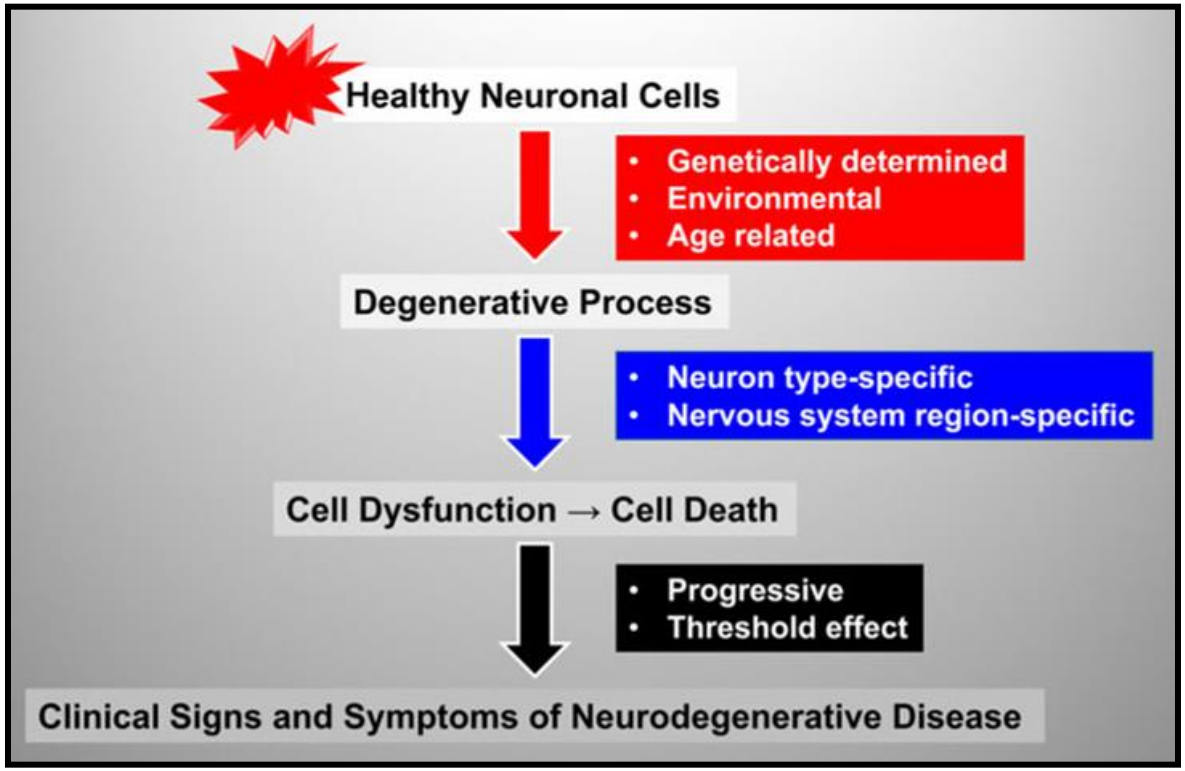
NEURODEGENERATIVE DISEASES

DEGENERATION DUE TO INTRA- AND/OR EXTRACELLULAR PROTEIN ACCUMULATION, FREQUENTLY ASSOCIATED WITH AGING

CLASSIFICATION: 1) CNS REGION; 2) INCLUSION TYPE (USEFUL FOR DIAGNOSIS)

III/IV AGE DISEASES: INCREASED PREVALENCE DUE TO INCREASED LIFE SPAN



Isaya, 2014

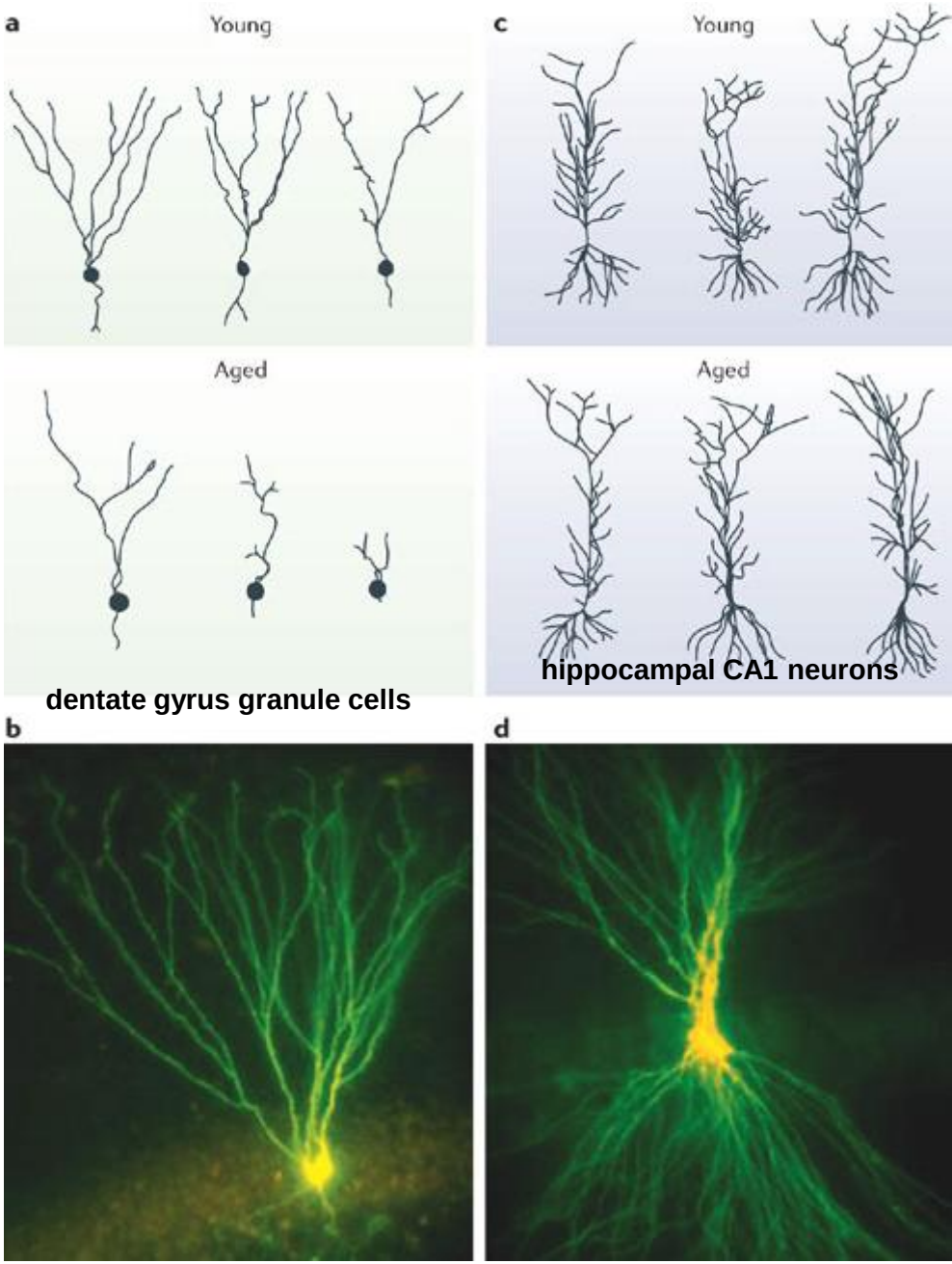
BRAIN AGING

A common misconception: significant cell loss and dramatic changes in neuronal morphology occur

a | progressive loss of the dendritic surface in aged human **dentate gyrus granule cells**.

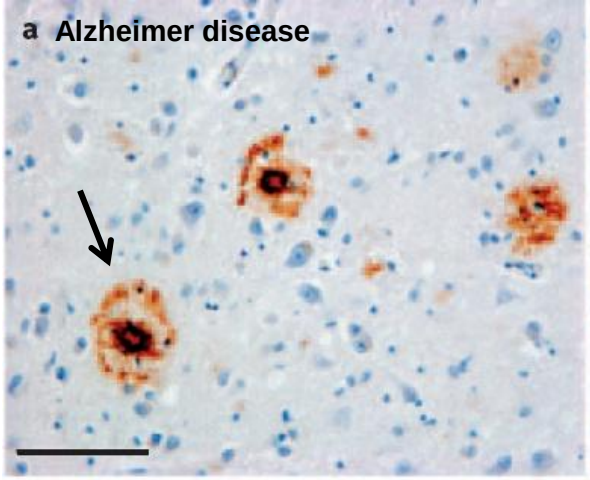
c | Reconstructions of representative **hippocampal CA1 neurons from young rats (2 months) and old rats (24 months).** There is **no reduction** in dendritic branching or length with age in area CA1.

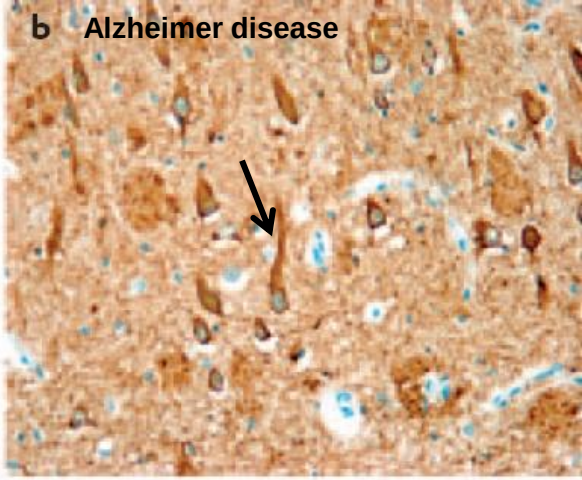
Carboxyfluorescein
b | granule cells filled with 5,6-carboxyfluore-scein from the **dentate gyrus of a 24-month-old rat**. In the rat dentate gyrus, there is no significant change in dendritic extent between young and old animals, but there is a significant increase in electrotonic coupling
d | A CA3 neuron filled with 5,6-carboxyfluo-rescein from a **24-month-old rat**. There is no regression of dendrites but the aged cells show a **significant increase in the number of gap junctions compared with young.**

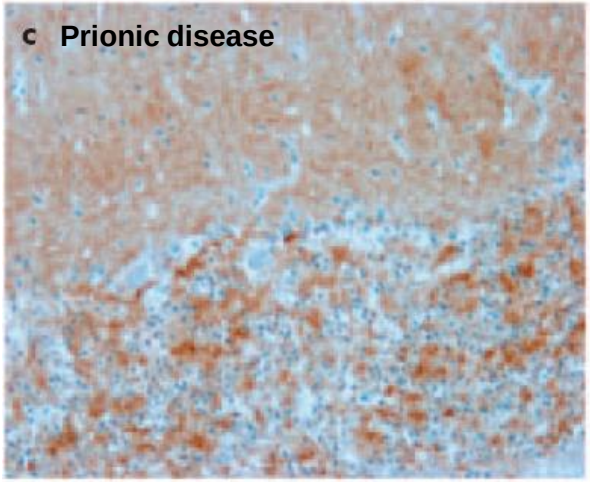


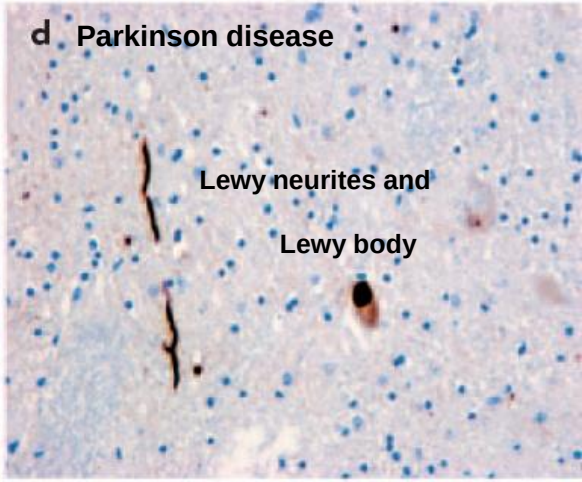
INCLUSION EXAMPLES

a Aβ → senile plaques
b tau → neurofibrillar tangles
c PrP
d α-sinuclein
e Ub-huntingtin

