Electrophysiology

Scientific Basis of Vision

Ophthalmology Department
UPMC
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Lecture Outline

- Overview of Visual Electrophysiology
  - Types of Clinical Visual Electrophysiology
  - Basic Neurophysiology
- ERG
- VEP
- EOG
- Clinical Application

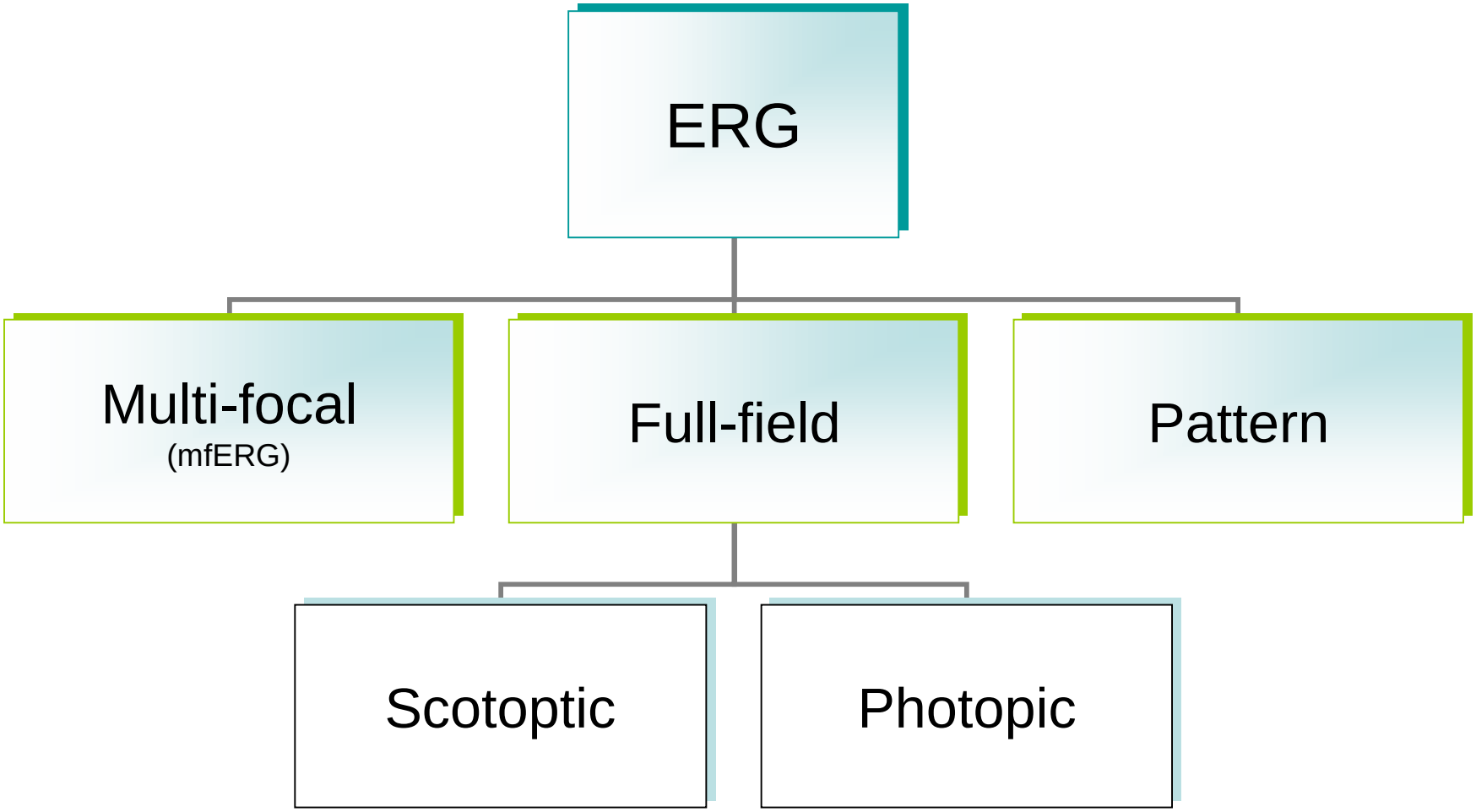
Visual Electrophysiology

- Study of the electrical signals that occur in the visual system in response to a visual stimulus.

Types of Visual Electrophysiology

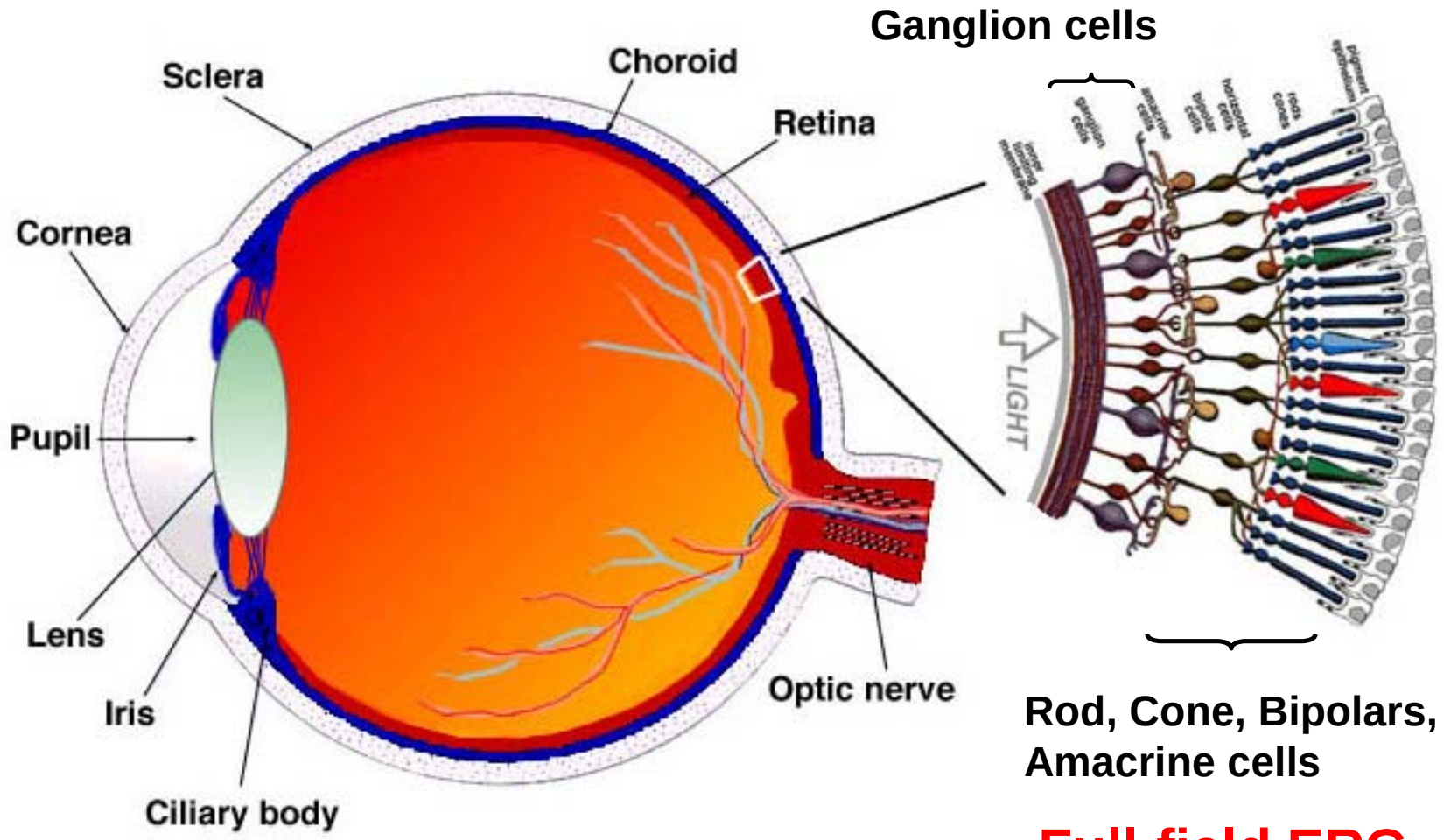
- Electroretinogram (ERG) usually represents the summed activity of the retina in response to a light flash or local electrophysiological responses from different regions of the retina
- Visual Evoked Potentials (VEP) are massed or local electrical signals generated by occipital cortical areas in response to visual stimulation.
- Electroculogram (EOG) is the measure of the functional state of the outmost retinal layers.

