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The Cellular Basis of Electroretinogram (ERG) Signals

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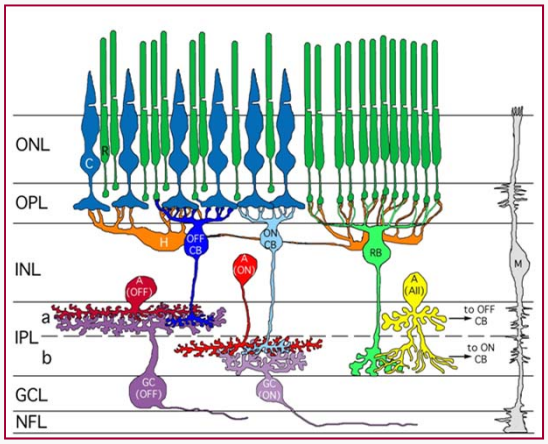
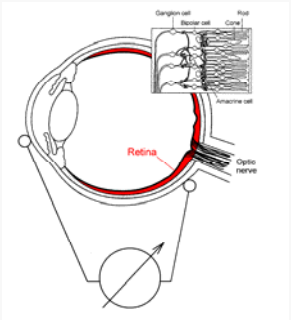
Cellular origins and mechanisms of generation of the various waves of the ERG



Sherry, 2002



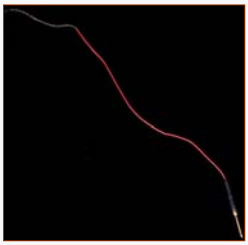
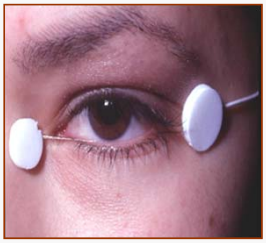
Modified from Dowling, 1987

The ERG – a non-invasive tool for assaying retinal function and integrity

- The ERG can inform us about the functional status of retinal cells and circuits
- The ERG is useful for detecting and monitoring the progression of diseases that affect the retina
- The ERG can be used to monitor effects of therapeutic interventions

Non-invasive recording of the ERG in man, monkey, mouse etc.

Electrodes: Conductive materials in contact with the cornea
Example: Contact lens electrodes and DTL fiber electrodes

- Pupils – fully dilated for full field flash ERG
- Corneas – covered with moistening/ionic conducting solution
- Fixation (spot or cross)
- Responses – repeated trials averaged when signals are small
- Animals and sometimes young children: generally anesthetized using drugs with minimal effects on retinal function

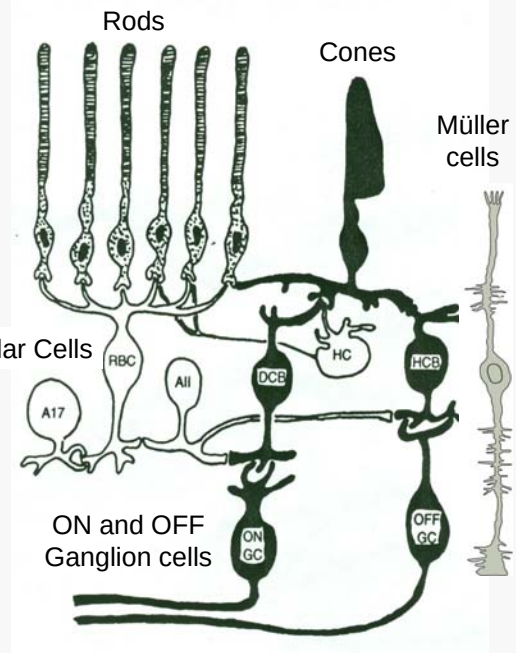
ERG Stimulus - The diffuse flash full-field or “Ganzfeld” ERG

Current LED-based Ganzfeld stimulators

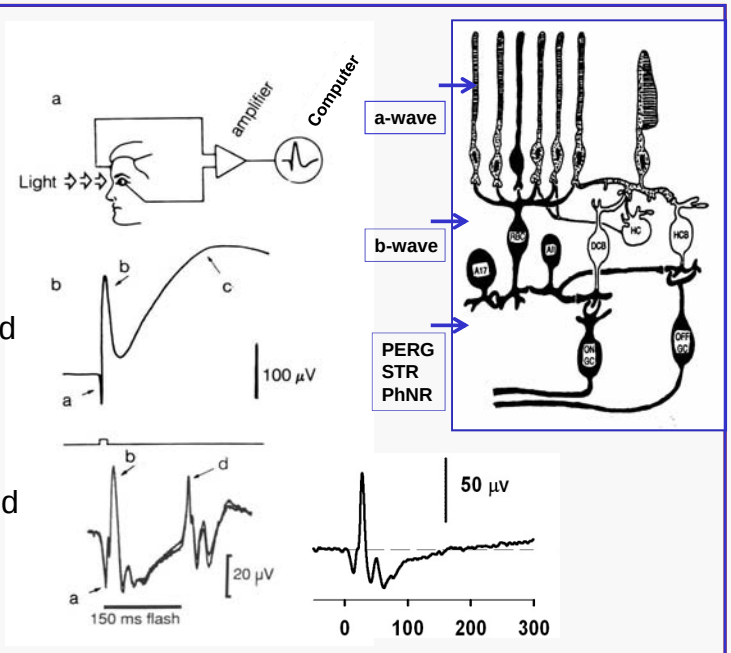
Traditional white light (xenon flash tube is common) within a Ganzfeld globe

