

**Astrocytic contribution to
deficient Ca²⁺ signalling and oxidative stress
mediated by TRPV4 channels
in A β ₄₀-induced hippocampal cell death**

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AD, A β , Astrocytes, TRPV4

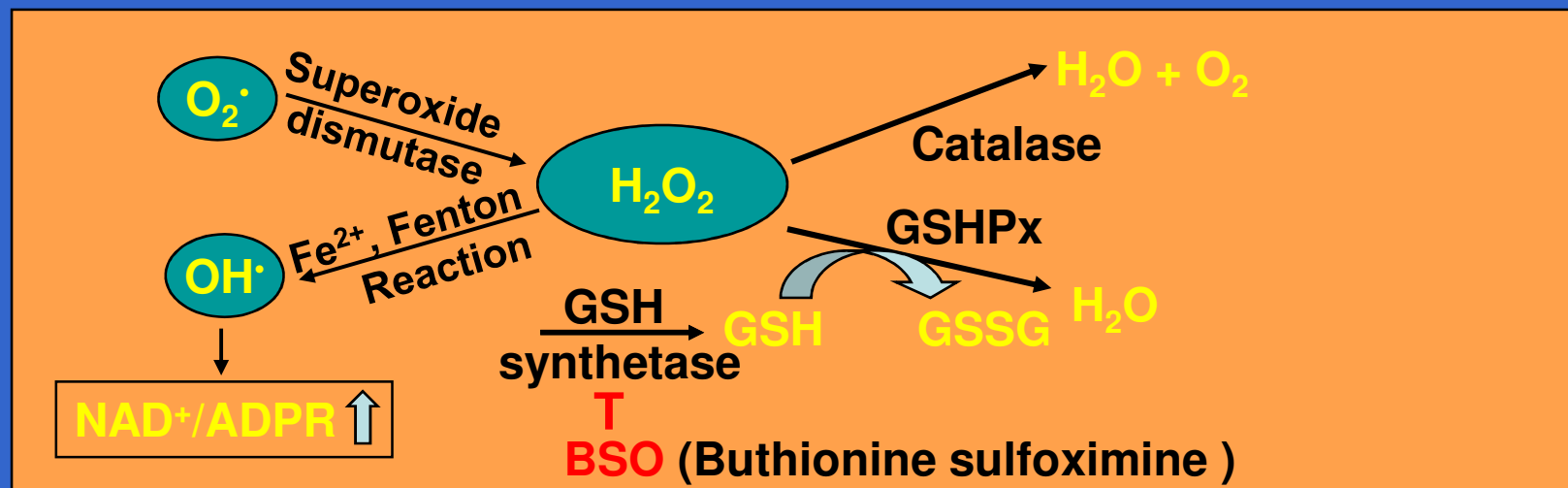
- **Brain cell death & amyloid deposition:**
pathological hallmarks of **Alzheimer's disease (AD)**
- Toxic **amyloid β (A β)** species:
primary factor in AD pathogenesis
- **Astrocytes** as primary target of toxic A β action:
Ca²⁺ signalling, oxidative states, brain cell death in AD
- **Ca²⁺-permeable TRPV4 channels :**
expression in rat hippocampal astrocytes
oxidative stress-induced cell damage
ischemia-evoked Ca²⁺ entry in reactive astrocytes.

Objective:

To investigate the potential role of TRPV4 channels in A β -evoked *in vitro* damage of the hippocampus, a brain region highly vulnerable in AD

Methods

- **Model systems:**
 - *Monolayer co-culture* of neurons and astrocytes
 - *Organotypic slice culture* of rat hippocampus
- **Cell death induction:**
 - Exogenous Amyloid β_{1-40} peptide (**A β_{40}**)
 - Endogenous increase in ROS evoked by **BSO**



Methods (cont.)

- **Detection of TRPV4 expression:**

RT-PCR, Western blotting, Immunocytochemistry

- **Detection of cell death:**

Uptake of fluorescent propidium iodide (**PI**)

