New Biotic INC.
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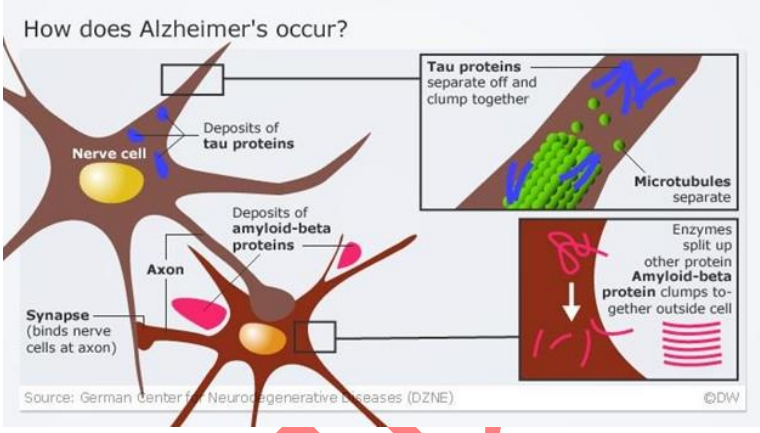
April 16, 2022

Synopsis of Alzheimer's Disease

Introduction:

Alzheimer's Disease (AD) afflicts 6.2 million Americans, is the most expensive disease in the United States and responds only **marginally and briefly** to currently available drugs that have been approved by the Food and Drug Administration for its treatment.

The AD brain is characterized microscopically by the combined presence of two classes of abnormal structures, extracellular amyloid plaques and intraneuronal neurofibrillary tangles, spread through the brain, both of which comprise highly insoluble, densely packed filaments. The soluble building blocks of these structures are amyloid-β (Aβ) peptides for plaques and tau for tangles. Amyloid-β peptides are proteolytic fragments of the transmembrane Amyloid Precursor Protein (APP), whereas tau is a brain-specific, axon-enriched microtubule-associated protein.



The behavioral symptoms of AD correlate with the accumulation of plaques and tangles, and they are a direct consequence of the damage and destruction of synapses that mediate memory and cognition. Synapse loss can be caused by the failure of live neurons to maintain functional axons and dendrites or by neuron death.

During the past dozen years, a steadily accumulating body of evidence has indicated that soluble forms of Aβ and tau work together, independently of their accumulation into plaques and tangles, to drive healthy neurons into the diseased state and that hallmark toxic properties of Aβ require tau. For instance, acute neuron death, delayed neuron death following ectopic cell cycle reentry, and synaptic dysfunction are triggered by soluble, extracellular Aβ species and depend on soluble, cytoplasmic tau. Therefore, Aβ is upstream of tau in AD pathogenesis and triggers the conversion of tau from a normal to a toxic state, but there is also evidence that toxic tau enhances Aβ toxicity via a feedback loop.

Because soluble toxic aggregates of both Aβ and tau can self-propagate and spread throughout the brain by prion-like mechanisms, successful therapeutic intervention for AD would benefit from detecting these species before plaques, tangles, and cognitive impairment become evident and from interfering with the destructive biochemical pathways that they initiate.

Extracted from: (Bloom, 2014)

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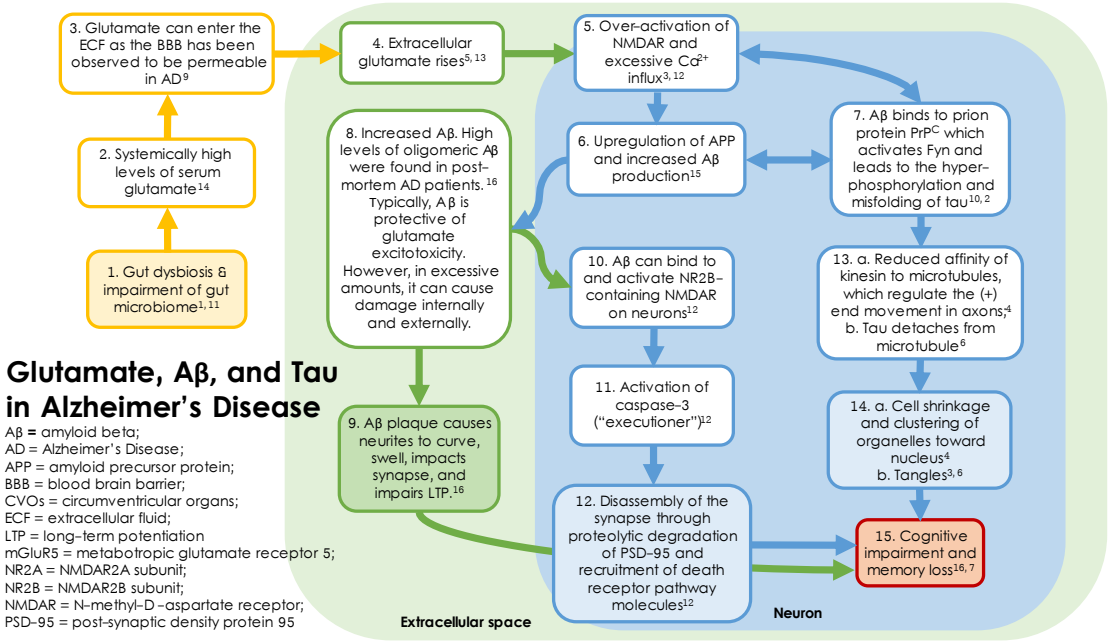
Synopsis:

