Prion pathogenesis and toxicity and pathophysiologic mechanisms in ADRD

10:00-11:15 am

- Christina Sigurdson, DVM, PhD, Department of Pathology, UC San Diego
- Adriano Aguzzi, MD, Director, Institute of Neuropathology, University of Zürich
- David Harris, MD, PhD, Department of Chemistry and Cell Biology, Boston University

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Synaptic signaling and excitotoxicity in prion disease

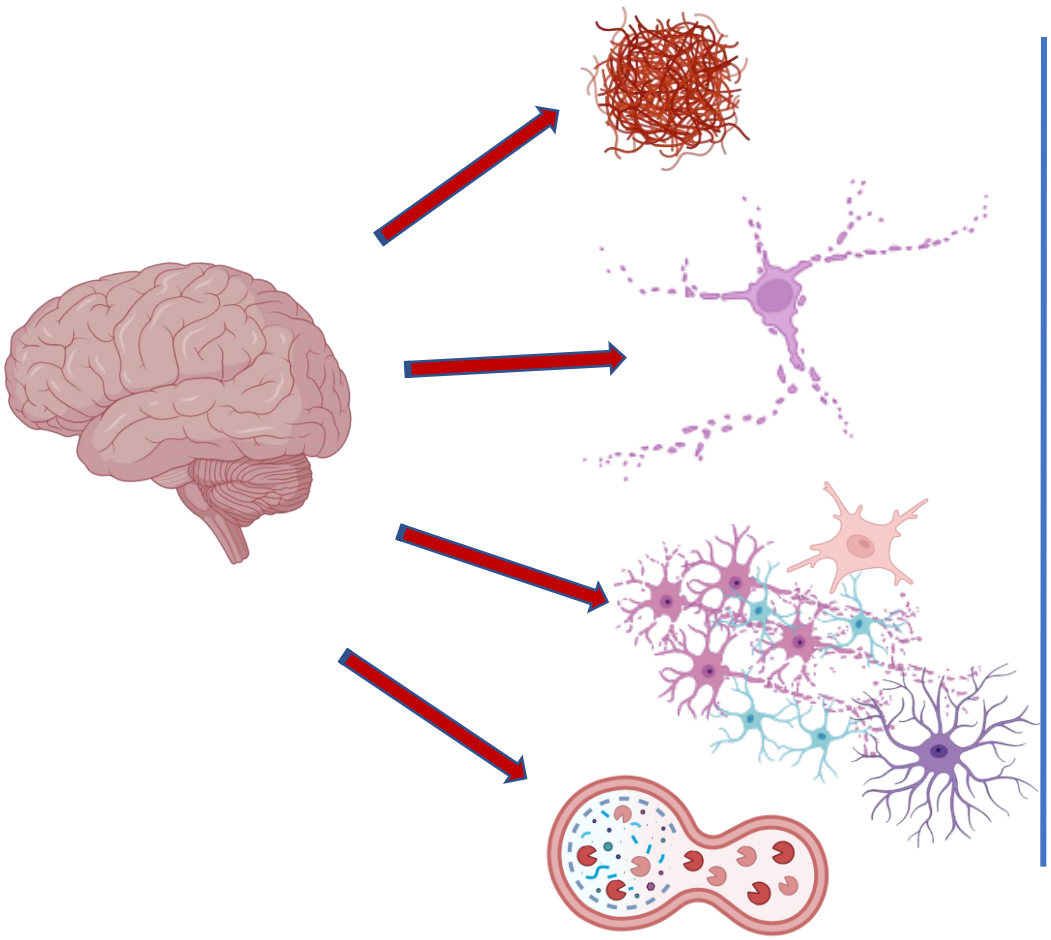
Christina Sigurdson
Department of Pathology
UC San Diego

Mechanisms of Neurodegeneration in Human Prion Diseases and their Intersection with AD/ADRD

NIH NINDS

November 14, 2024

Pathological hallmarks of prion disease



Prion protein aggregates

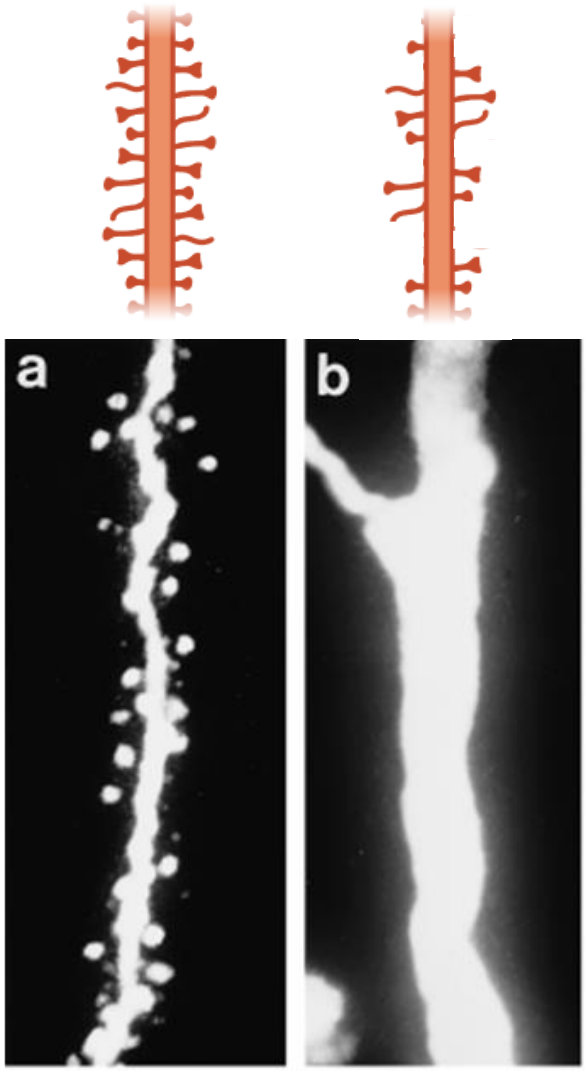
Spongiform degeneration, synapse loss and neuron death ->loss of connectivity

Neuroinflammation:
- Astrogliosis
- Microgliosis

Proteostatic dysfunction

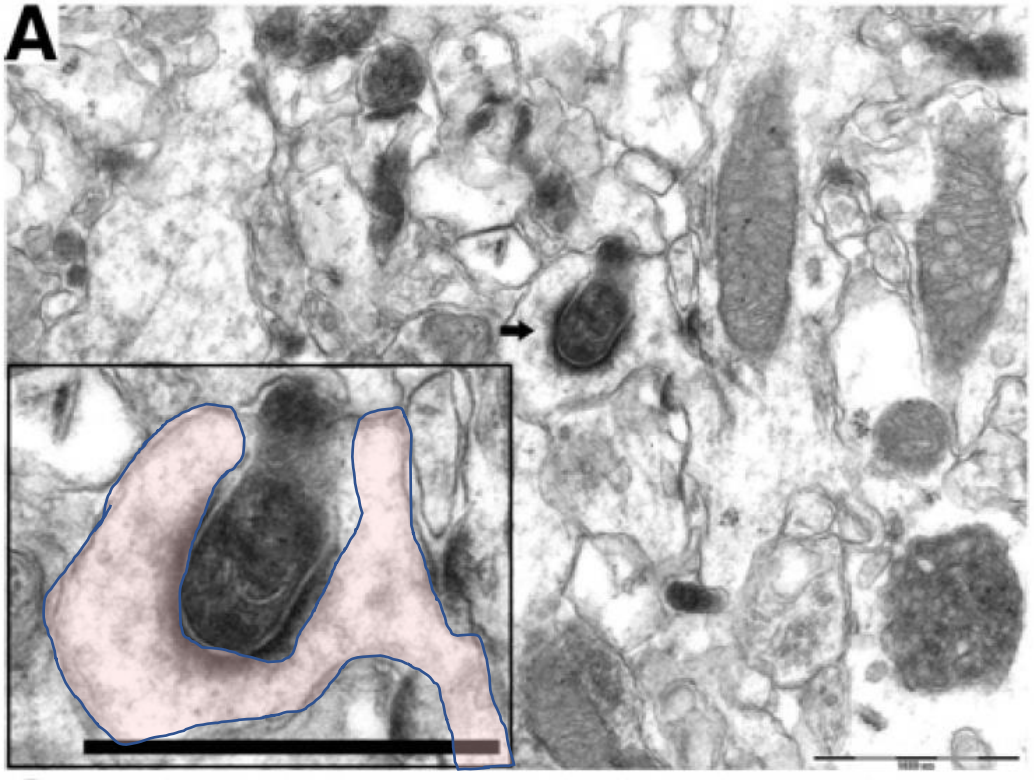
What molecular processes are early disease drivers?

Synapse loss is an early feature of prion disease



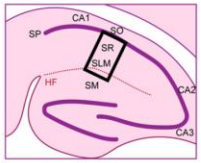
Belichenko, PV, Fraser *et al*, *Neuropathology Appl Neurobio*, 2001

ME7



Jeffrey, M, *et al*, *Neuropathology Appl Neurobio*, 1997
Cunningham, C, *et al, Eur J Neurosci,* 2007
Chiesa, R, *et al, PNAS*, 2005
Mallucci, G, *et al, Neuron*, 2007
Fuhrmann, M, *et al, J Neurosci,* 2007
Fang, *et al, PLoS Pathogens,* 2016, 2018

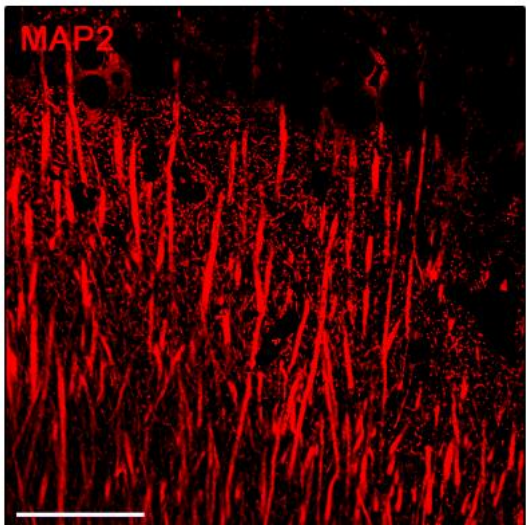
Prion- infected brain:
Loss of dendritic structure and synapses