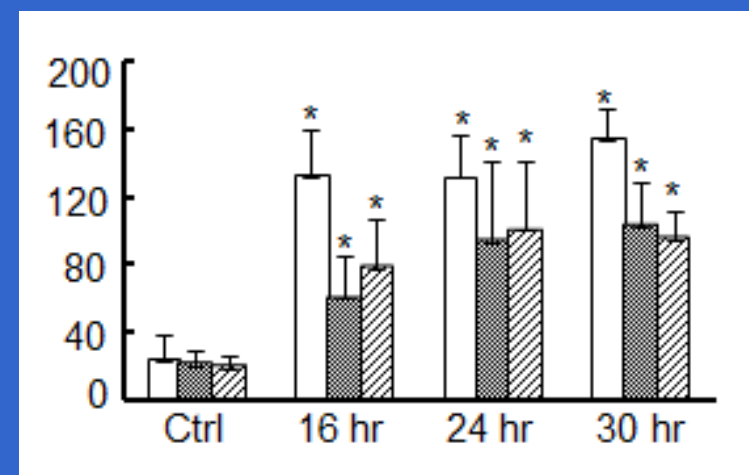
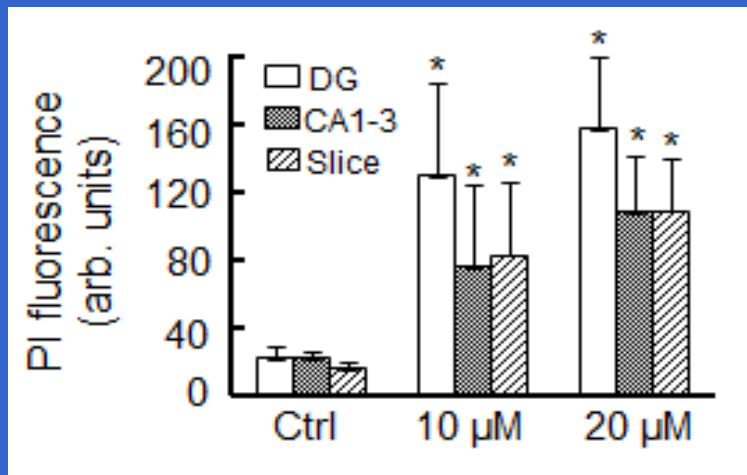
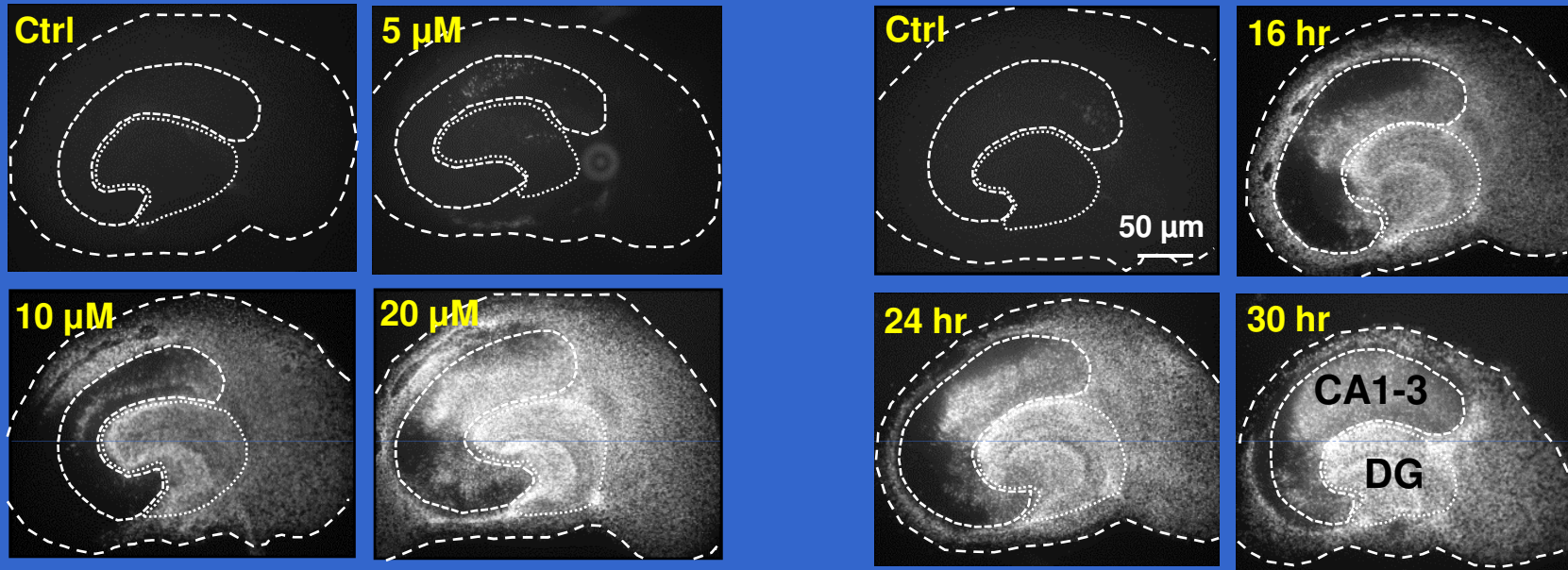
- **Effect of $A\beta_{40}$ on activation of TRPV4 channels:**

