Evidence for Abnormal Protein Processing in AD

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Examples of Abnormal Protein Processing

- APP generating β-amyloid
- Cytoskeletal proteins: hyperphosphorylation and proteolysis

Proteins implicated in AD are targets of caspases

Amyloid precursor protein (APP)

Gervais et al. (1999) *Cell* **97**: 395-406

Lu et al. (2003) *J Neurochem* **87**: 733-41

Presenilin-1 (PS1)

van de Craen et al. (1999) *FEBS Lett* **445**: 149-54

Fluhrer et al. (2004) *J Biol Chem* **279**: 1585-93

Tau

Canu et al. (1998) *J Neurosci* **18**:7061-74

Fasulo et al. (2000) *J Neurochem* **75**: 624-33

Gamblin et al. (2003) *PNAS* **100**: 10032-7

Caspase activation in the AD brain: usually chronic

Stadelmann (1999) *Am J Pathol* 155:1459-1466

Su et al. (2001) *Brain Res* 898:350-7

Rohn et al. (2001) *Neurobiol Dis* 8:1006-16

Rohn et al. (2002) *Neurobiol Dis* 11:341-54

Su et al. (2002) *Acta Neuropathol* 104:1-6, 2004

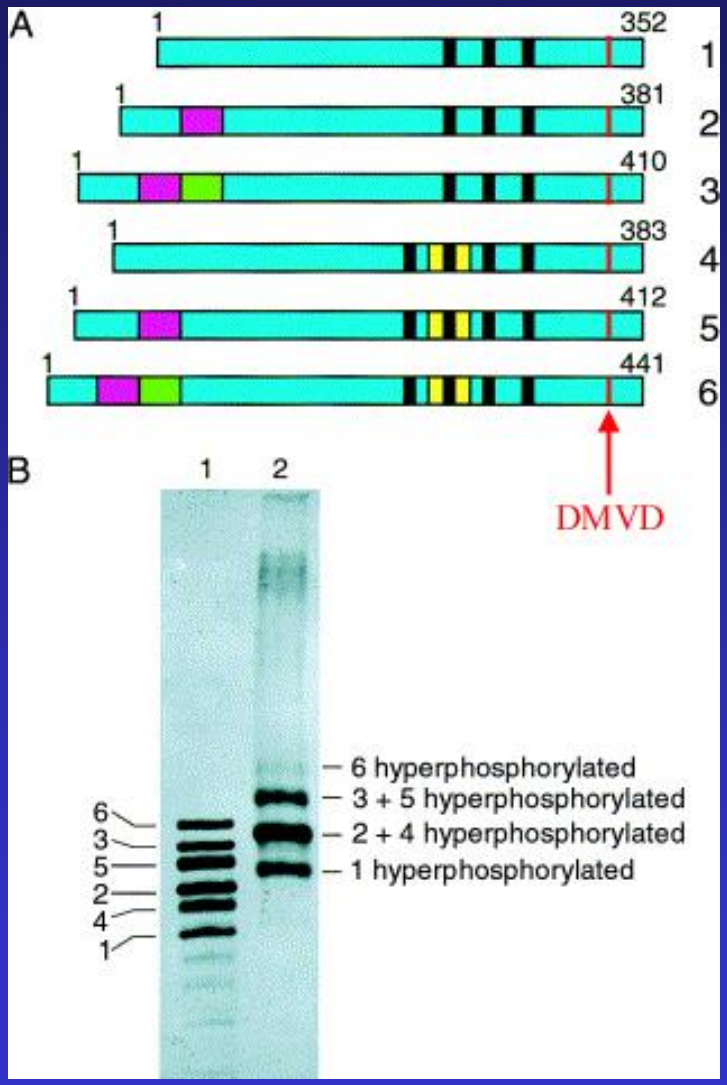
Gastard et al (2003) *Ann Neurol* 54:393-8

Pompl et al. (2003) *Arch Neurol* 60: 369-76.

Biology of Tau

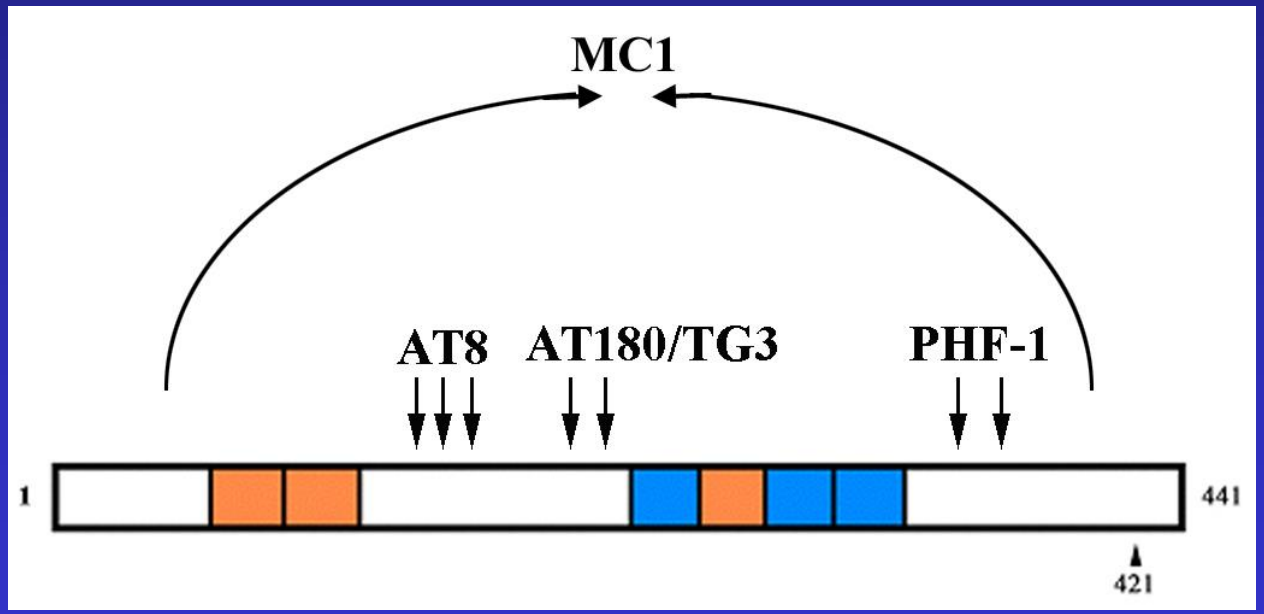
- Tau is a microtubule associated protein that drives microtubule assembly therby stabiizing the cytoskeleton,
- tau also participates in vesicular transport and axonal polarity.
- 6 isoforms of Tau exist in the adult human brain all of which are produced by alternative splicing from one gene located on chromosome 17.
- These isoforms of tau differ by the inclusion or exclusion of 1 or 2 n-terminal inserts and/or a fourth microtubule binding domain (3R vs 4R).
- All 6 isoforms of tau contain a caspase 3 and 7 consensus sequence (DMVD).