CA1

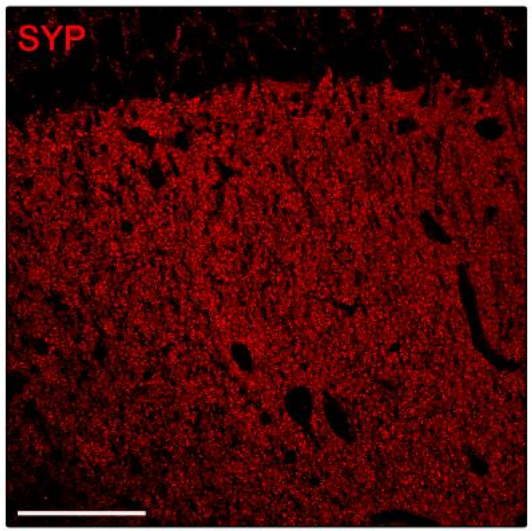
MAP2

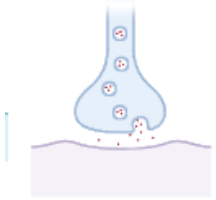
Mock



Synaptophysin

Mock

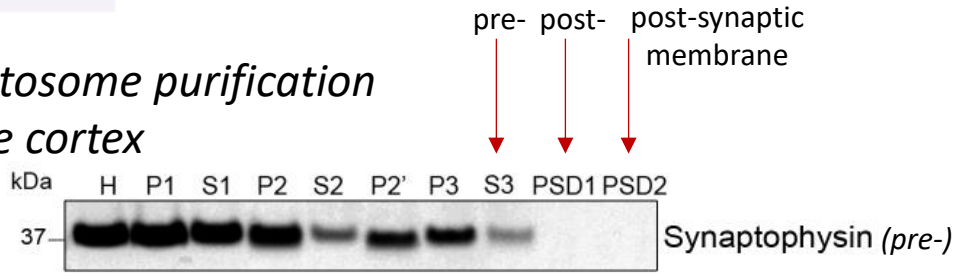


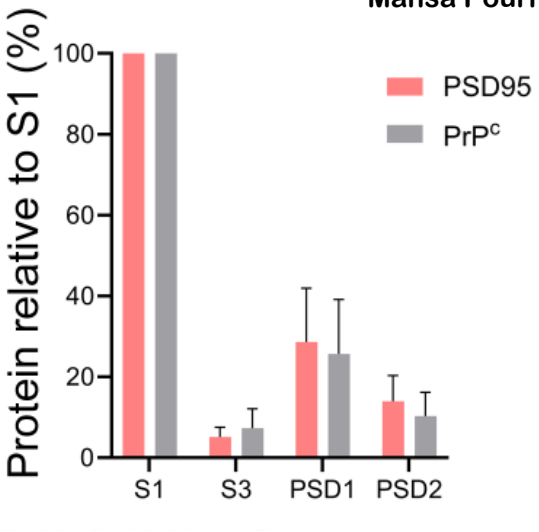


PrP^C is a post-synaptic protein

Mahsa Pourhamzeh, PhD

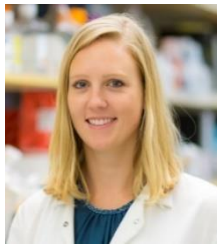
Synaptosome purification
Mouse cortex



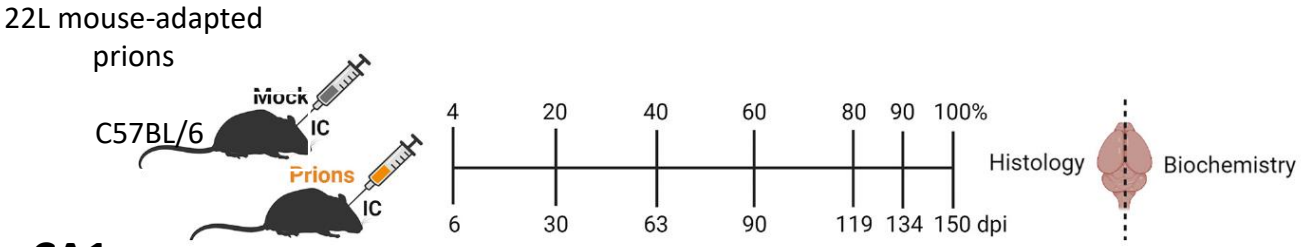


Early prion disease: Ubiquitinated protein aggregates

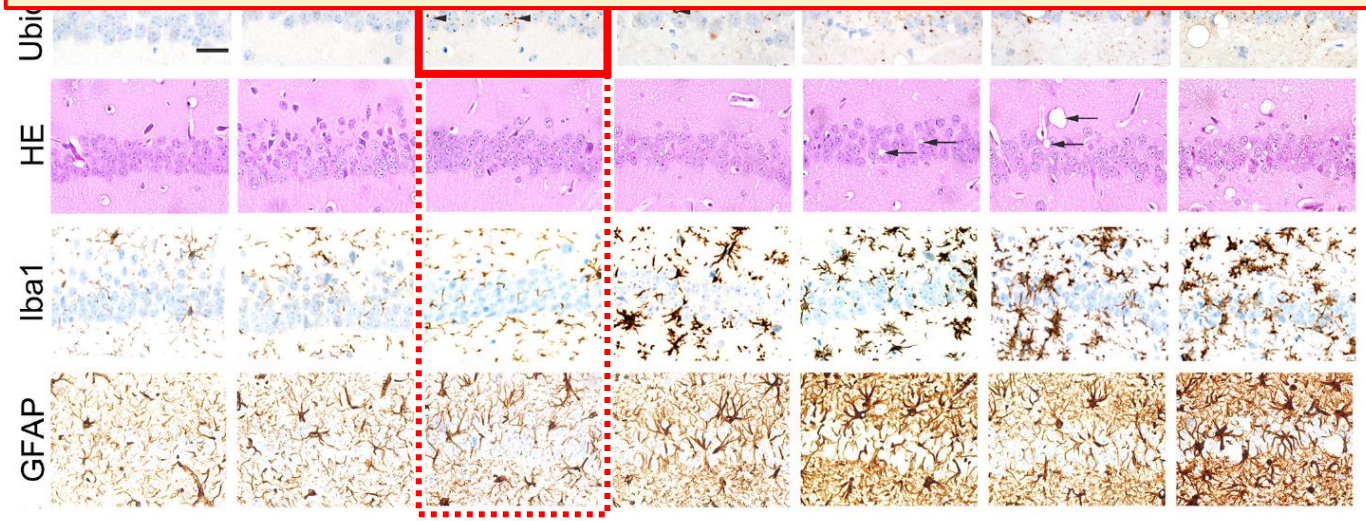
Daniel Ojeda-Juárez

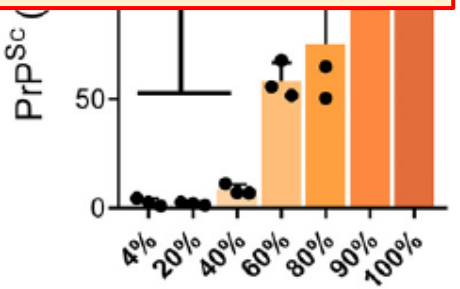


Jessica Lawrence



Increase in ubiquitinated proteins in early prion disease

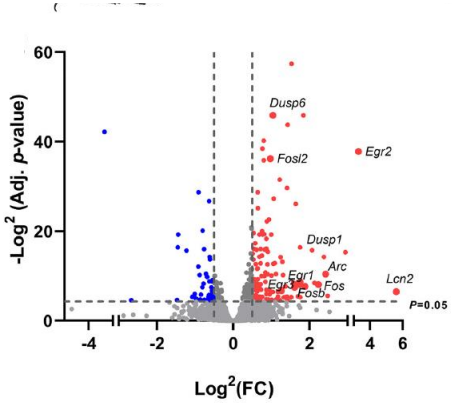


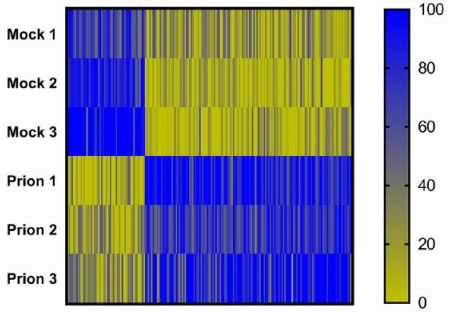


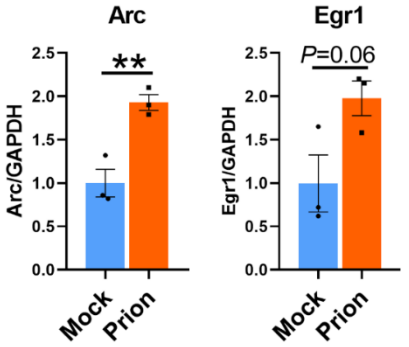
Ojeda-Juárez, D, Lawrence, J *et al*. *Neurobiology of Disease*, 2022

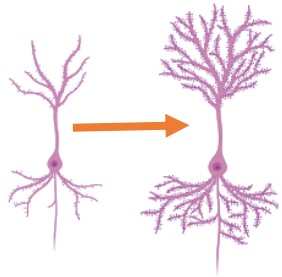
Activation of immediate early genes

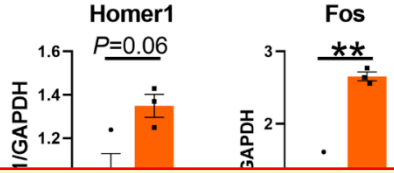
40% = early disease: RNAseq of hippocampus





