Patch clamping in the retina

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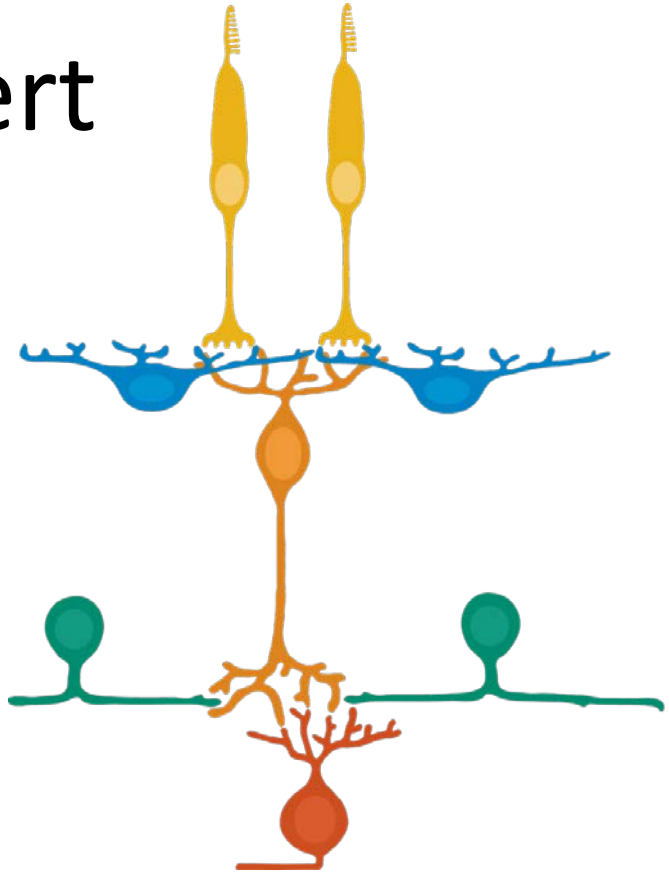
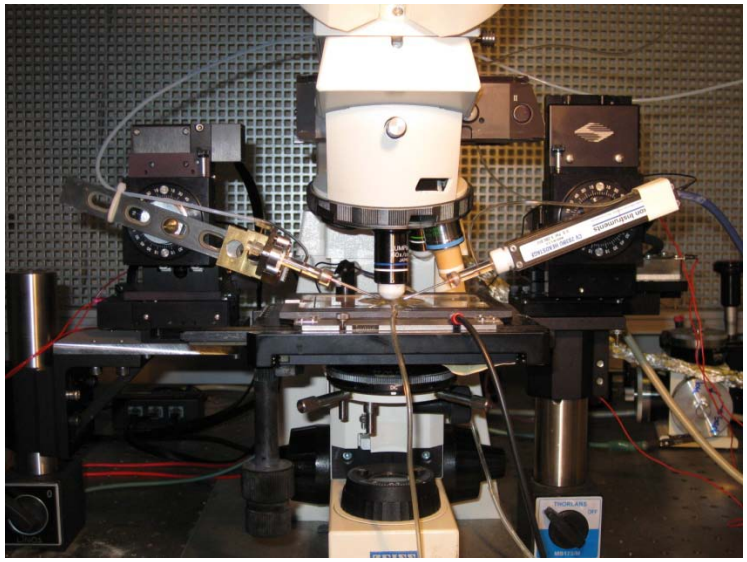
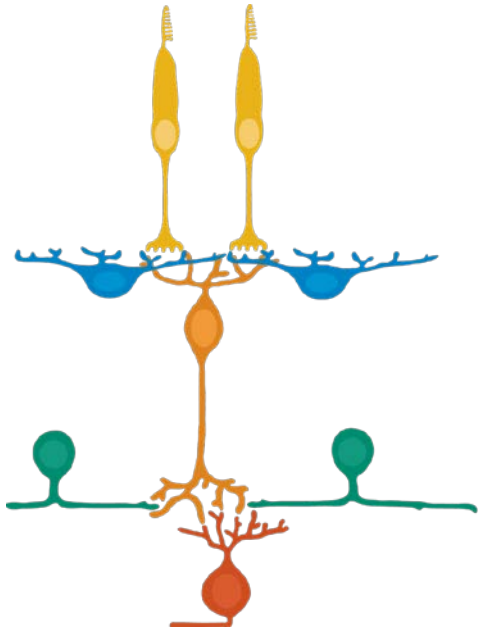
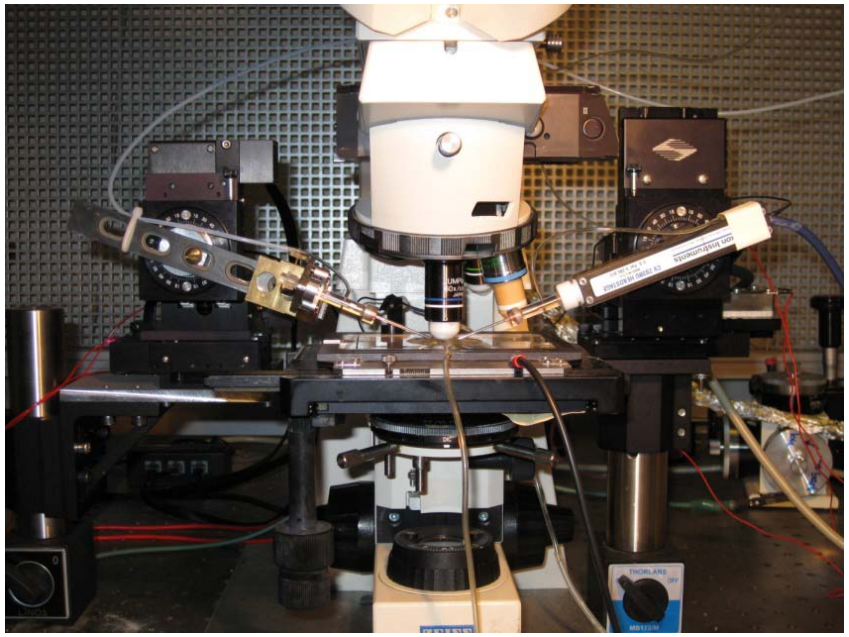


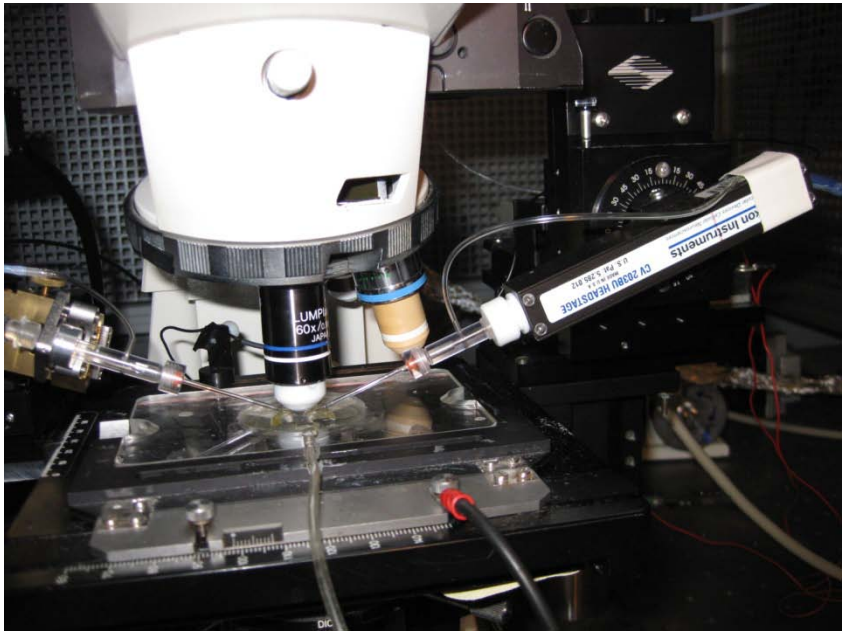




Outline

- general principle of the patch clamp method
- basic ideas behind the experiments
- two easy-to-follow examples how to record currents through voltage-gated and transmitter-gated ion channels in retinal neurons





