A background image showing a network of neurons with various colored axons and dendrites, some appearing in shades of red and orange against a light grey background.

Development of Novel AD Therapeutics: Targeting Calcium Channels

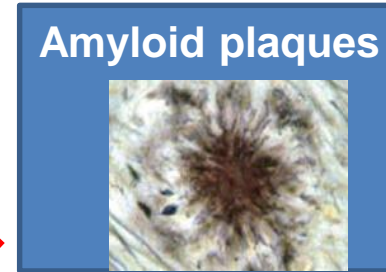
Beth Stutzmann, Ph.D

Professor / Department of Neuroscience

Director, Center for Neurodegenerative Disease and Therapeutics

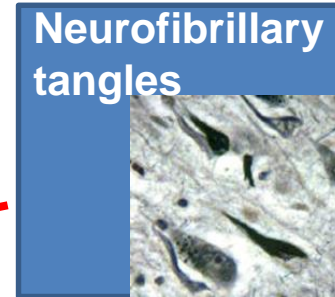
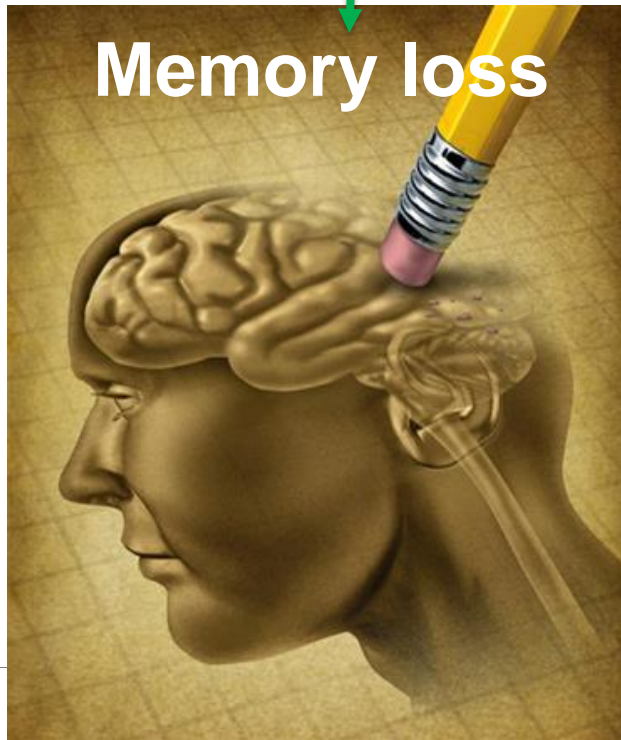
***Rosalind Franklin University of Medicine and Science
The Chicago Medical School***

Where is the field today in terms of understanding and treating cognitive deficits in AD?



Amyloid hypothesis under scrutiny

The only feature of AD that directly correlates with cognitive impairment. No known therapeutics.

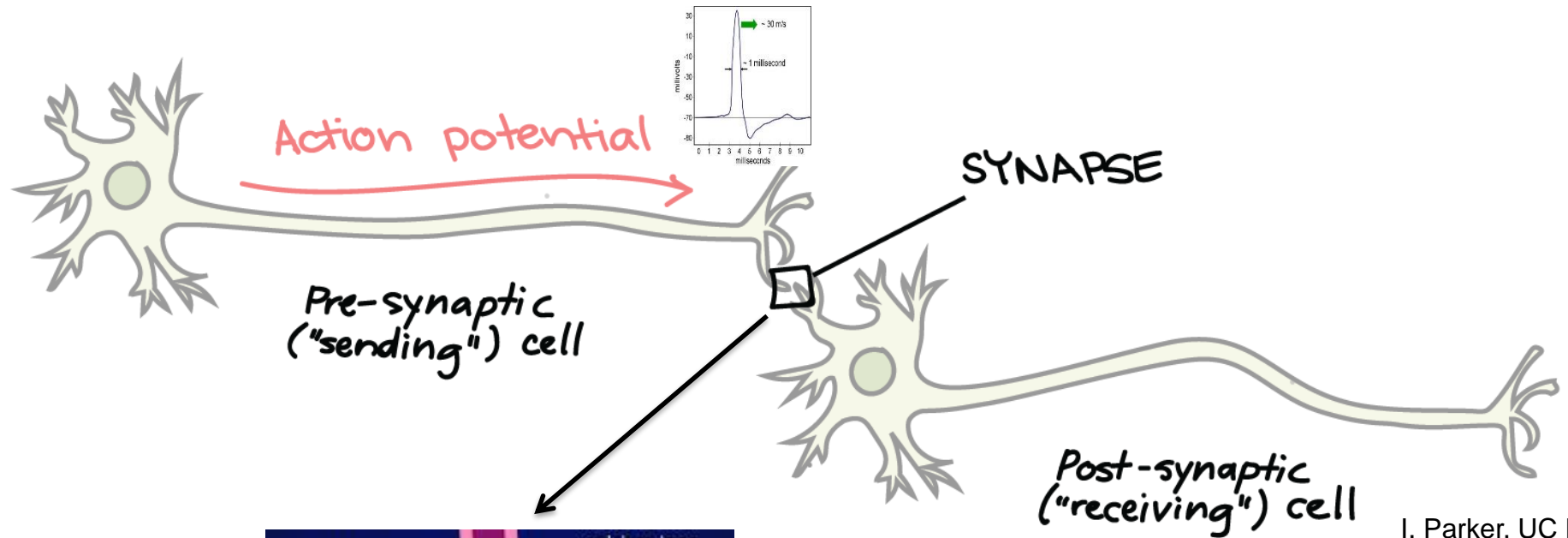


Association with memory loss unclear but correlations exist, later stage feature.