Tau is a microtubule associated protein

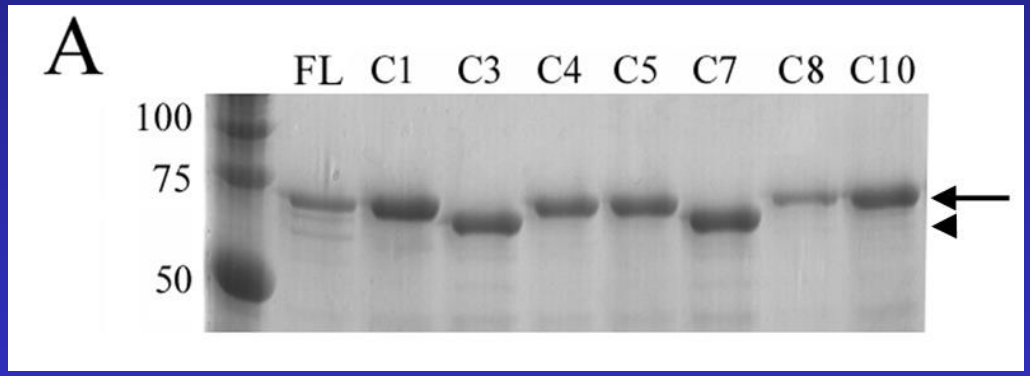


Spillantini et al., (1998) *Trends in Neuroscience* **21**: 428-33

Alterations of tau conformation and phoshphorylation in AD
Sequence:1.MC1, 2. AT8, 3.PHF-1
Caspase clavage?