Recording chamber

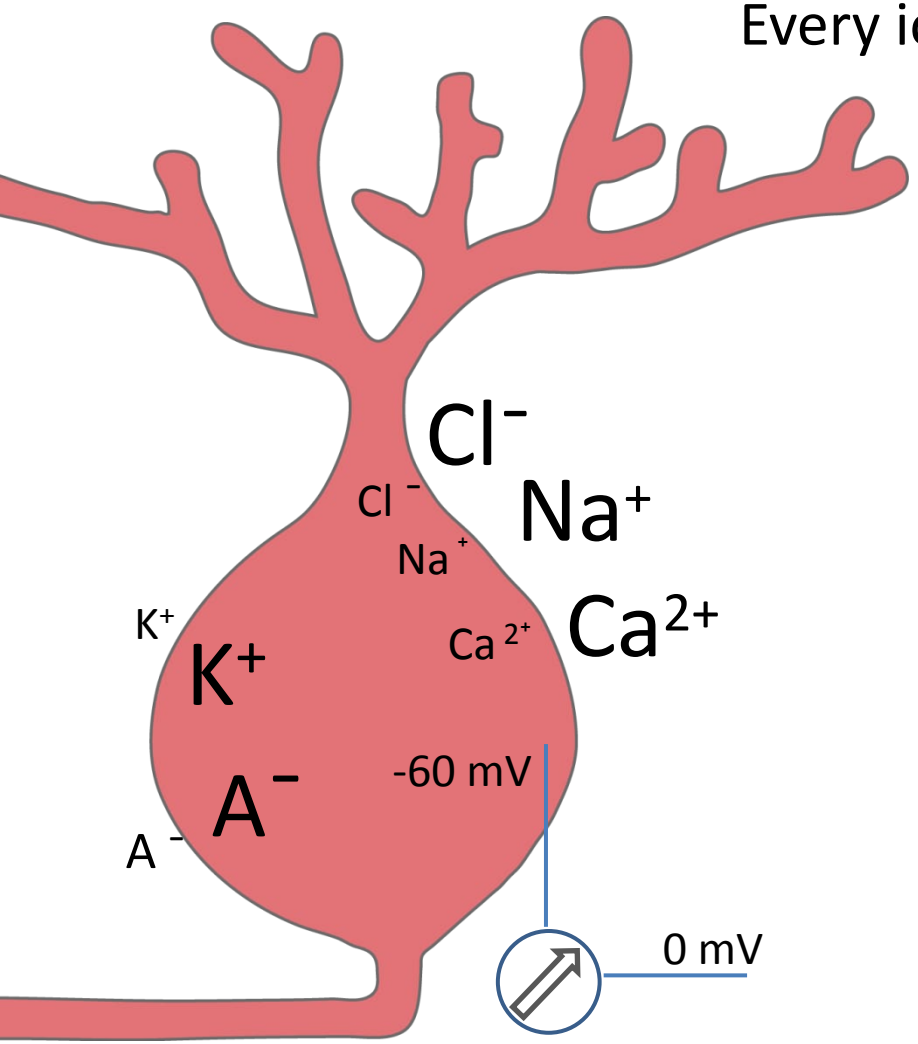
Pre-amplifier

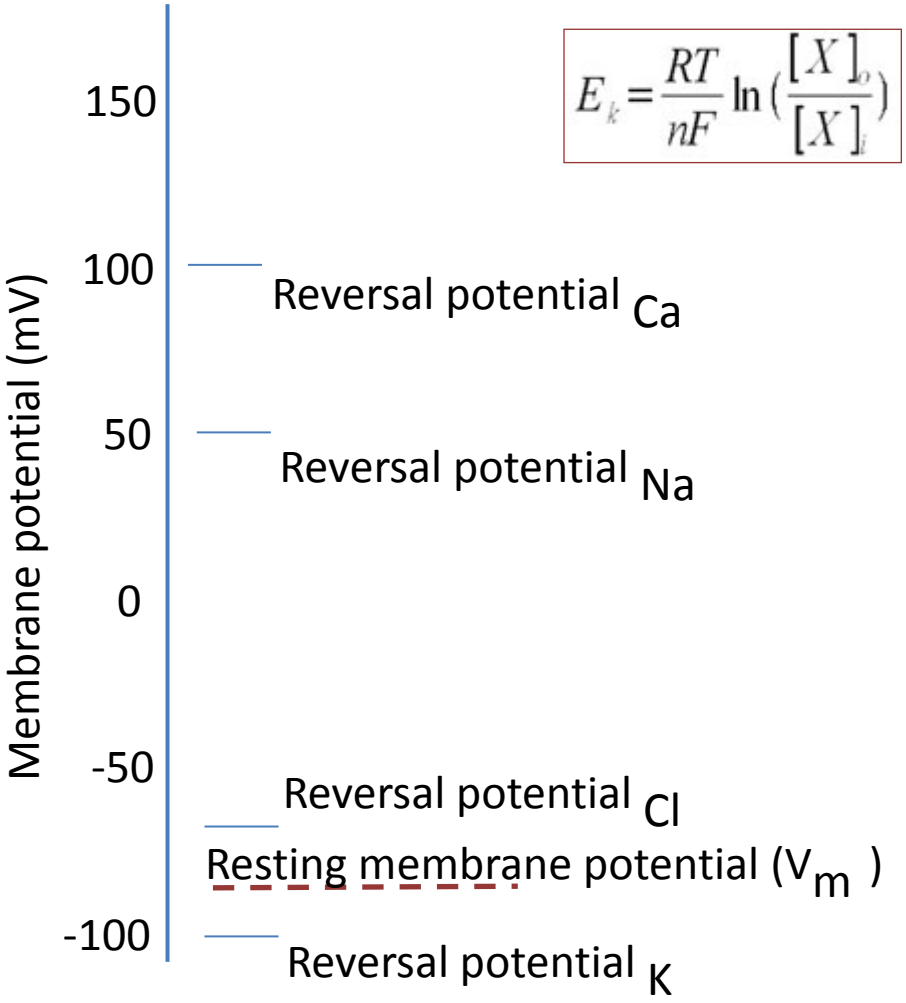
Amplifier
(Axon or HEKA)

A/D converter

Computer
Software

Every ion has ist own reversal potential



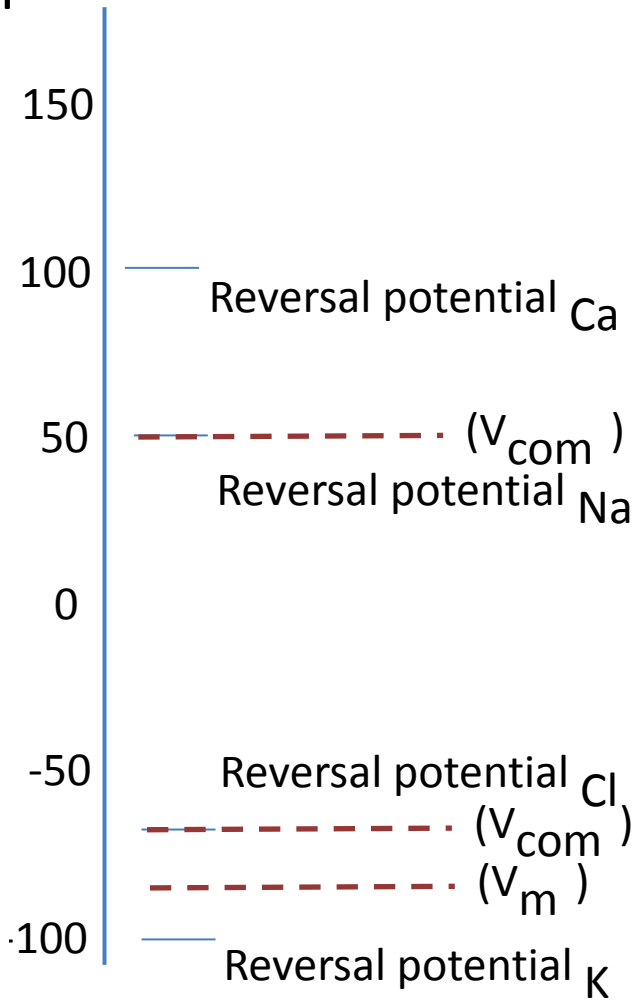


$$E_k = \frac{RT}{nF} \ln\left(\frac{[X]_o}{[X]_i}\right)$$

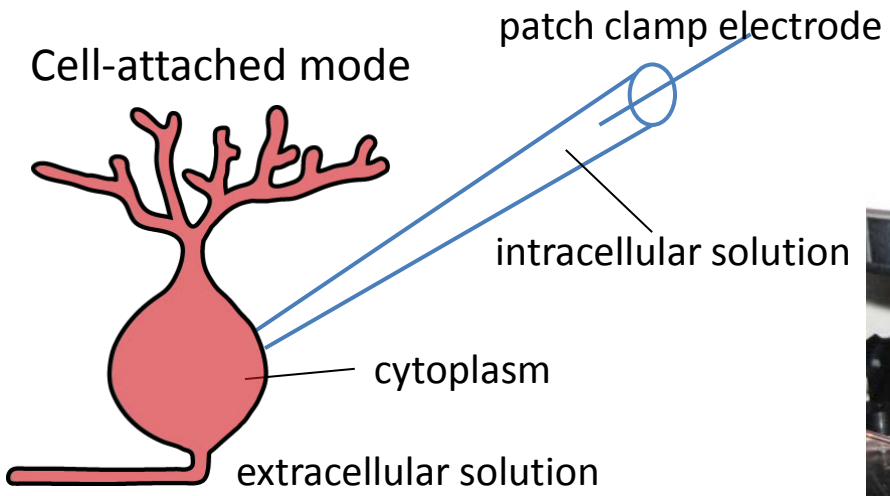
- ion pumps (proton-driven)
- ion transporters (3Na/2K)

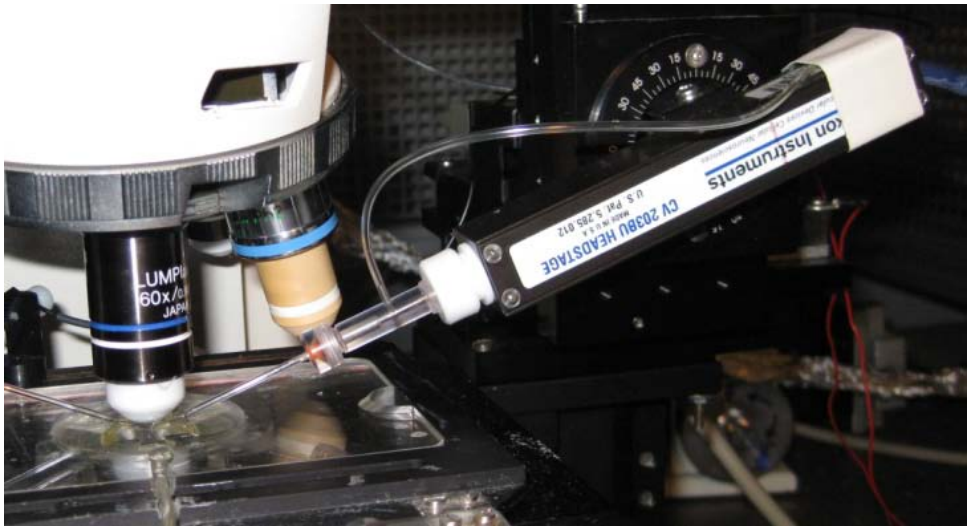
$$V_m = \frac{RT}{F} \ln\left(\frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o}\right)$$