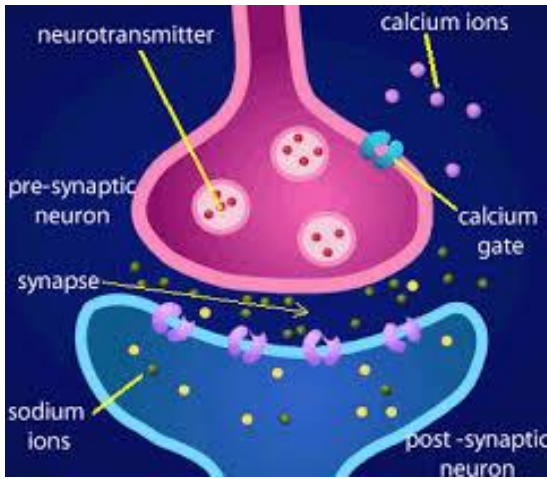


➤ \$4B is spent annually on AchE inhibitors that only have symptomatic effects

Synaptic transmission and plasticity



I. Parker, UC Irvine

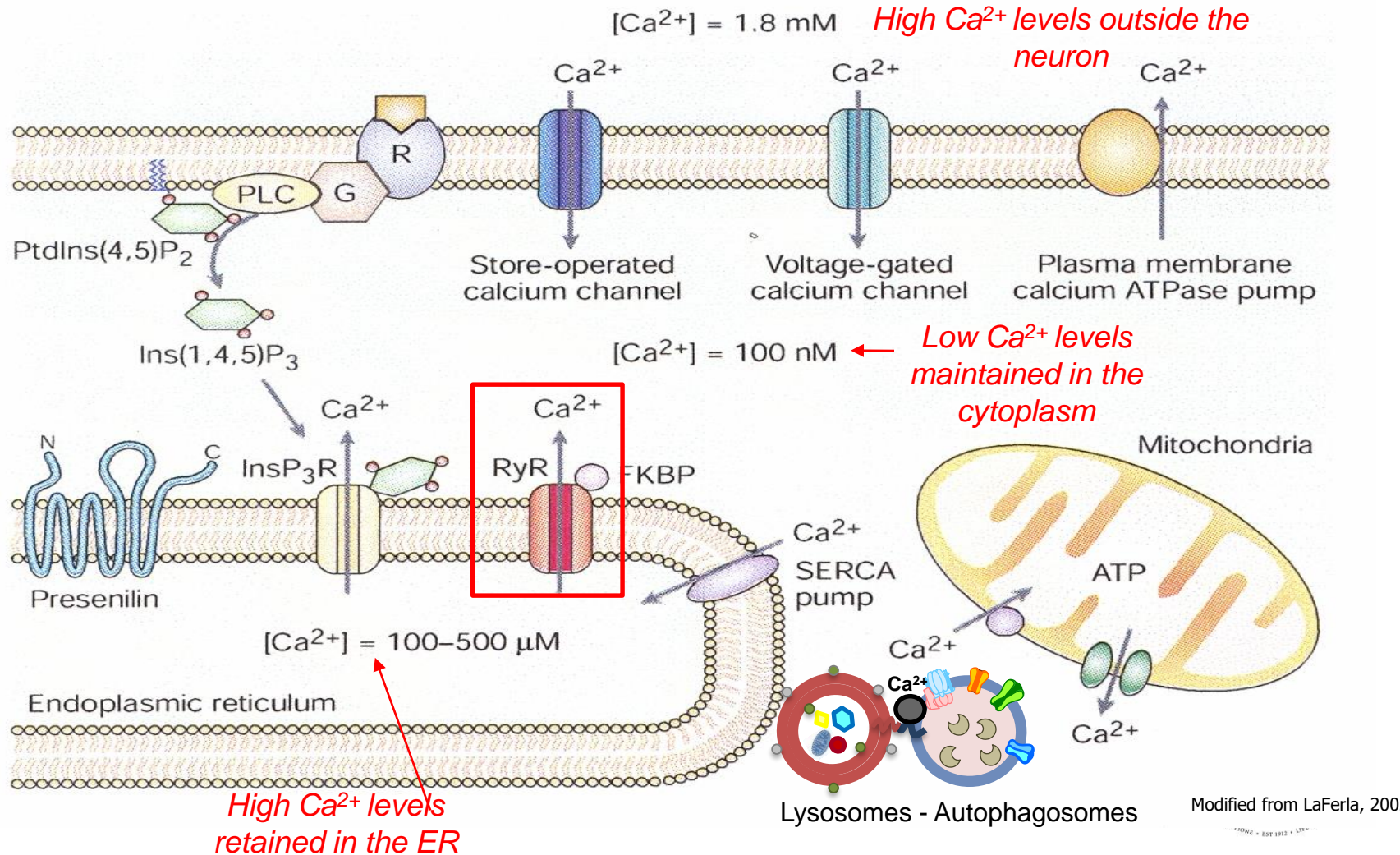


Changes in the signaling strength at the synapse underlies learning and memory. This is dependent on the signaling ion, **calcium**.



www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/a/the-synapse

Ca²⁺ signaling is critical for neuronal health and synaptic function



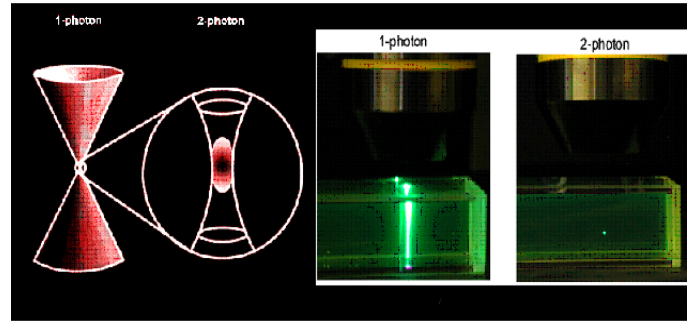
Modified from LaFerla, 2002

Technical approaches used to study AD mechanisms in the Stutzmann lab:

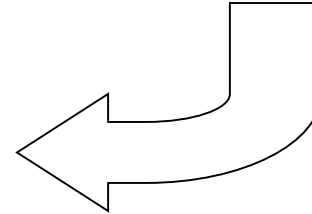
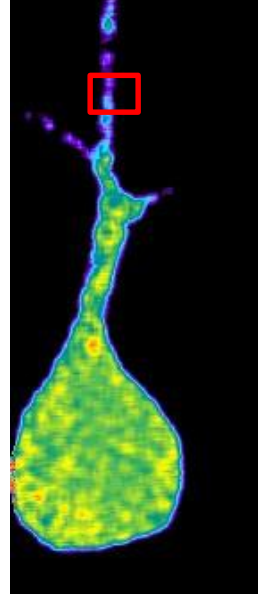
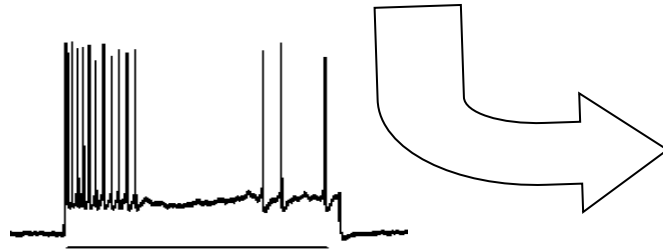
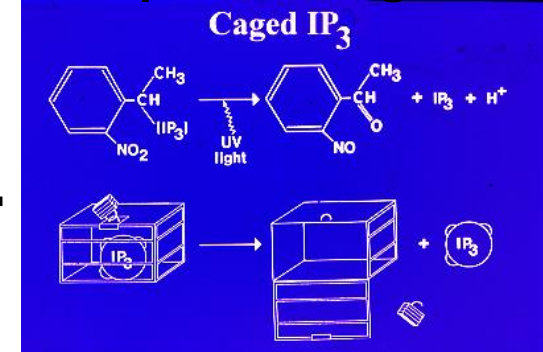
***In vitro* patch clamp electrophysiology**



