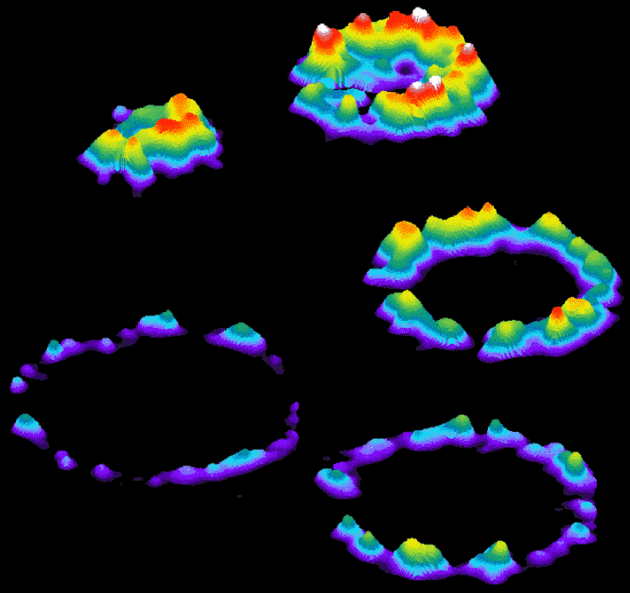
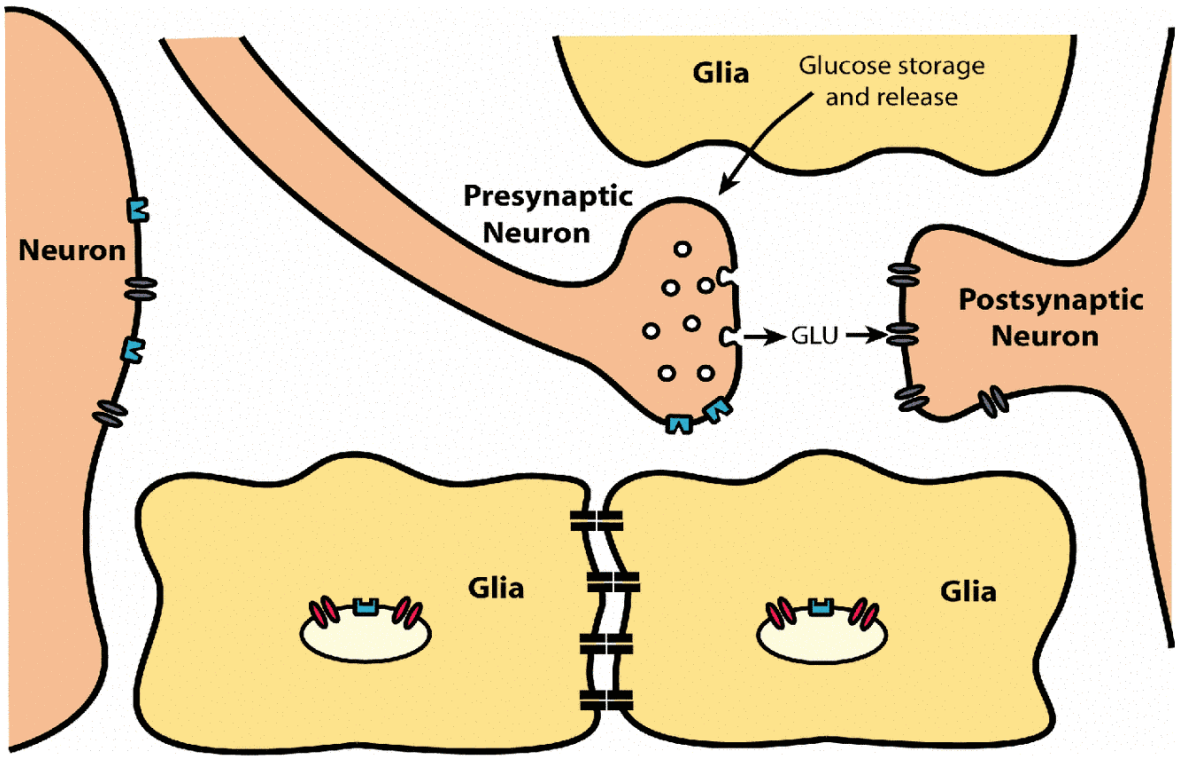
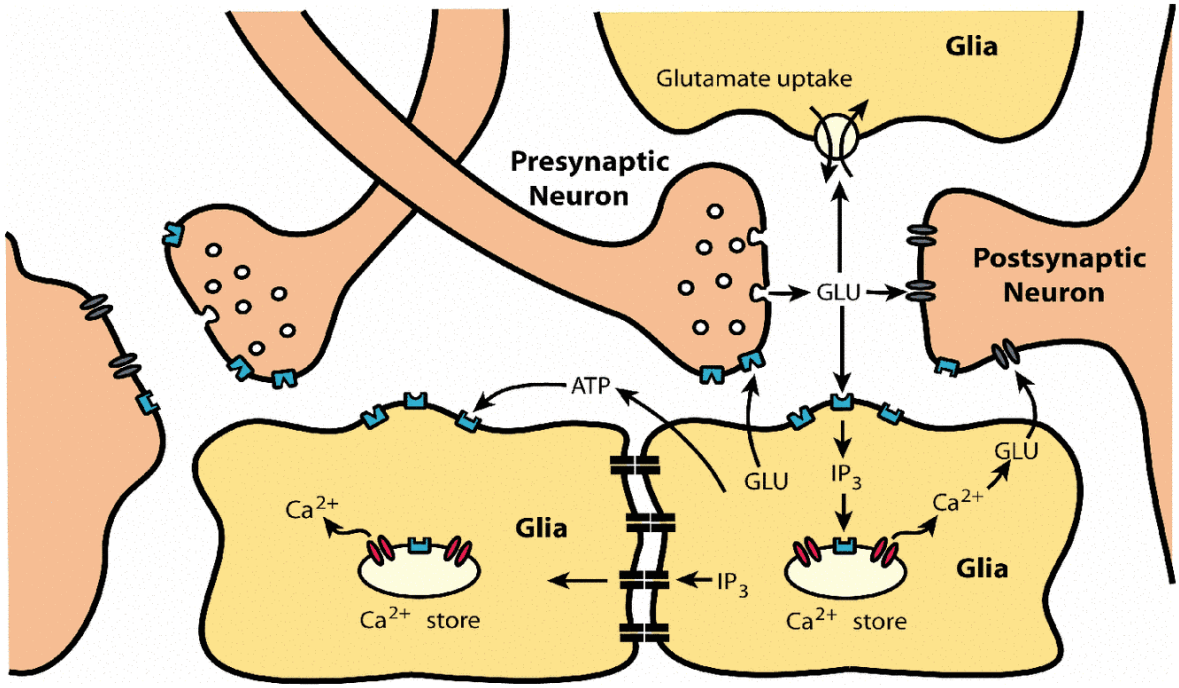
Glial Cell Regulation of Synaptic Transmission in the Retina



Eric A. Newman, Department of Neuroscience,
University of Minnesota

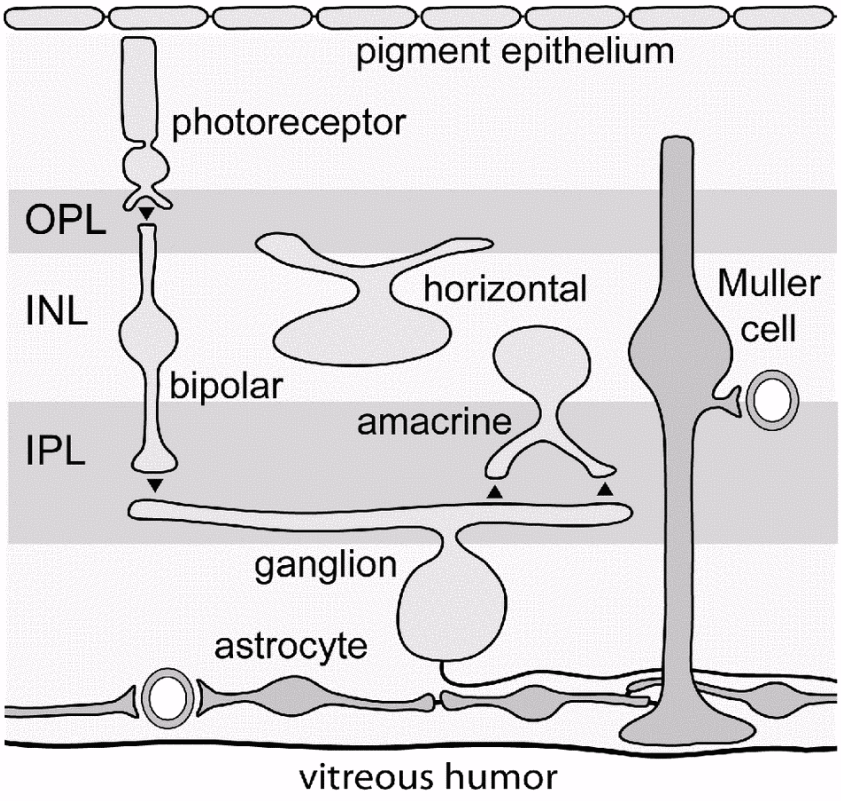




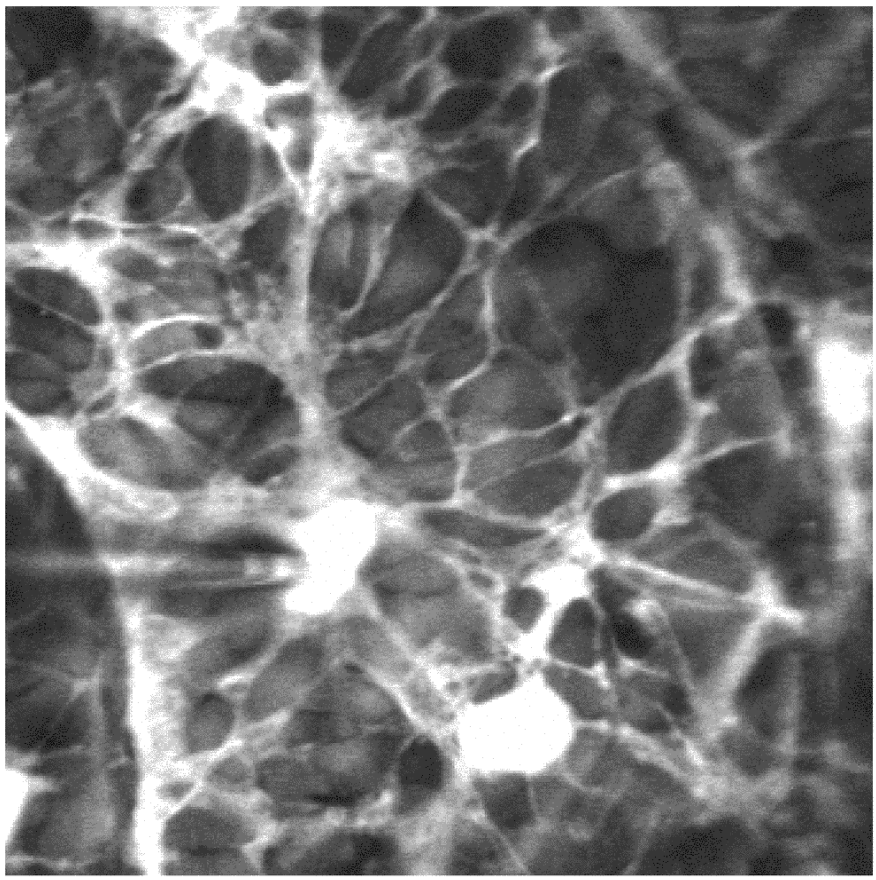
Do glial cells modulate neuronal activity in intact CNS tissue?