Amyloid-beta plaque production and tau hyper-phosphorylation are two key hallmarks of Alzheimer's Disease (AD). Amyloid-beta is a protein that regulates synaptic plasticity and health. However, excessive amyloid-beta over time can 1) form plaques that fill the extracellular space and affect the morphology of neurons, 2) activate a cellular pathway that leads to the disassembly of the synapse, and 3) trigger tau hyper-phosphorylation and mis-folding. Tau is a protein that typically regulates organelle movement along the microtubule. However, the hyper-phosphorylation of tau can lead to cell shrinkage and tangle formation (first in axons then in dendrites). Together amyloid-beta and tau hyper-phosphorylation can lead to the cognitive impairment observed in AD. This synopsis outlines how these two mechanisms interact and specifically illuminates the role gut dysbiosis and glutamate excitotoxicity in initiating them. The numbers guiding the outline correlate with the cell numbers in the diagram below.



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The synopsis is divided into the following five sections:

A. Disruption of the gut's glutamate metabolic efficiency leads to the over-expression of amyloid-beta (Aβ)
B. Over-expression of Aβ leads tau to enter a toxic state
C. Tau and Aβ influence each other in a vicious cycle
D. The elevation of Aβ and tau toxicity eventually leads to the pathological effects of Aβ in the extracellular space and tau in axons
E. Both events lead to the loss of neurotransmission and can clinically manifest as cognitive impairment.

A. Disruption of the gut's glutamate metabolic efficiency leads to over-expression of amyloid beta

1) Gut dysbiosis in AD

a) "Our data demonstrated a remarkably reduction in the bacterial diversity and alterations in the taxonomic composition of the fecal microbiota of the AD patients." (Ling, et al., 2021)

b) How is gut dysbiosis relevant to glutamate excitotoxicity?

As gut bacteria can alter the bioavailability of amino acids, (Neis, Dejong, & Rensen, 2015), a disruption of resident bacteria due to gut dysbiosis can lead to the abnormal metabolism of dietary glutamate.

2) Elevated serum glutamate.

Abnormal metabolism of dietary glutamate can lead to increased absorption of glutamate into the serum. As such, elevate plasma glutamate has been seen in AD. (Miulli, Norwell, & Schwartz, 1993)

3) How does elevated serum glutamate cross into the brain?

Because the blood brain barrier (BBB) prevents the translocation of peripheral metabolites into the brain, a breach in the BBB or an alternate route of entry would be necessary for plasma glutamate to cause neuronal glutamate excitotoxicity as commonly seen in ALS. Intriguingly it has been observed that the **BBB is compromised** in several neurological disorders including ALS**, Alzheimer's Disease**, Parkinson's Disease and Huntington's Disease (Kakaroubas, Brennan, Keon, & Saksena, 2019).

4) Elevated ECF and CSF glutamate seen in AD

a) "Alzheimer's disease (AD) is characterized by excitotoxic levels of extracellular glutamate alongside accumulation of soluble Aβ and hyperphosphorylated tau protein leading to neuronal cell death" (Findley, Bartke, Hascup, & Hascup, 2019)

b) "Mean CSF glutamate levels were significantly higher in patients with probable AD compared to healthy controls and hydrocephalus patients ($F = 50.8$, $p < 0.0001$)" (Madeira, et al., 2018)

5) Extracellular glutamate over activates NMDA receptors and cause excessive calcium influx

Glutamate is a neurotransmitter that stimulates several post-synaptic receptors, one of which is NMDA receptor (NMDAR). NMDARs usually benefit memory formation and long-term synaptic plasticity. However, this is dependent on the location of NMDAR. The activation of extrasynaptic NMDARs has been shown to have excitotoxic effects. Two downstream effects of the activation of extra-synaptic NMDAR are: amyloid-beta (Aβ) production (Pearson & Peers, 2006) (Liu, et al., 2010) and the hyper-phosphorylation of tau. (Chohan & Iqbal, 2006).