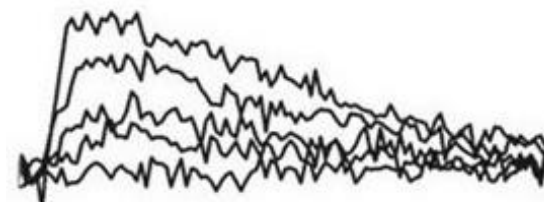
Video rate 2-photon calcium imaging



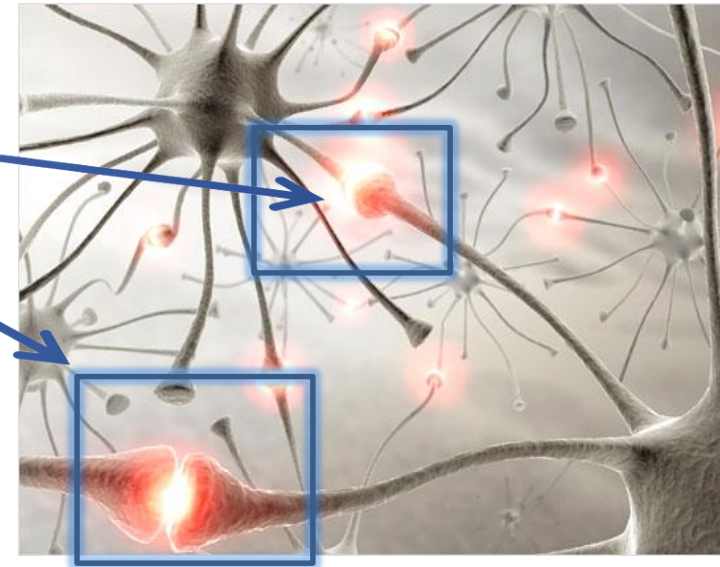
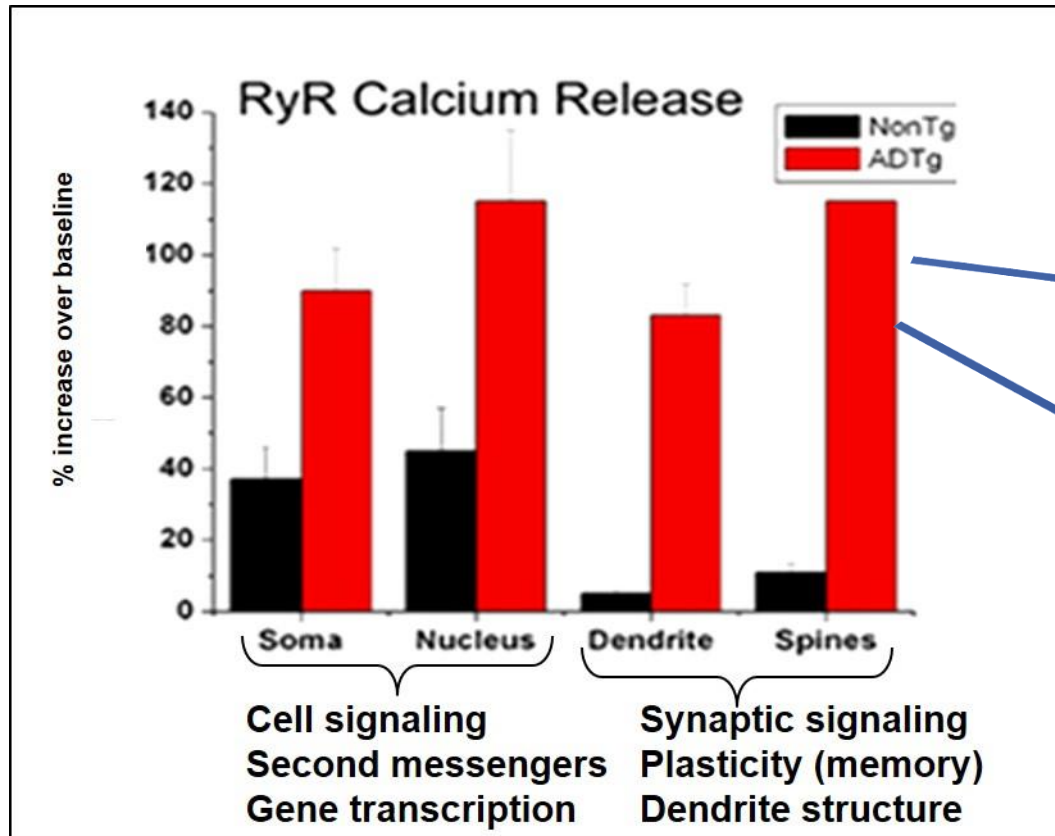
UV photolysis of caged compounds / agonists



Calcium response



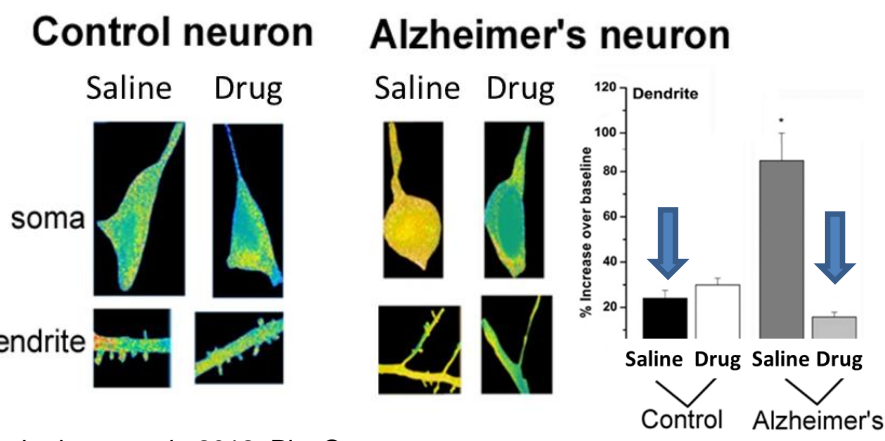
ER calcium release in AD mouse models is greatest in synapses relative to control neurons.



(Goussakov et al., 2010)

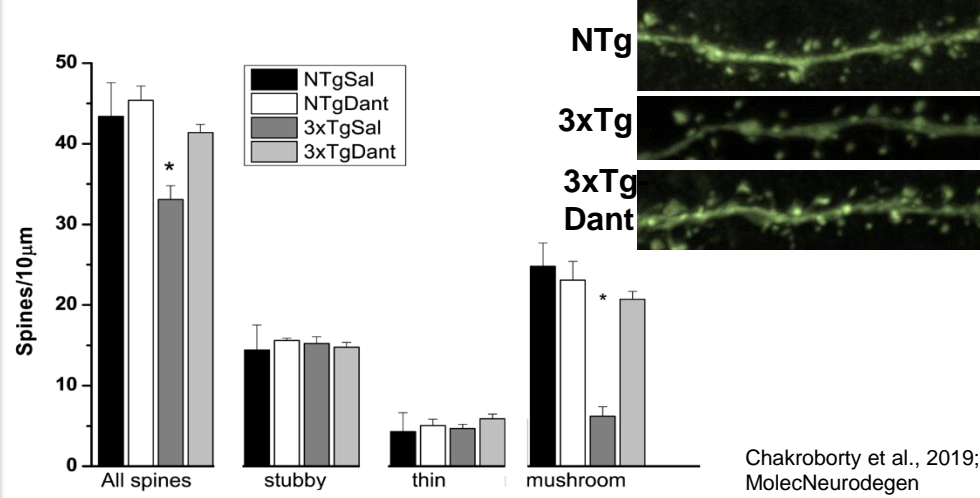
In Vivo Proof of Concept–Dantrolene (RyR inhibitor) as AD Therapeutic?

1. Calcium signaling normalized

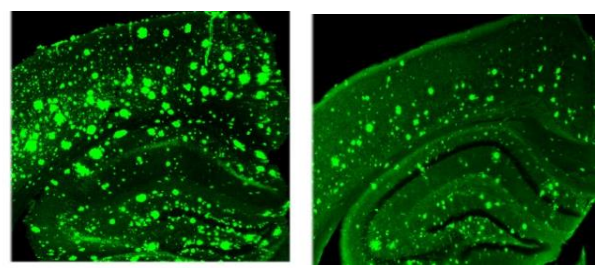


Chakroborty et al., 2012; PlosOne

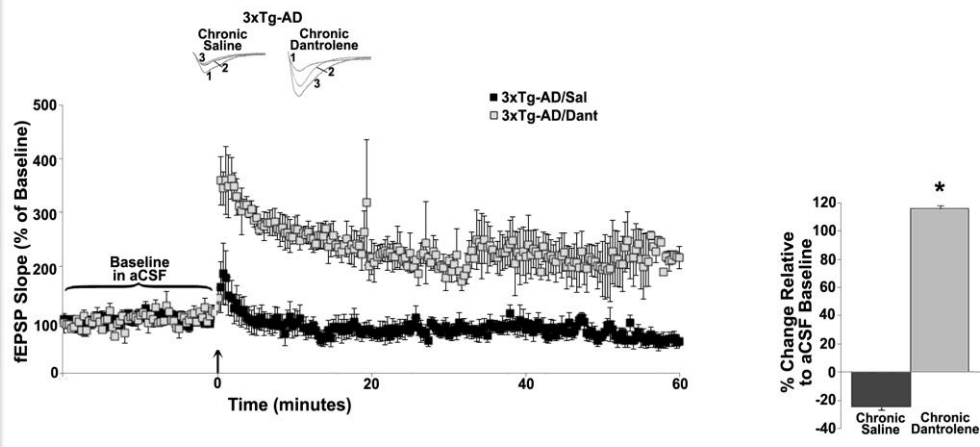
2. Restoration of stable dendritic spines and synapses in AD models