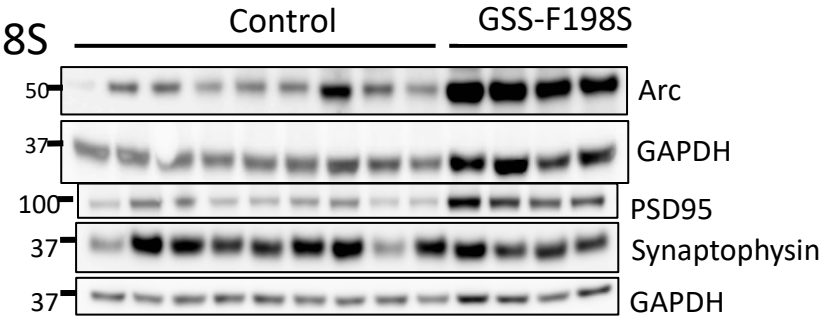


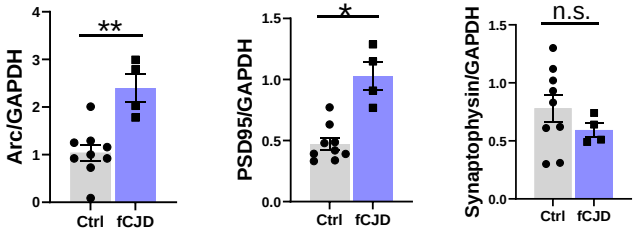




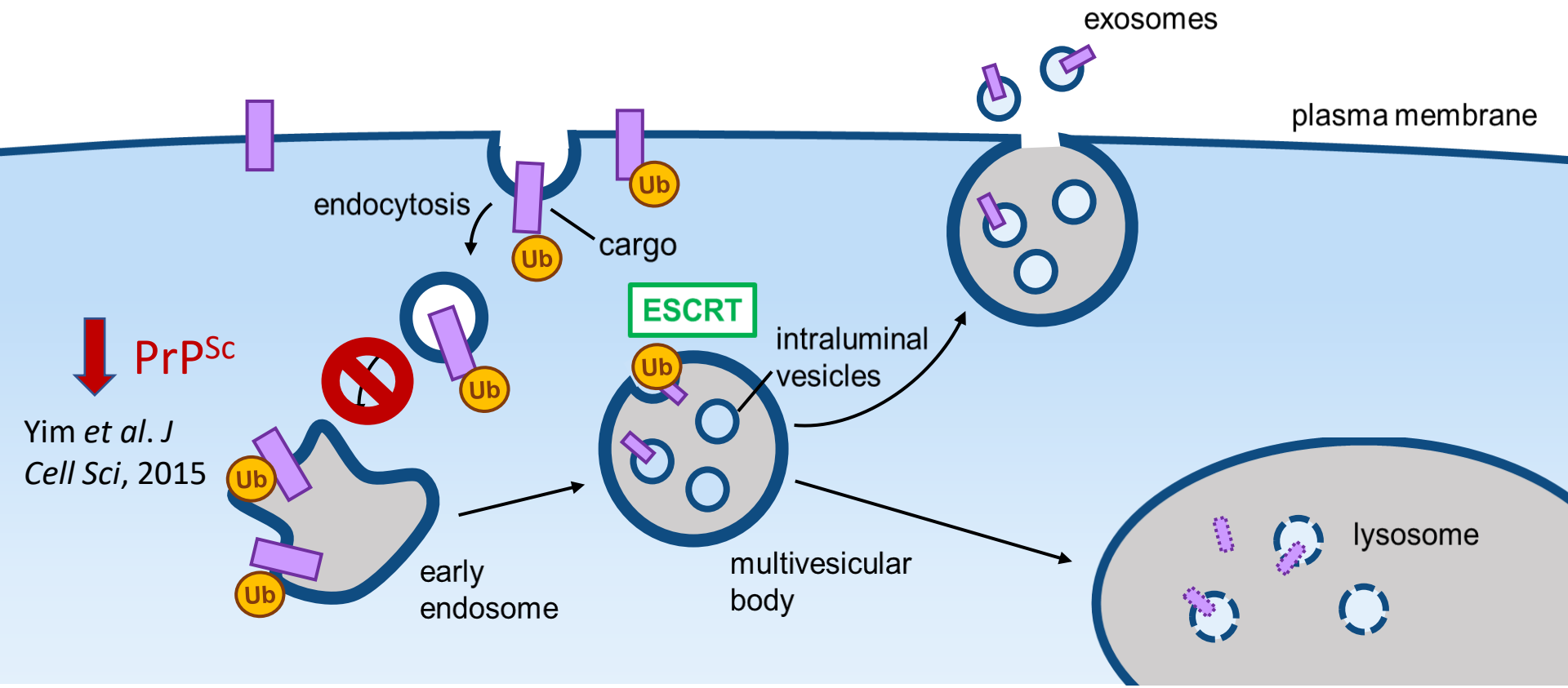
Neuronal activity and proteostatic stress develop in early disease

GSS:
PRNP-F198S





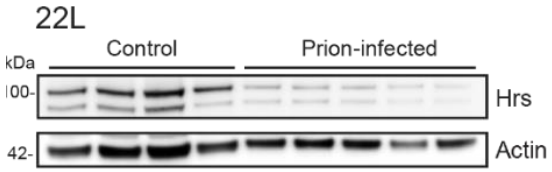
ESCRT pathway: clusters ubiquitinated membrane proteins for clearance through exosomes or lysosomes

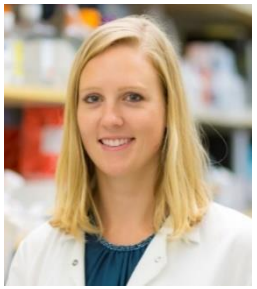


- HRS and STAM1: ancient highly conserved proteins
- FYVE domain to bind early endosomes
- Ubiquitin-interacting motifs
- Sorts ubiquitinated proteins

What are the levels of ESCRT-0 in prion-affected brain?

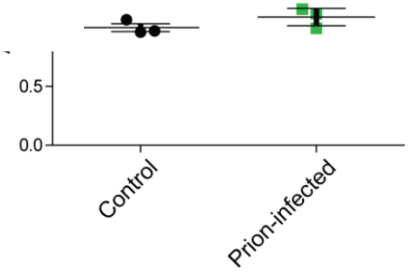
Massive reduction of *ESCRT-O* in the terminal prion-infected brain





Jessica Lawrence

Hrs



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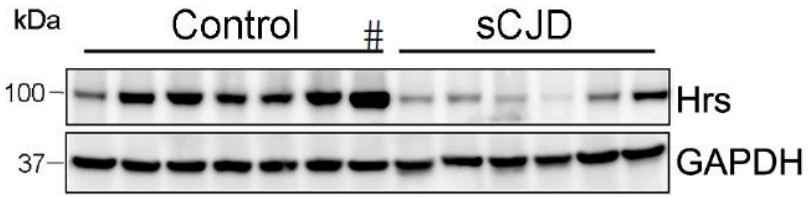
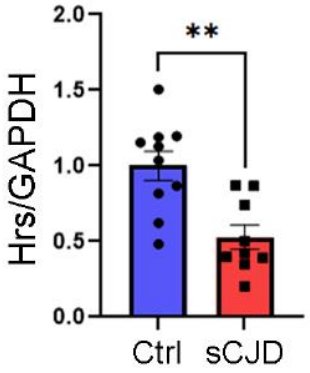




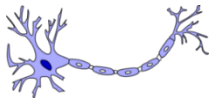
Lawrence, JA *et al*, *J Neurosci.,* 2023

How does Hrs depletion impact prion conversion and disease progression *in vivo*?

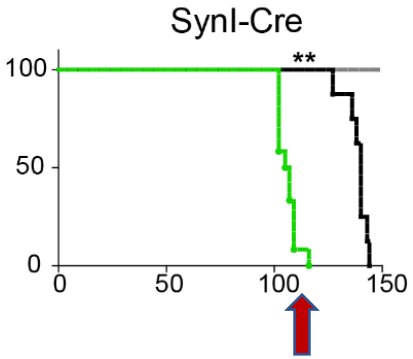
Hrs$^{f/f}$

Hrs$^{f/f}$Syn1-Cre

Neurons



Prion inoculation
IC (22L)



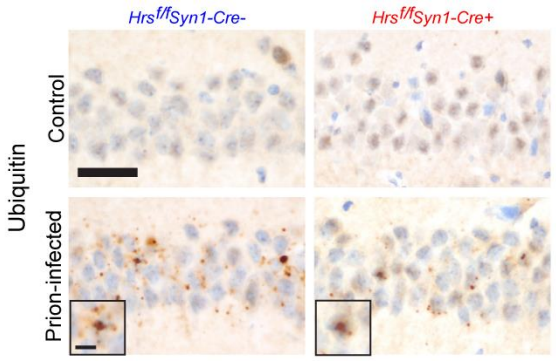
Accelerated disease

Tamai et al., Am J Pathol, 2008

What process(es) drives the disease acceleration?

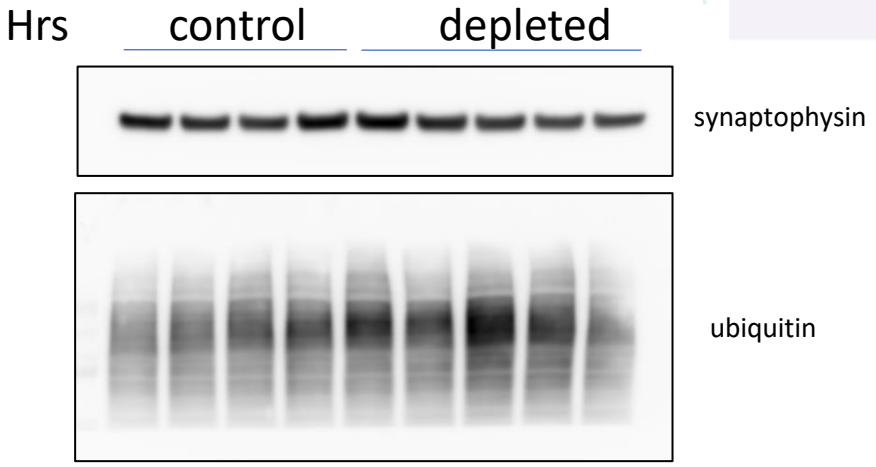
Lawrence, JA *et al*, *J Neurosci.*, 2023

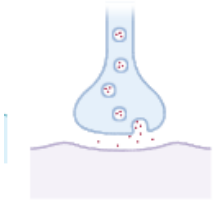
Proteostasis: Hrs depletion *accelerates* ubiquitinated protein accumulation at synapses