$[Ca^{2+}]_i$ changes in neurons and astrocytes

by fluorescence digital imaging

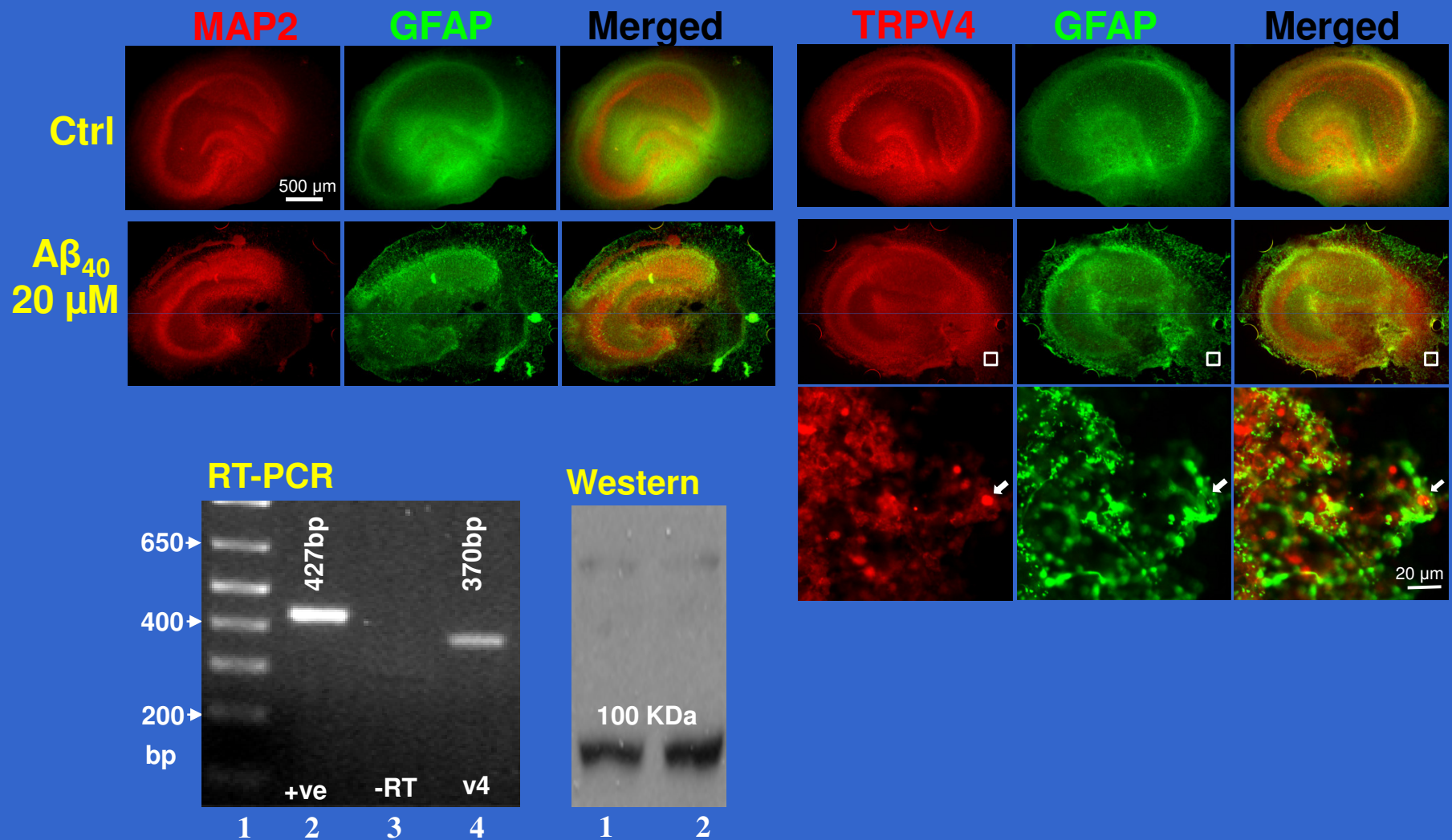
(Olympus Live Cell Confocal System)