3. Soluble and Insoluble Amyloid Reduced by >45%



4. Synaptic plasticity is restored



5. Cognitive function preserved, and other pathogenic features reversed (Chakroborty et al., 2012; Oules et al., 2012; Peng et al., 2012)

Dantrolene not suitable for CNS targets:

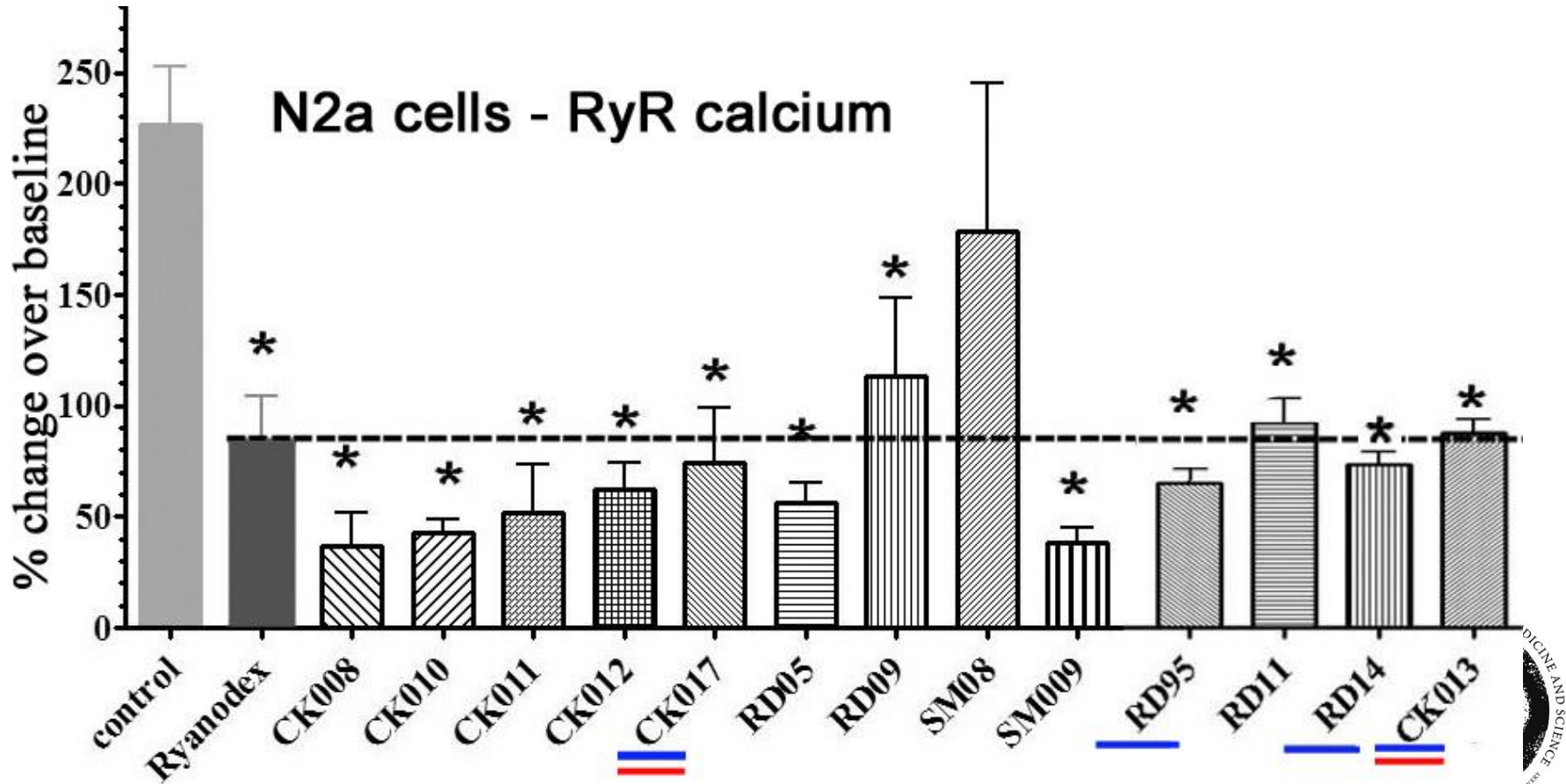
Development of Novel RyR2 Allosteric Regulators for AD

- We have developed a series of small molecule compounds designed as allosteric modulators of the RyR2 (with John Buolamwini, Ph.D; Chair, Pharmaceutical Sciences, CoP).
- Over 100-fold more brain penetrant than dantrolene
- Currently developed compounds have reasonable PK and no detectable cardiovascular or adverse effects in animal models.
- Stable, water soluble, small molecules (MW ~250-420 kD)
- Do not function as channel blockers or antagonists, rather as allosteric modulators that maintain RyR function within physiological levels.



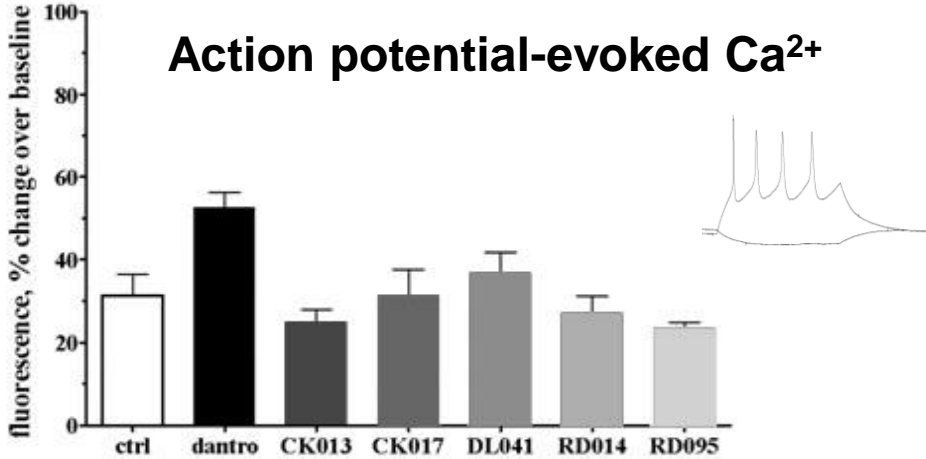
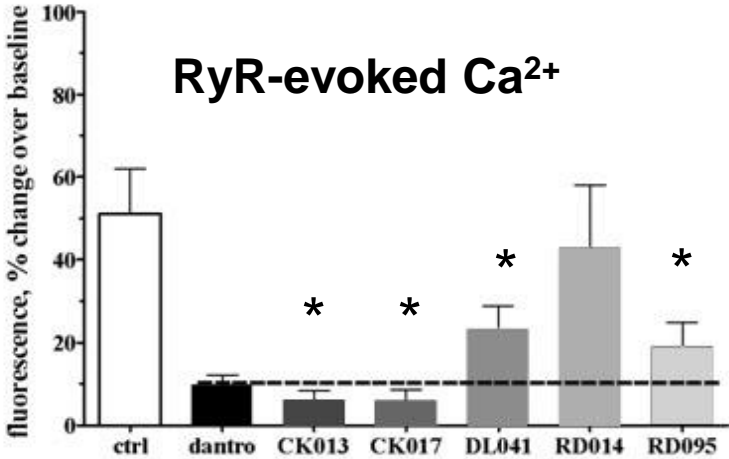
Ligand Based Design: Our RyR2 stabilizers prevent excessive Ca²⁺ release in model cell systems