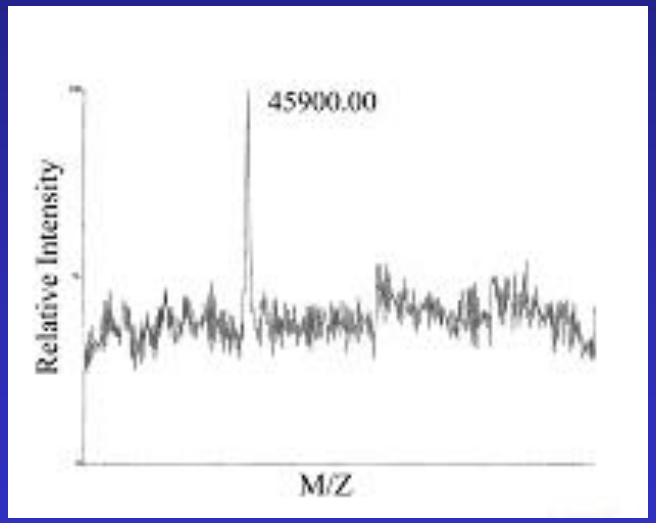


Executioner caspases cleave tau *in vitro*

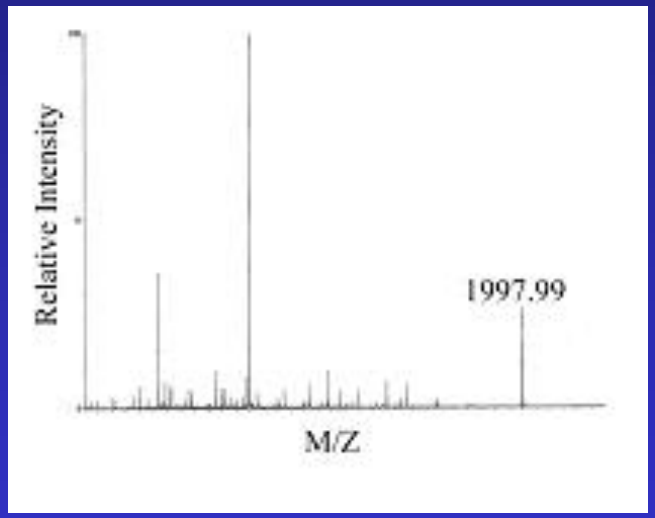


Tau is cleaved at Asp^{421}

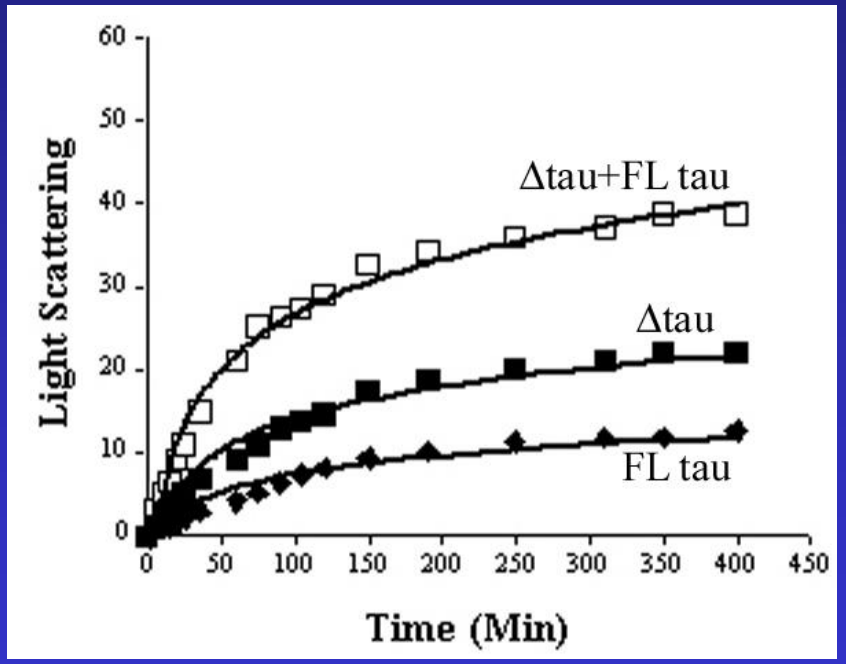
Δtau



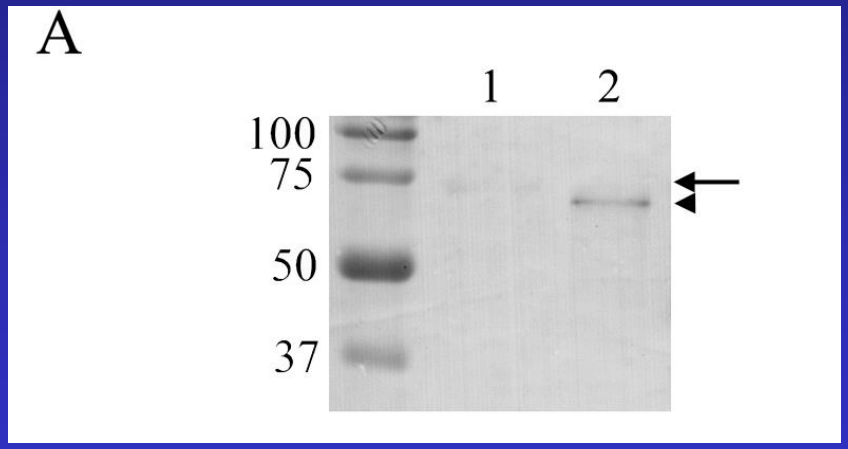
C-terminus



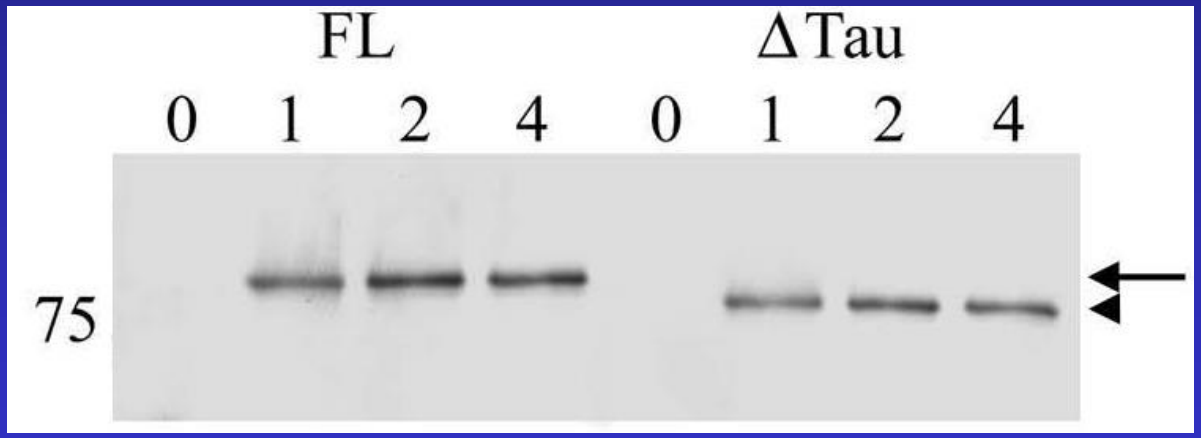
Δtau is involved in nucleation-dependent filament formation



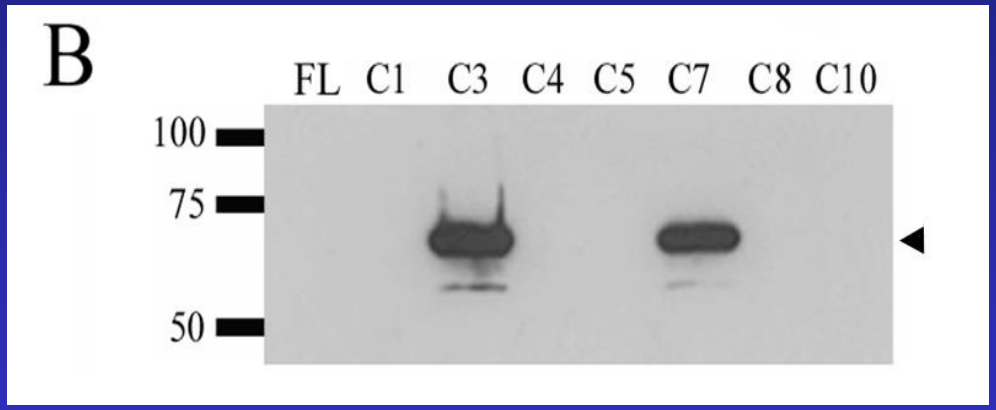
Caspase-cleavage of tau induces a conformational change recognized by the early-tangle marker MC1



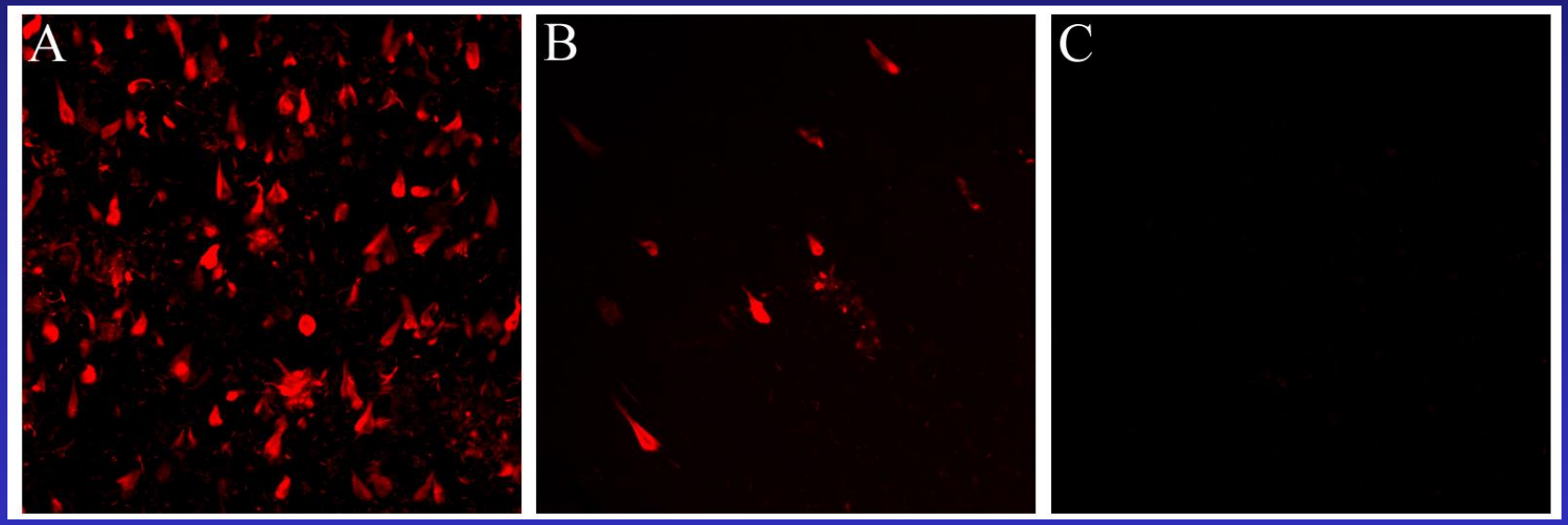
Δtau is hyperphosphorylated *in vitro* by GSK-3β: PHF-1 positive



Antibody generated is specific for tau cleaved after Asp^{421} generated by executioner caspases