6) NMDAR activation leads to increased production of amyloid-beta

Steinbach et al. showed that the activation of NMDAR upregulates the production of Aβ's precursor, APP. In addition to upregulating APP, the activation of NMDAR also inhibited alpha-secretase, thereby promoting the production of Aβ over other peptides. (Pearson & Peers, 2006). Figure 1 shows how Aβ is produced in neurons.

B. Over-expression of Aβ leads tau to enter a toxic state (and a brief mention of the basic functions of Aβ and tau)

Under normal circumstances…

a) Aβ suppresses excessive synaptic activity and actually serves as a safety against excitotoxicity. (Pearson & Peers, 2006), and is suggested to play a role in synaptic plasticity (Spires-Jones & Hyman, 2014).

b) Tau is a protein that stabilizes microtubule tracks and are largely localized in the axon of neurons. Movement of organelles along microtubule tracks are regulated by kinesin, which induces plus-end movement toward the peripheral of the cell, and dynein, which induces negative-end movement toward the cell's nucleus. The activity of kinesin and dynein usually depend on the phosphorylation of tau, which is determined by a fine balance of kinase and phosphatase concentrations.

7) However, under circumstances of excessive Aβ, Aβ oligomers can trigger tau pathology

a) "Aβ is upstream of tau in AD pathogenesis and triggers the conversion of tau from a normal to a toxic state..." (Bloom, 2014)

How: AB oligomer dimers specifically bind to prion protein PrP^C and activate Fyn, which in turn triggers tau aberrant missorting and hyperphosphorylation (Larson, et al., 2012)

C. Tau and Aβ influence each other in a vicious cycle

a) "Aβ is upstream of tau in AD pathogenesis and triggers the conversion of tau from a normal to a toxic state, **but there is also evidence that toxic tau enhances Aβ toxicity via a feedback loop**." (Bloom, 2014)

b) Figures 2, 3 and 4 depict the interaction between tau and amyloid-beta

D. The elevation of Aβ and tau toxicity eventually leads to the pathological effects of Aβ in the extracellular space and tau in axons

Amyloid-Beta

8) Amyloid-beta accumulation in ECF
Amyloid-beta accumulation in the ECF can have two effects: 1) an external effect on neuron morphology and 2) an internal effect on synaptic dysfunction

9) First, the external effect of insoluble Aβ on neuron morphology (Aβ plaque in ECF)

a) Overexpression of APP can lead to Aβ plaque formation within 24 hours. This can lead to neurites curving and degeneration a few days later (Spires-Jones & Hyman, 2014).

b) Plaque causes neurites to exhibit swollen and dystrophic morphologies and disrupt axon and dendrite trajectories which are usually fairly straight (Spires-Jones & Hyman, 2014).

10) through 12) Second, the internal effects of soluble AB.

a) Soluble Aβ rapidly instigates the degradation of post-synaptic proteins and **reduces synaptic stability** through the mediation of NR2B-containing NDMAR" (Liu, et al., 2010)

b) How: Soluble Aβ → NR2B-containing NMDAR over-activation → increased Ca2+ → activation of calpain → calpain cleaves caspase-8 and caspase-3 and contributes to the disassembly of the synapse through proteolytic degradation of PSD-95 and recruitment of death receptor pathway molecules (Liu, et al., 2010)

c) "Our finding that soluble Aβ induces **synaptic dysfunction [not synaptic loss]** by concomitantly suppressing NR2A and activating NR2B is in accord with a previous demonstration that neuronal cell survival and death are mediated by NR2A- and NR2B-containing NMDARs, respectively." (Liu, et al., 2010)

Tau hyperphosphorylation affects axonal and dendritic structure

13) Reduced affinity of kinesin to microtubules

In normal conditions tau is a protein that stabilizes microtubule tracks and are largely localized in the axon of neurons. Movement of organelles along microtubule tracks are regulated by kinesin, which induces plus-end movement toward the peripheral of the cell, and dynein, which induces negative-end movement toward the cell's nucleus. The activity of kinesin and dynein usually depend on the phosphorylation of tau, which is determined by a fine balance of kinase and phosphatase concentrations. However, under conditions where that balance may be disturbed, hyper-phosphorylation occurs. As a result, the hyper-phosphorylation of tau reduces the affinity of kinesin to the microtubule, thereby affecting plus-end movement.

