Potential role of extracellular tau in development and spread of tauopathy: Effects of anti-tau antibodies

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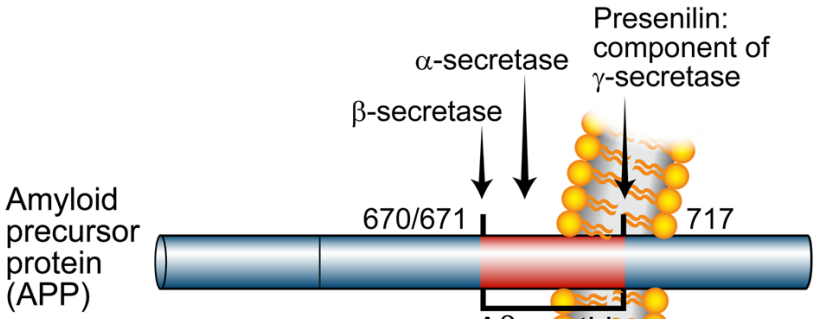
Role of Amyloid-β, apoE, and tau metabolism in pathogenesis of Alzheimer's disease and tauopathies



cellular factors
affecting Aβ processing,
levels, and production:
synaptic activity
LRP1
SORL1

Aβ38,40,42
(Aβ42 prone to aggregate)

Toxicity

Aβ aggregates

Inflammation

Aβ
interacting
molecules

ApoE
Clusterin

Aβ

molecules &
processes
that affect
Aβ aggregation
and clearance

blood brain barrier
LRP1
RAGE
Neprilysin
IDE
CSF, ISF flow

In certain forms of FTD,
PSP, CBD, and TBI,
tau aggregation appears to
be the key event in disease

soluble
Tau

levels, sequence,
and
phosphorylation
of Tau

insoluble
Tau → toxicity

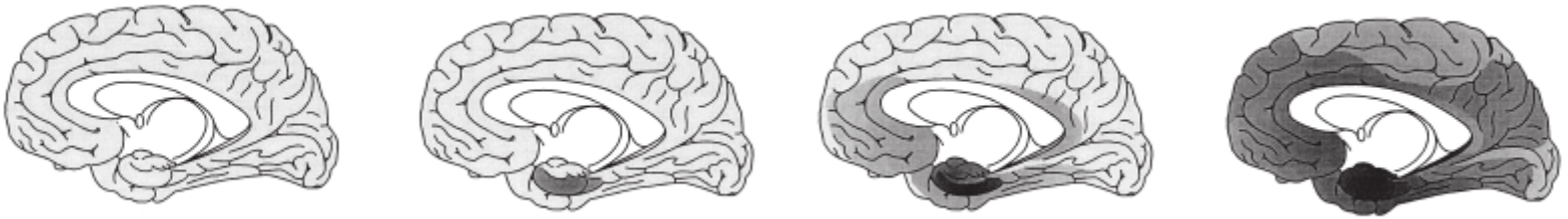
Neurodegenerative Diseases and tau: Potential contribution of protein aggregate spreading

- Proteopathies: Aggregation of misfolded proteins

- Tau aggregation in neurons and glia:

  - Sporadic: Alzheimer Disease (AD), Frontotemporal Dementia (FTD), Progressive Supranuclear palsy, (PSP), Cortico-basal degeneration (CBD)

  - Acquired: Chronic Traumatic Encephalopathy (CTE)



- Trans-cellular propagation of tau: a potential prion-like mechanism for disease progression

Braak and Braak Staging, 1991

Outline

1. **Studies of ISF tau metabolism demonstrate the normal presence of both extracellular monomeric tau and suggest the presence of extracellular tau aggregates in vivo.**

2. Certain anti-tau can bind to and sequester extracellular tau aggregates in vitro and block the cell to cell transfer of tau and subsequent tau aggregation.

3. Effects of certain anti-tau antibodies on tauopathy in vivo.

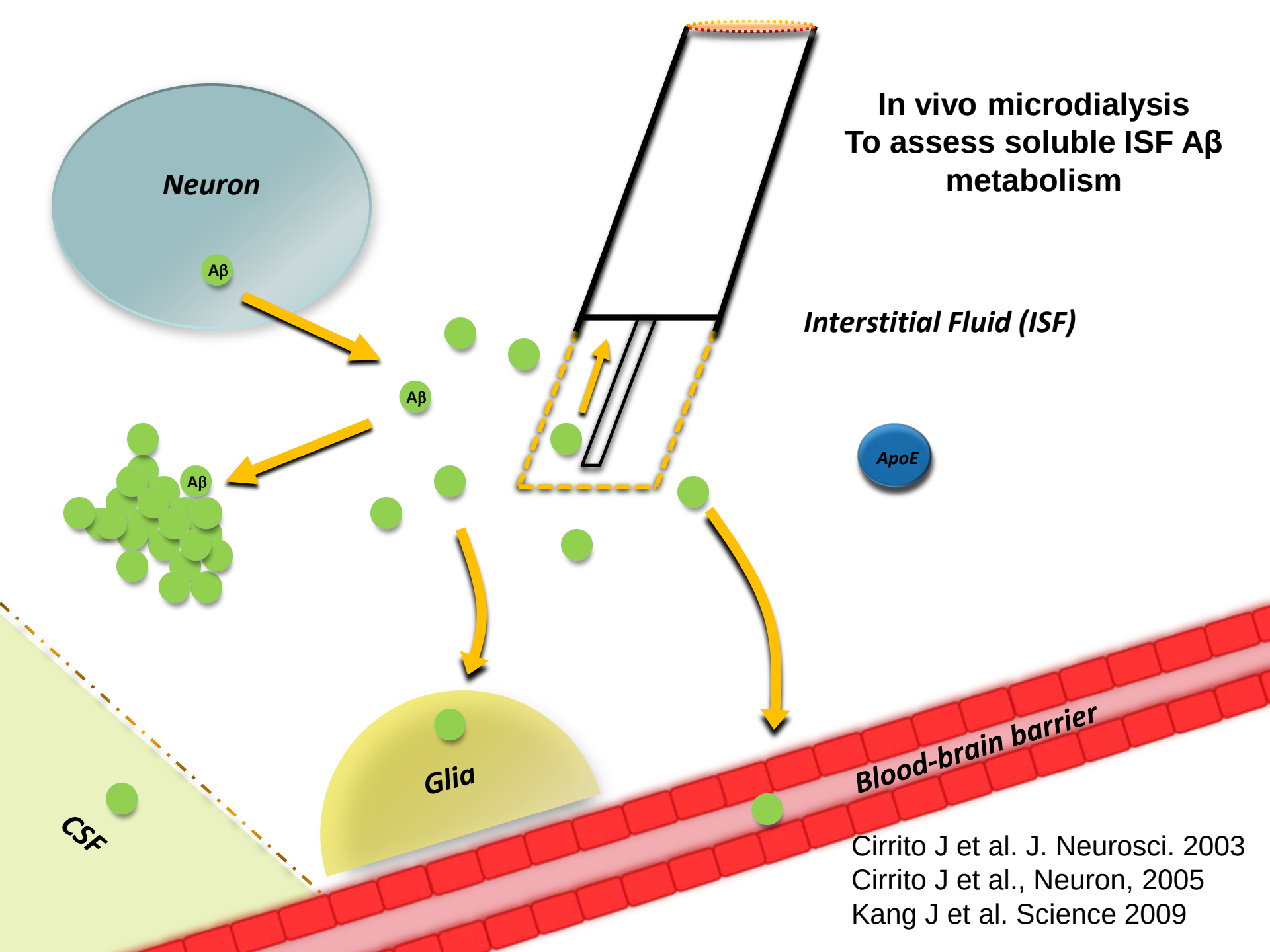
Tau metabolism

Tau plays a key role in both AD and other neurodegenerative diseases such as FTD, PSP, CBD, and TBI. We have a poor understanding of tau metabolism in vivo. Tau is an intra-cellular protein, yet it is found in CSF and is elevated in some tauopathies such as AD but not others such as FTD.

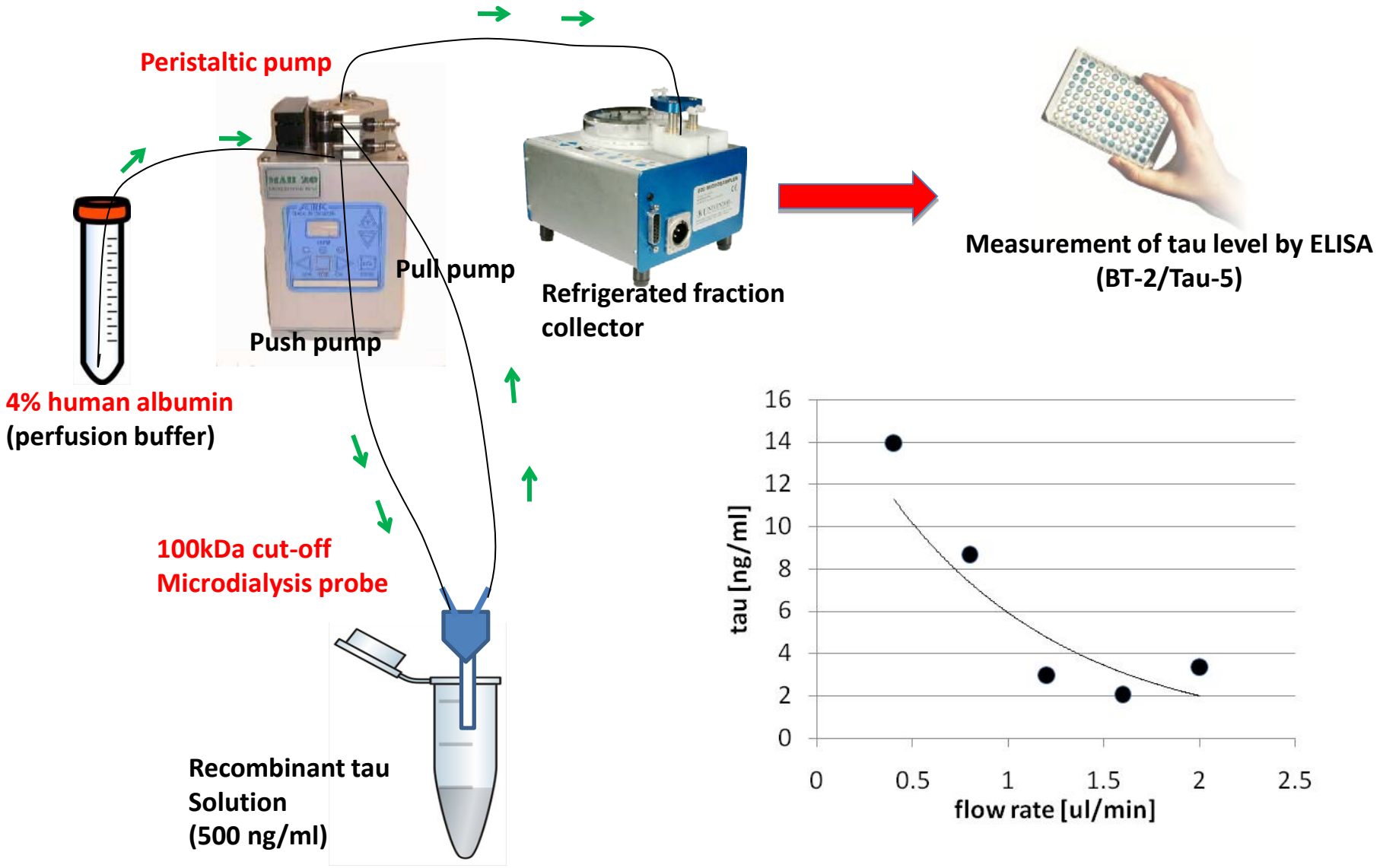
Extracellular tau appears to be involved in spreading of tau aggregation. In addition, tau is increased in the CSF in the setting of "neurodegeneration" (stroke, CJD, AD). Developing a way to measure the concentration of tau in the extracellular space of the brain should provide insight into tau physiology and pathophysiology in vivo and may provide an in vivo drug screening tool for future studies.

To better study tau metabolism in vivo:

We have developed a tau microdialysis technique to begin to examine the changes that occur in the normal brain and animal models of AD and tauopathy.


In vivo microdialysis
To assess soluble ISF Aβ metabolism
Neuron
Aβ
Aβ
Aβ
Interstitial Fluid (ISF)
ApoE
Glia
CSF
Blood-brain barrier
Cirrito J et al. J. Neurosci. 2003
Cirrito J et al., Neuron, 2005
Kang J et al. Science 2009

In vitro microdialysis of tau with 100kDa probes



P301S mutant tau transgenic mice

P301S mutation

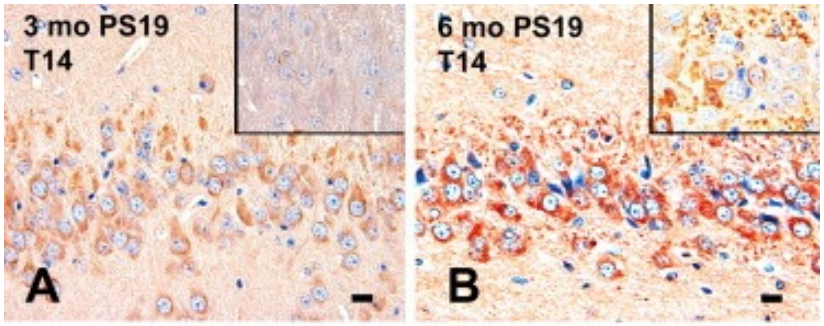
In humans causes early-onset FTDP-17.

P301S tau transgenic mice (express human P301S tau)

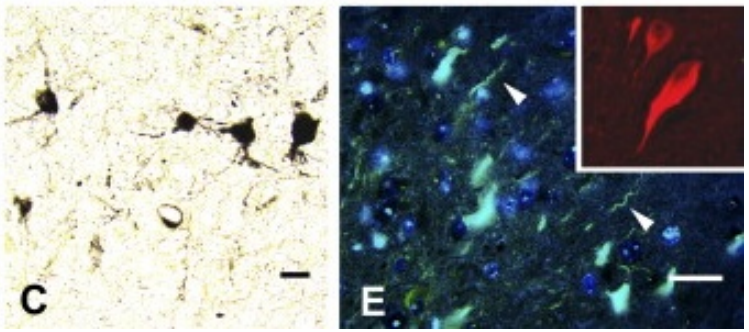
Strong tau positive neuronal staining at 6 months of age.

Show neuronal loss ~ 8months of age.