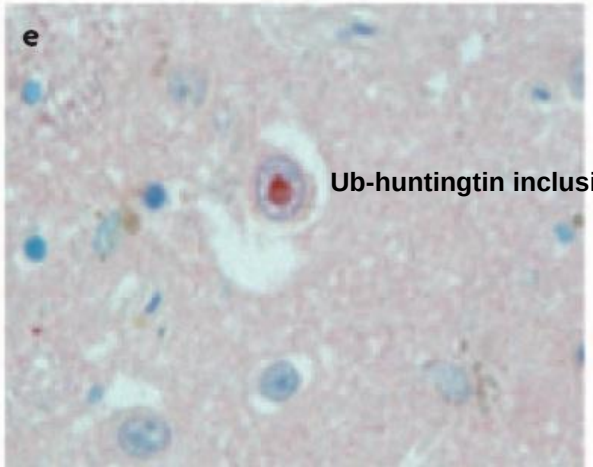








Huntington disease

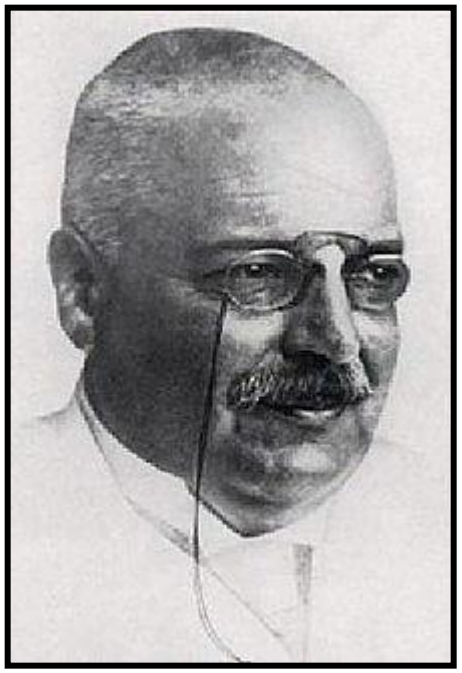


Haass & Selkoe, 2007

Neurodegenerative diseases associated with aggregated proteins

Disease	*Protein*	*Normal Structure*	*Aggregate/ Inclusion*	*Location*
Alzheimer disease	Amyloid precursor protein (APP)	**α-**Helix and random coil	**β**-pleated sheet, amyloid (fragment of APP)	Extracellular
Tauopathies and Alzheimer disease	Tau, microtubule binding protein	3 and 4 repeat isoforms	hyperphosphorylated aggregated protein	Intracellular
Parkinson disease	α-Synuclein	Random coil, repeats	Aggregated, Lewy bodies	Cytoplasmic
Multiple system atrophy	α-Synuclein	Random coil, repeats	Aggregated, Glial cytoplasmic inclusions	Cytoplasmic
Huntington disease	Huntingtin	Trinucleotide repeats	Insoluble aggregates	Nuclear
Spinocerebellar ataxias	Ataxins	Trinucleotide repeats	Insoluble aggregates	Nuclear
Transmissible spongiform encephalopathies (Prion disease)	Prion protein (PrP)	**α**-Helix and random coil	**β**-pleated sheet, proteinase K-resistant	Extracellular

ALZHEIMER DISEASE



ALZHEIMER DISEASE IS THE MOST FREQUENT AND THE MAIN CAUSE OF COGNITIVE IMPAIRMENT IN THE ELDERLY

PROGRESSIVE DISEASE

INCLUSION TYPE: 1) β-AMYLOID (Aβ; extracellular, most abundant); 2) PHOSPHORYLATED TAU (intracellular, nuclear and/or cytoplasmatic); 3) VASCULAR AMYLOIDOSIS

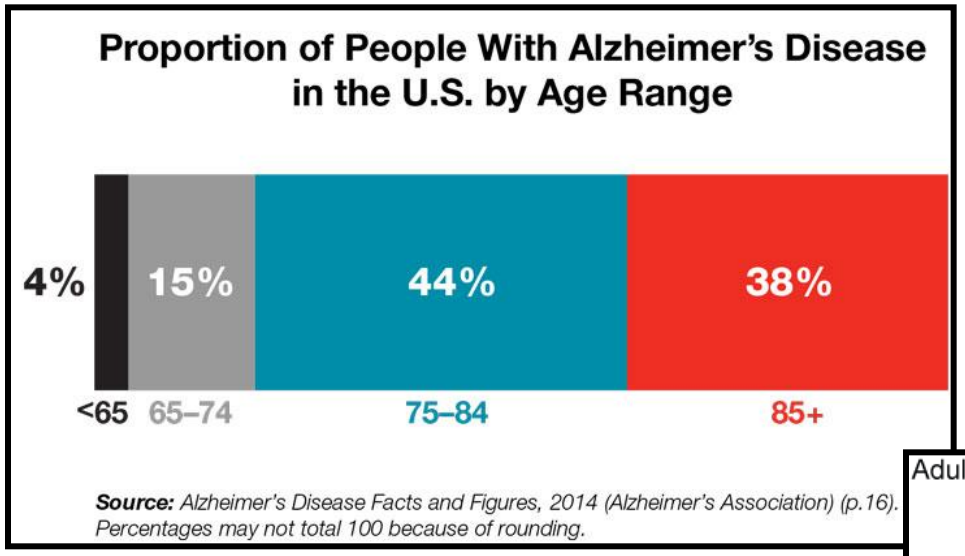
<u>MAIN FEATURES</u>

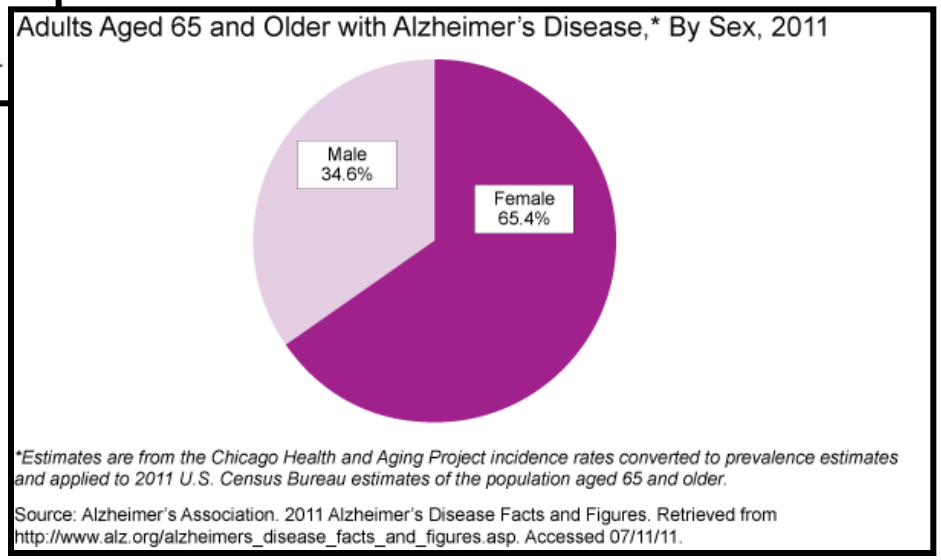
- Loss of: memory and speech, judgment capacity, spatial and temporal orientation
- Histopathologic alterations
- Neuron loss, mainly from cortex and hippocampus
- Plaque formation

ABSOLUTELY SURE DIAGNOSIS ON BRAIN HISTOLOGY (post-mortem)
HOWEVER PET AND FUNCTIONAL TESTS QUITE GOOD

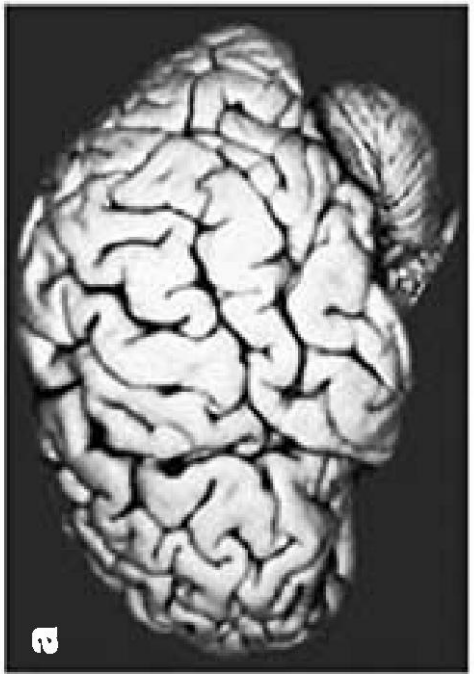
In November 1906, at a German psychiatrists meeting, **Alois Alzheimer** presented the pathological findings on a brain of a 56 y.o. woman who died after a progressive dementia

FIRST SYMPTOMS AFTER 60 yr, IF BEFORE CONSIDERED EARLY ONSET
PROGRESSIVE DECLINE (5-10 yr)
INCIDENCE INCREASES WITH AGE
SPORADIC: 90-95%