$A\beta_{40}$ -evoked hippocampal damage is region-specific

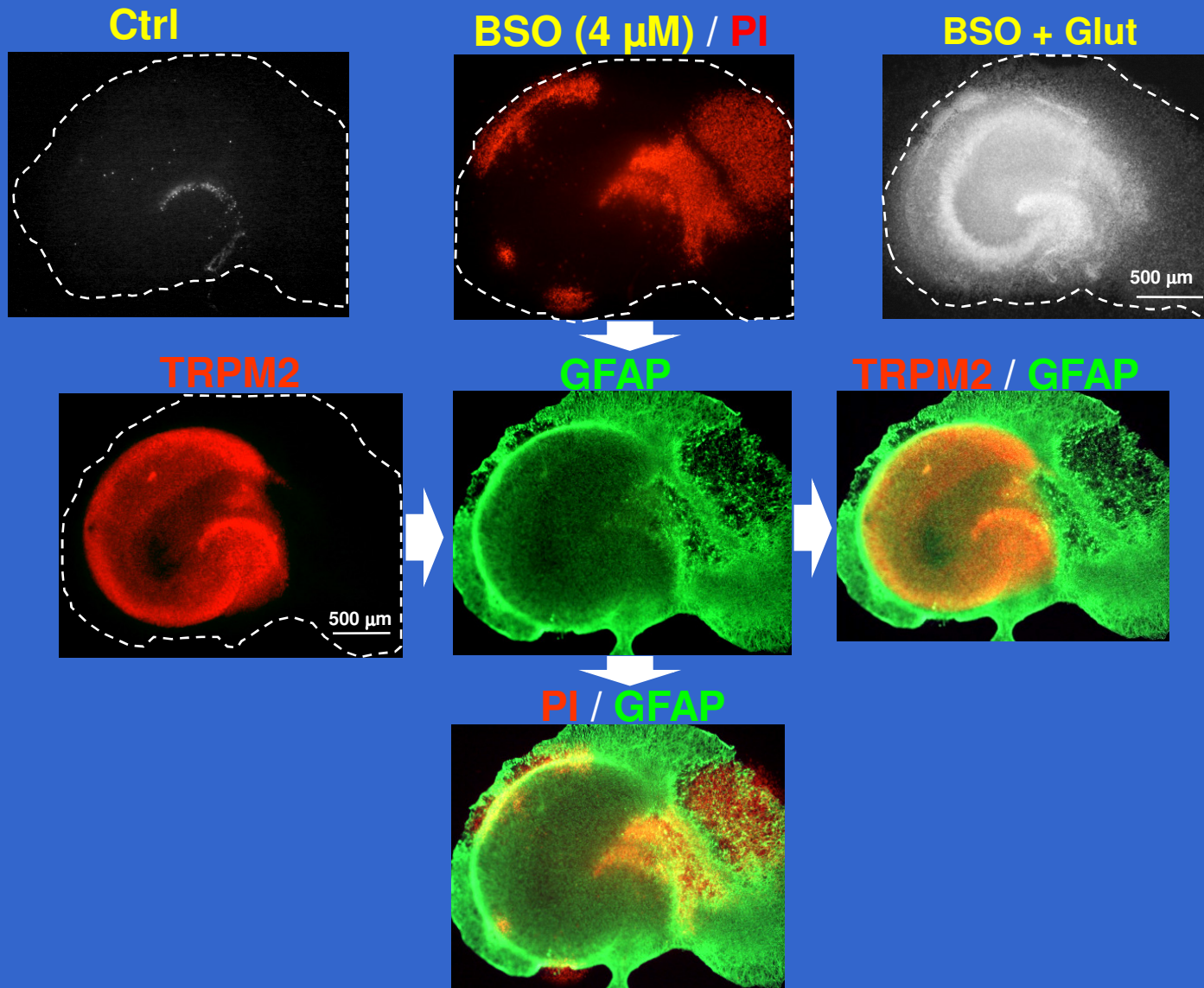


N = 7-16, $p < 0.001$ vs corresponding controls

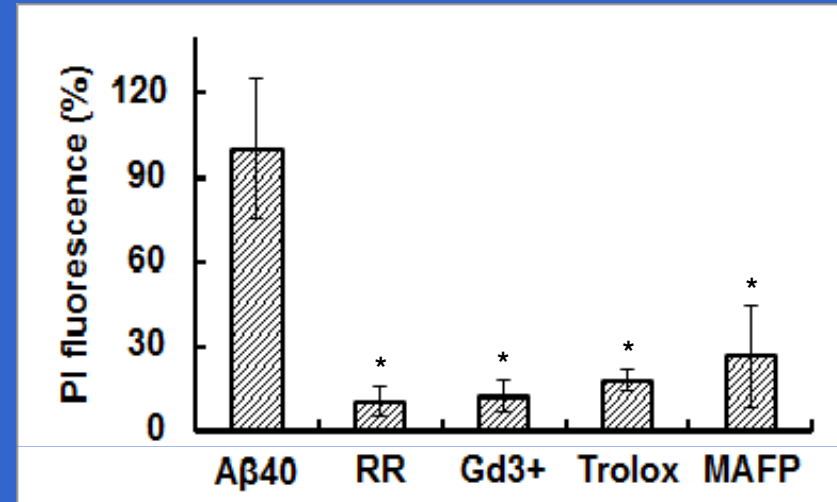
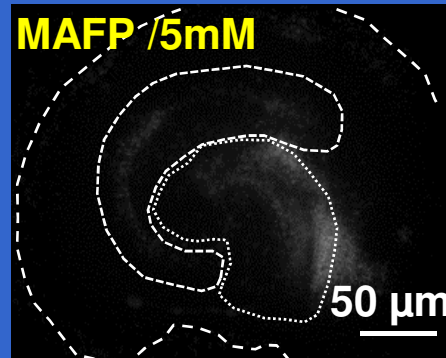
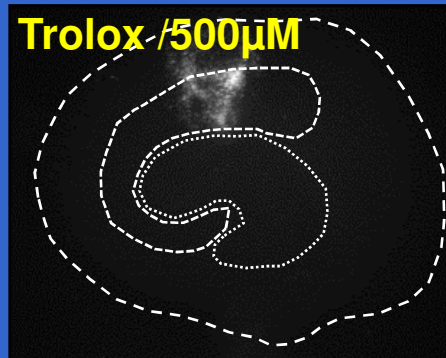
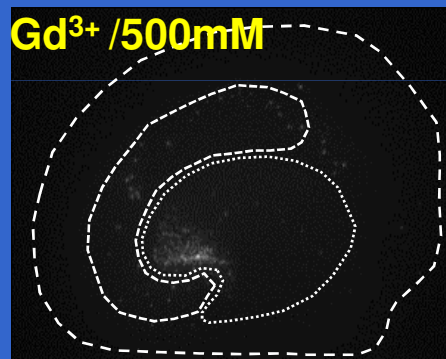
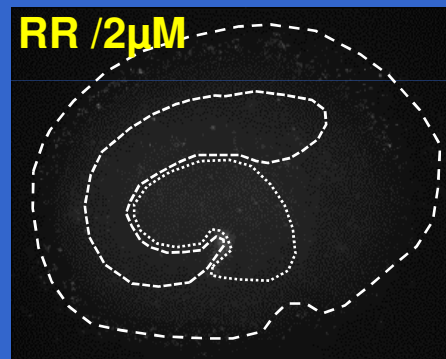
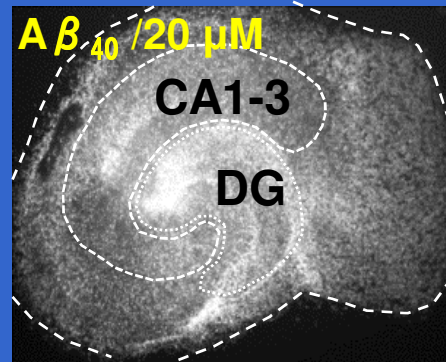
$A\beta_{40}$ -evoked hippocampal damage is region-specific with altered TRPV4 and GFAP expression