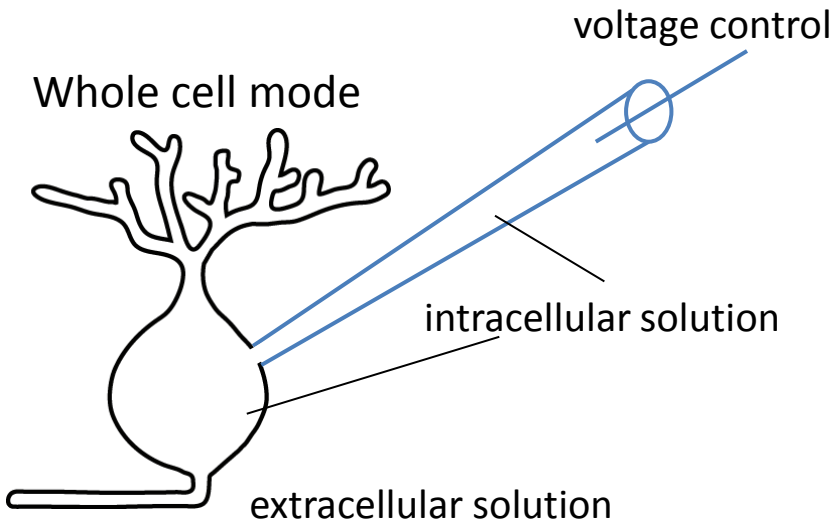
Idea: Adjust membrane potential, prevent or block most currents, isolate and measure the remaining specific ion channel current



Advantages of the patch clamp technique

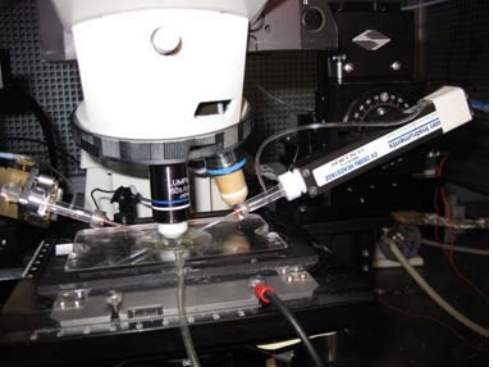






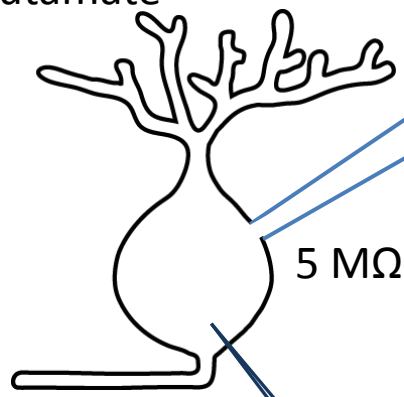
- record specific **ion currents** through voltage-gated channels or ligand-gated channels
- in combination with specific agonists and antagonists

Basic patch clamp circuit



light

glutamate



5 MΩ

350 MΩ

Feedback resistance

Rf

U pip

OPA

U hold

Current measurement
(Na current / glu R)

CM

Command voltage
(from amplifier)

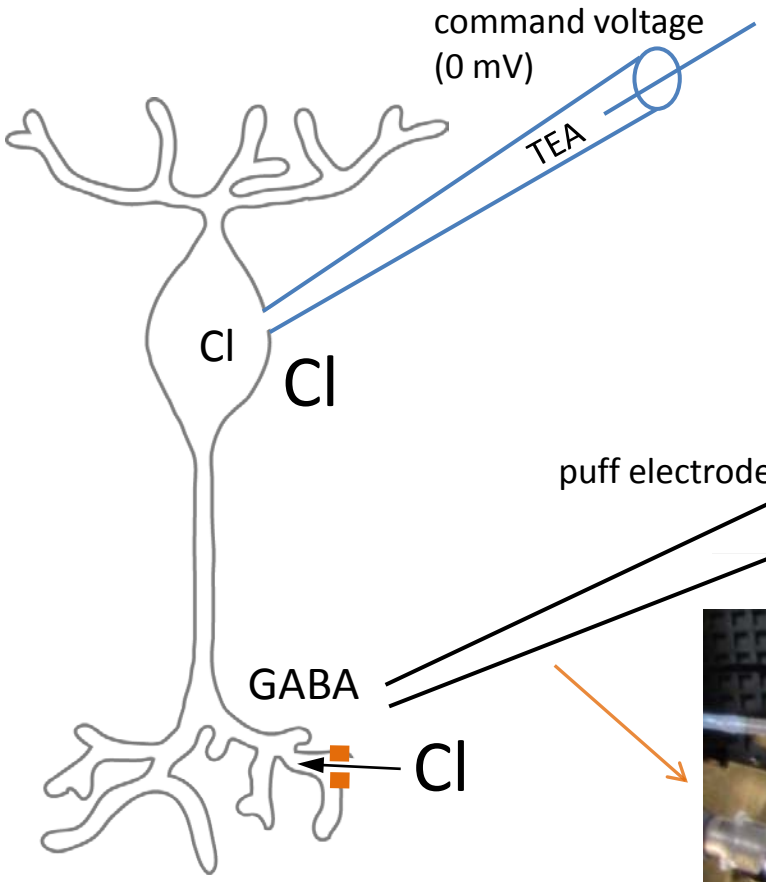
U = R x I (Ohm's law)

- membrane potential can be adjusted to command voltage
- injected compensation current (needed to adjust cell to command current) is measured

(old-school) Intracellular recordings

- record membrane potential only
- cannot control membrane potential (no current measurement)

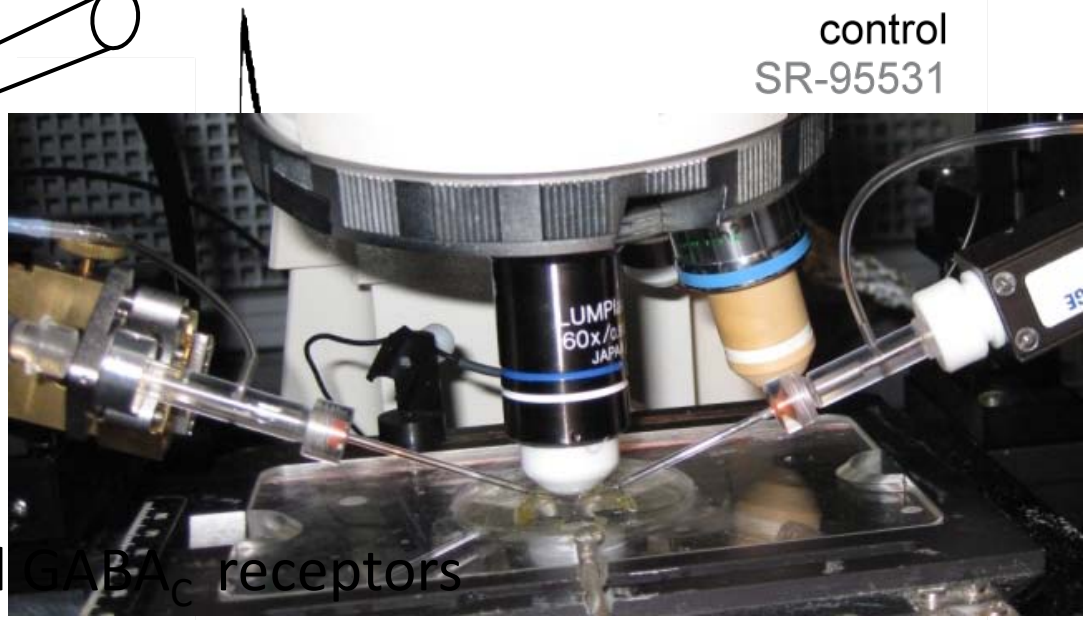
Quick example of GABA-evoked currents in bipolar cells