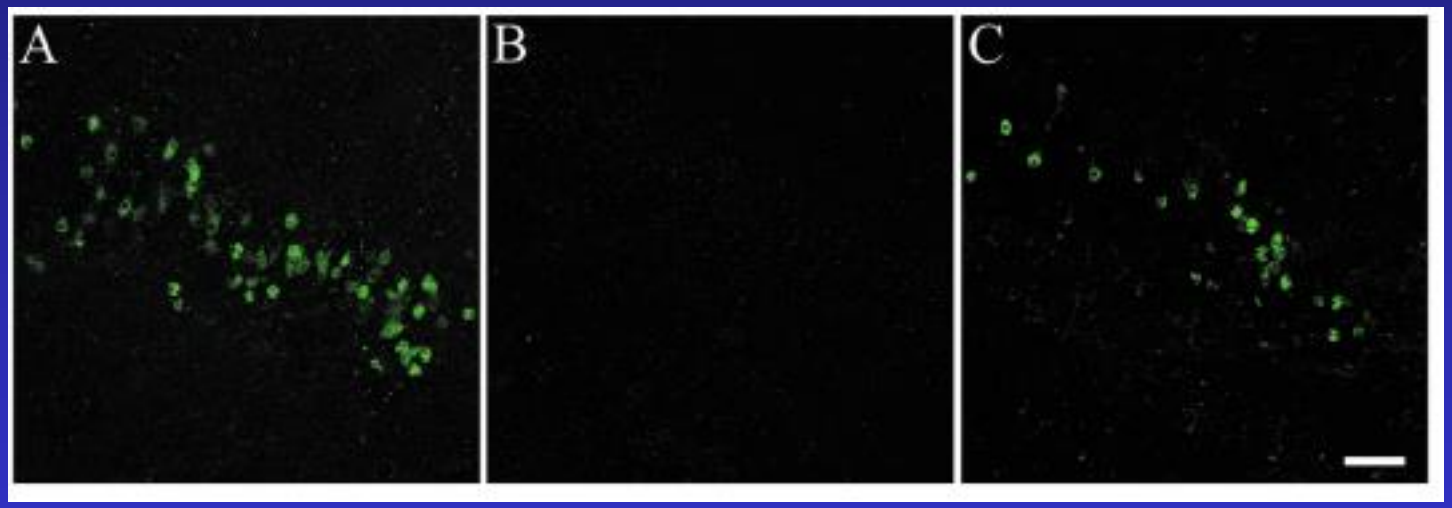
Δtau is detected in the AD brain



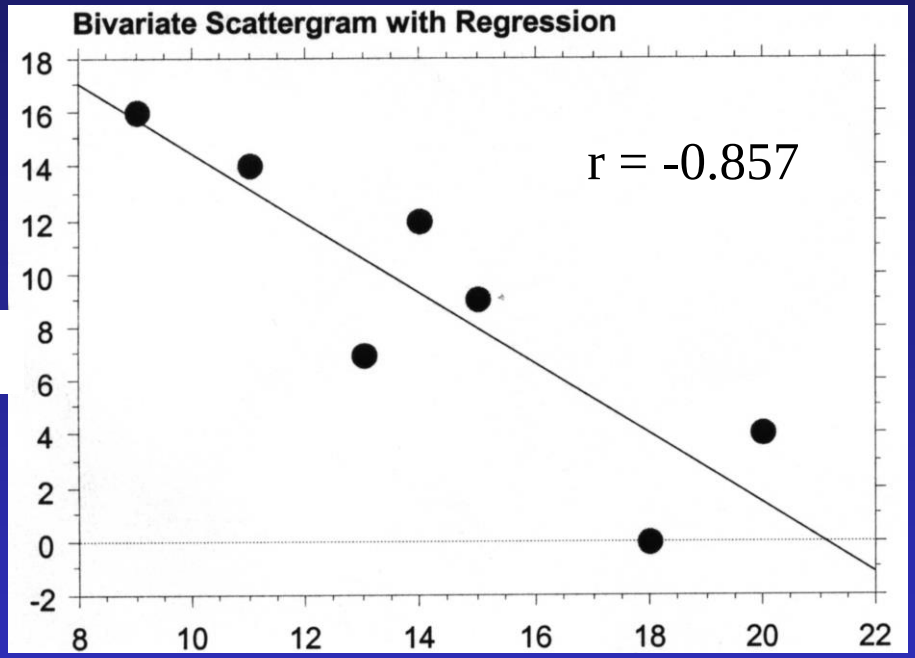
AD Control Preadsorption

The α-ΔTau antibody specifically recognizes caspase-cleaved tau *in vivo:* does not stain tau-/- mouse brain after head injury

Caspase-3 ΔTau Fodrin-CCP

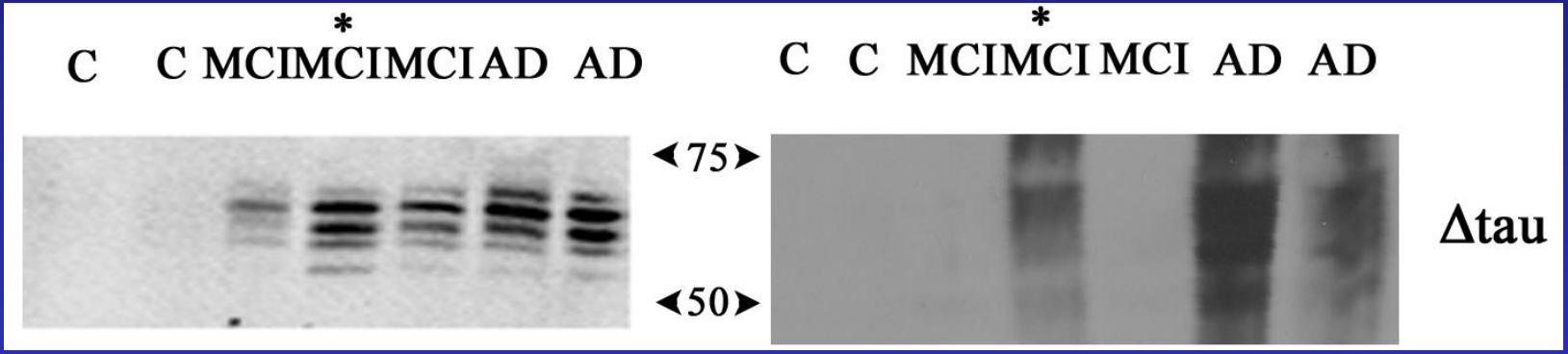


Caspase-Cleavage of Tau is Correlated with Cognitive Decline



The increased presence of the number of ΔTau positive cells was inversely correlated with cognitive decline, as determined by MMSE score

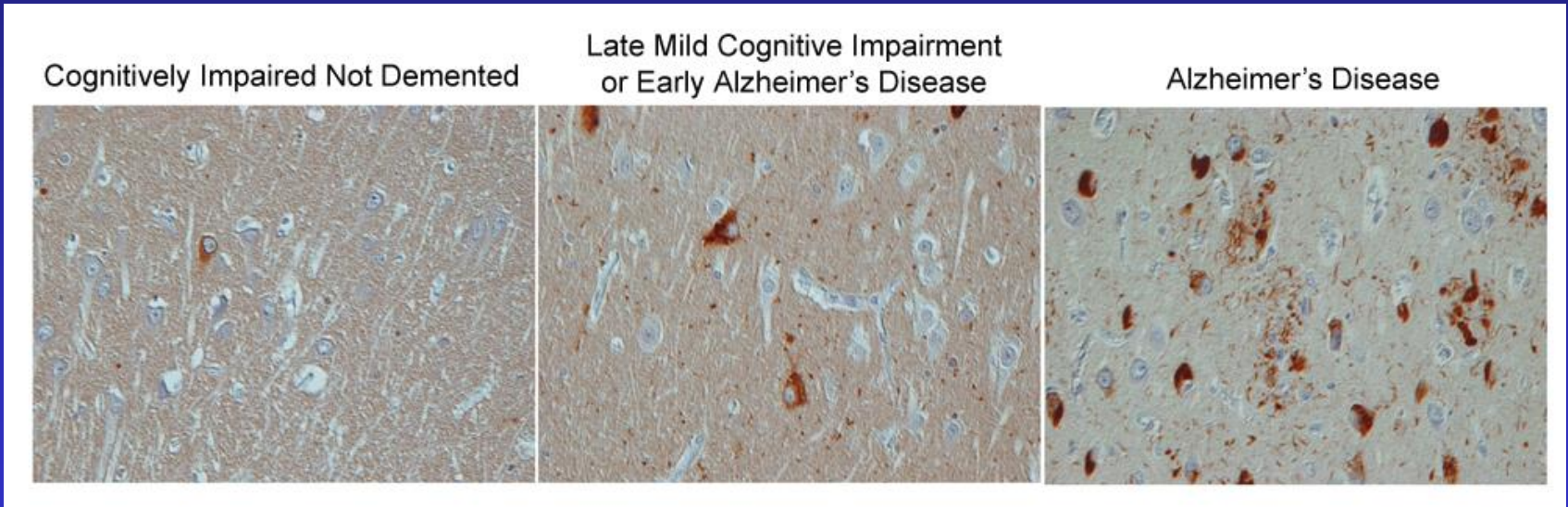
Δtau becomes increasingly insoluble with AD progression



