UCL

BIOS 3001 Advanced Visual Neuroscience

Advanced Retina

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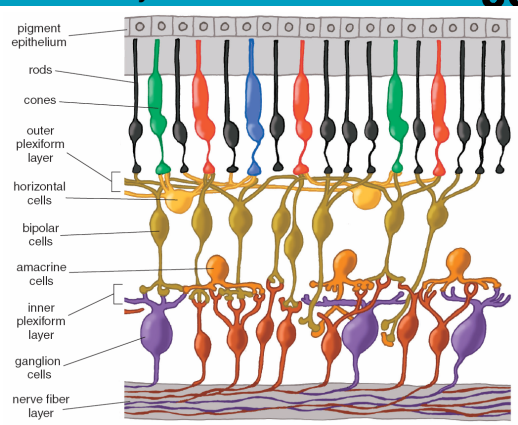
Institute of Ophthalmology

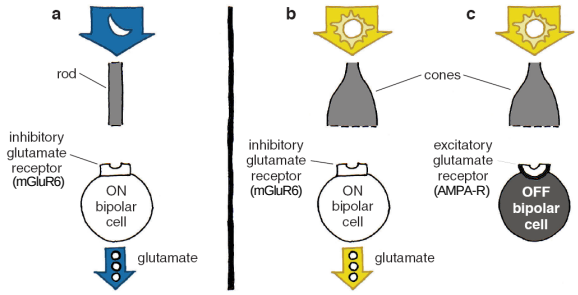
Retinal Circuitry



Summary of pathways through the retina

- Photoreceptors always respond to light ON with membrane potential hyperpolarisation, resulting in a reduction of neurotransmitter (Glutamate) release onto Bipolar Cells.
- Bipolar Cells respond to light with either **ON** or **OFF** responses. This is due to the expression of different Glutamate receptor types at the photoreceptor-bipolar cell synapse.
- Bipolar Cells utilise glutamate to synapse onto Retinal Ganglion Cells, conferring them with either **ON** or **OFF** responses.
- Retinal Ganglion Cells (RGCs) generate action potentials in responses to graded synaptic input potentials. Action potentials are conducted to the brain along the axons of RGCs running in the optic nerve.

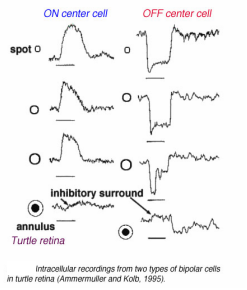
ON and OFF bipolar cells



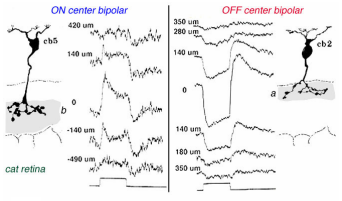
Photoreceptors release the transmitter glutamate in the dark, and stop releasing glutamate when stimulated by light.

Different bipolar cells respond differently to glutamate, depending on their type of glutamate receptor:
- ON bipolar cells have a depolarising receptive field (a, b)
- OFF bipolar cells have a hyperpolarising receptive field (c).

Bipolar Cell Responses

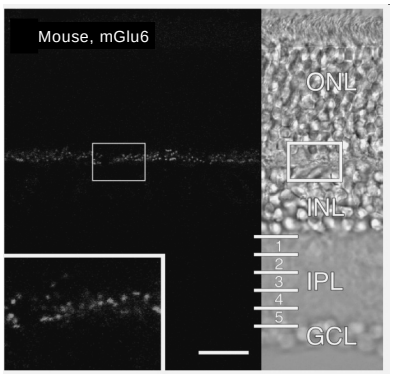


Intracellular recordings from two types of bipolar cells in turtle retina (Ammermuller and Kolb, 1995).