BC in vertical retina slice

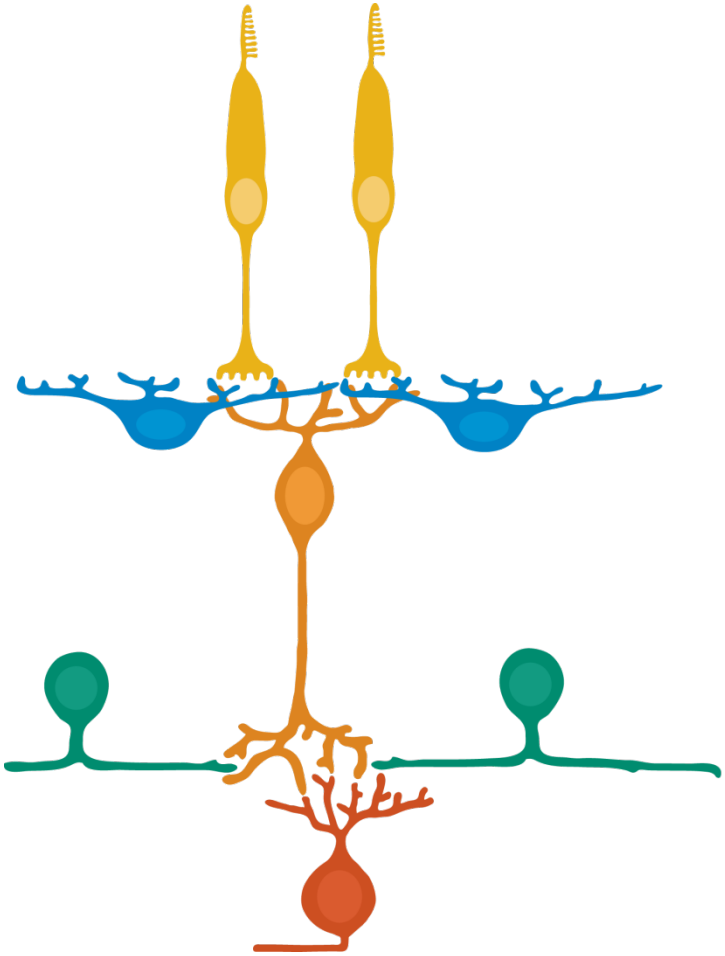
- BC voltage-clamped at 0 mV
- Na,Ca reversal potential: 0 mV
- Cl reversal potential: - 60 mV
- potassium channels blocked with TEA

- GABA application evokes current
- $i = (V_{com} – E_{Cl}) \times g$ indicates current



- BC axons express $GABA_A$ and $GABA_C$ receptors

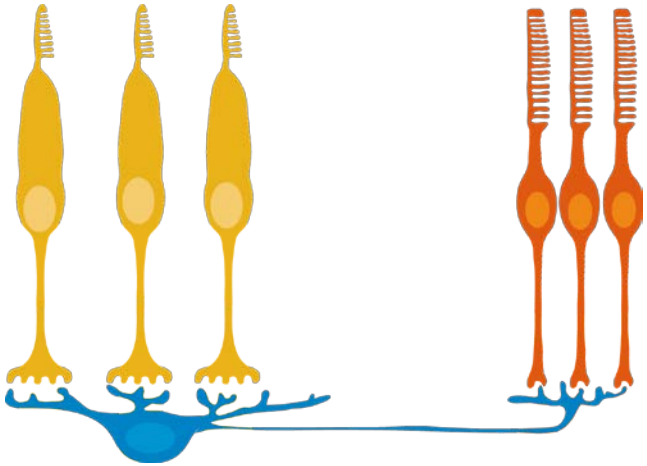
Two real examples of patch clamp recordings in the mouse retina:



Identification of voltage-gated calcium channels in horizontal cells

Development of inhibitory synaptic input from amacrine cells to bipolar cells

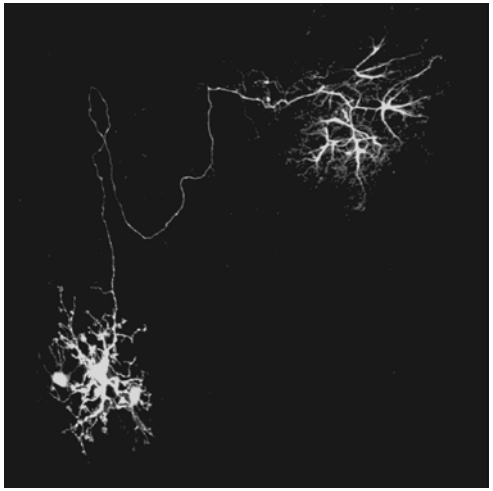
Yet unidentified mechanism of feedback inhibition from horizontal cells to photoreceptors



Horizontal cells regulate glutamate release from Photoreceptors (feedback)

Horizontal cell to cone feedback:

- ephaptic feedback (hemichannels)
- pH mediated feedback (proton release)
- non-vesicular GABA release (transporter)
- GABA release via vesicles

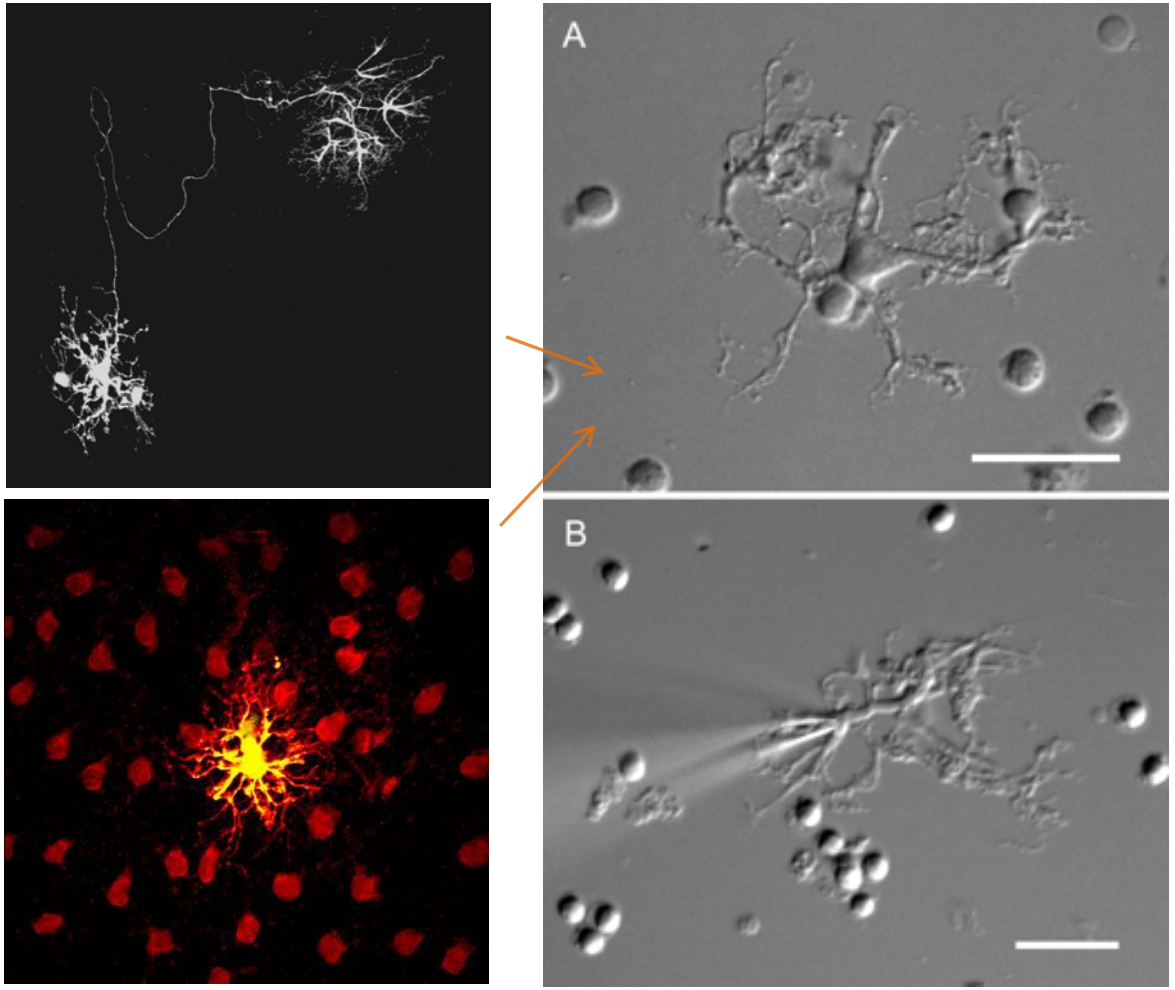


high voltage-activated calcium channels
are linked to syntaxin and iniate vesicle release

- actived at -30 mV
- permeable only for calcium
- non-activating currents
- tail currents
- can be pharmacologically distinguished

Morphology of horizontal cells

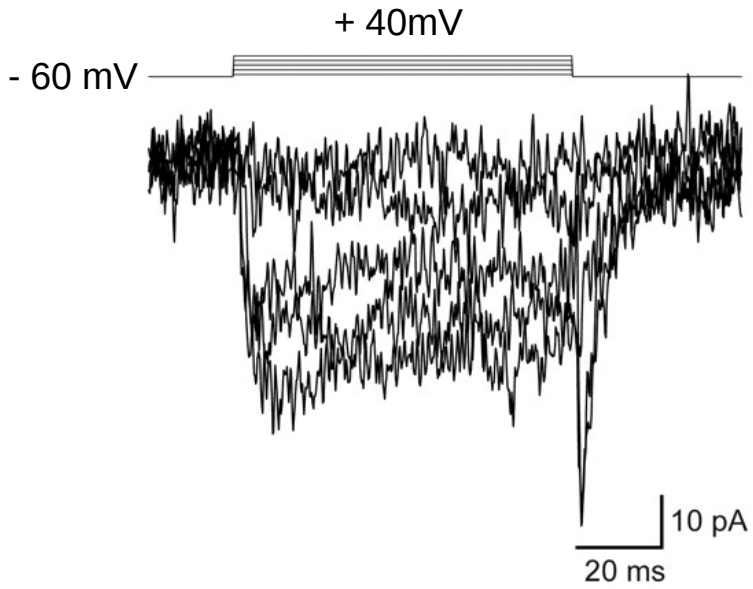
Acutely dissociated HCs



HCs injected with sharp electrode in flat mount retina

(Schubert, Weiler, Feigenspan, 2006)