Electroretinogram



Visual Physiology

Pattern ERG

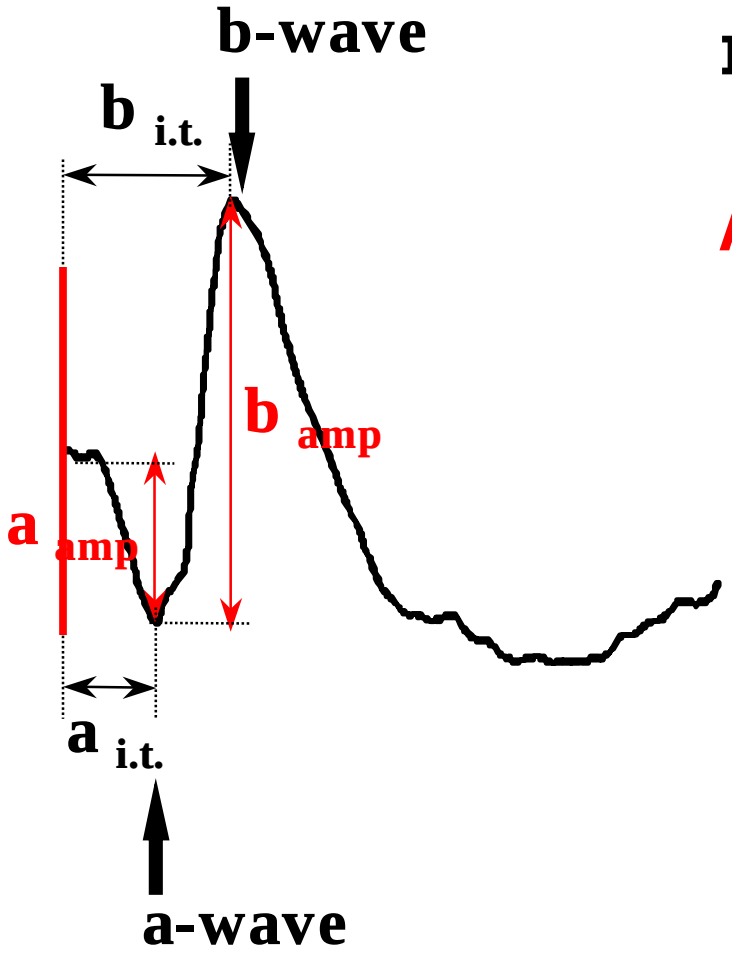


Full-field ERG
Multi-focal ERG

Full-Field Electroretinogram (ERG)

- Represents the summed activity of the retina in response to a light flash.
- Isolate responses of different retinal cells
- Dominated by extramacular rods and cones, it is most useful in patients suspected of having widespread retinal disease.
- Essential in diagnosis of numerous disorders including cone dystrophy, retinoschisis, congenital stationary night blindness, Leber congenital amaurosis, rod monochromatism, and paraneoplastic retinopathies.

Basic ERG waveform & components



Implicit time (i.t.)= time from stimulus until peak of activity

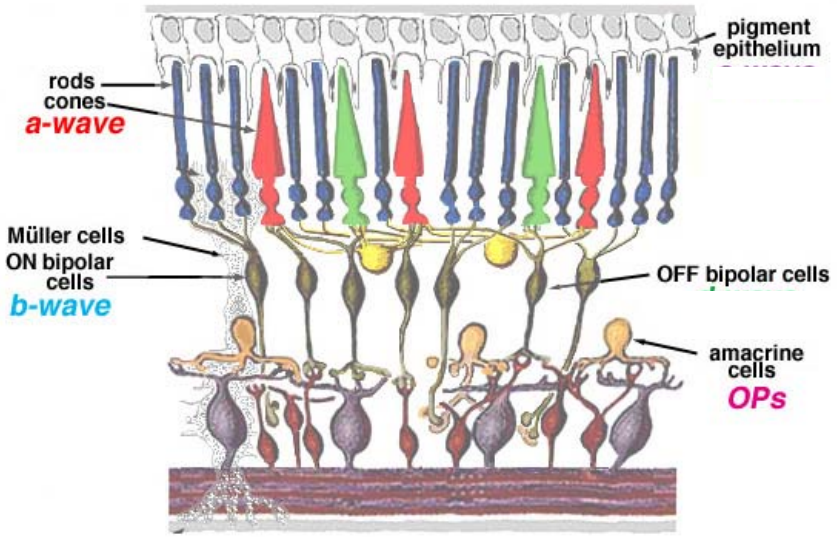
Amplitude (amp)= voltage magnitude at peak of activity

a typical response to white light contains an early, corneal-negative component (**a-wave**) and

A slower, corneal-positive component (**b-wave)**

Various Layers of the Retina

- **a-wave** is know to be generated within the photoreceptor layer and directly reflects photoreceptor activity.
- **b-wave** is generated at the level of the inner nuclear layer, probably by Muller cells, and indirectly reflects bipolar cell activity.
- **Oscillatory Potentials (OPs)** are thought to reflect activity initiated by **amacrine cells** in the inner retina (Wachtmeister& Dowling 1978).



Physiology related to ERG

- Events leading to the full-field ERG response begin at the photoreceptors, the light-detecting cells in the retinal outer layer.
- Each photoreceptor cell consists of an inner and outer segment.
- The inner segments of the photoreceptors contain the cell nuclei and make up the outer nuclear layer of the retina.
- The outer segments : - are cellular extensions contacting the retinal pigment epithelium.
  - contain intracellular membranes where initial light activation of the retina takes place.
- Light activates the light-sensitive visual pigments in the outer segment of the photoreceptors and trigger events leading to the ERG response.

Physiology

Phototransduction

- initiated by the light-activated visual pigment that decrease sodium (Na^{+}) and calcium (Ca^{2+}) ion permeability of the photoreceptor plasma membrane leading to a lower rate of release of the photoreceptor neurotransmitter, glutamate.
- In darkness, Na+ and Ca^{2+} channels of the outer segment of the rod are open, allowing Na+ and Ca^{2+} into the cell.
- The Na+ / Ca^{2+} -K^{+} (potassium) exchange pumps at the outer segment cellular membrane and a compensatory extrusion of -K^{+} at the inner segment maintain the intracellular and extracellular cation concentration.
- This circulating *dark current* maintains the rod in a relatively depolarized state.
- With light, phototranduction causes the closure of the outer segment Na+ and Ca^{2} channels, and the release of glutamate is diminished.
- Light-activated rhodopsin activates transducin that in turn activates phosphodiesterase which subsequently hydrolyzes cyclic guanosine monophosphate (cGMP).

Physiology

Phototransduction

- With a decrease in intracellular cGMP, the cellular membrane Na+ and Ca^{2+} channels close and the rate of glutamate is decrased. This hyperpolaization of the photoreceptor caused an increase of predominantly extracellular Na+ as well as Ca^{2+} . This relative increase in other retina positivity is measured indirectly at the cornea as the initial negative portion of the **ERG a-wave**.