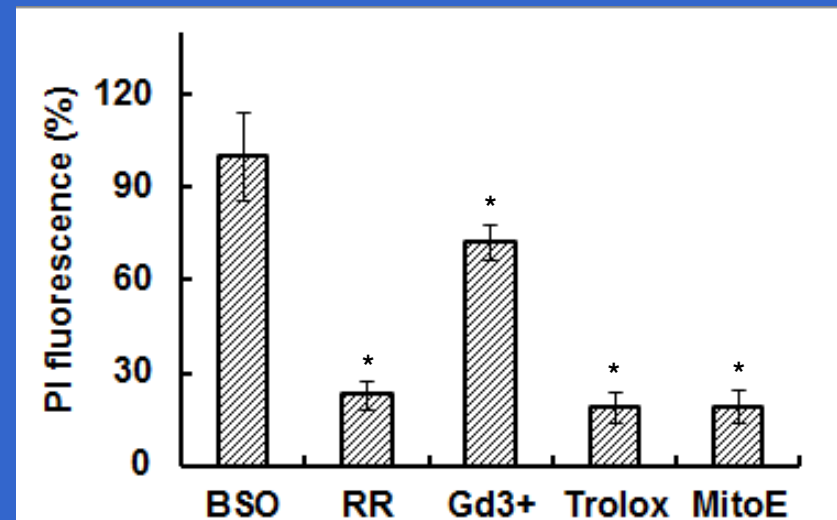
Oxidative stress induces astrocytic damage in organotypic hippocampal cultures



