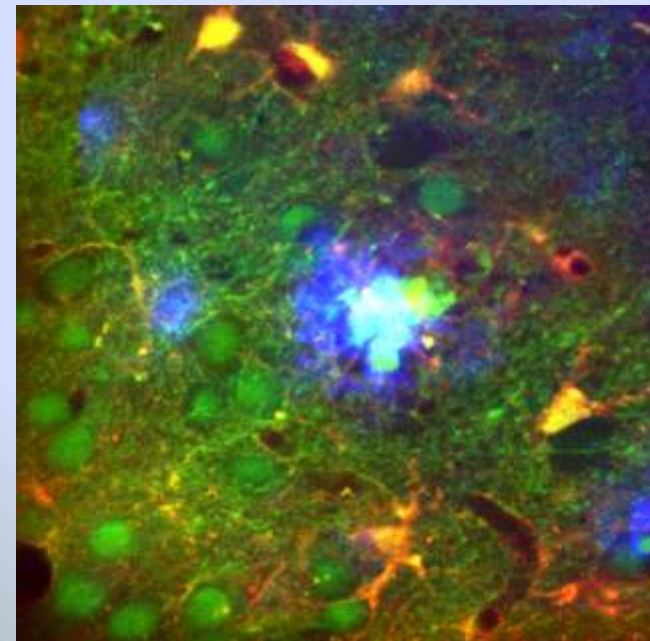


## Techniques for imaging inflammation in vivo :

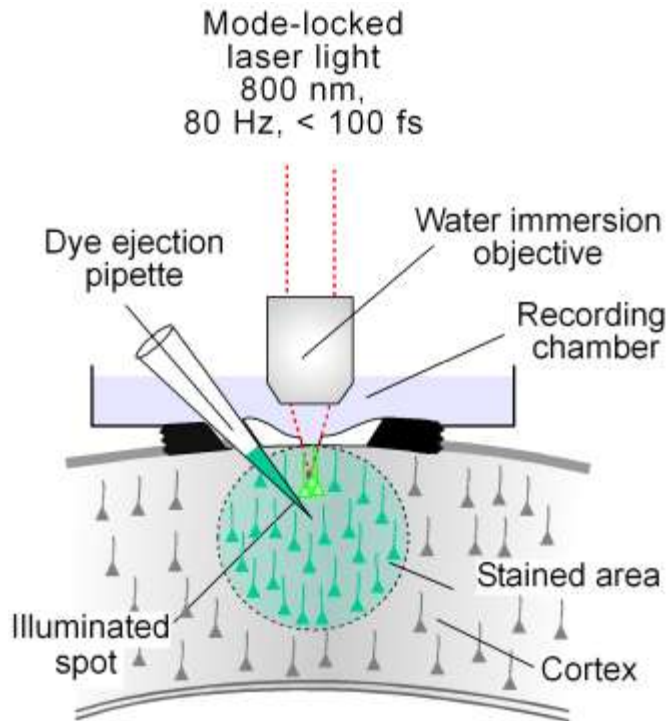
- Tools for high resolution in vivo imaging of neural function in acute and chronic preparations
- Functional imaging of astrocytes
- High resolution in vivo imaging of microglia
- An application example



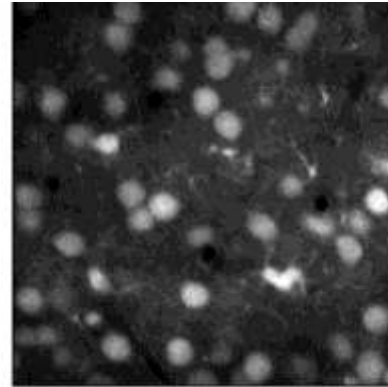
■ - neurons ■ - glia ■ - plaques

# Multi-cell bolus loading technique

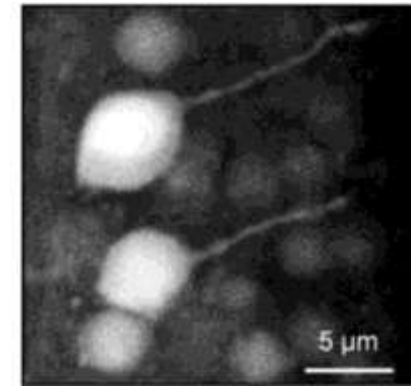
## Two-photon imaging



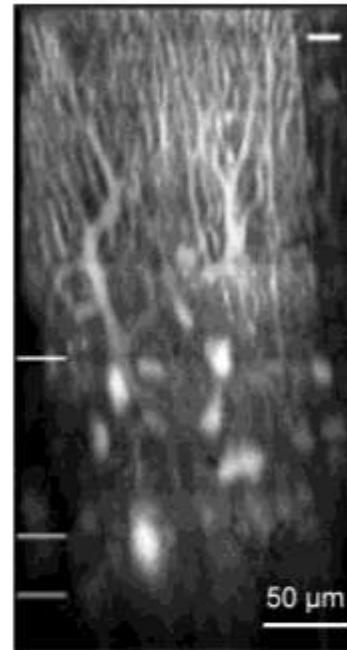
## Cortex ( Mouse, Rat, Cat)



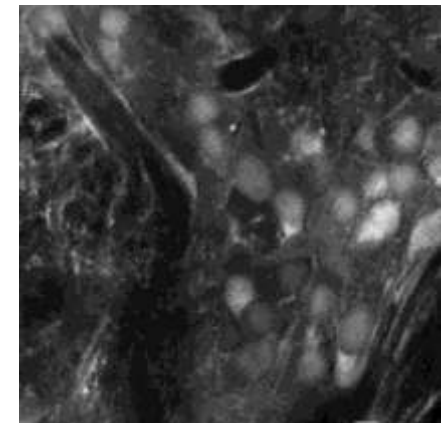
## Spinal cord ( Zebrafish)



## Cerebellum (Rat)



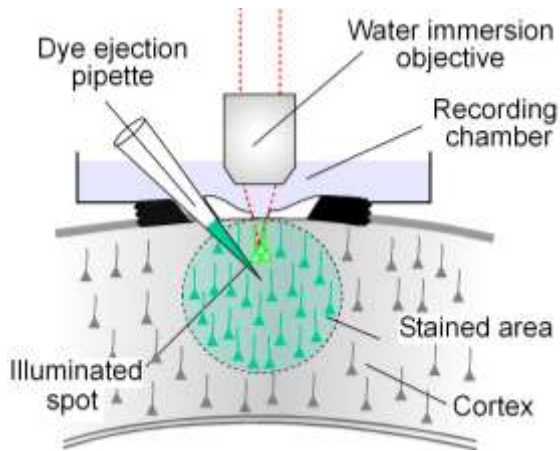
## Olfactory bulb (Mouse)



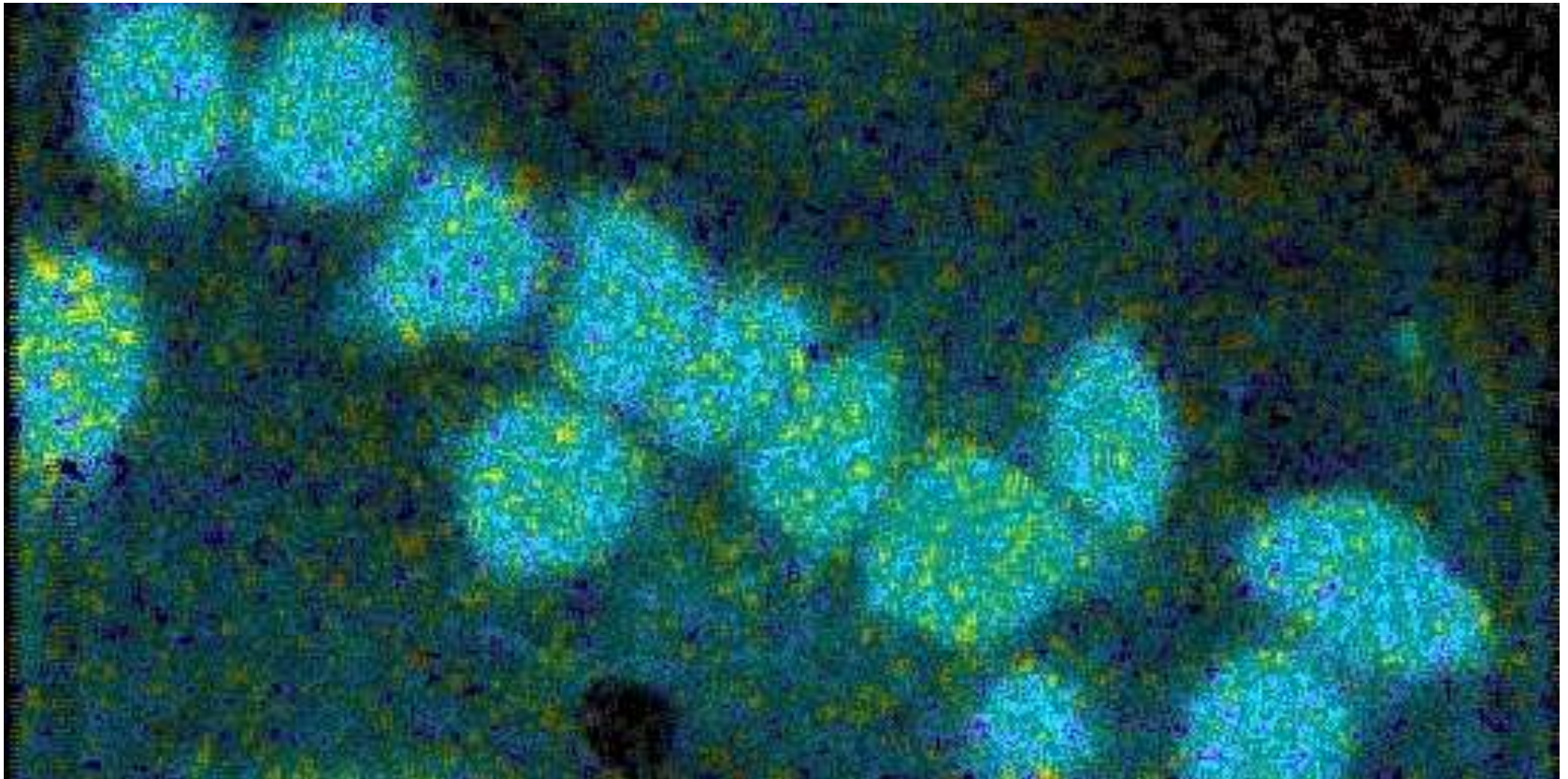
Stosiek et al., *PNAS USA*, 2003  
Garaschuk et al., *Nature Protocols*, 2006