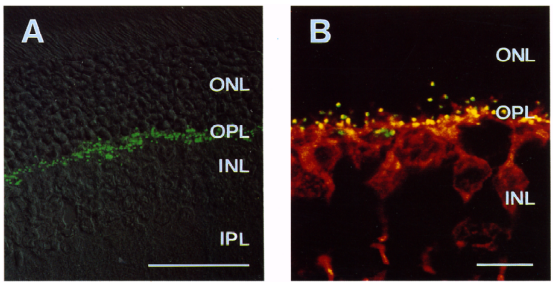
Intracellular recordings from ON and OFF bipolar cells in cat retina. Size of the receptive field measured in μm.

mGlu6 localisation in retina



Quraishi S., Gayet J., Morgans C.W., and Duvoisin R.M. (2007)
Distribution of group-III metabotropic glutamate receptors in the retina. J Comp Neurol 501, 931-43.

mGlu6 in bipolar cells

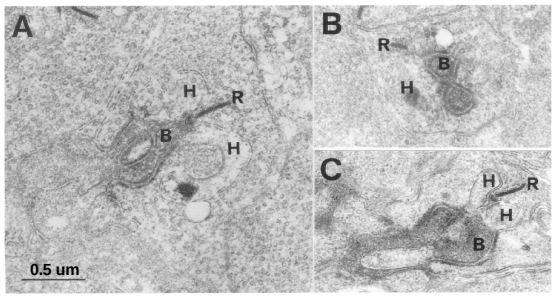


(A) Vertical sections of the adult rat retina were immunostained with the mGluR6 antibody, and aconfocal micrograph superimposed on the Nomarski image of an immunostained section is indicated. A punctate labeling pattern (green) is restricted to the OPL. IPL, inner plexiform layer.

(B) Sections of the adult rat retina were immunostained with the mGluR6 antibody (green and yellow) and the PKC antibody(red), and a confocal micrograph of a double-stained section is presented. Intense punctate mGluR6 immunoreactivity is seen only at the dendritic termini of the PKC-immunoreactive bipolar cells.

Nomura *et.al.* (1994) Developmentally regulated postsynaptic localization of a metabotropic glutamate receptor in rat rod bipolar cells. Cell 77:361-369.

EM of Rod Bipolar Cells Stained for mGluR6



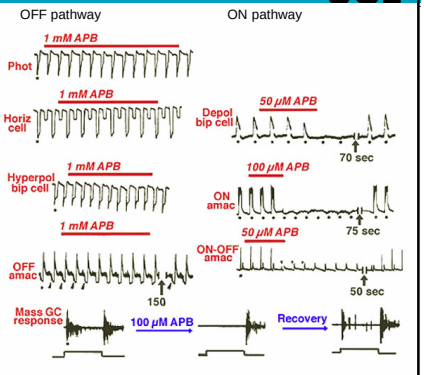
Rod bipolar cells were sectioned parallel (A and C) and perpendicular (B) to the bipolar cell dendrite. mGluR6 immunoreactivity (A and B) is observed only on a dendritic terminus of a rod bipolar cell, which branches off into two parts. PKC immunoreactivity (C) is seen in a branched dendritic terminus and its proximal dendritic shaft of a rod bipolar cell. R, synaptic ribbon; H, horizontal cell process; B, rod bipolar cell process.

Nomura *et.al.* (1994) Developmentally regulated postsynaptic localization of a metabotropic glutamate receptor in rat rod bipolar cells. Cell 77:361-369.

L-AP4 effects in the Retina

Note

- L-AP4 (formerly known as APB) is a Group III mGlu *AGONIST*.
- L-AP4 is a functional antagonist of the ON pathway.



intracellular recordings from different retinal neurons show that APB selectively antagonizes the ON pathway (right column). From Slaughter and Miller, 1981.

mGlu6 Receptor gated currents

Note

- mGlu6 is a Group III metabotropic glutamate receptor
- L-AP4 (formerly known as APB) is a Group III mGlu *AGONIST*.
- mGlu6 activation closes cation channels

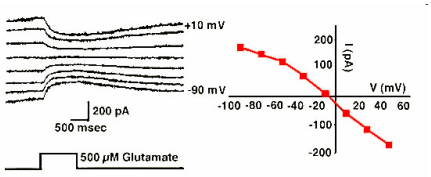


Fig. 10. Whole cell current traces (left) and the calculated current-voltage relationship (right) for APB receptor-gated currents. Glutamate acting on APB receptors closes non-selective cation channels resulting in a conductance decrease (from Grant and Dowling, 1996).