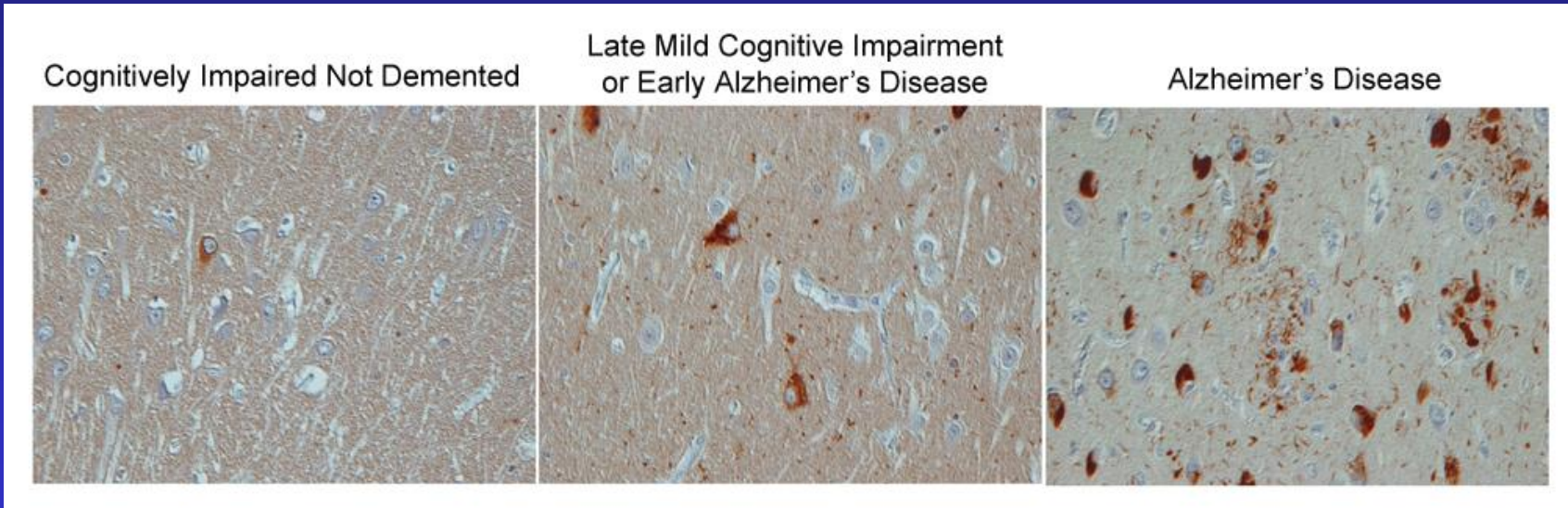
RAB

RIPA

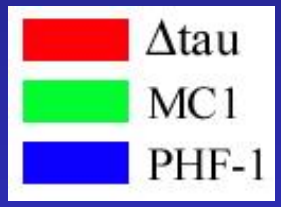
Tau Pathology in Nondemented and Early AD Cases in area CA1 of the hippocampus