Retinal cells and circuits

All are represented in the ERG



Rod- and cone-driven ERGs

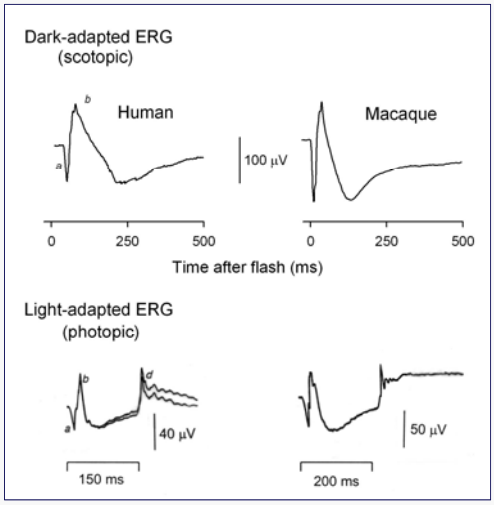


Modified from Dowling, 1987

Origins of the ERG waves

- Intraretinal localization
- Pharmacological dissection
- Modeling
- Site-specific lesions/pathology
- Targeted mutations
- New stimulation/analysis techniques

Animal model for necessarily invasive experiments: macaque monkey (primate) retina and ERG are very similar to those of humans

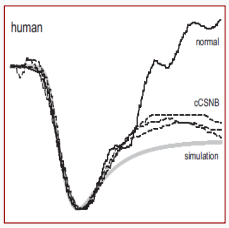


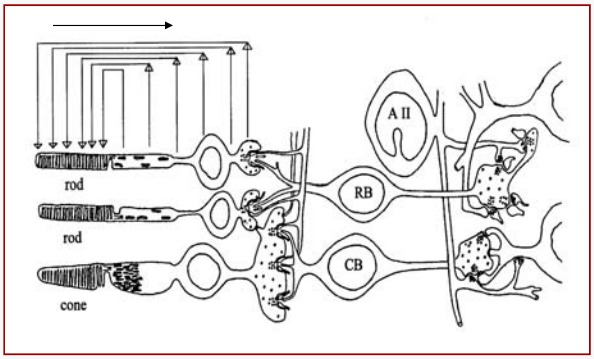
Intraretinal localization: Radial Currents

Generation of the a-wave (PIII)

Circulating current and resulting a-wave from the rods

A-wave

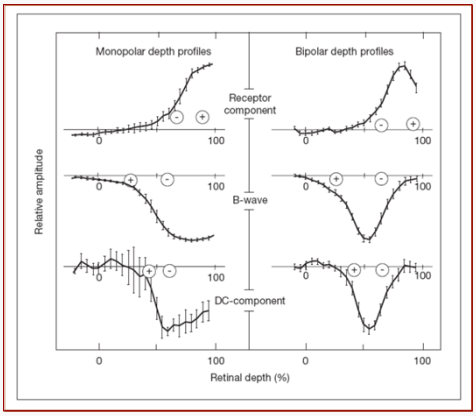




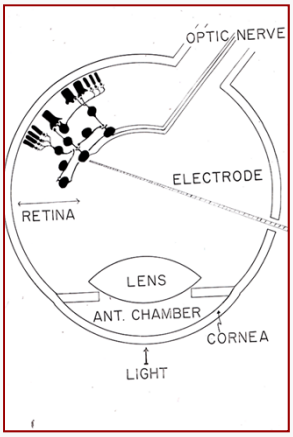
After Penn & Hagins, 1972; Pugh et al., 1998

Intraretinal localization

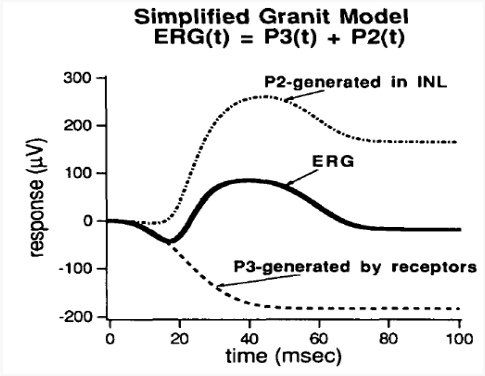
Current source density analysis in monkey retina localizing a-waves to photoreceptors, b-waves to bipolar cells



The pluses and minuses indicate the current sources (+) and sinks (–) for the components as calculated from current source-density analyses based on the coaxial electrode recordings and resistance measurements.