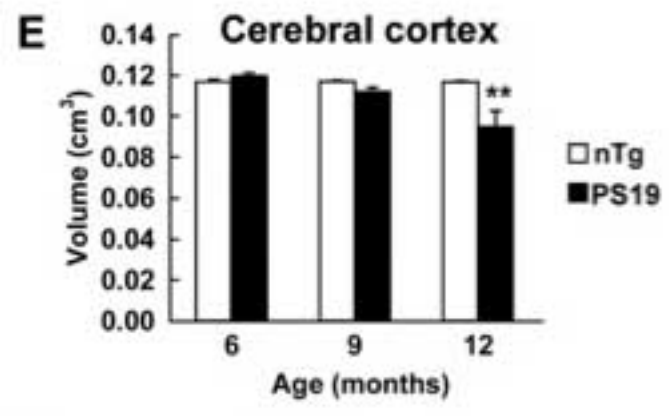
Yoshiyama *et al*., Neuron, 2007

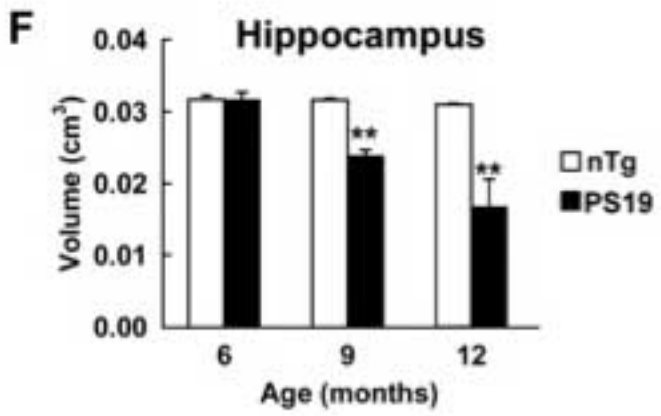


Hippocampus staining of tau

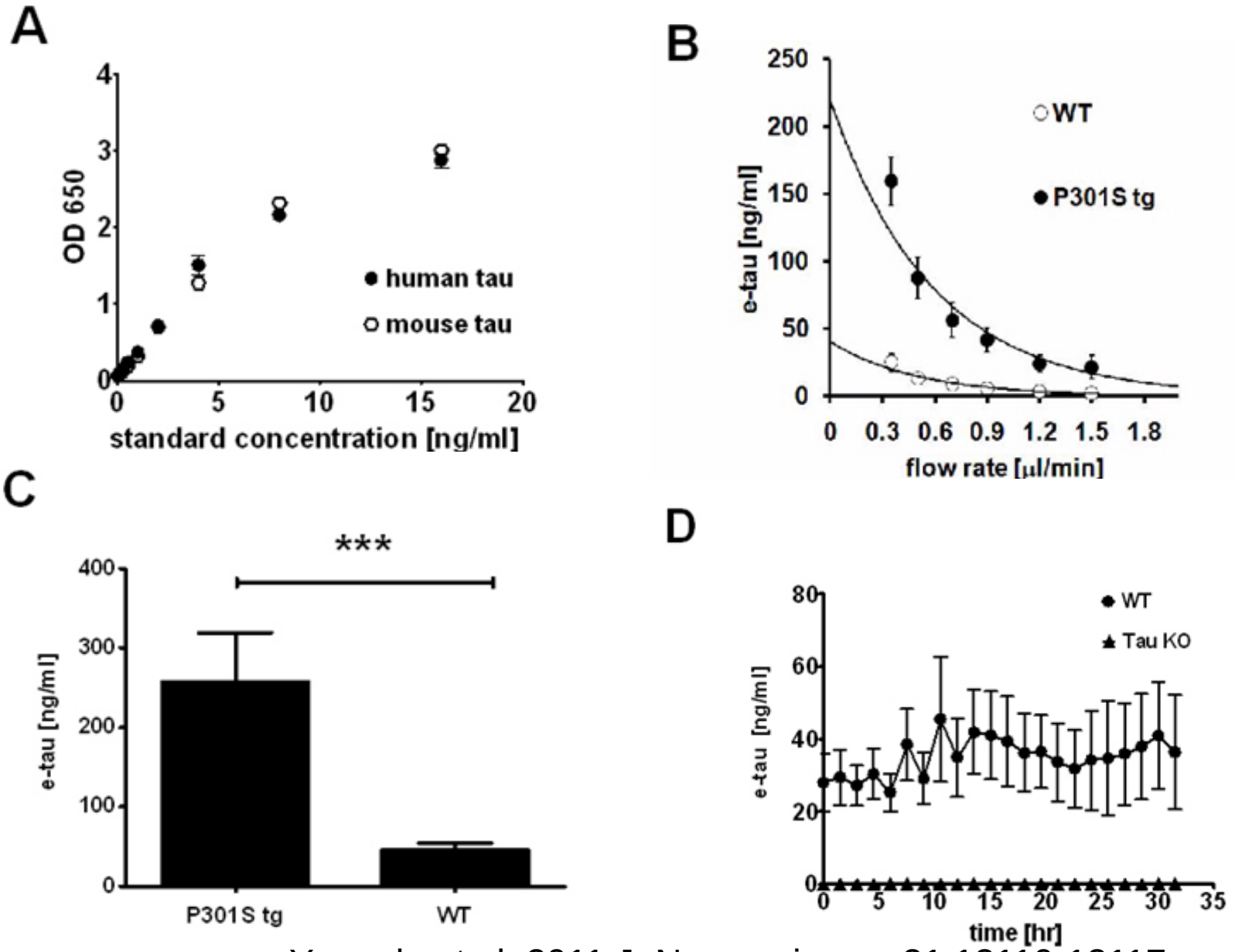


Gallyas silver staining (C) and thioflavin S staining (E)



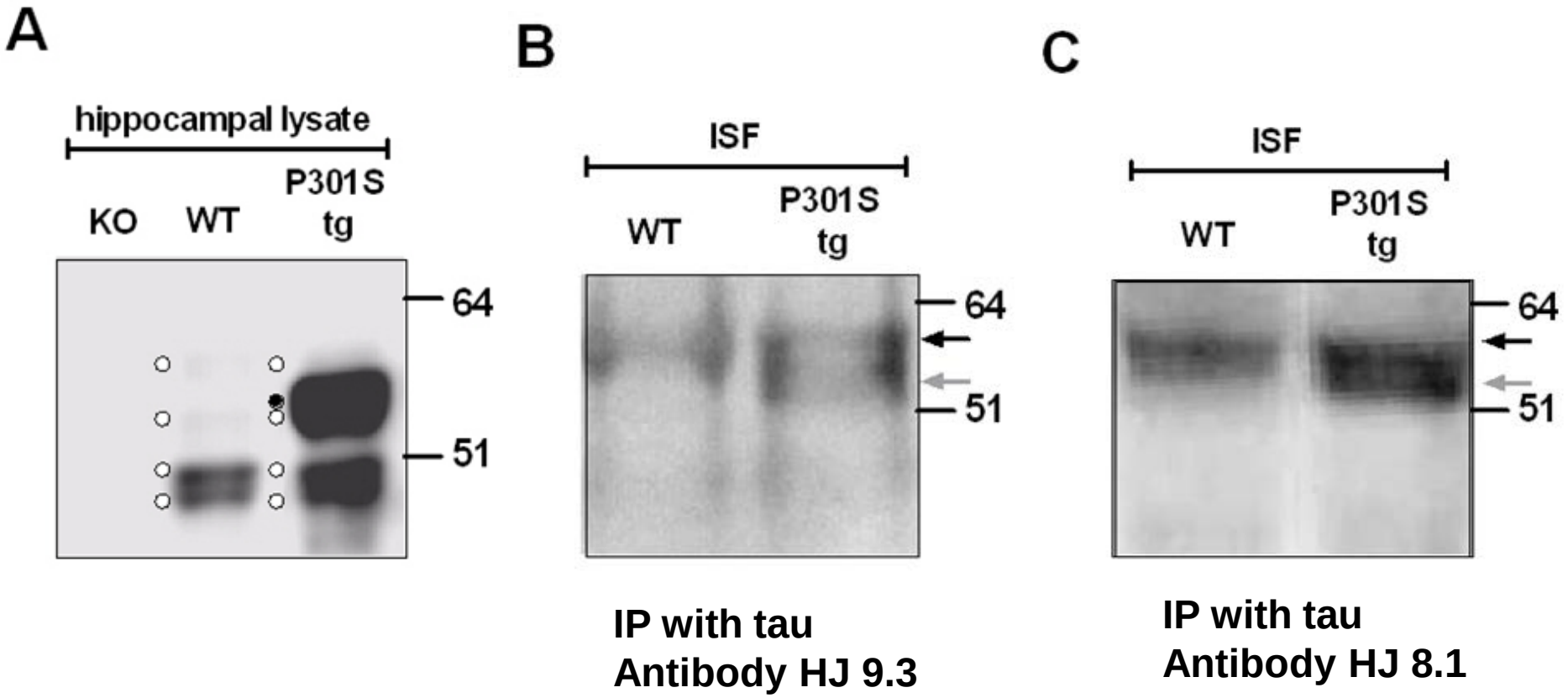


Tau is present and can be measured dynamically in the ISF

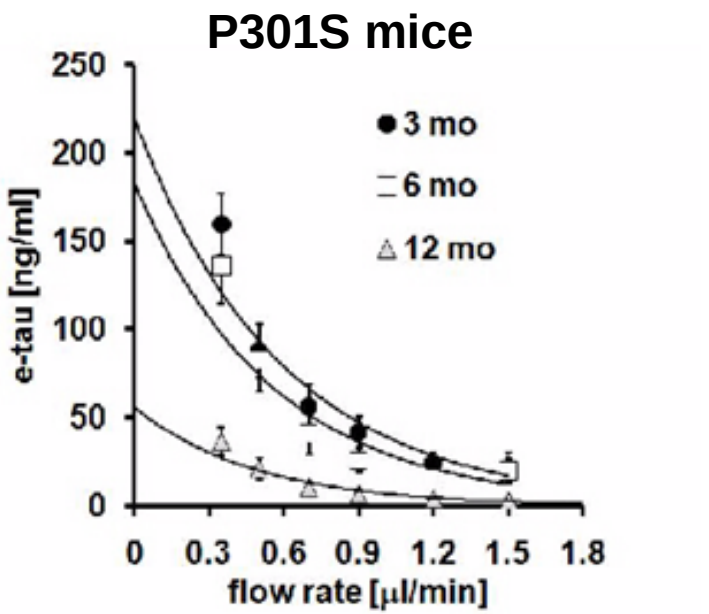


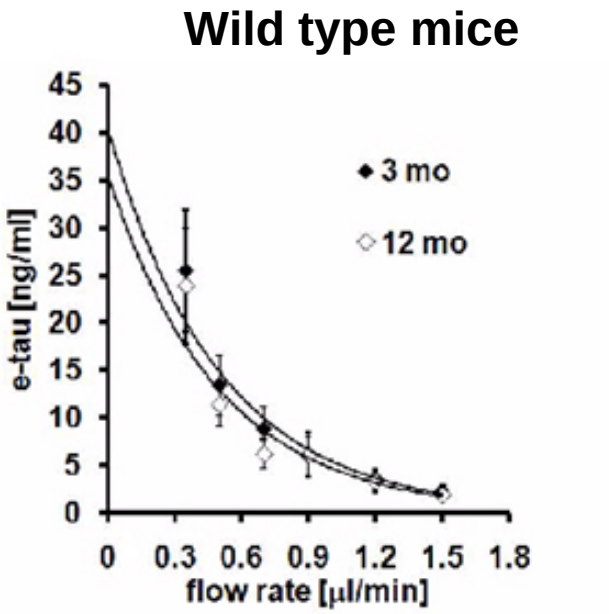
Yamada et al. 2011 J. Neuroscience. 31:13110-13117

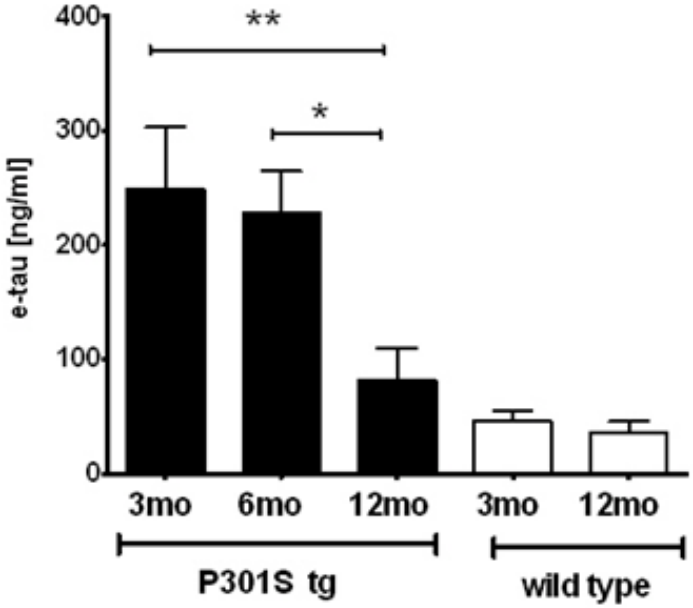
Full length murine and human Tau is present in the ISF

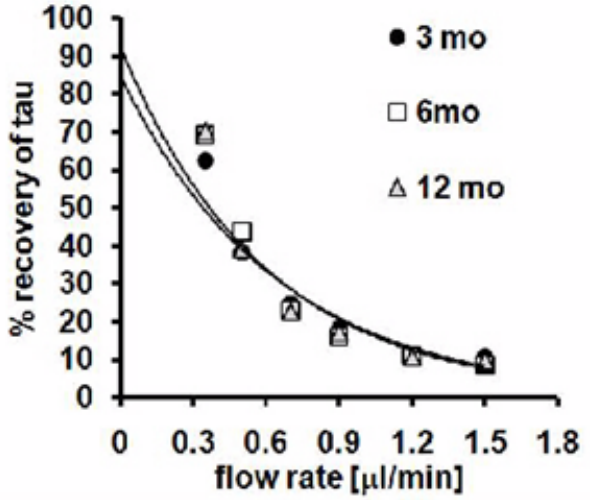


P301S Tau Tg mice have high levels of human Tau in ISF: Marked decrease in ISF Tau with age in Tau Tg mice