Organization of the Retina

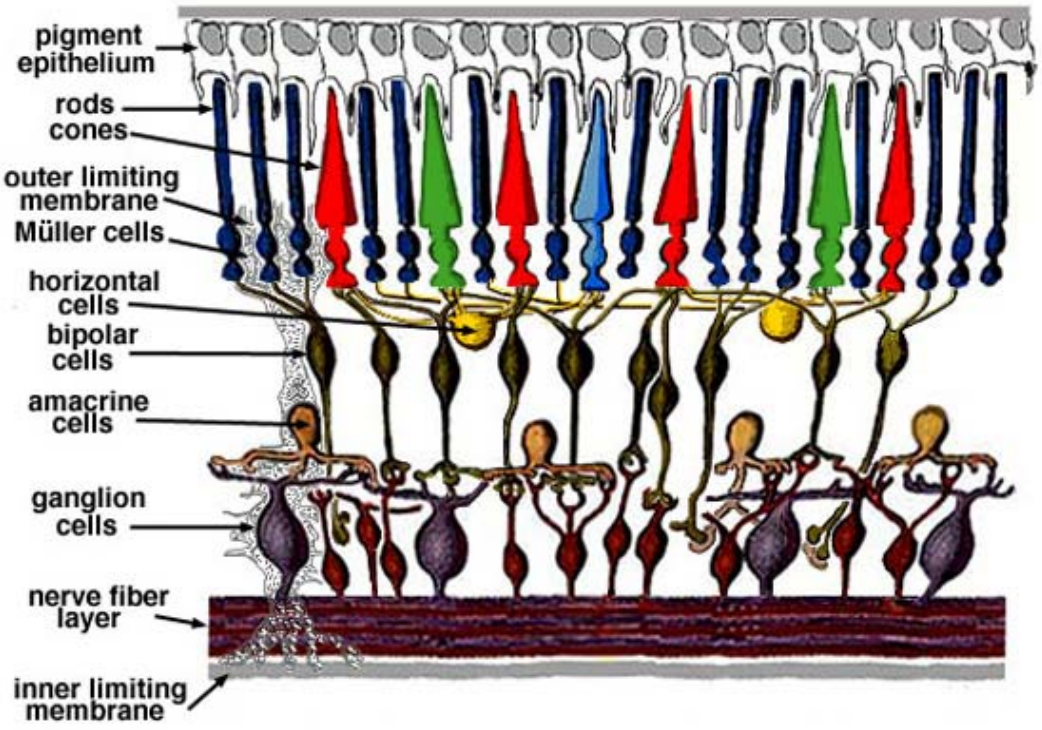
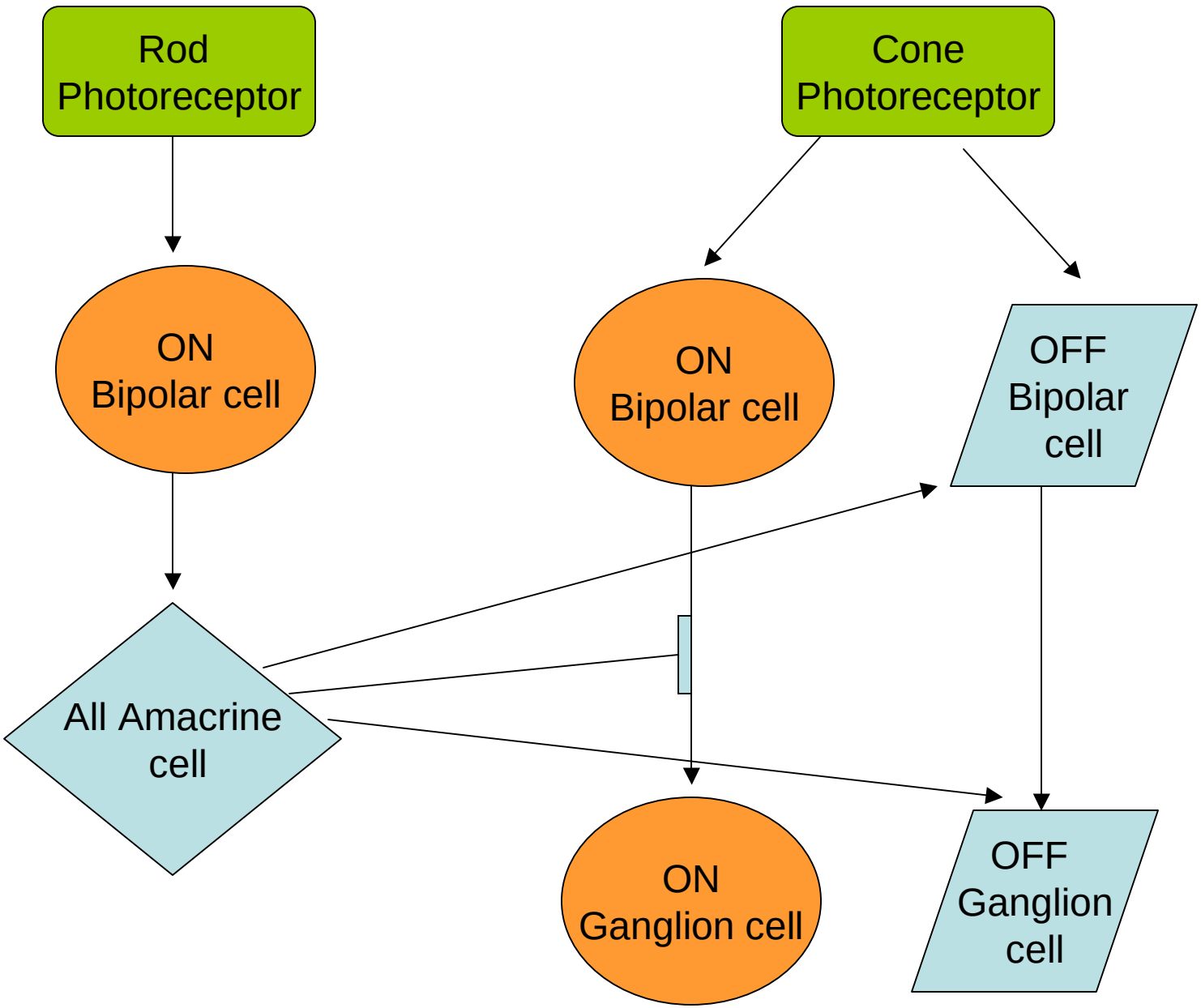


Fig. 2. Simple diagram of the organization of the retina.

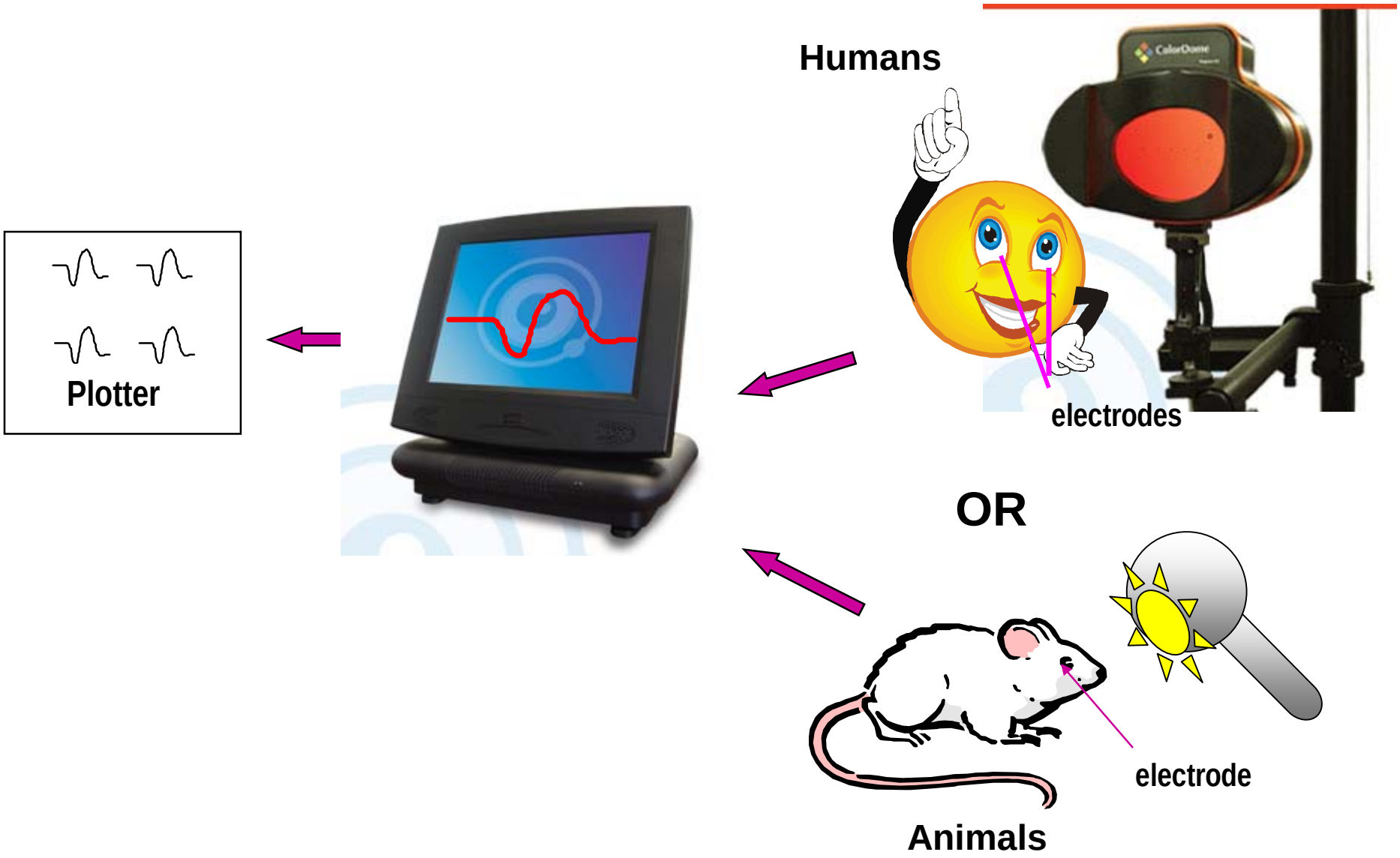

Rod Photoreceptor
Cone Photoreceptor
ON Bipolar cell
ON Bipolar cell
OFF Bipolar cell
All Amacrine cell
ON Ganglion cell
OFF Ganglion cell

Physiology

Processing photoreceptor signals in the inner retina

- The inner retinal ERG signal is a summation of all of the cells of the inner retina with primary contributions from depolarizing ON-bipolar cells and hyperpolarizing OFF-bipolar cells.
- The predominantly depolarization process results in an out flow of intracellular -K^+ in the outer plexiform layer.
- This produces a depolarization of the Müller cells generating a transretinal potential that is measured as the corneal positive **ERG b-wave**.

Recording the ERG



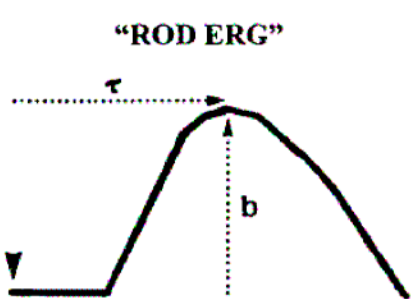
Standard Full-field ERG Protocol
ISCEV Standard

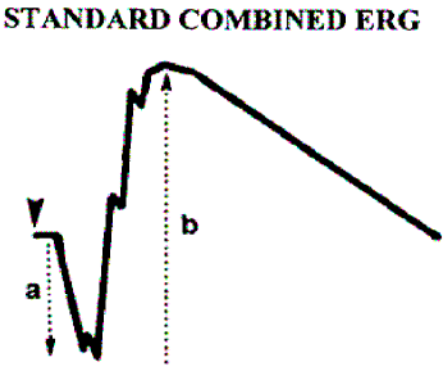
Step	Condition	Interval	Response
30 minutes Dark Adaptation			
1	Scotopic -24 dB Flash	**2** seconds	Rod Response
2	Scotopic 0 dB Flash	**10** seconds	Standard Combined Response
3	Scotopic OP	**15** seconds	Oscillatory Potentials
10 minutes Light Adaptation			
4	Photopic 0 dB Flash	**1** second	Cone Response
5	Photopic 30 Hz Flicker		Flicker Response