(10 μ M; 30 minute incubation)

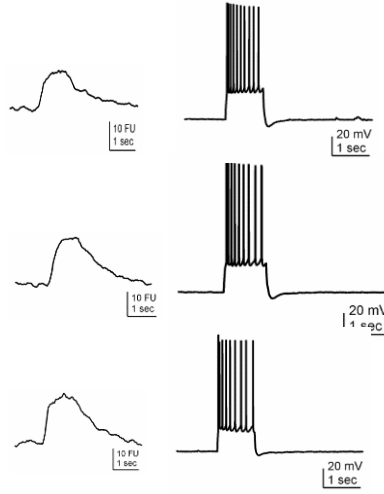
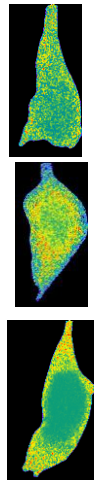
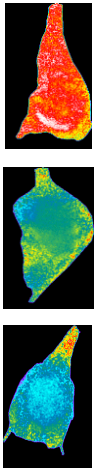


The RyR2 compounds selectively normalize pathogenic RyR-calcium responses in neurons from AD mice

(hippocampal slices incubated for 1 hour; 10 μM)

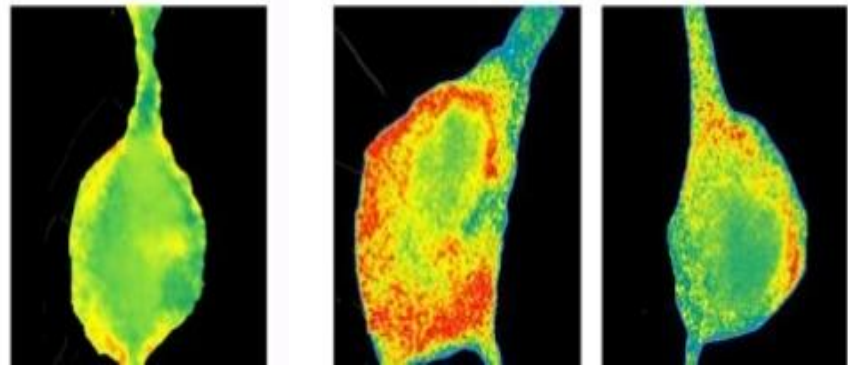
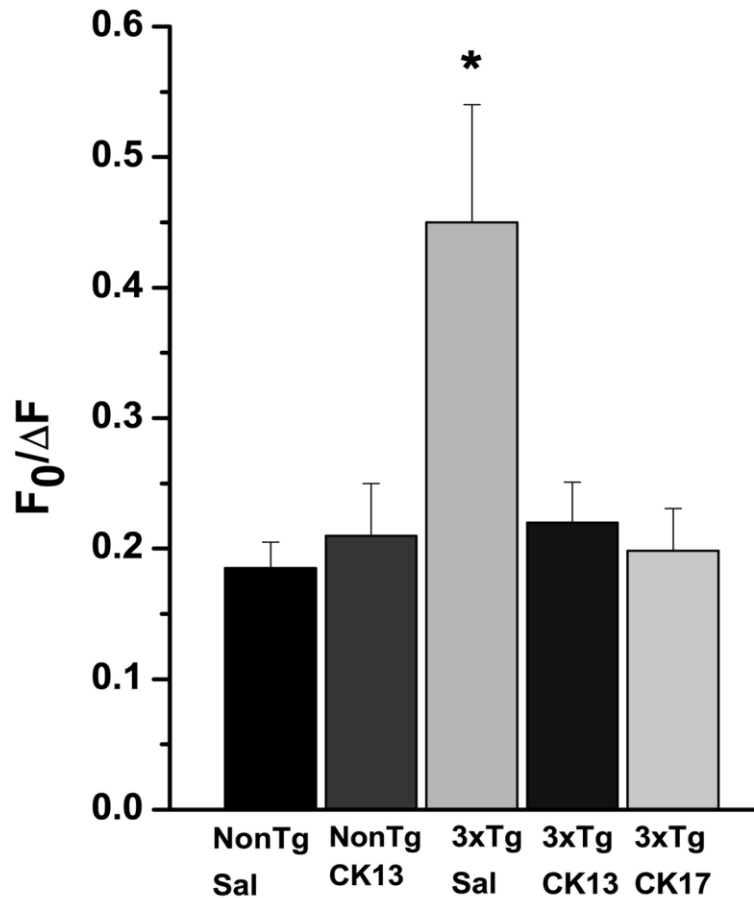


control
dantrolene
CK013



Chronic Treatment in AD and NonTg mice reverses RyR-calcium abnormalities

(3 and 9 months of age, 4 week treatment, IP, 10mg/kg)