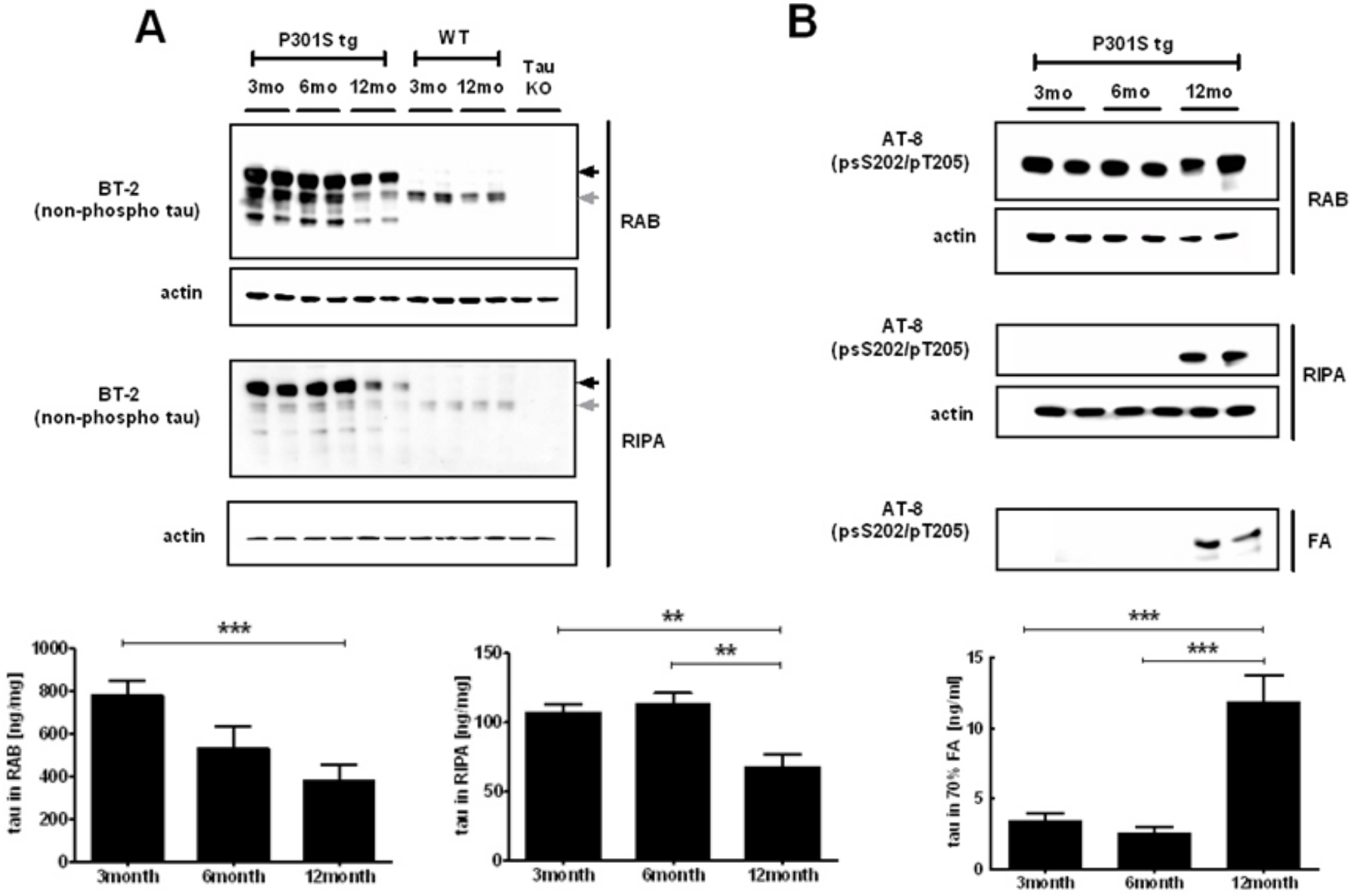




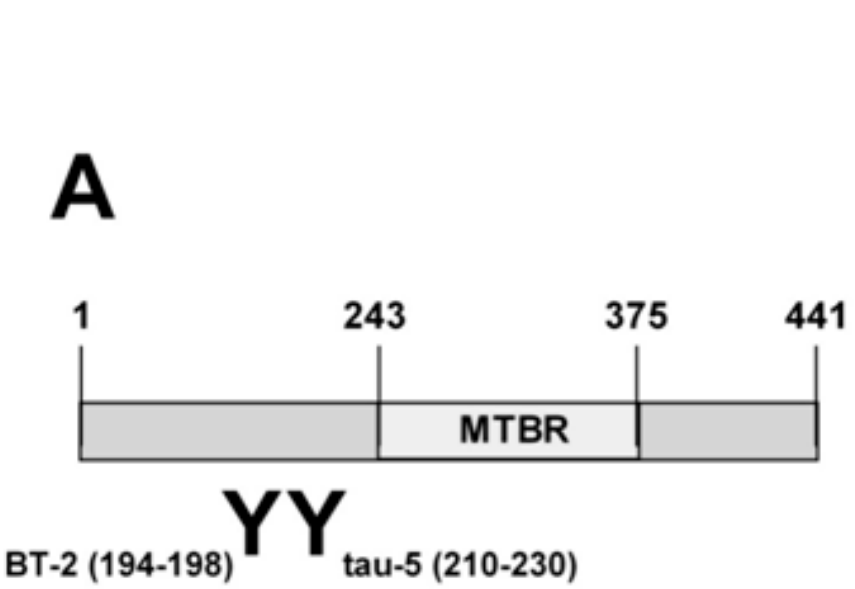


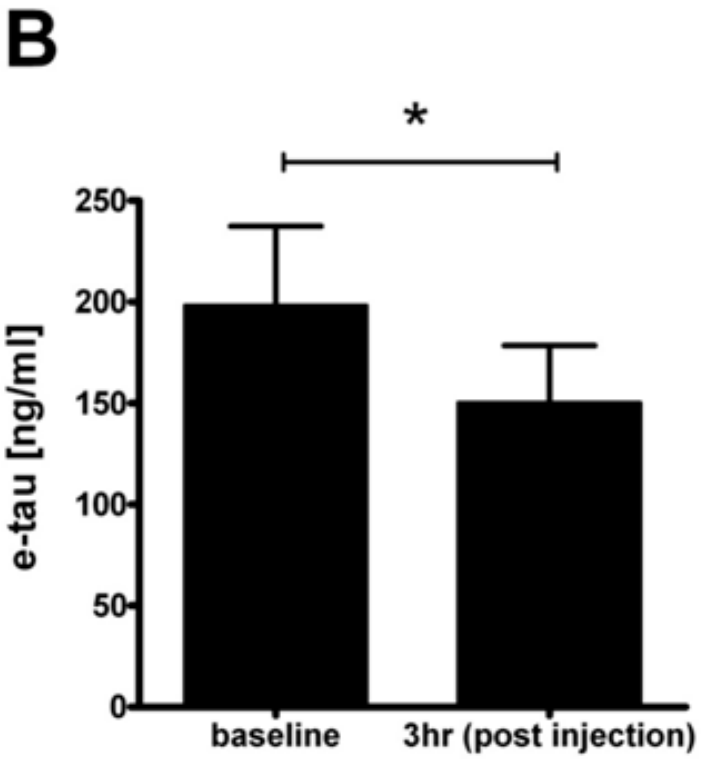


Decrease in soluble Tau and increase in insoluble Tau with age in P301S Tg mice



Decrease in ISF tau following infusion of Tau fibrils in P301S Tg mice

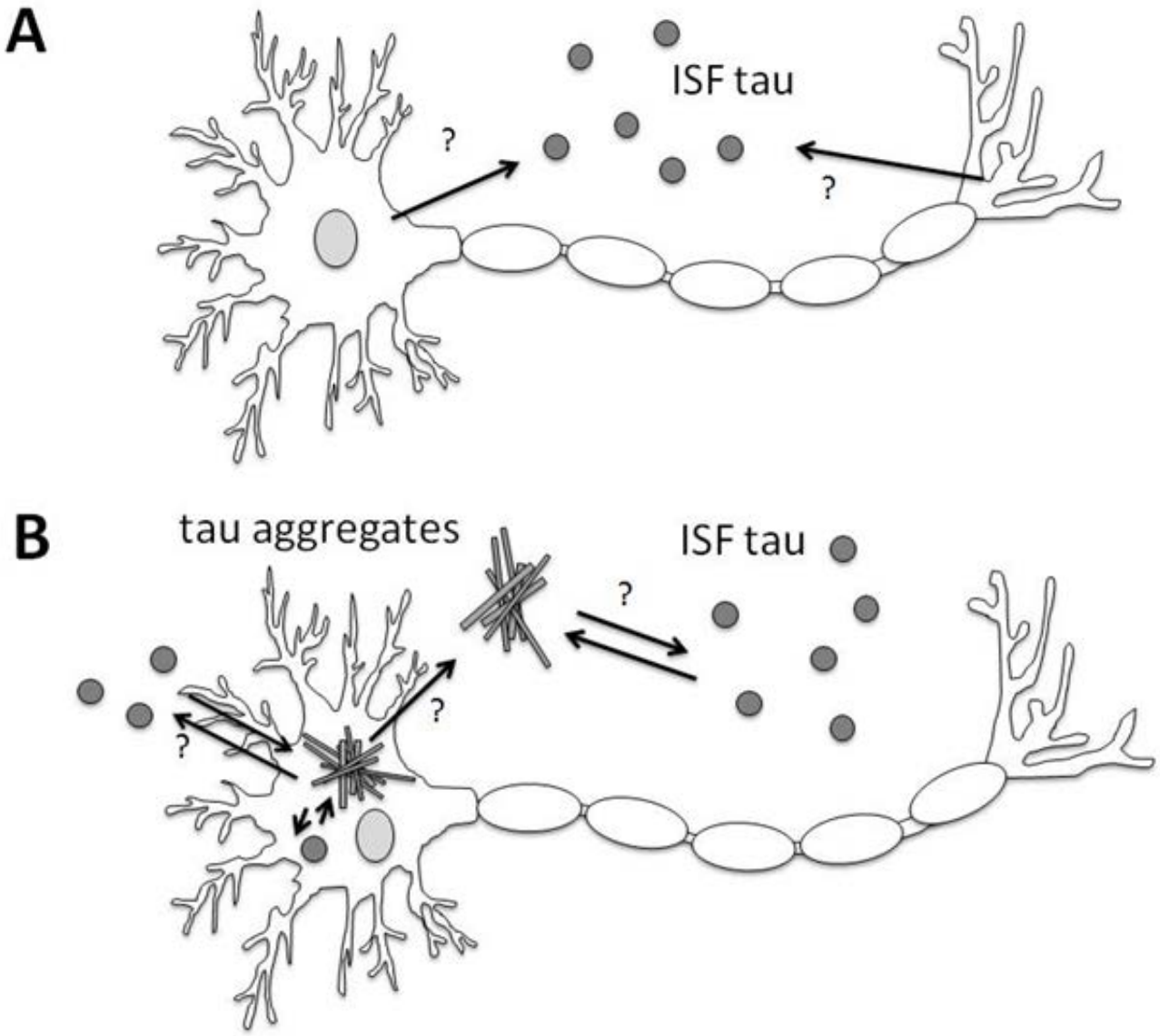




Yamada et al. 2011 J. Neurosci. 31:13110-13117

Collaboration with lab of Marc Diamond

Model of Tau metabolism:
Equilibrium between soluble and insoluble Tau



Outline

1. Studies of ISF tau metabolism demonstrate the normal presence of both monomeric tau as suggest the presence of extracellular tau aggregates in vivo.

2. **Certain anti-tau can bind to and sequester extracellular tau aggregates in vitro and block the extracellular induced intracellular aggregation of tau.**

3. Effects of certain anti-tau antibodies on tauopathy in vivo.

Papers showing that active or passive immunization against tau have effects in mouse models of tauopathy

Active Immunization studies:

Bi, M., Ittner, A., Ke, Y.D., Gotz, J., and Ittner, L.M. (2011). PLoS One 6, e26860.

Boimel, M., Grigoriadis, N., Lourbopoulos, A., Haber, E., Abramsky, O., and Rosenmann, H. (2010). Exp Neurol 224, 472-485.

Asuni, A.A., Boutajangout, A., Quartermain, D., and Sigurdsson, E.M. (2007). J Neurosci 27, 9115-9129.

Boutajangout, A., Quartermain, D., and Sigurdsson, E.M. (2010). J Neurosci 30, 16559-16566.

Troquier, L., Caillierez, R., Burnouf, S., Fernandez-Gomez, F.J., Grosjean, M.E., Zommer, N., Sergeant, N., Schraen-Maschke, S., Blum, D., and Buee, L. (2012). Curr Alzheimer Res 9, 397-405.

Passive immunization studies

Boutajangout, A., Ingadottir, J., Davies, P., and Sigurdsson, E.M. (2011). J Neurochem 118, 658-667.

Chai, X., Wu, S., Murray, T.K., Kinley, R., Cella, C.V., Sims, H., Buckner, N., Hanmer, J., Davies, P., O'Neill, M.J.*, et al.* (2011). J Biol Chem 286, 34457-34467

Evidence for spreading of tau between cells is involved in tauopathy

Frost, B., Jacks, R.L., and Diamond, M.I. (2009). J Biol Chem 284, 12845-12852.

Clavaguera, F., Bolmont, T., Crowther, R.A., Abramowski, D., Frank, S., Probst, A., Fraser, G., Stalder, A.K., Beibel, M., Staufenbiel, M.*, et al.* (2009). Nat Cell Biol 11, 909-913.

Guo, J.L., and Lee, V.M. (2011). J Biol Chem 286, 15317-15331.

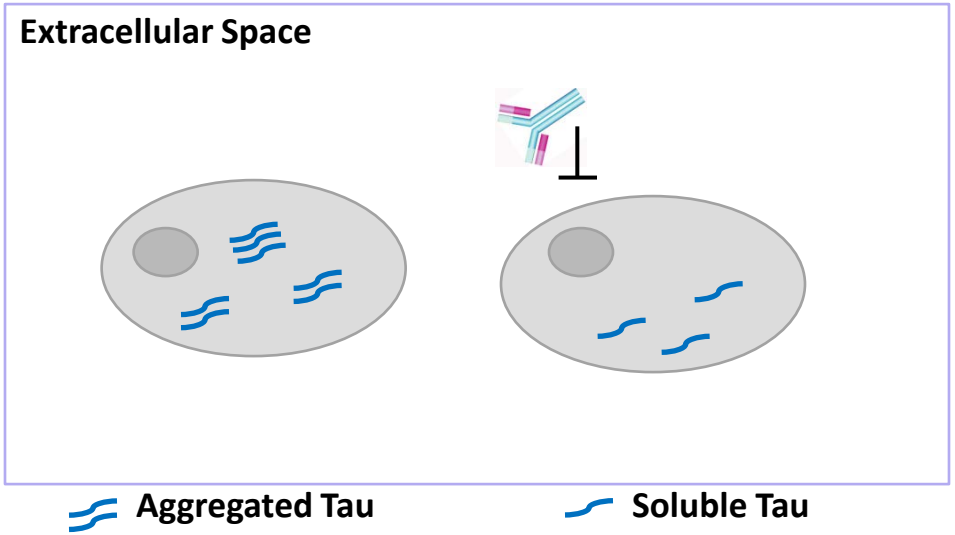
Santa-Maria, I., Varghese, M., Ksiezak-Reding, H., Dzhun, A., Wang, J., and Pasinetti, G.M. (2012). J Biol Chem 287, 20522-20533.

de Calignon, A., Polydoro, M., Suarez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina, K.J., Pitstick, R., Sahara, N., Ashe, K.H., Carlson, G.A.*, et al.* (2012). Neuron 73, 685-697.