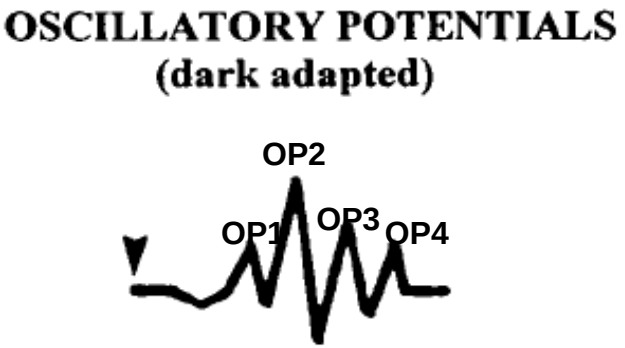
Scotopic ERG: obtained in the dark

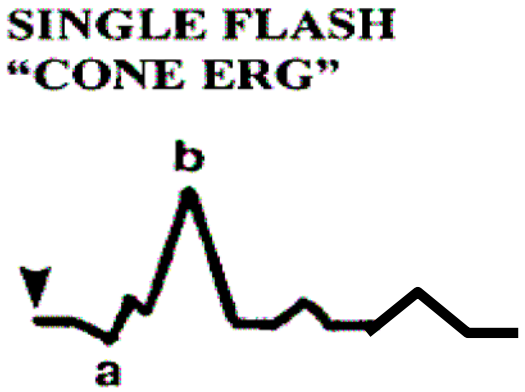
Photopic ERG: obtained in the light

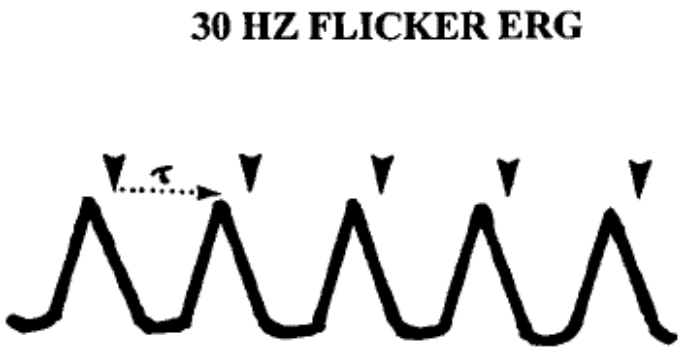
ERG Response



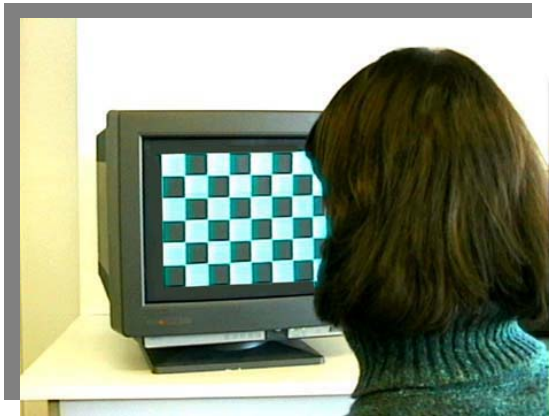








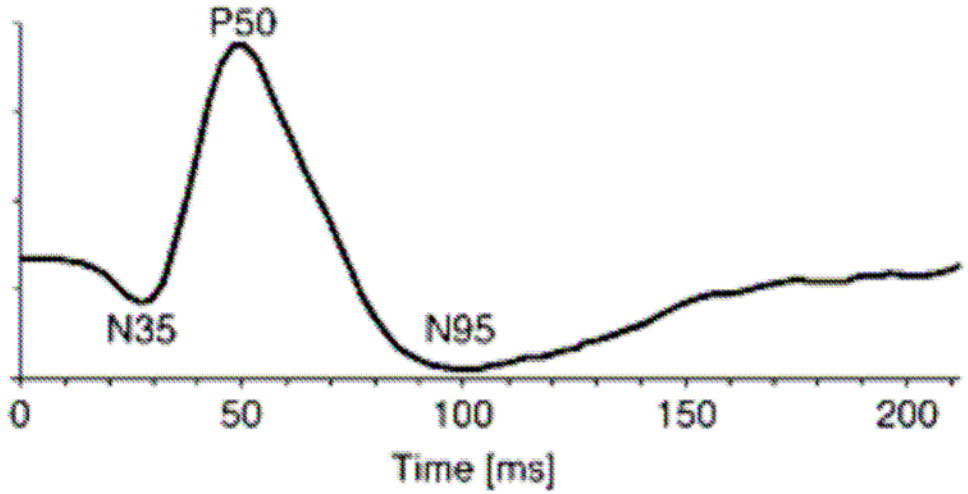
Pattern ERG (PERG)



- Retinal response evoked by viewing a pattern stimulation (usually black and white checkerboard or bar gratings)
- Provide information of the retinal ganglion cell function and function of macular.
- Clinically, the PERG can be used in a patient with an abnormal visual evoked potential to establish whether a retinal (macular) disorder is present, and thus differentiate between macular and optic nerve dysfunction as a cause for the VEP abnormality.
- It can also directly demonstrate retinal ganglion cell dysfunction.

PERG Waveform

- N35: small negative peak at 35 ms
- P50: large positive peak at 45-60ms
  - Produced by retinal ganglion cells and other retinal cellular elements
- N95: large negative peak at 90-100ms
  - Predominantly by retinal ganglion cells.

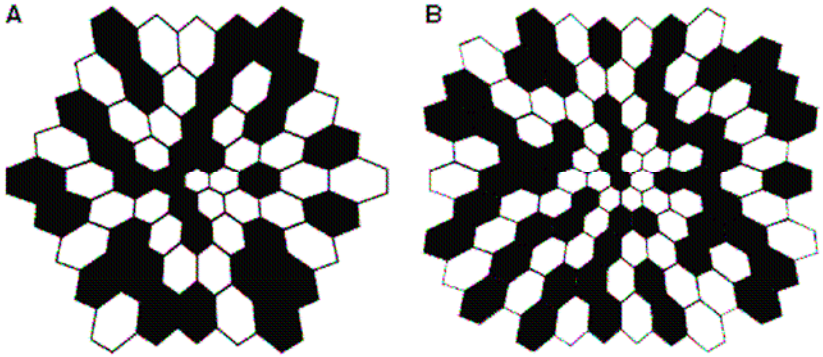


PERG Components

- Amplitudes: voltage magnitude between peaks and troughs.
  - P50: from the trough of N35 to the peak of P50
  - N95: from peak of P50 to the trough of N95.
- Implicit time: time from the onset of the contrast reversal to the peak of the component concerned

Multifocal ERG (mfERG)

- A method of recording local electrophysiological responses from different regions of the retina
- Provides a topographic measure of light-adapted (photopic) retinal electrophysiological activity
- Retina is stimulated with an array of hexagonal elements (typically 61 or 103, occasionally 241), each of which has a 50% chance of being illuminated every time the frame changes.
- Each hexagon goes through a pseudo-random sequence (the m-sequence) of black and white presentations and has the probability of 0.5 of reversing on any frame change



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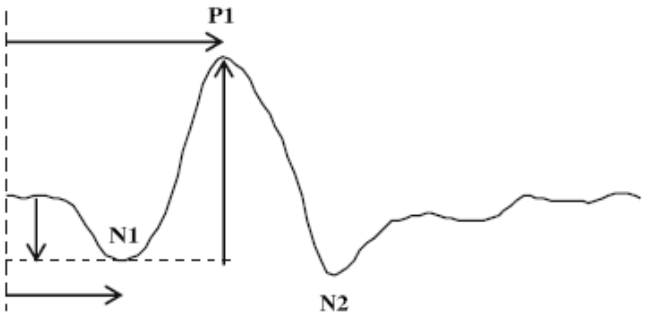
mfERG

- By correlating the continuous ERG signal with the sequence of on- and off-phases of each element, the local ERG signal is calculated.
- mfERGs are a mathematical extraction of the signal and are not direct electrical potentials from local regions of retina.
- The waveform of the local mfERG response can be influenced both by
  - preceding (adaptation effects)
  - subsequent stimuli (induced effects)
  - the response to light scattered on other retinal area.
  - Quality of fixation

mfERG Waveform

- First-order response or first-order kernel
- A biphasic wave with an initial negative deflection (**N1**) followed by a positive peak (**P1**). There is usually a second negative deflection after the positive peak (**N2**).



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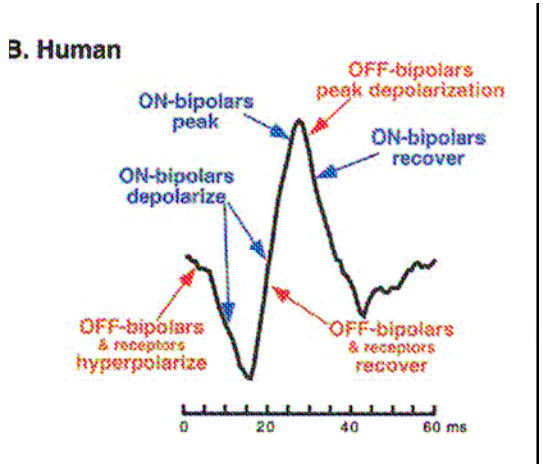
- N1- cells contribute to a-wave of full-field cone ERG
- P1- cells contribute to cone b-wave and oscillatory potentials
- mfERG responses are not “little ERG” responses. Therefore, it is inappropriate to use “a-wave” and “b-wave” to describe features of mfERG waveform.