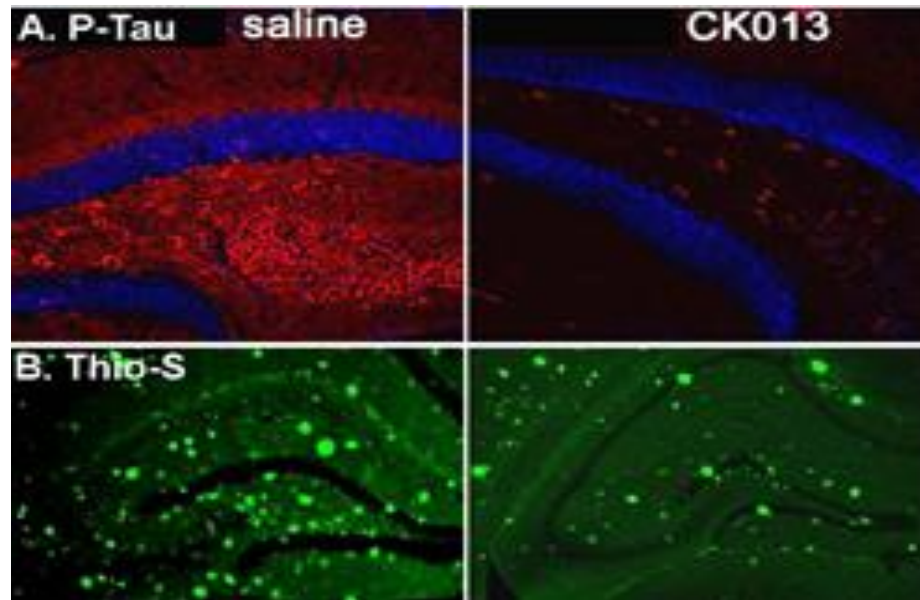
NonTg Sal

3xTg Sal

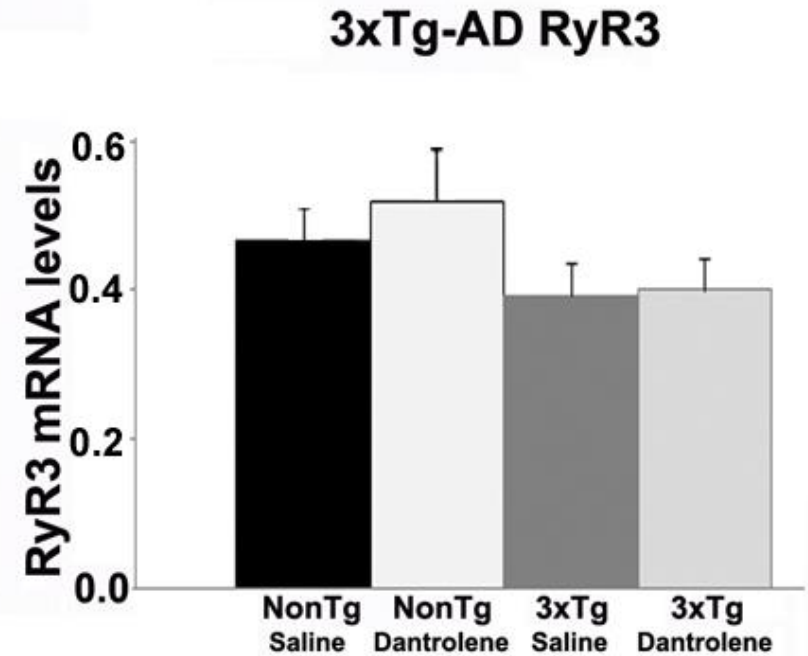
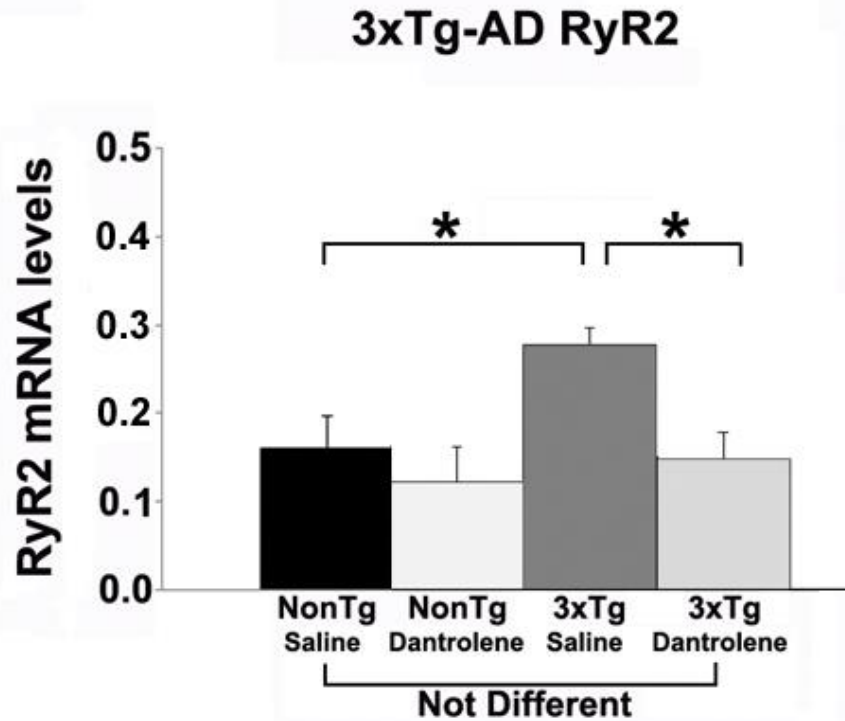
3xTg CK13



Amyloid and tau histopathology are reduced with chronic CK13 treatment



RyR2 expression is normalized in dantrolene-treated AD mice

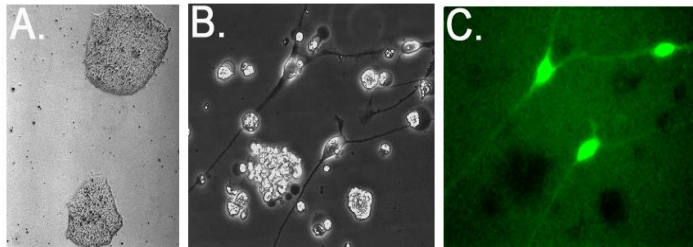


Recent Phase III Clinical Trials and Outcomes

Agent	Target/Mechanism	Outcome
• Atorvastatin HMG	CoA reductase	Failed
• Dimebon	Mitochondrial function	Failed
• Rosiglitazone	PPAR gamma agonist	Failed
• Pioglitazone	Mitochondrial function	Failed
• AC-1204	Ketone energy metabolism	Failed
• NSAIDs	Inflammation	Failed
• Flurizan	Inflammation	Failed
• Azeliragon	RAGE receptor inhibitor	Failed
• Xaliproden	Serotonin 5HT6 antagonist	Failed
• Idalopirdine	Serotonin 5HT6 antagonist	Failed
• Intepirdine	Serotonin 5HT6 antagonist	Failed
• TauRx0237	Tau immunotherapy	Failed
• LY450139	Gamma secretase	Failed
• Phenserine	Cholinesterase/Amyloid	Failed
• Tarenflurbil	Gamma secretase	Failed
• Gammagard	Gamma secretase	Failed
• AN1792	Active amyloid immunization	Failed
• Bapineuzumab	Passive amyloid immunization	Failed
• Solanezumab*	Passive amyloid immunization	Failed (3x)
• IVIG	Passive amyloid immunization	Failed
• Crenezumamab	Passive amyloid immunization	Failed
• Aducanumab	Passive amyloid immunization	Failed (2.5x)
• Verubecestat	BACE inhibitor	Failed (2x)
• Lanabecestat	BACE inhibitor	Failed
• Umibecestat	BACE inhibitor	Failed
• Elenbecestat	BACE inhibitor	Failed

A better assay system? Induced human neurons :

Direct (fibroblast → neuron) and indirect (fibroblast → iPSC → neuron) transformation of human fibroblasts (control and AD patients) to neurons



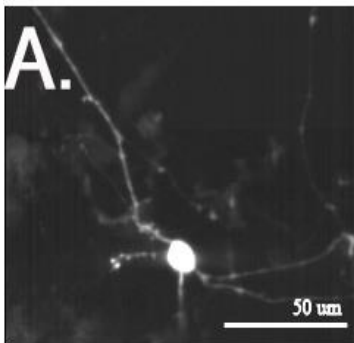
Production of iN from fibroblasts from human FAD patients