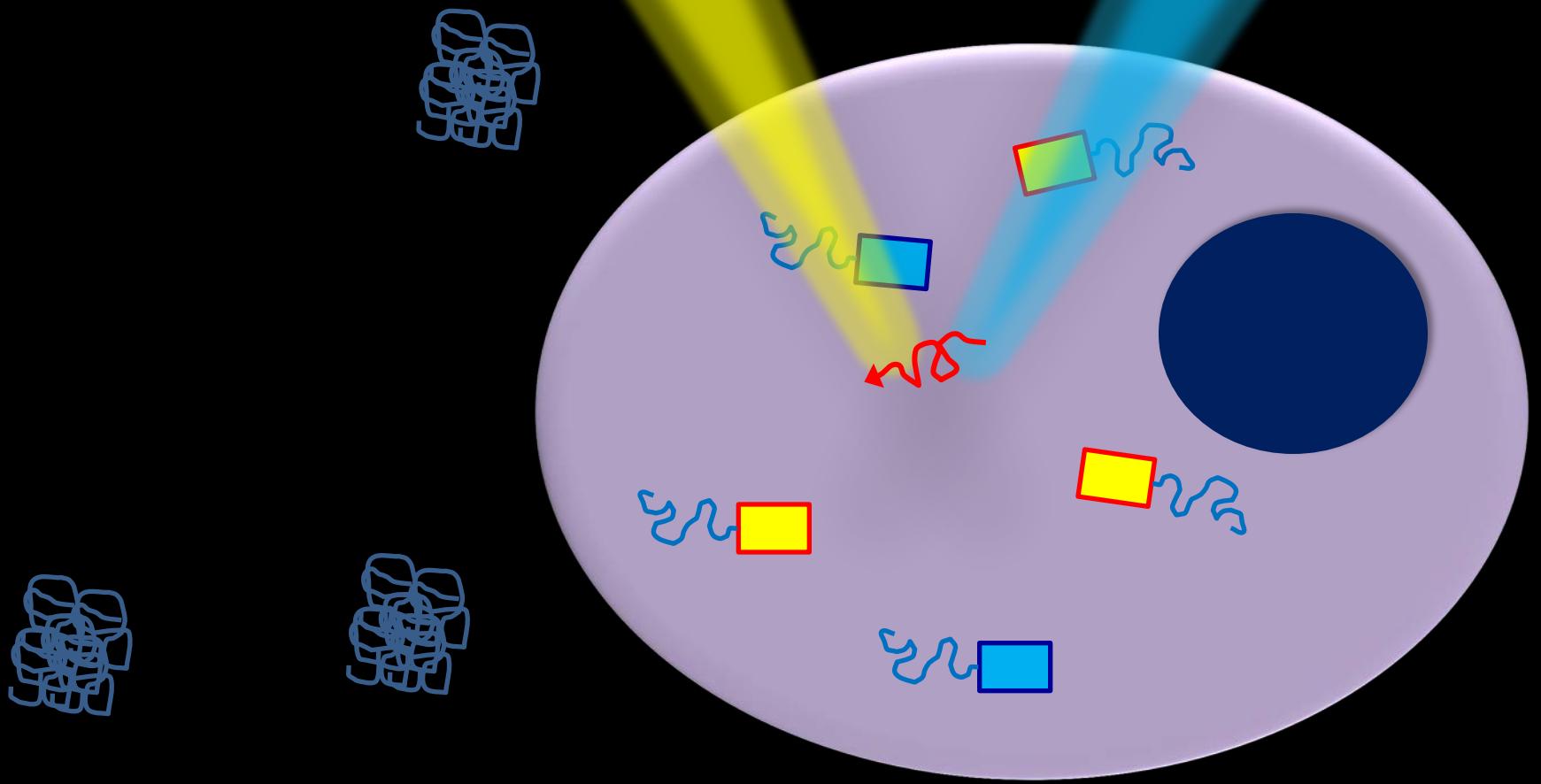
Liu, L., Drouet, V., Wu, J.W., Witter, M.P., Small, S.A., Clelland, C., and Duff, K. (2012). PLoS One 7, e31302.

Iba, M., Guo, J.L., McBride, J.D., Zhang, B., Trojanowski, J.Q., and Lee, V.M. (2013). J Neurosci 33, 1024-1037.

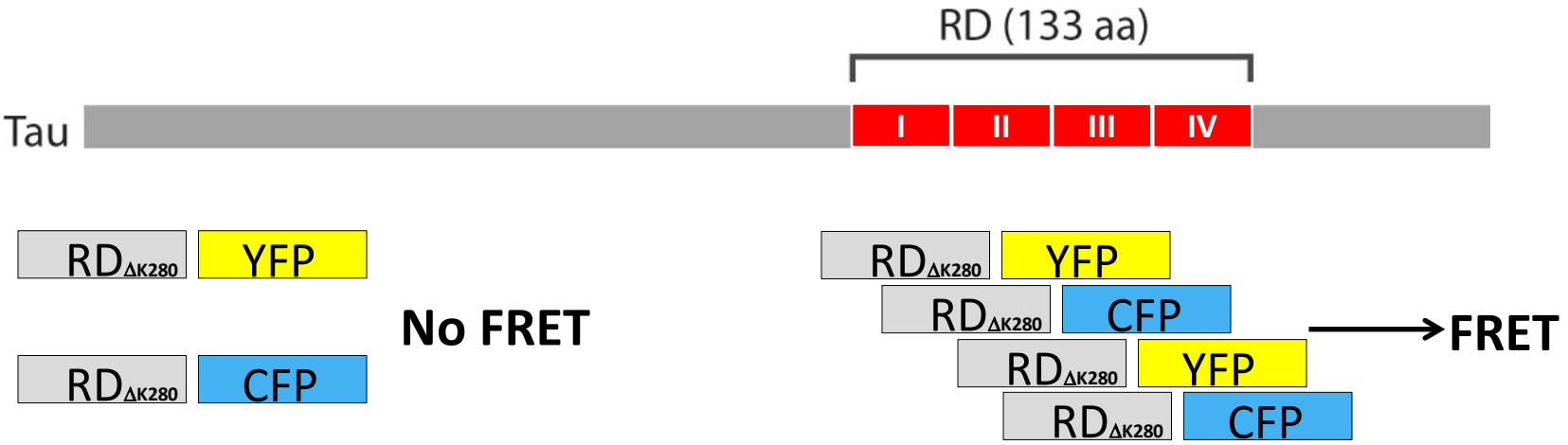
Progression of Disease - Prion Mechanism

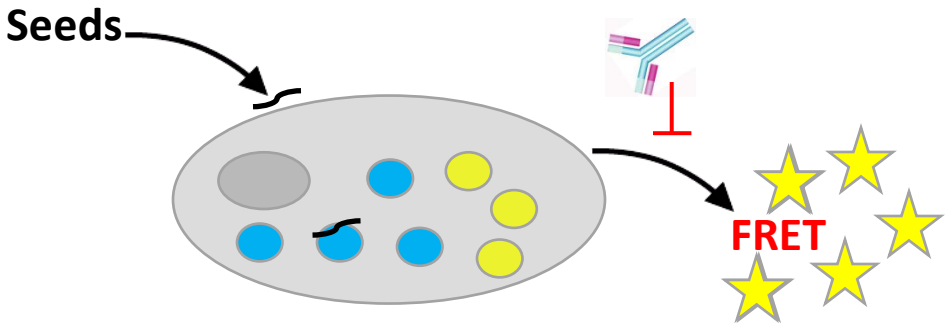


Diamond Lab Tau FRET Seeding Assay

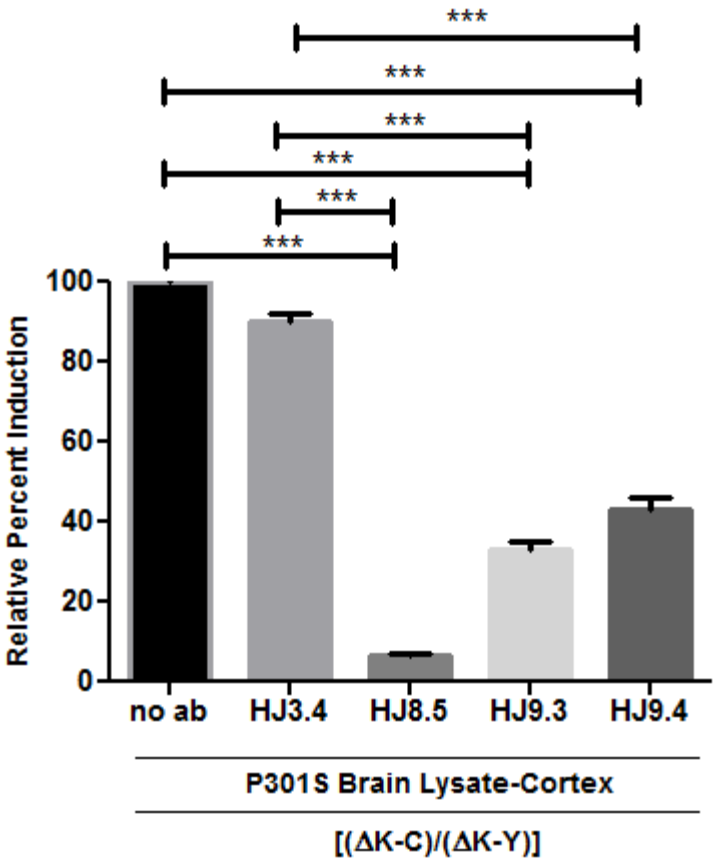


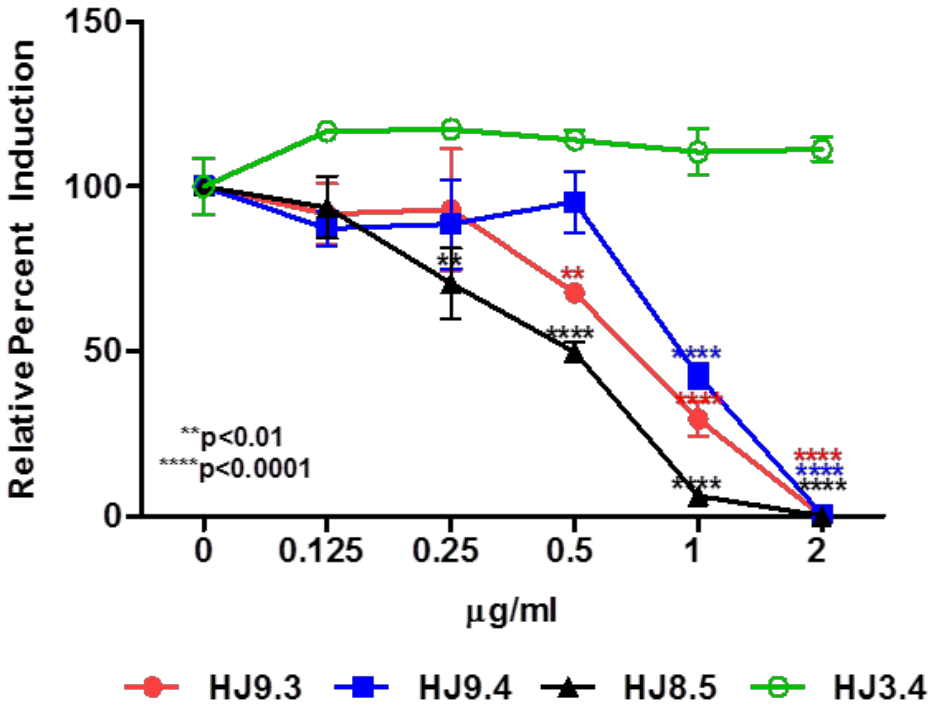
Seeding Assay: FRET-Based





Tau antibodies block the tau aggregate inducing activity of P301S brain lysates





Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, Wozniak DF, Diamond MI, **Holtzman DM**. Neuron. 2013 Oct 16;80(2):402-14.

Outline

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2. Certain anti-tau can bind to and sequester extracellular tau aggregates in vitro and block the cell to cell transfer of tau and subsequent tau aggregation.