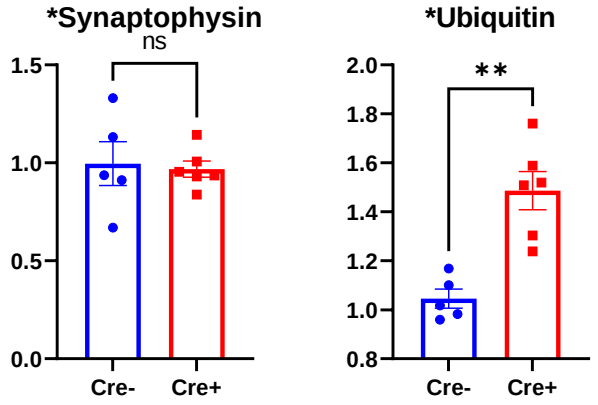


Prion-infected synaptosomes

106 dpi

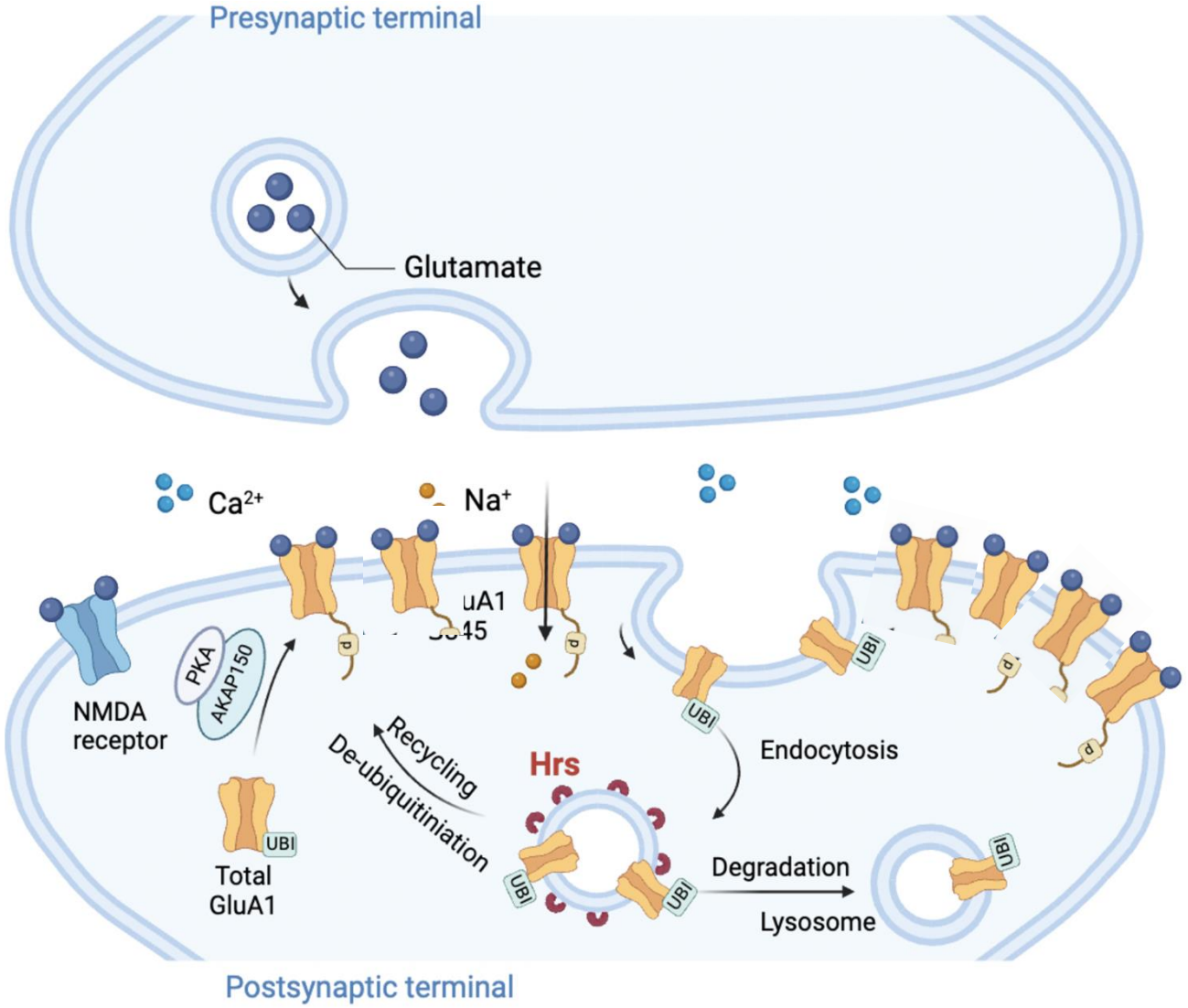






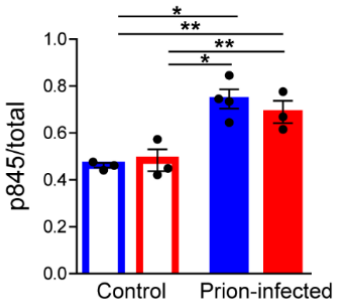
No difference in pre-synaptic synaptophysin, but ubiquitinated protein accumulation was accelerated

Glutamate receptors: Hrs depletion *accelerates* build-up of surface AMPA receptors



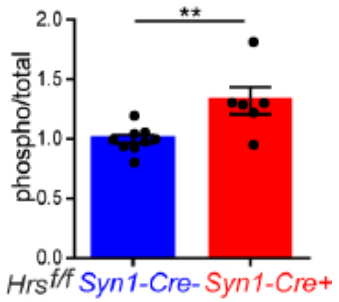
terminal

pGluA1-S845

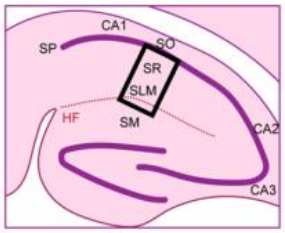


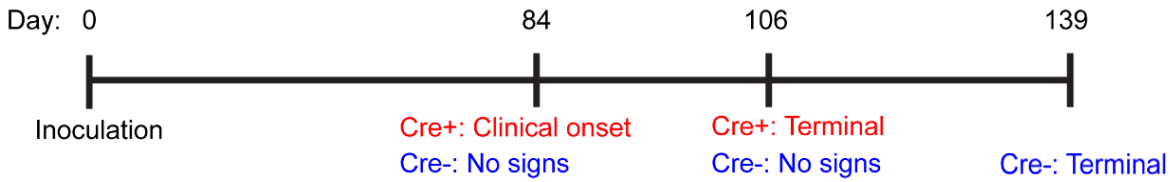
106 days post-inoculation

pGluA1-S845

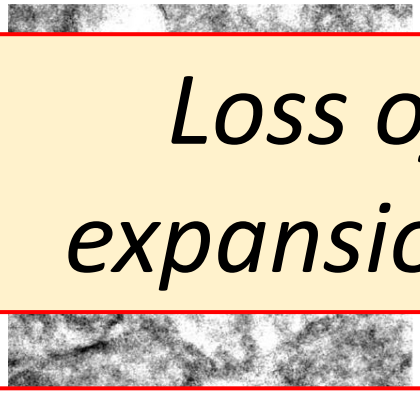


Neuronal Hrs depletion *accelerates* synaptic structural changes in prion-infected mice





84 dpi

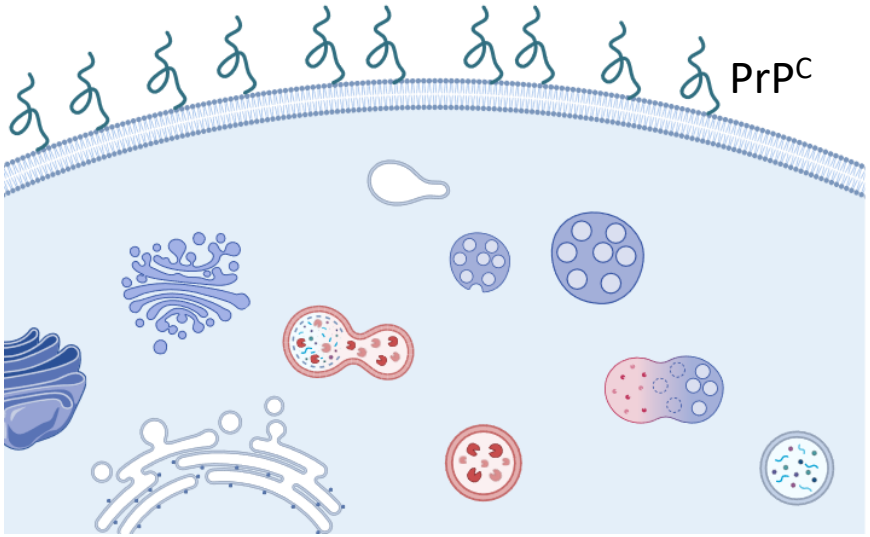


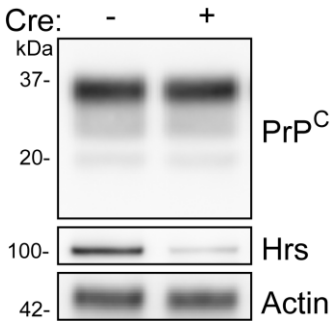
Loss of Hrs accelerates synaptic expansion and reduces survival time

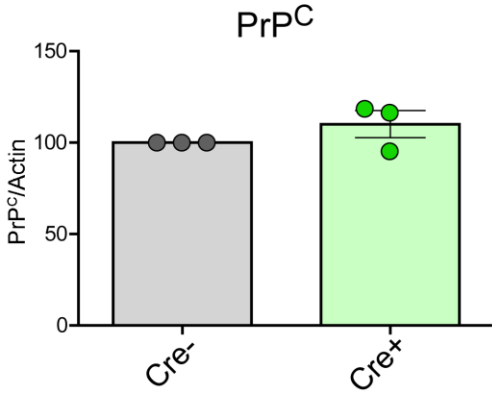
How does Hrs affect cell surface PrP^C levels?