14) Cell shrinkage and tangles

a) This causes organelles and vesicles to cluster around the nucleus, which inevitably leads to the shrinking of the cell. (Ebneth, et al., 1998)

b) The abnormal phosphorylation of tau also results in neurofibrillary tangles, a characteristic of AD. (Chohan & Iqbal, 2006)

E. Both events lead to loss of neurotransmission and can clinically manifest as cognitive impairment

15) Both amyloid-beta and tau pathology lead to neurodegeneration and cognitive impairment. (Spires-Jones & Hyman, 2014)

Amyloid-beta (from #12)

a) A 2017 study measured cognition over 72 months in cognitively normal older adults (n=335). Cognition was compared between people with low (Aβ-) levels and high (Aβ+) levels. They found that "compared to the Aβ- group, the Aβ+ group showed no cognitive impairment at baseline but showed substantial decline in verbal learning, episodic memory and attention **over 72 months**." (Harrington, et al., 2017)

b) These observations suggest that Aβ may play a role in affecting cognition in early or pre-clinical AD over time.

Tau (from #14)

a) "Knocking out the tau genes in the APP/PS1 mice conferred protection not only against memory impairment, but against synaptic loss, neuron loss, and premature death as well." (Bloom, 2014)

Comment:

Effective AD treatment requires the following steps:

1. Reducing glutamate excitotoxicity by restoring glutamate metabolize bacteria plus limiting consumption of dietary glutamate.
2. Normalize NMDAR's synaptic function which avoid production of Amyloid-β's precursor-APP, and tau hyperphosphorylation; this avoids AD and/or stop of progression of AD.
3. Dissolving soluble amyloid- β (monomer and oligomer) to prevent excess soluble amyloid-beta from activating NR2B-containing NMDA receptors.
4. Dissolving insoluble amyloid-β (fibrils and amyloid plaque, also call Lewy Body) throughout the entire brain, which prevent AD from progressing to Dementia.
5. Dissolve Tau tangle to prevent it from turning into toxic states.
6. Restore normal synaptic function that mediate memory and cognitive function.
7. Regenerate neuronal axons and dendrites so it can perform its synaptic function.

Reference Figures:

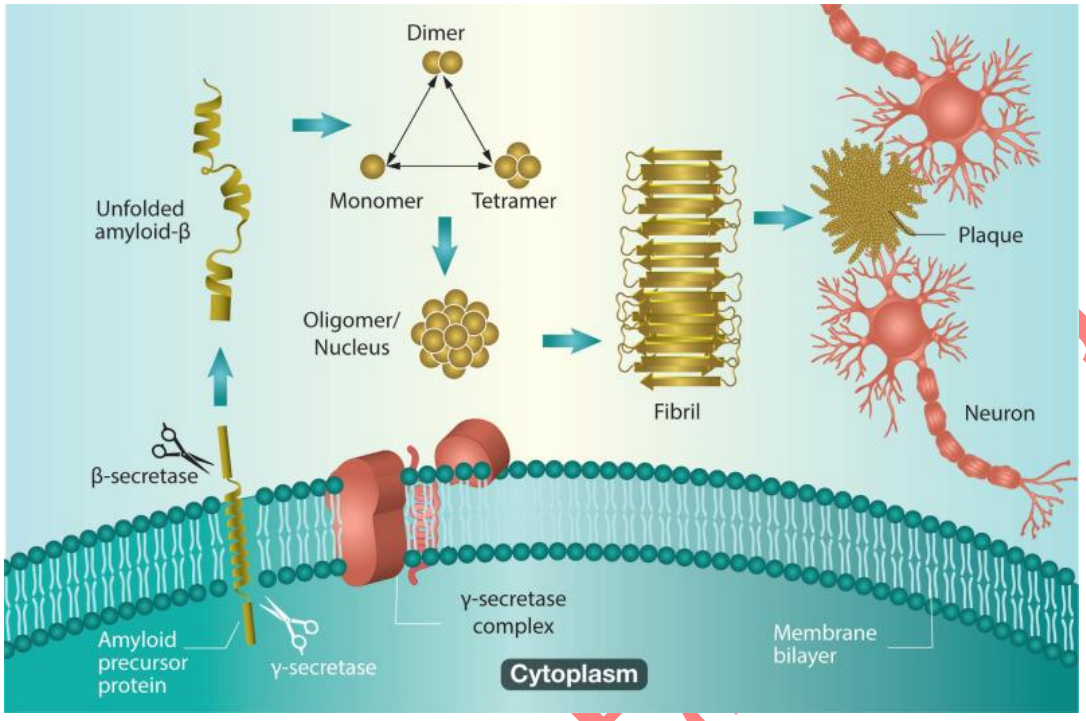

Figure 1: Formation of amyloid-beta

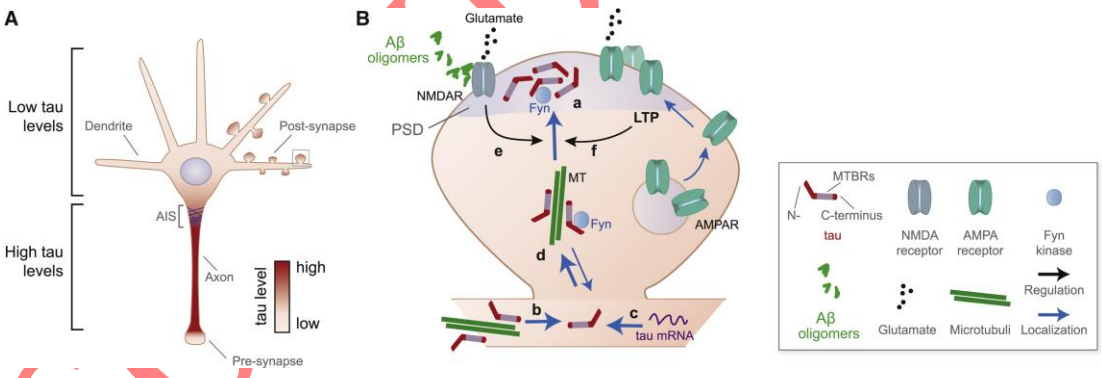

Figure 2: Interaction between tau and amyloid-beta oligomers via Fyn

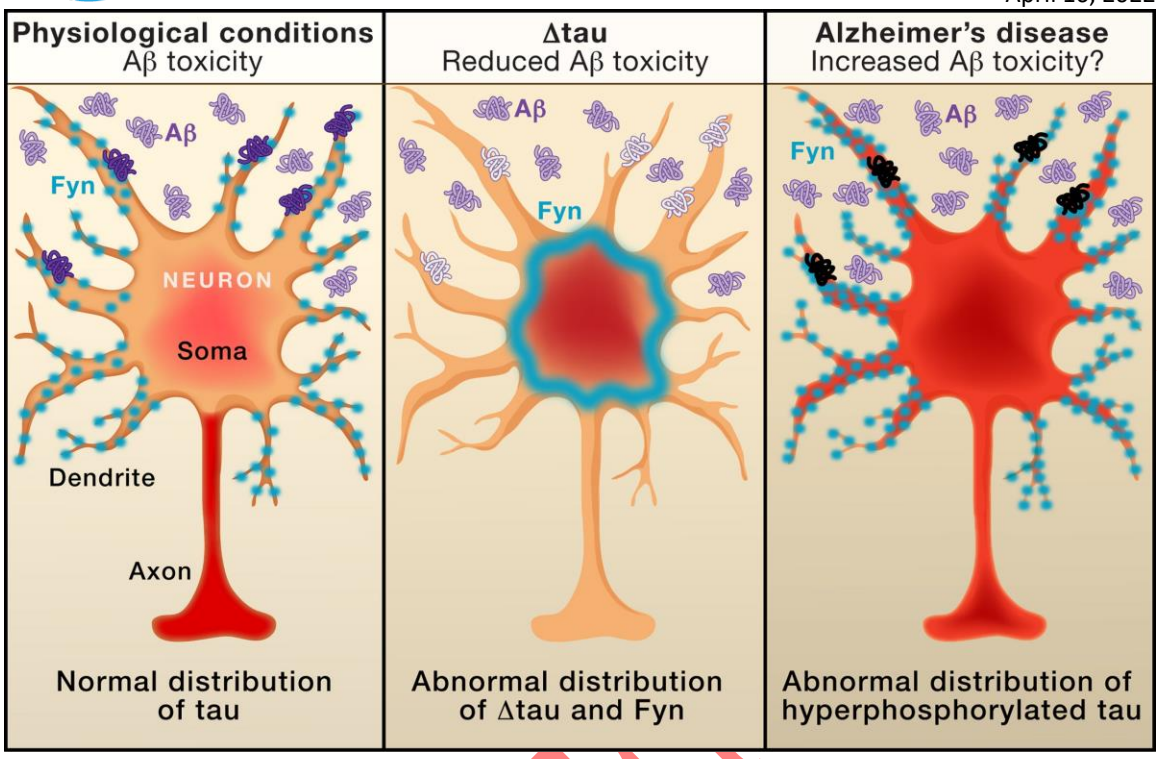

Figure 3: Distribution of tau, amyloid-beta and fyn in normal and pathological conditions