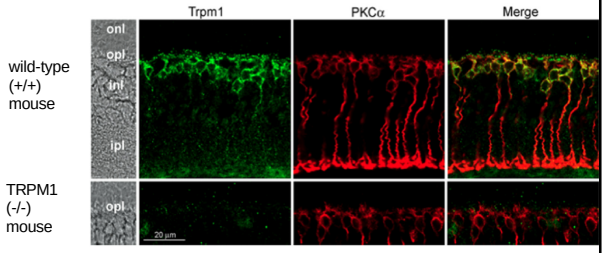
What is the Identity of the Cation Channel that mGluR6 gates/modulates?

- mGluR6-coupled current of ON-bipolar cells is inhibited by TRP* channel antagonists
- Congenital night blindness in Appaloosa horses linked to TRPM1 gene
- Electroretinograms on these horses indicate defective transmission between photoreceptors and ON-bipolar cells.

* Transient Receptor Potential

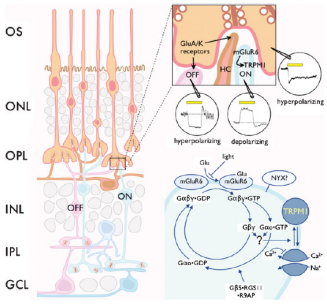
TRPM1 is expressed by ON-bipolar cells

Vertical sections from a wild-type (*Top*) and TRPM1-/- (*Bottom*) retina were immunofluorescently labeled by antibodies directed against TRPM1 (green) and PKC (red). Areas of colocalization appear yellow in the merged images



Morgans, C. W., *et al.* (2009). *Proc Natl Acad Sci U S A* **106**(45): 19174-19178.

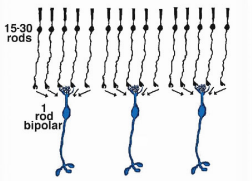
Summary: ON Bipolar Synapse



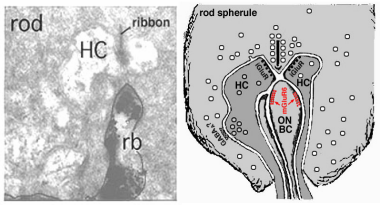
- All photoreceptors respond to light by hyperpolarization, as do horizontal cells (HC) and OFF bipolar cells (pink), which express AMPA and kainate-type glutamate receptors (GluA and GluK).
- ON-bipolar cells depolarize in response to light, a mechanism involving mGluR6 and TRPM1 channels.
- The G protein signaling cascade activated by mGluR6 is represented at the bottom right.
- The function of nyctalopin (NYX) and the second messenger signal between Go and the TRPM1 channel remain unknown.

Morgans, C. W., et al. (2010). Bioessays 32(7): 609-614.

Rod Bipolar Cells



Convergence of rods onto rod bipolars

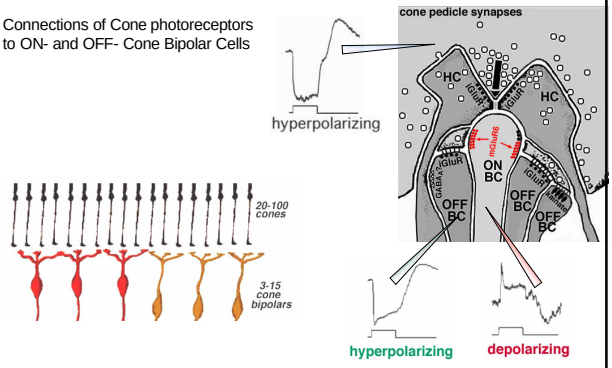


Electron micrograph and schematic of a rod spherule

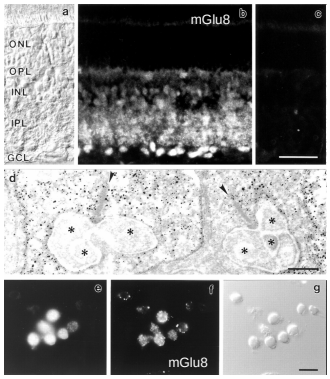
BC – Bipolar Cell
HC – Horizontal Cell

Cone Bipolar Cells

Connections of Cone photoreceptors to ON- and OFF- Cone Bipolar Cells



mGlu8 receptor location in retina



(a) Retinal layers (ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer).