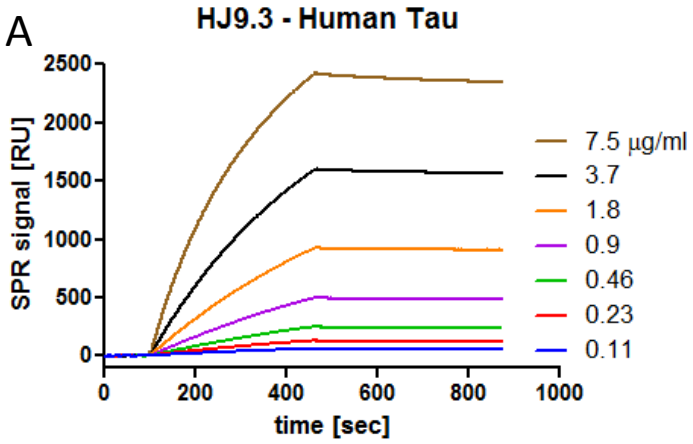
3. **Effects of certain anti-tau antibodies on tauopathy in vivo.**

Experiments: Led by Kiran Yamanandra, Najla Kfoury, Hong Jiang, Tom Mahan

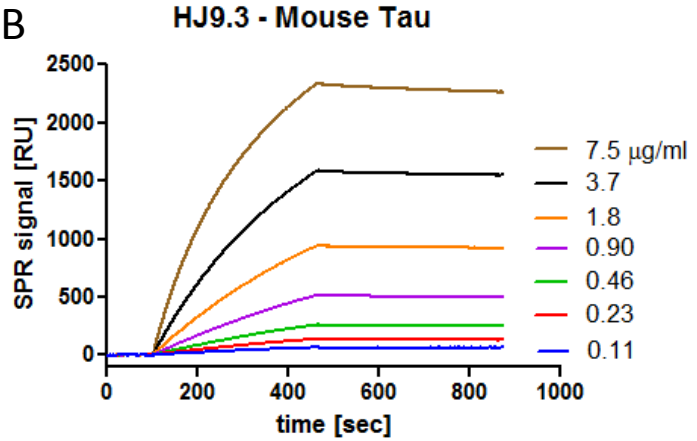
Holtzman lab and Diamond lab

Characterization of anti-tau antibodies by using Biacore (surface plasmon resonance)

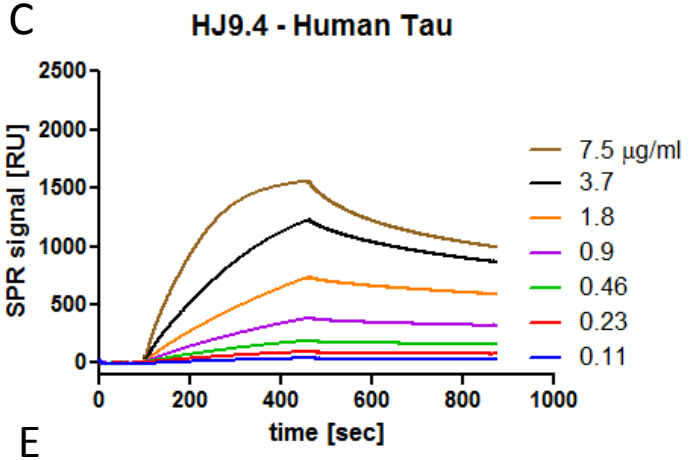
A



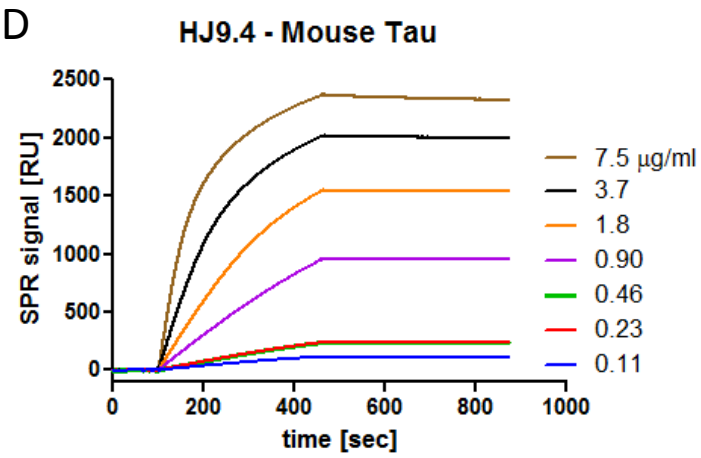
B



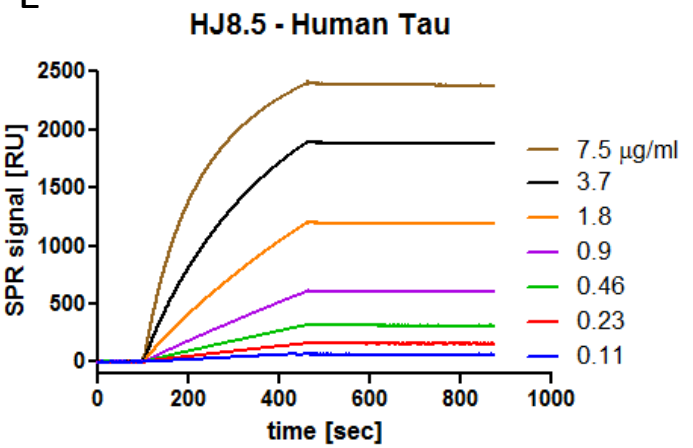
C



D



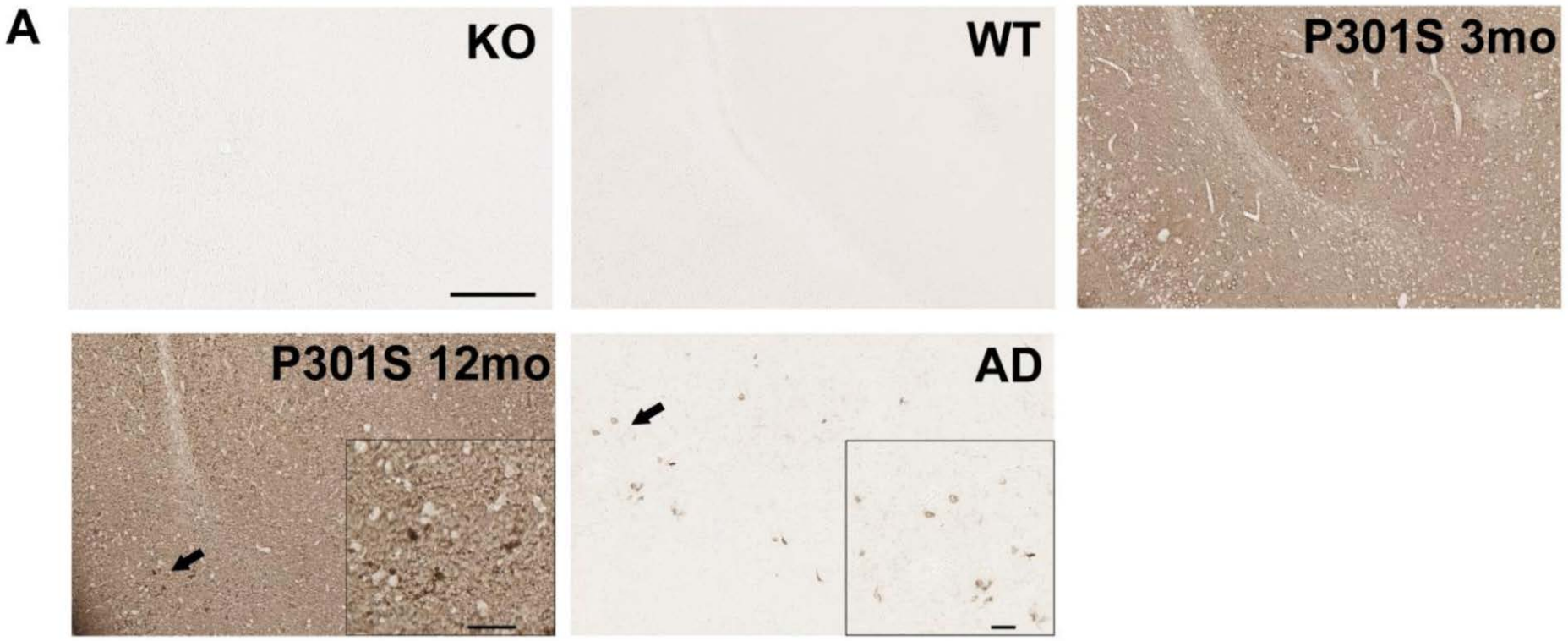
E



F

	HJ8.5			HJ9.3			HJ9.4		
	K_a (1/Ms)	K_d (1/s)	**K_D (M)**	K_a (1/Ms)	K_d (1/s)	**K_D (M)**	K_a (1/Ms)	K_d (1/s)	**K_D (M)**
Human Tau	1.3×10^{5}	4.34×10^{-8}	**0.3 pM**	7.55×10^{4}	7.51×10^{-6}	**99 pM**	1.53×10^{5}	1.07×10^{-3}	**6.9 nM**
Mouse Tau	-	-	-	8.61×10^{4}	9.16×10^{-6}	100 pM	2.28×10^{5}	5.1×10^{-7}	2.2 pM

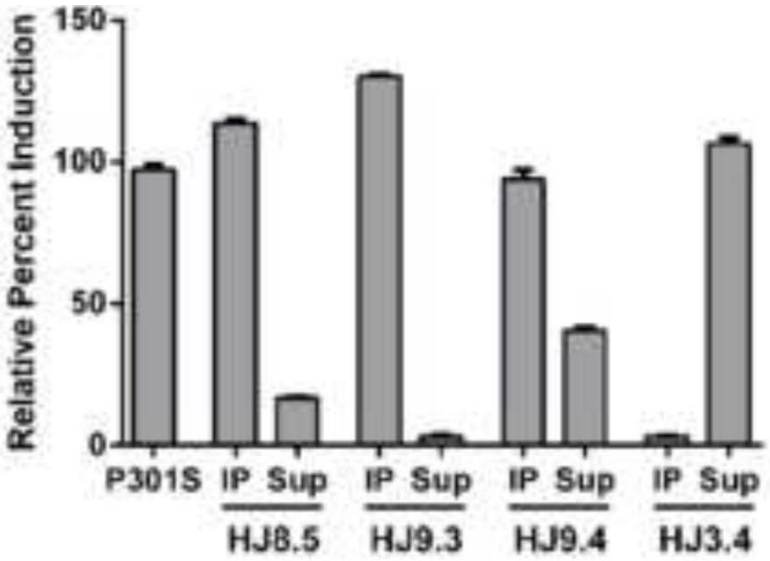
Anti-Tau antibodies HJ8.5, HJ9.3, and HJ9.4 stain both soluble and aggregated forms of tau (HJ8.5 staining shown here)



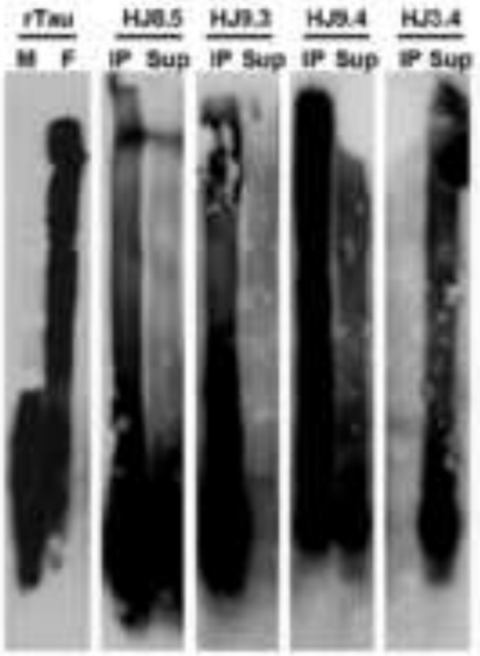
Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, Wozniak DF, Diamond MI, **Holtzman DM**. Neuron. 2013 Oct 16;80(2):402-14.

Anti-Tau antibodies IP cellular tau seeding activity: Evidence that oligomeric Tau Is Involved

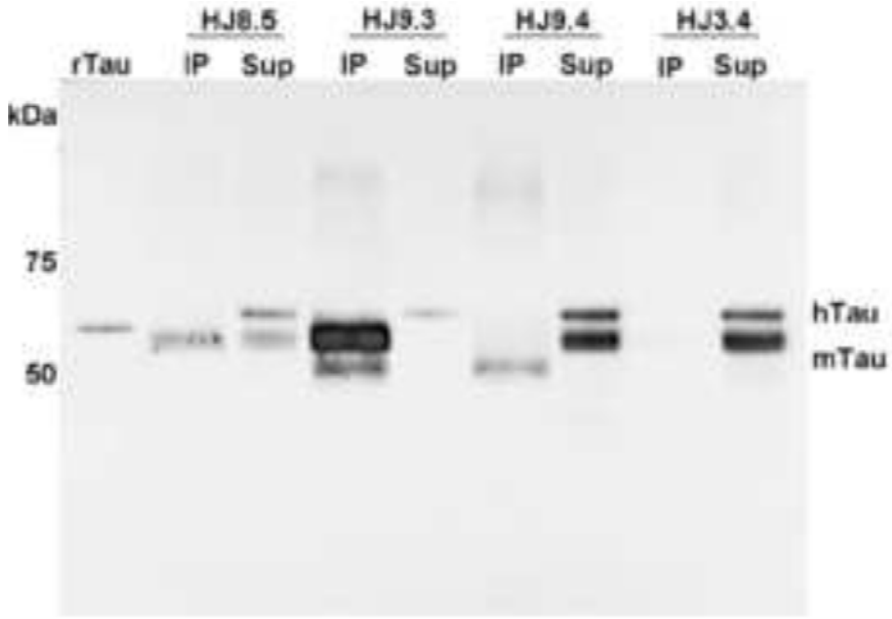
Tau antibodies IP seeding activity



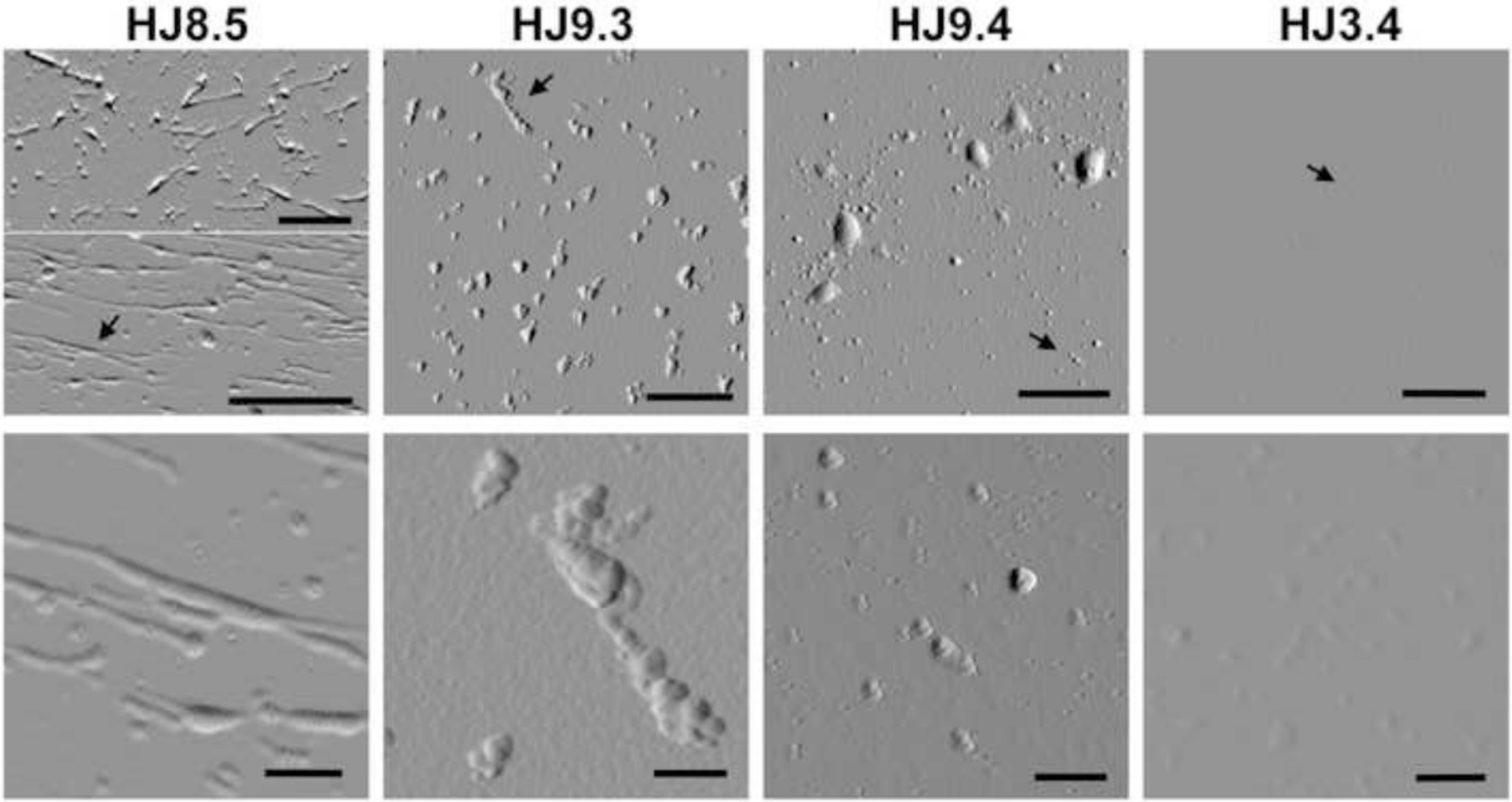
SDD-AGE reveals oligomeric tau in TBS-soluble P301S brain lystates