Increased surface PrP^C in Hrs-depleted neurons

Total PrP^C levels are unchanged

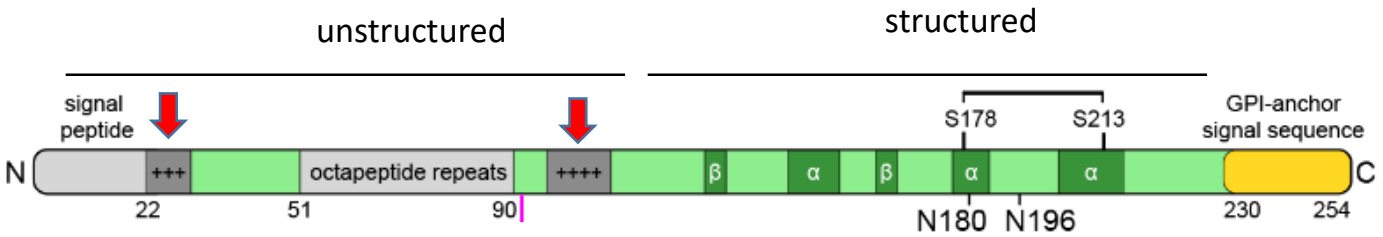






Working model: Loss of Hrs in prion disease exacerbates retention of surface proteins at excitatory synapses, including AMPAR and PrP^C, accelerating synaptic expansion and excitotoxicity

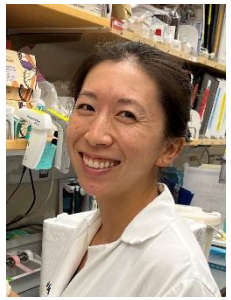
PrP^c N-terminus is implicated in neurotoxicity



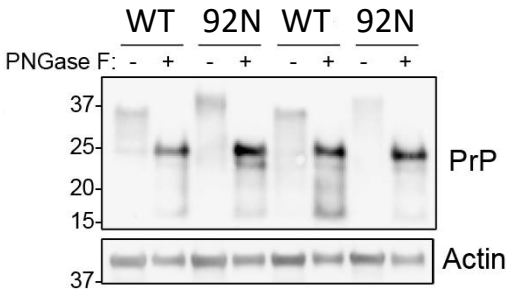
N-terminus is linked to toxic signaling

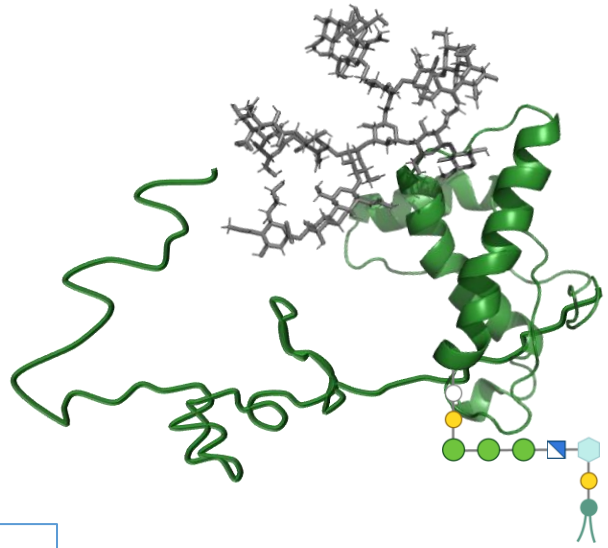
Knockin mice: Spontaneous

Julia Callender, PhD



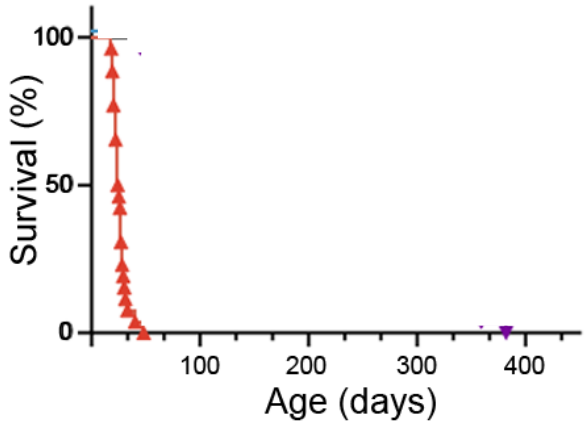
Joie Lin, DVM

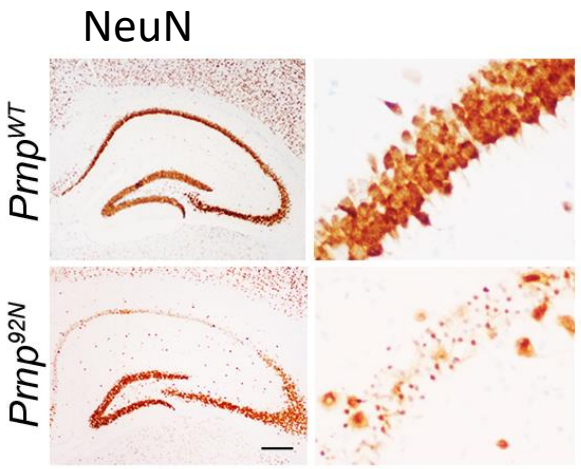


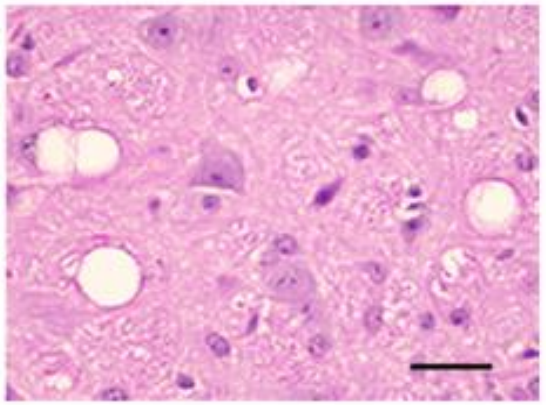


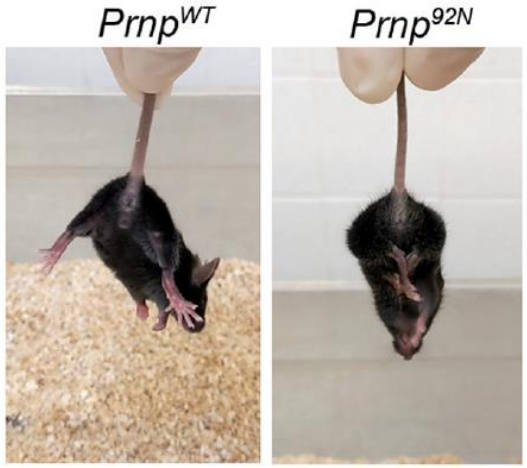
Indistinguishable after deglycosylation

Prnp^{92N} mice develop rapidly progressive neurodegenerative disease









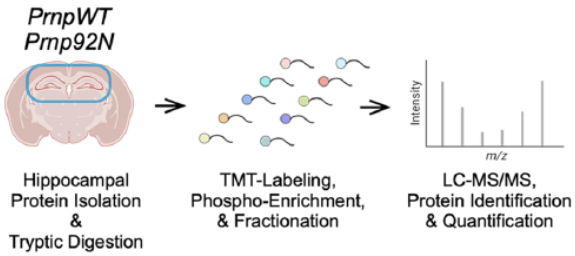
+ seizures, tremors

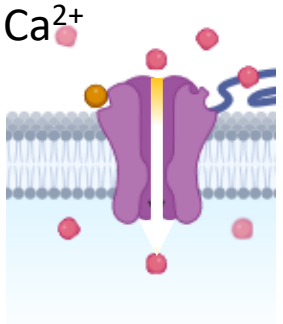
- PrP^{92N} traffics to cell surface
- **Does not aggregate, seed conversion, or spread to mice**

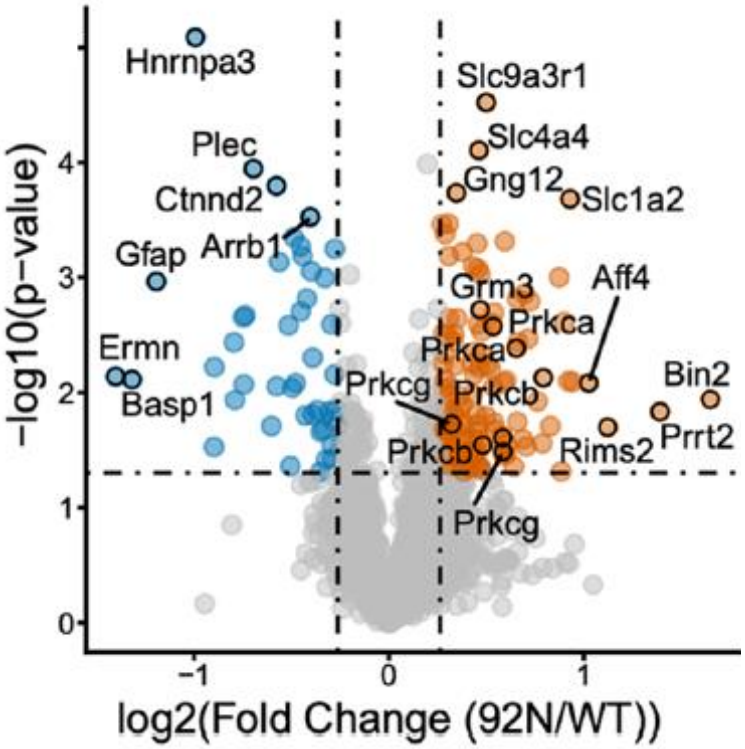
PrP^{92N} knockin model: uncouples PrP aggregation from neurotoxicity