(b) Retina section stained with the antiserum against mGluR8.

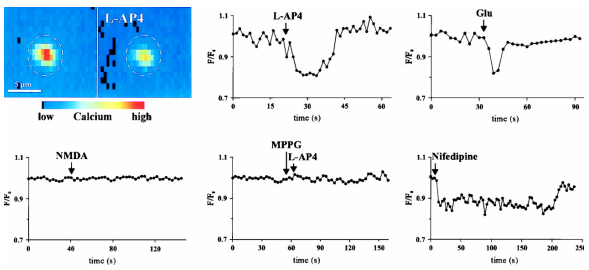
(c) Control; the anti-mGluR8 antiserum was preadsorbed with the antigen,

(d) Electron micrograph showing the presynaptic localization of mGluR8 immunoreactivity in rod spherules. The presynaptic ribbon in the rod spherule is marked by an arrowhead. Postsynaptic processes are marked by stars.

(e-g) Double labeling of isolated photoreceptors with an antibody against arrestin (e) and the antiserum against mGluR8 (f). (g) Nomarski micrograph showing the isolated cells.

Koulen etal. (1999) Proc Nat Acad Sci (USA) 96, 9909-9914

mGlu8 presynaptic function on photoreceptors



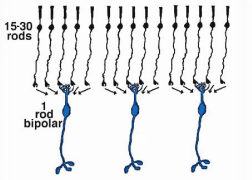
The colour figure shows a typical calcium imaging experiment performed on a freshly isolated photoreceptor loaded with the calcium indicator fluo-3. The high [Ca2+]i in the photoreceptor before the application of L-AP4 (Left) significantly drops after the application of L-AP4 (Right).

As shown in the graphs representing single experiments, L-AP4 and glutamate lead to a decrease in the $[Ca^{2+}]i$ in photoreceptors; NMDA does not.

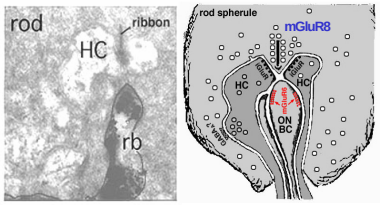
The decrease of the $[Ca^{2+}]i$ by L-AP4 can be specifically blocked by the group III mGluR antagonist MPPG. Application of the L-type calcium channel blocker nifedipine leads to a decrease of the $[Ca^{2+}]i$ in a range comparable to that elicited by L-AP4 or glutamate.