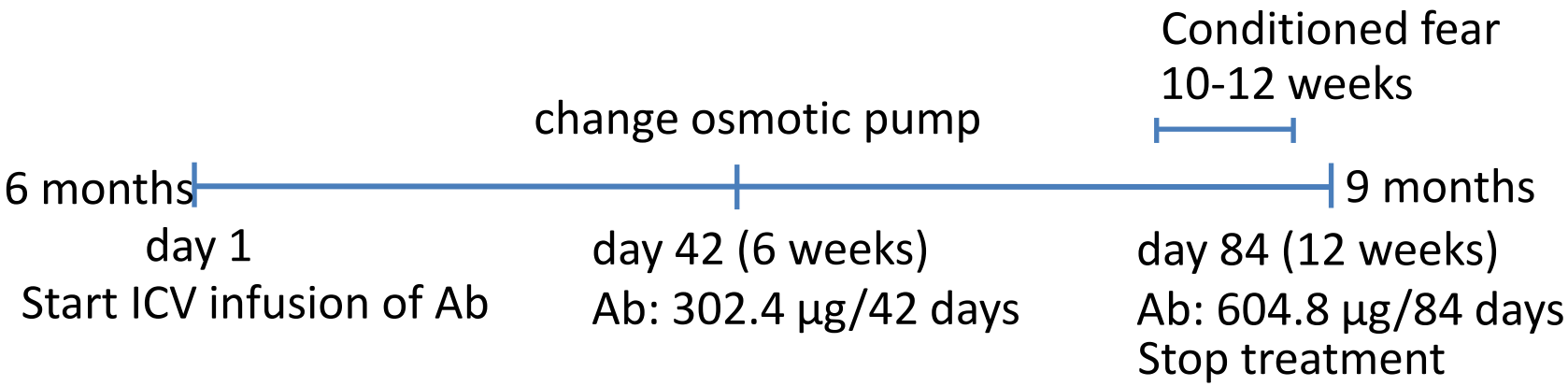
SDS-PAGE reveals Anti-tau antibodies IP human and mouse Tau from P301S brain lystates



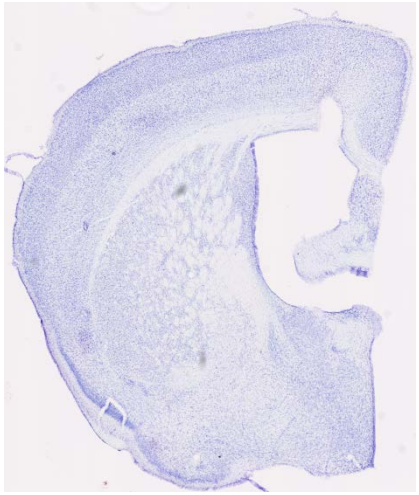
Anti-Tau antibodies IP tau fibrils and other aggregates from TBS-soluble P301S Brain Lysates



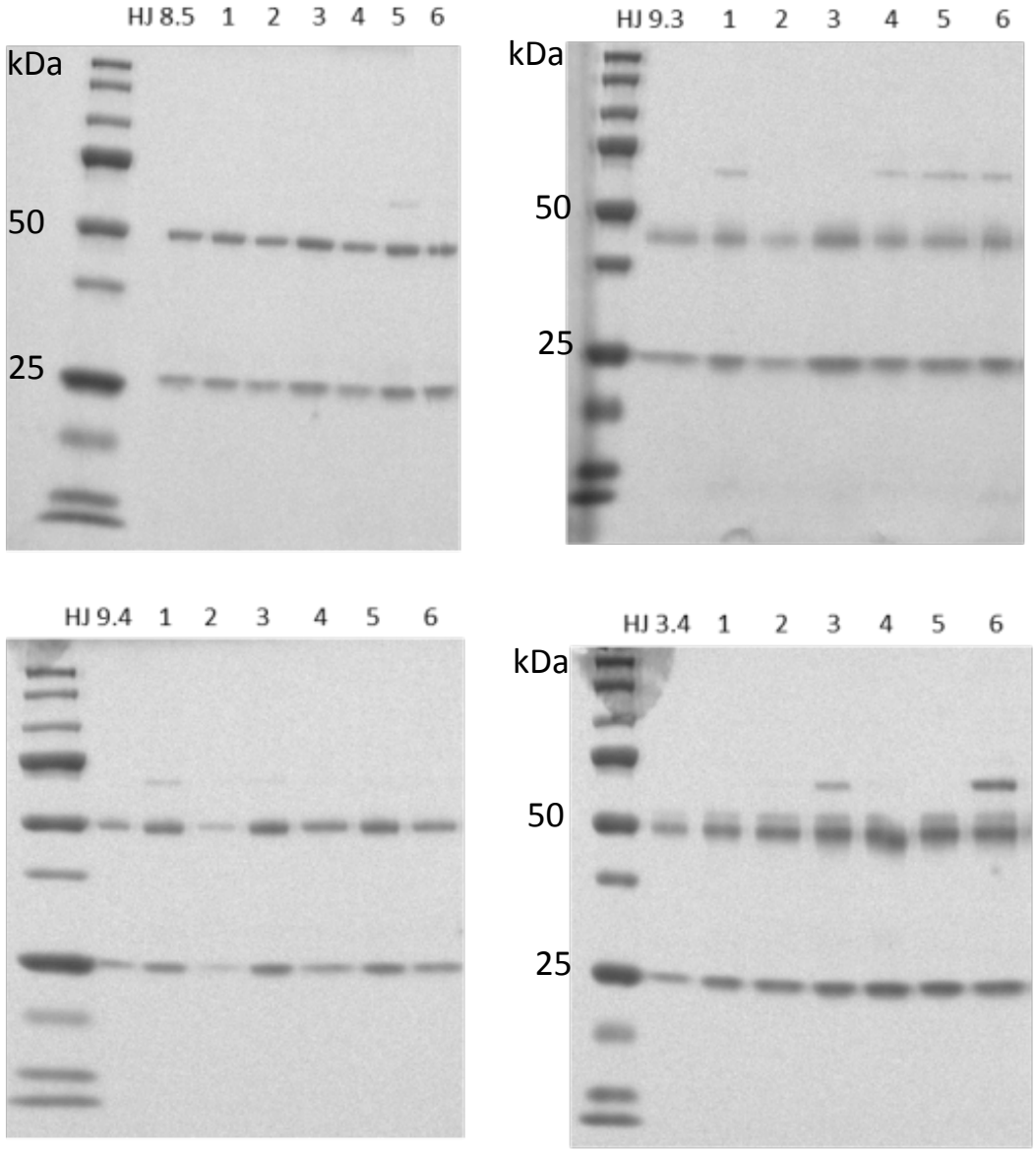
Experimental plan for ICV infusion of anti-Tau antibodies (Abs)



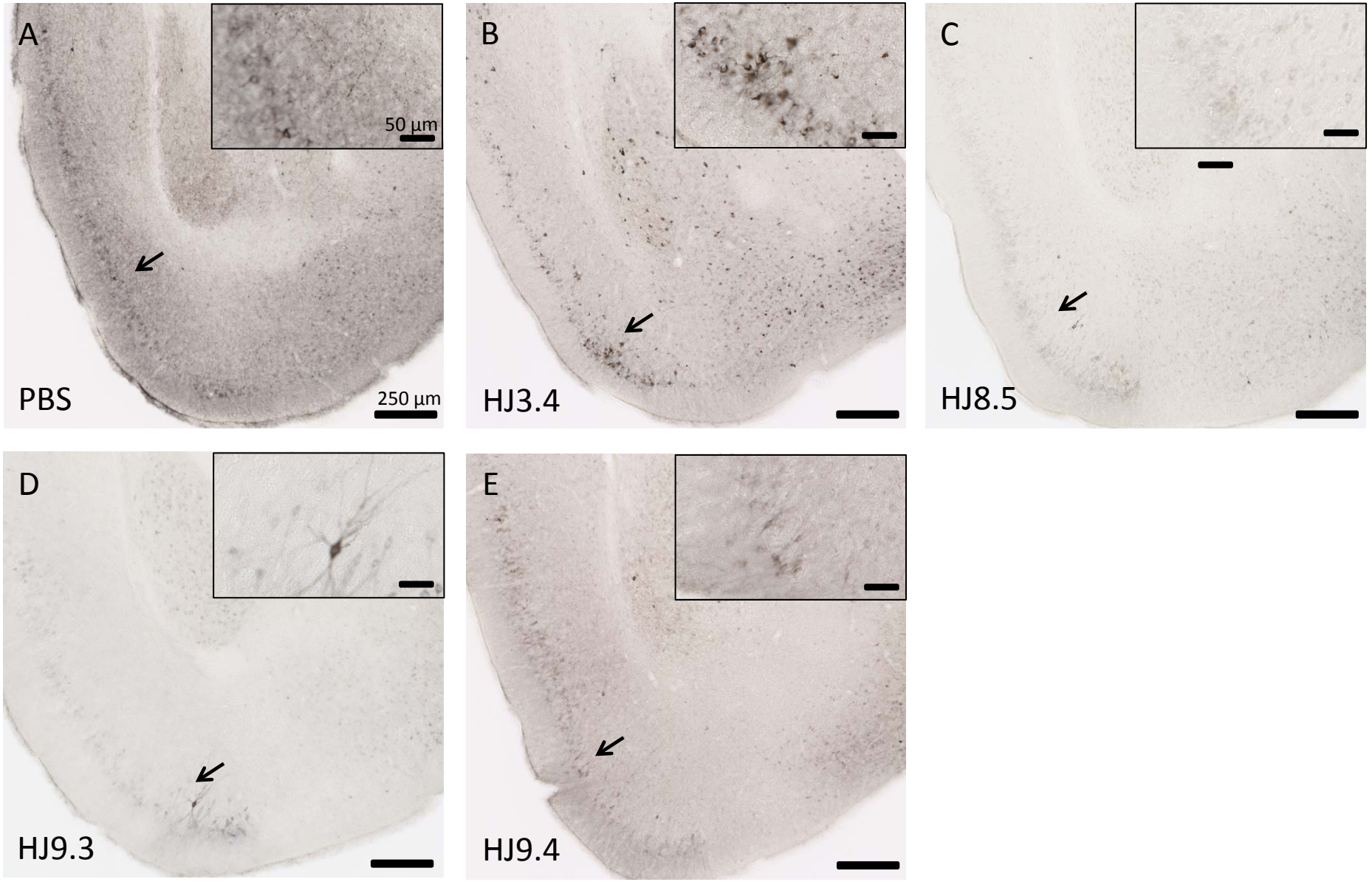
Rate of infusion: 3.6 µl/day – 0.15 µl/hour,
2 µg/µl concentration of antibody = 7.2 µg/day



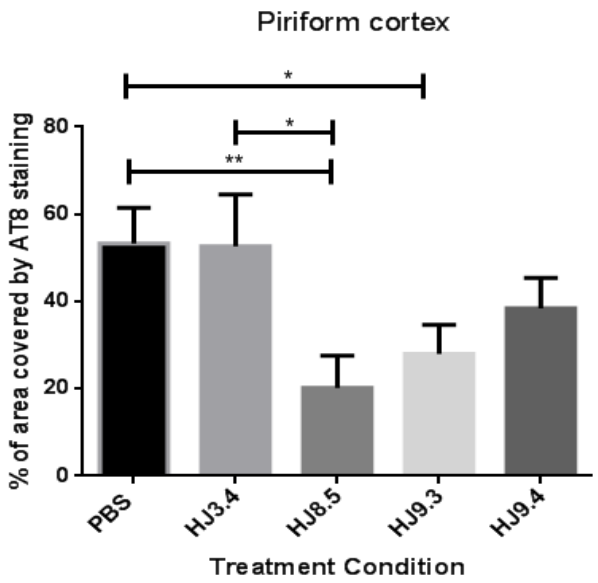
Anti-tau and Aβ antibodies are stable after their presence for 6 weeks in osmotic pumps in vivo