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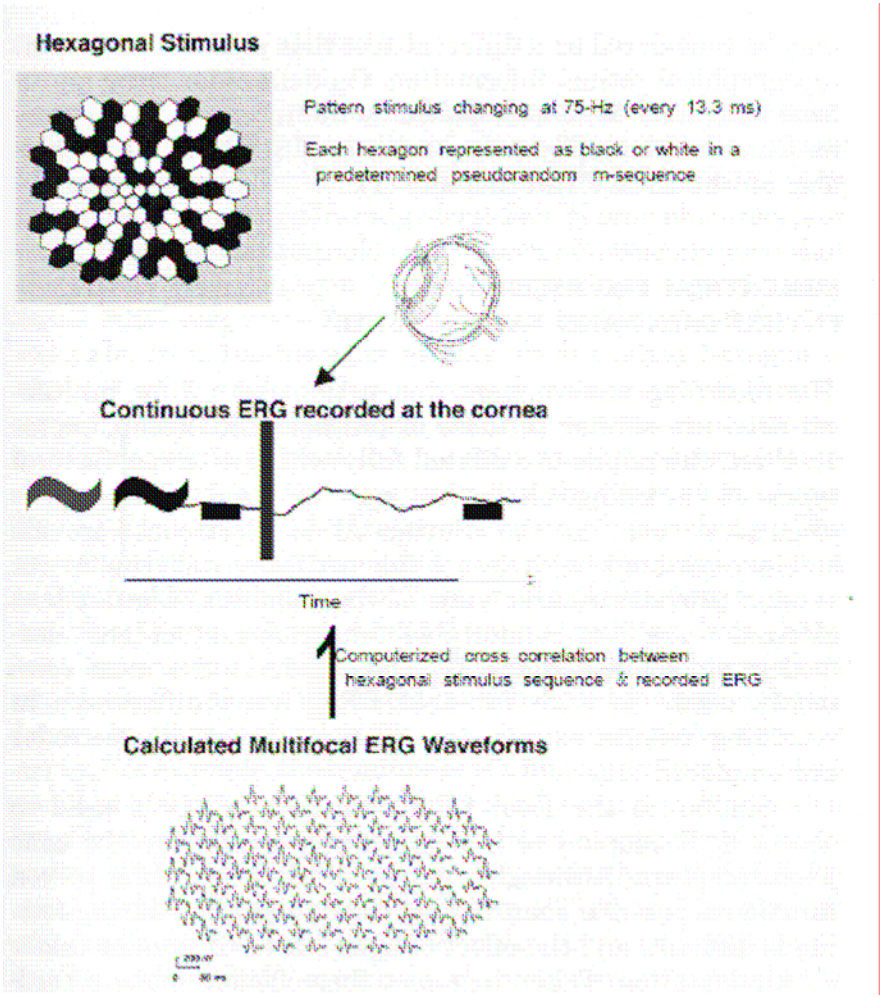
Schematic diagram (basic principles of mfERG)

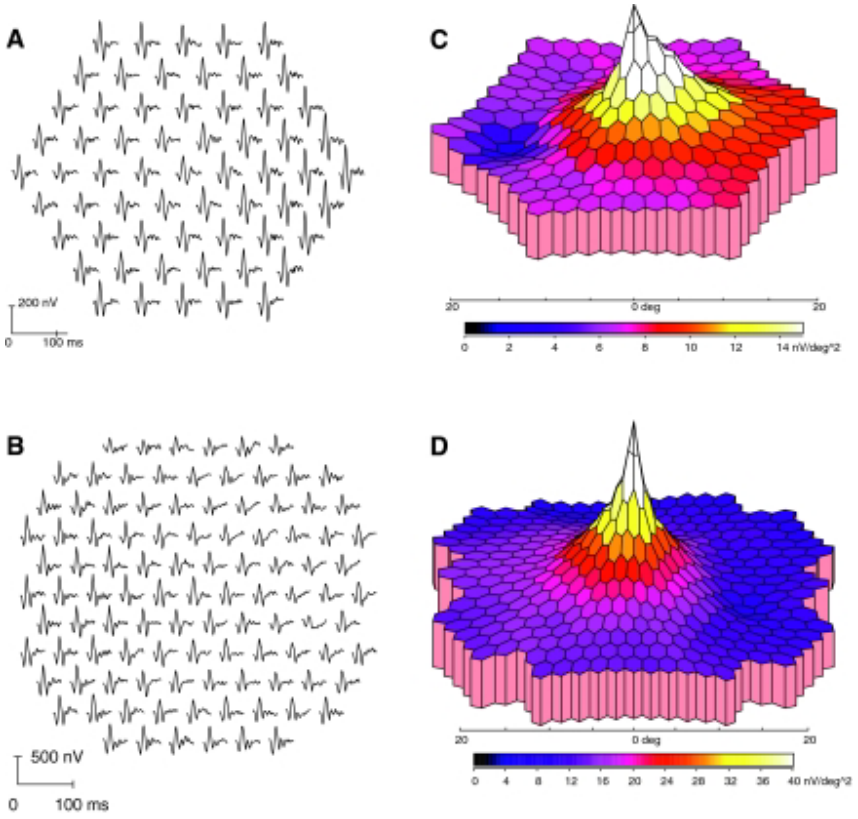


Lam B. 2005

mfERG Traces:

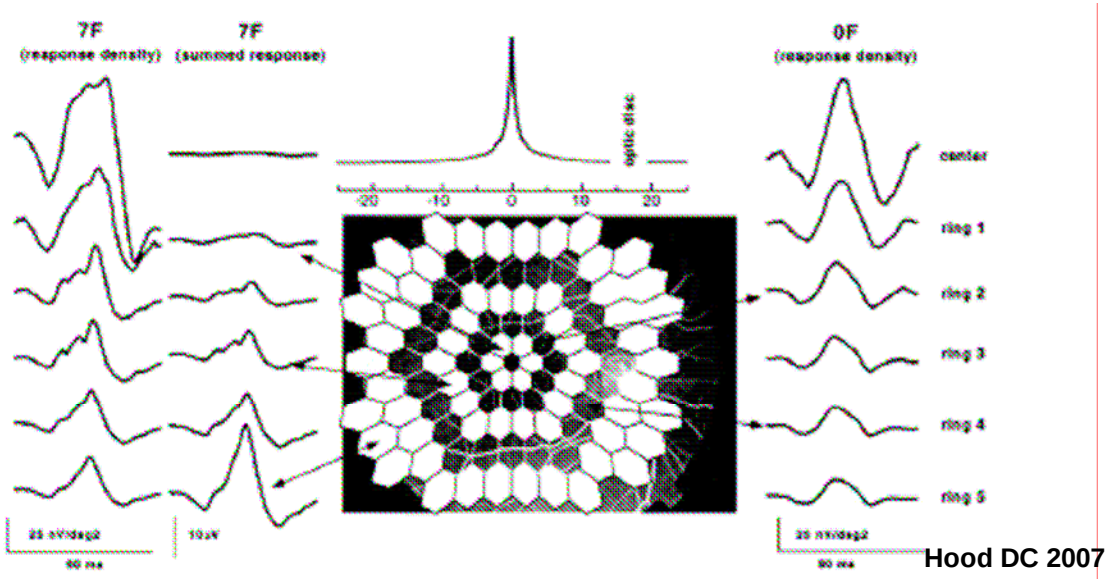
3D Response Density Plots

- Shows the overall signal strength per unit area of retina
- Advantages: for visualizing areas of abnormality and for comparing the mfERG results to visual fields from perimetry.
- Disadvantage:
  - information about the waveform is lost
  - A central peak in the 3-D plot can be seen in some records without any retinal signals
  - Highly dependent on how the local amplitude is measured.



mfERG: Group Averages

- Most commonly used display is response density in which the responses from each elements in each ring are summed and then divided by the area of the elements responses by rings (from center to peripheral)
- Helpful for comparing quadrants, hemiretinal areas, normal and abnormal regions of two eyes, or successive rings from center to periphery.