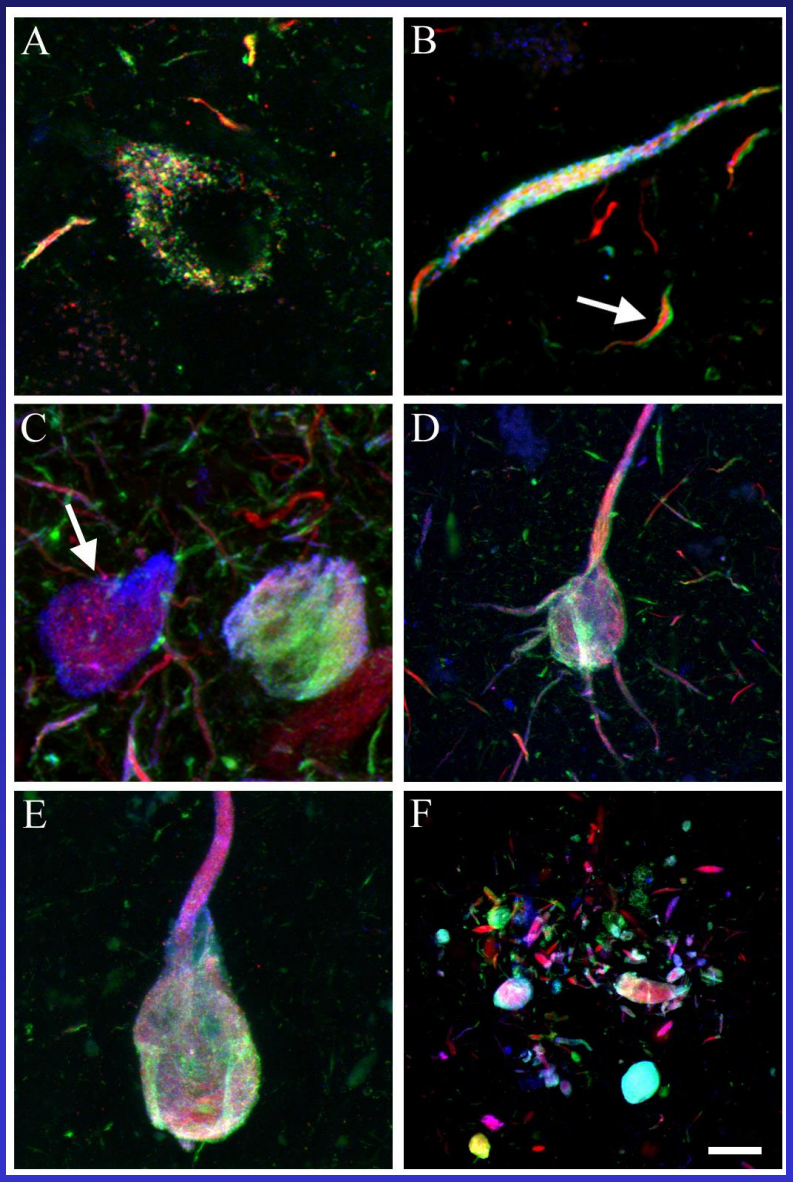


Tau Pathology in Nondemented and Early AD Cases in area CA1 of the hippocampus

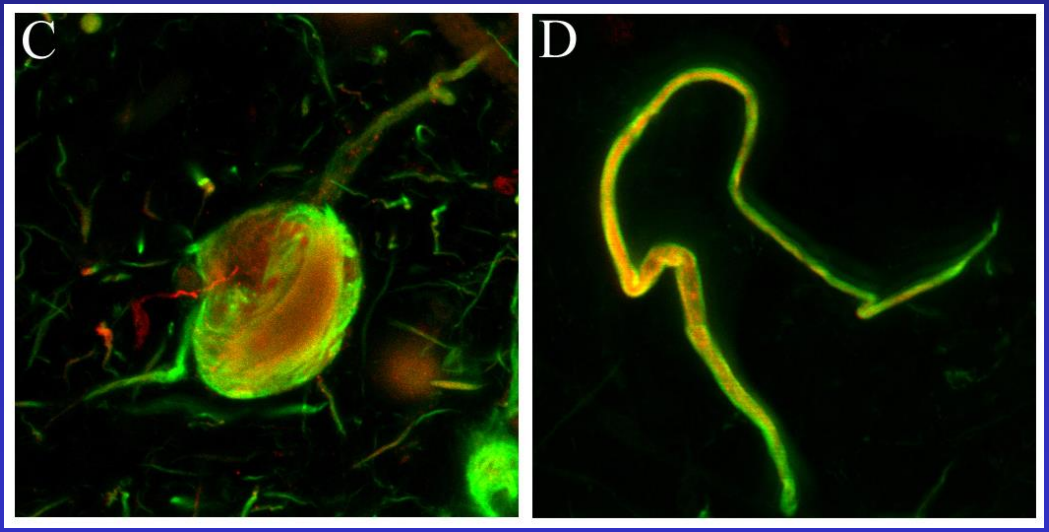


Δtau is present throughout the evolution of NFTs

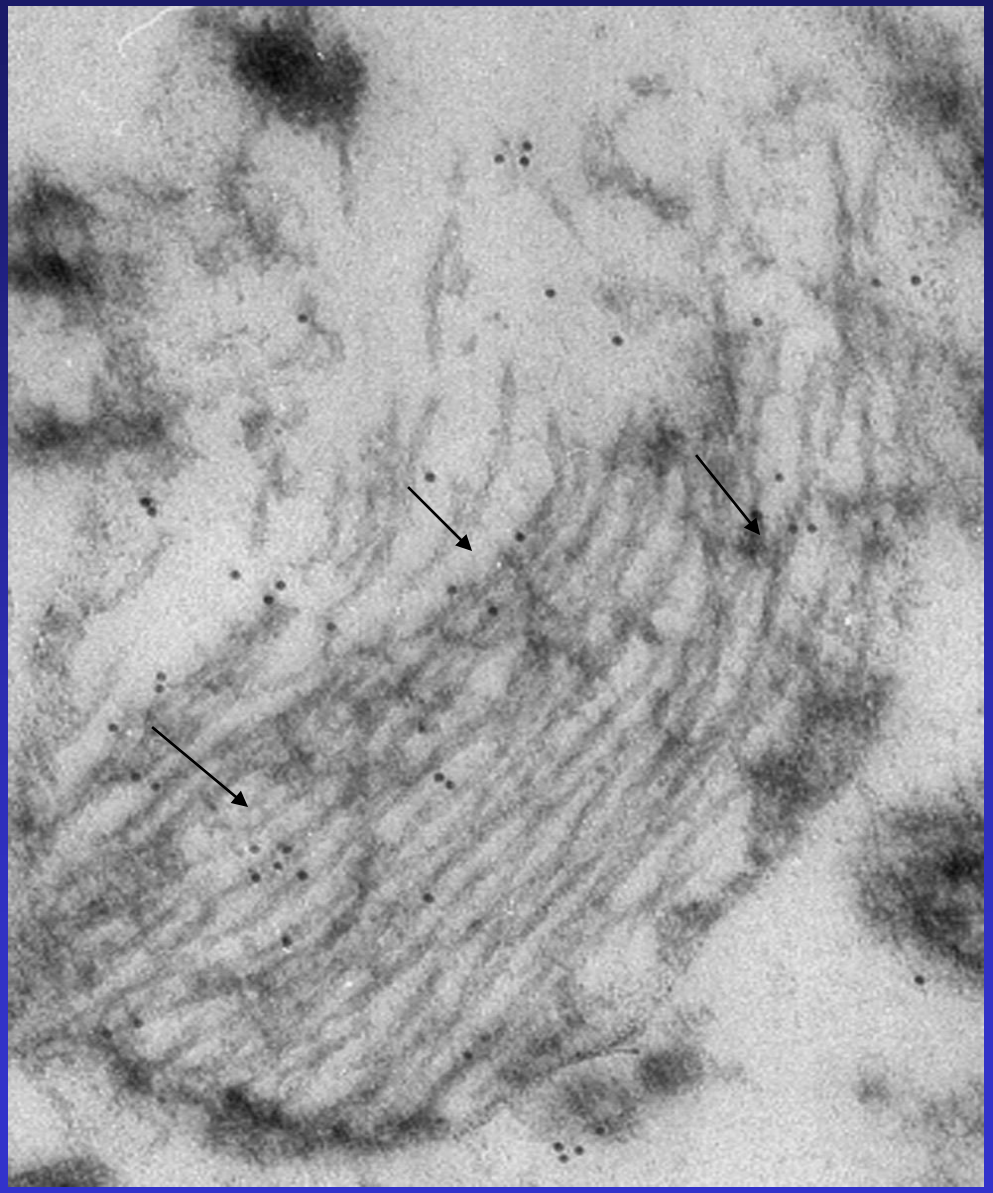




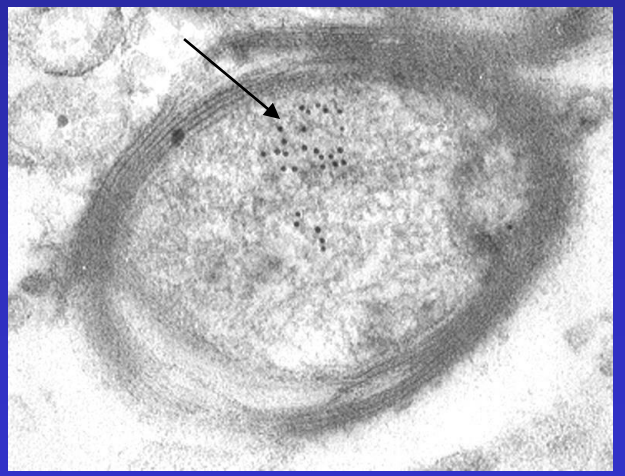
Δtau and full-length tau are both present within NFTs and dystrophic neurites



TAU-CCP immunoreactivity recognizes tangles



AXON



What leads to Δtau in AD? Does Aβ drive tau pathology?