Table. Tau-Dependent Effects of Aβ

Study	System	Summary of Main Results
Götz et al,[5] 2001	Mouse	Tangle formation accelerated by injection of Aβ fibrils into the brain
Lewis et al,[6] 2001 and Hurtado et al,[7] 2010	Mouse	Mutant APP expression accelerates tangle formation by mutant tau
Roberson et al,[8] 2007	Mouse	Tau required for learning and memory deficits when plaques are present
Leroy et al,[9] 2012	Mouse	A feedback loop connects Aβ and tau pathologies
Ittner et al,[10] 2010	Mouse	Aβ causes tau-dependent excitotoxicity at NMDA receptors
Rapoport et al,[11] 2002	1° Neurons	Aβ fibrils are cytotoxic
King et al,[12] 2006	1° Neurons	AβOs cause tau-dependent MT loss
Nussbaum et al,[13] 2012	1° Neurons	Pyroglutamylated AβOs cause tau-dependent cytotoxicity
Seward et al,[14] 2013	1° Neurons	AβOs cause tau-dependent, ectopic cell cycle reentry
Shipton et al,[15] 2011	Brain slice	AβOs cause tau-dependent impairment of long-term potentiation
Vossel et al,[16] 2010	1° Neurons	AβOs cause tau-dependent inhibition of mitochondrial transport on MTs
Zempel et al,[17] 2013	1° Neurons	AβOs cause tau-dependent MT severing and synaptic damage in dendrites

Abbreviations: Aβ, amyloid-β; AβO, amyloid-β oligomer; APP, amyloid precursor protein; MT, microtubule; NMDA, *N*-methyl-D-aspartate.

Figure 4: Studies that show the tau-dependent effects of amyloid-beta. Extracted from (Bloom, 2014).