Pharmacological dissection: Origins of the dark-adapted ERG: Ragnar Granit, 1933



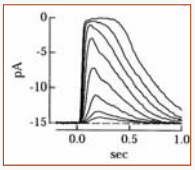
Component processes:
Disappearing waves – during ether induction:
P1- RPE: c
P2- Bipolar cells: b
P3- Photoreceptors: a

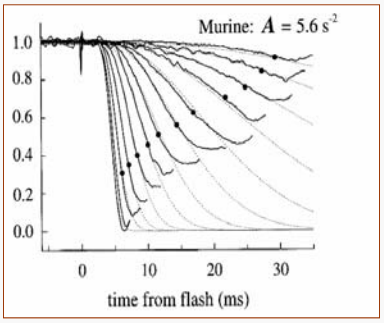
First pharmacological dissection of the ERG:
order of disappearance of "processes" P1, P2, P3 following induction of ether anesthesia

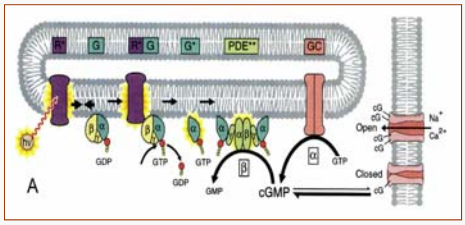
Hood & Birch, 1992

Modeling and interpreting – transduction cascade* & the a-wave

Mouse rod OS photocurrent recording (Field & Rieke, 2002)



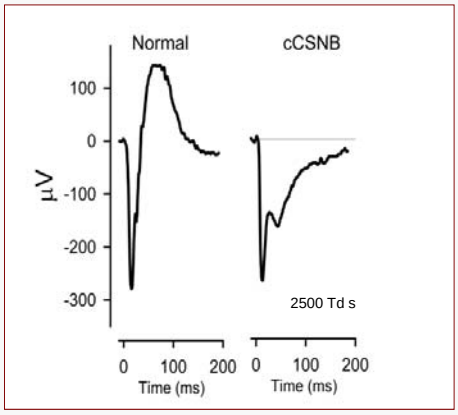




Lamb & Pugh, 1992; Pugh et al., 1998
Hood & Birch, 1990

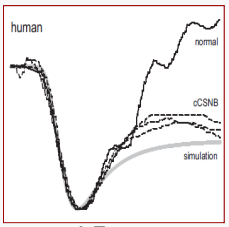
*a-wave generated by outer segment, OS current ?

The isolated a-wave, is not identical to OS current



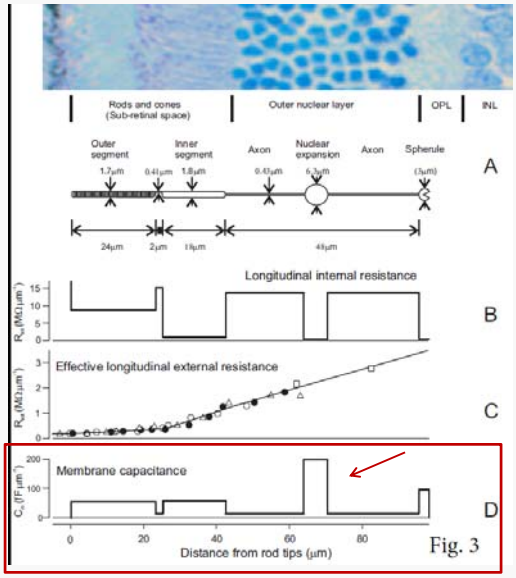
P3 revealed by removing the b-wave

- Pharmacologic blockade
- Anoxia – ischemia – occlusion of inner retinal circulation
- cCSNB in human



l 5 ms

A model of the a-wave that includes capacitance of ONL

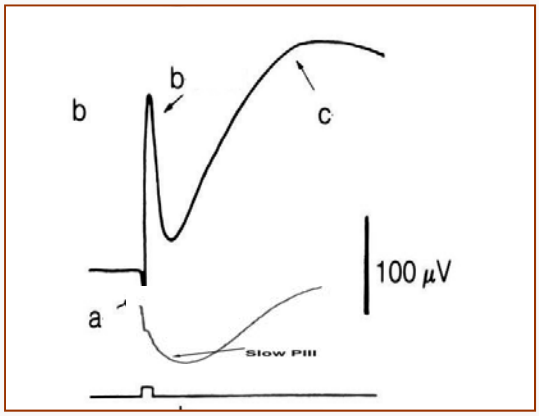


Model includes OS currents, and longitudinal resistances and ONL capacitance seen in the rat retinal slice (Penn & Hagins, 1970)

Robson & Frishman, 2014

Other photoreceptor-dependent events:

The c-wave and slow P3: Photoreceptor response-dependent $[K^+]_0$ changes in subretinal space between photoreceptors and RPE affect RPE cells (TEP c-wave) and Müller cells (slow P3)

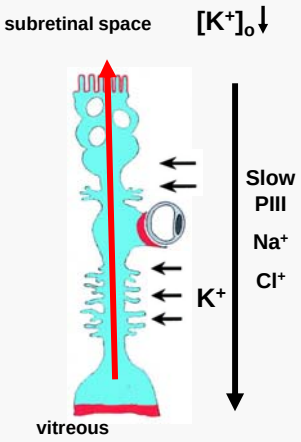


Spatial buffer currents in Müller cells move K^+ from areas of high extracellular to areas of low extracellular concentration

Slow P3

- A corneal negative subcomponent of the C-wave
- A major photoreceptor-dependent component generated by Müller cell currents
- The K^+ channel blocker, barium (Ba^{2+}) eliminates slow P3
- Knocking out Kir4.1channels in mice eliminates slow P3 (the b-wave remains intact)