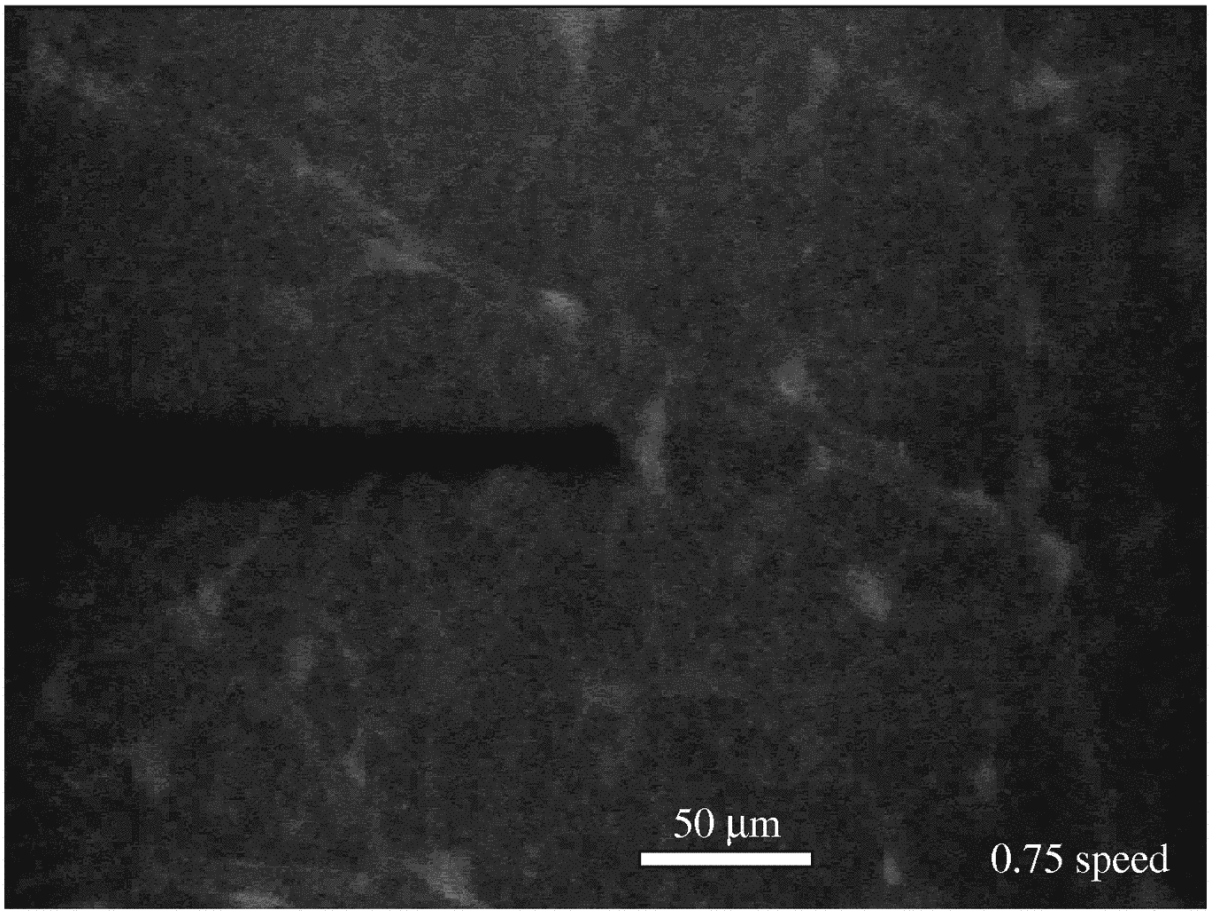
- Retinal model: acutely isolated rat retina
- Questions
  - Do glial Ca^{2+} waves occur *in situ*?
  - What is the mechanism of propagation of Ca^{2+} waves *in situ*
  - Does light stimulation elicit glial Ca^{2+} increases?
  - Do activated glial cells modulate retinal neurons?

Mammalian retina

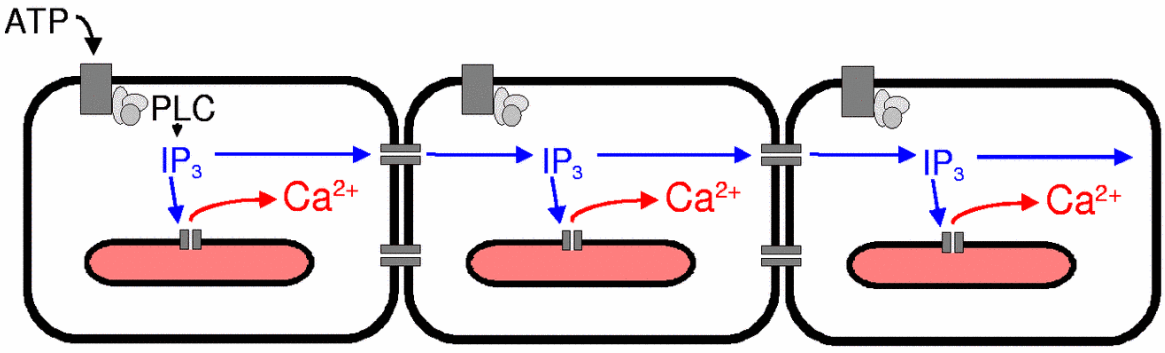


Rat retina labeled with Calcium Green-1 or Fluo-4

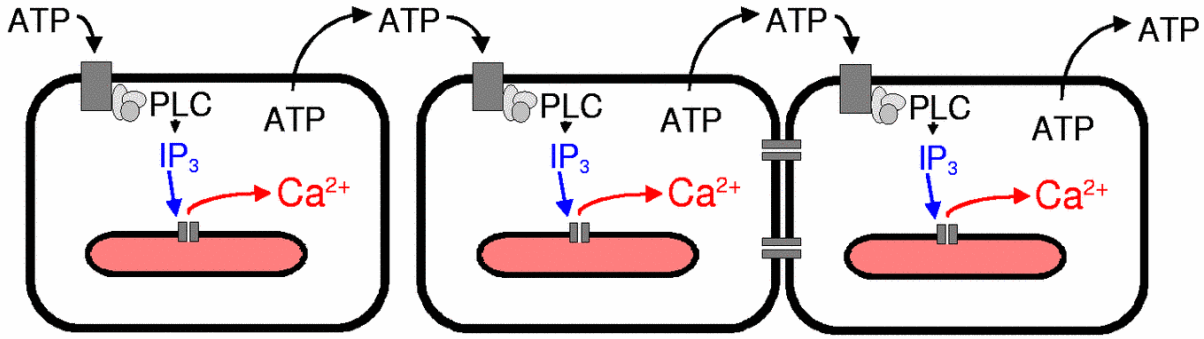


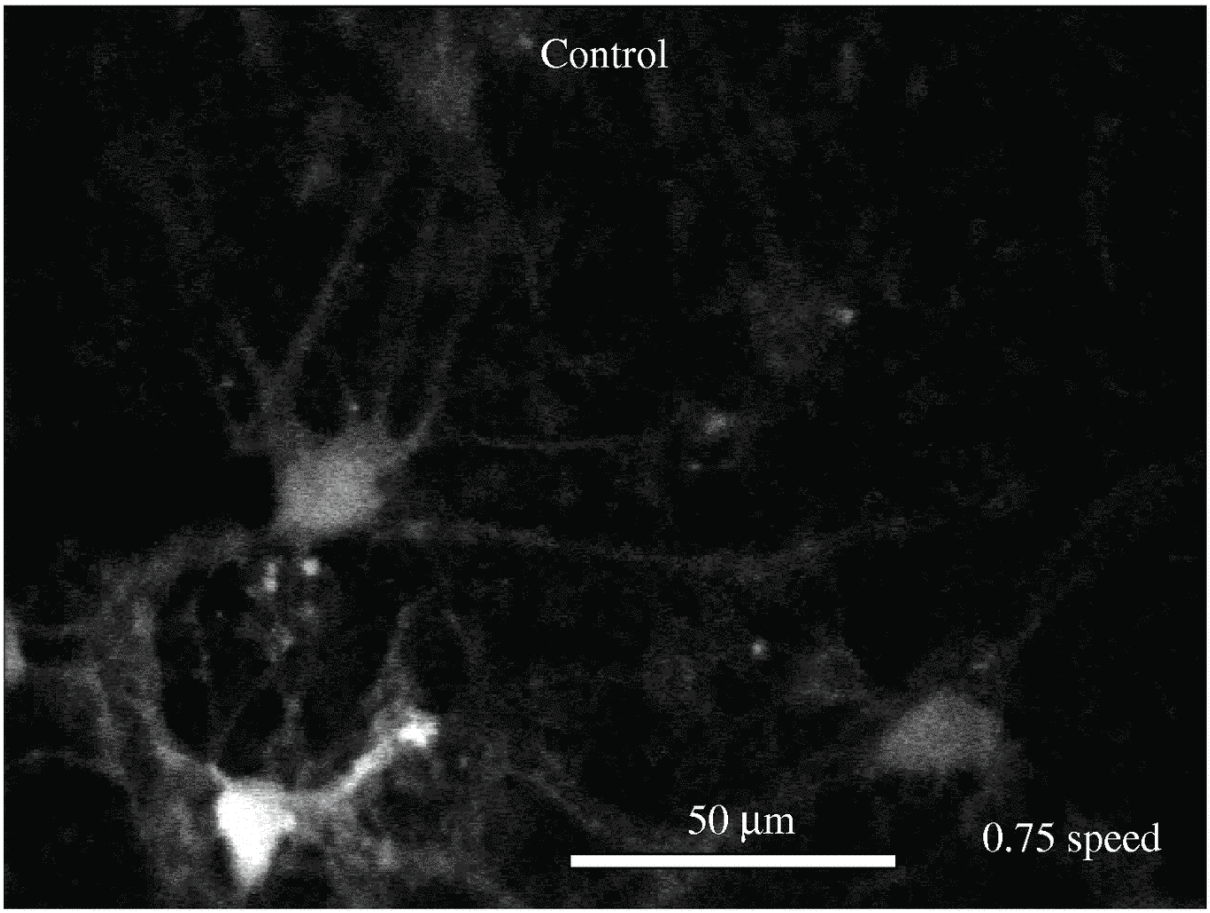


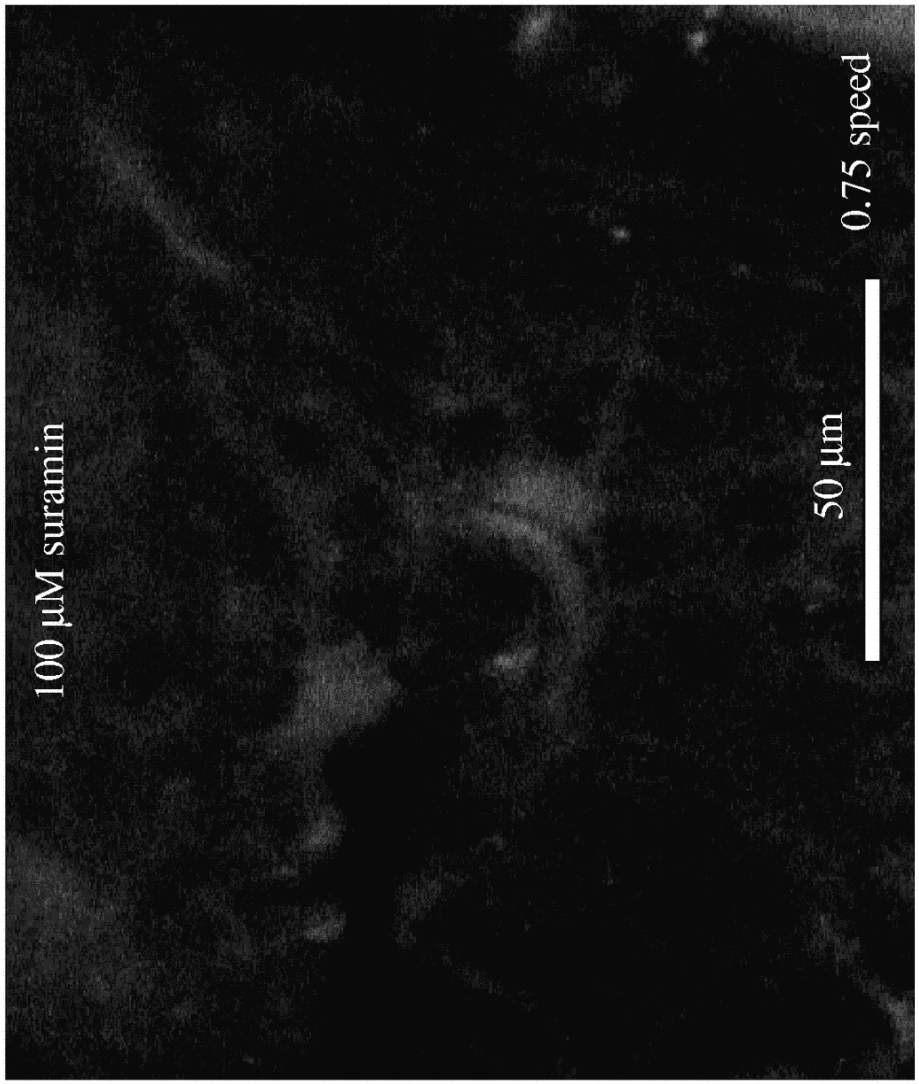
- What is the mechanism of Ca^{2+} wave propagation?
  - Diffusion of IP_3 through gap junctions