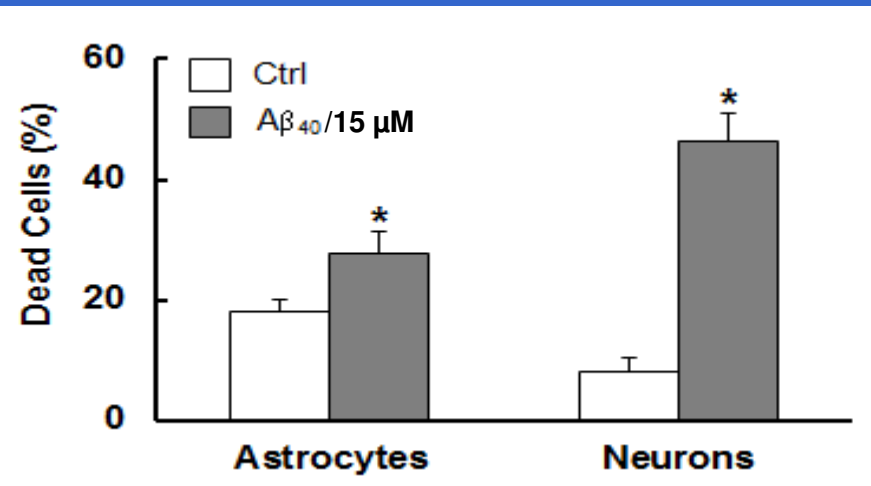
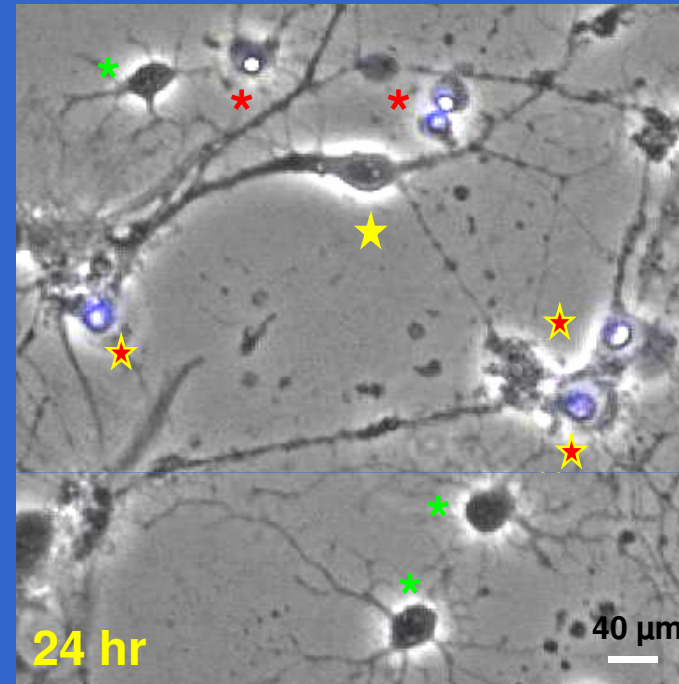
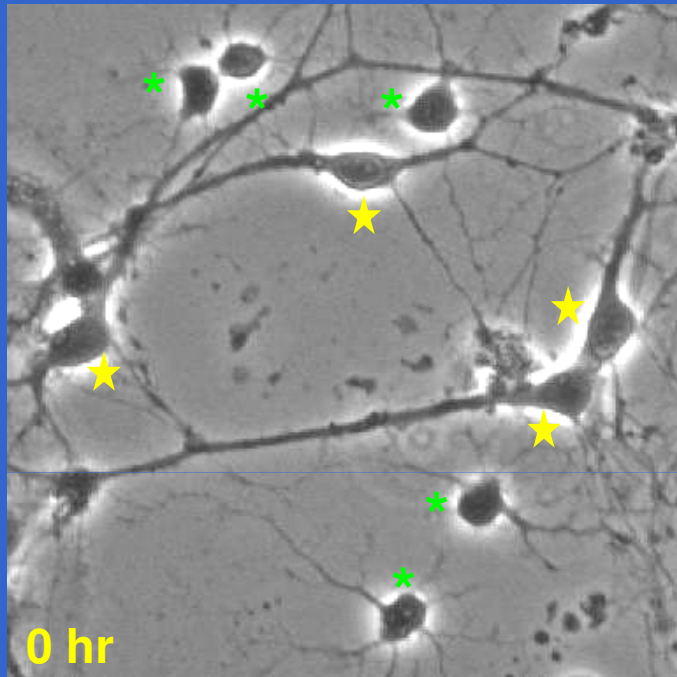
Cell death induced by $A\beta_{40}$ (or oxidative stress) is attenuated by TRPV4 blockers and antioxidants