Koulen etal. (1999) Proc Nat Acad Sci (USA) 96, 9909-9914

Rod Bipolar Cells



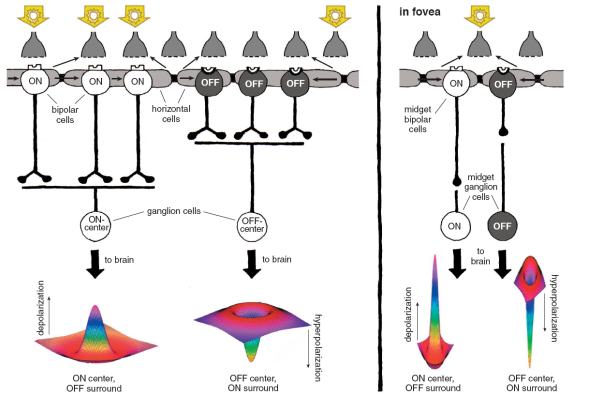
Convergence of rods onto rod bipolars



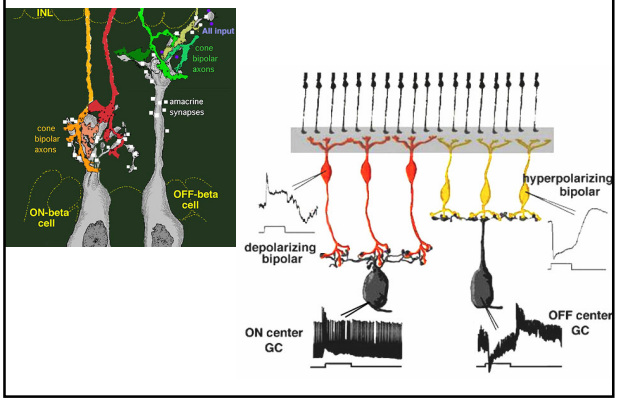
Electron micrograph and schematic of a rod spherule

BC – Bipolar Cell
HC – Horizontal Cell

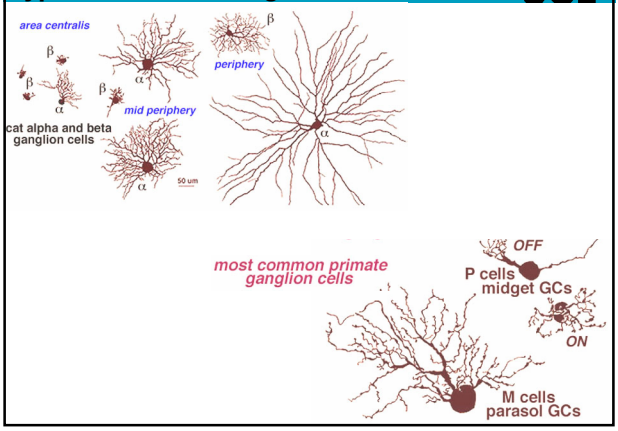
ON and OFF Cone paths to Ganglion Cells



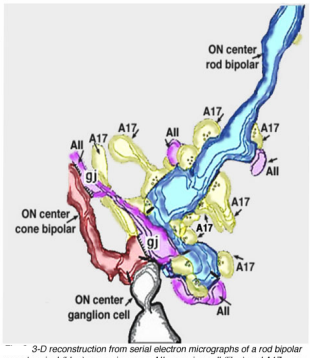
Cone Bipolar Termination on RGC



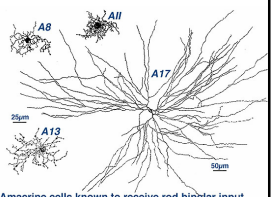
Types of Retinal Ganglion Cells



Rod Pathways to RGCs

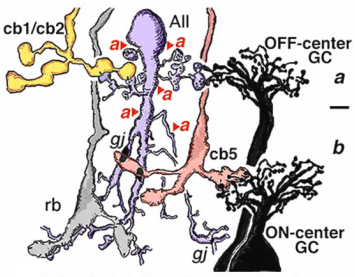


3-D reconstruction from serial electron micrographs of a rod bipolar axon terminal (blue) synapsing upon AII amacrine cell (lilac) and A17 amacrine cell (yellow) profiles. A17 processes make reciprocal synapses. AII amacrine cells make gap junctions on ON center cone bipolar axons.

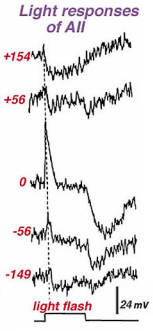


Amacrine cells known to receive rod bipolar input

Amacrine Cell Function (AII)

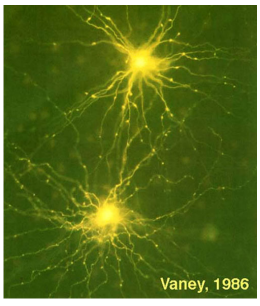


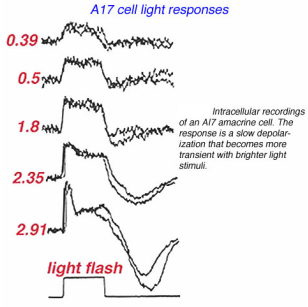
Drawing to show the circuitry of the AII amacrine cell with pre and post synaptic neurons. Sublaminas a and b are indicated.