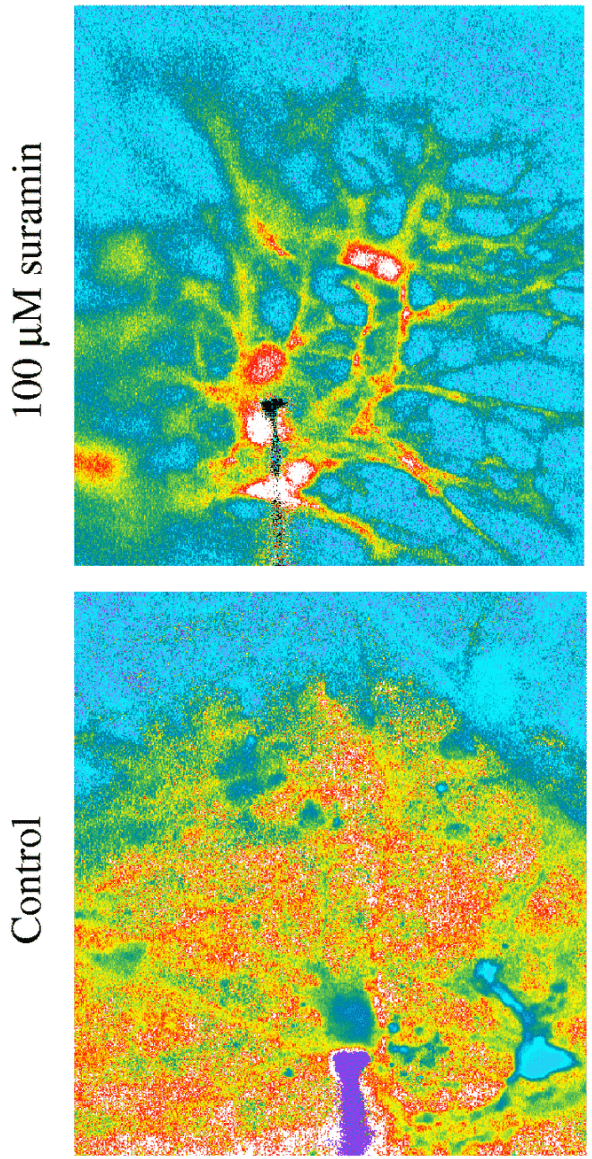


– Release of ATP, which functions as extracellular messenger stimulating glial purinergic receptors