Ohki et al., *Nature* 2005  
Sullivan et al., *J Neurophysiol.* 2005  
Brustein et al., *Pflugers Arch.* 2003  
Homma et al., *Front Neural Circ.* 2013

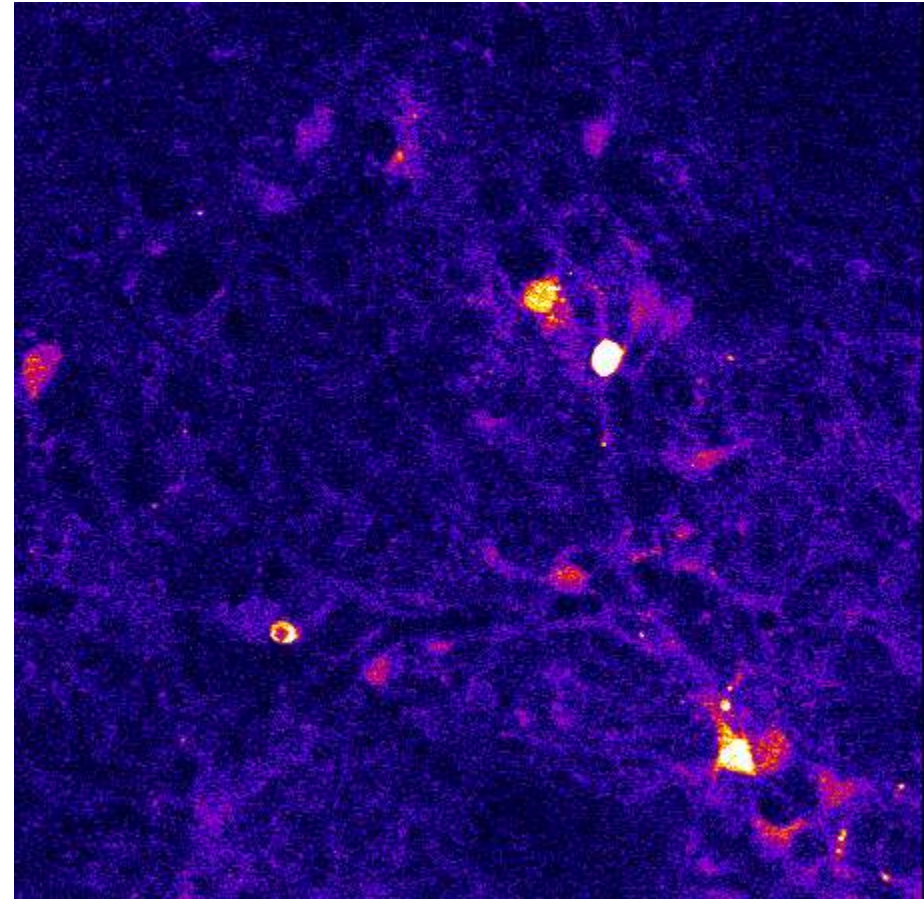
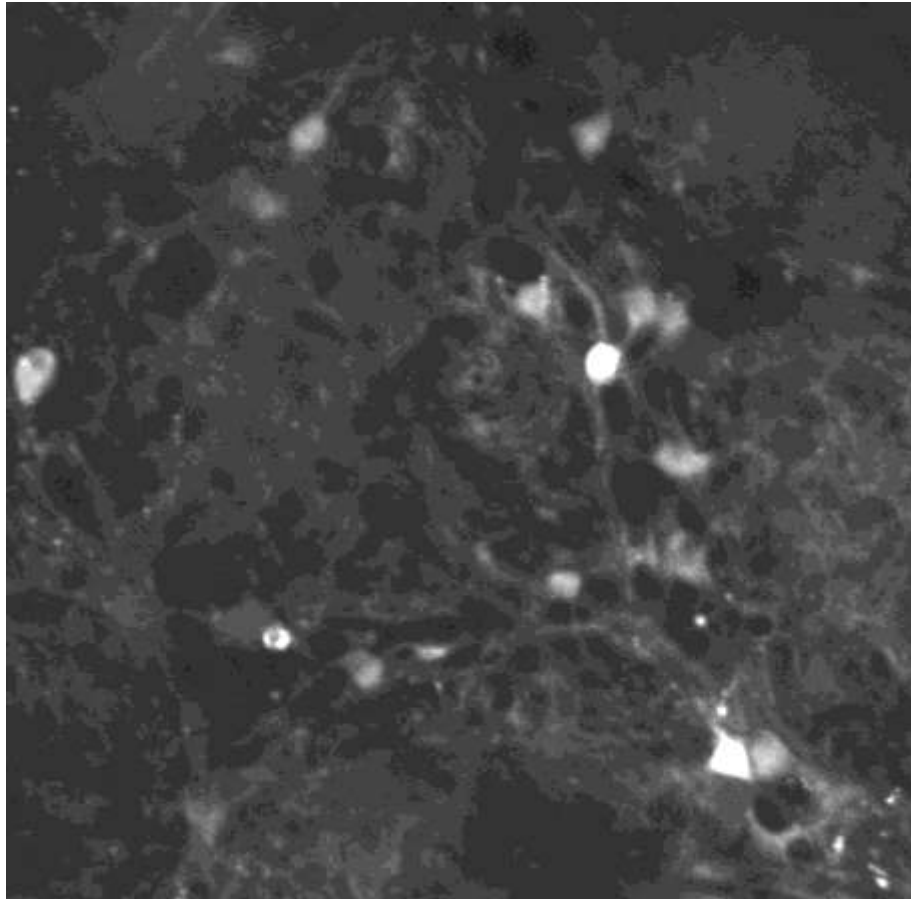
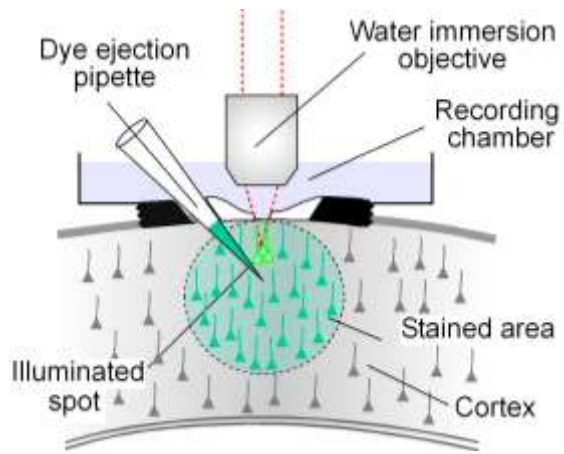
# Spontaneous calcium transients in a WT mouse



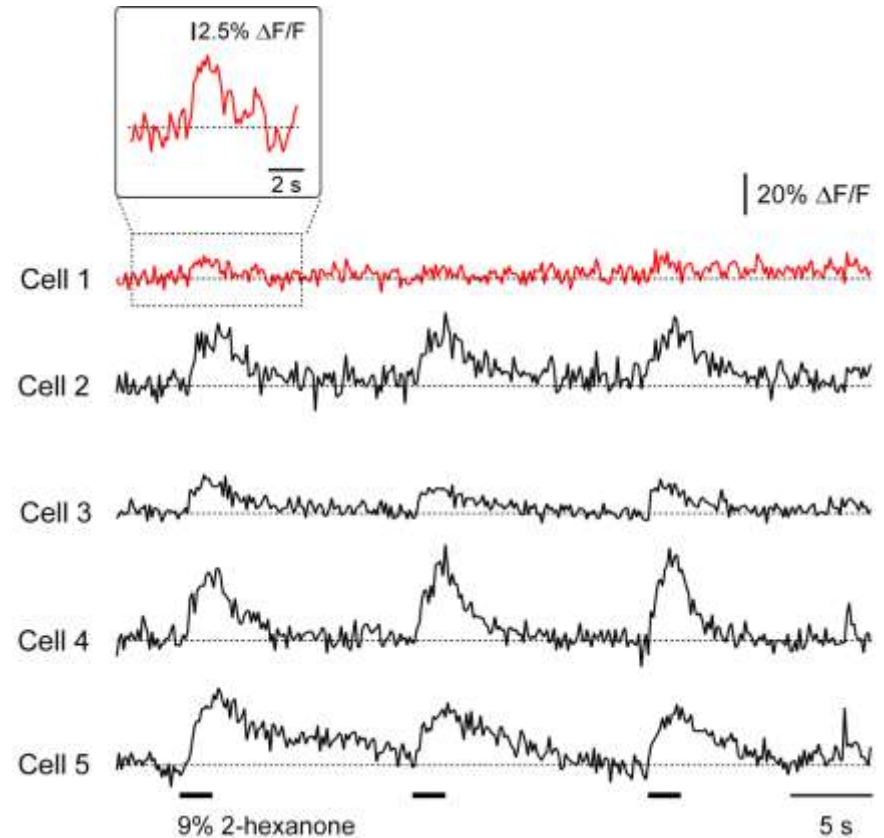
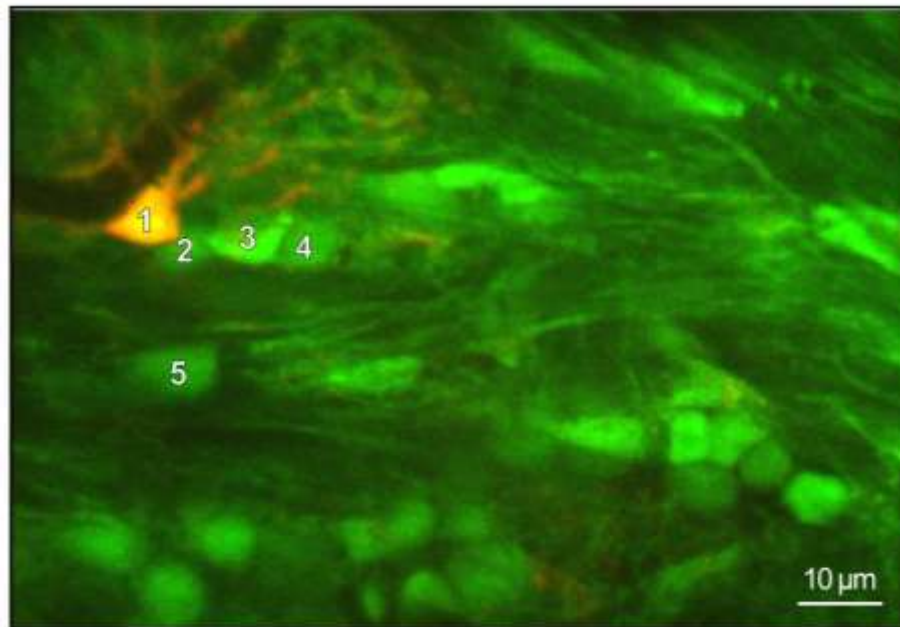
## Acute preparation