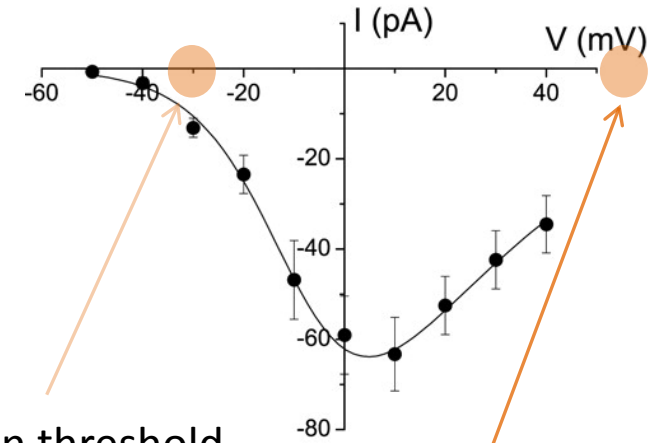
Voltage steps from -60 mV to higher potentials reveal high voltage-activated calcium channels



- potassium currents blocked with CsCl, TEA
- no Na channels in HCs
- calcium currents can be determined:

$i = (V_{com} - E_{Ca}) \times g$

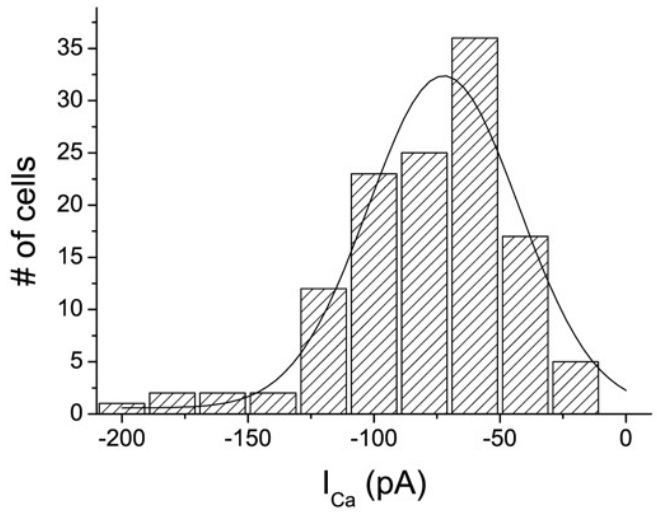
Determining the I/V curve and the relative conductance

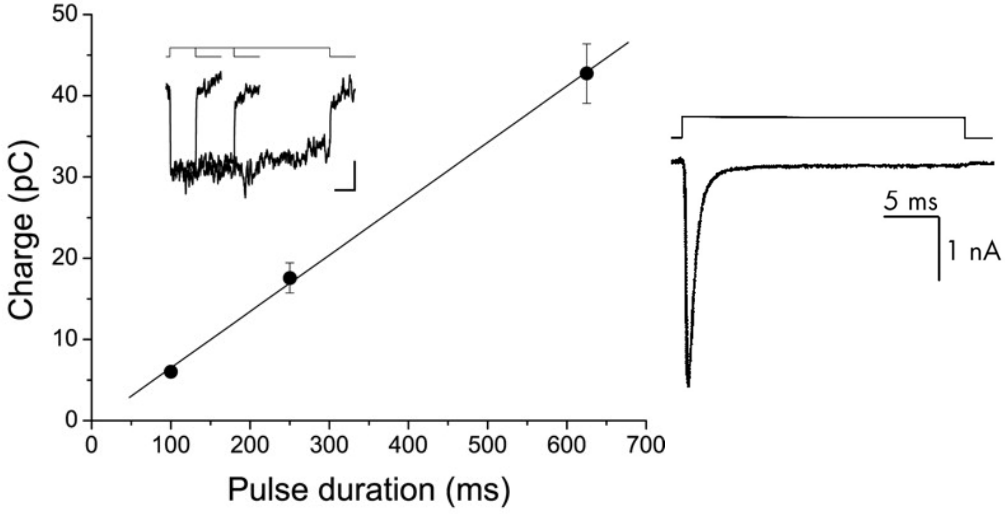


Activation threshold
of hva calcium channels

Calcium reversal
potential (+ 80 mV)

Voltage steps from -60 mV to -10 mV reveal high voltage-activated calcium channels in a homogenous horizontal cell population





Steps from -60 to +10 mV indicate a homogenous horizontal cell population (no amacrine cells)

Linearity of charge excludes Na-channels or low voltage-activated calcium channels (both transient)

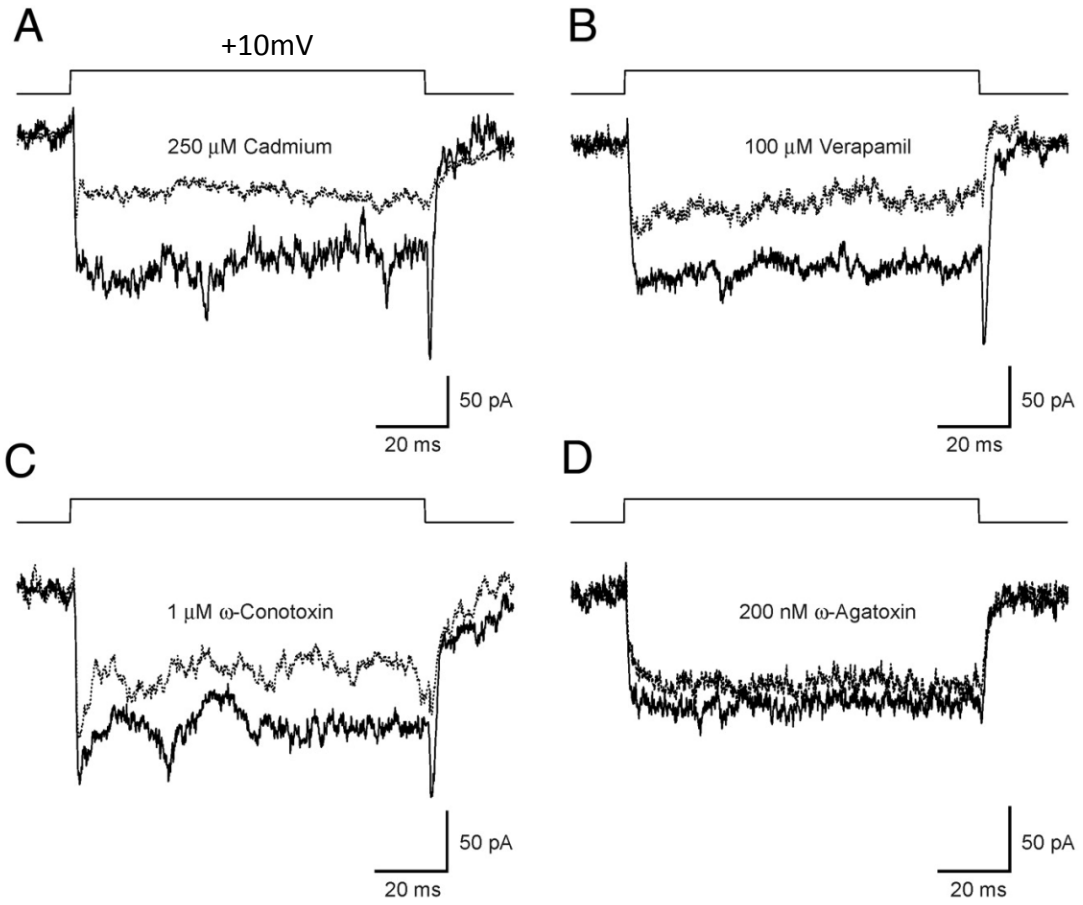
HVA calcium channel nomenclature and selective blockers

- No difference in kinetics but functional differences

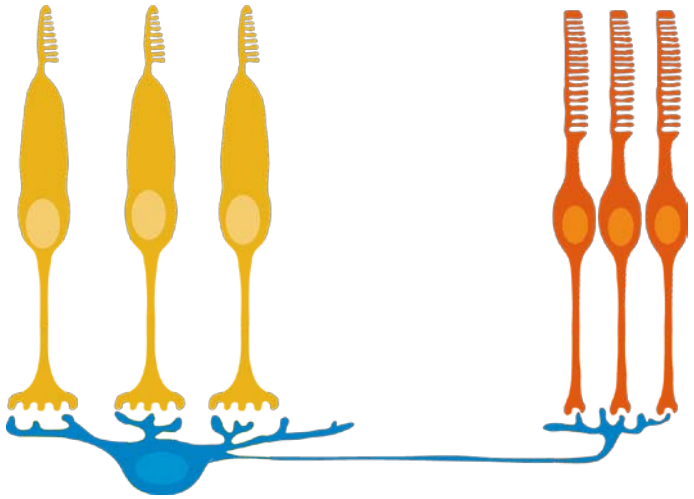
- L-type channels: soma/gene expression (dihydropyridines/verapamil)
- P/Q/R-type channels (ω-agatoxin IV4)
- **N-type channels**: axon terminal/transmitter release (ω-conotoxin GVIA)

- Cadmium and cobalt block
  all high-voltage activated calcium channels.