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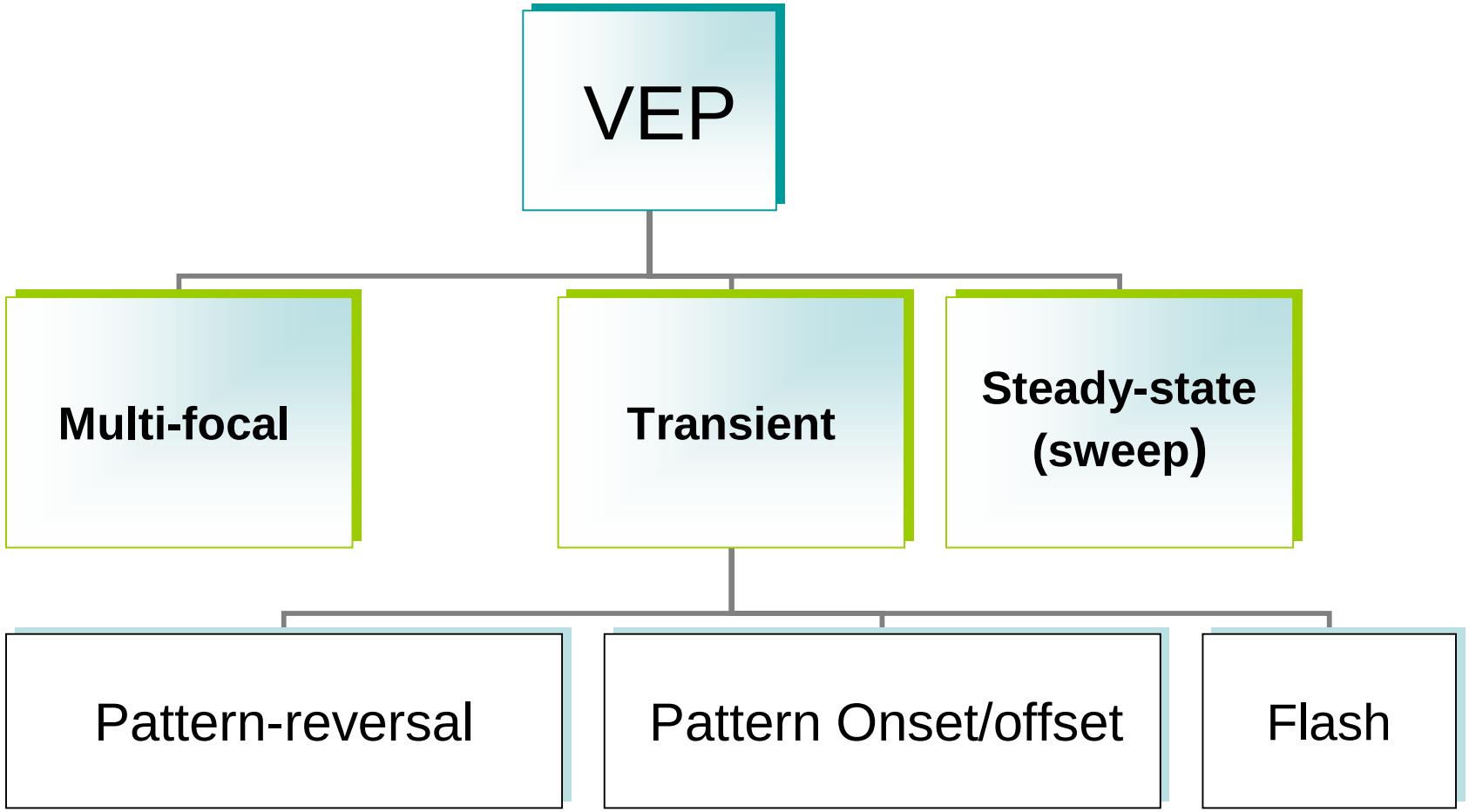
Visual Evoked Potential

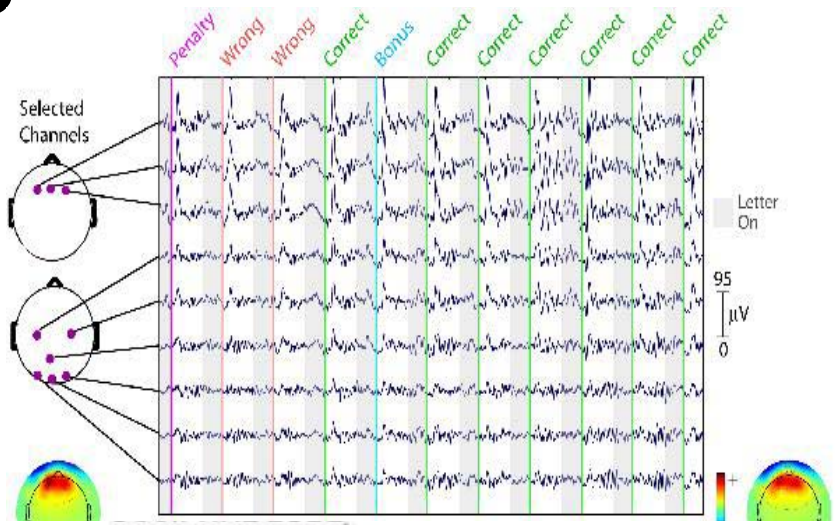


Origin of the VEP

- VEPs are visually evoked electrophysilogical signals extracted from the electroencephalographic activity (Electroencephalogramm (EEG)) in the visual cortex record from the overlying scalp in the Occipital Lobe.

- VEP is the summation of a large number of excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potentials (IPSPs) in pyramidal cells
  - EPSPs generate surface negativity
  - IPSPs generates surface positvity

- The main components of the VEP are believed to be generated in the striate cortex (Primary visual cortex, V1 or Brodmann area 17)



Striate cortex **(V1)**

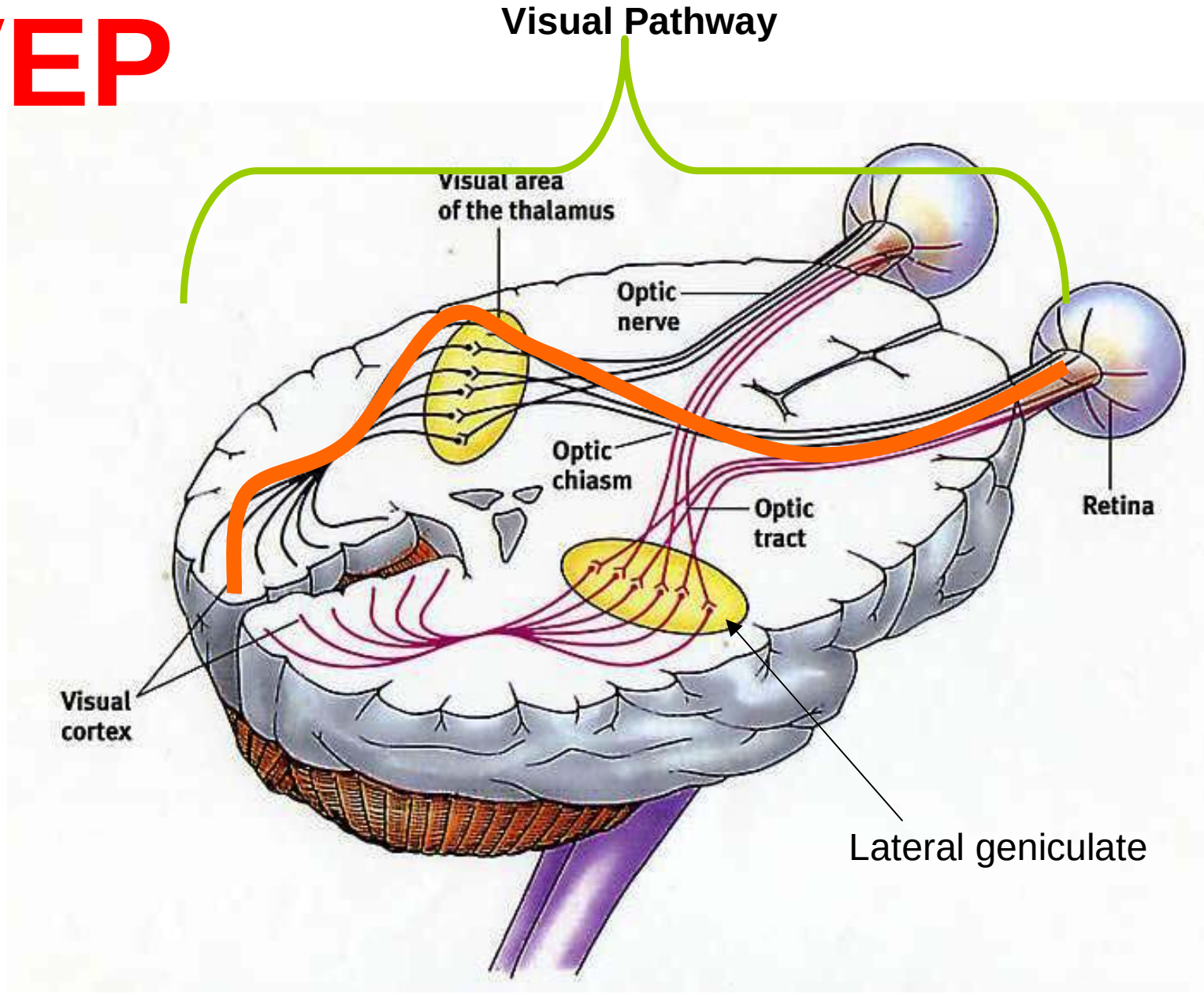
Basic Functions of V1

- Neurons in V1 respond to:
  - Orientation
  - Direction
  - Motion
  - Edges
  - Drifting grating
  - Depth
  - Color