# Specific staining and functional imaging of astrocytes



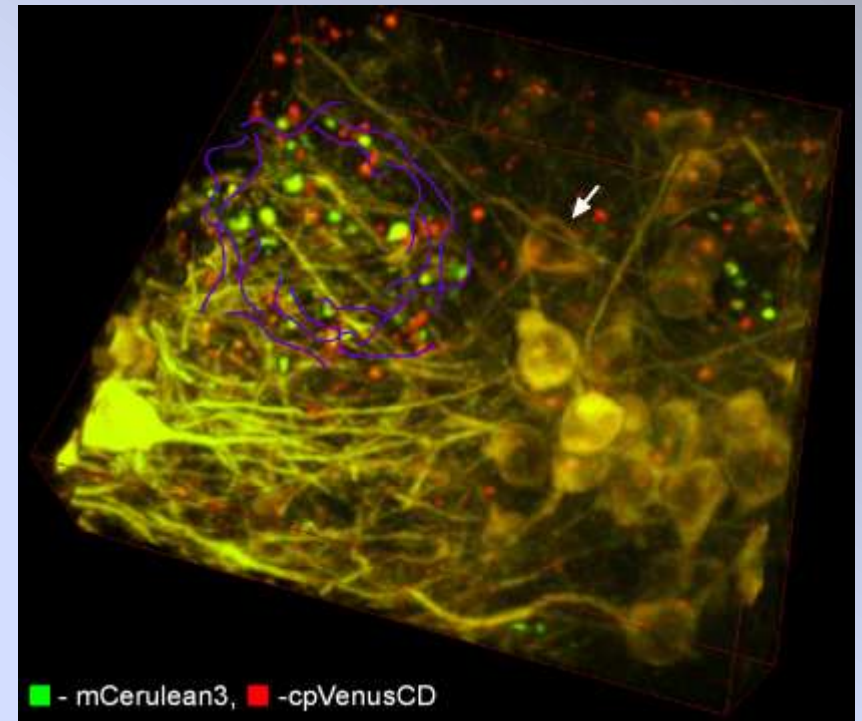
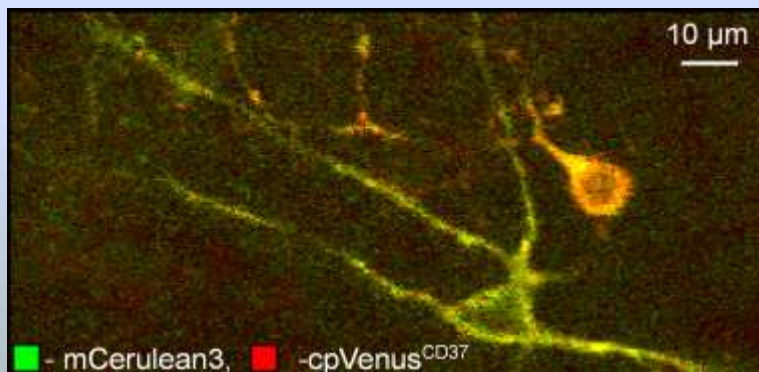
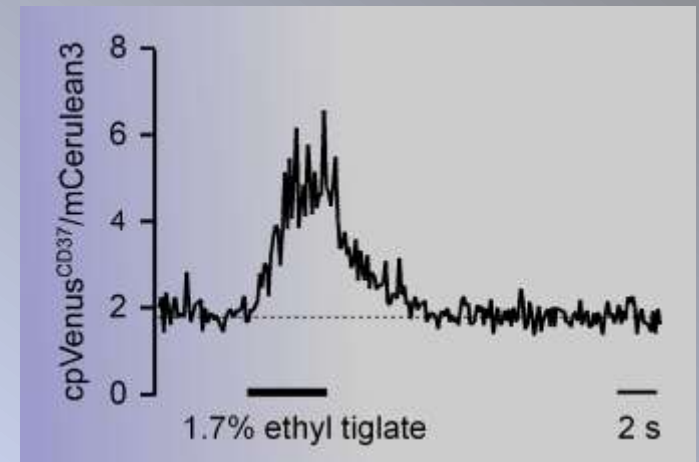
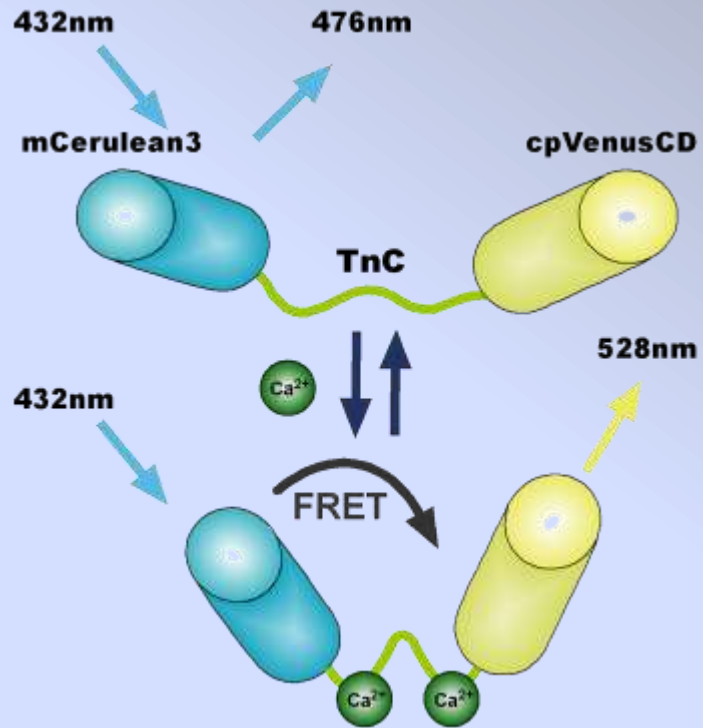
# Sensory-evoked calcium signals in genetically-labeled astrocytes



## Advantages:

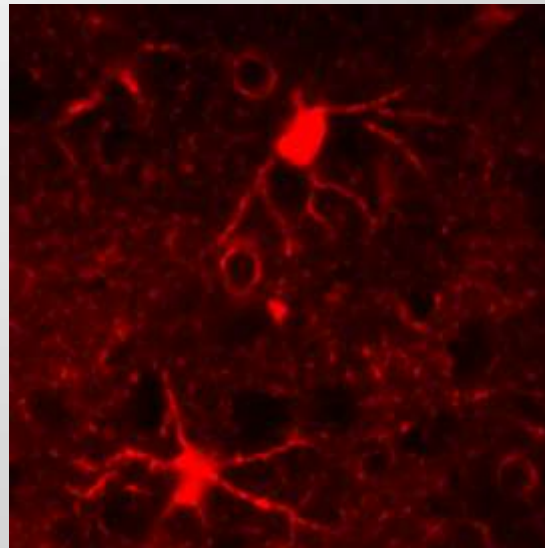
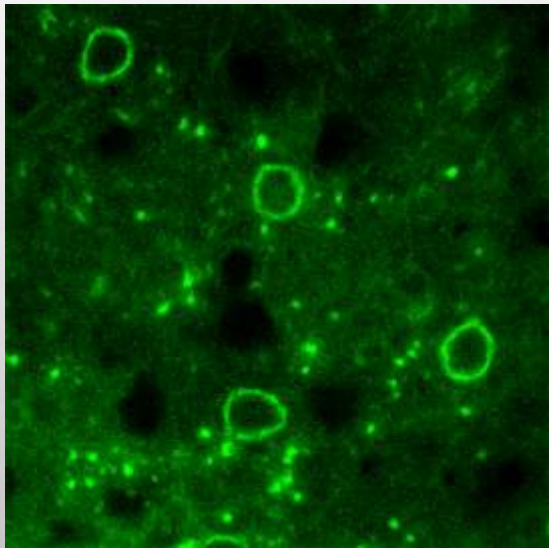
- MCBL can be targeted to any brain area
- It requires only 5-10  $\mu\text{l}$  of staining solution per experiment
- It provides long lasting staining of neurons *in vivo* and *in vitro*
- It allows to apply dye mixtures
- In combination with cell type-specific markers allows to study defined neuronal populations