n = 9 - 30, p < 0.001 vs $A\beta_{40}$ or BSO

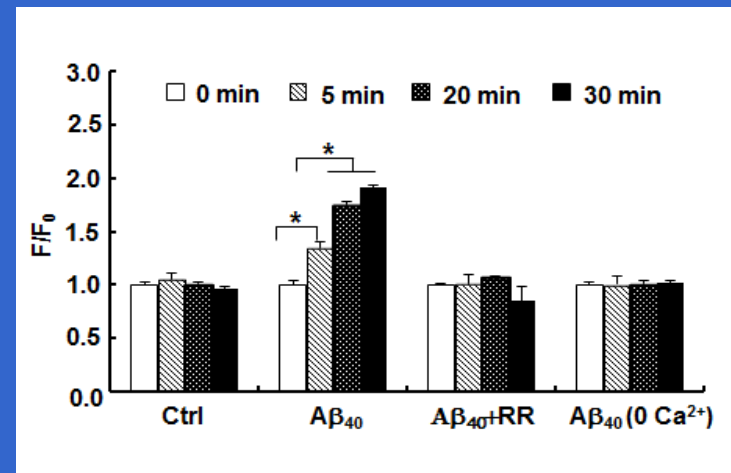
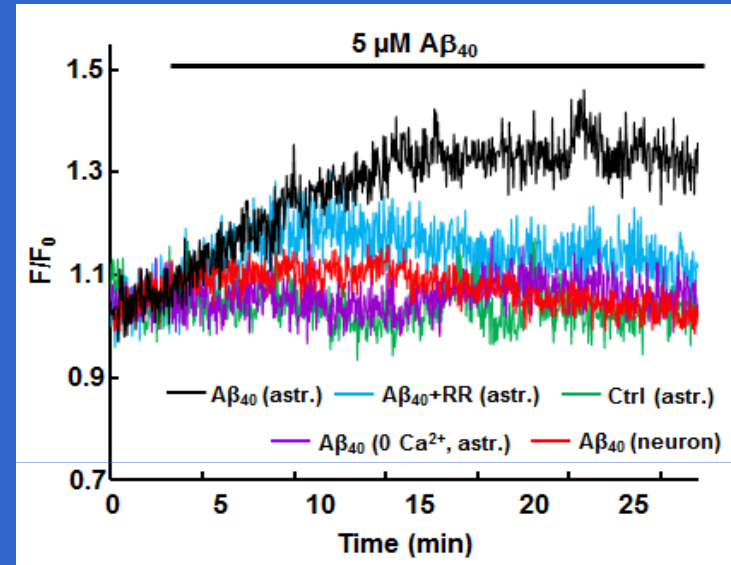
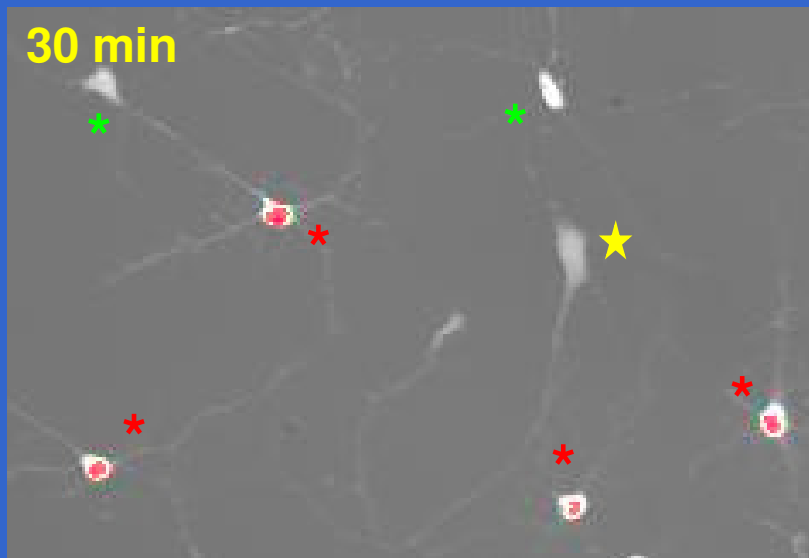
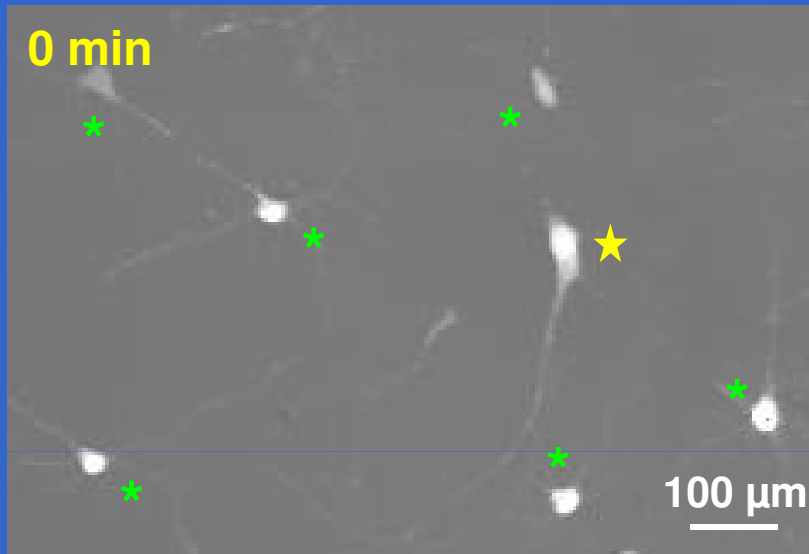


$A\beta_{40}$ evoked mainly neuronal damage in co-cultures of hippocampal neurons and astrocytes