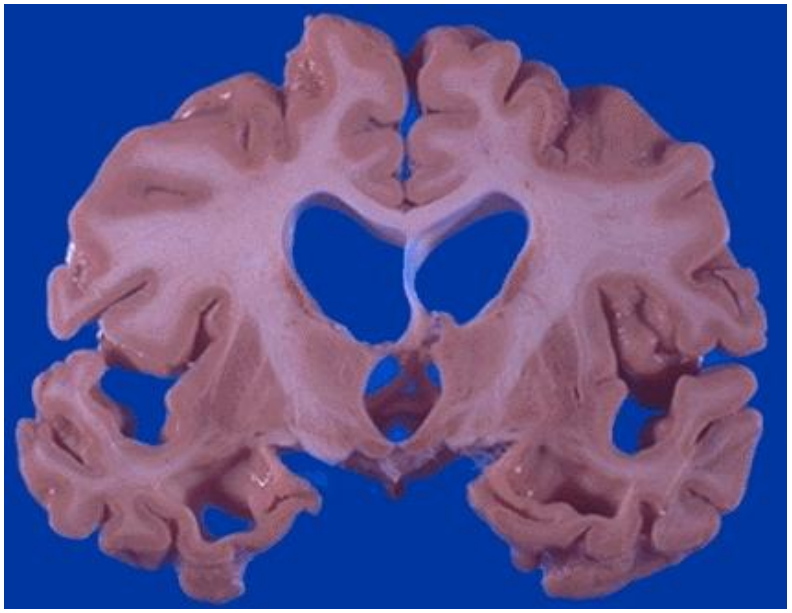




Normal brain

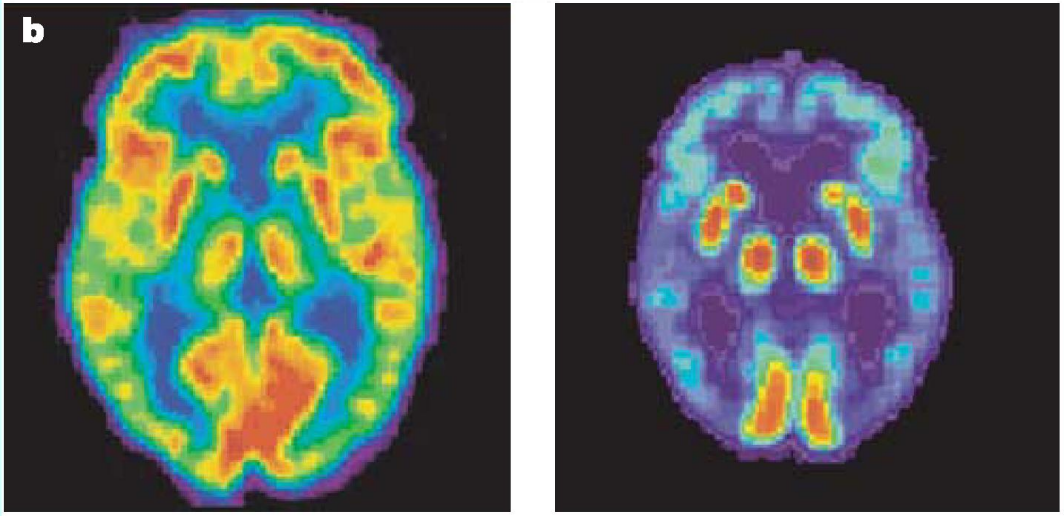


Alzheimer's brain



Normal brain

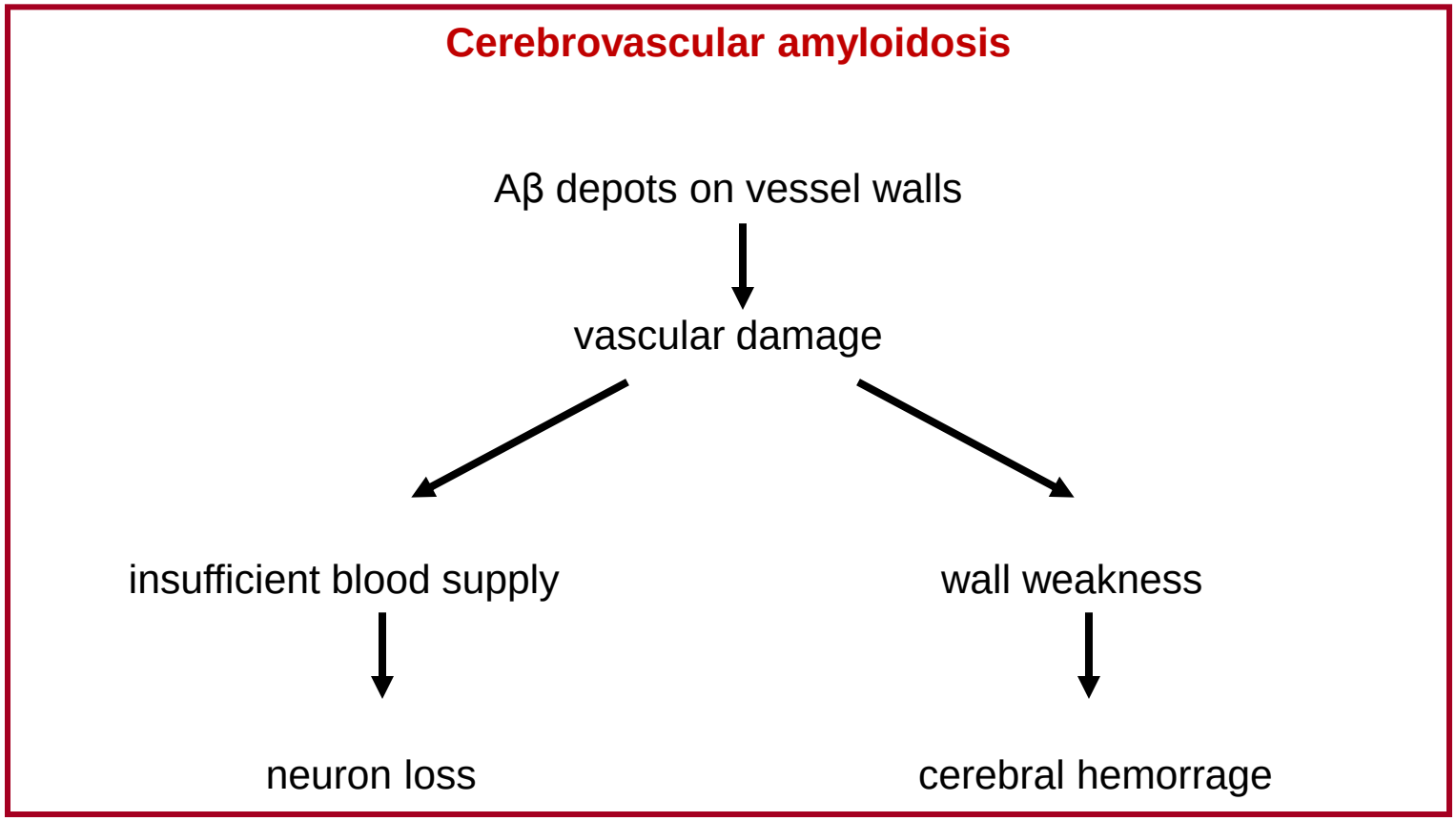
Alzheimer's brain



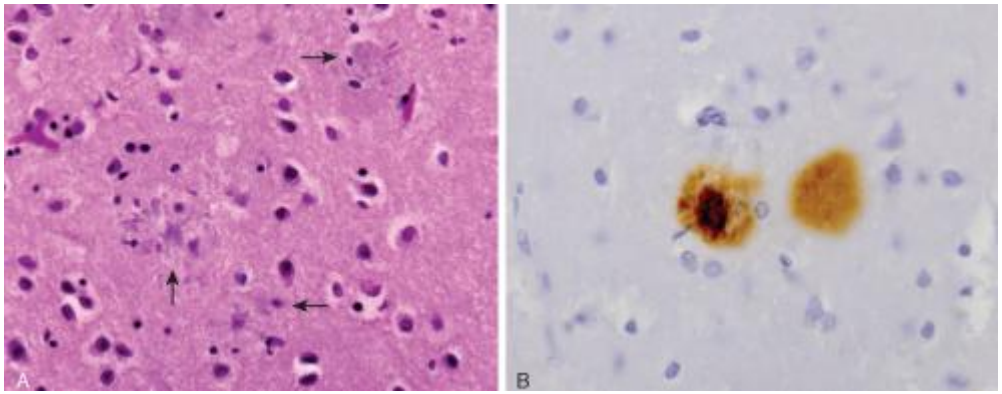
Normal brain

Alzheimer's brain

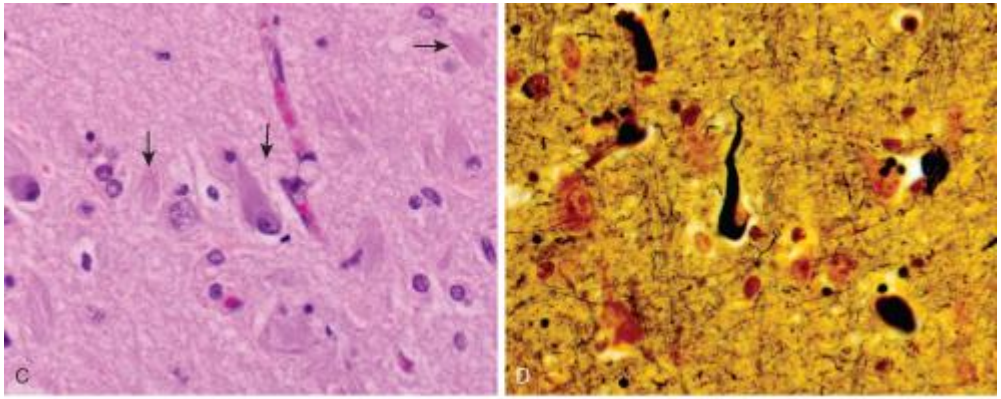
Pet scans (glucose utilization)


Cerebrovascular amyloidosis
Aβ depots on vessel walls
vascular damage
insufficient blood supply
wall weakness
neuron loss
cerebral hemorrage

Alzheimer disease, microscopy

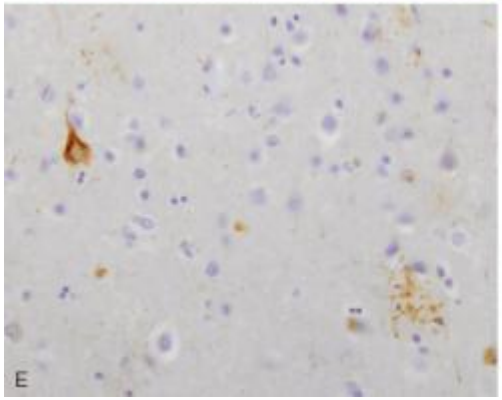


A, Plaques with dystrophic neurites surrounding amyloid cores (*arrows*).
B, Plaque core and surrounding neuropil are immunoreactive for Aβ.

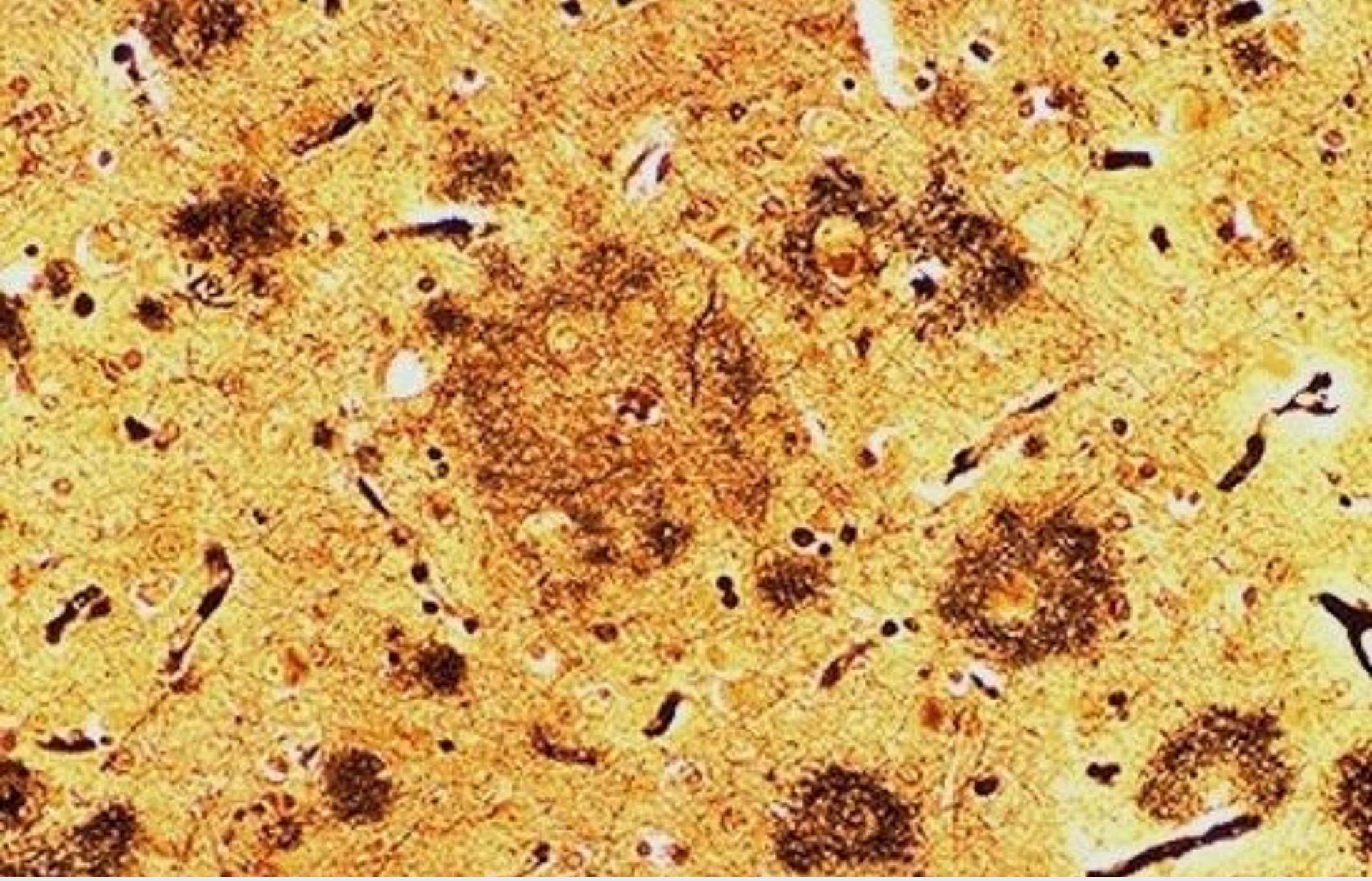


C, Neurofibrillary tangle is present within one neuron, and several extracellular tangles are also present (*arrows*).

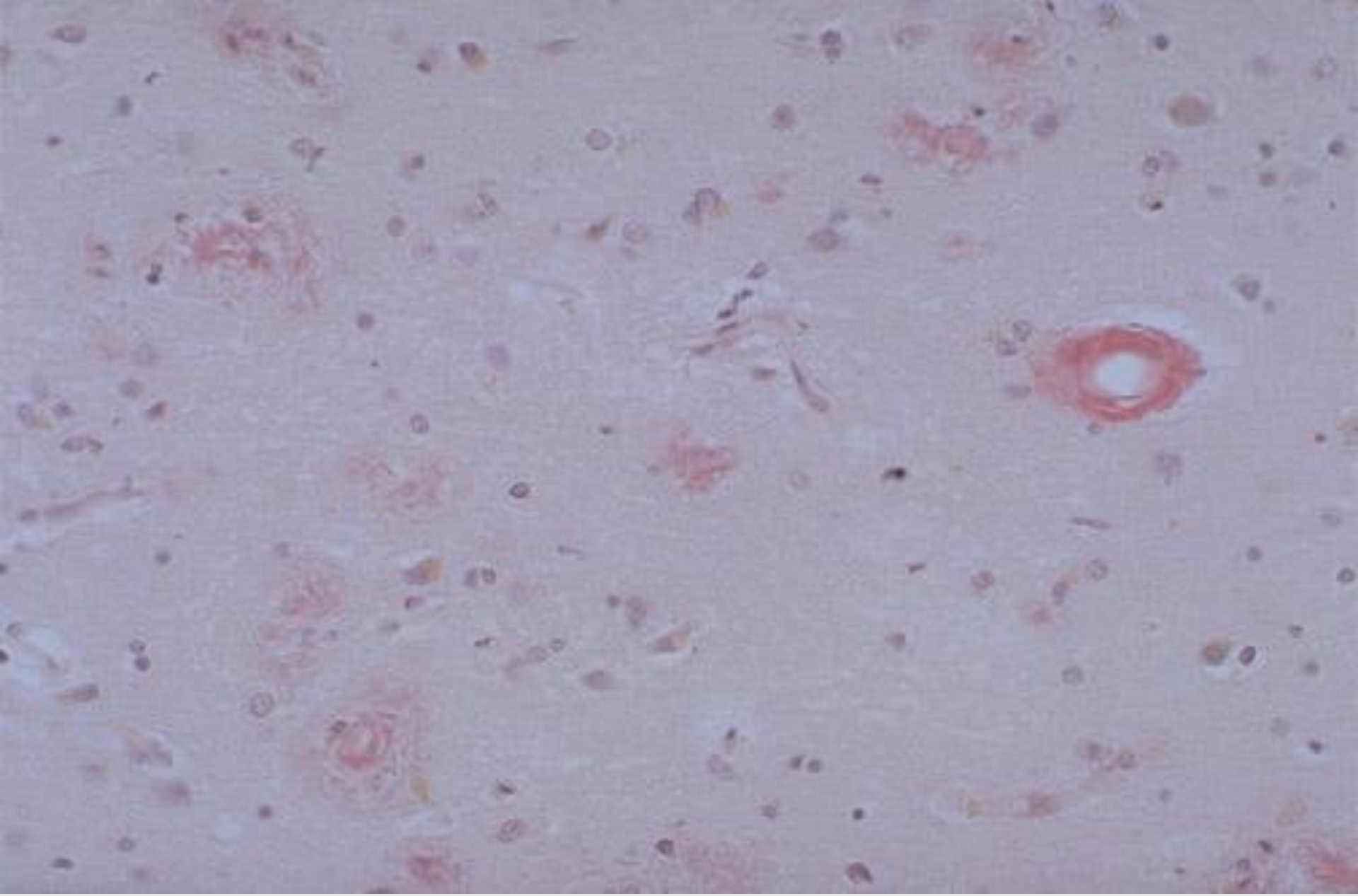
D, Silver stain showing a neurofibrillary tangle within the neuronal cytoplasm.