# Chronic imaging of neural function using novel FRET-based sensors

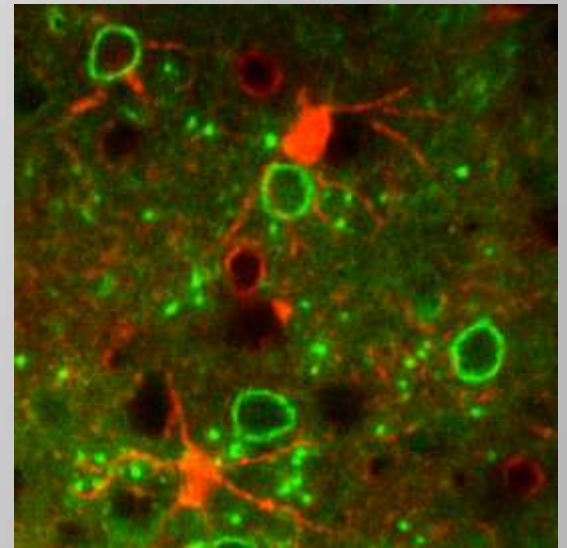


# Specific neuronal staining

Mouse 2 months old, zoom x120



165  $\mu\text{m}$  depth



 - neurons  - astrocytes

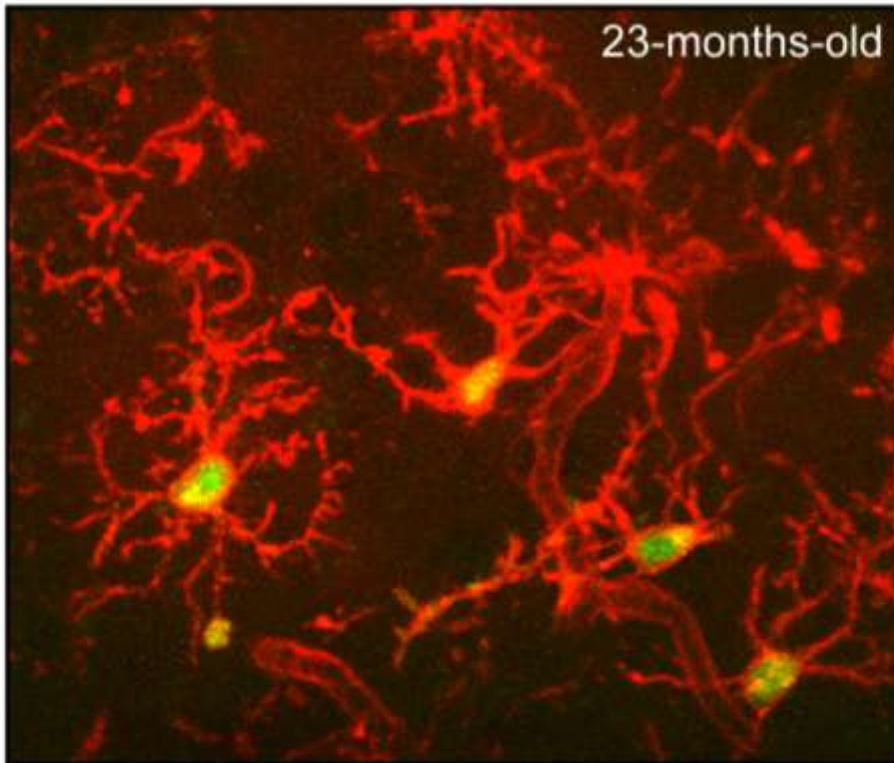


## Advantages:

- Targeted transgenic expression of the fluorophore / sensor
- RGB labeling allows long-time monitoring of many identified cells
- Twitch sensors allow repeated ratiometric calcium measurements
  - High  $\text{Ca}^{2+}$  sensitivity, high brightness, low toxicity
  - Provide visualisation of the cell morphology down to dendritic spines
  - Allow in vivo functional analyses of axons and spiny dendrites

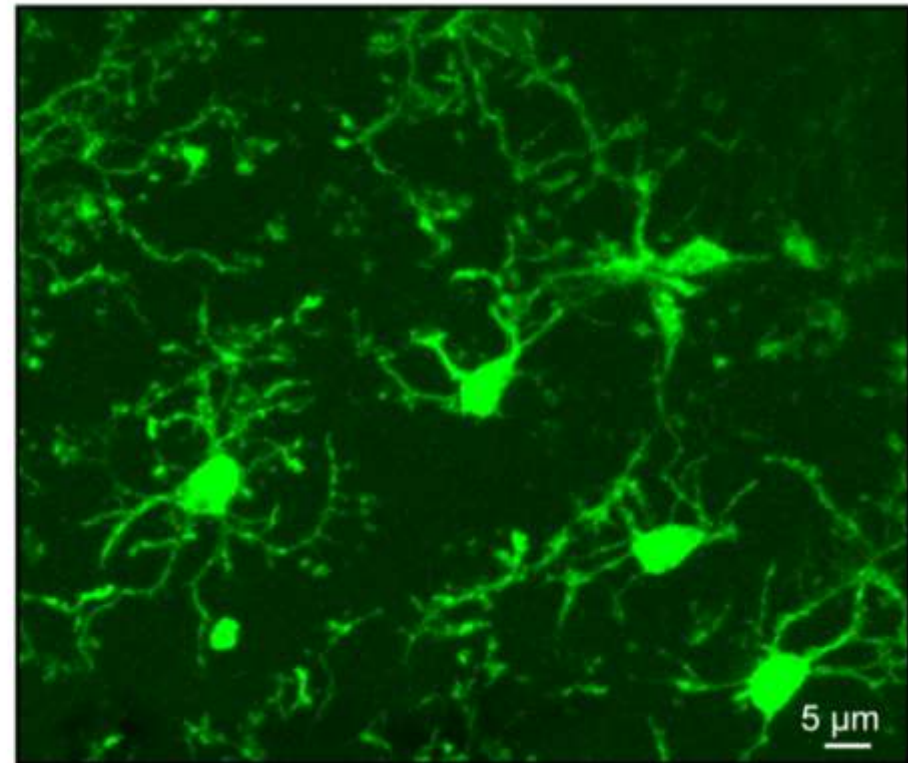
# Visualization of microglia in the cortex of living mice

800 nm excitation wavelength



■ -GFP, ■ -DyLight 594-conj. Tomato lectin

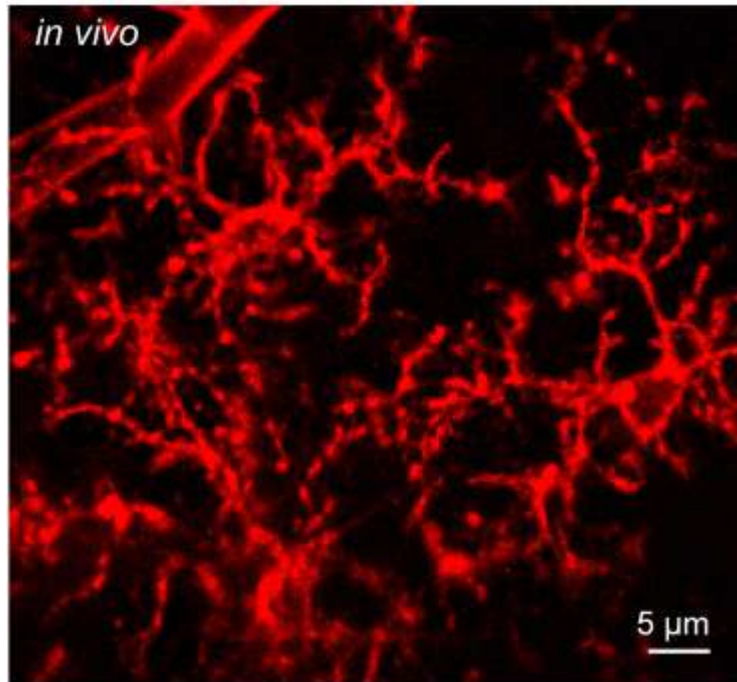
930 nm excitation wavelength



■ -GFP

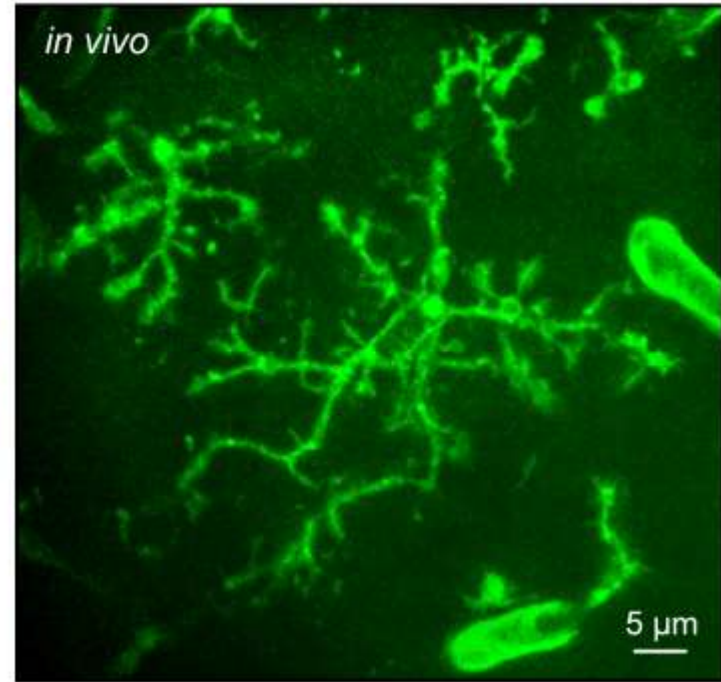
# Tomato lectin (from *Lycopersicon esculentum*) is an effective marker of microglial cells