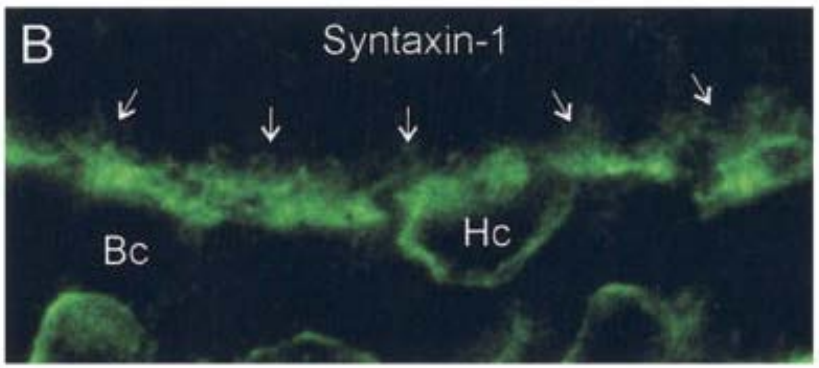
Pharmacology indicates that horizontal cells express L- and N- tpye channels



Finding N-type calcium channels support idea of vesicular GABA release in horizontal cells

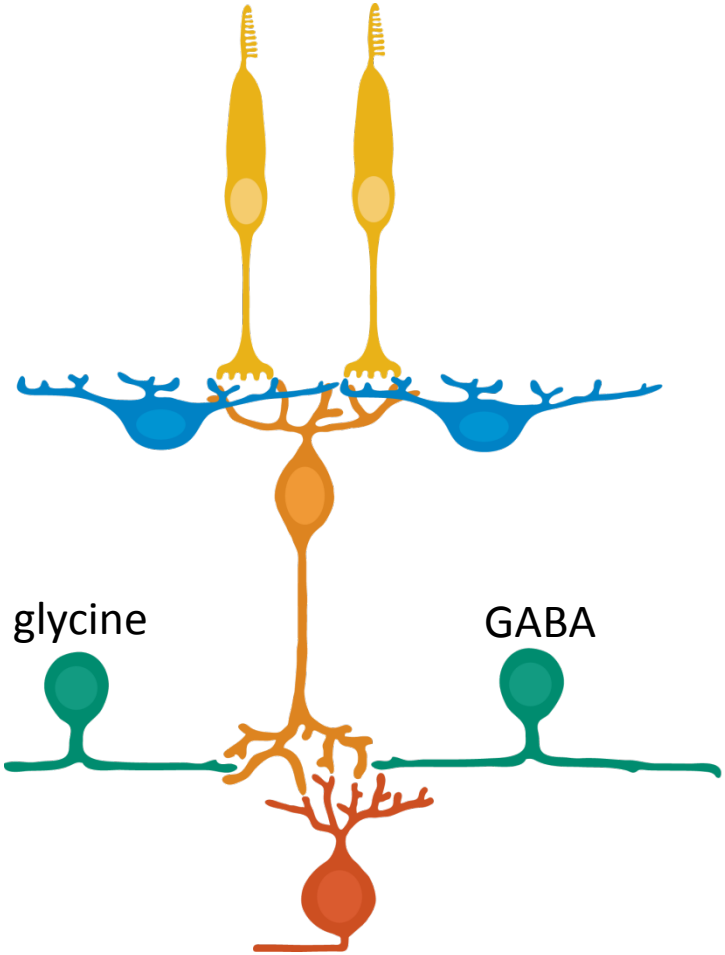


- ephaptic feedback (hemichannels)
- pH mediated feedback (proton release)
- non-vesicular GABA release (transporter)
- GABA release via vesicles



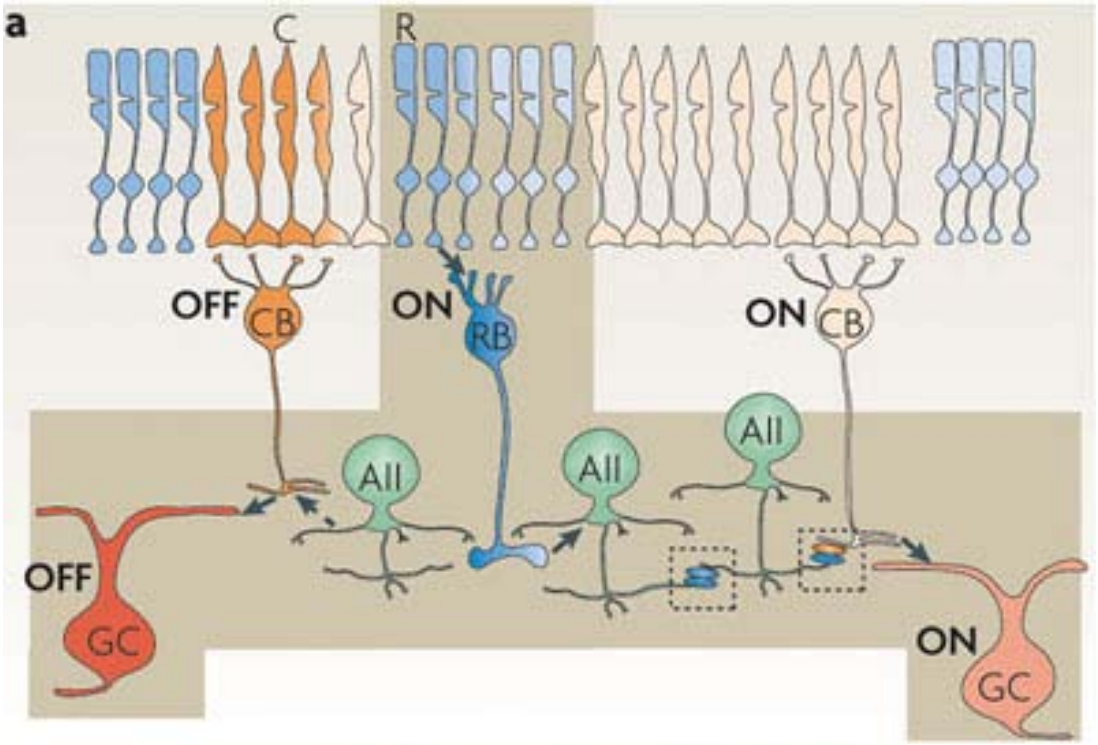
Hirano et al., 2005

High voltage-activated N-type calcium channel in horizontal cells (connected to syntaxin-1)



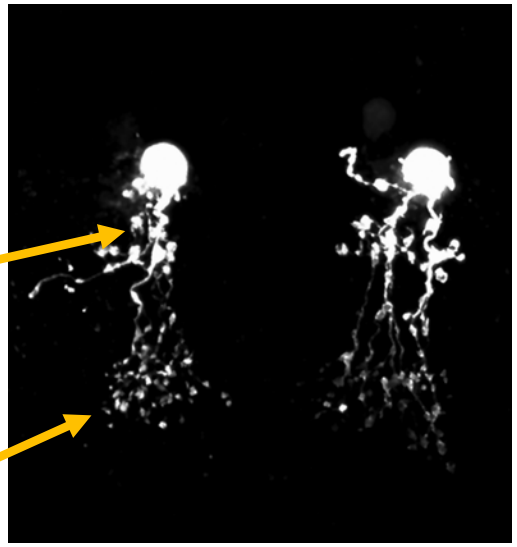
When does inhibitory synaptic input from amacrine cells to bipolar cell axons develop?
Is it light-dependent ?

Connections at the RBC and OFF-CBC axon terminals



- Rod bipolar cell axons receive little GABAergic and glycinergic input

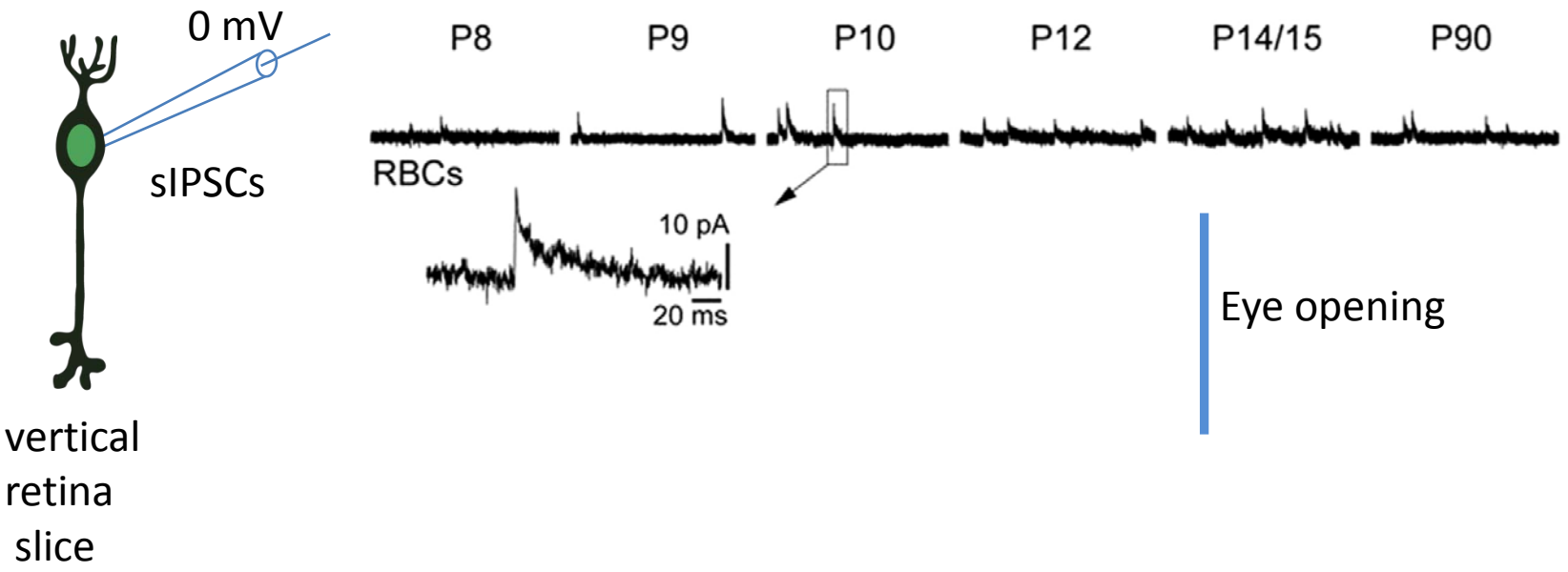
- OFF-CBC axons receive little GABAergic and massive glycinergic AII amacrine cell input



lobular appendages (glycinergic synapses to OFF-CBCs)