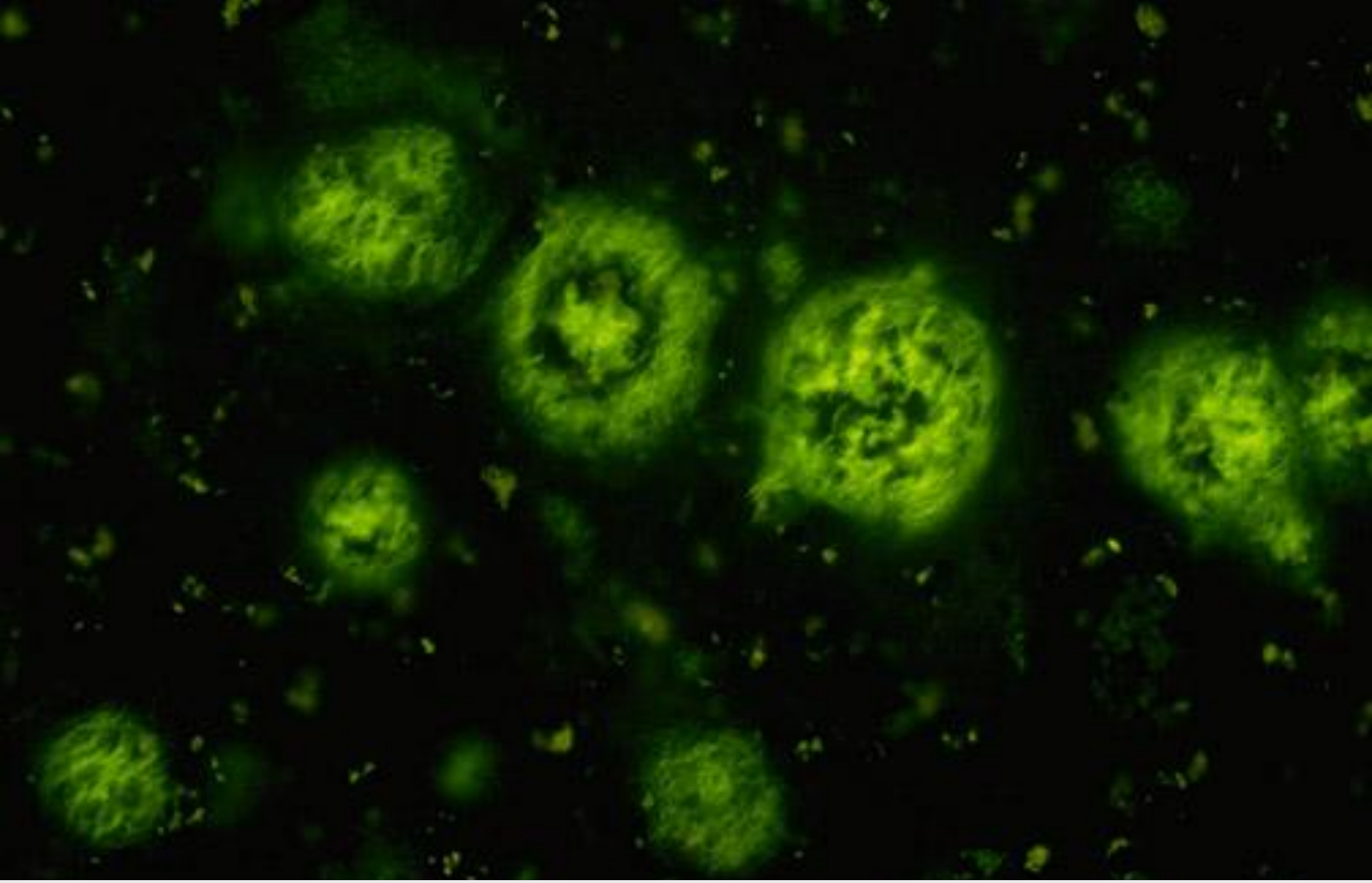
E, Tangle *(upper left)* and neurites around a plaque *(lower right)* contain tau, demonstrated by immunohistochemistry.



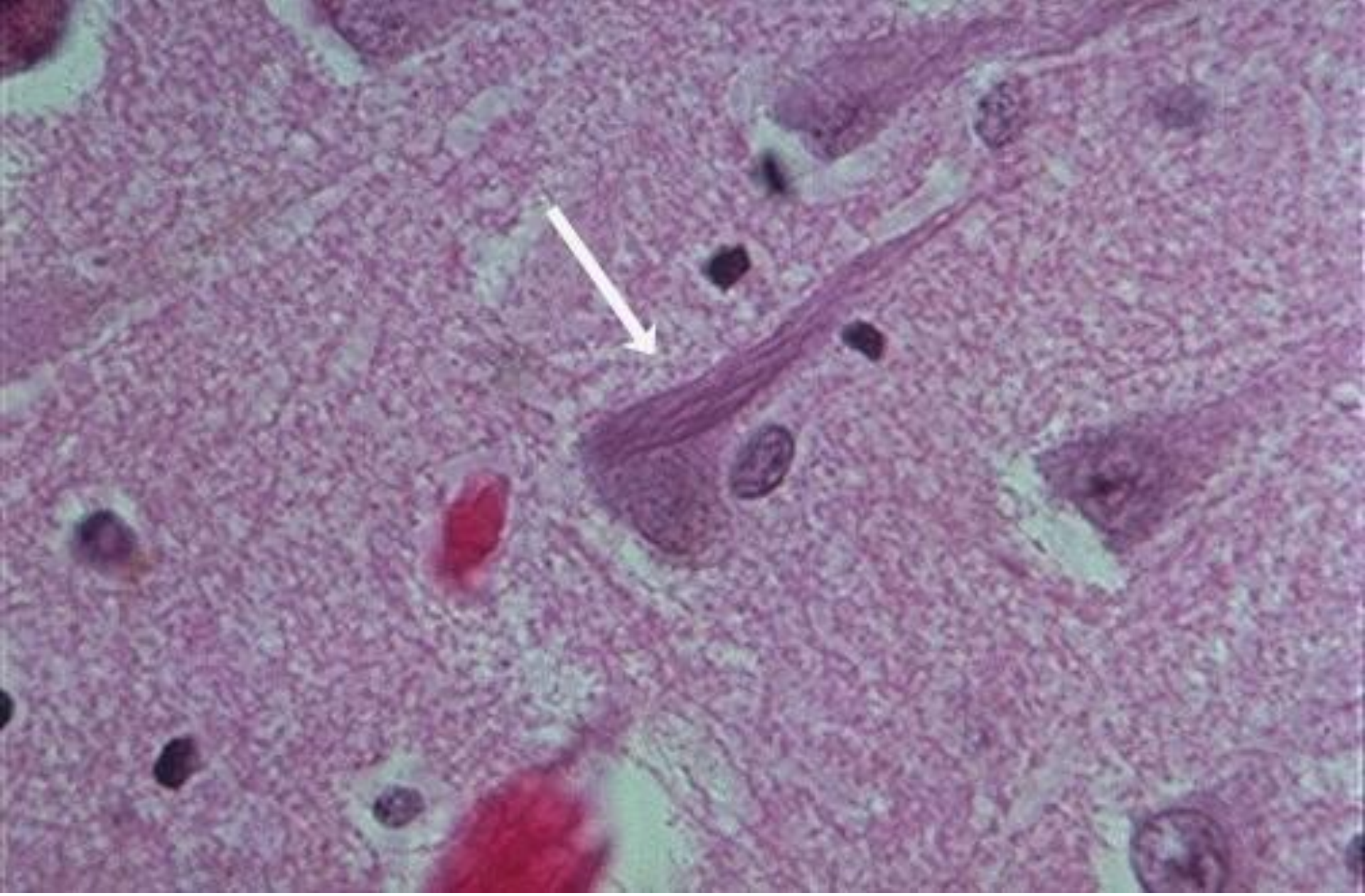
The characteristic microscopic findings of Alzheimer's disease include "senile plaques", of varying size, which are collections of degenerative presynaptic endings along with astrocytes and microglia (silver stain)



A number of neuritic plaques. They have an amyloid core (Congo red stain). Small peripheral cerebral arteries may also be involved.



Thioflavin T stain, viewed with fluorescence microscopy, highlights the neuritic plaques of Alzheimer's disease with amyloid deposition which fluoresces bright green.



Neurofibrillar tangles in Alzheimer's disease. The tangles appear as long pink cytoplasmatic filaments.

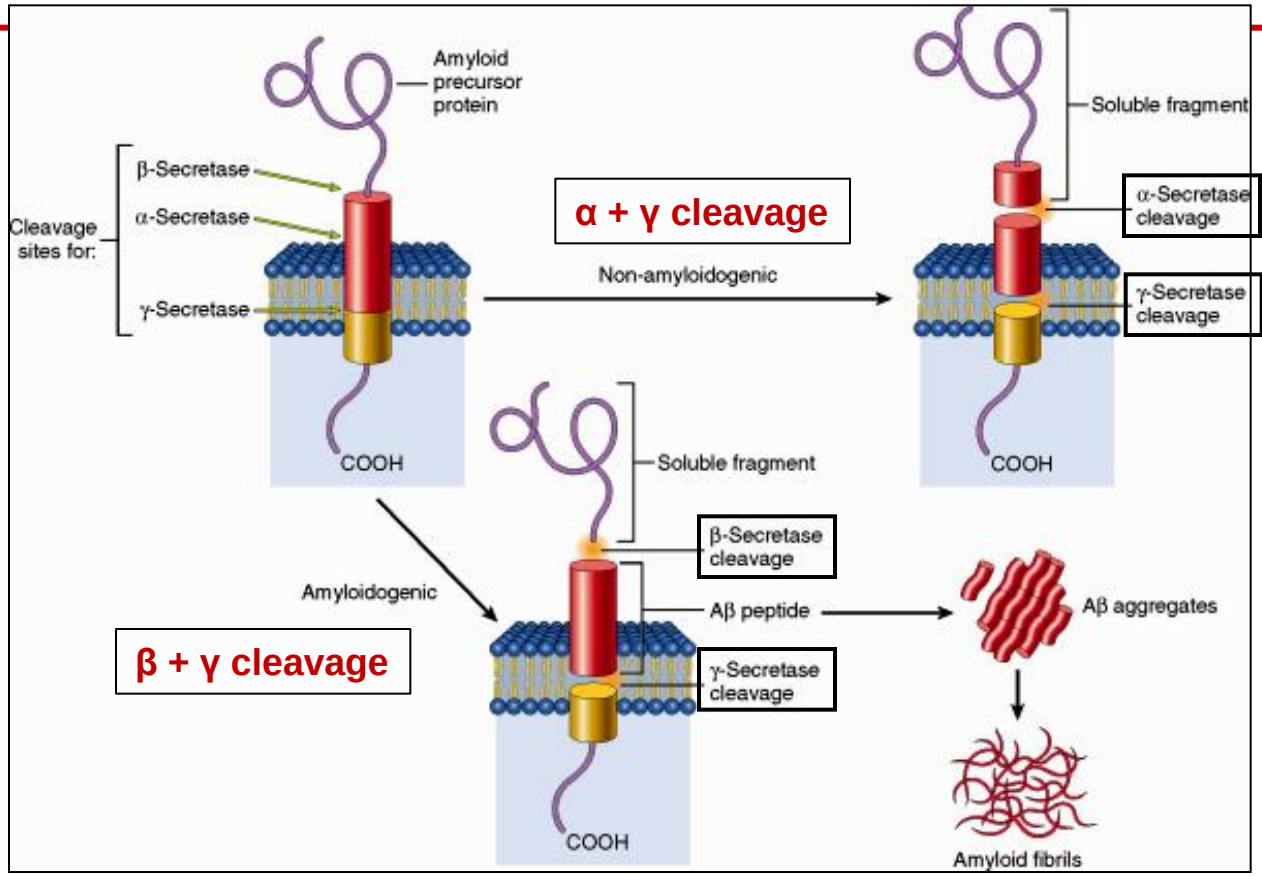
Aβ: WHERE IS IT FROM?