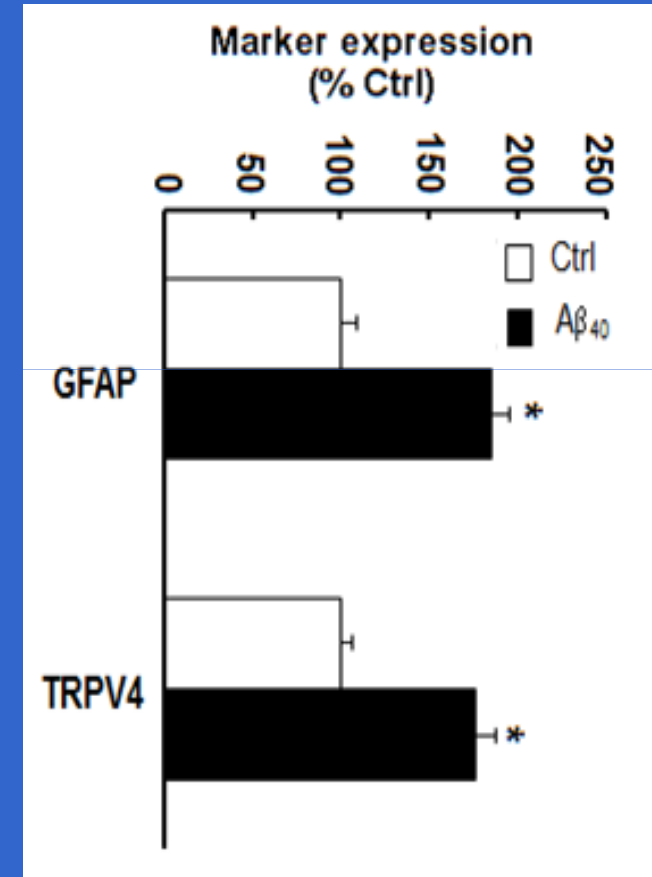
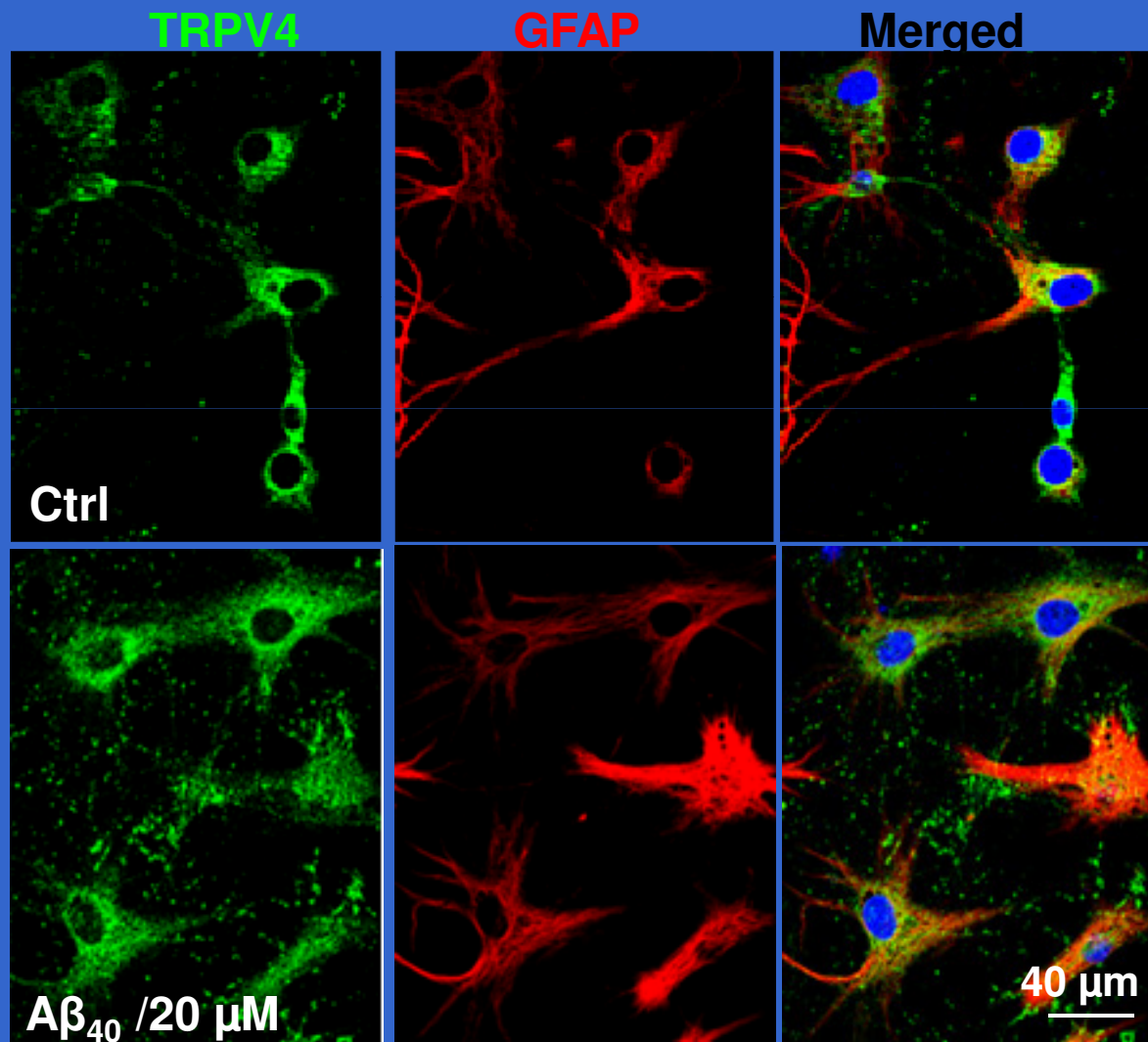
n = 3 - 4,
p < 0.001 vs
relative controls

$A\beta_{40}$ enhanced- $[Ca^{2+}]_i$ in astrocytes is attenuated by RR and in Ca^{2+} -free media



n = 16 - 26, p < 0.001 relative to the period before $A\beta_{40}$ treatment

$A\beta_{40}$ enhanced the expression of TRPV4 and GFAP proteins in astrocytes



n = 18 - 31, p < 0.001 relative to corresponding controls

Summary

- TRPV4 modulators inhibit $A\beta_{40}$ -evoked:
 - region-specific damage that alters TRPV4 & GFAP expression
 - astrocytic Ca^{2+} influx, while $A\beta_{40}$ damages more neurons
- **TRPV4-expressing astrocytes protect Hippocampal pyramidal neurons against $A\beta_{40}$ and oxidative damage**
- **$A\beta_{40}$ primarily activates astrocytic TRPV4 channels, leading to neuronal death with limited astrocyte damage**

We propose that the altered astrocytic state affects neuronal survival due to lack of trophic and other support, forming a link between **astrocyte dysfunction** and **neurodegeneration in AD**.



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