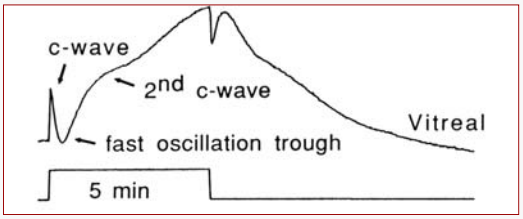
Kofuji et al (2000)



Other outer retinal events

Waves in the DC ERG with origins in the retinal pigment epithelium (RPE)

TEP C-wave
FOT
Light Peak



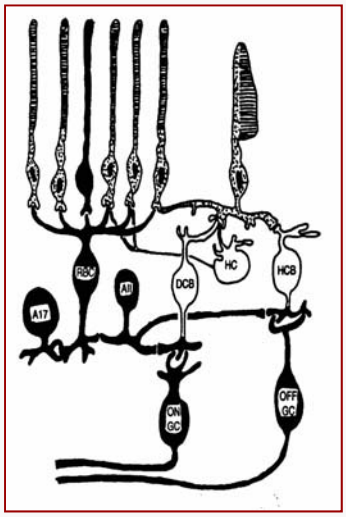
Measure with the electrooculogram (EOG) in awake humans

Cat: Steinberg et al (1985)

Postreceptoral contributions to the ERG

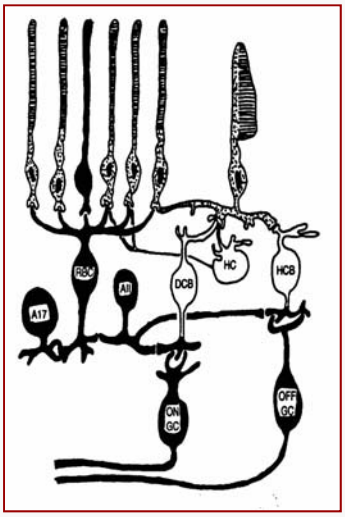
Pharmacological dissection studies

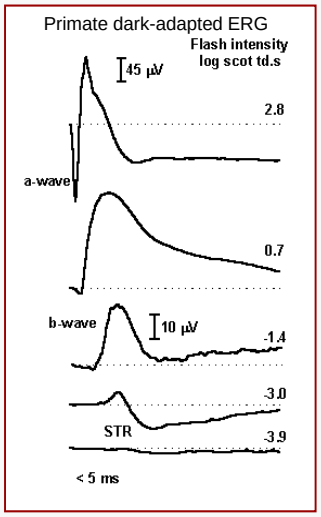
Dark-adapted ERG
The rod pathway: a simple circuit sensitive to single photon events



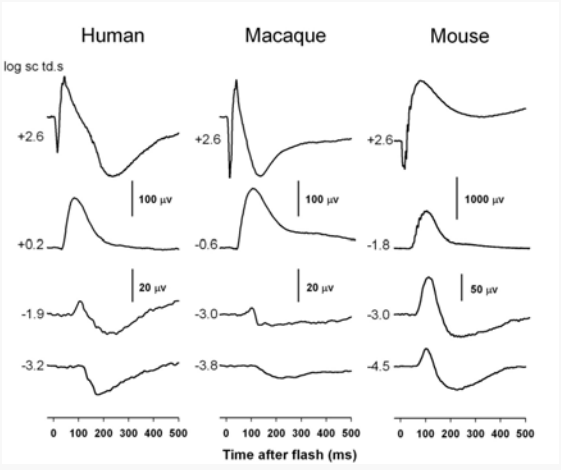
- The rod pathway
  - Rods
  - Rod bipolar cells
  - AII amacrine cells
  - Cone bipolar cells
  - Ganglion cells

Origins of the dark-adapted ERG from postreceptoral neural retina

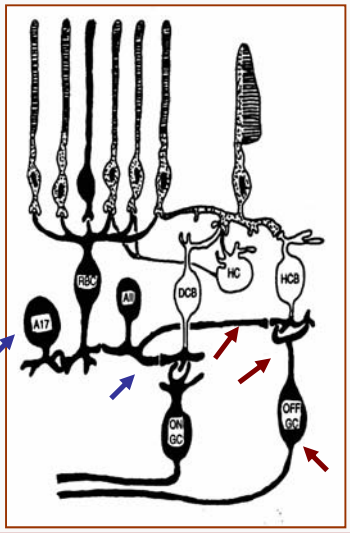




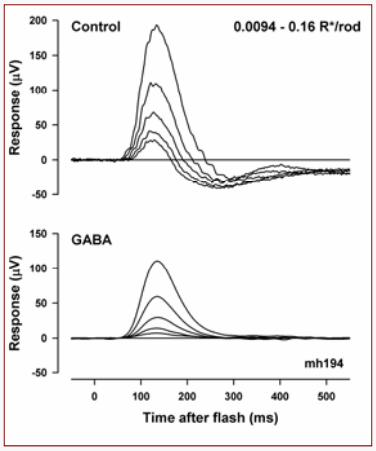
Dark-adapted ERGs are similar in primates and rodents



Removal of amacrine and ganglion cell contributions to the ERG to isolate the RBC component (PII)



GABA
NMDA



Remove scotopic threshold response (STR) pharmacologically

TTX (Na^+-dependent spikes)