AMYLOID PRECURSOR PROTEIN (APP)

APP knock-out mice do not show any phenotype (not necessary for development and survival)

PROTEOLYSIS DUE TO α, β e γ-SECRETASES (TGN/endosome lumen)

- α + γ → soluble peptides
- **β + γ → Aβ42 and Aβ40 (amyloidogenic), rapidly released outside the cell**



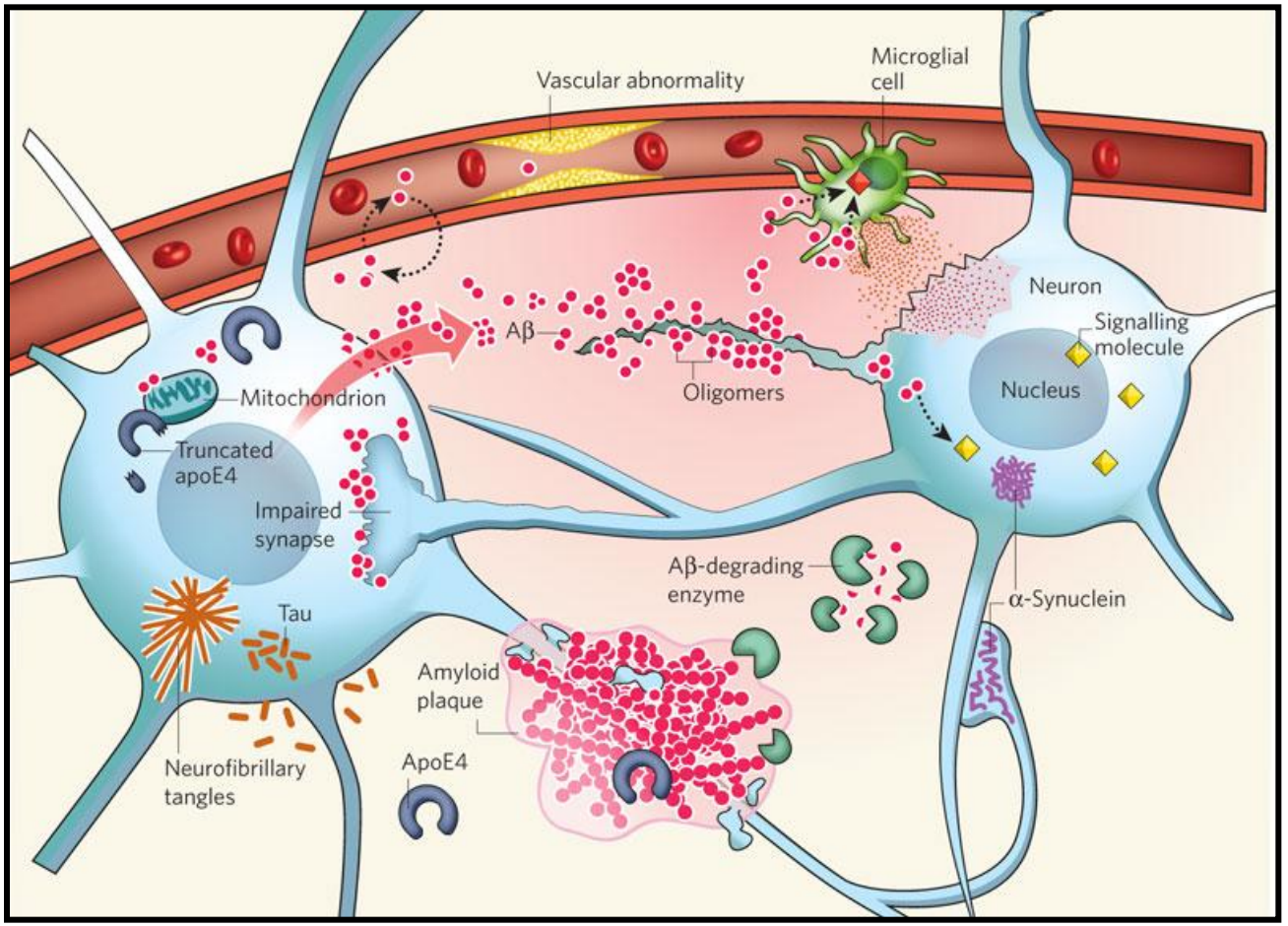
The role of Aβ in AD

- **Large amyloid plaques**, a hallmark of Alzheimer's disease, **displace and distort neuronal branches.**

- **Smaller Aβ assemblies** (oligomers) have been much harder to detect in brain tissues but **may be even more detrimental** than plaques.

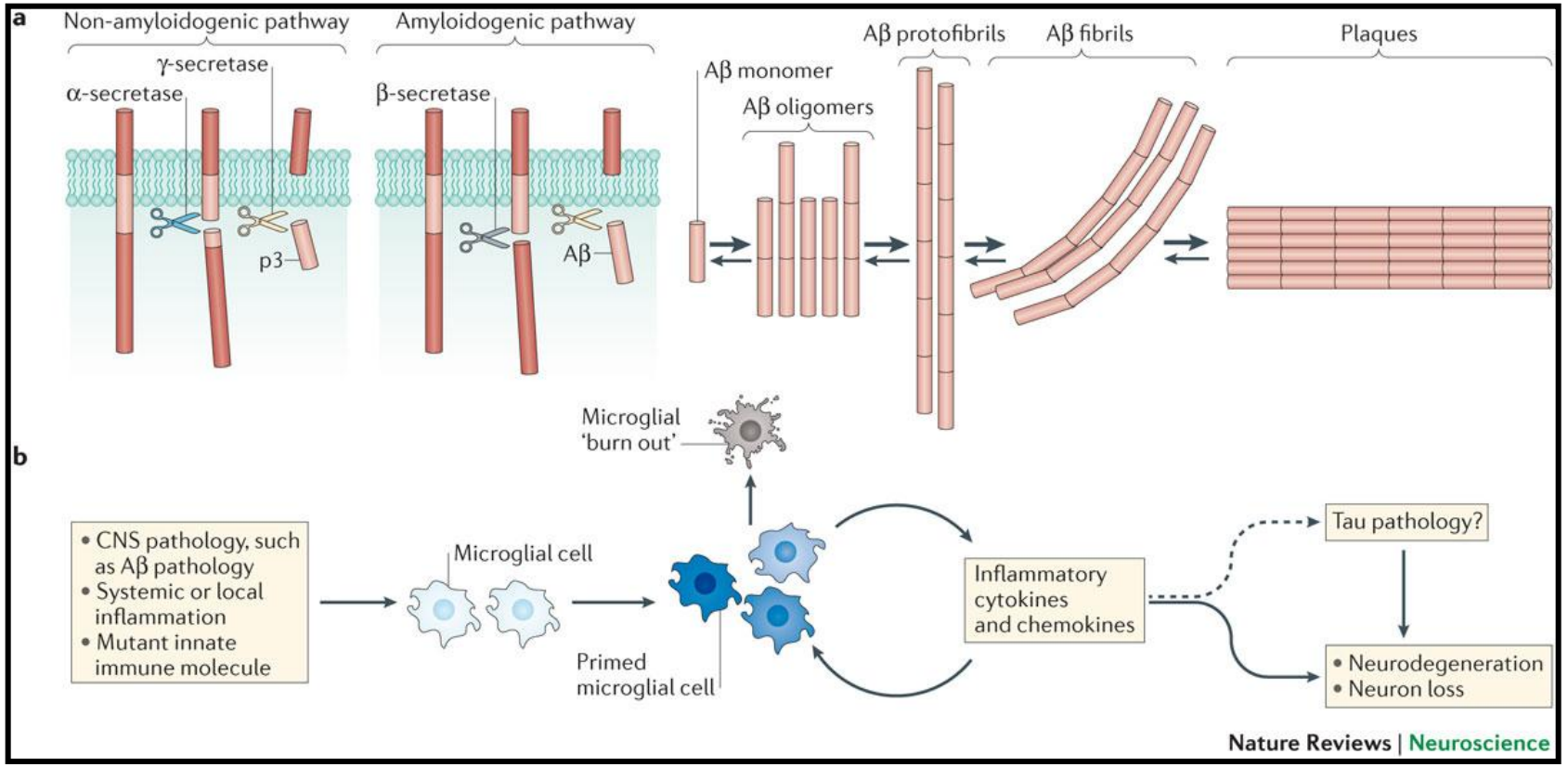
- **Aβ oligomers may impair** synapses or alter neuro-transmission by other mechanisms.

This would deplete signalling molecules that are dependent on synaptic activity and are required for memory and other brain functions.



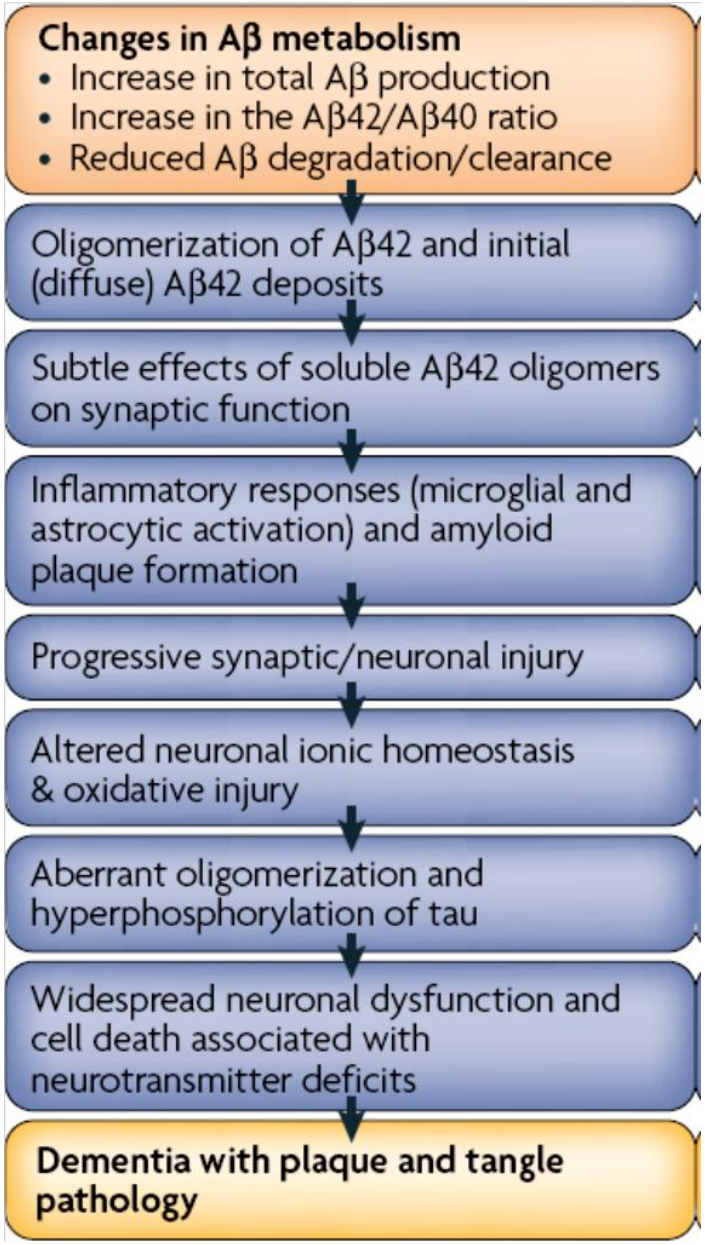
Mucke, 2009

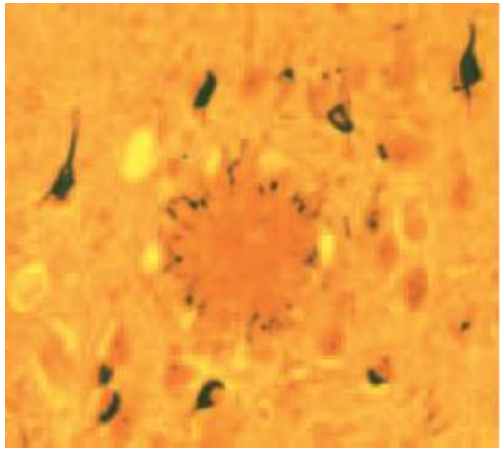
Aβ and inflammation



Heppner et al., 2015

The amyloid β cascade hypothesis





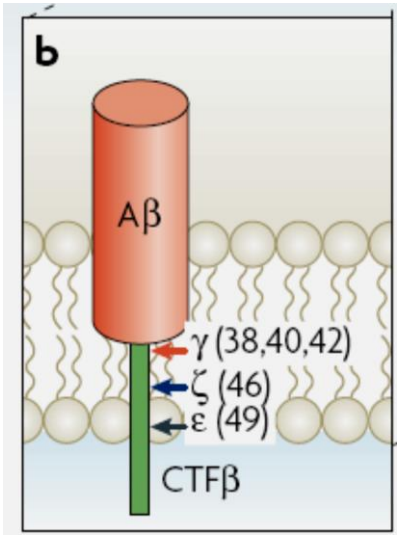
Haass & Selkoe, 2007

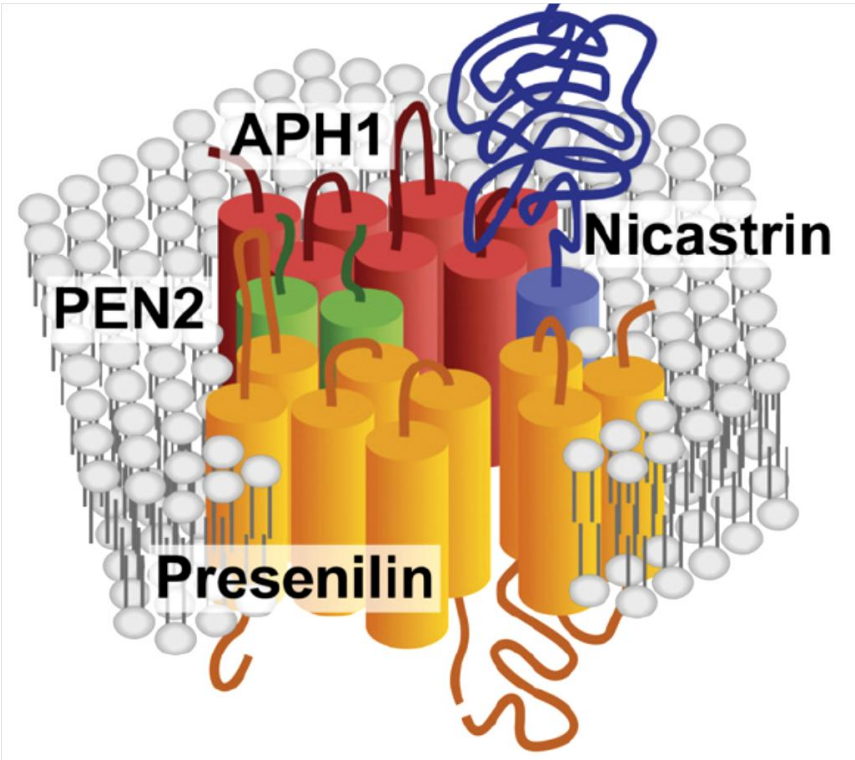
γ-secretase

Produces several peptides, among which Aβ40 and Aβ42

Part of a complex containing 4 transmembrane proteins:
presenilins (9 TransMembrane Domains), APH1 (7 TMD), nicastrin (1 TMD), PEN2 (2 TMD): 19 TMD that provide a hydrophilic environment necessary to hydrolase function