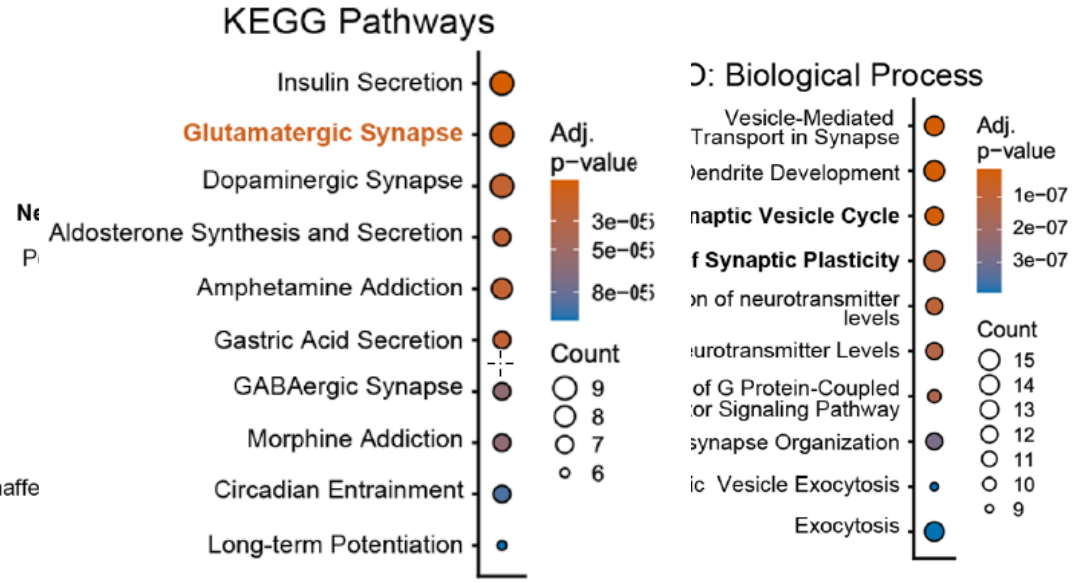
Phosphoproteomics of hippocampus implicate protein kinase C and glutamatergic signaling

20 day old



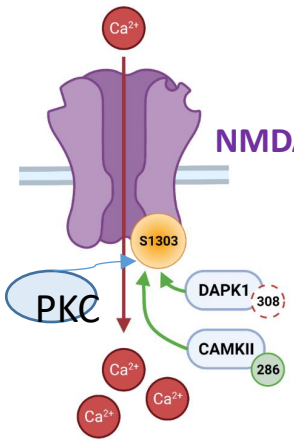


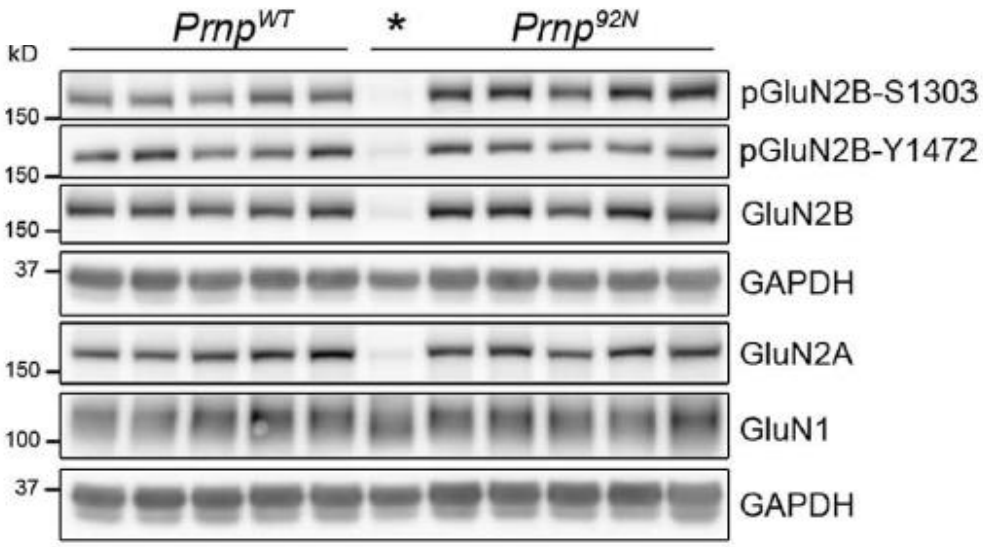


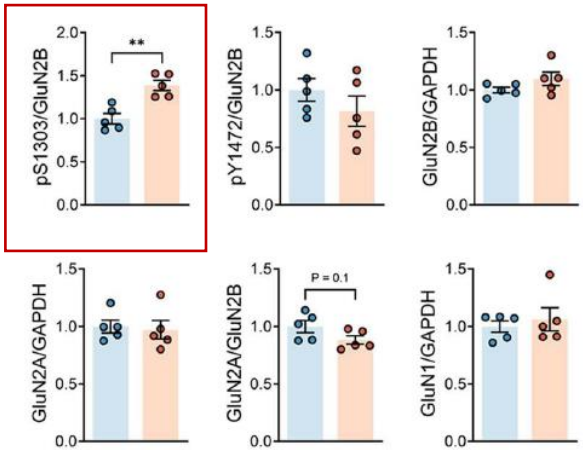


John R. Yates, III, Dan McClatchy, Joshua Mayfield

Phosphoproteomics: Increased S1303-GluN2B in *Prnp*^{92N} mice



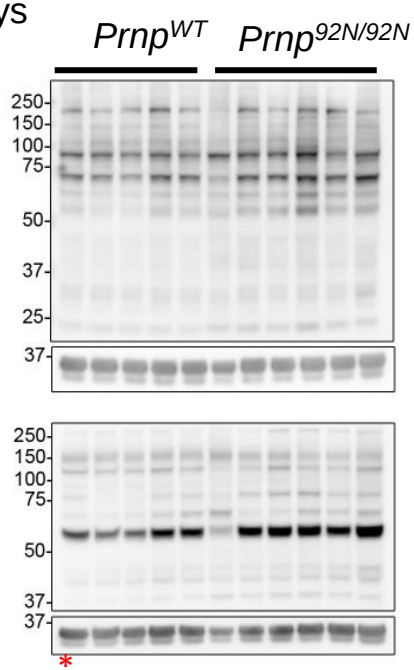


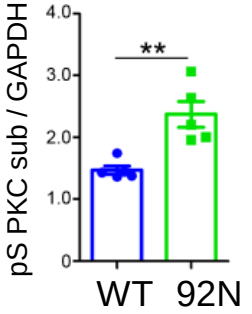


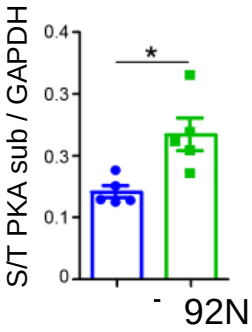
What kinases phosphorylate NMDA receptor subunit, GluN2B, at 1303?

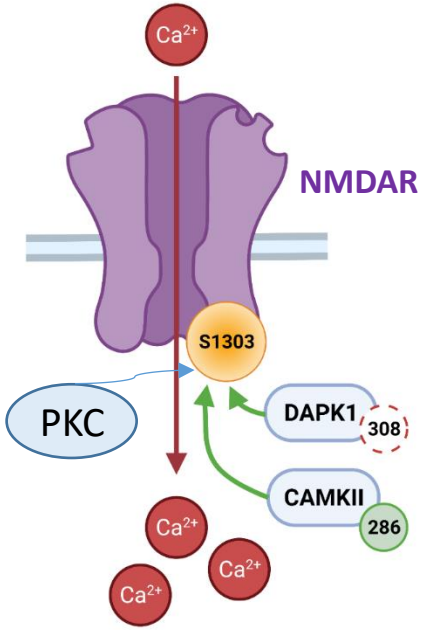
Active calcium responsive kinases

Age: 20 days





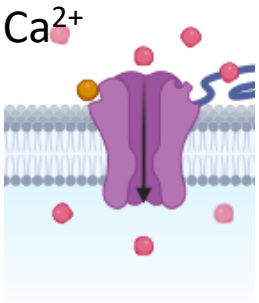




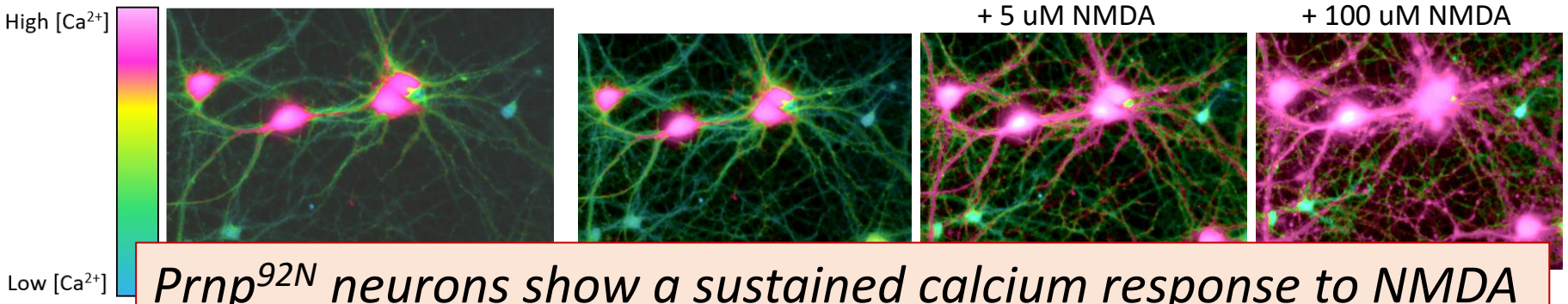
Increased NMDAR-2B channel conductance
Increased excitotoxicity
Decreased recovery from stroke (Tu et al. *Cell* 2010)

KC, PKA, and ERK activity

Altered calcium response to NMDA

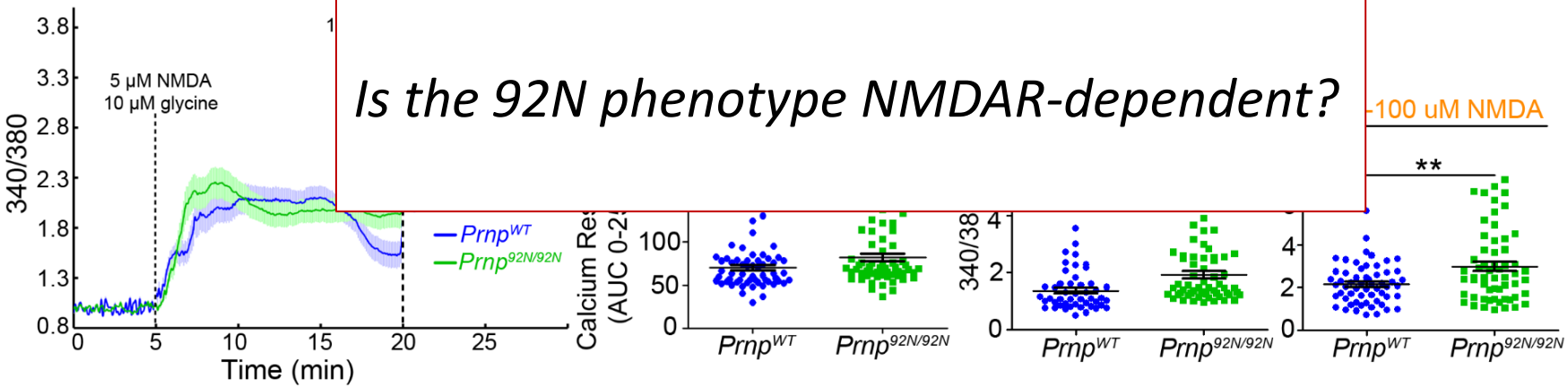


Cortical neurons, DIV 14-16
Loaded with Fura2 (calcium sensitive)