800 nm excitation wavelength



■ -DyLight 594-conj. Tomato lectin

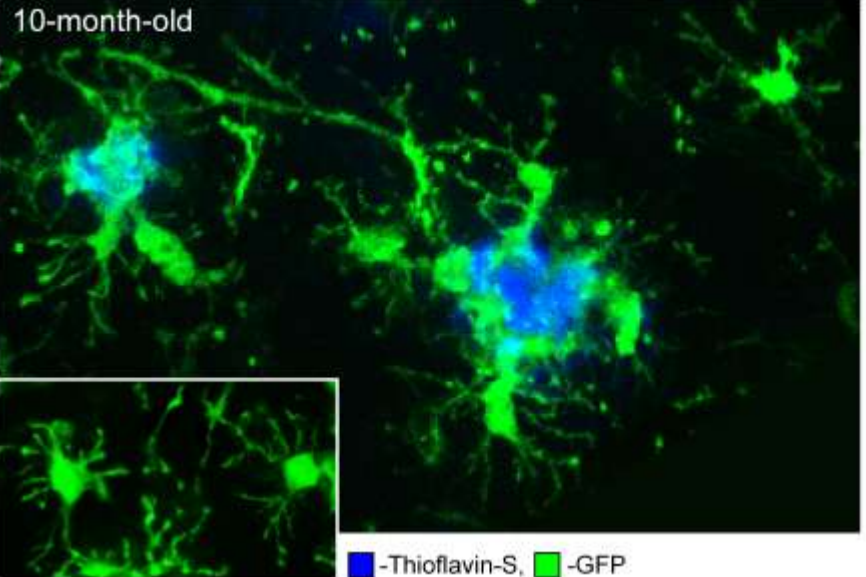
800 nm excitation wavelength



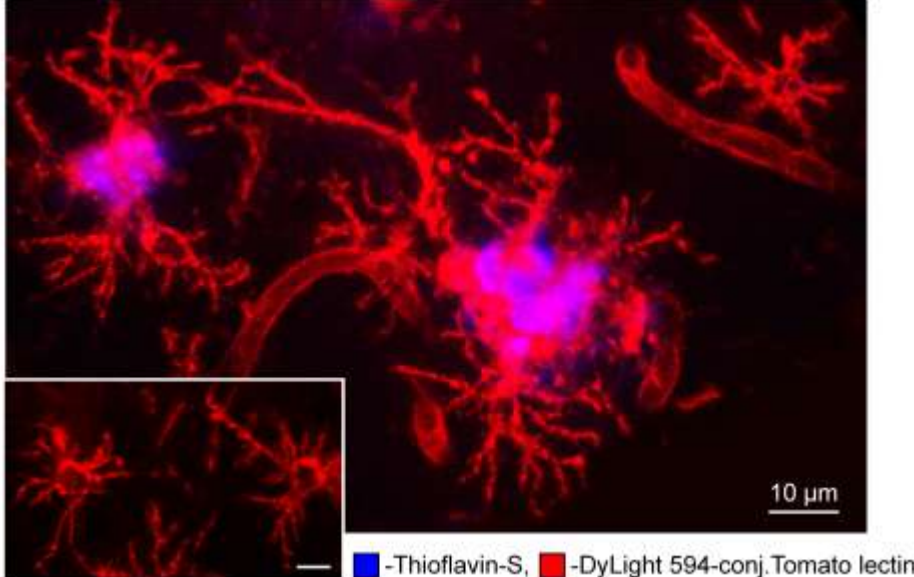
■ -Fluorescein-conj. Tomato lectin

# Microglia labeling in a mouse model of Alzheimer's disease

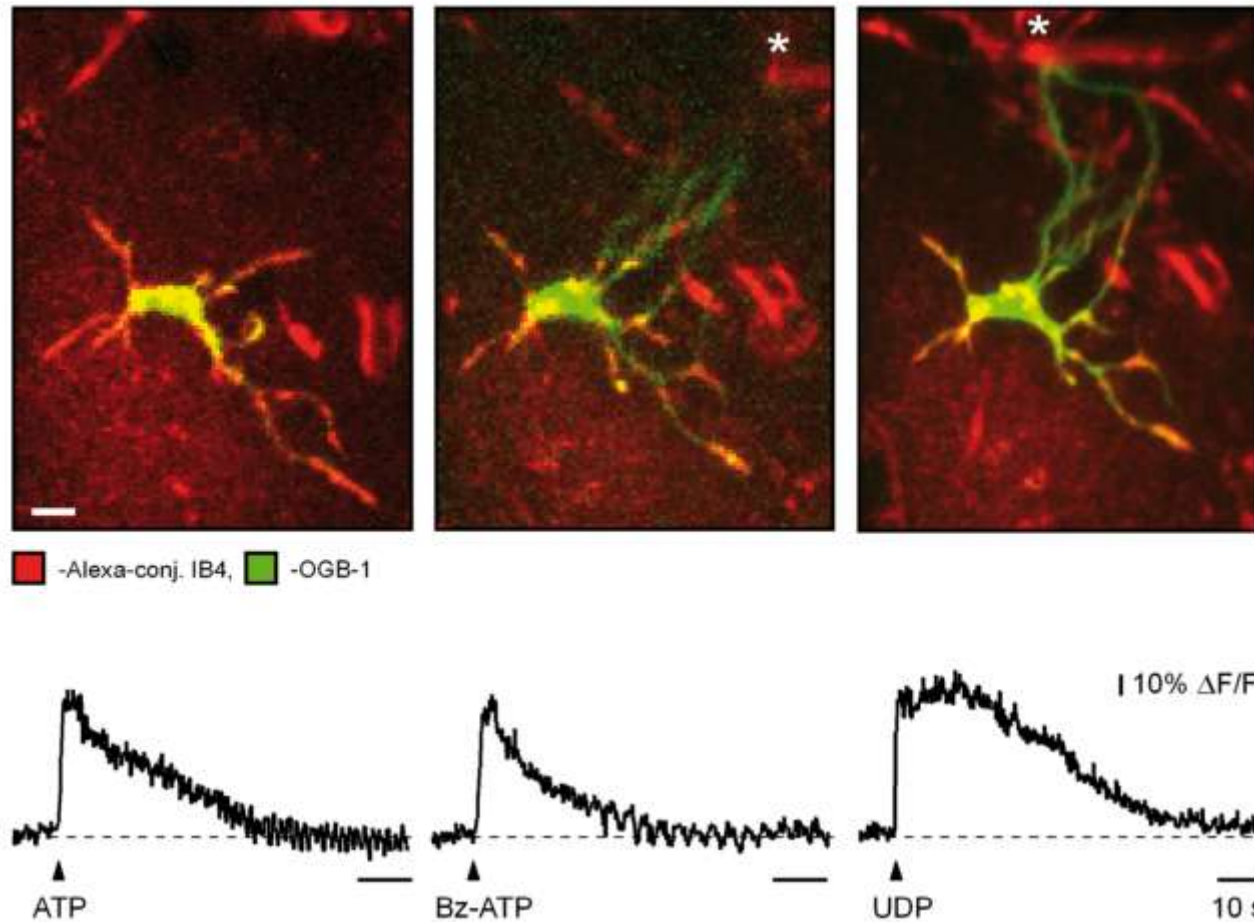
930 nm excitation wavelength



800 nm excitation wavelength



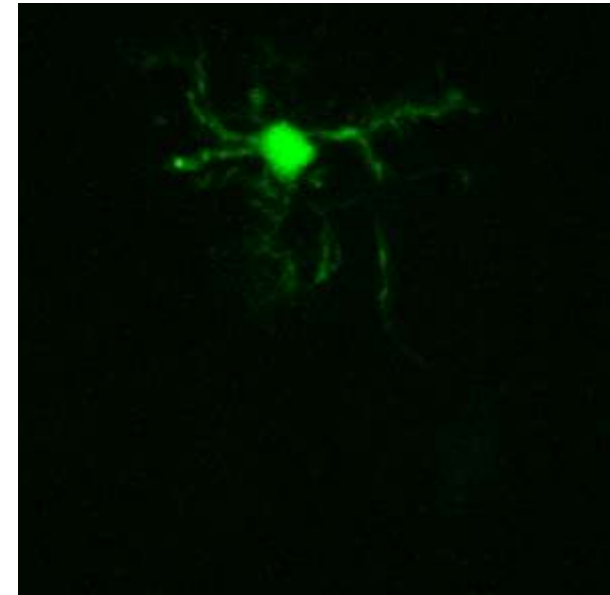
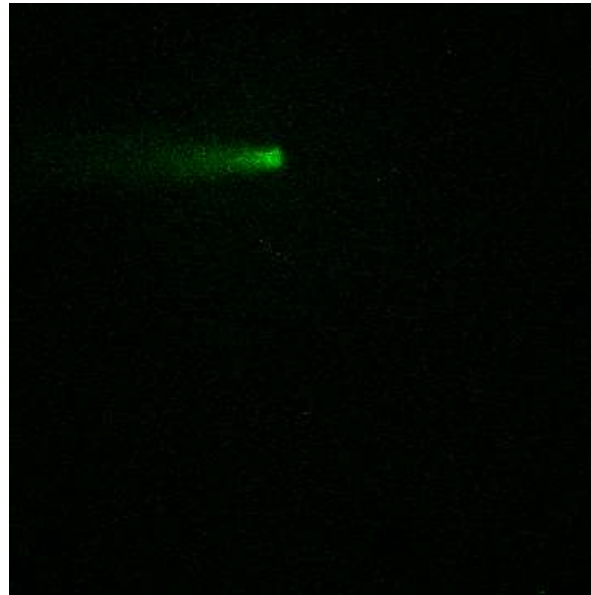
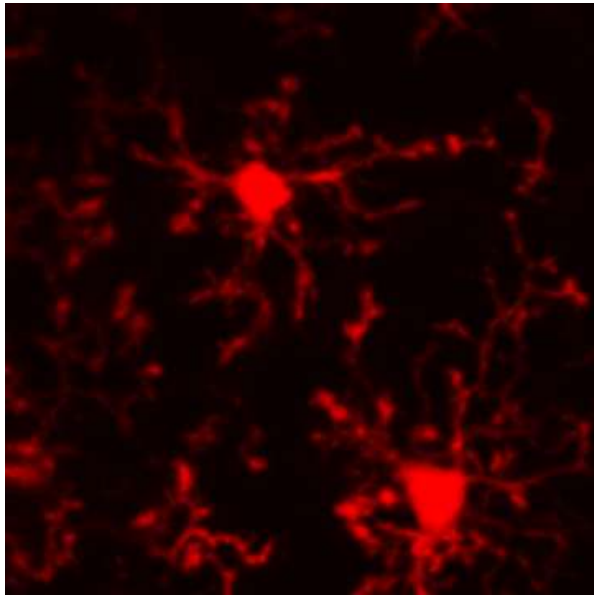
# The use of lectins for in vivo labeling of macrophages