γ-dependent cleavage due to presenilins 1-2 autoactivation. Presenilins are aspartic proteases (as β-secretase)





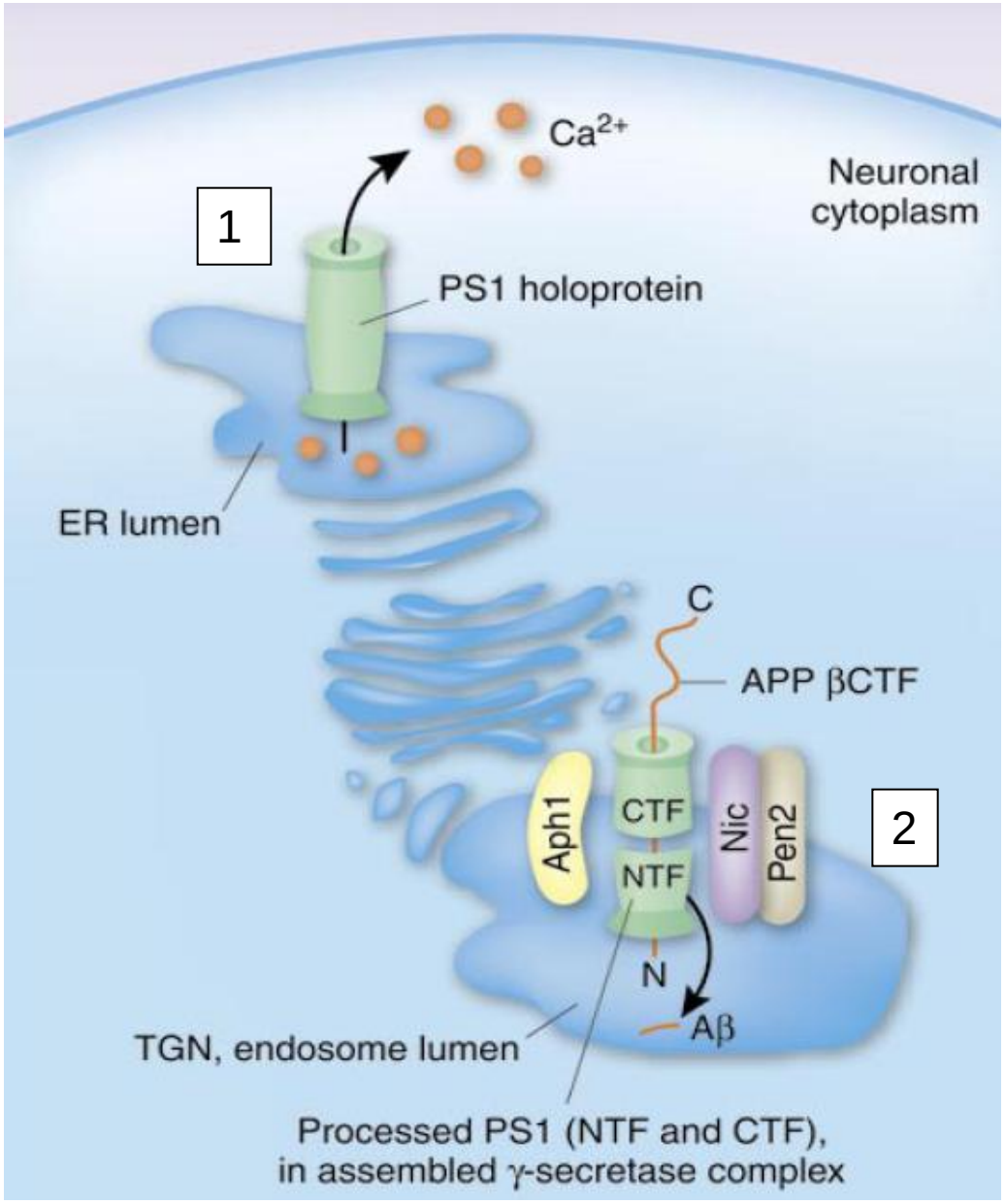
Calcium, β-amyloid, presenilin and AD

1 **Presenilin holoproteins** **modulate calcium leakage from the endoplasmic reticulum (ER),** whereas

2 **their cleaved derivatives** (PS1 NTF,; CTF,) **assemble with partners (Aph1, Pen2, and nicastrin (Nic) to form the γ-secretase complex,** which is concentrated **in the *trans*-Golgi network (TGN) and endocytic system.**

γ-Secretase cuts β-cleaved C-terminal fragments (βCTFs) of the amyloid precursor protein (APP), **releasing amyloid-β peptide (Aβ),** indicated in orange.

- Aβ is initially localized in the TGN lumen (dark blue), endosome lumen (dark blue) or both, but is rapidly released from the cell through the constitutive secretory pathway.



Gandy et al., 2006

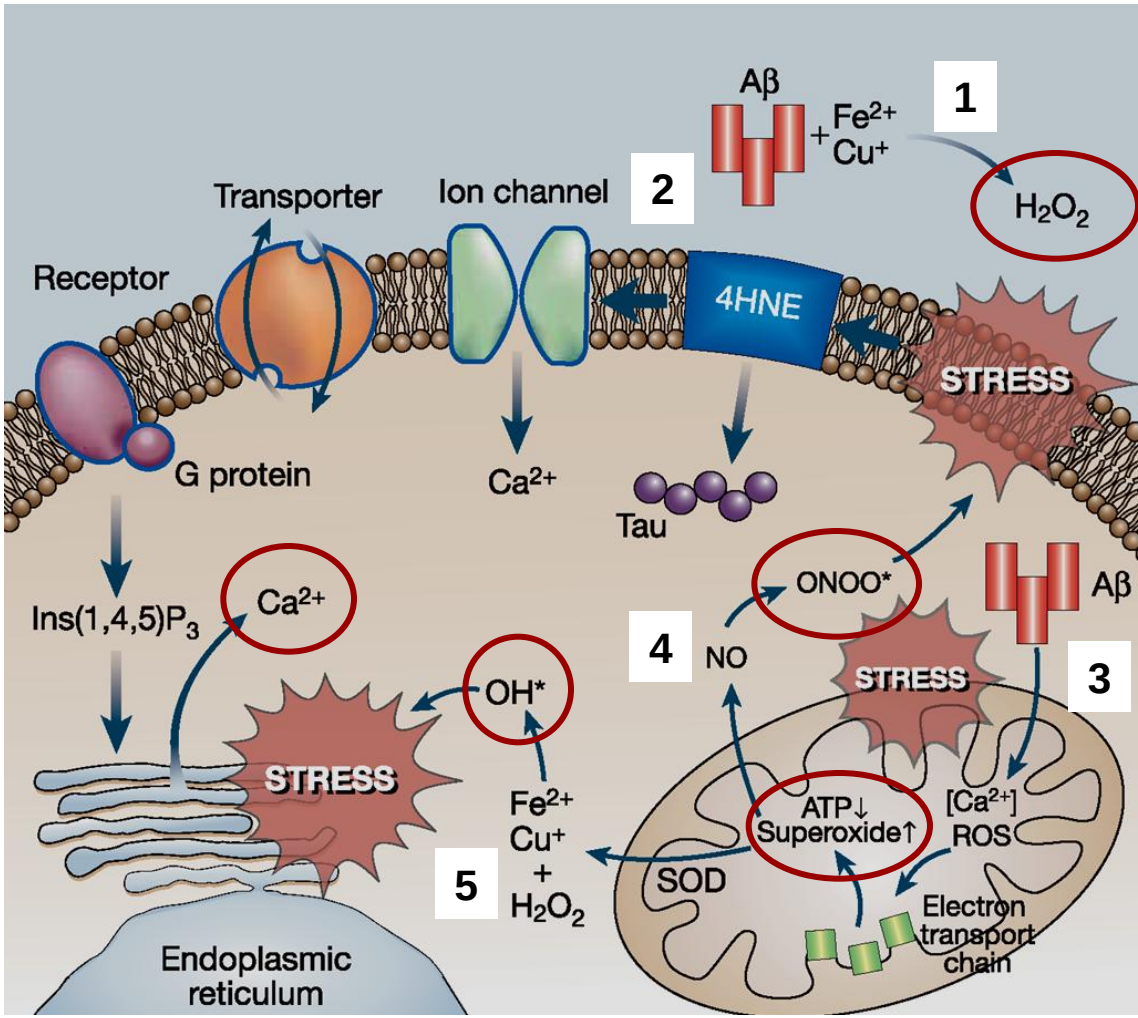
Neurotoxic action of Aβ involves ROS generation and disruption of calcium homeostasis

1. **Aβ oligomers** and Fe^{2+} or Cu^{+} generate H_2O_2

2. **Aβ aggregation at the cell membrane** → lipid peroxidation → generation of 4HNE that covalently modifies proteins: transporters, Rs, G proteins and ion channels

3. **Aβ → mitochondrial oxidative stress** → impairment of the electron transport chain, increased production of $O_2^{\cdot -}$ and decreased production of ATP.

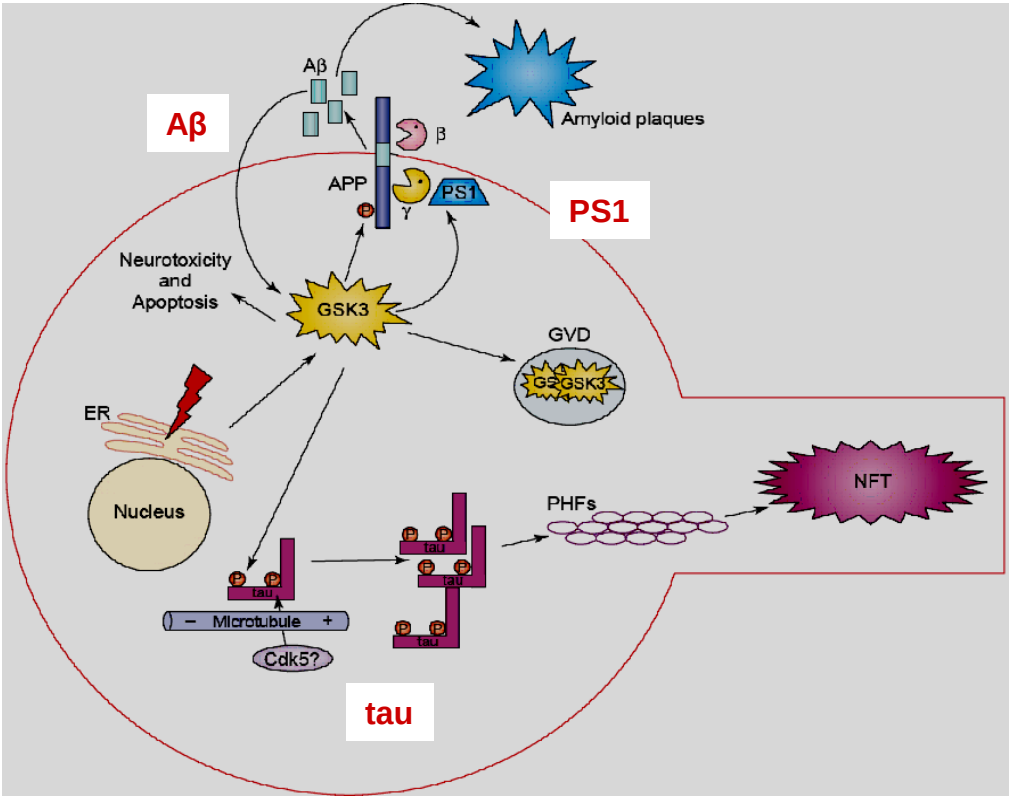
4. **$O_2^{\cdot -}$** → H_2O_2 (by the activity of SOD) and interacts with NO (via NOS) to produce peroxynitrite (ONOO*).



5. **Interaction of H_2O_2 with Fe^{2+} or Cu^{+}** generates the hydroxyl radical (OH*), a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the ER.

Mattson , 2004

GSK3β is associated with the neuropathology of AD



Links between glycogen synthase kinase-3β (GSK3) and neuropathological features of Alzheimer's disease.

1. Tau is a substrate of GSK3β, phosphorylated tau: low microtubule binding, enhanced PHF formation → neurofibrillary tangle (NFT) formation.

- GSK3 might also contribute to hyperphosphorylated tau deposits found in other neurodegenerative diseases.

- Transgenic mouse models in which GSK3β expression is increased have increased tau phosphorylation and deficits in spatial learning.

2. GSK3β is involved in amyloid-associated processes:

- GSK3β facilitates the production of Aβ

- **Treatment of neurons with Aβ activates GSK3β** and **inhibition of GSK3β protects from Aβ toxicity**.

- GSK3β also phosphorylates APP.