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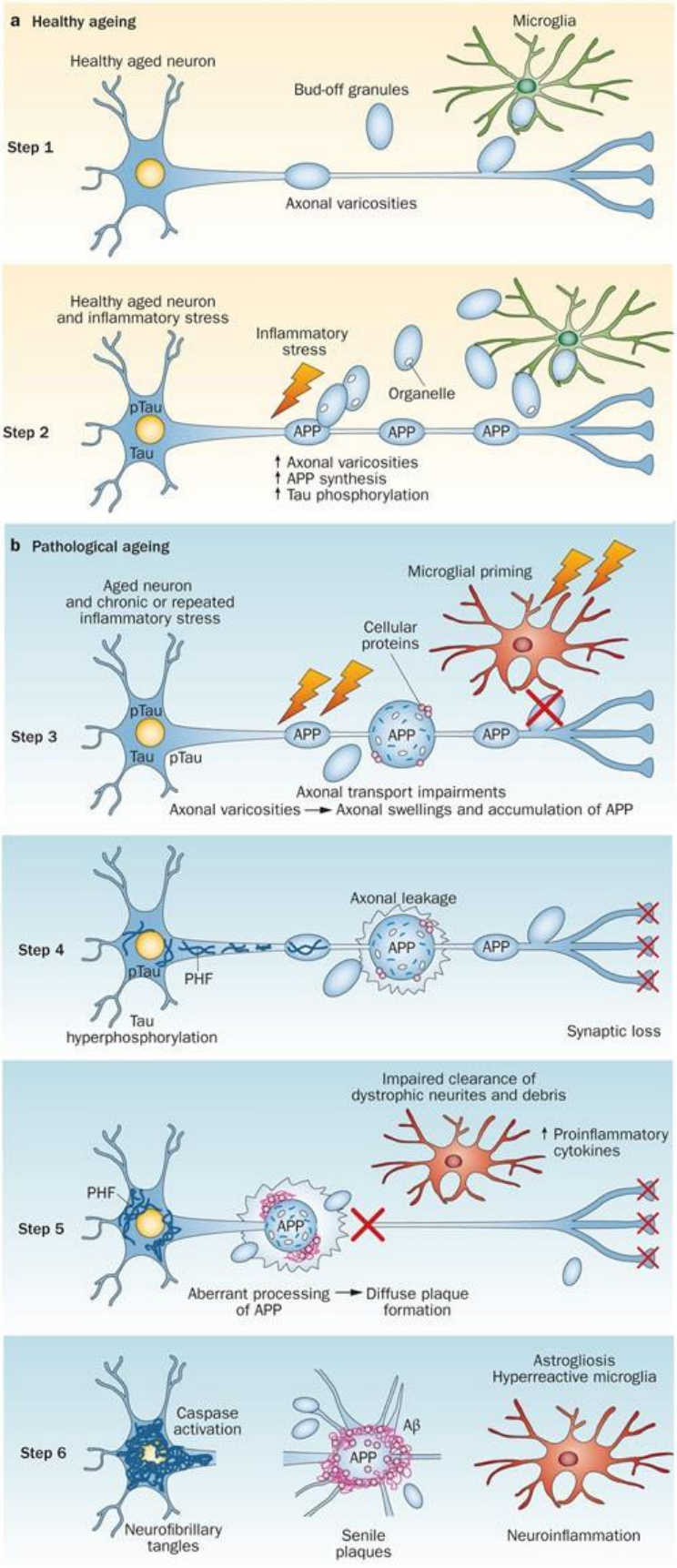
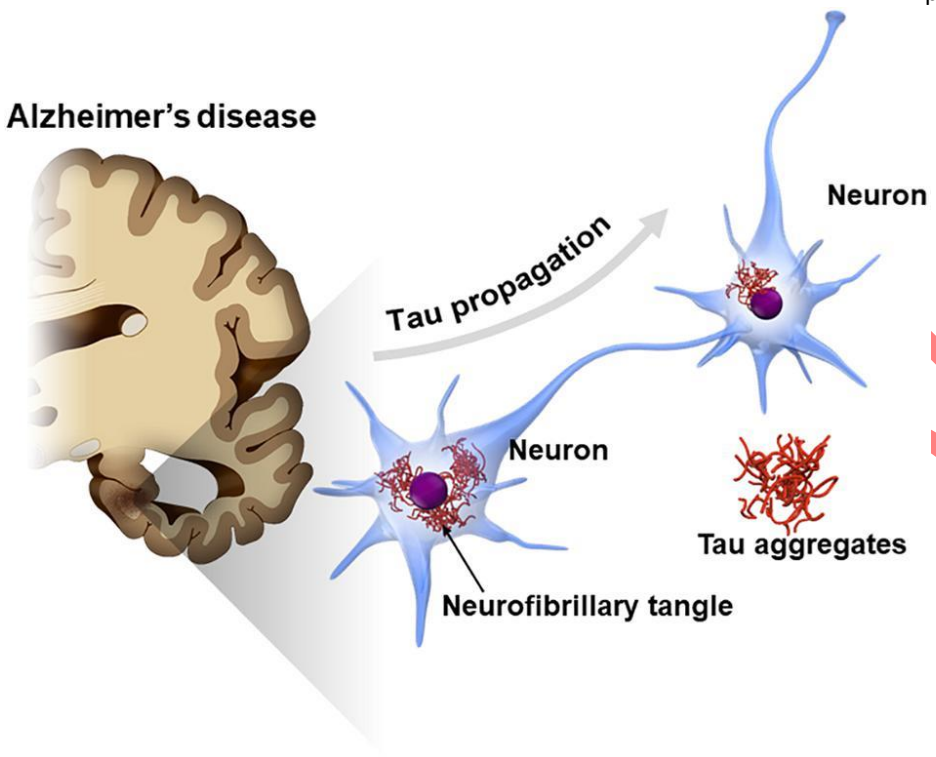
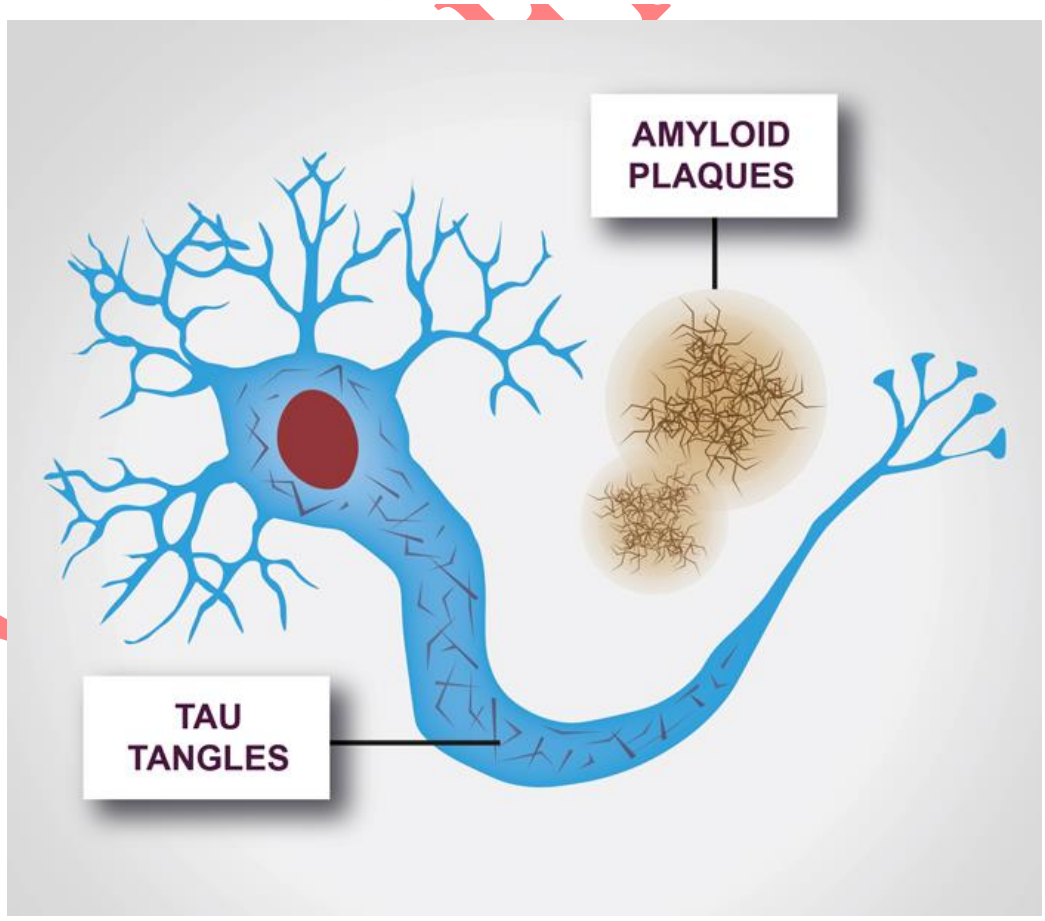