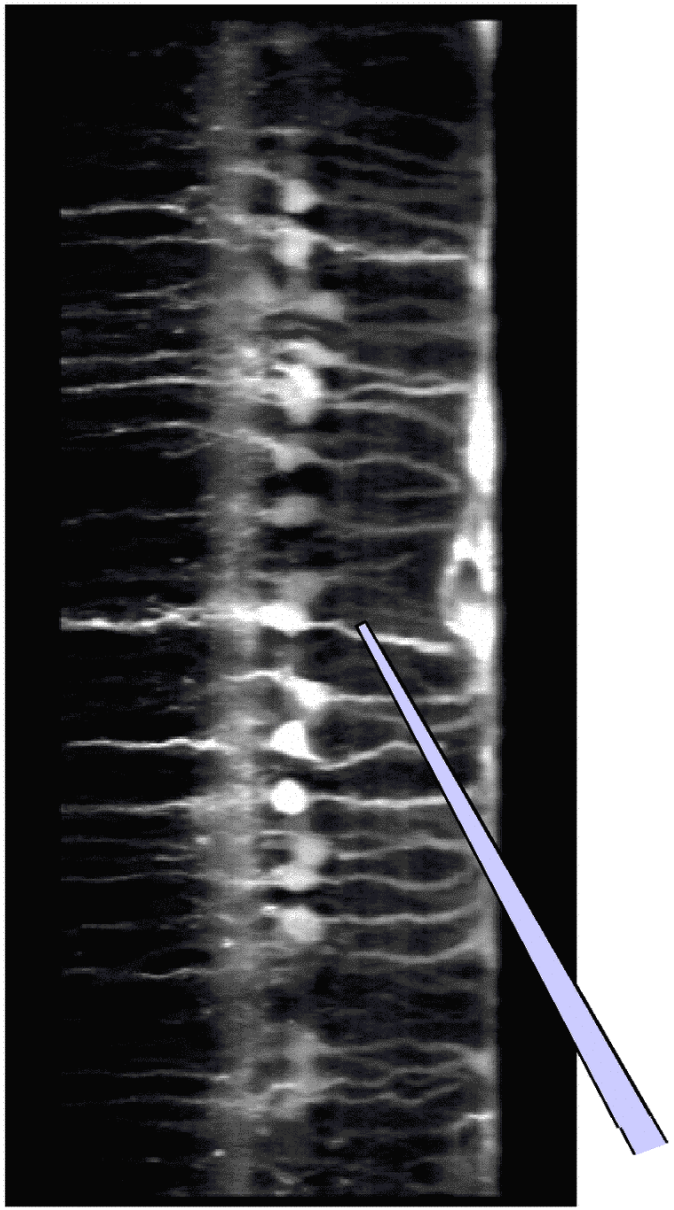




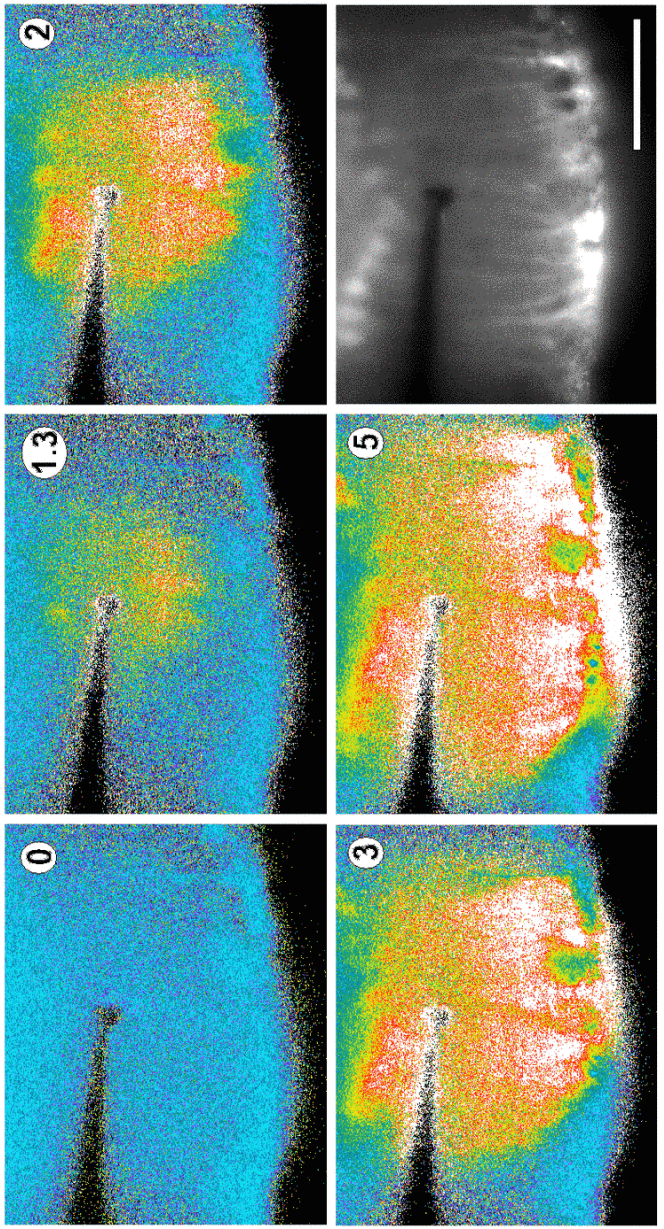

100 µM suramin
50 µm
0.75 speed


100 µM suramin
Control

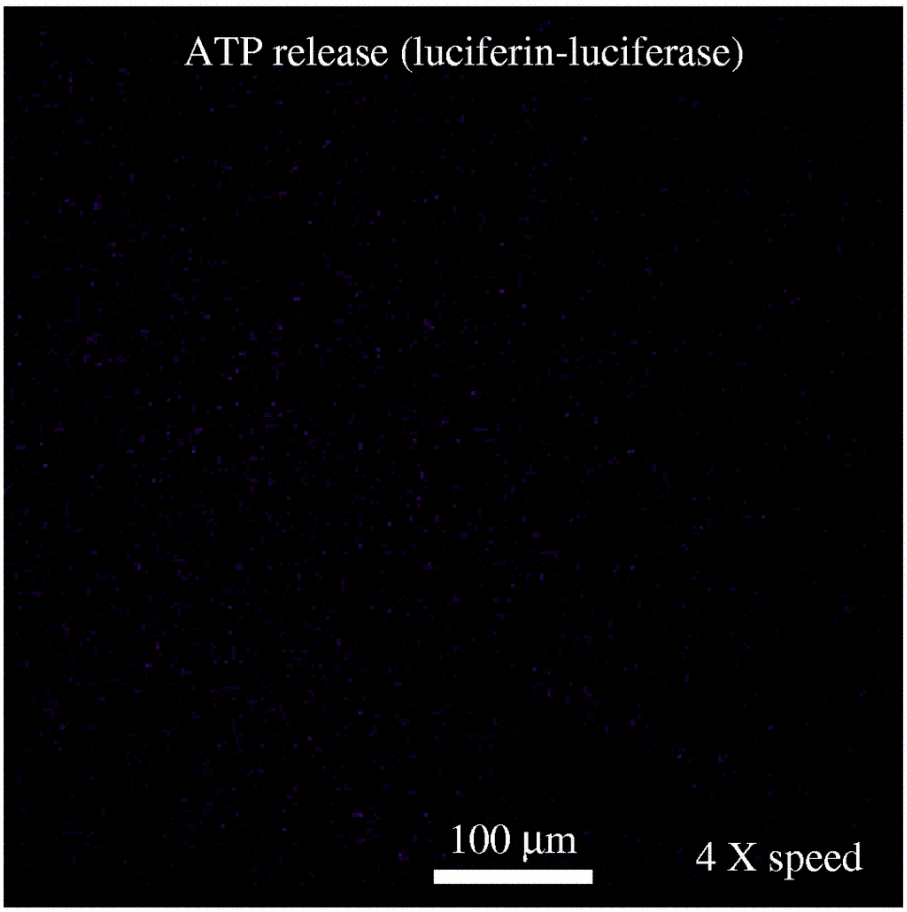
Muller cell stimulation



Retinal slice

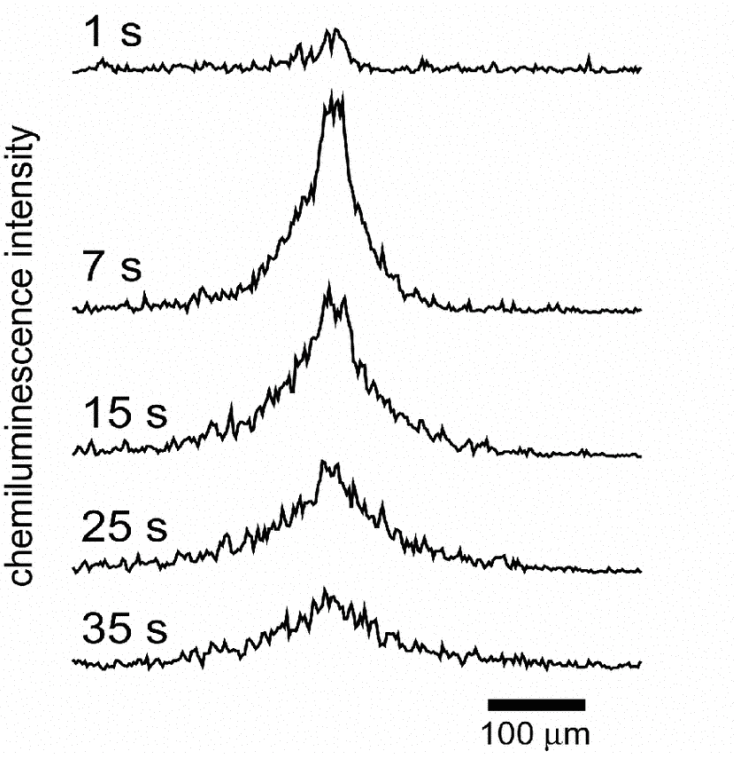


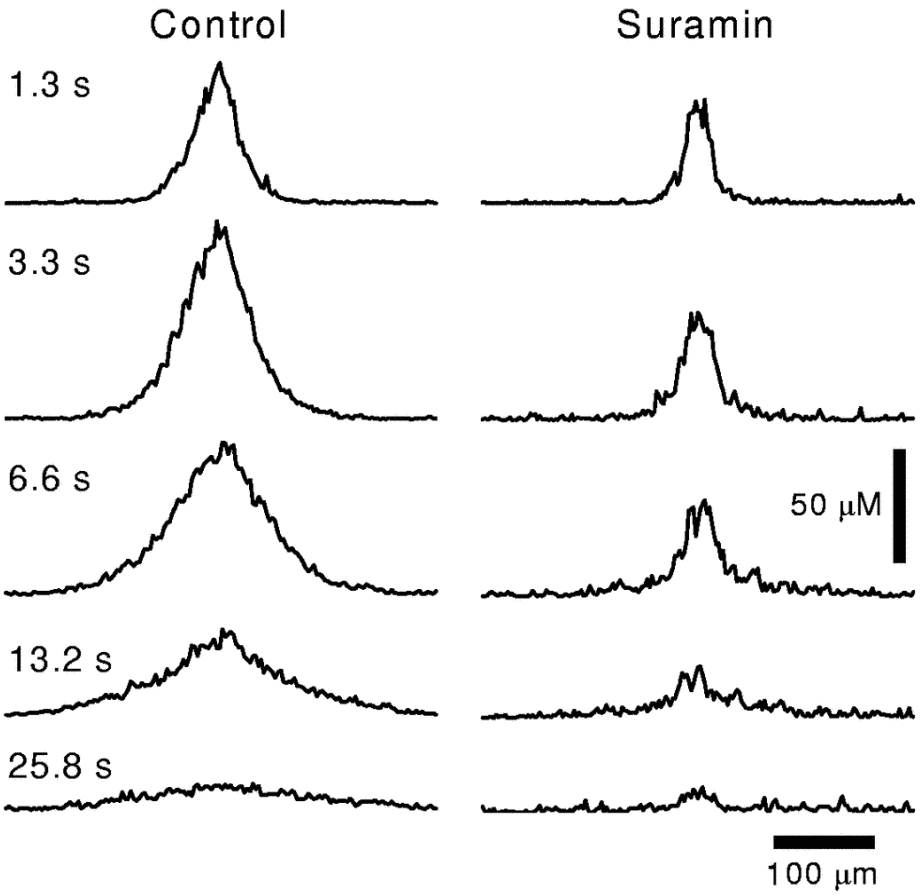
- Muller cell to Muller cell propagation of Ca^{2+} waves
  - Propagation blocked by suramin
  - Propagation occurs along the length of the cells
    - ATP released from all regions of Muller cells
    - Purinergic receptors in all Muller cell regions

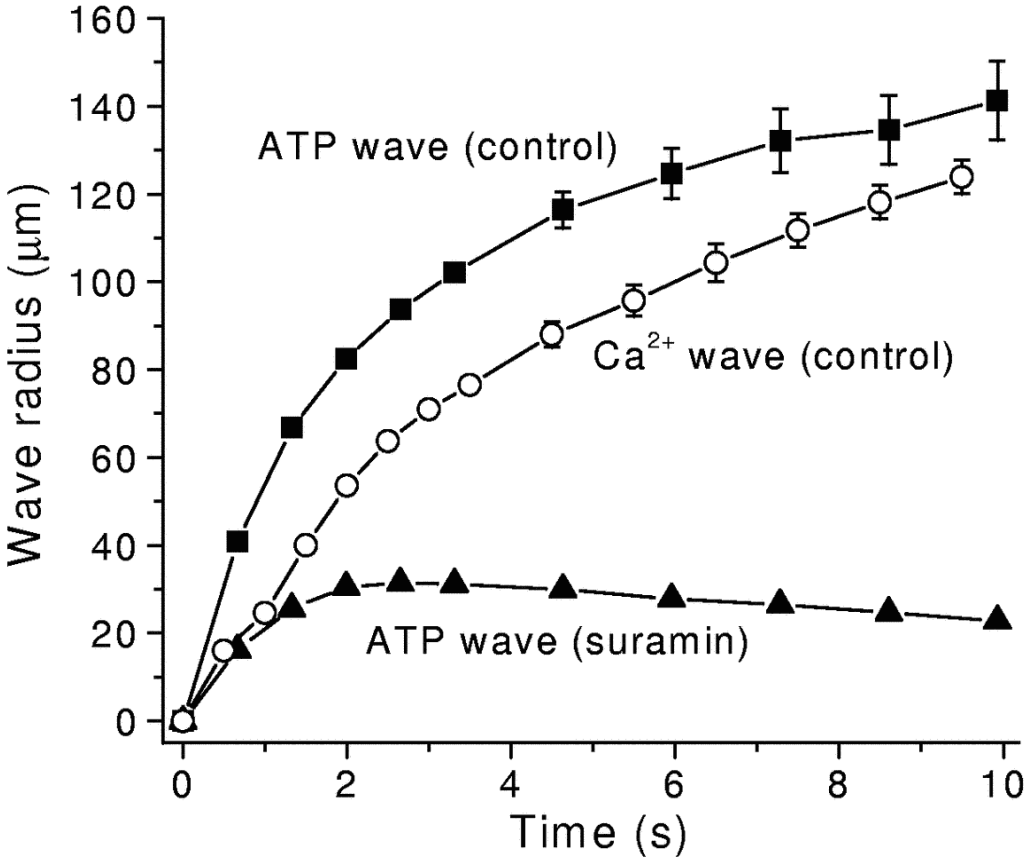


ATP release at the retinal surface

(100 μM Cd^{2+} in solution)





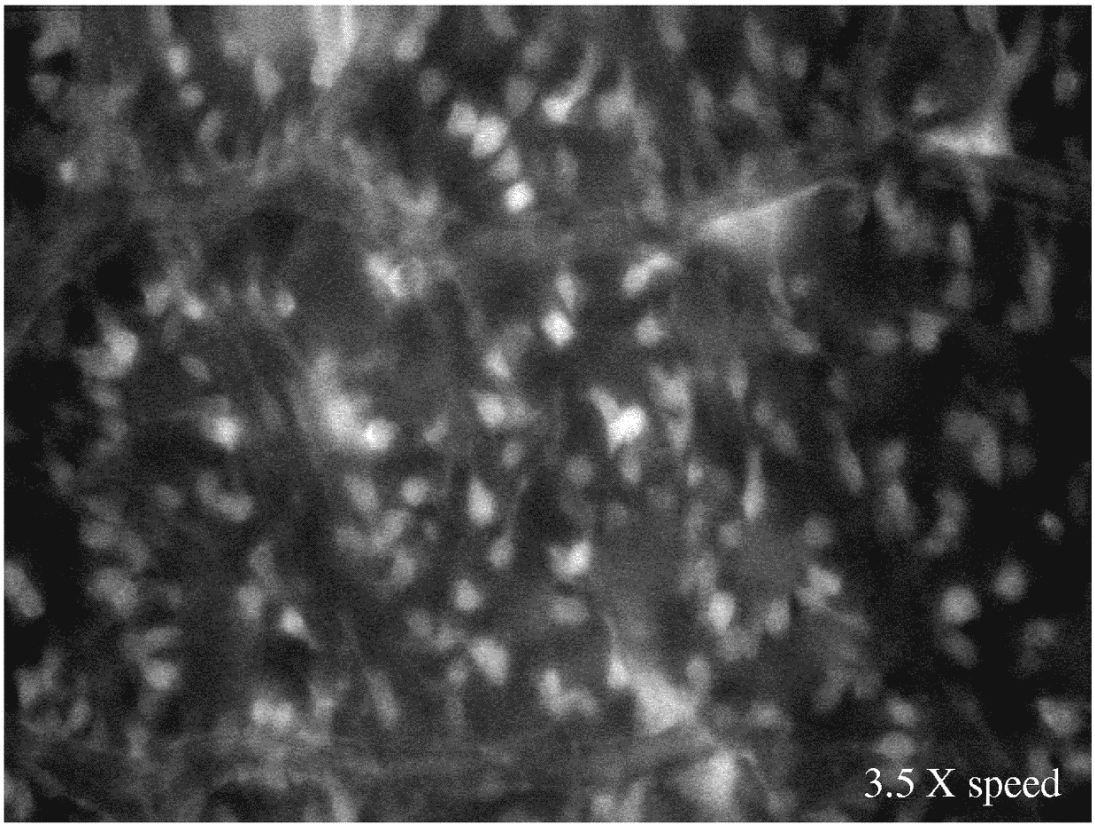


- Calcium waves propagate through retinal glial cells by two mechanisms
  - Diffusion of an internal messenger (IP_3) through gap junctions
    - Astrocyte to astrocyte propagation
  - Release of extracellular messenger (ATP)
    - Astrocyte to Muller cell propagation
    - Muller cell to Muller cell propagation
    - Augments astrocyte to astrocyte propagation

Activation of Glial Cells by Light Stimulation

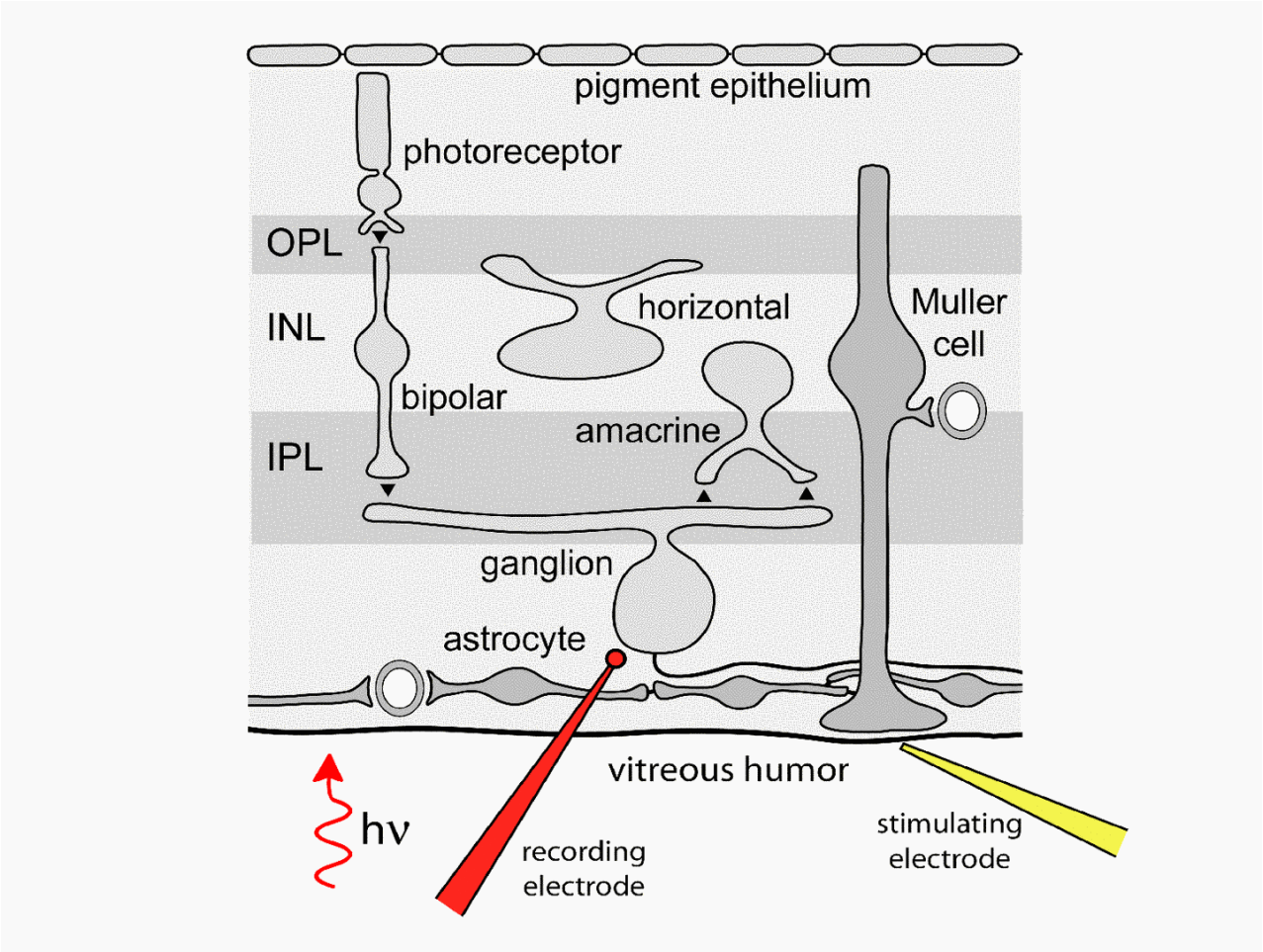
- Brain slices: neuronal activity elicits Ca^{2+} increase in glial cells
  - Mediated by neuronal release of glutamate, GABA, acetylcholine and nitric oxide
- Does light-evoked neuronal activity in the retina elicit Ca^{2+} increases in astrocytes and Muller cells?