# Labeling approaches for *in vivo* imaging of microglia

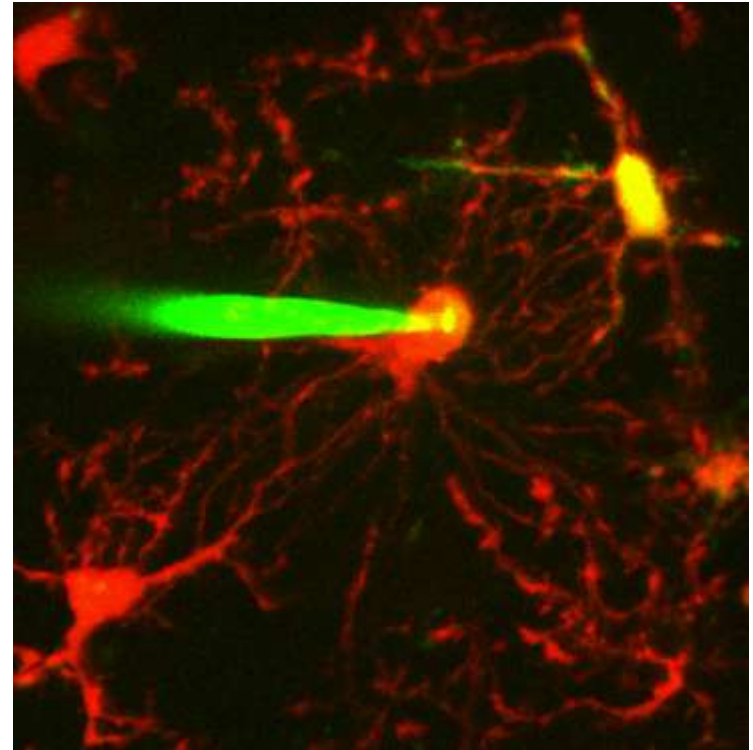
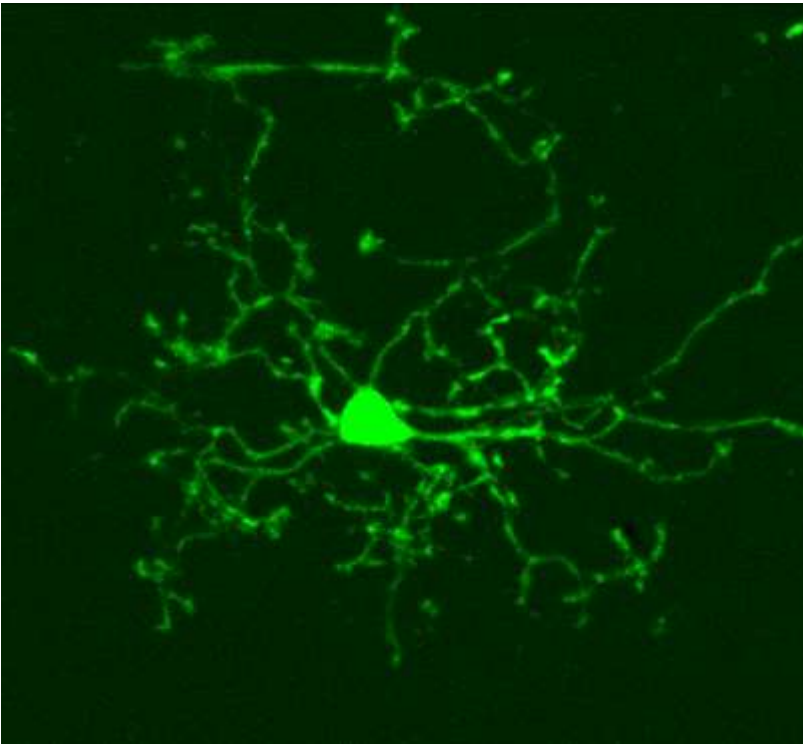
In vivo labeling technique	Labeling of cell body	Labeling of microglial processes	Overall image quality	Chronic imaging	Applicable to WT mice	Applicable to AD mice	Versatility of the approach
<b>GFP,</b> chronic expression	yes	yes	excellent	feasible	yes	yes	requires breeding of experimental animals
<b>Tomato lectin,</b> acute application	yes	yes	very good	requires re-labeling	yes	yes	applicable to any mouse strain at any experimental age
<b>Isolectin IB<sub>4</sub>,</b> acute application	yes	poor	medium	requires re-labeling	yes	no	works in juvenile, adult and aged WT but not AD mice

## *In vivo* electroporation of microglial cell



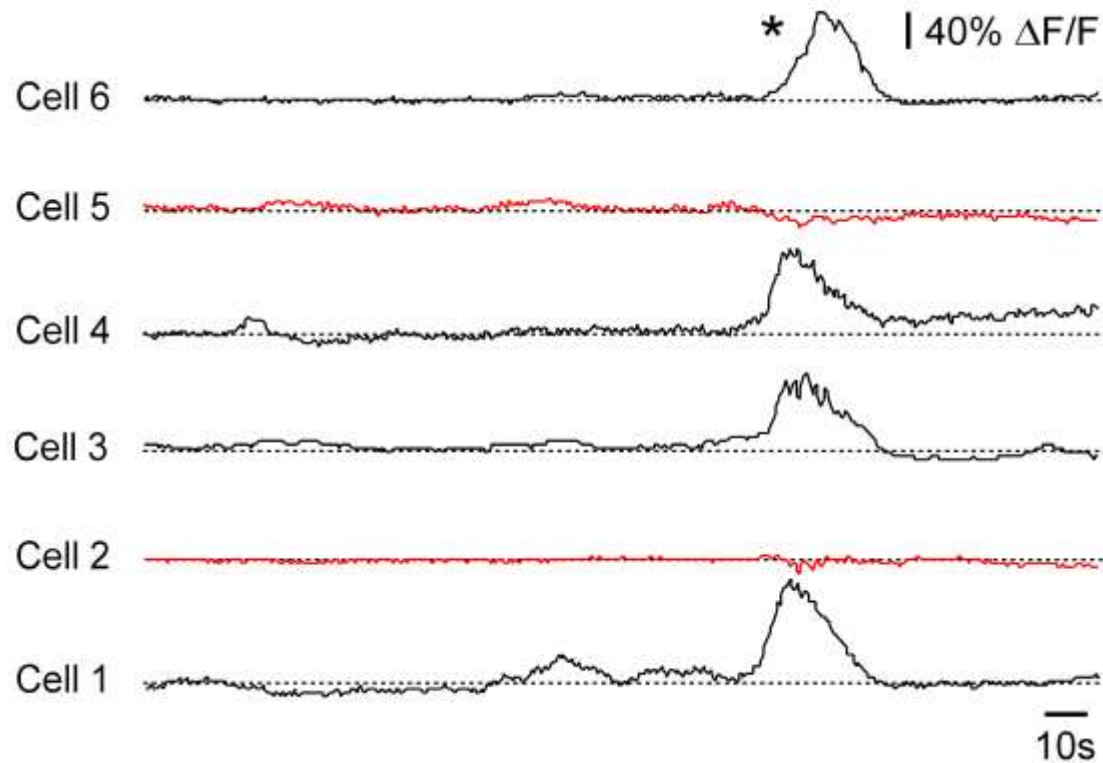
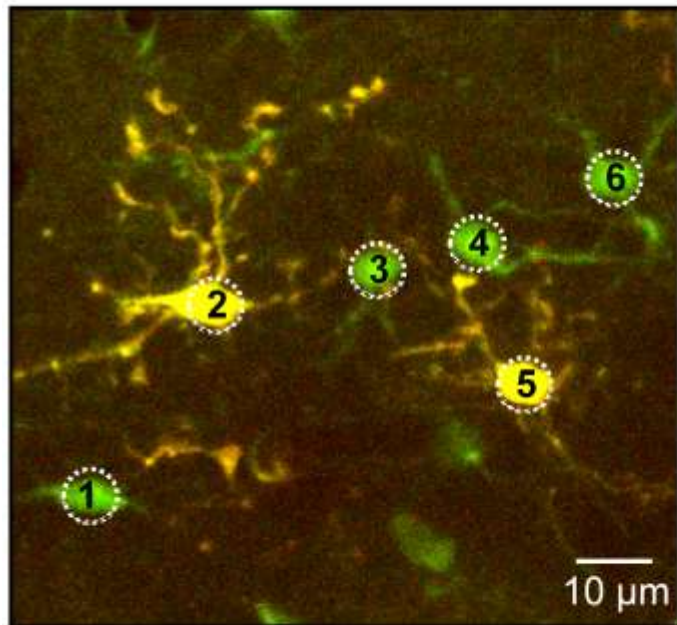
■ - Oregon green BAPTA 1, ■ - eGFP

# Does electroporation harm microglial cells?



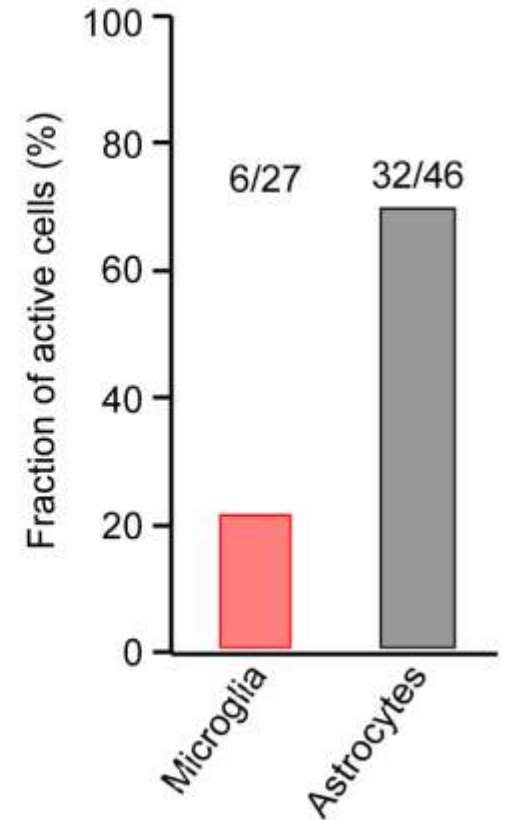
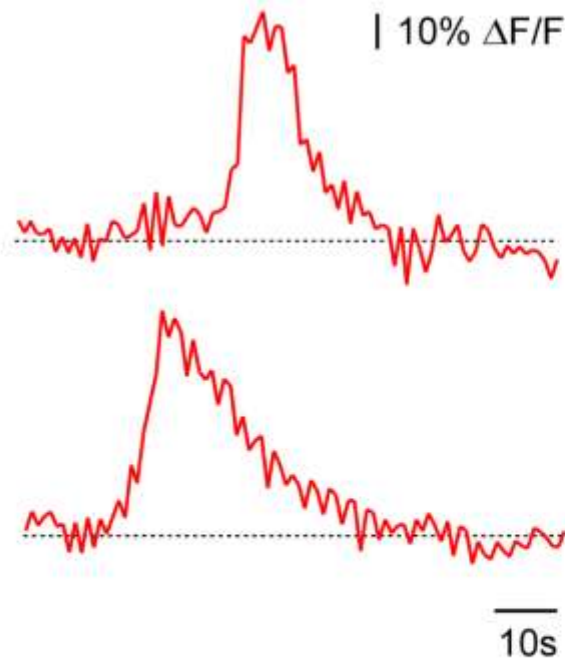
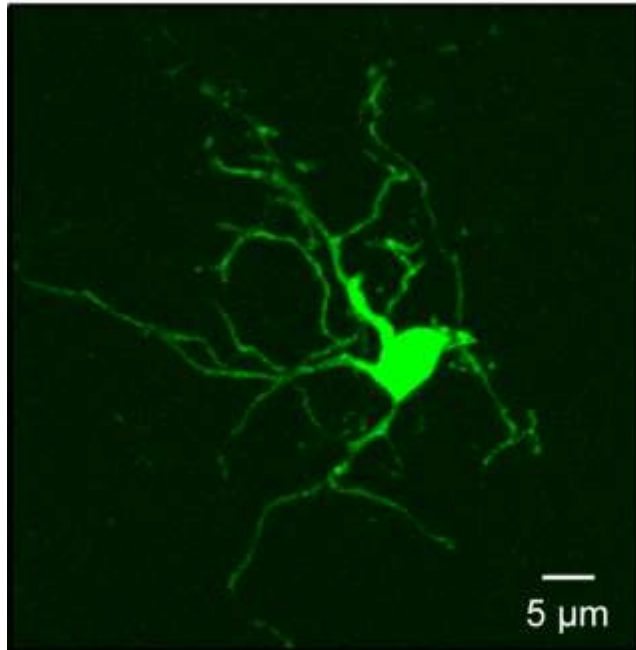