Aβ activates caspases *in vitro:*

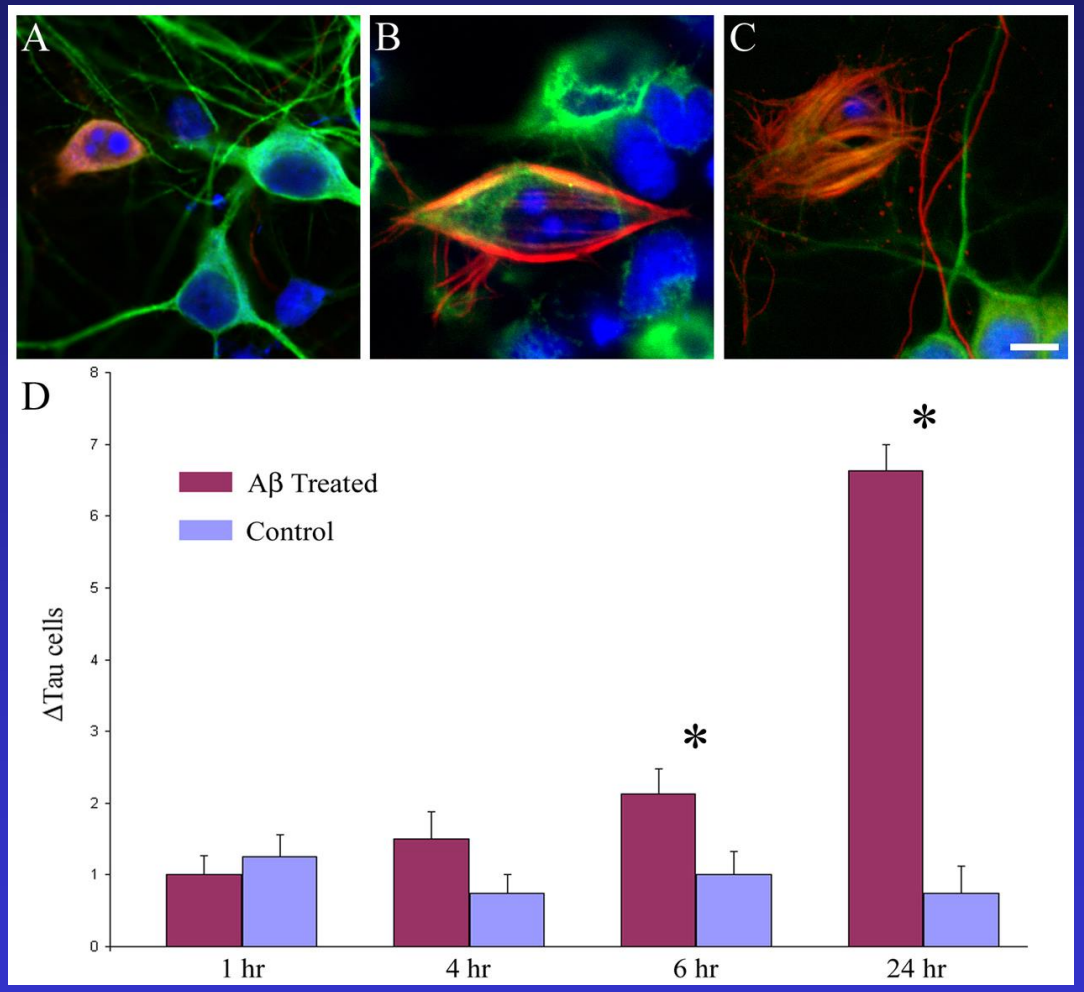
Loo et al. (1993) PNAS 90:7951-5

Ivins et al. (1998) Neurobiol Dis 5:365-78

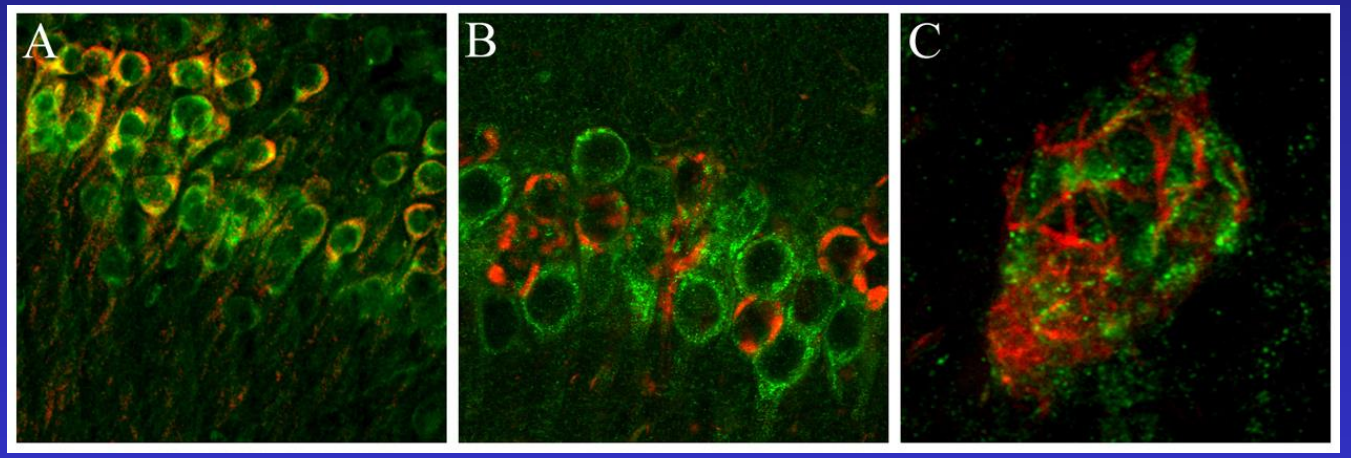
Oxidative stress activates caspases *in vitro:*

Camondola et al. (2000) *J Neurochem* 74:159-68

$A\beta_{1-42}$ treatment leads to Δtau in primary cortical neurons

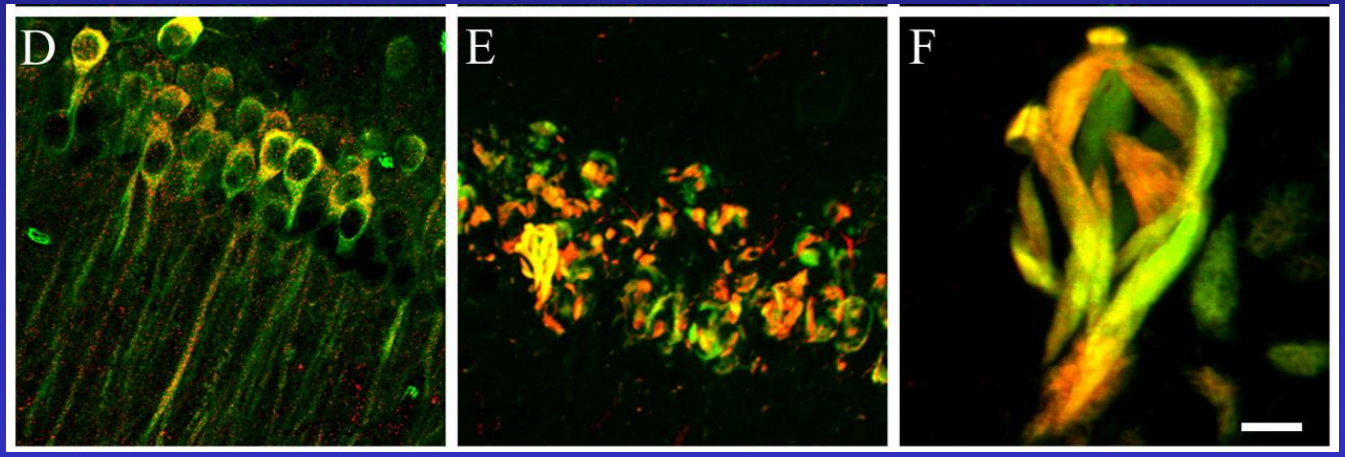


Is cleaved tau present in 3xTg-AD mouse and does Δtau co-localize with $A\beta_{1\text{-}42}$?