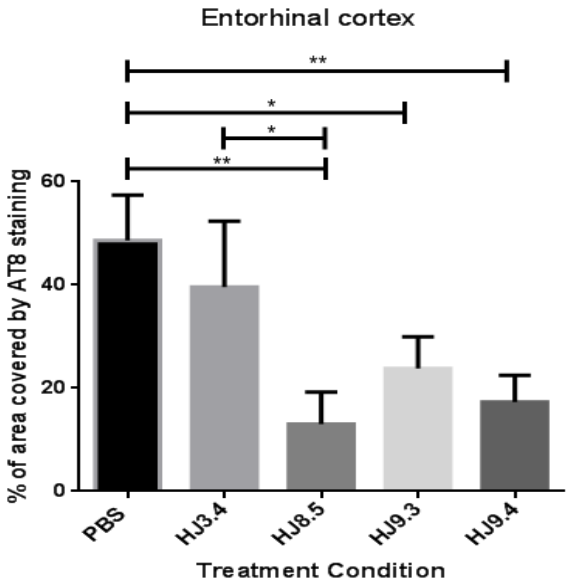


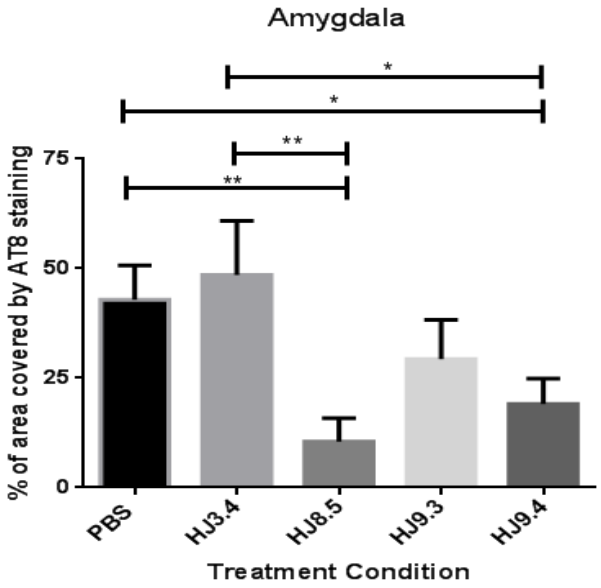
Anti-Tau Antibody treatment reduces pathological tau staining in P301S mice

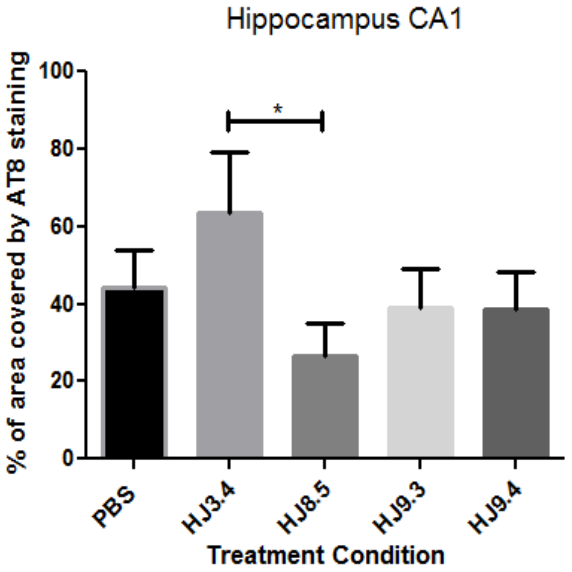


Quantification of AT8 staining in P301S mice

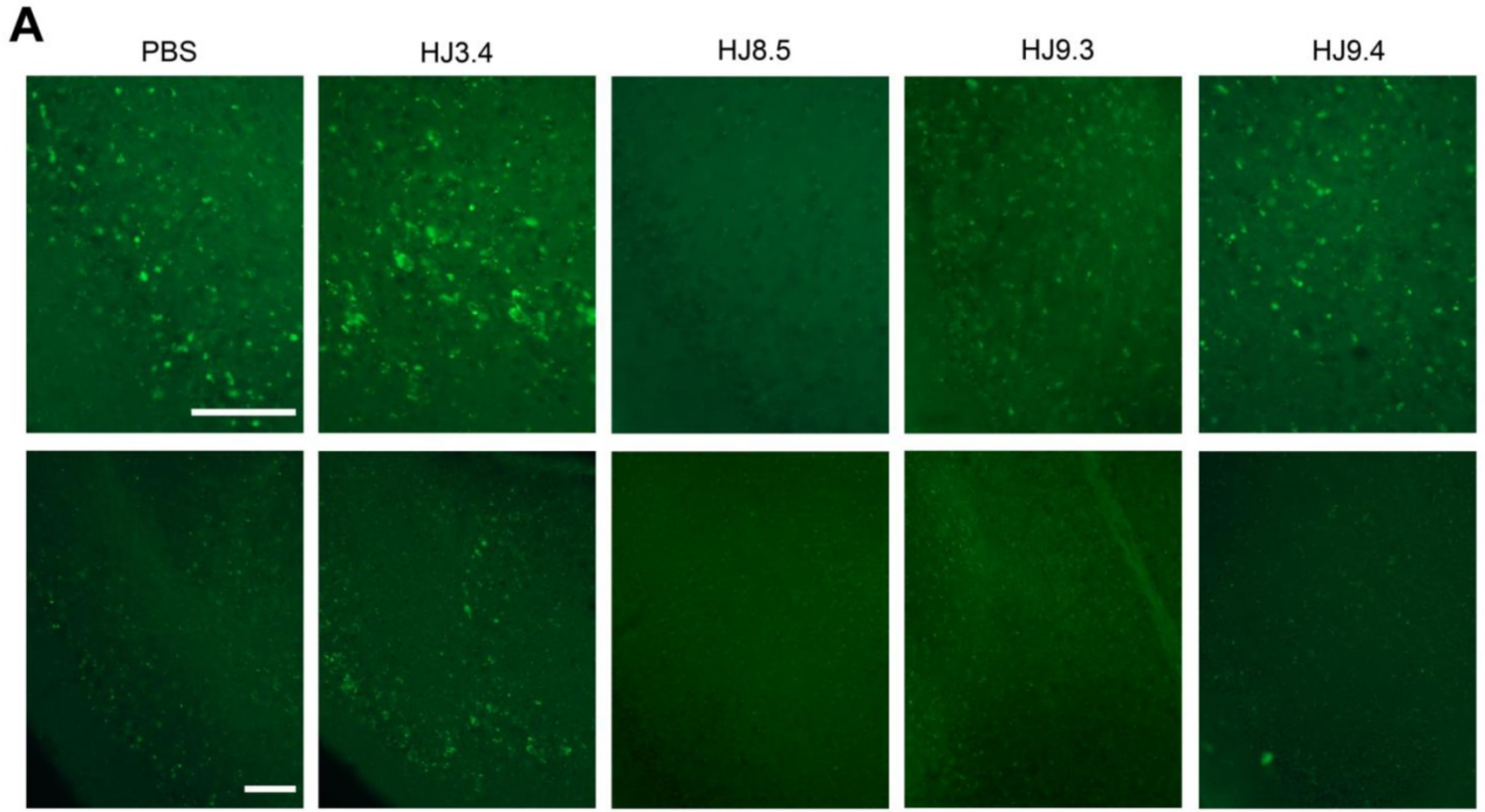


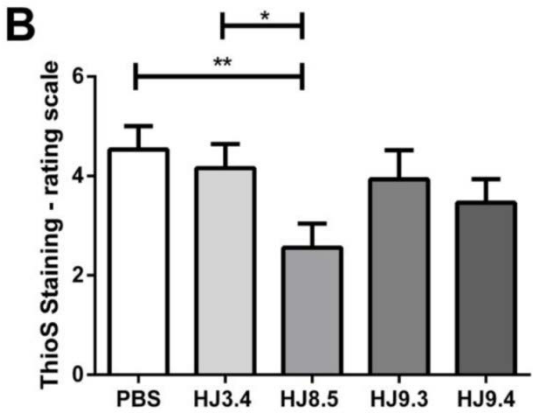




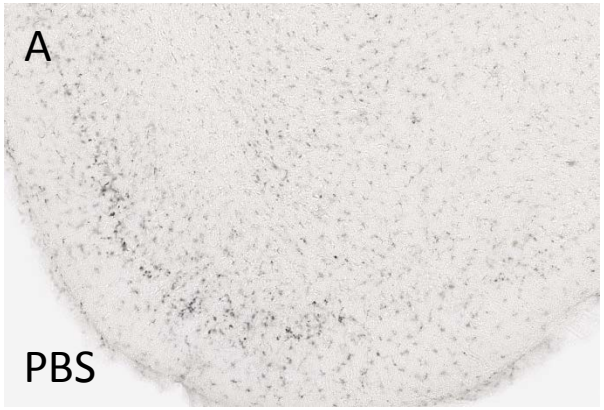


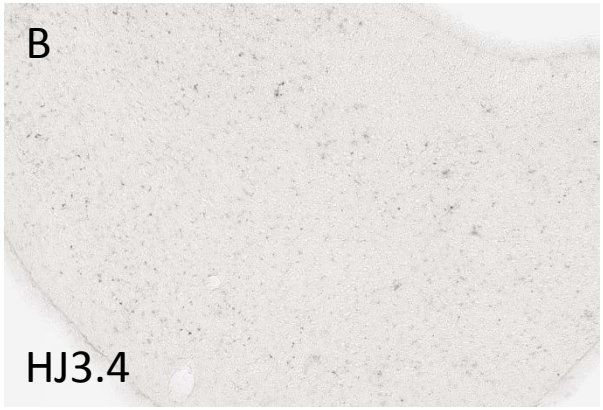
HJ 8.5 Antibody treatment reduces ThioS staining in P301S mice

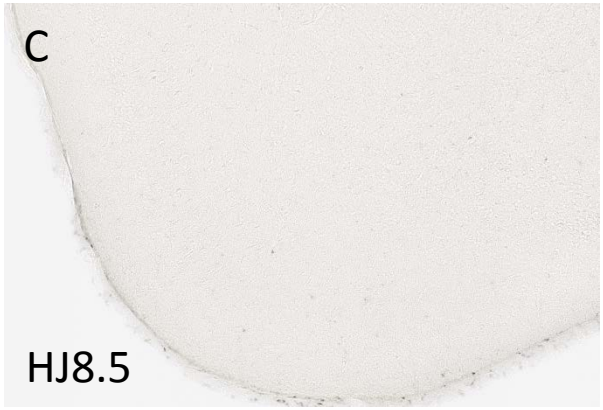


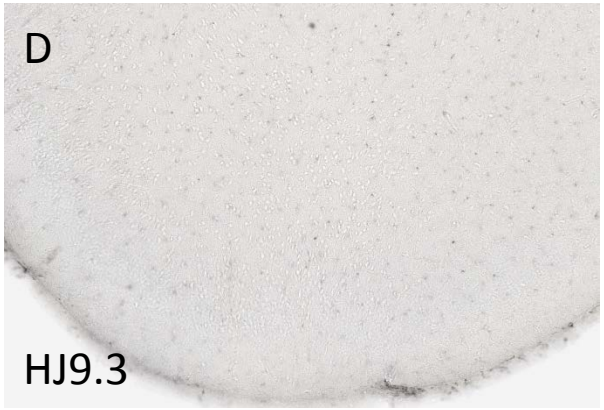


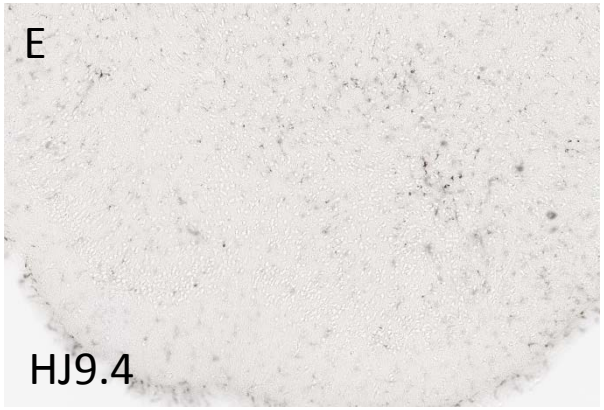
Anti-Tau antibodies reduce activated microglia as marked by CD68 staining in P301S mice