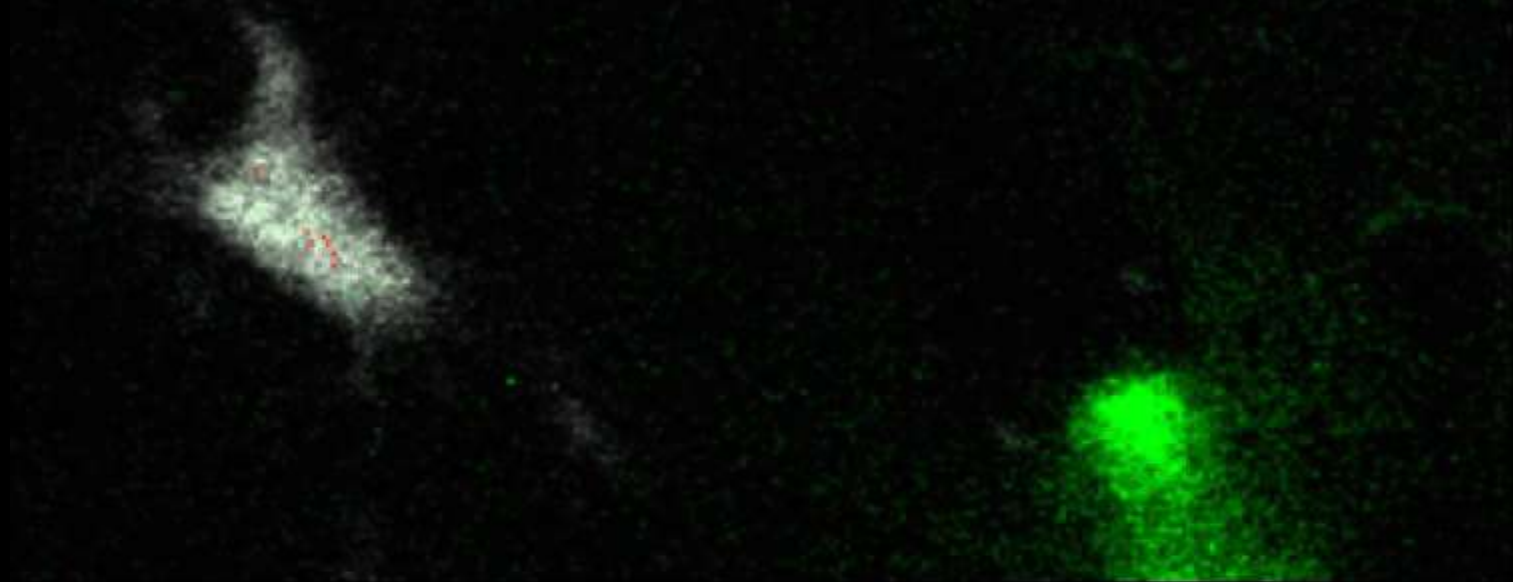
# WT microglia show very little spontaneous activity and do not participate in astrocytic calcium waves



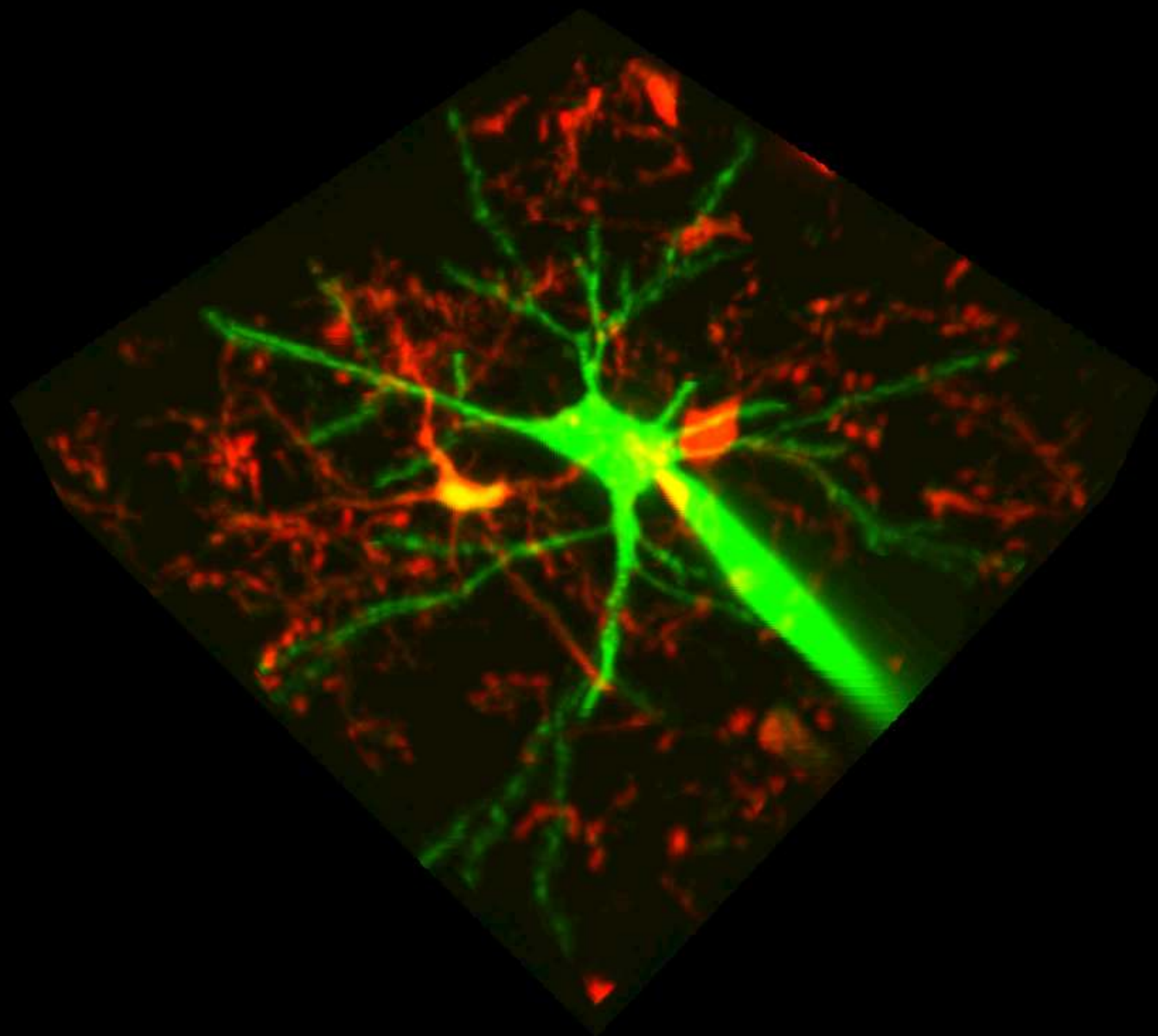
# Spontaneous calcium transients in WT microglia

WT





10 s



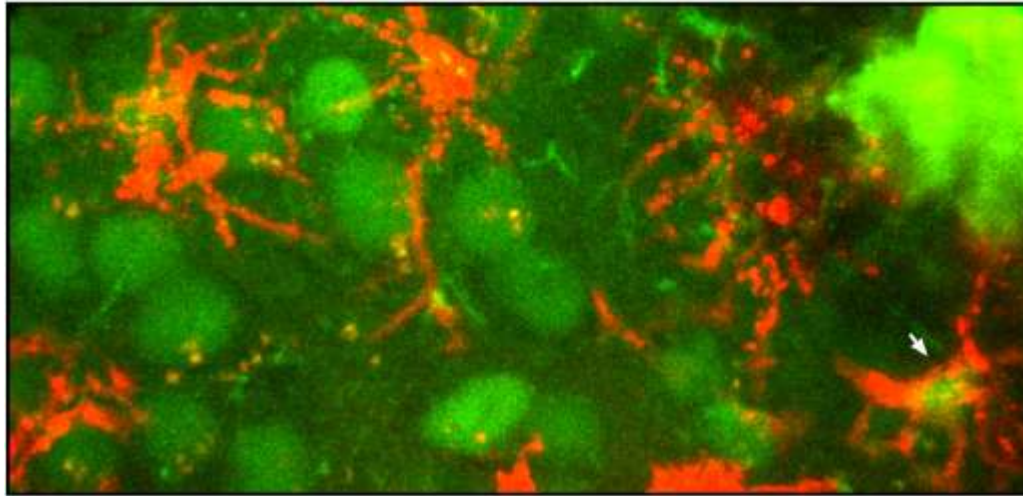
**In young WT mice:**

**Somatic Ca<sup>2+</sup> signaling in microglia is not involved in the surveillance of the extracellular milieu or in the detection of physiological levels of neuronal/astroglial activity**

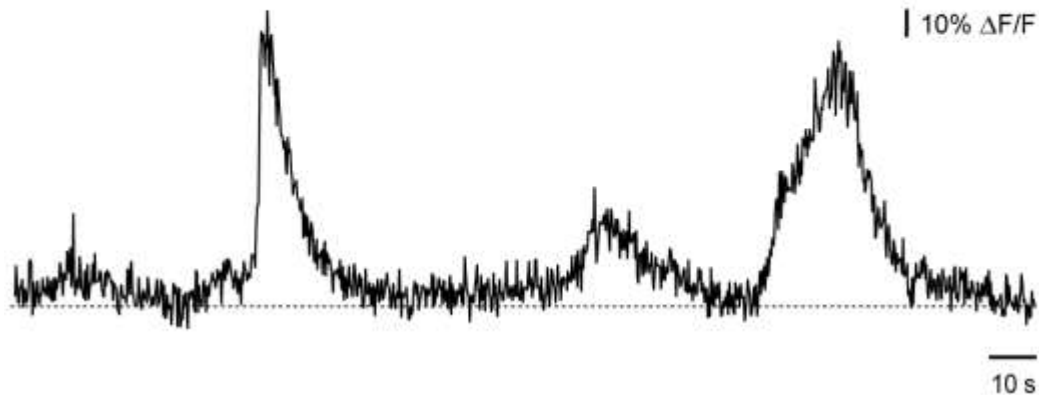
**Rather, it functions as highly sensitive and specific signal for recognition of the damage in the microglial microenvironment**