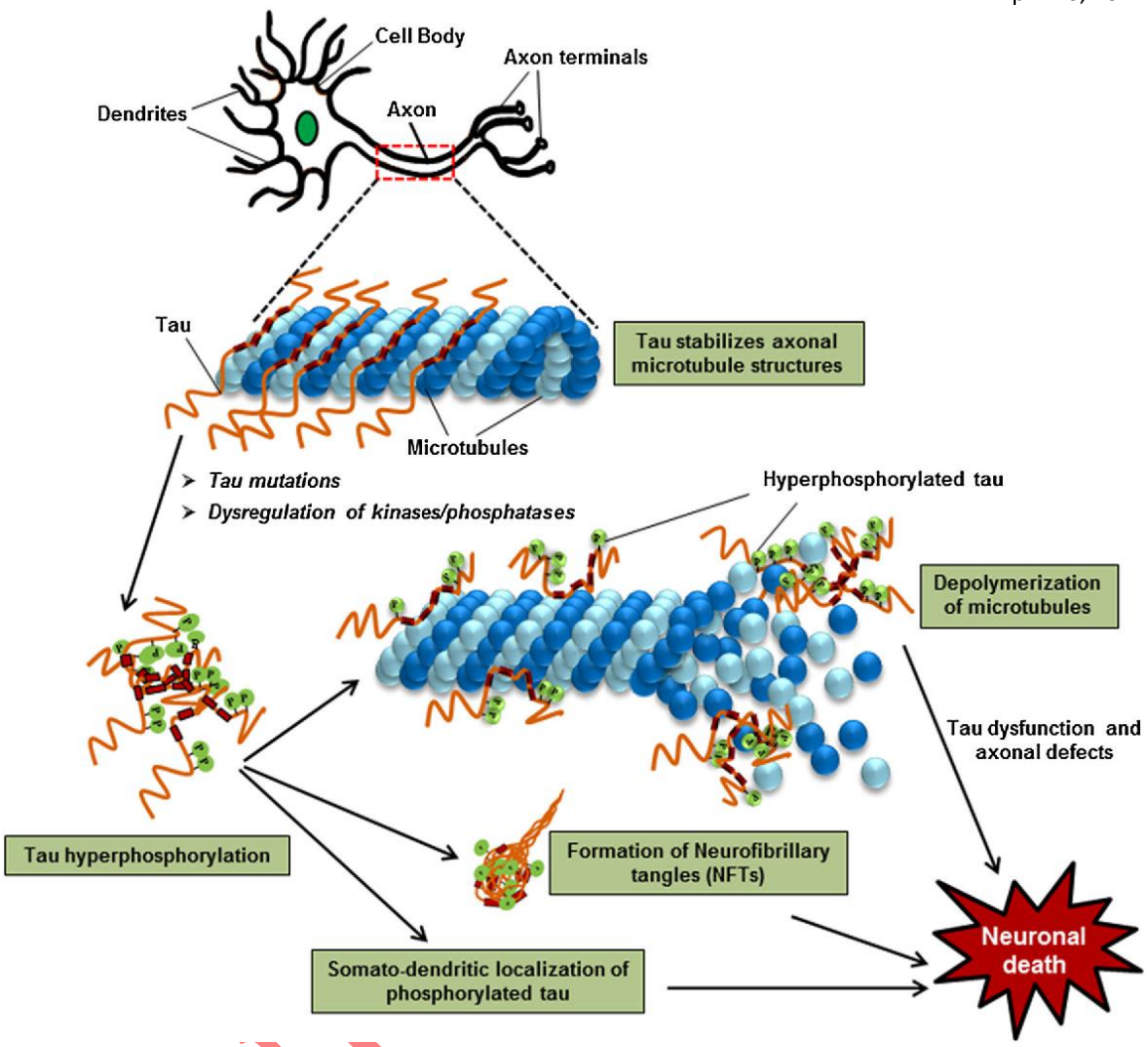



Alzheimer's disease
Tau propagation
Neuron
Neuron
Tau aggregates
Neurofibrillary tangle


AMYLOID PLAQUES
TAU TANGLES



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