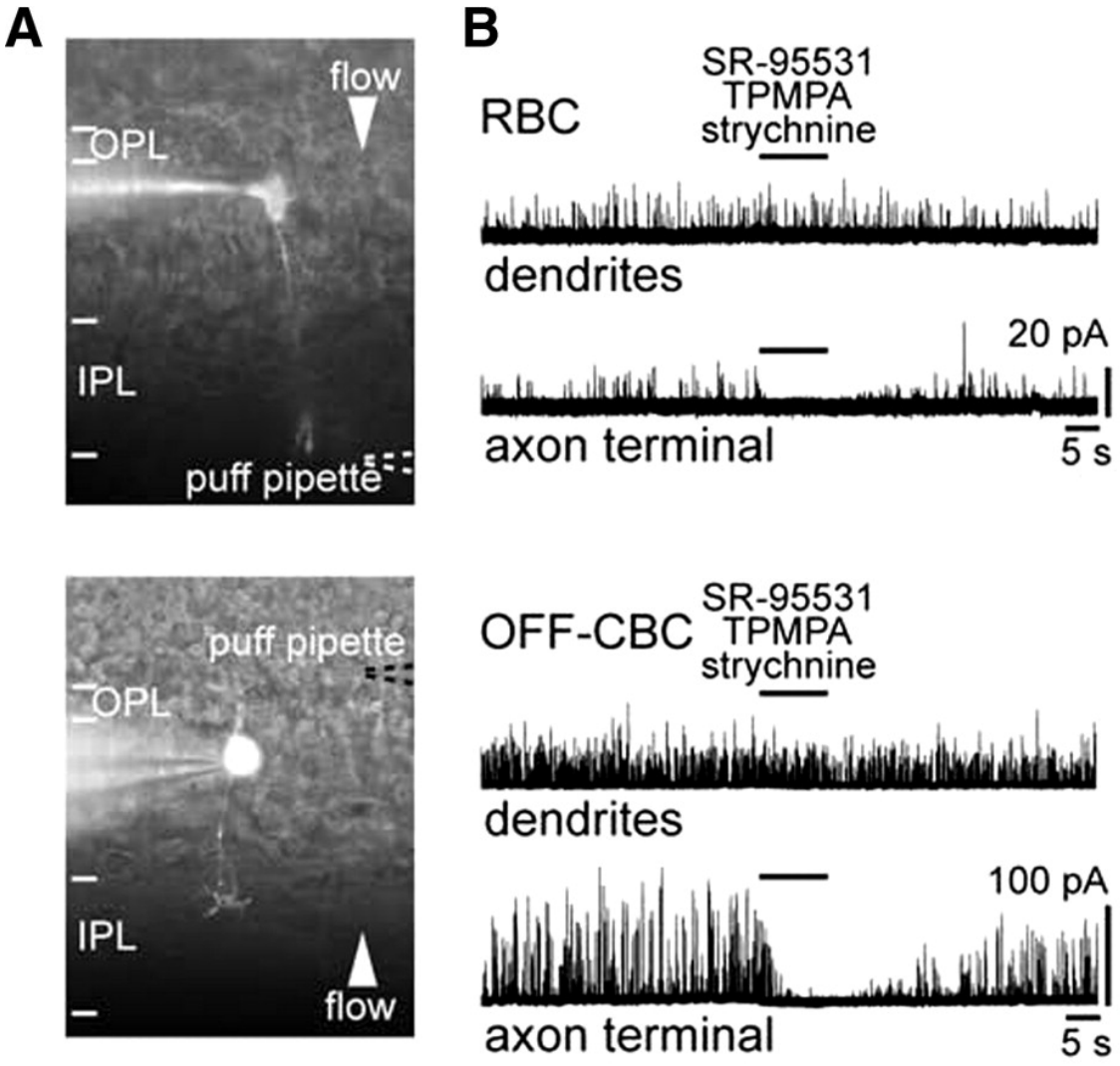
distal appendages (gap junctions to ON-CBCs,other AIIs)

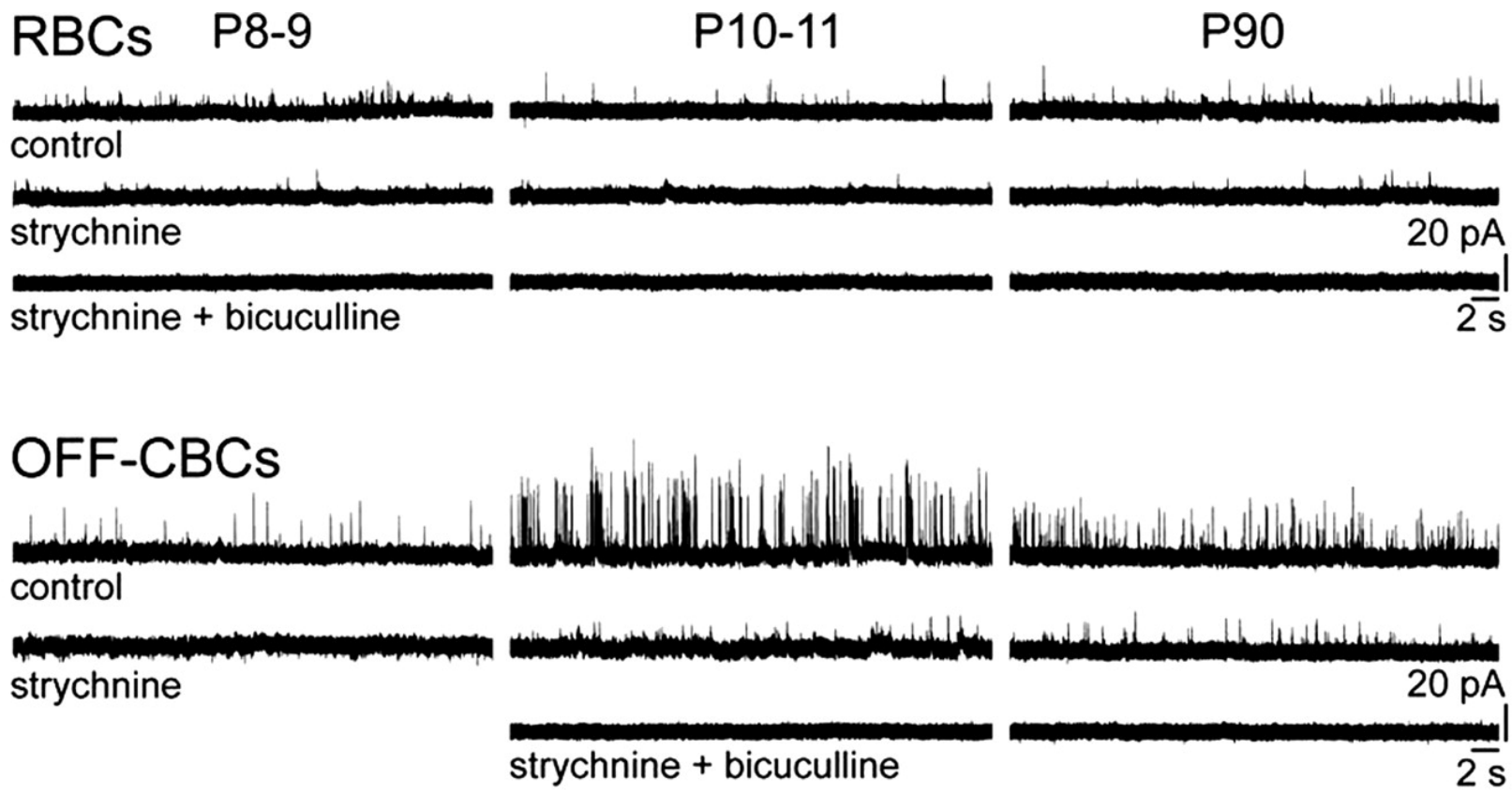
Inhibitory input to axon terminals of OFF-CBCs and RBCs develops differently and before eye opening



sIPSCs = spontaneous inhibitory (glycinergic/GABAergic) postsynaptic currents (accidental vesicle release events)

Sponateous inhibitory input is provided by amacrine cells but not by horizontal cells