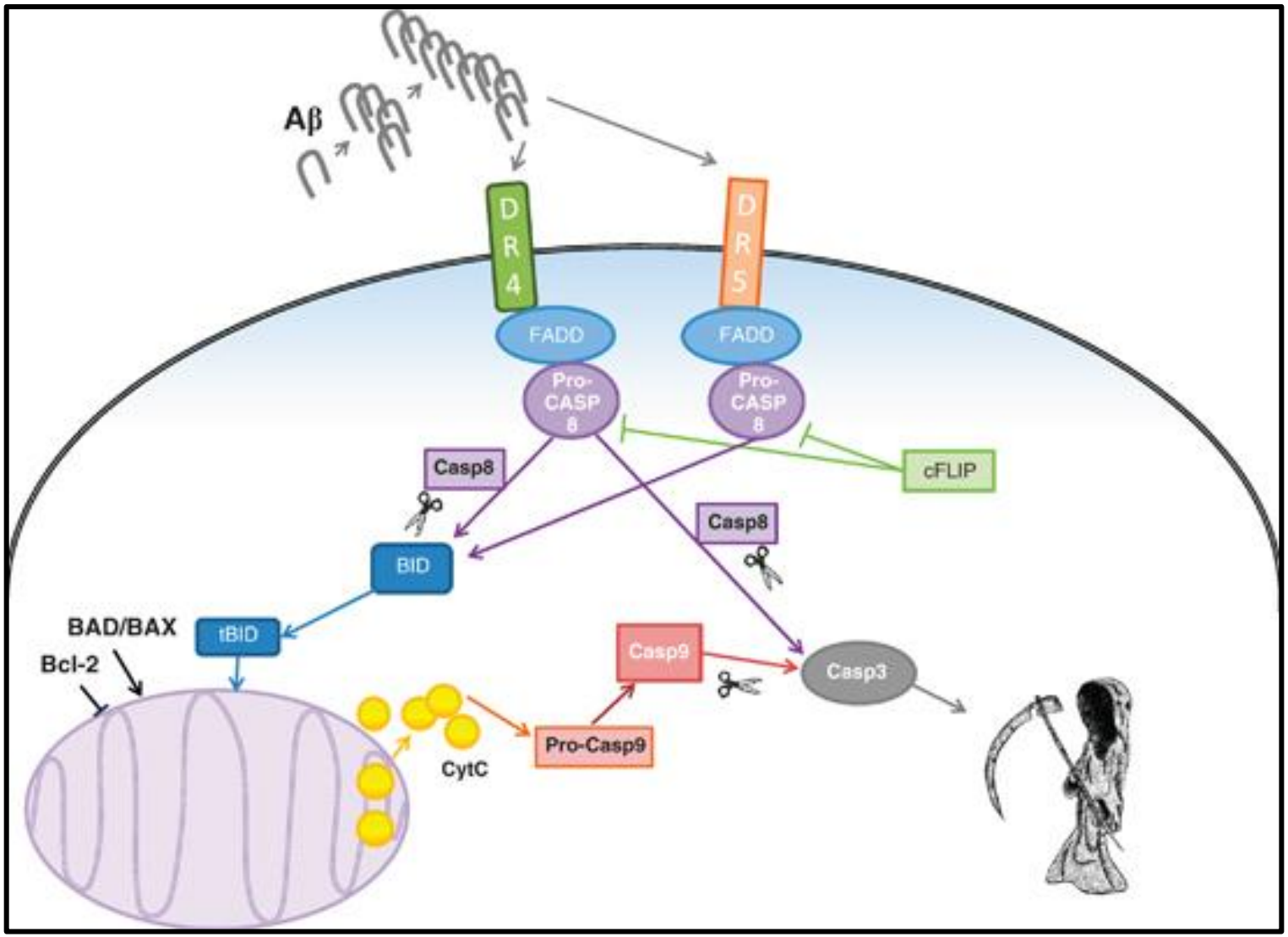
Thus, GSK3 contributes to the neurotoxicity and production of Aβ, potentially influencing the production of amyloid plaques.

3. Presenilin-1 (PS1) directly binds GSK3β: this association is altered by mutations of presenilin-1 (PS1) associated with FAD (familiar AD).

ER stress and the accumulation of misfolded proteins contributes to the neurotoxicity in AD, and ER stress causes activation of GSK3β.

Jope & Johnson, 2004

Apoptosis in AD



Fossati et al., 2012

Familiar Alzheimer Disease (FAD)

EARLY ONSET

GENETIC BASIS mutations on APP (Hsa21), PS 1 (Hsa14) and PS2 (Hsa1), APOEε4 allele (Hsa19)

Most frequent mutations (gain of function: γ-secretasi hyperactivation) on PS1 and PS2

Chr.	*Gene*	*Mutations/Alleles*	*Consequences*
21	**Amyloid precursor protein (*APP*)**	**Single missense mutations Double missense mutation Trisomy 21 (gene dosage effect)**	**Early-onset FAD Increased Aβ production**
14	**Presenilin-1 (*PS1*)**	**Missense mutations. Splice site mutations**	**Early-onset FAD Increased Aβ production**
1	**Presenilin-2 (*PS2*)**	**Missense mutations**	**Early-onset FAD Increased Aβ production**
19	**Apolipoprotein E (*ApoE*)**	**Allele ε4 (aa substitutions at positions 112 and 158 define 3 isoforms: ε4 associated with sporadic AD, ε2 may be protective)**	**Increased *risk* of development of AD, decreased age at onset of AD**

Allele	*112 residue*	*158 residue*
E2 (5-10%)	Cystein	Cystein
E3 (60-70%)	Cystein	Arginine
E4 (15-20%)	Arginine	Arginine

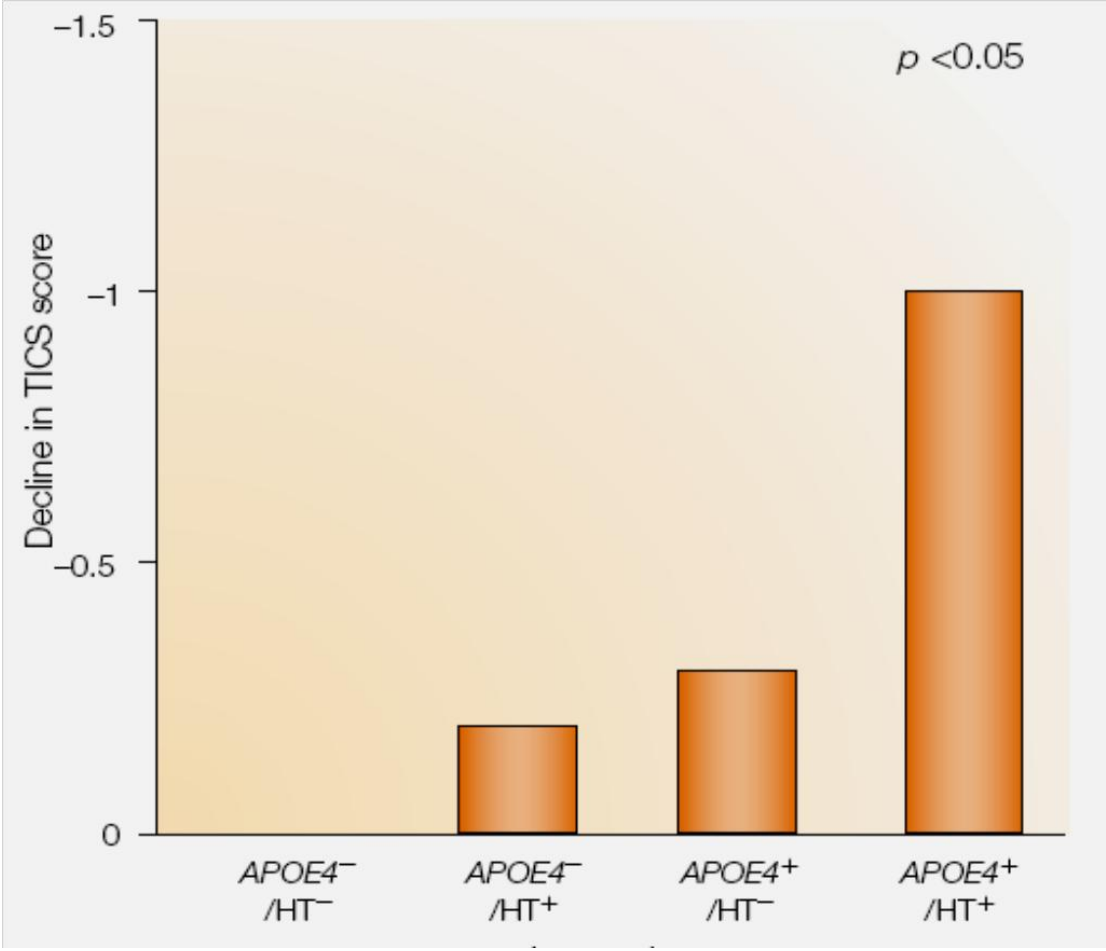
Examples of gene–environment interactions from epidemiological studies.

Perhaps the most robust association of a common variant with a serious disease is the **association of the apolipoprotein E4 (*APOE4*) allele with risk of cognitive decline and Alzheimer disease.**

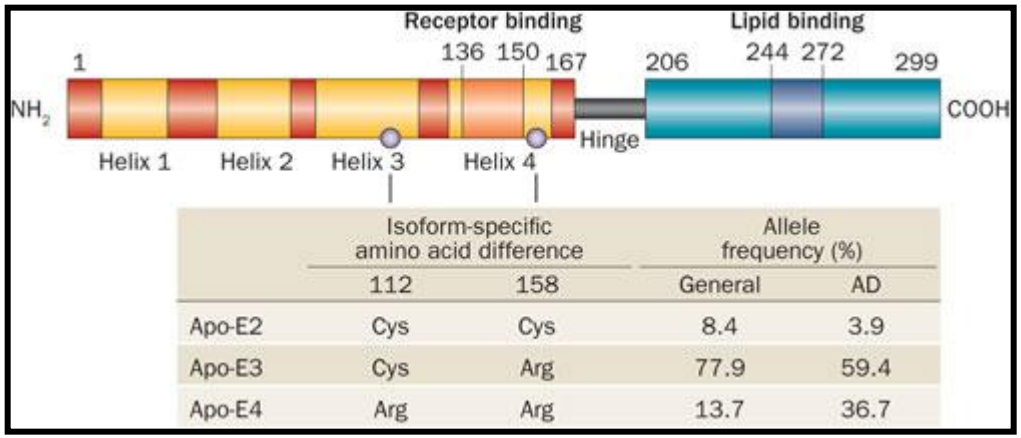
Recent studies suggest that **the risk of cognitive decline is particularly**

high in *APOE4* carriers who have untreated hypertension (the *APOE4*+/HT+ group).

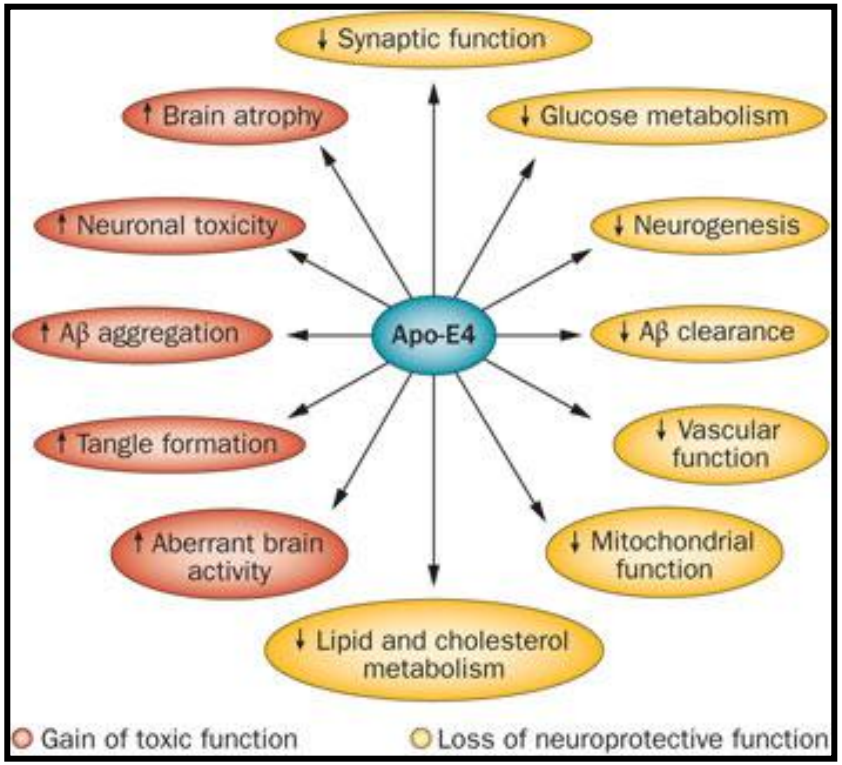
(TICS is a test of cognitive function)



APOE4



	Isoform-specific amino acid difference		Allele frequency (%)	
	112	158	General	AD
Apo-E2	Cys	Cys	8.4	3.9
Apo-E3	Cys	Arg	77.9	59.4
Apo-E4	Arg	Arg	13.7	36.7