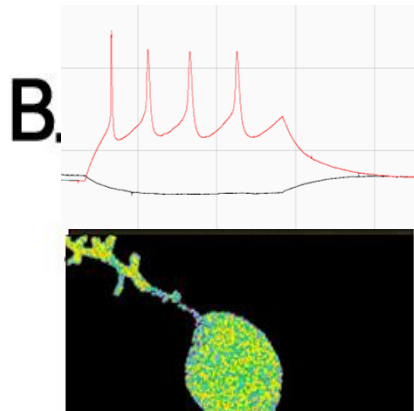
Sarah Mustaly



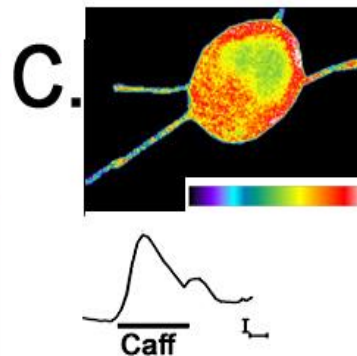
Bob Marr, Ph.D



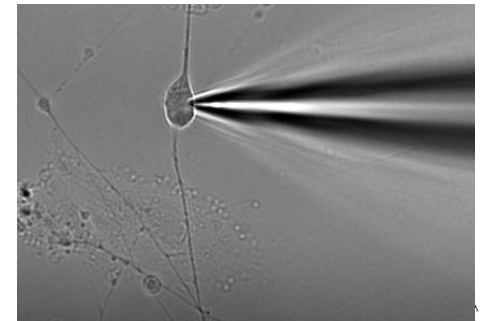
Dye-filled neuron via patch pipette



Spikes and voltage-gated calcium signals

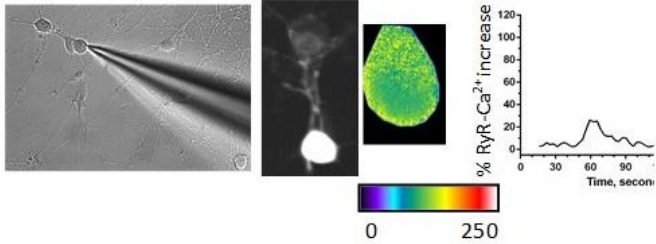


RyR-Ca²⁺ responses

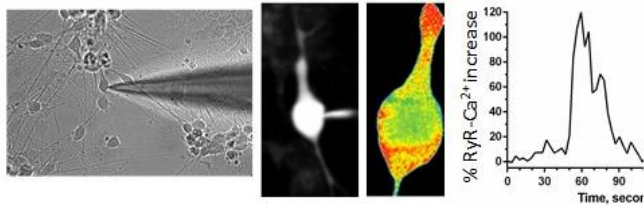


RyR-evoked Ca^{2+} signals are increased in human AD neurons

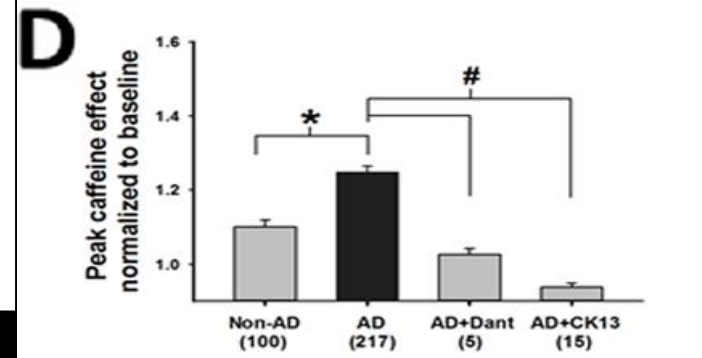
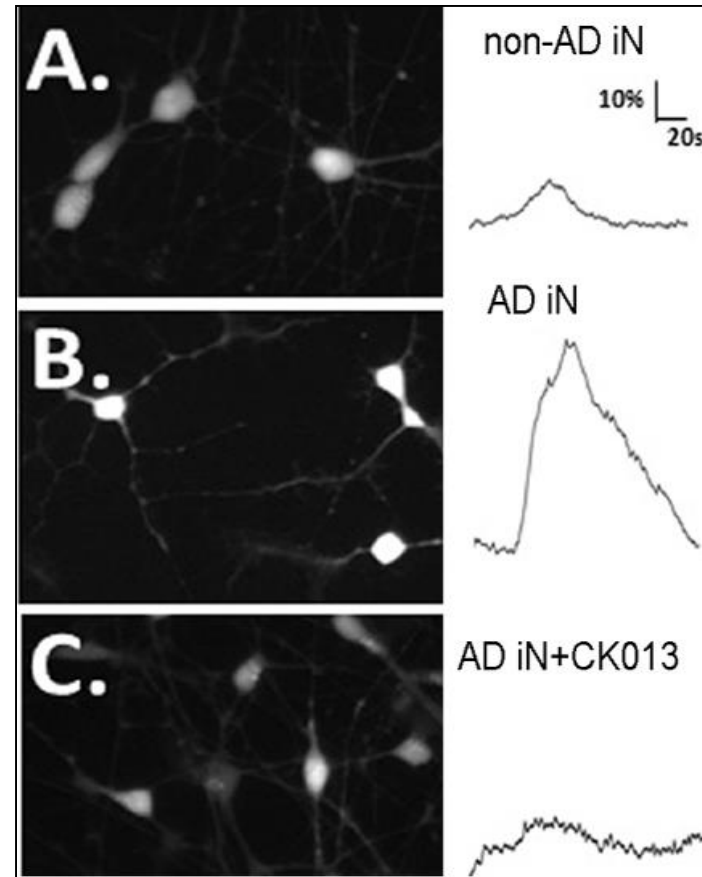
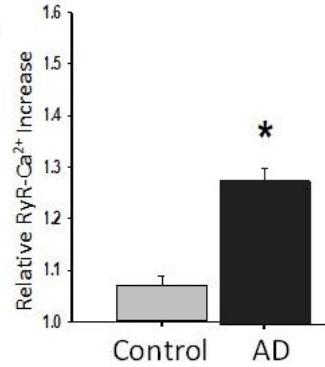
A. Control neuron



B. AD neuron



C.

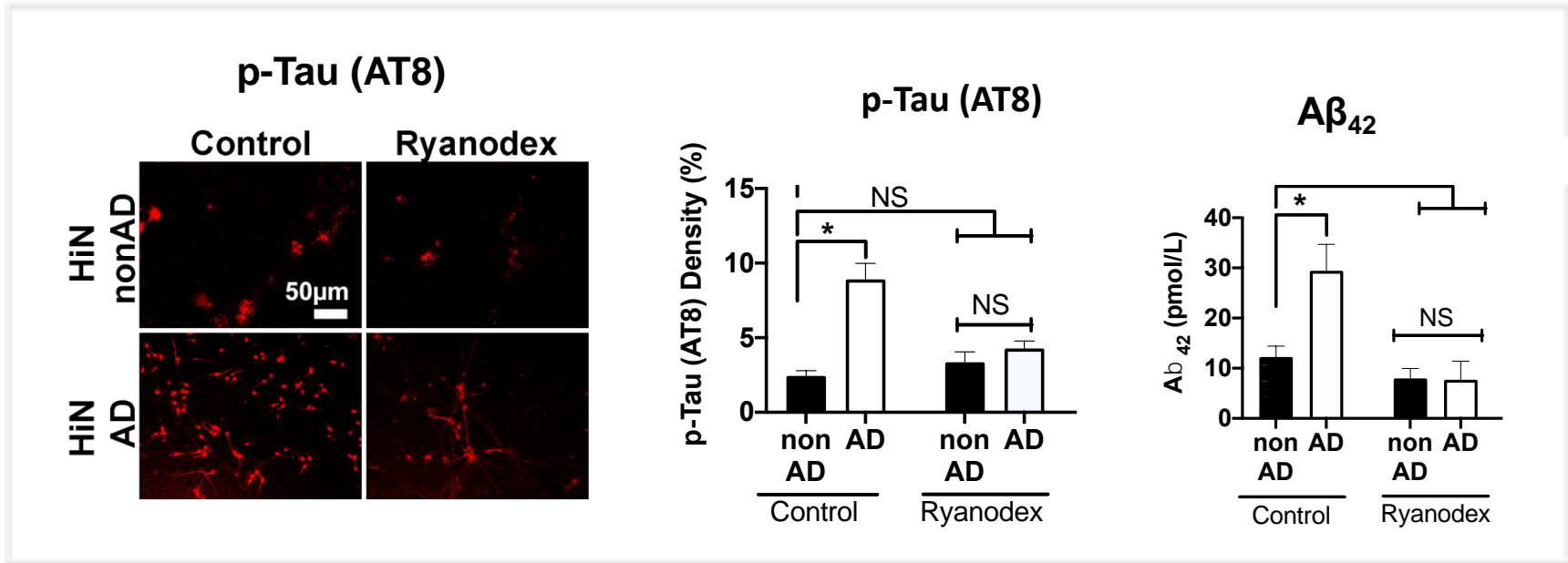


And reduced with dantrolene
and our novel compounds



Proof of principle in human AD neurons:

Stabilizing ER calcium abolishes amyloid and tau pathology in AD and reduces superoxide production