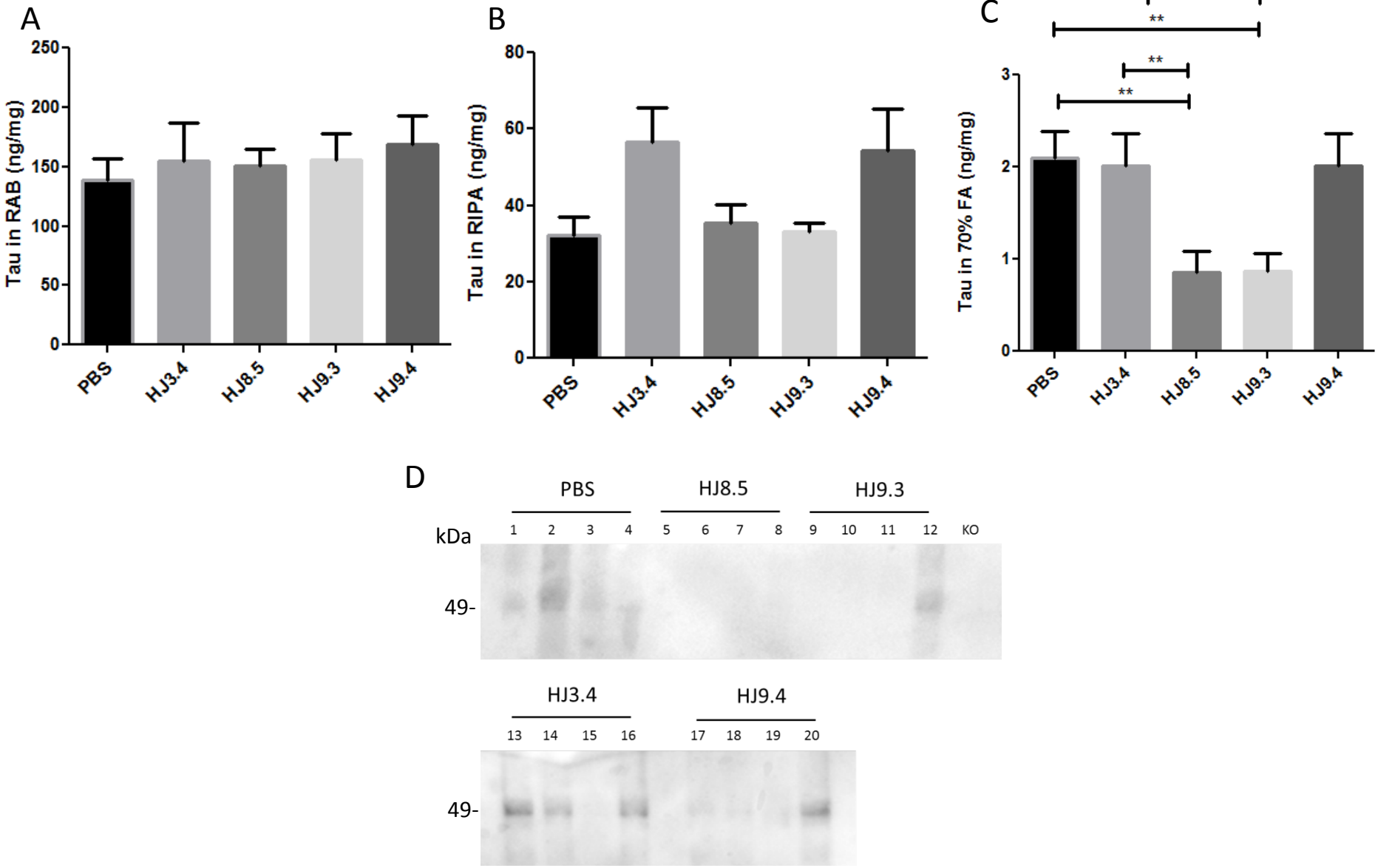




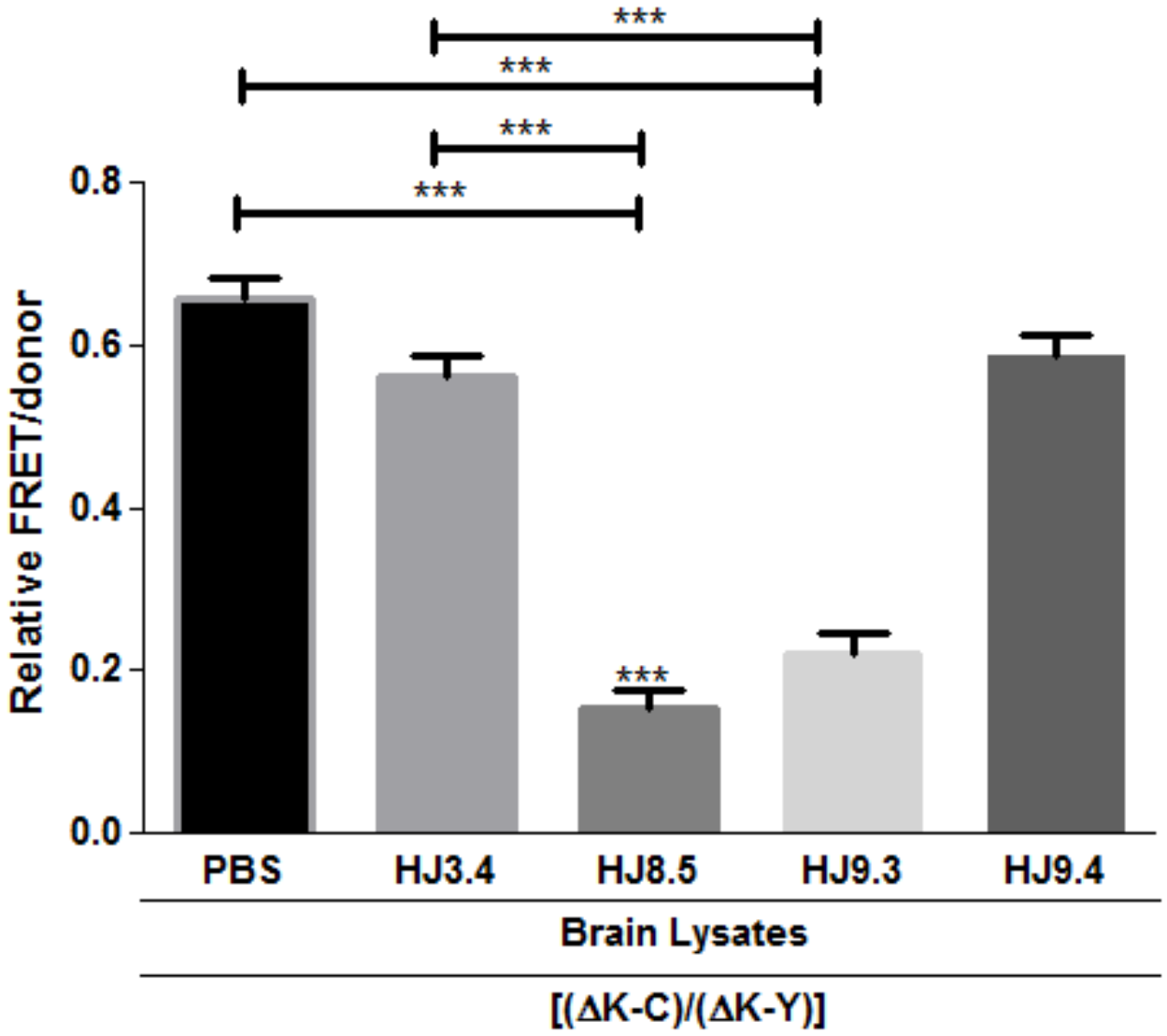




HJ8.5 and HJ9.3 strongly decrease insoluble tau in P301S mice

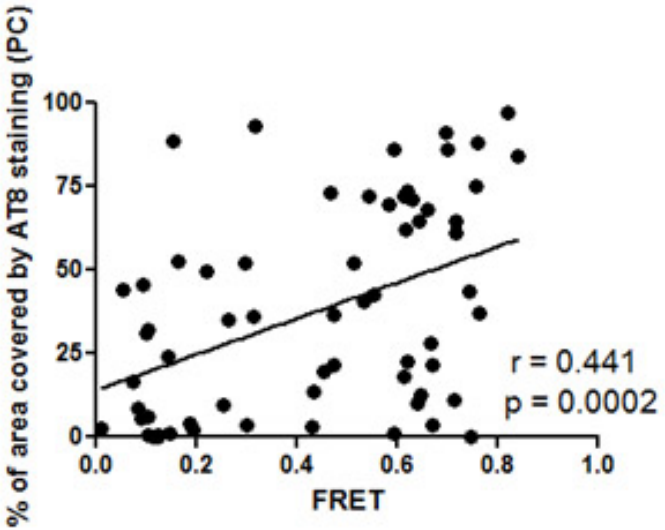


HJ8.5 and HJ9.3 treated brain lysates are strongly suppressed in their ability to induce intracellular tau aggregation

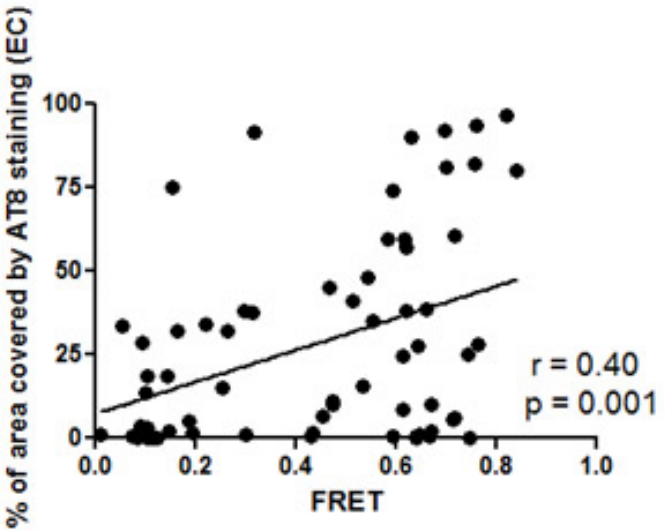


Correlation between AT8 staining and tau seeding activity

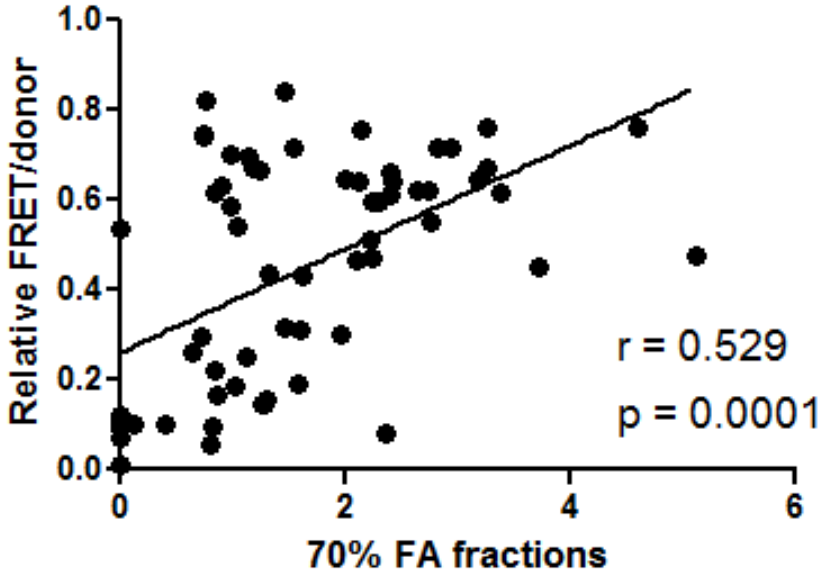
Piriform cortex

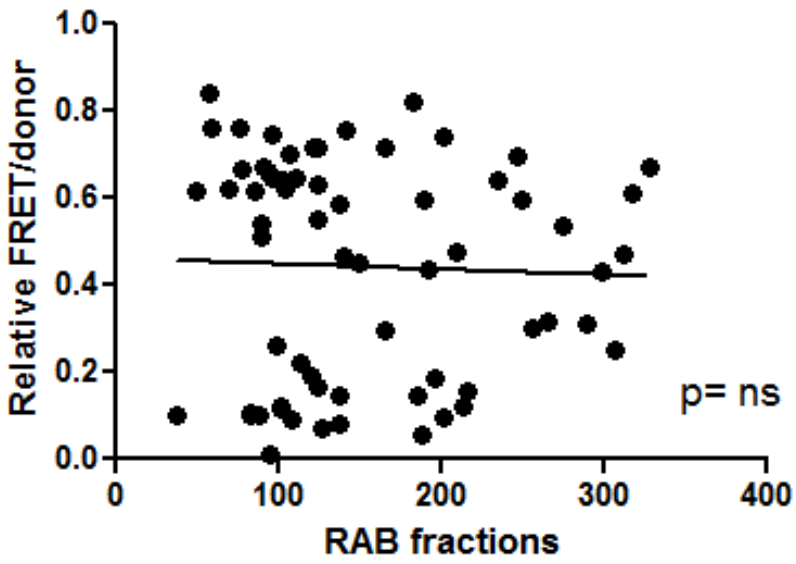


Entorhinal cortex

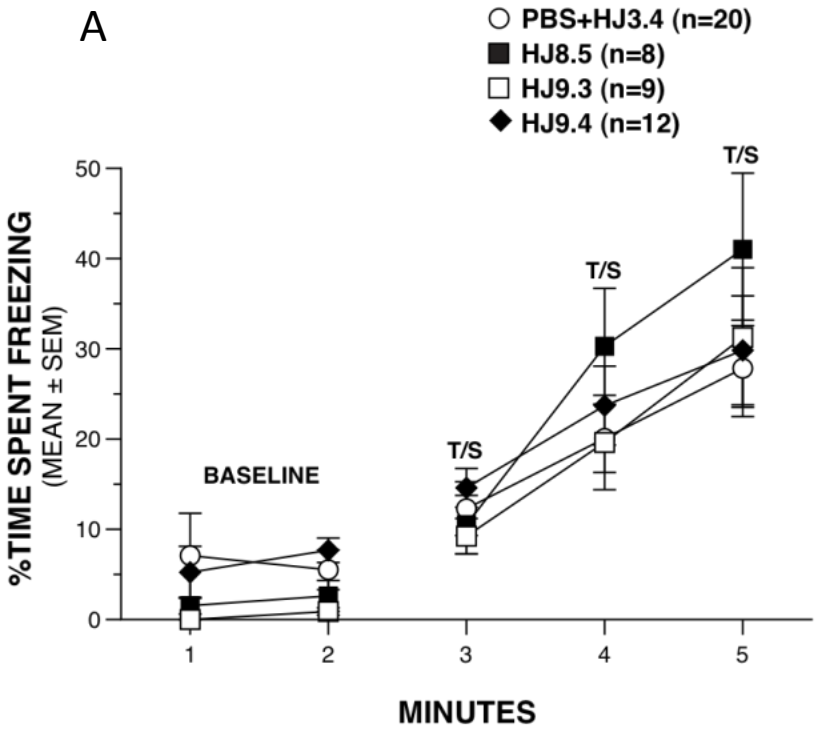


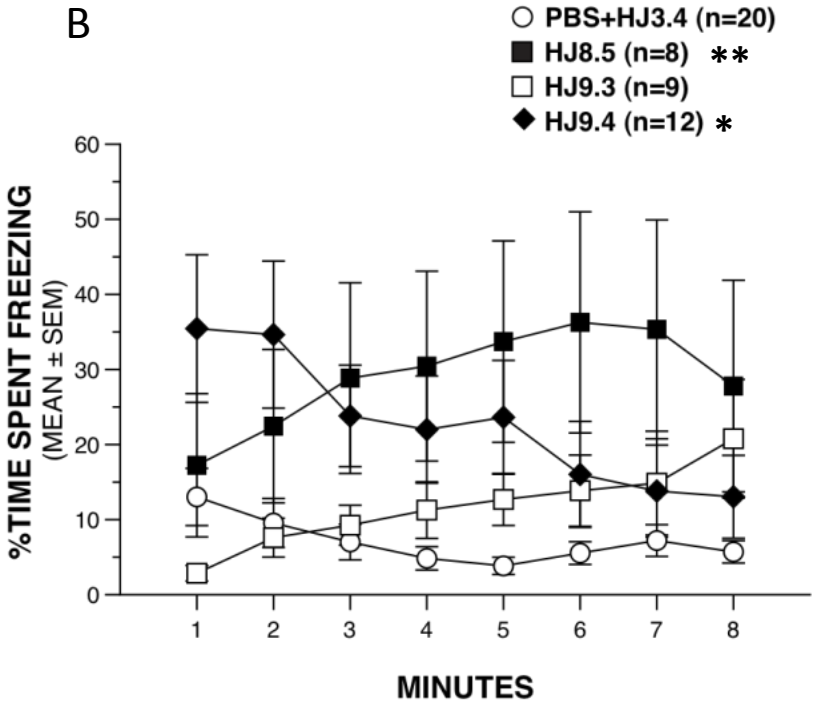
Significant correlation between brain tissue Tau seeding activity and insoluble but not soluble Tau





HJ8.5 and HJ9.4 treated mice have significantly better performance in fear conditioning task versus controls





Summary

1. In vivo experiments with anti-tau antibodies show we are strongly decreasing tau pathology and improving function with ICV infusion. This suggests that this type of approach has promise therapeutically.

2. Evidence suggests that tau aggregate spreading may contribute importantly to tauopathy. We can use in vitro seeding assays that we can apply to the in vivo setting to both further test this hypothesis, develop novel treatments, and test them in vivo.