# Spontaneous calcium signaling in activated microglia

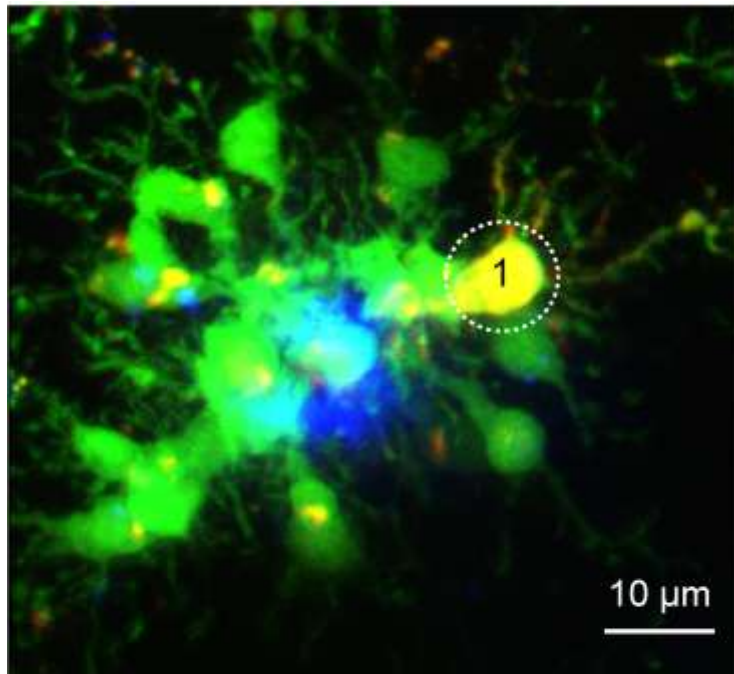
800 nm excitation wavelength



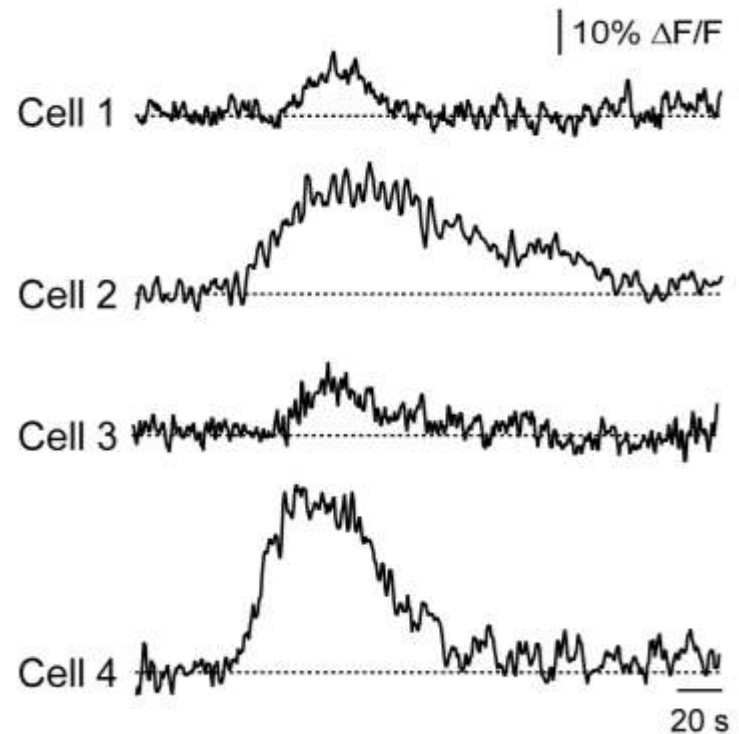
■ -OGB-1, ■ -DyLight 594-conj. Tomato lectin



# Spontaneous $\text{Ca}^{2+}$ signaling in plaque-associated microglia in AD mice

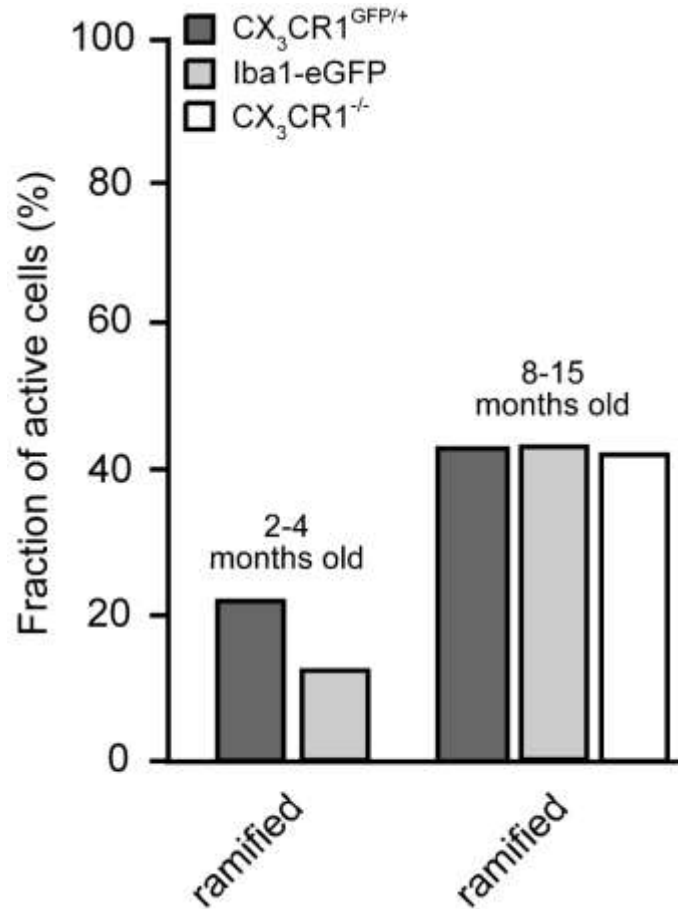


■ -GFP, ■ -Thioflavin-S, ■ -OGB-1

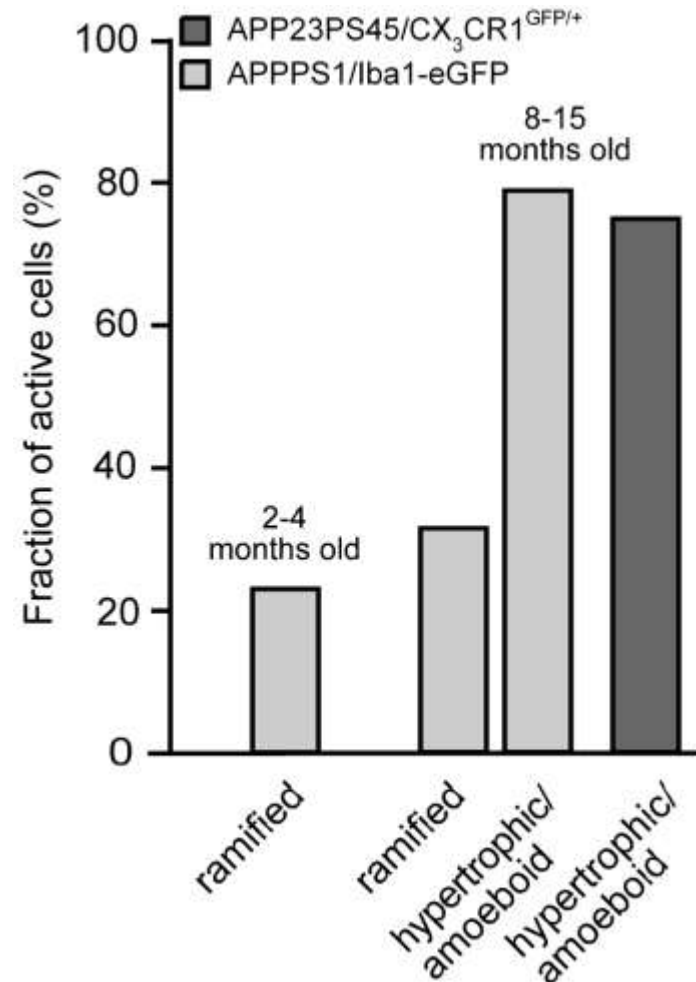


# Spontaneous calcium signaling is increased in the course of aging and AD pathology

## Wild Type

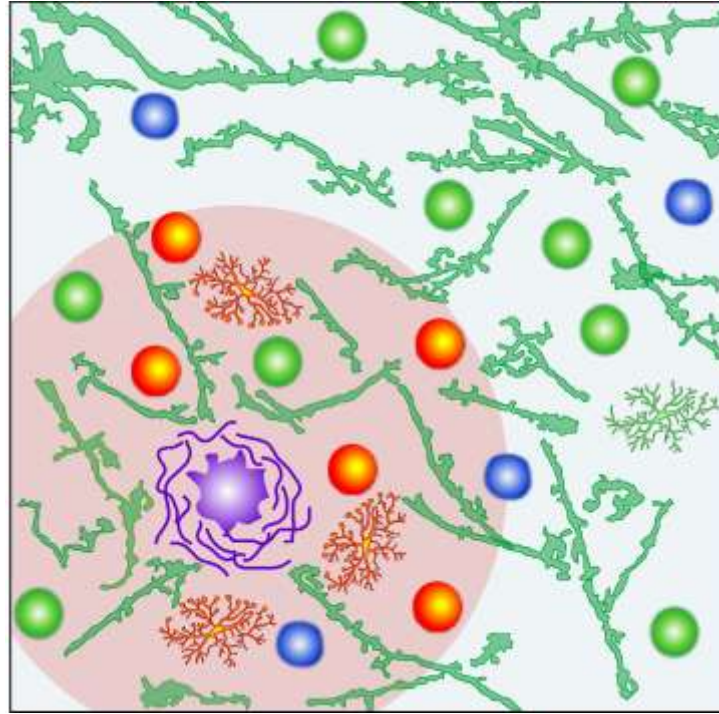


## Alzheimer's disease





# Impaired functional properties of microglia in amyloid-depositing mice



- impaired ability of hypertrophic and amoeboid cells to respond to “danger” stimuli in their microenvironment
- as a result, microglia in AD brain might fail to establish the firm barrier between healthy tissue and the one undergoing a minute damage
- plaque-associated microglia show a much higher incidence of “spontaneous”  $\text{Ca}^{2+}$  transients. These signals are likely causing a  $\text{Ca}^{2+}$ -dependent release of cytokines from microglia thus inducing/exacerbating neuronal hyperactivity

**Thanks to:**



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**LMU München**

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Hirnforschung**

**Mathias Jucker**