Scotopic b-wave originates from rod-driven bipolar cells

Rod bipolar cell current from patch recordings in mouse retinal slice (Field & Rieke, 2002) compared with isolated human (by weak light adaptation) and mouse scotopic b-waves (ERG RBC response, PII)

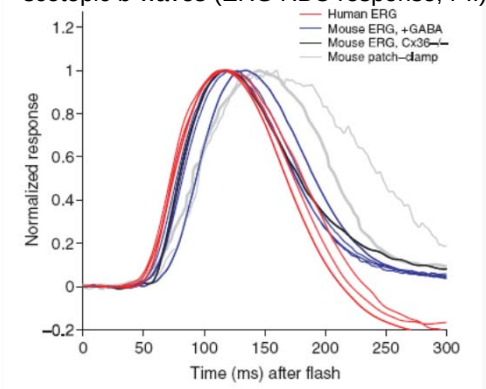
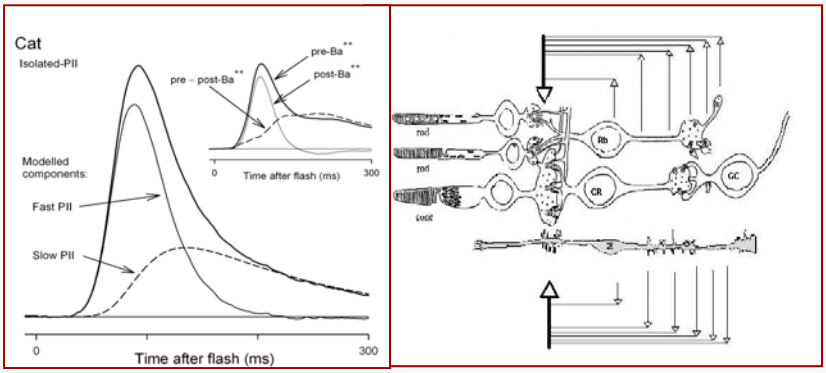


Figure 4. Comparison of rod bipolar cell responses estimated here, with those reported in the literature, for flashes of about 1 photoisomerization per rod

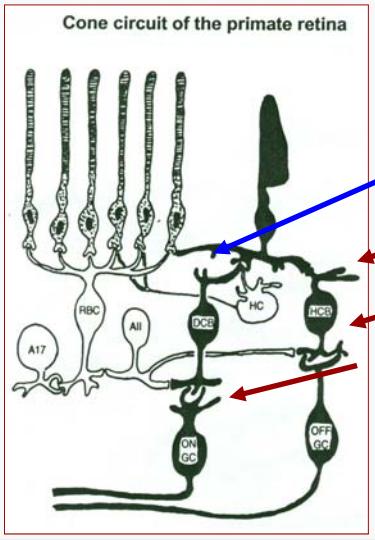
From Cameron, Mahroo & Lamb (2006) modified from Robson et al (2004)

The scotopic b-wave (PII):
mainly from radial currents around bipolar cells - late Müller cell contribution



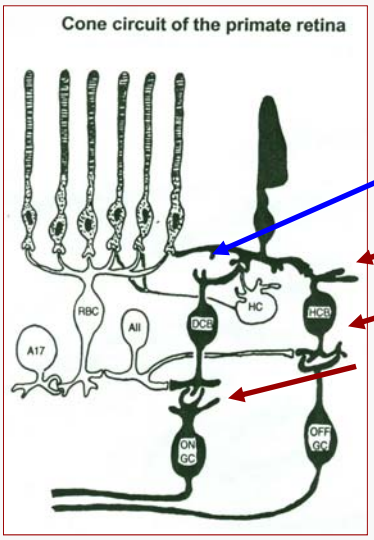
Barium ($BaCl_2$) an inward rectifying K^+ channel blocker removes a slow component of the b-wave by blocking Kir channels in Müller cell membranes

Origins of the light-adapted waves of the ERG from postreceptoral neural retina – using glutamate pharmacology



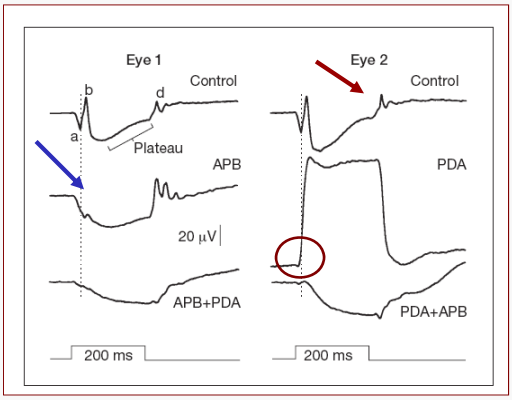
- Metabotropic glutamate receptor: ON bipolar cell
- Ionotropic glutamate receptor OFF bipolar (& HzCs), amacrine and ganglion cells

Pharmacological dissection of On and Off pathways through the retina



- APB: elimination of ON bipolar cell activity (b-wave) via agonist effect on metabotropic GluRs
- PDA: Blockade of ionotropic GluRs on OFF bipolar (& Hz cells), amacrine and ganglion cells