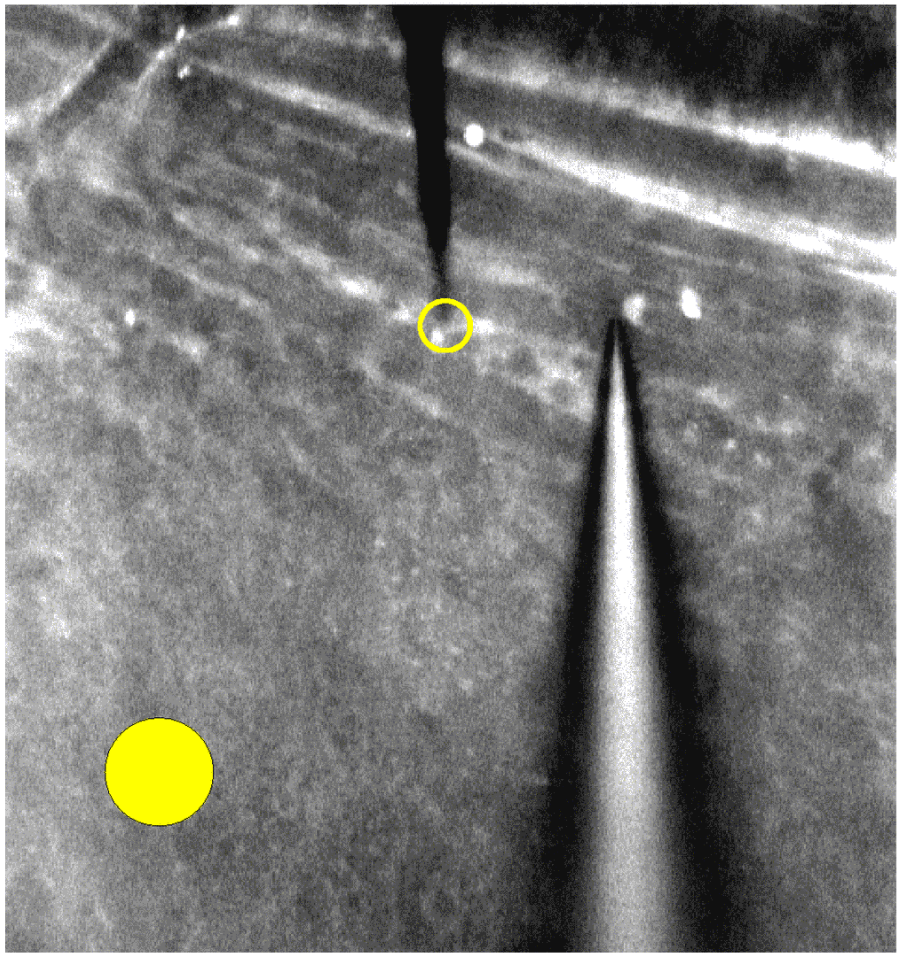
Ca^{2+} transients in Muller Cells

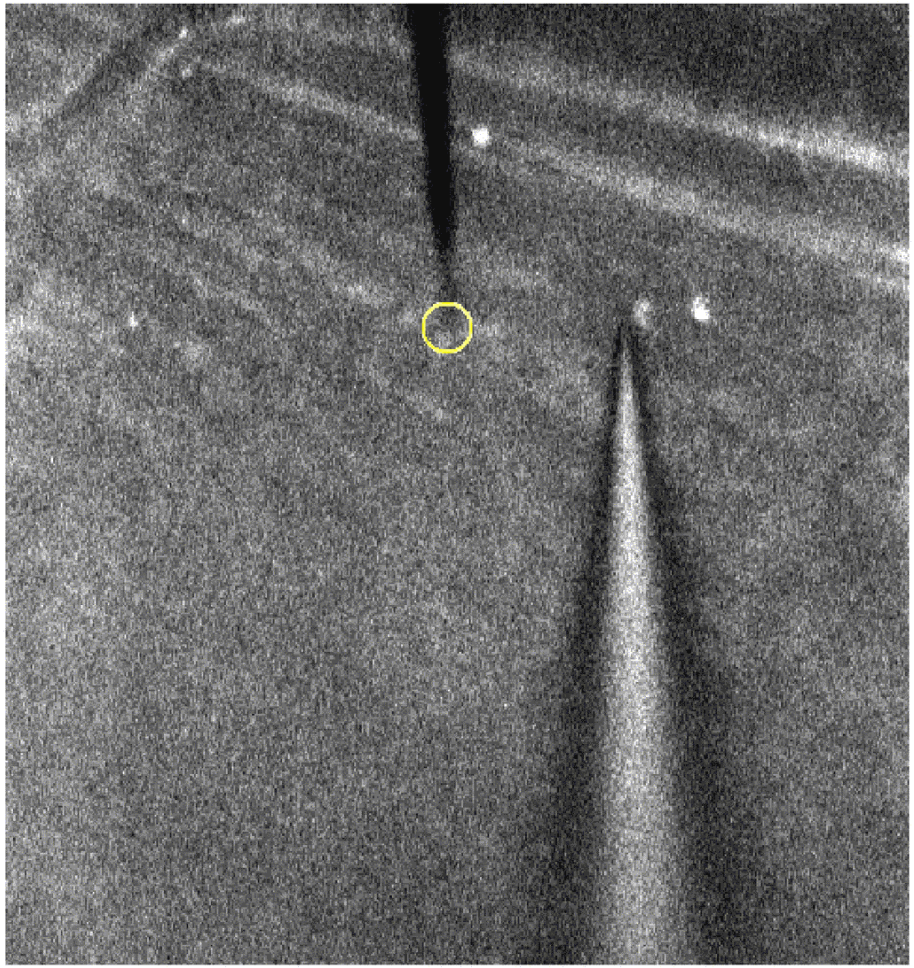


Do glial cells modulate neuronal activity?

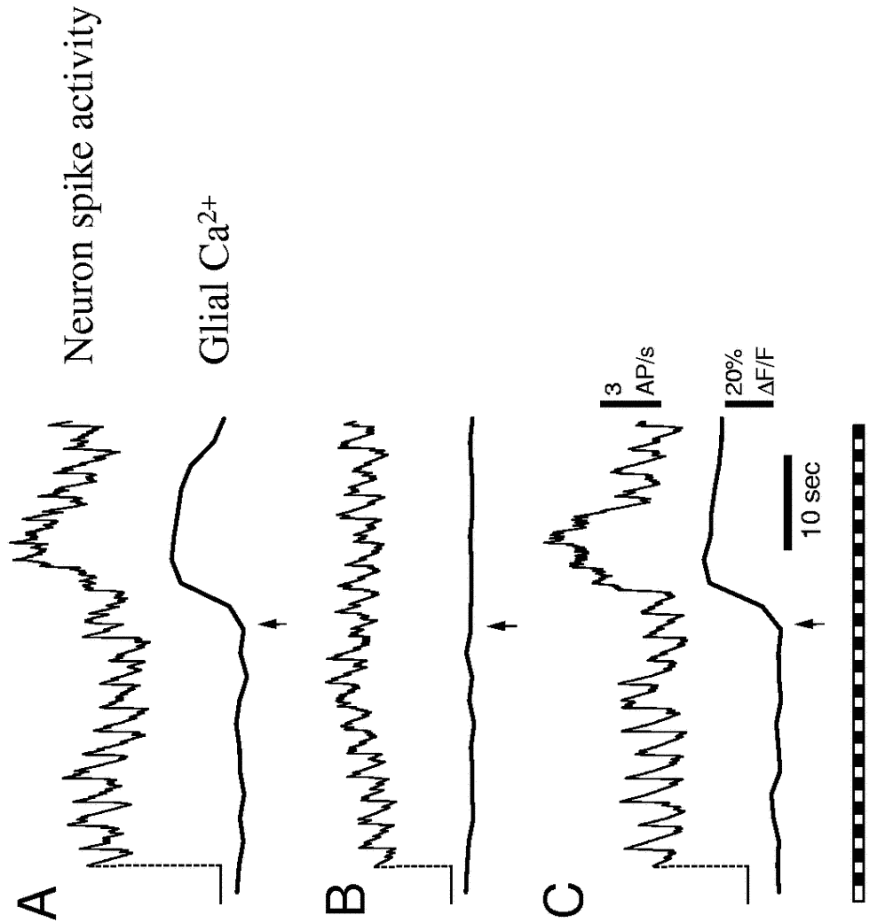
- Is the spike activity of retinal neurons modulated when glial cells are activated and generate Ca^{2+} waves?
- Is the modulation excitatory or inhibitory?