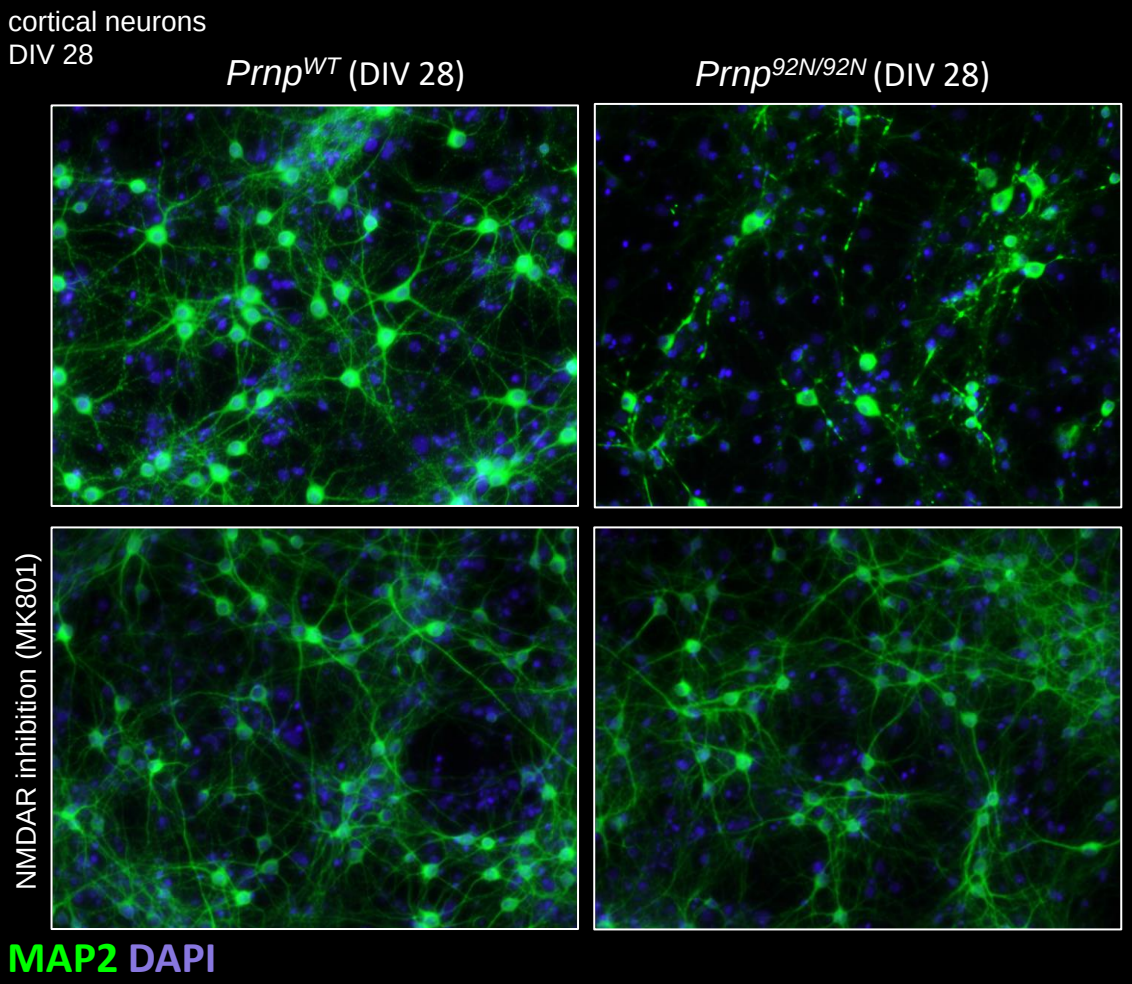


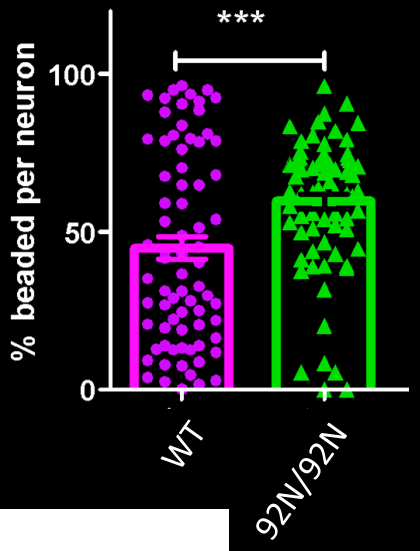
Prnp^{92N} neurons show a sustained calcium response to NMDA

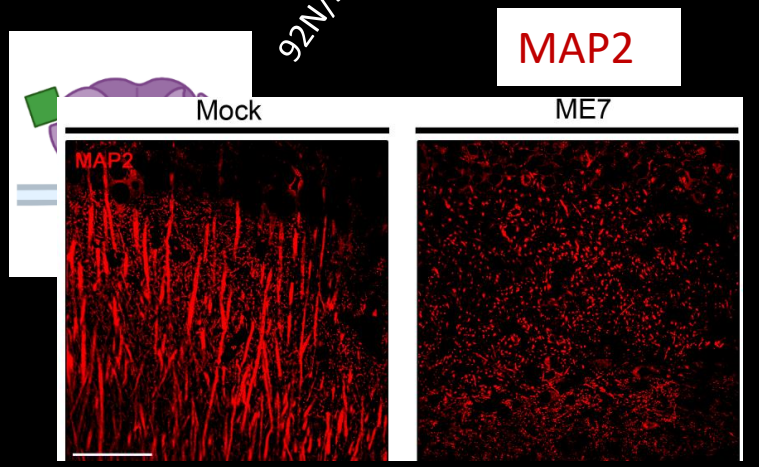
Is the 92N phenotype NMDAR-dependent?



Increased excitotoxicity displayed by *Prnp*^{92N} neurons is NMDAR-mediated

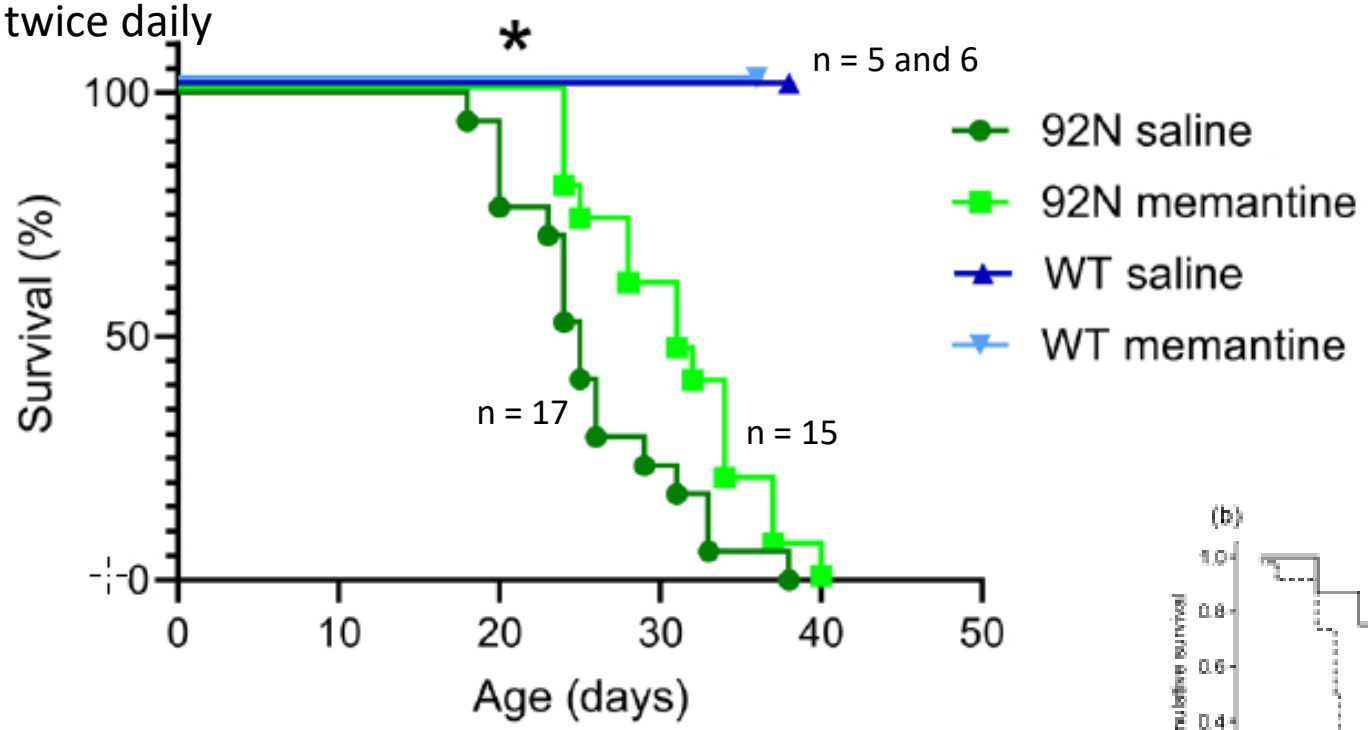






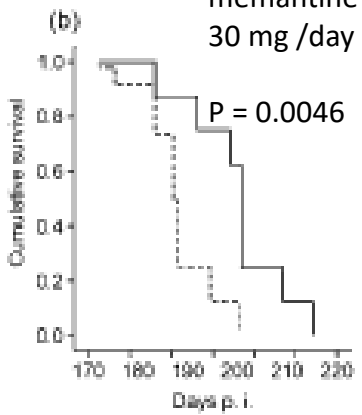
Prolonged survival in 92N mice treated with NMDA antagonist, memantine

10 mg/kg IP, twice daily



…relevance of the Prnp^{92N} to prion disease?