Physiology related to VEP

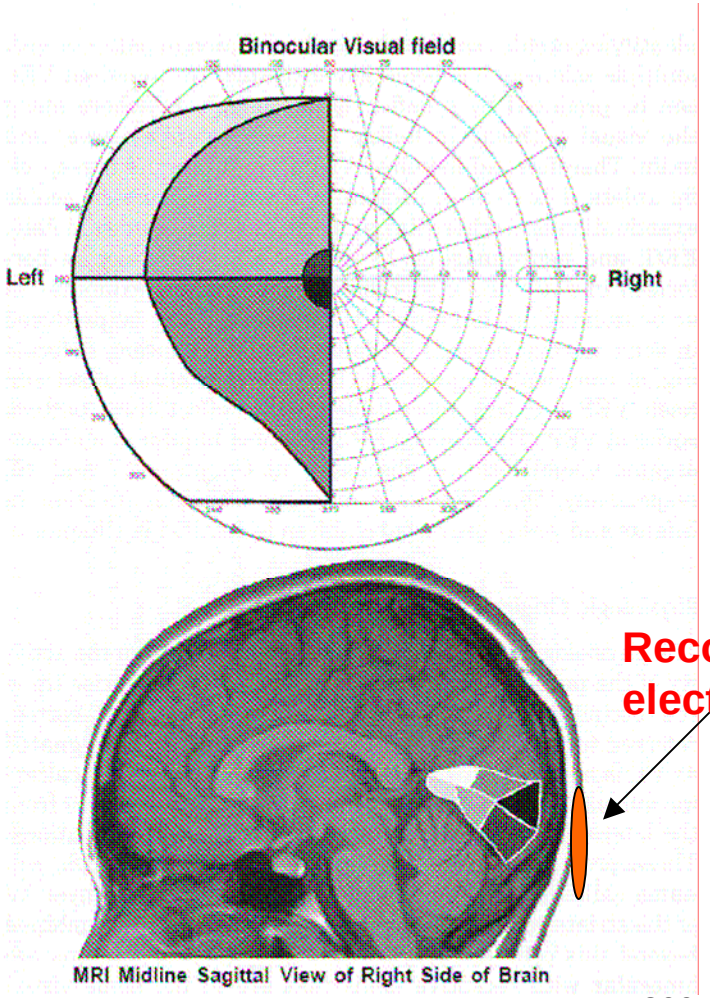
- The physiologic basis of VEP is predominantly from the activity of the primary visual cortex.
- The visual cortex receives visual projections from the lateral geniculate neurons by way of the optic radiations.
- These projections terminate in alternating eye-specific columns called ocular dominance columns of cortical layer IV of the striate cortex.
- The signals of the 2 eyes are combined in layer 4B so that most cortical neurons are binocular.
- As a consequence of the semi-decussation of the retinal ganglion cell fibers at the optic chiasm, the right hemifield is represented in the left striate cortex. In addition, the upper visual field is represented below the calcarine sulcus, and the lower visual field is represented above the calcarine sulcus.

VEP


Visual Pathway
Visual area
of the thalamus
Optic
nerve
Optic
chiasm
Optic
tract
Retina
Visual
cortex
Lateral geniculate

Visual Field Representation

- The fovea or central vision is represented disproportionately by a large cortical area occupying the posterior portion of the striate cortex, near to VEP is dominated by activity from the central visual field
- This disproportionate representation of central vision reflects the high density of photoreceptors and the high number of retinal ganglion cell projections from the fovea.
- At least 50% of the cortical neurons of the striate cortex have receptive fields in the central 10 deg of the visual field..



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Physiology related to VEP

- VEP stimulus consisting of alternating black and white checkerboard stimulate different areas of the afferent visual system.
- The cells of the retina and the lateral geniculate body respond well to a change in luminance in their receptive field
- Cortical neurons of the striate cortex respond more actively to light-dark edges and orientation of the stimulus.

VEP

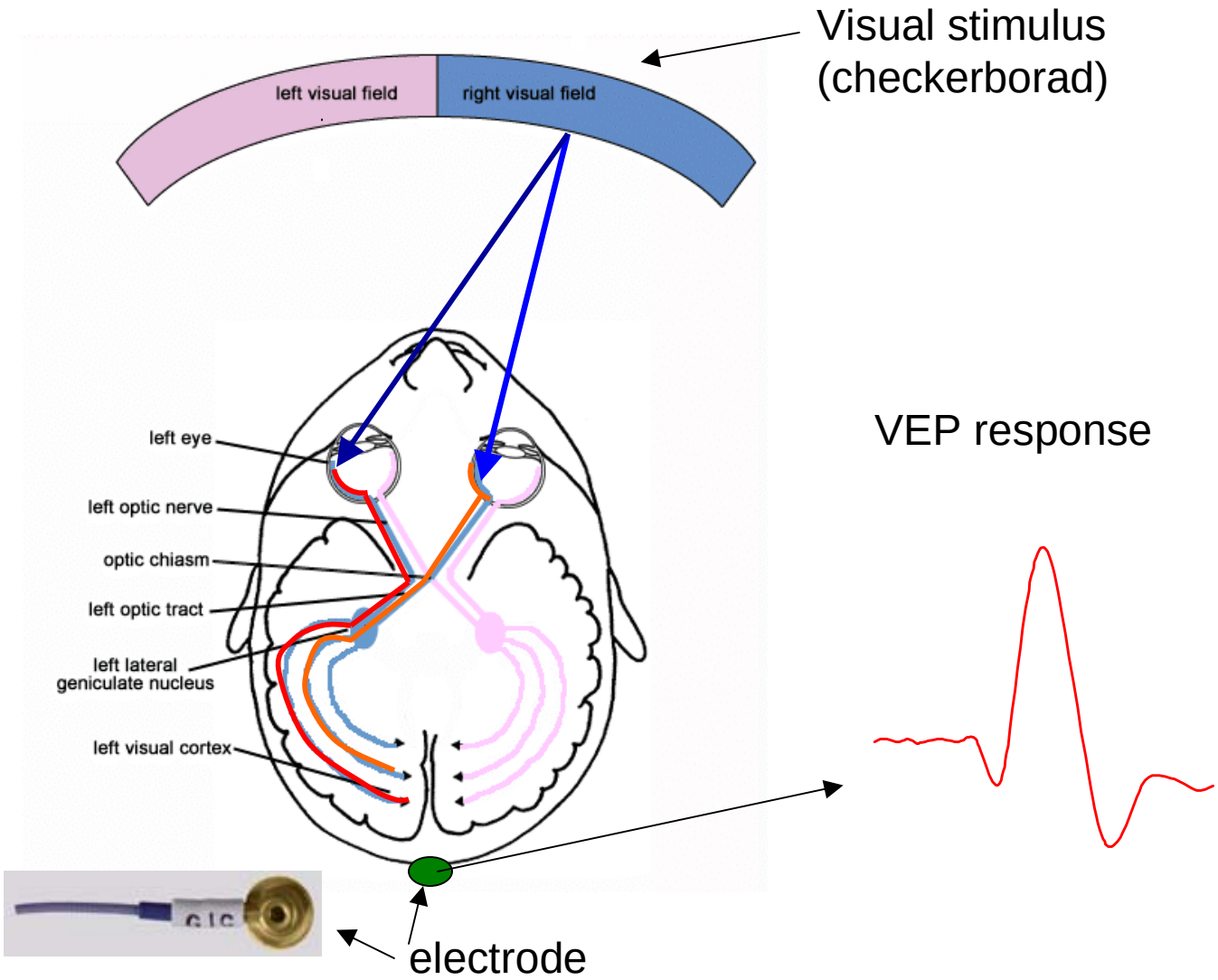
- As visual cortex is activated primarily by the central visual field, VEPs depend on functional integrity of central vision at any level of the visual pathway including the eye, retina, the optic nerve, optic radiations and occipital cortex.

- Clinical VEPs are used to
  - evaluate the integrity of the visual pathway from the retina to the occipital cortex,
  - identify the site and nature of neurological lesions
  - evaluate different aspects of visual function such as **acuity**, contrast sensitivity, and **binocular vision**

Transient vs Steady-state

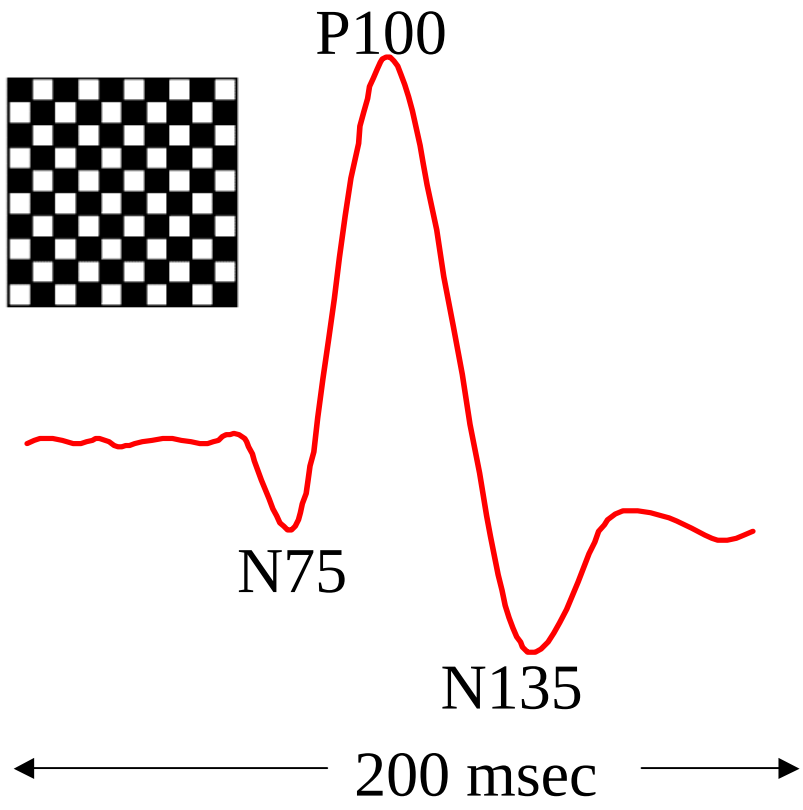
- Transient: relevant brain mechanism must return to its resting state before the next stimulus
- Steady-state: repetitive potential whose discrete frequency components remain constant in amplitude & phase over a long time period

Recording VEP



Transient: Pattern-reversal VEP

- VEP Is elicited by a checkerboard-like stimulus of alternating black and white square checks that reverse in a regular phase frequency
- Waveform consists of a N75, P100 and N135 peaks.
- P100 is usually a prominent peak that used for analysis.
- P100 shows relatively little variation between subjects, minimal within subject interocular difference, and minimal variation with repeated measurement over time.
- P100 peak time is affected by non-pathophyiologic parameters such as pattern size, pattern contrast, mean luminance, signal filtering, patient age, refractive error, poor fixation and miosis.