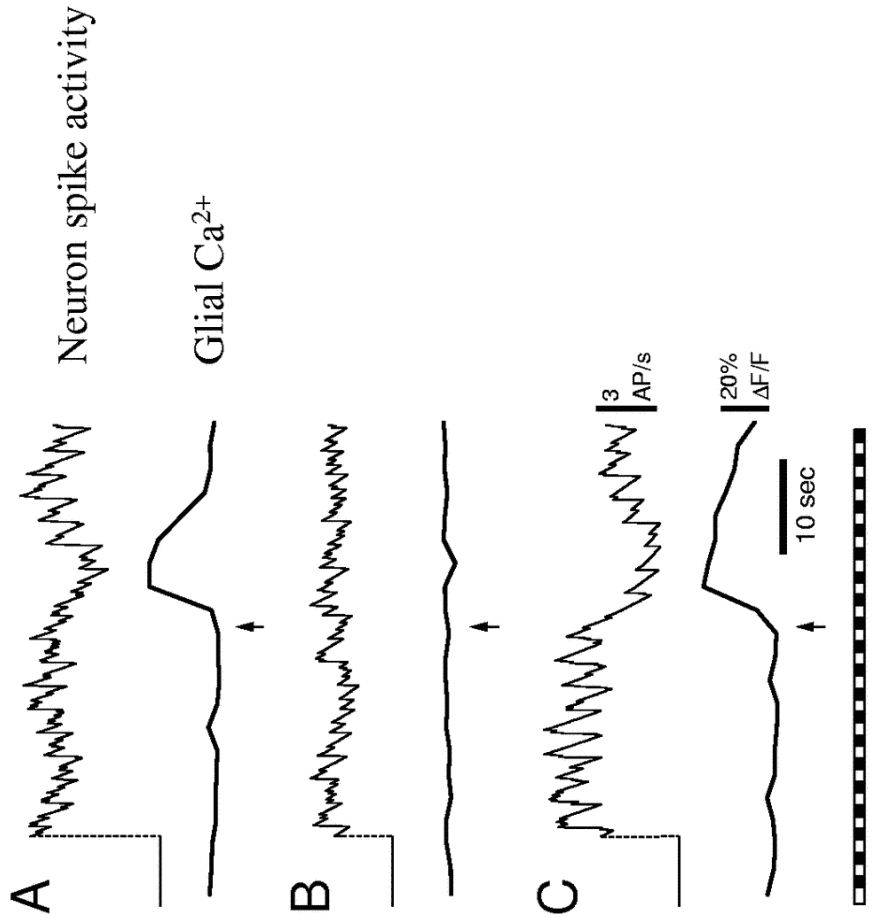






normal speed


A
Neuron spike activity
Glial Ca2+
B
C
3
AP/s
20%
ΔF/F
10 sec


A
Neuron spike activity
Glial Ca2+
B
C
3
AP/s
20%
ΔF/F
10 sec

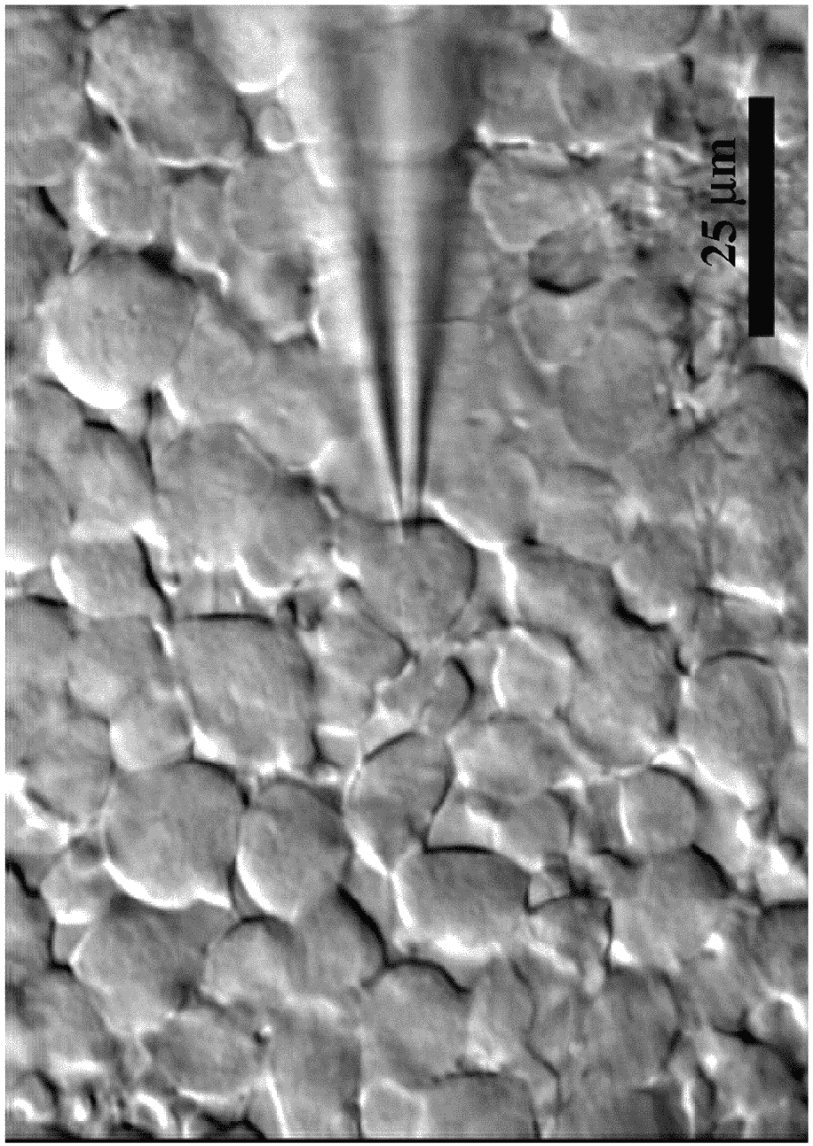
Glial modulation of neurons

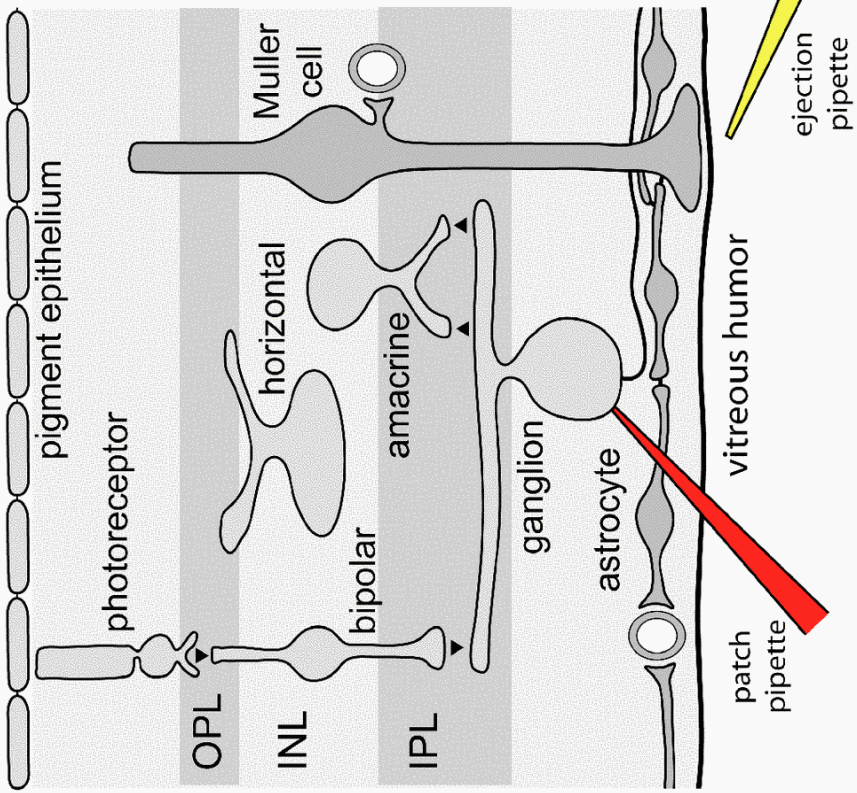
- Light-evoked activity of neurons modulated by glial cell activation
- 57% of neurons show significant change in spike activity
  - 17% of cells excited
  - 83% of cells inhibited
- Inhibitory modulation blocked by neurotransmitter antagonists
  - Glutamate: NBQX + D-AP7
  - GABA-glycine: bicuculline + strychnine

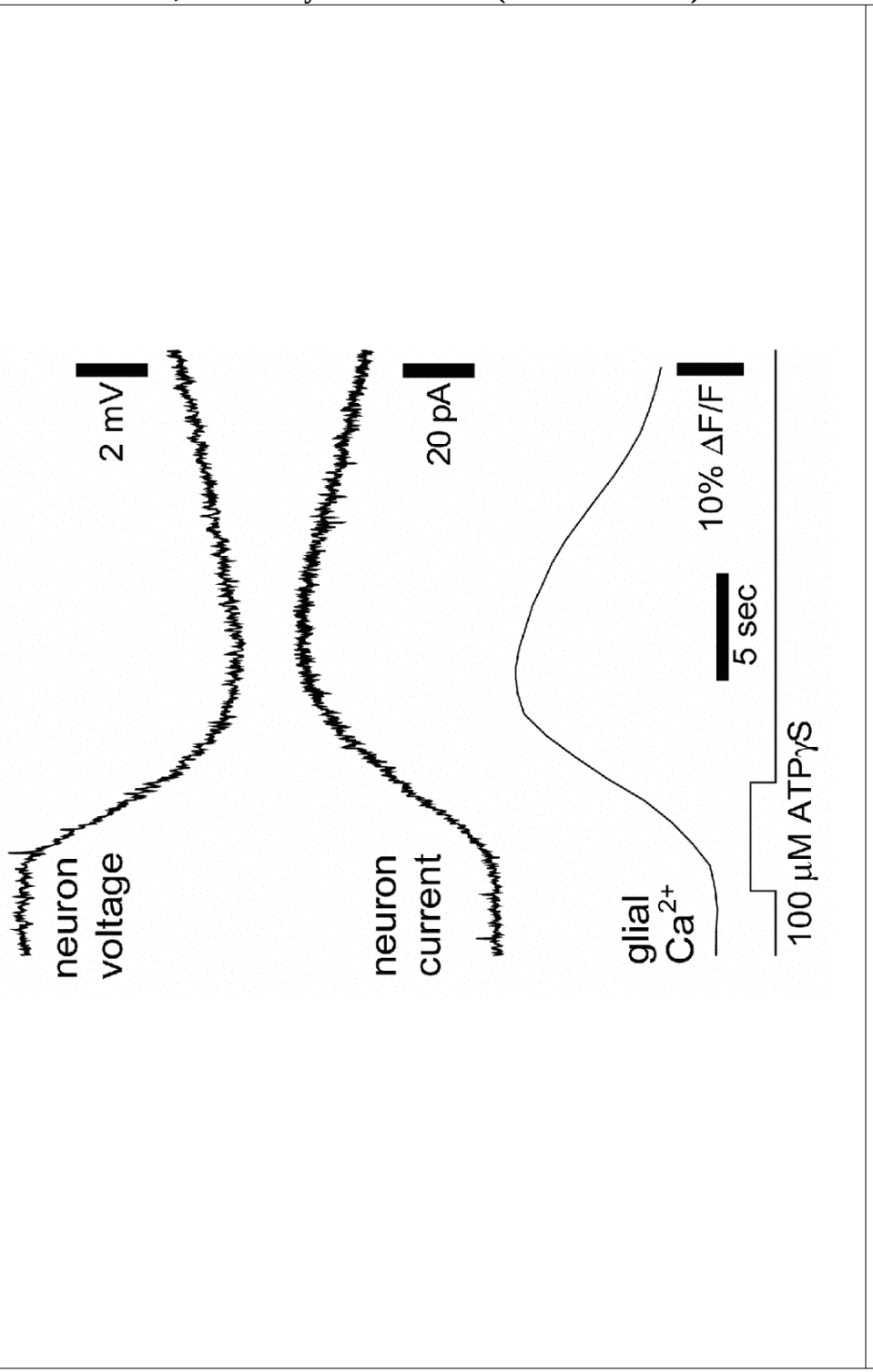
How do glial cells modulate neuronal activity?

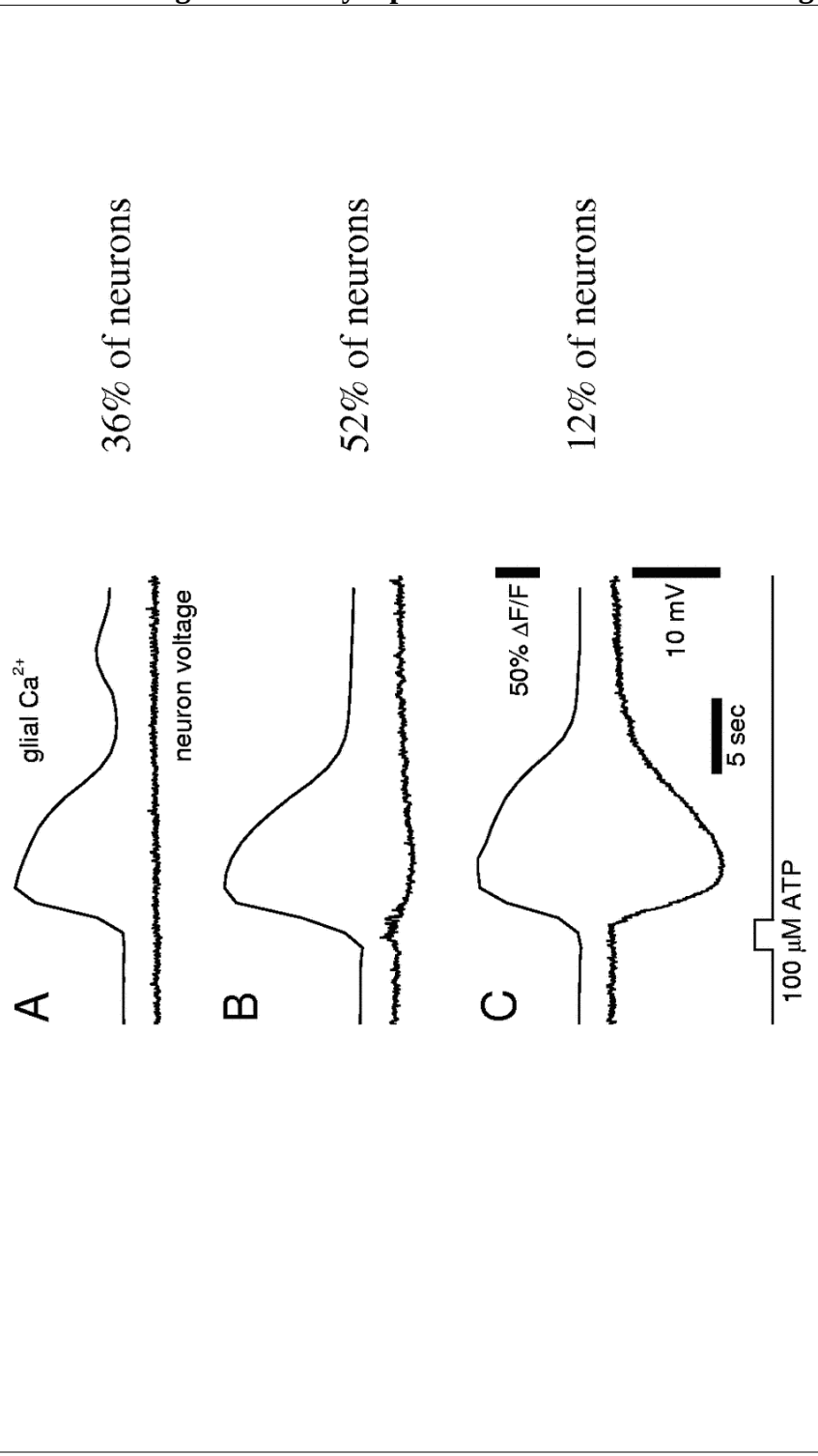
- Whole-cell patch recordings from neurons in isolated rat retina
- Glial cells activated by ejection of ATPγS and other agonists
- Glial Ca^{2+} increases monitored with Fluo-4