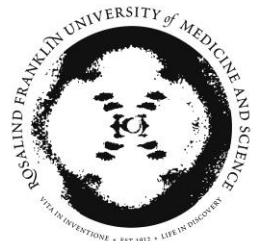
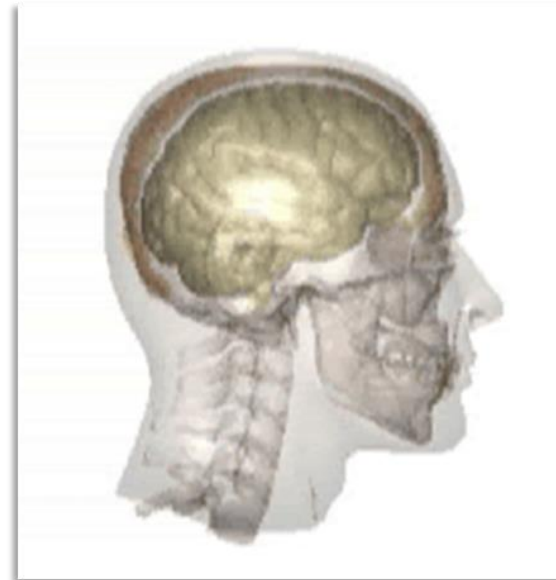
Mustaly et al., 2022; BioRx

Mitochondrial findings – not published yet.



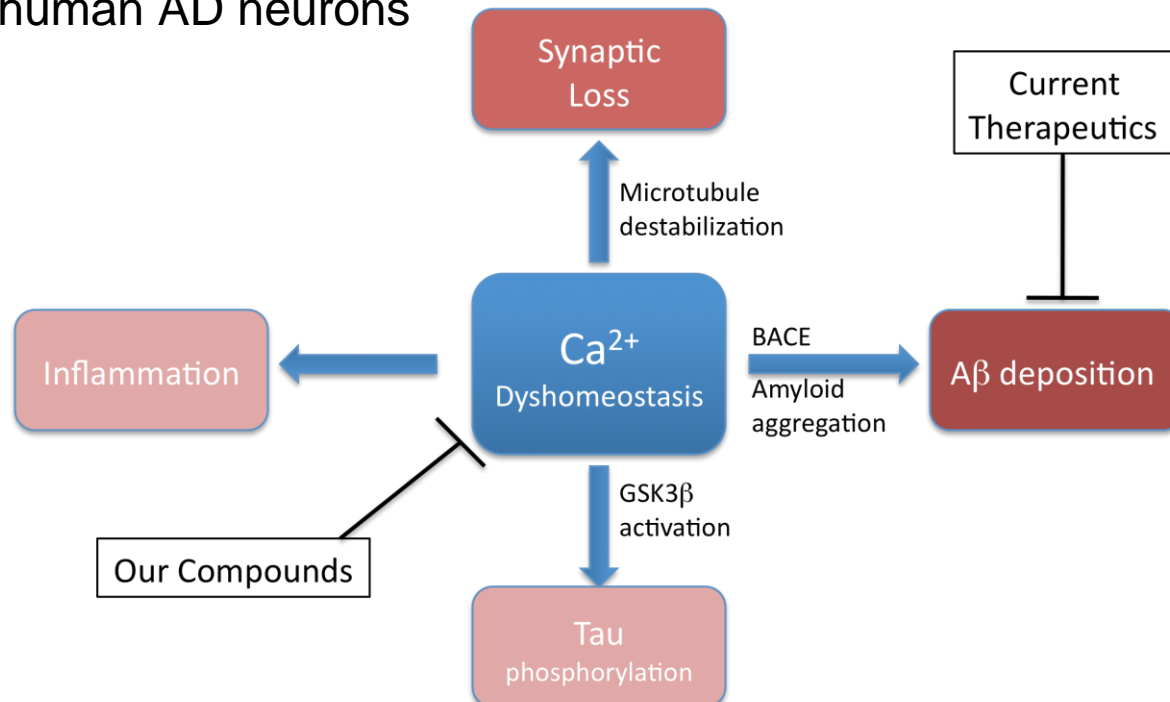
Related applications and diseases:

- **Traumatic Brain Injury**
- **Down Syndrome**
- **Huntington's disease**
- **Parkinson's disease**



Reason for optimism?

- We are targeting an early, upstream pathogenic mechanism that taps multiple aspects of AD pathology, including synaptic pathophysiology.
- There is an identifiable drug-able target that is linked to all the major features of AD pathology
- Importantly, there are no detectable effects on 'normal' RyR-mediated calcium signaling or downstream pathways with our small molecule compounds.
- We can validate using approaches beyond the usual cellular assays, including detailed neurophysiological, network, and imaging assays in mouse models and human AD neurons



Thank you-

Stutzmann Lab:

- Sarah Mustaly
- Noah Muller
- *Sean Schrank, Ph.D*
- *Clark Briggs, Ph.D*
- *John McDaid, Ph.D*
- *Shreaya Chakroborty, Ph.D*
- *Rosalind Helfrich*
- *Alyssa Littlefield*

RFUMS Collaborators:

CNDT

- Robert Marr, Ph.D

College of Pharmacy

- John Buolamwini, Ph.D
- *Mithun Raju*
- *Shannon Riley*
- *Russell Dahl, Ph.D*
- *Christopher Kaiho*



Ron Kaplan

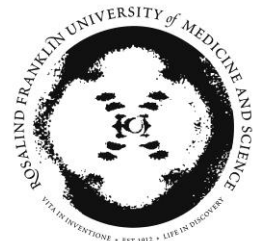


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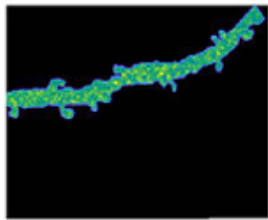
afar

american federation
for aging research

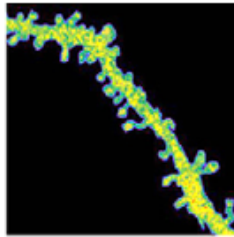
The Ca^{2+} increases in the AD models alter synaptic plasticity and structure

Postsynaptic Ca^{2+} response:
Significant INCREASE in
AD neurons

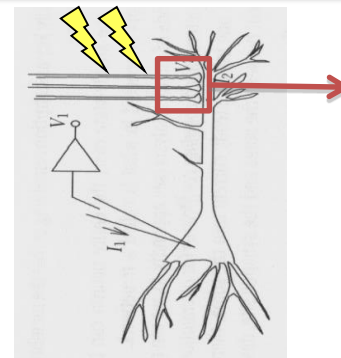
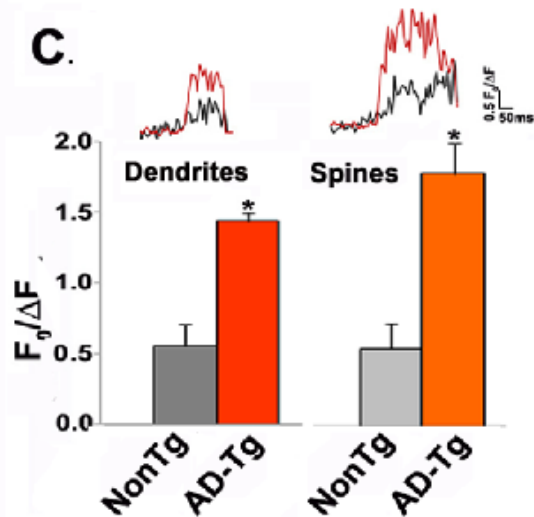
A. NonTg



B. AD-Tg



C.



Synaptic plasticity:
Strengthening (or weakening) of
synaptic associations:
Cellular correlate of learning
and memory

Postsynaptic electrophysiological response:
Significant DECREASE in synaptic plasticity