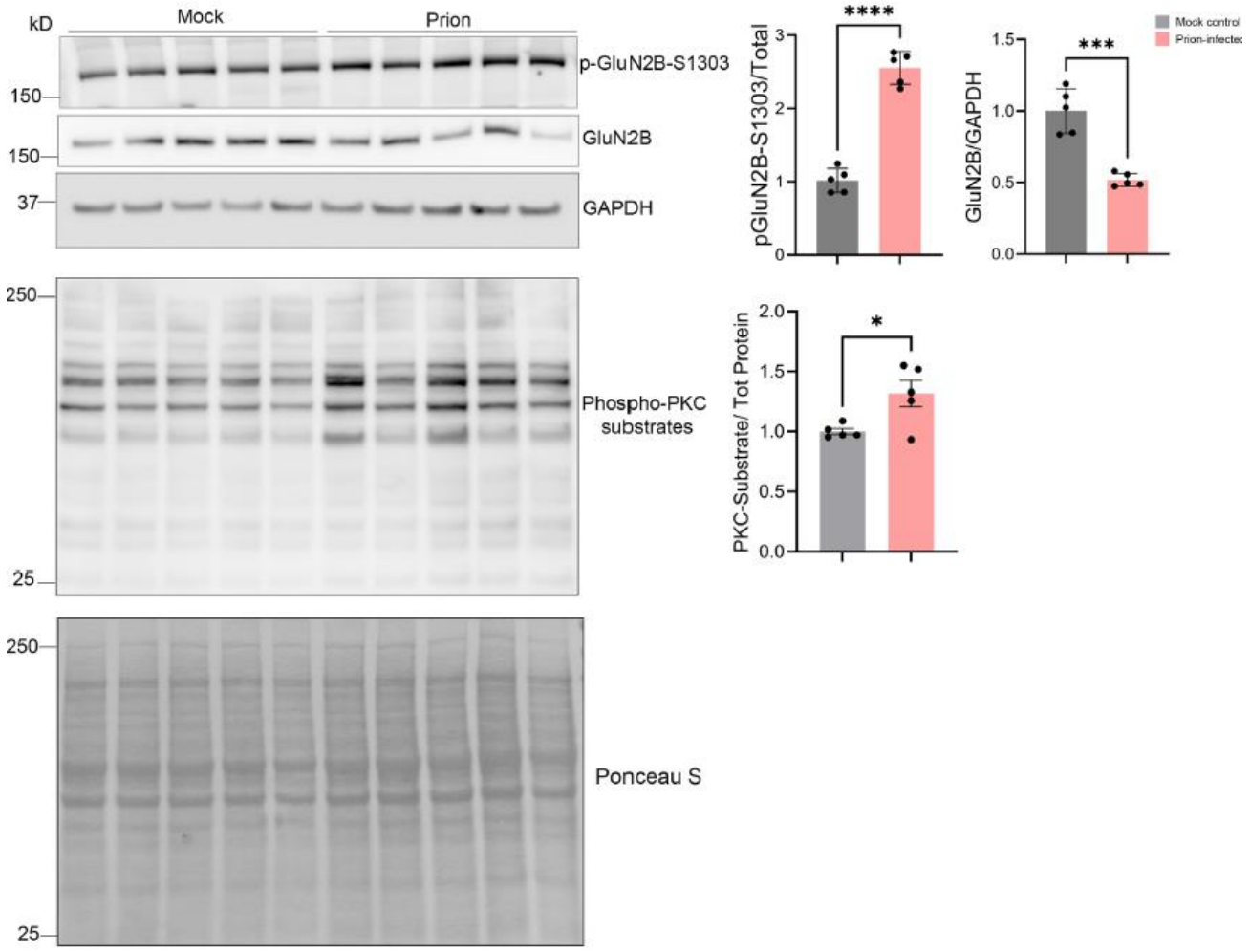
100dpi, (139A)
memantine
30 mg /day PO

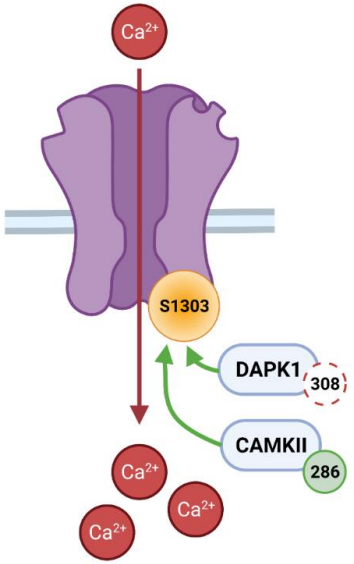


Riemer…Baier, 2008, J Gen Virol

Prion-infected mice also show increased pGluN2B-1303 and PKC substrates

22L cortex
80% timepoint

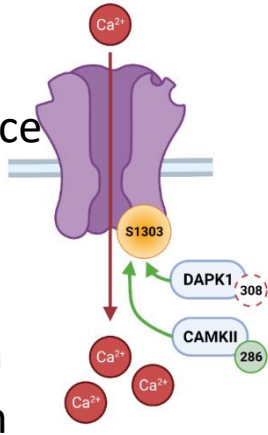




Summary: Disrupted synaptic activity and PKC activity in prion disease

- PrP^C localizes to the post-synaptic membrane

- Early increases in IEGs, Arc and cFos, are suggestive of increased neuronal activity



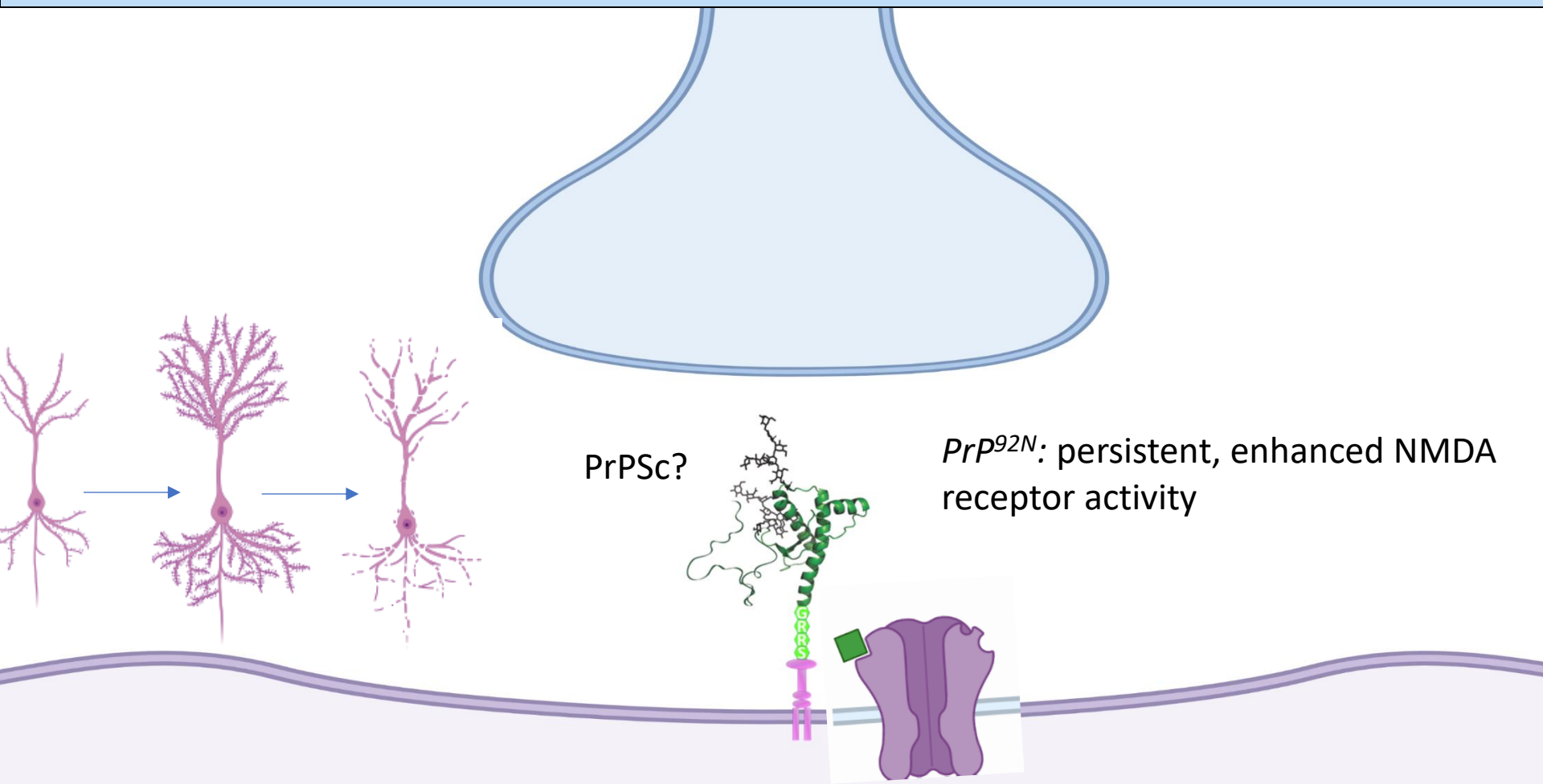
- Ubiquitinated proteins build early, while ESCRT-0 decreases in prion disease. Depleting ESCRT-0 *raises surface PrP^C*, potentiates large post-synapses and surface AMPAR, and accelerates disease.

- *$Prnp^{92N}$* knockin model of prion excitotoxicity shows spongiform change, gliosis, neuronal loss *in absence of aggregates*. Neurons show sustained high calcium in response to NMDAR activation. NMDAR are phosphorylated, to increase calcium influx. The disease is delayed by NMDA antagonist, memantine (also used in patients)

- Protein kinase C activity (calcium-sensitive kinase) is increased, also seen in prion disease

- PKC could exacerbate calcium influx through NMDAR-phospho. – TBD…

Model: prion excitotoxicity



PrPSc?

PrP^{92N}: persistent, enhanced NMDA receptor activity

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