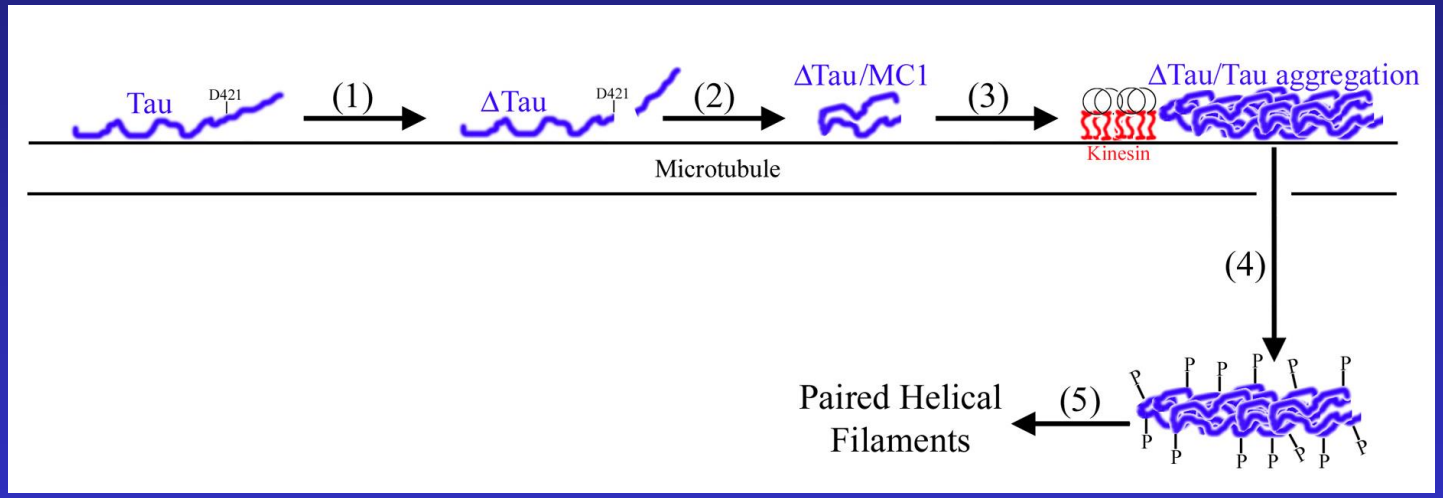


Does Δtau co-localizes with MC1 in 3xTg-AD mouse?

Δtau
MC1



Proposed role of Δtau in NFT pathology



Summary

- Several proteins get cleaved by caspases and appear to be present in neurons for prolonged periods of time, e.g., fodrin, actin and tau as well as APP
- Tau is cleaved by executioner caspases initiated by β-amyloid, oxidative damage
- Cleaved tau seeds (nucleates) the assembly of tau into PHF-1 like assemblies and assumes an MC-1 conformation.
- Cleaved tau is present in pre-tangle and tangle neurons
- Cleaved tau neurons inversely correlate with cognitive function
- Chronic abnormal protein processing may be a new mechanism catalyzing AD pathology

Acknowledgements

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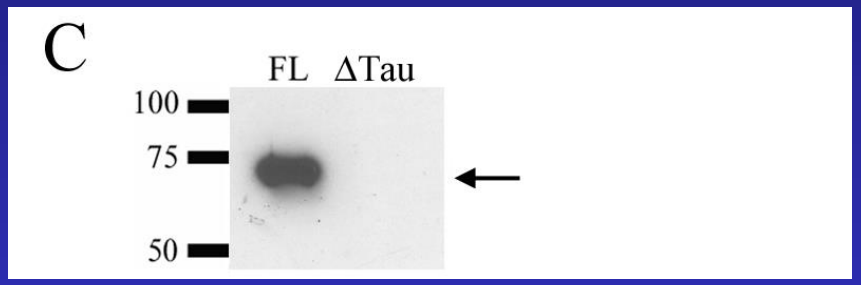
Dr. Troy Rohn

Mass spectroscopy
Nemone Muster

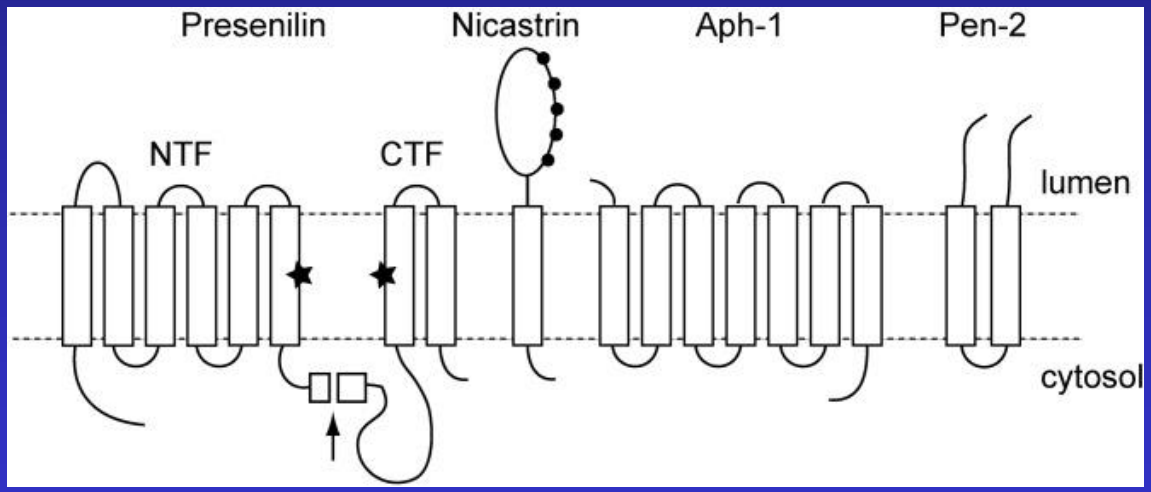
Laser Light Scattering
Dr. Wytze van der Veer

The C-terminal-specific antibody T46 does not recognize Δtau



γ-Secretase substrates integral to AD Pathogenesis

Components of the γ-Secretase Complex



Substrates

APP & APPLPs

E-Cadherin

Notch 1-4

ERB-4

Nectin-1α

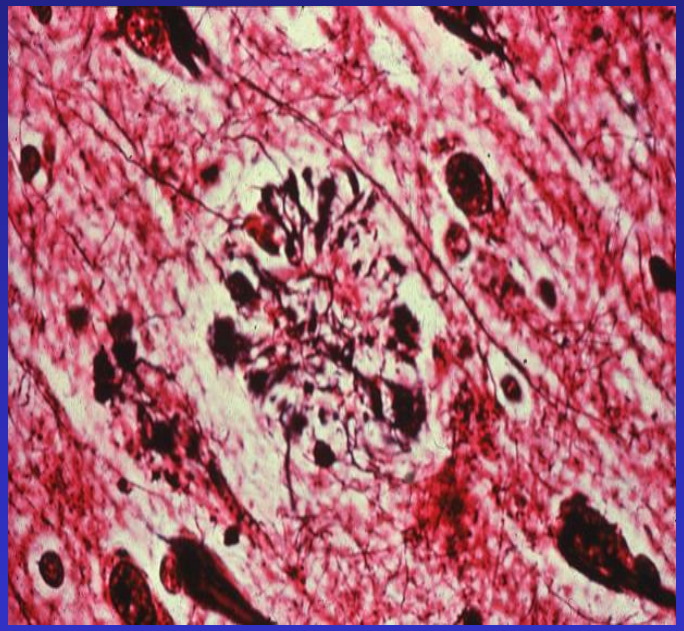
CD44

LRP

P75

γ-Secretase Complex adapted from Kimberly & Wolfe, 2003

AD pathologic hallmarks: Senile plaques and neurofibrillary tangles



Δtau is inversely correlated with cognitive function