Liu et al., 2013

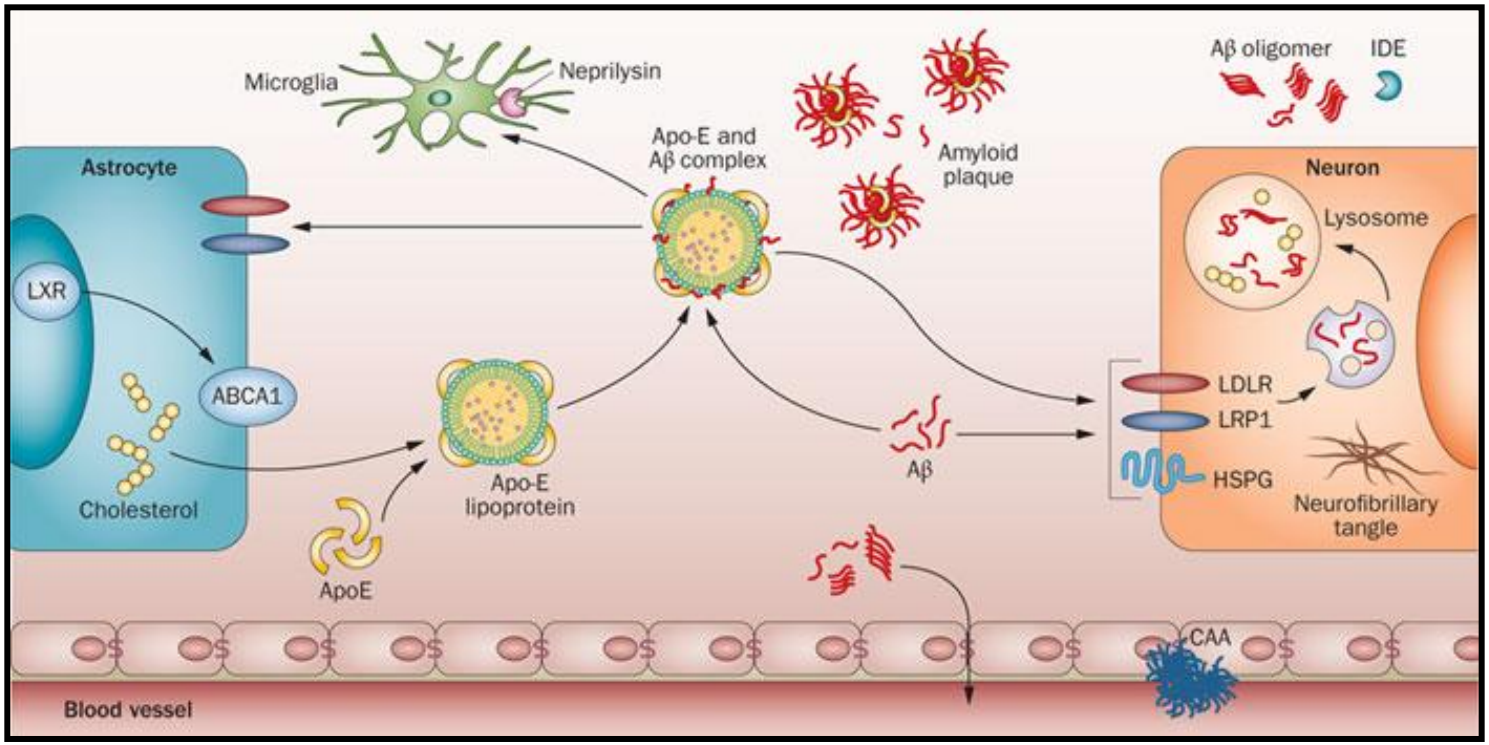
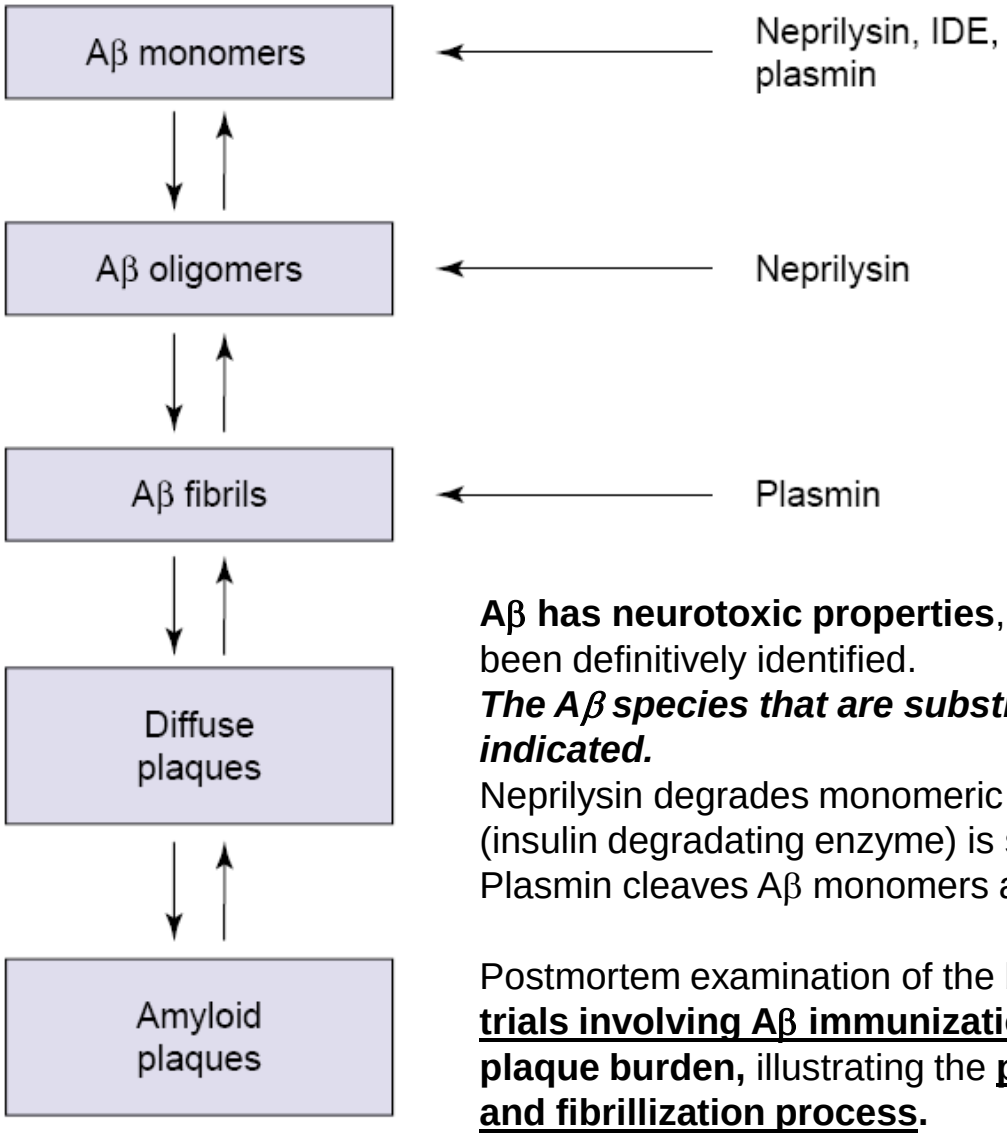


Figure 2: Apolipoprotein E and amyloid-β metabolism in the brain.Major Aβ clearance pathways include receptor-mediated uptake by neurons and glia, drainage into interstitial fluid or through the BBB, and proteolytic degradation by IDE and neprilysin. Impaired Aβ clearance can cause accumulation in brain parenchyma, triggering formation of Aβ oligomers and amyloid plaques. Perivascular Aβ accumulation leads to CAA, which disrupts blood vessel function. Apo-E is primarily synthesized by astrocytes and microglia, and is lipidated by the ABCA1 transporter, forming lipoprotein particles. Lipidated Apo-E binds soluble Aβ and facilitates Aβ uptake through cell-surface receptors, including LRP1, LDLR, and HSPG.175, 177 Apo-E facilitates binding and internalization of soluble Aβ by glial cells, disrupts Aβ clearance at the BBB in an isoform-dependent manner (Apo-E4 > Apo-E3 > Apo-E2) and influences CAA pathogenesis. Abbreviations: Aβ, amyloid-β; ABCA1, ATP-binding cassette A1; Apo-E, apolipoprotein E; BBB, blood–brain barrier; CAA, cerebral amyloid angiopathy; HSPG, heparan sulphate proteoglycan; IDE, insulin-degrading enzyme; LDLR, low-density lipoprotein receptor; LRP1, low-density lipoprotein receptor-related protein 1; LXR, liver X receptor (Liu et al., 2013).



Aβ aggregation and fibrillization inhibition

Aβ initially exists as a monomer, and a chain of events leads to its final deposition as an amyloid plaque.

Aβ has neurotoxic properties, although the pathogenic form has not as yet been definitively identified.

The Aβ species that are substrates for the various proteases are indicated.

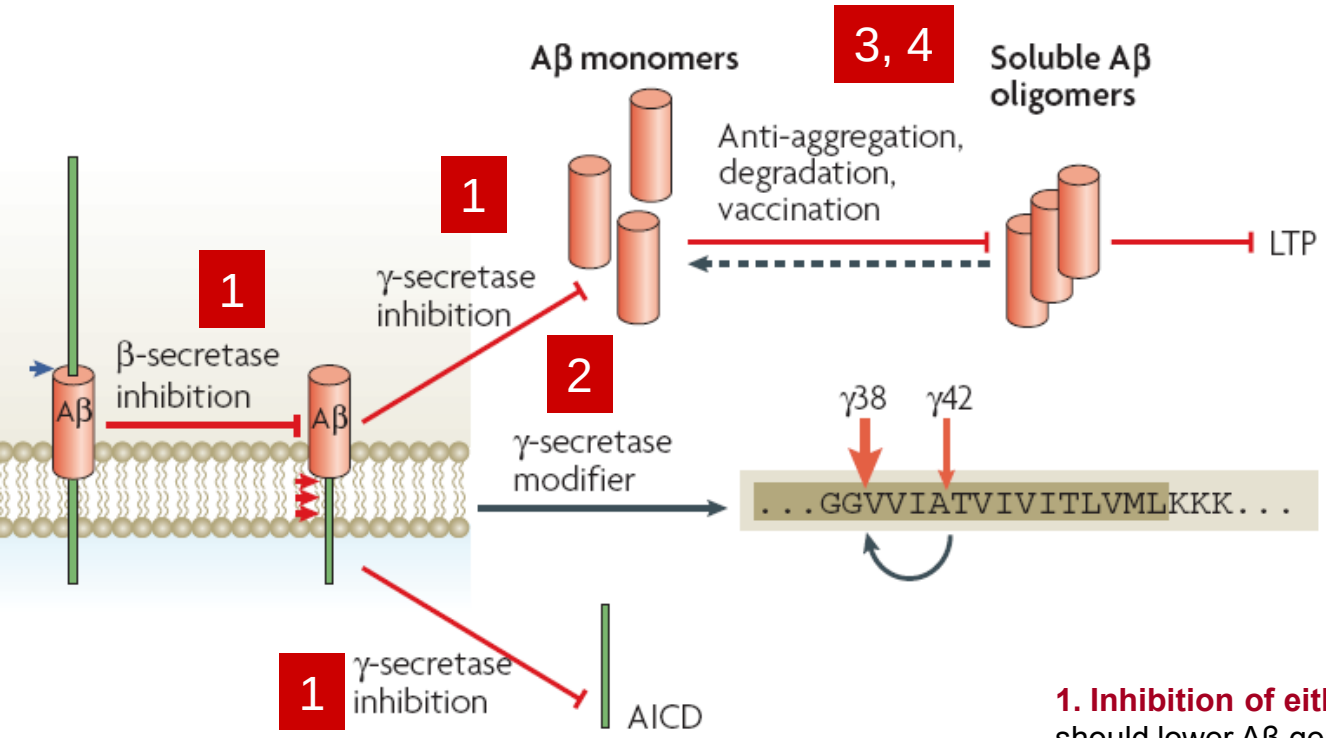
Neprilysin degrades monomeric and oligomeric forms of Aβ, whereas IDE (insulin degradating enzyme) is selective for monomers.

Plasmin cleaves Aβ monomers and also Aβ fibrils.

Postmortem examination of the brains of AD sufferers participating in **clinical trials involving Aβ immunization** has revealed a **significant reduction in plaque burden,** illustrating the **potential reversibility of the aggregation and fibrillization process.**

Vardy et al., 2005

Therapeutic approaches targeting Aβ production and oligomerization



Several therapeutic strategies (some already in early clinical trials) have emerged from knowledge of Aβ generation and the effects of soluble Aβ oligomers on synaptic function.

1. Inhibition of either β- or γ-secretase should lower Aβ generation. However, it might cause unwanted side effects.

2. γ-secretase modulators such as certain non-steroidal anti-inflammatory drugs (NSAIDs) and their derivatives, on the other hand, will not block the γ-secretase cleavage but rather shift its cleavage site from the rapidly aggregating 42-residue variant to the far less amyloidogenic 38-residue form.

3. Anti-aggregation drugs, which prevent or even disrupt oligomers, will shift the pool of oligomeric Aβ to the benign Aβ monomers (dashed arrow).

4. Aβ immunotherapy might also allow the selective neutralization of Aβ oligomers.
Any Aβ-related treatment strategy will lower the amyloid plaque load as well.

Haass & Selkoe , 2007