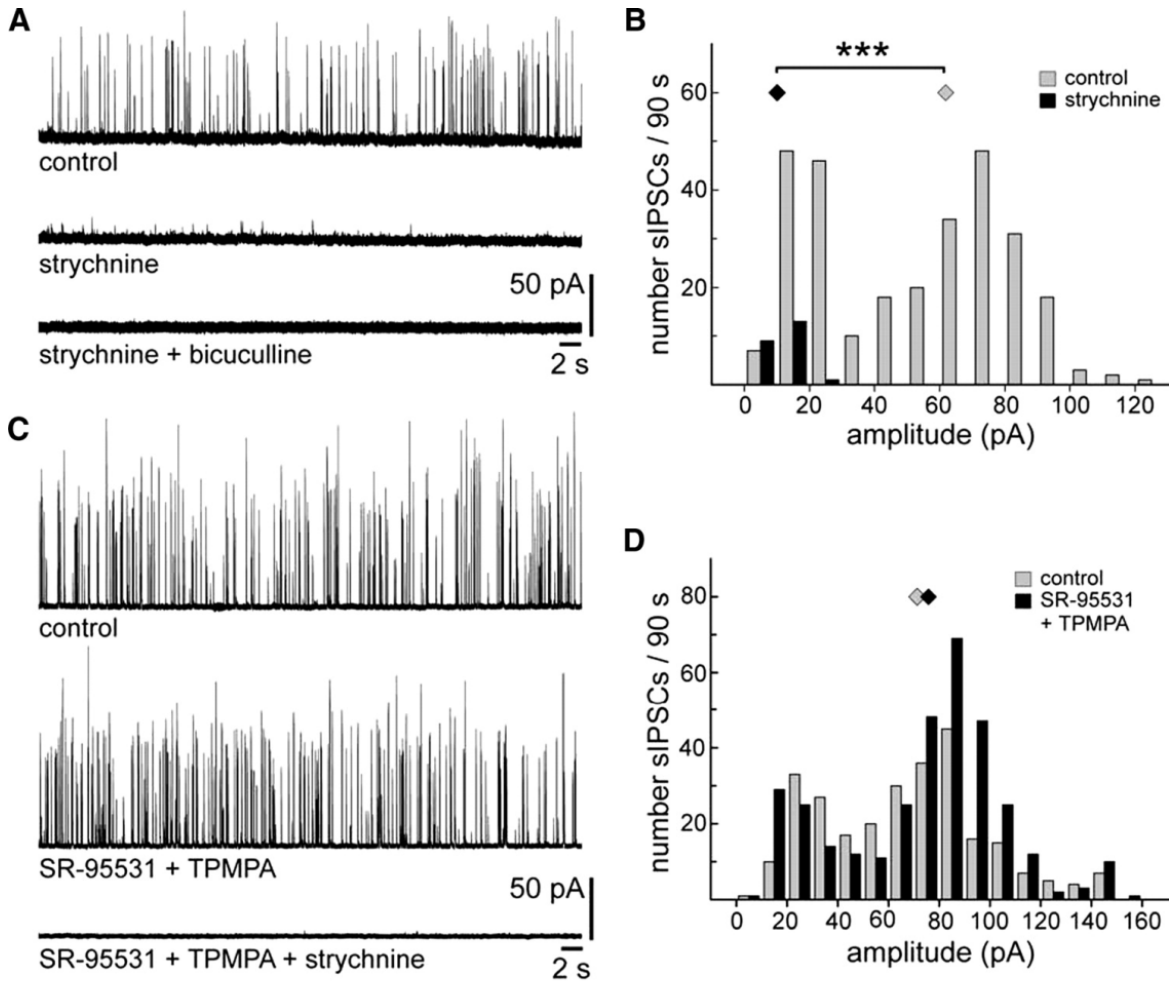




strychnine: glycine rec blocker
Bicu. : GABA rec blocker

- inhibitory input to RBCs is established around P8 and does not change too much during development
- inhibitory amacrine cell input to OFF CBCs develops successively

P11



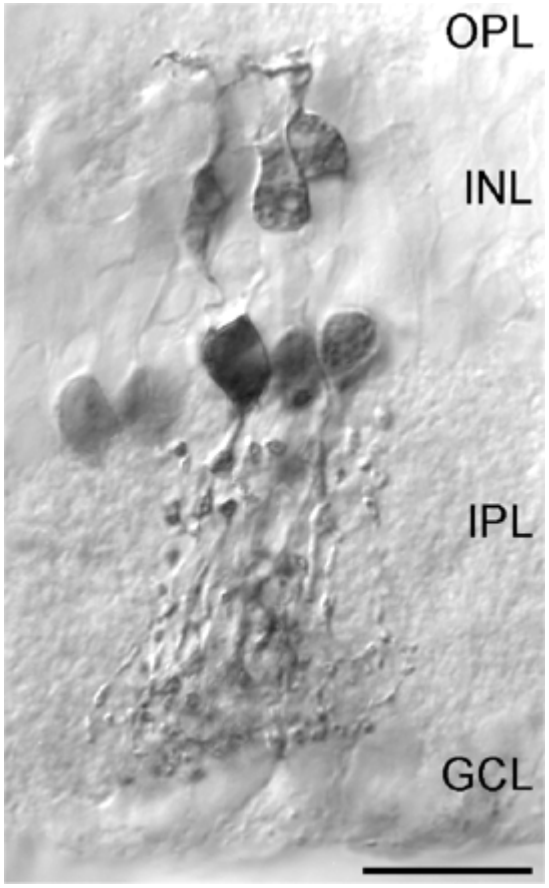
3 types of inhibitory input to OFF-CBCs

• OFF CBCs receive GABAergic and glycinergic small- amplitude-input from unknown amacrine cells

• **large-amplitude input** presumably from **AII amacrine cells**

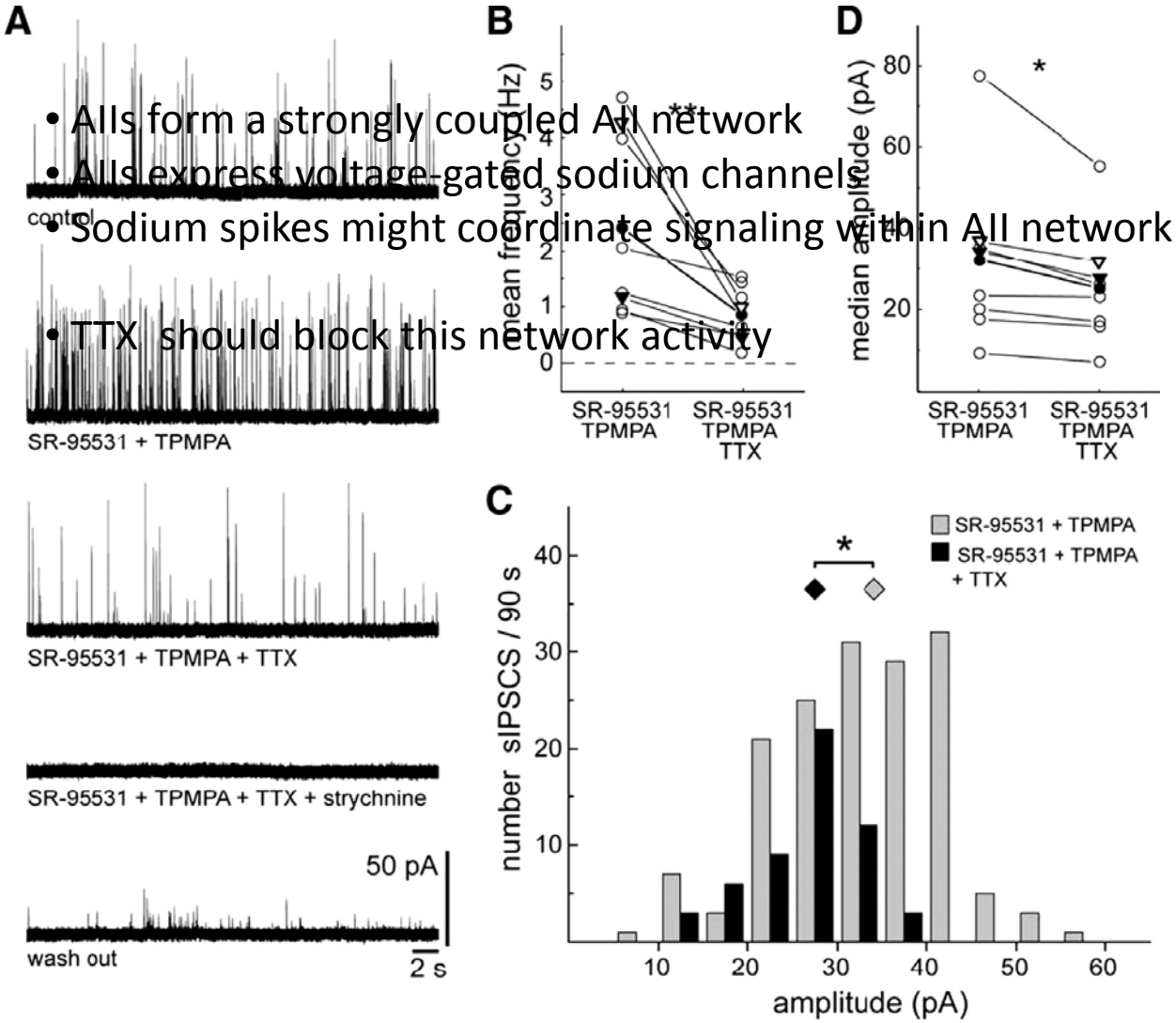
The voltage-gated Na channel blocker TTX decreases frequency and amplitude of sIPSCs

Neurobiotin-injected AIIs



(Habermann et al., 2003)

- AIIs form a strongly coupled AII network
- AIIs express voltage-gated sodium channels
- Sodium spikes might coordinate signaling within AII network
- TTX should block this network activity



TTX blocks multi vesicle events

- Inhibitory connections at BC axon terminal are established before eye opening (in particular AII – OFF-CBC synapse)
- Formation unlikely to be light-dependent