Origins of waves in the light-adapted ERG of the primate



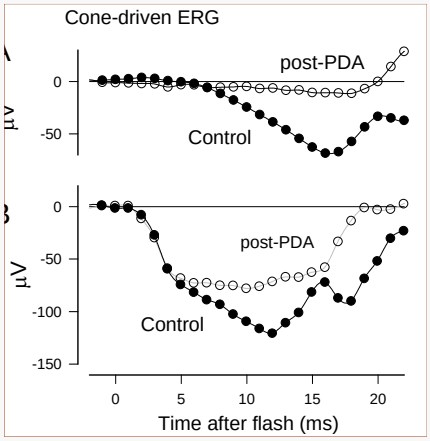
Sieving et al., 1994 *Bush & Sieving, 1994*

- b-wave: ON cone bipolar cells – removed by APB.
- d-wave: OFF bipolar cells + cone offset – reduced by PDA
- push-pull or inhibition makes b-wave small
- a-wave: partially OFF bipolar cell response

Monkey: Origins of a-waves in the light-adapted ERG

weak

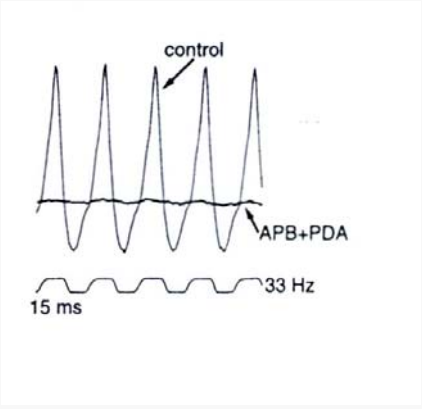
stronger



- a-wave: OFF cone bipolar cell contribution removed by PDA

Robson et al., 2003

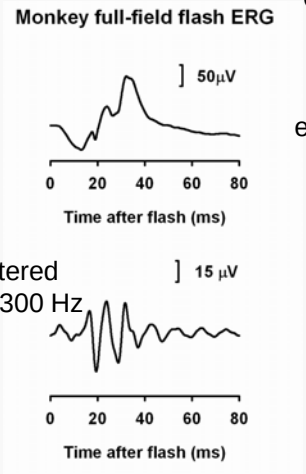
Postreceptoral origins of "30 Hz" fast flicker ERG in nonhuman primates



APB+PDA ON and OFF bipolar cell responses removed

Bush & Sieving, 1996

Oscillatory potentials (OPs)



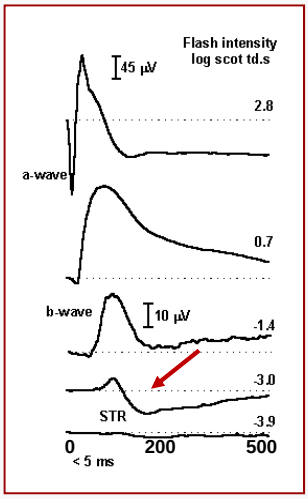
OPs: wavelets superimposed on the b-wave. occur in scotopic and photopic ERG

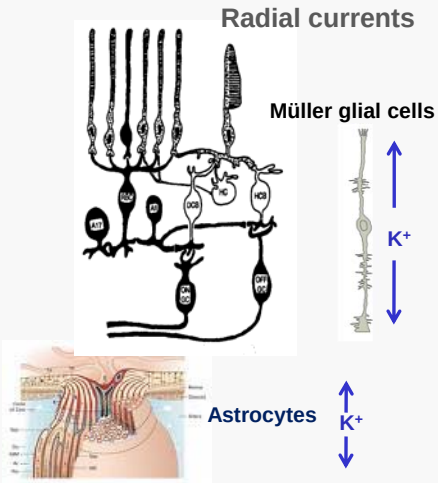
Involvement of amacrine cells is well established;mechanisms of generation are not fully understood

OPs are reduced in eyes with diabetic retinopathy

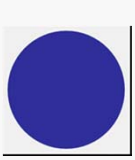
High frequency OPs (peak at 150 Hz) are related to ganglion cell function.

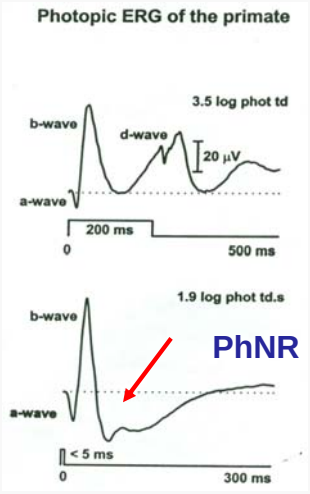
Retinal ganglion cells – contributions to flash ERG? The scotopic threshold response (STR) originates from amacrine cells and retinal ganglion cells