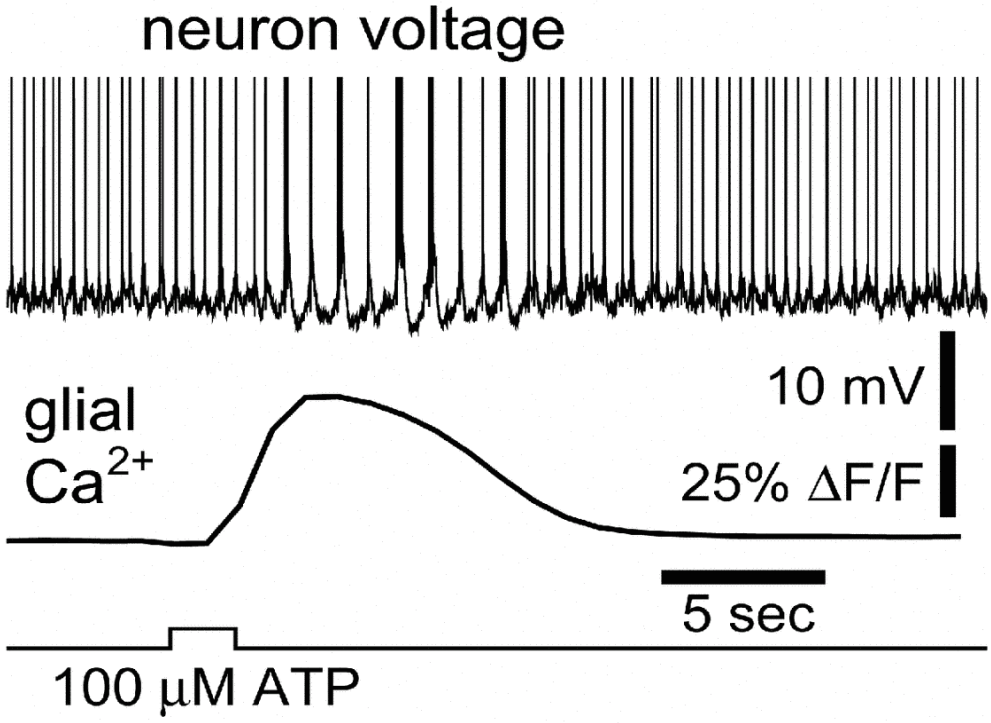
IR-DIC image of ganglion cell layer






neuron voltage
2 mV
neuron current
20 pA
glial Ca^{2+}
10% ΔF/F
5 sec
100 μM ATPγS

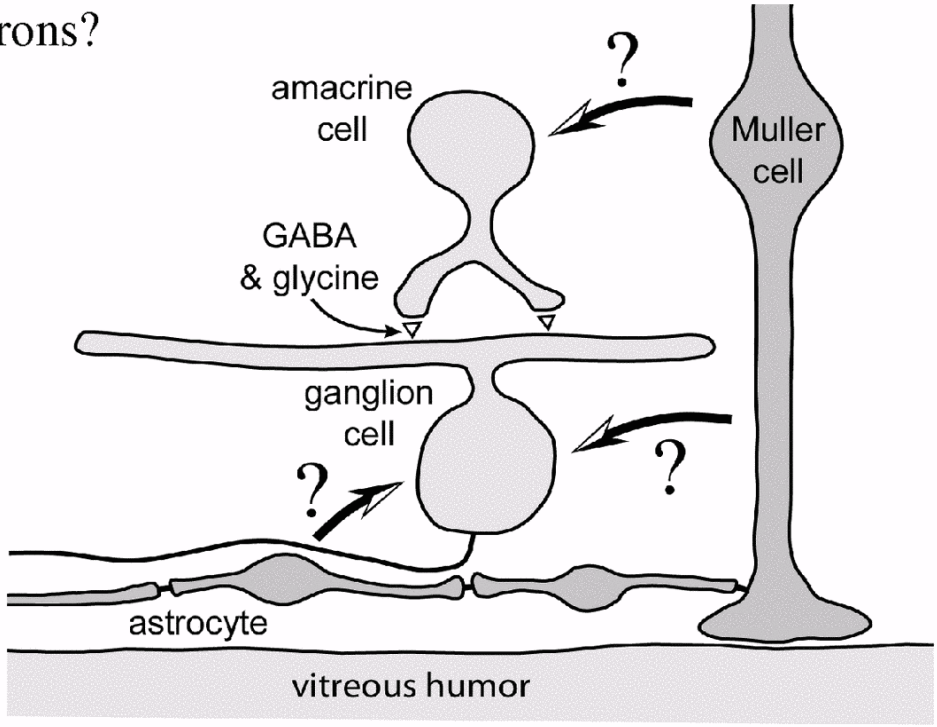

A
glial Ca^{2+}
neuron voltage
36% of neurons
B
52% of neurons
C
50% ΔF/F
10 mV
5 sec
12% of neurons
100 μM ATP

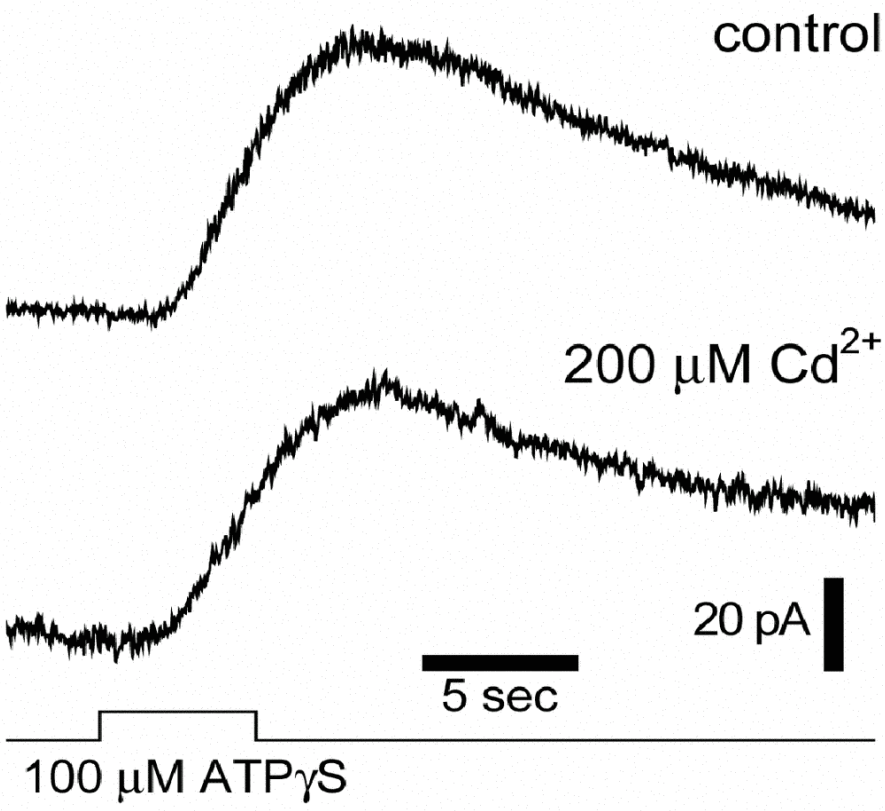


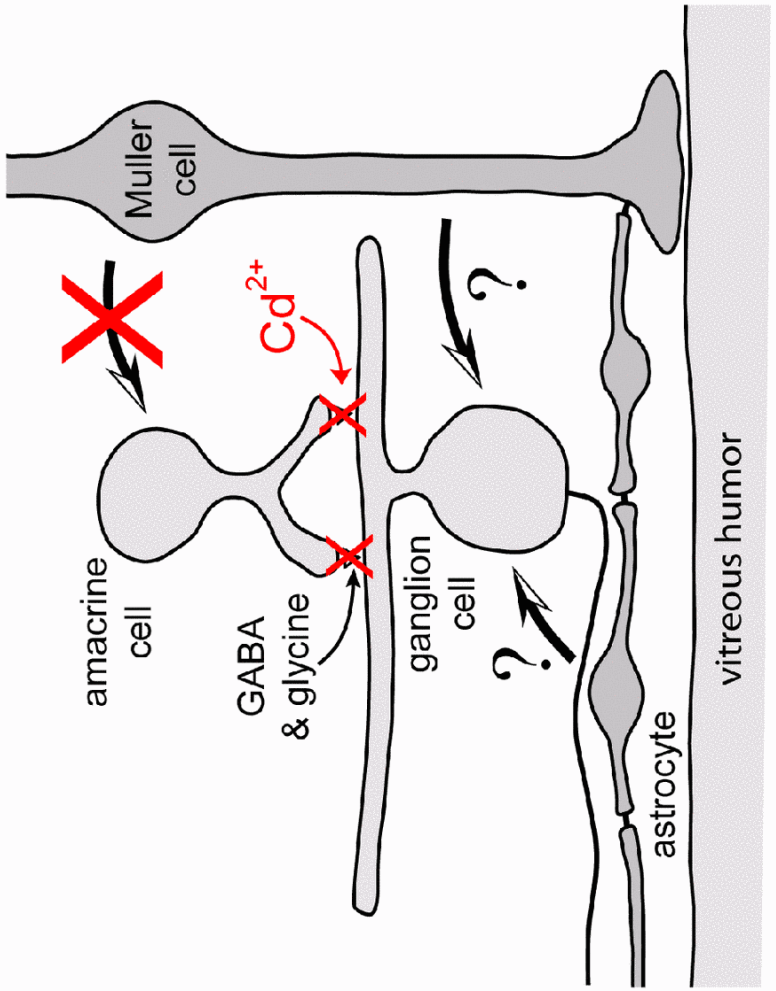
Neuronal inhibition

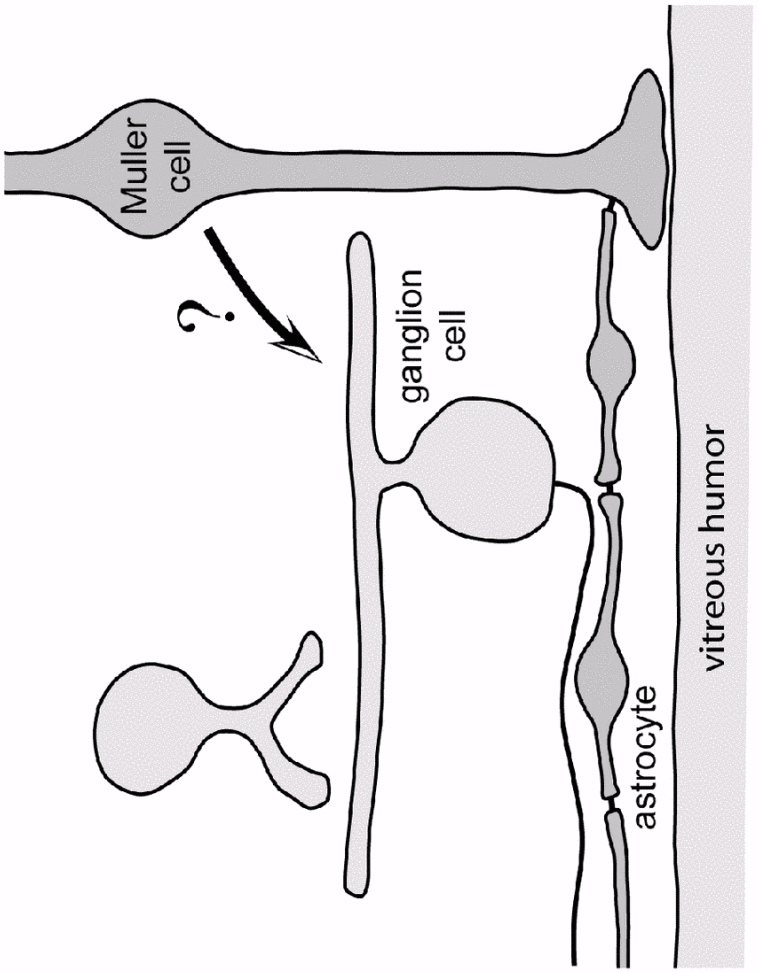
- Glial activation and neuronal hyperpolarization evoked by
  - ATP (100 μM)
  - ATPγS (100 μM)
  - Dopamine (1 mM)
  - Thrombin (100 units/ml)
  - Lysophosphatidic acid (500 μM)
  - Mechanical stimulation

Do glial cells inhibit ganglion cells directly or do they activate inhibitory interneurons?