Intracellular recordings of AII amacrine cells. AII amacrine cells respond to light with a depolarizing response (ON center response) in their centers (Nelson, 1982; Dacheux and Raviola, 1986).

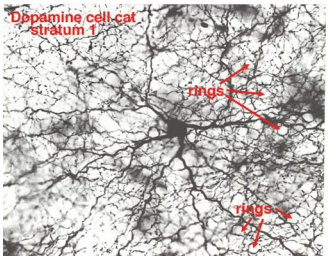
Amacrine Cell Function (A17)





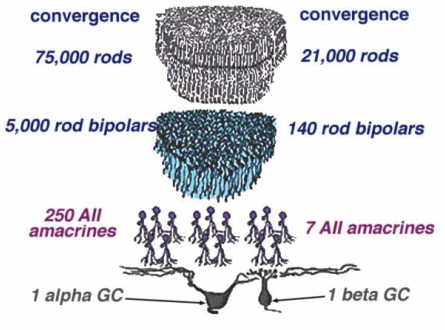
Intracellular recordings of an A17 amacrine cell. The response is a slow depolarization that becomes more transient with brighter light stimuli.

Dopamine containing (A18) cells



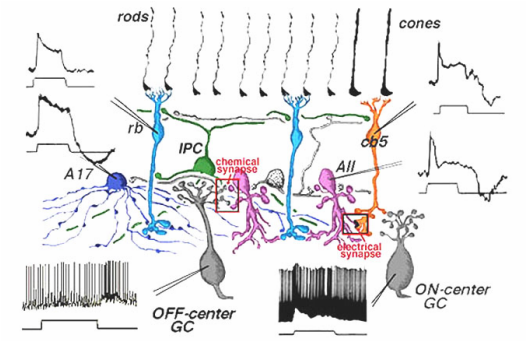
Immunostaining for tyrosine hydroxylase. A18 Amacrine cells have overlapping dendrites that form into rings.

Convergence of the Rod Pathway

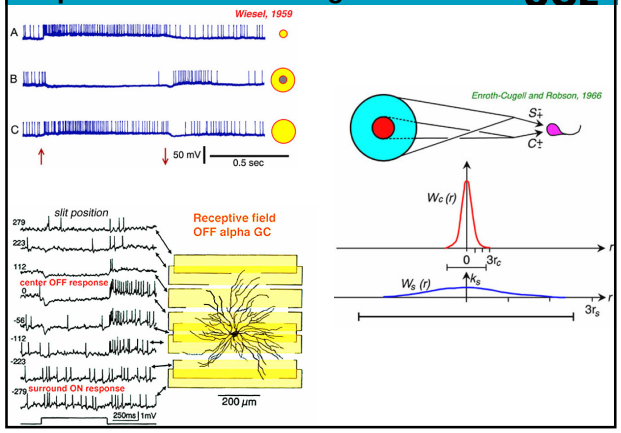


Cat retina

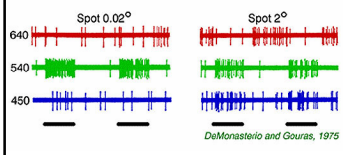
Summary of Rod-driven pathways



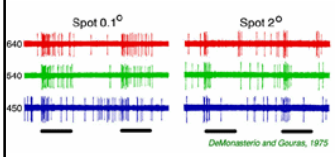
Responses of Retinal Ganglion Cells



Responses of primate RGCs



Spectral responses of tonic ganglion cell in rhesus monkey retina. Stimulus wavelength in nm is given to the left of impulse discharge responses. The light stimulus is ON during the black bars at the bottom of the figure. This cell is a green ON-center red OFF-surround unit.