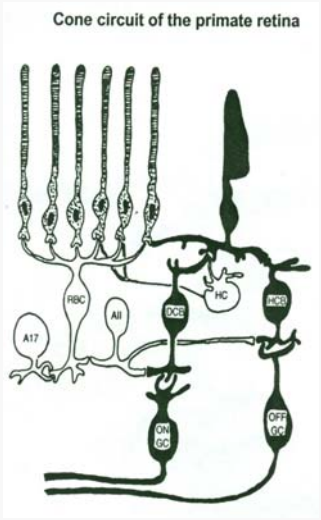




Photopic flash ERG in monkey – photopic negative response (PhNR) after b-wave

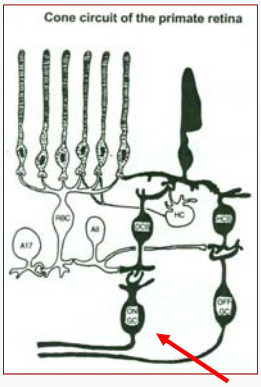


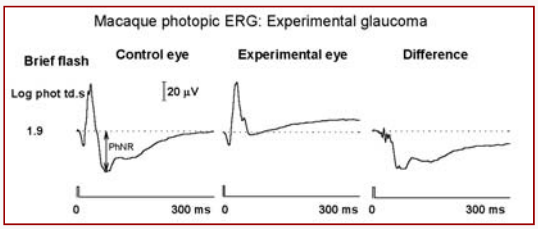




Glial mediation?

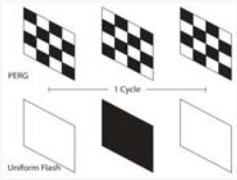
The PhNR (and PERG) in primates is selectively and similarly reduced by experimental glaucoma which causes retinal ganglion cell death



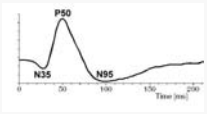


Viswanathan et al., 1999

Pattern ERG (PERG)

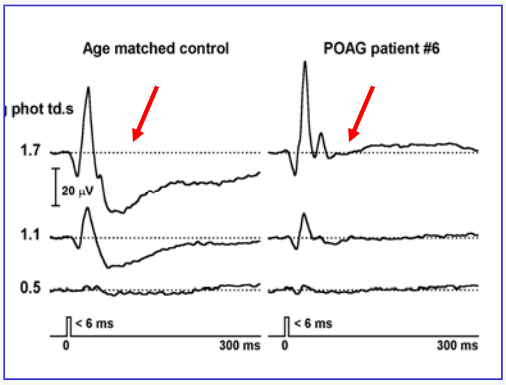


Ganglion cell origin well established



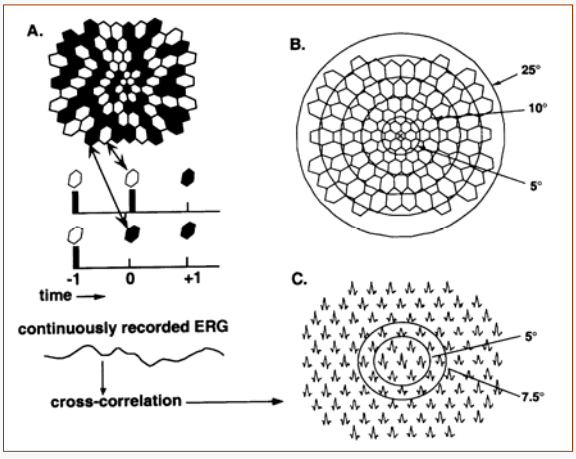
PhNR amplitude is reduced in humans and animals with optic neuropathies

- Open angle glaucoma
- Optic atrophy
- NAION
- Optic neuritis (MS)



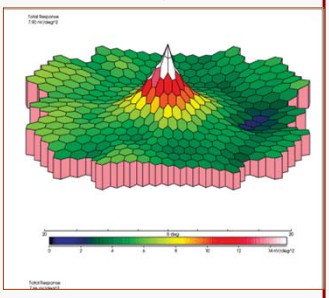
Viswanathan et al.,2001

Multifocal ERG*
(many small focal ERGs recorded simultaneously)



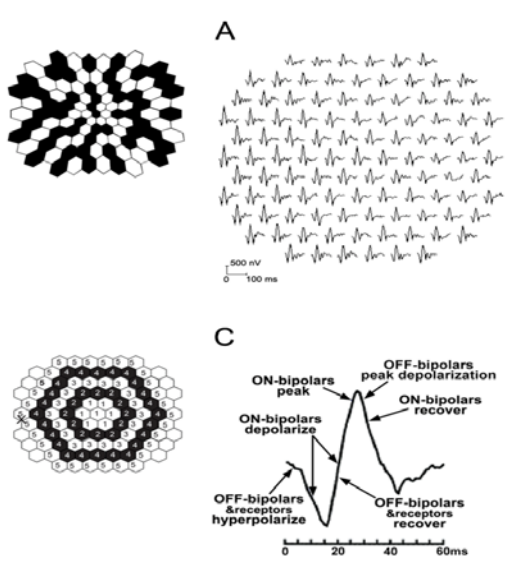
Hood (2000)

Central fixation



**Developed in late 20th century by Dr. Erich Sutter*

Multifocal ERG – cellular origins in primates



APB
Positive potential:
ON bipolar cells

APB + PDA
Initial negative potential
OFF bipolar cells and
cone photoreceptors

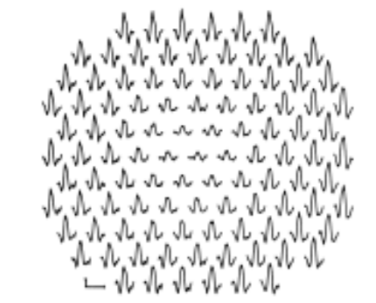
TTX+NMDA
Oscillatory activity from
amacrine and ganglion cells
removed