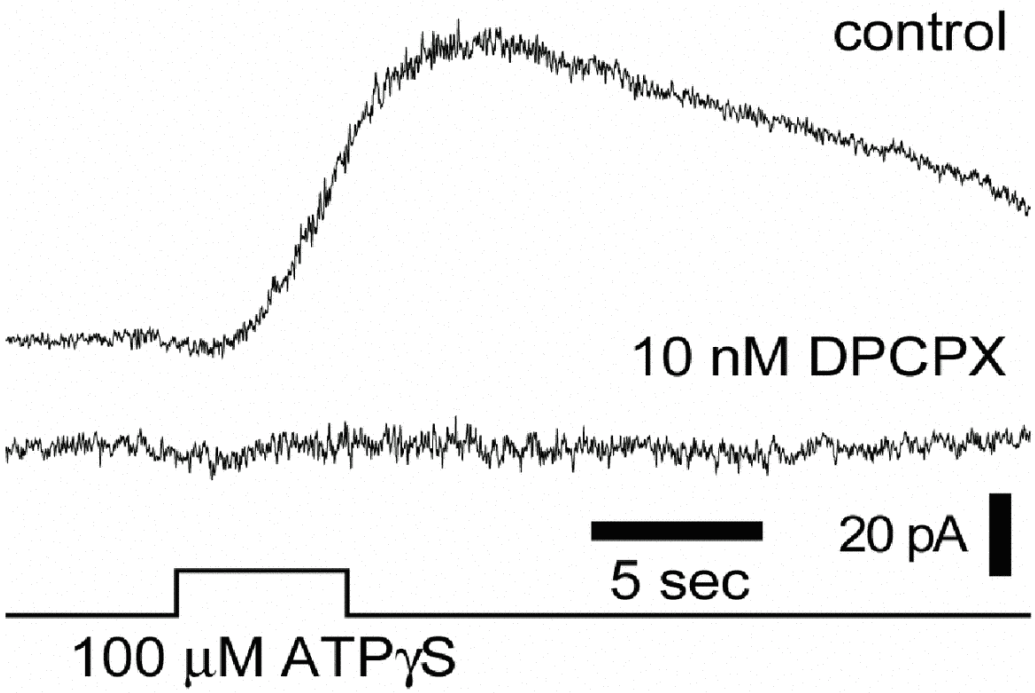

Muller cell
Cd^{2+}
?
amacrine cell
GABA & glycine
ganglion cell
?
astrocyte
vitreous humor

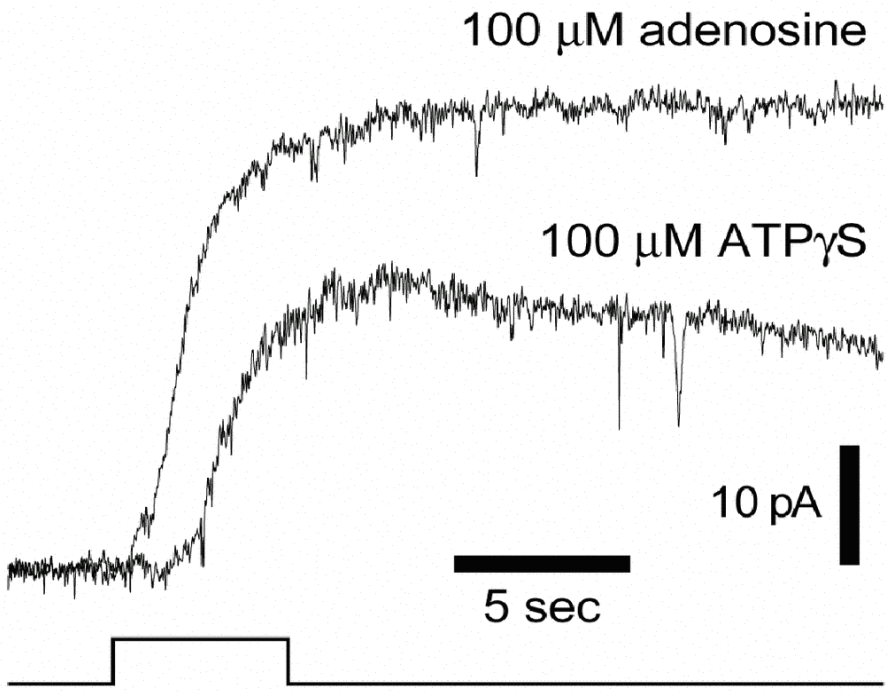

Muller cell
?
ganglion cell
astrocyte
vitreous humor

Neuronal inhibition

- Outward current *not* blocked by
  - Glutamate antagonists
    - NBQX (10 μM), DL-AP7 (100 μM)
  - GABA & glycine antagonists
    - bicuculline (5 μM), saclofen (200 μM), strychnine (1 μM)
  - Acetylcholine antagonist
    - Scopolamine (10 μM)

A_1 adenosine receptor antagonist





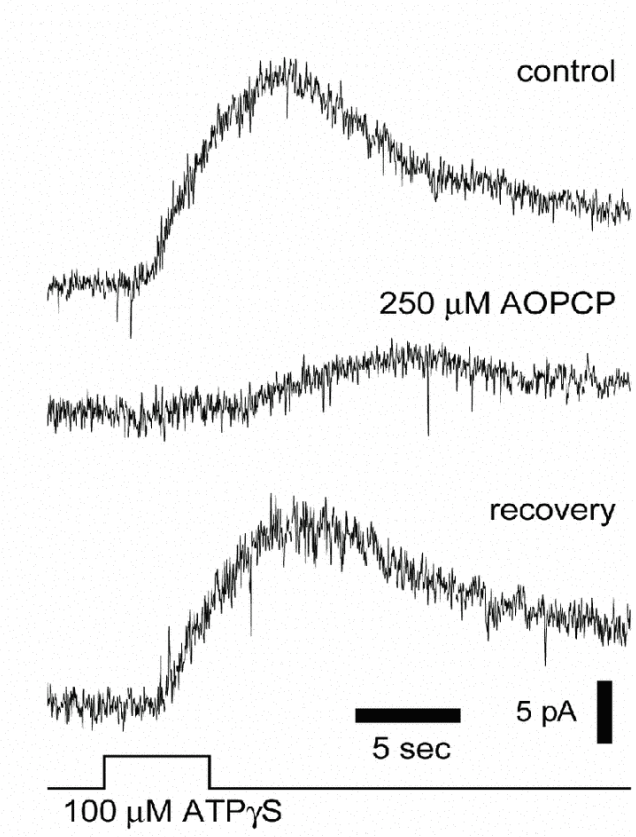
ATP metabolism in extracellular space

ARL-67156 AOPCP

ATP → ADP → AMP → adenosine

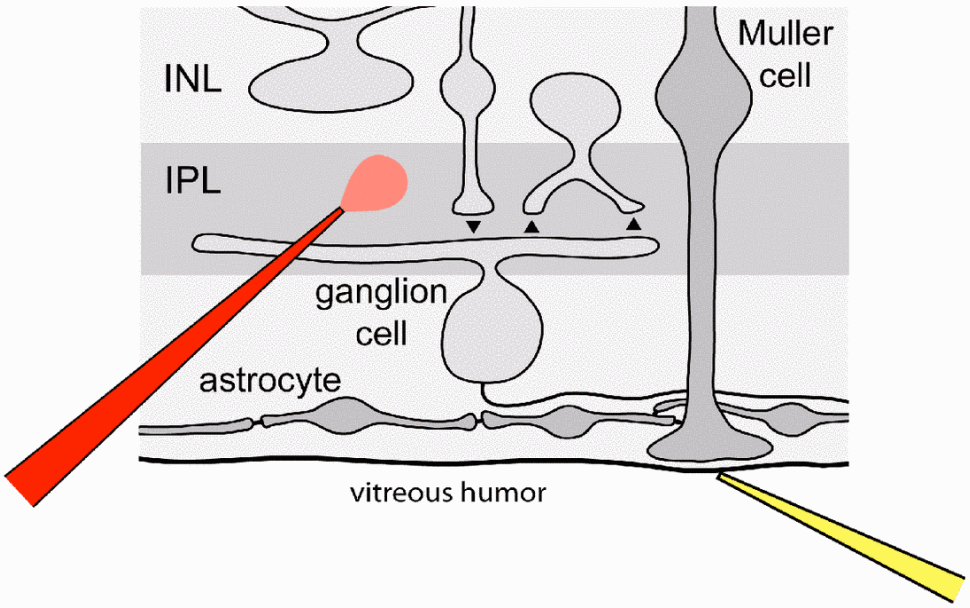
ecto-ATPase ecto-nucleotidase

Ecto-nucleotidase inhibitor AOPCP

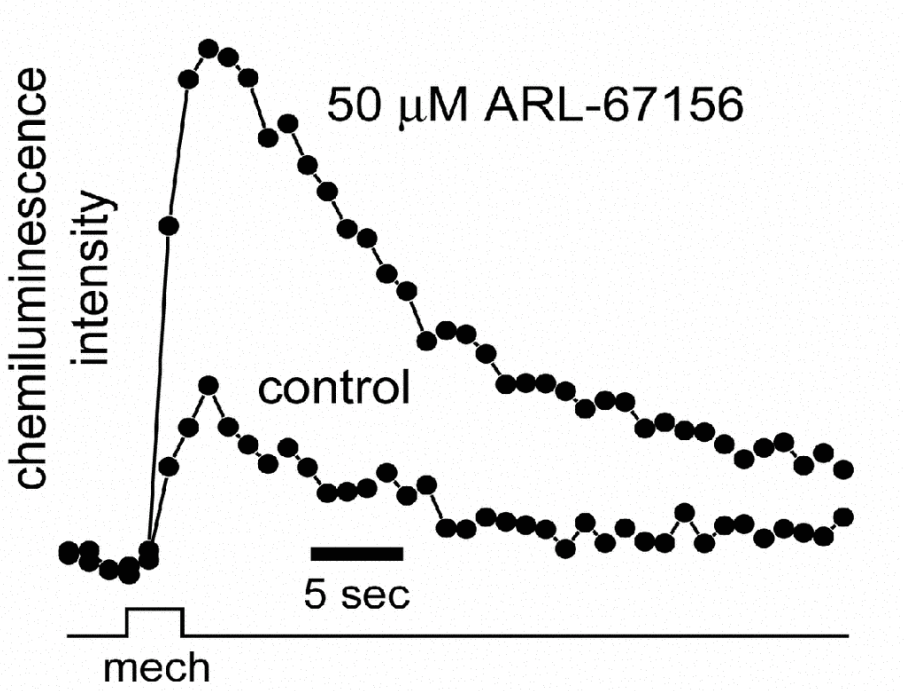


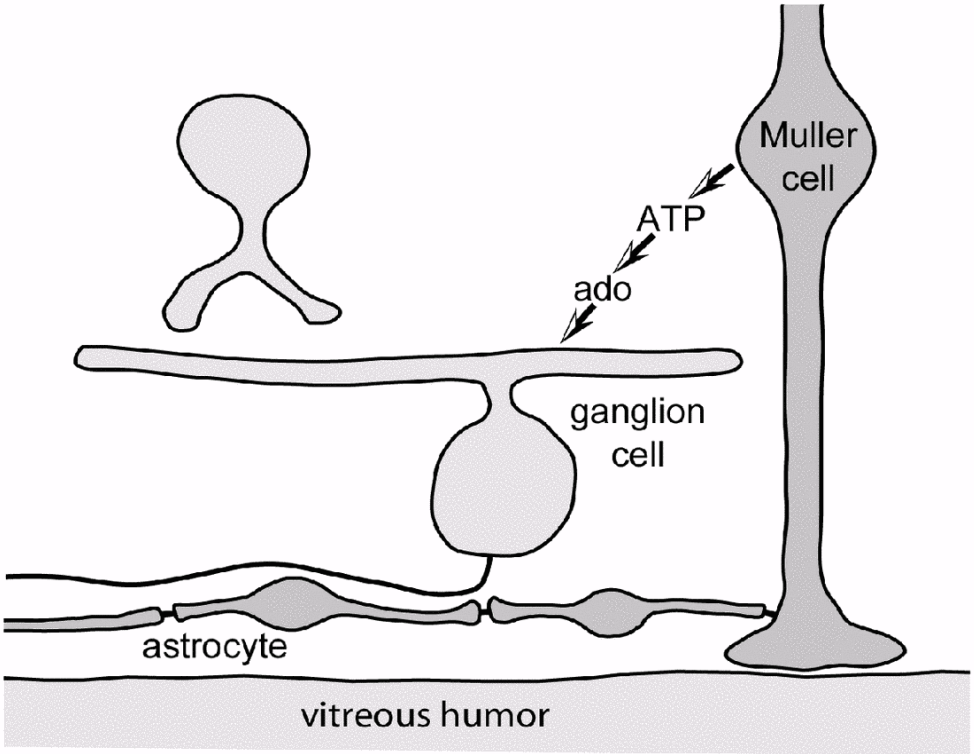
Luciferin-luciferase assay of ATP release in the inner plexiform layer

100 μM Cd^{2+} in solution



ATP release in the inner plexiform layer





Summary

- Ca^{2+} signals are propagated through retinal glial cells *in situ*
  - Propagation occurs both by diffusion of an intracellular messenger (IP_3) and release of an extracellular messenger (ATP)
- Light-stimulation of the retina evokes Ca^{2+} increases in glial cells
- Glial cell activation can either increase or decrease light-evoked neuronal spiking
  - Collaboration with Kathleen R. Zahs

- Glial activation can either potentiate or depress light-evoked EPSCs in neurons
- Müller cells can directly inhibit neurons by release of ATP and the subsequent activation of neuronal adenosine receptors
- Glia may participate in information processing in the retina