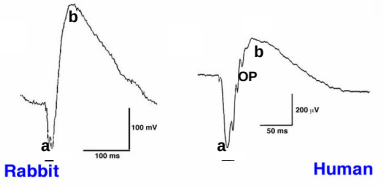


Spectral responses of phasic ganglion cell in rhesus monkey retina. Stimulus wavelength in nm given to left of impulse records. The light stimulus is ON during the black bars at the bottom of the figure. This cell is spectrally a luminosity type with ON-center and OFF-surround responses.

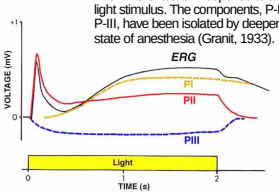
Electroretinogram (ERG)

ERG responses of rabbit and human, in addition to those recorded from other vertebrate species, are characterized by the basic features of a negative a-wave followed by a positive b-wave.

Responses to brief flashes in dark adapted state. Longer stimuli can also evoke a c-wave.



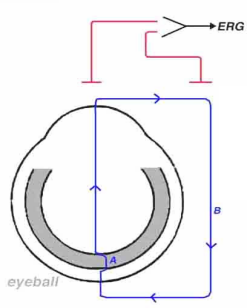
The ERG of a cat in response to a 2 sec light stimulus. The components, P-I, P-II and P-III, have been isolated by deepening the state of anesthesia (Granit, 1933).

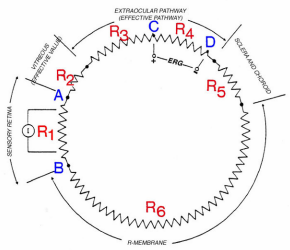


Ragnar Granit, winner of the Nobel Prize for Physiology and Medicine in 1954

Recording the ERG

A schematic representation of the extracellular currents that are formed following light stimulation. Pathway A represents local currents within the retina, while pathway B shows the currents leaving the retina through the vitreous and the cornea and returning to the retina through the choroid and the pigment epithelium.



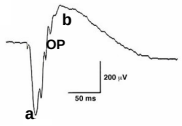


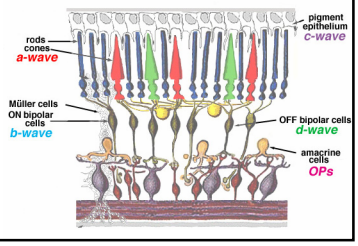
An electrical scheme of the resistances through which currents IA and IB flow when the retina is stimulated with light. The current source I, represents the electrical current that is generated in the retina in response to a light stimulus. Pathway A is the local intra-retinal route of current flow and pathway B is the remote route going from the retina and through the vitreous, lens, cornea, extra-ocular tissues and back to the retina through the sclera, choroid and pigment epithelium.

Cellular Origins of the ERG

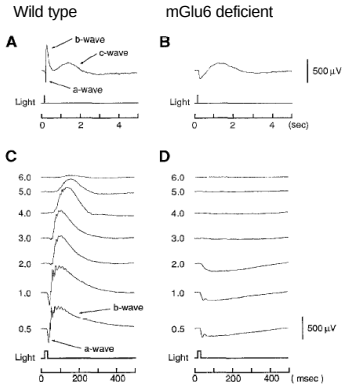
ERG components

- **a-wave**: photoreceptors
- **b-wave**: ON bipolars (?Mueller Cells)
- **c-wave**: pigment epithelium
- **d-wave**: OFF bipolars
- **OP** (oscillatory potentials): amacrine cells



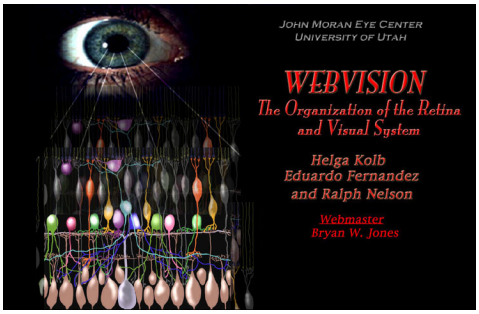


ERG in mGlu6 deficient mice



Masu et.al. (1995)
Cell 80[5], 757-765.

http://webvision.med.utah.edu/



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