Hood et al (2002)

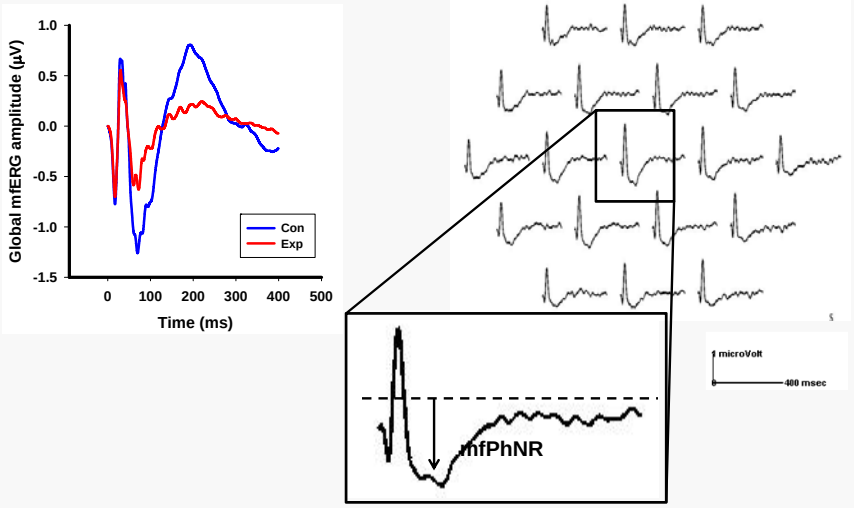
Detecting local functional losses

Normal

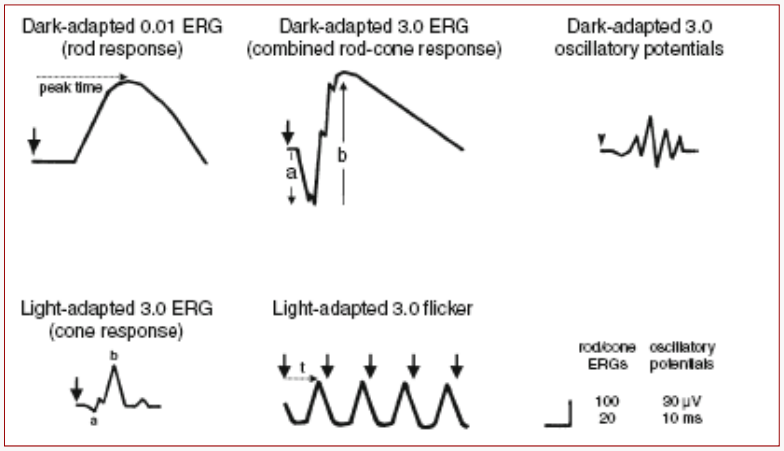
Stargardt's macular dystrophy



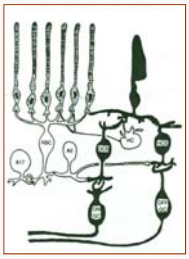
mfPhNR in glaucoma



ISCEV "Standard" Clinical Testing



Retinal cells contributing to waves of the flash and flicker ERG



ISCEV “Standard” clinical testing

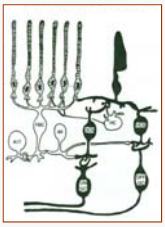
a-wave
- Photoreceptors (rods and cones) and late postreceptoral OFF bipolar, and ON pathway contributions

b-wave / (d-wave)
- Bipolar cells – scotopic b-wave: rod bipolar cells (RBC), slow Müller cell; photopic b-wave: ON cone bipolar -DCB) and OFF cone bipolar (HCB) bipolar cells, horizontal (Hz) cell feedback; photopic d-wave: OFF bipolar and offset of cone photoreceptor response

Oscillatory potentials (OPs)
- Amacrine and ganglion cells (membrane oscillations, feedback loops)

“30 Hz” fast flicker
- ON and OFF cone bipolar cells, small cone photoreceptor contribution (small contribution from spiking activity)

Retinal cells contributing to waves of the ERG



More specific tests

PhNR, STR, PERG
- Ganglion cells and their axons; amacrine cells as well for STR; glial currents (astrocytes?)

c-wave
- Retinal pigment epithelial (PPE) cell slow potentials; photoreceptor-dependent changes in $[K_o]^+$. Corneal signal has slow PIII subtracted from the RPE signal, which is generally larger.

Slow P3
- Müller (glial) cell currents across the neural retina; light-evoked photoreceptor-dependent changes in $[K_o]^+$

The end

References:

Useful website for knowledge about the visual system, includes retinal structure and function.

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

Review chapters with reference lists for papers on ERG origins:

Frishman L.J. (2013) Electrogenesis of the ERG In: Ryan Retina 5th edition, edited by D.R.Hinton, MD. Elsevier Mosby, Chapter 7, pp 177-2013.

Frishman L.J. (2011) ERG in man, monkey and mouse. Adler's Physiology of the Eye, 11th edition, edited by Kaufman & Alm.

Frishman, L.J, (2006) Origins of the ERG. In: Principles and Practice of Clinical Electrophysiology of Vision 2nd edition, edited by J. Heckenlively, & G. B. Arden, Ch 12, pp. 139-183.

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