Transient: Onset/offset VEP

•VEPs are elicited by checkerboard pattern abruptly exchanged with a diffuse gray background.

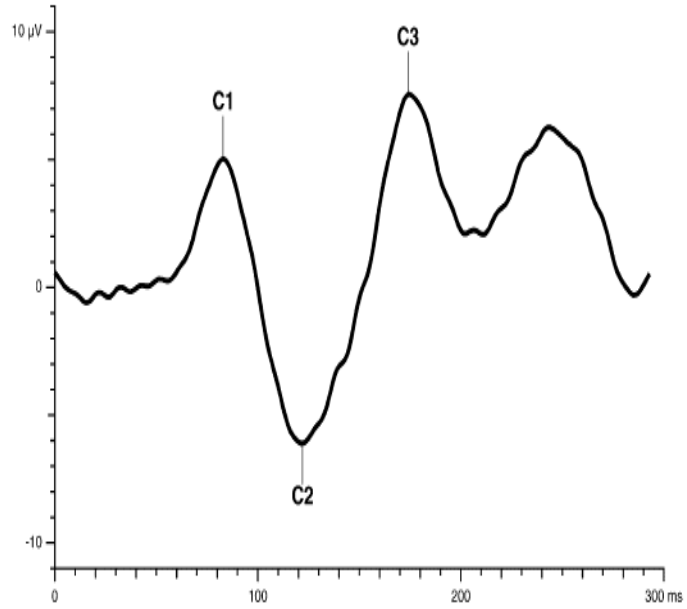
•This stimulus is best suited for the detection/confirmation of malingering and for use in patients with nystagmus.

•Less sensitive to confounding factors such as poor fixation, eye movements or deliberate defocus.

• Response consists of

- •C1 (positive ~75 ms)
- •C2 (negative ~ 125 ms)
- •C3 (positivee ~ 150 ms)

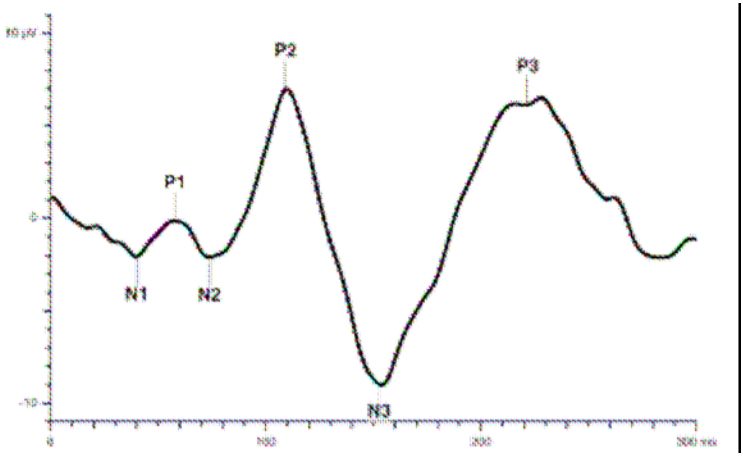
Amplitudes are measured from the preceding negative peak.



Transient: Flash VEP

•VEP is elicited by a flash stimulus

•Useful to test uncooperative children, poor fixation due to poor visual acuity, and when optical factors such as media opacities prevent the valid use of pattern stimuli.

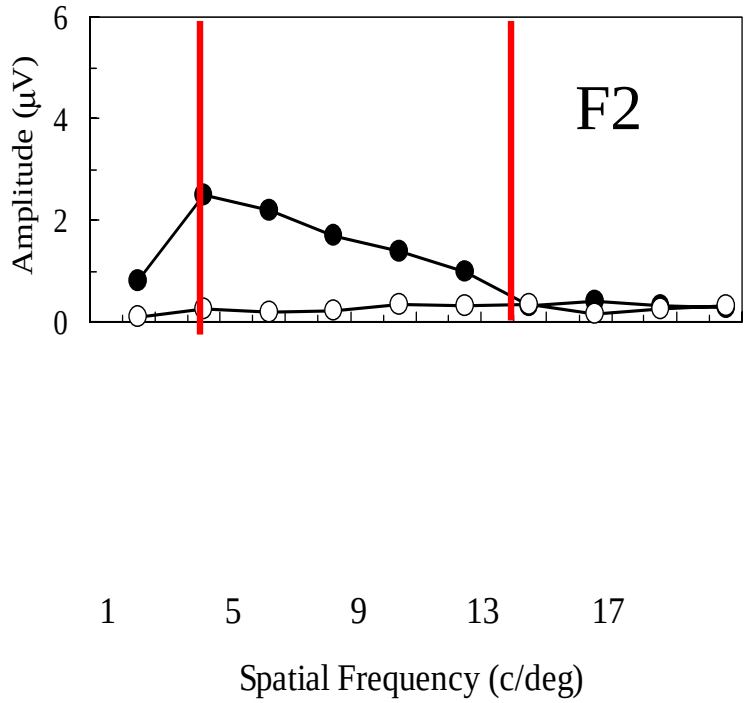


•Waveform consists of a series of negative and positive peak . The most robust components are the N2 and P2 peaks.

• Flash VEP reflects the activity of striate and extrastriate cortex but does not provide an assessment of visual function

Steady-state: Sweep VEP

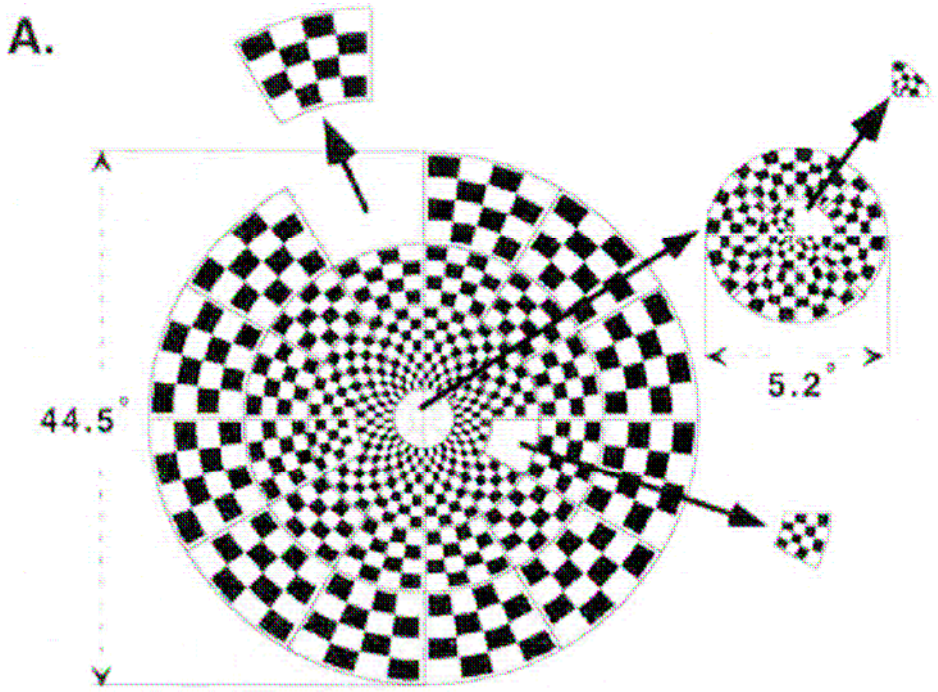
- Useful for assessing visual acuity especially for infants.
- Patients are presented with 10 to 20 pattern-reversal stimuli ranging from coarse to fine in rapid succession during a 10-second test period.
- Visual acuity are estimated by extrapolation of the high signal-to-noise portion of the curve to zero amplitude



Multifocal VEP (mfVEP)

- Electrophysilogical test that provides local VEP response from the visual field
- Multiple individual VEP responses are generated simultaneously from 60 or so regions of the central 20 to 25 deg radius of the visual field.,

• From a single, continuous EEG signal, a sophisticated mathematical algorithm extracts the VEP response generated by each of the independent regions.

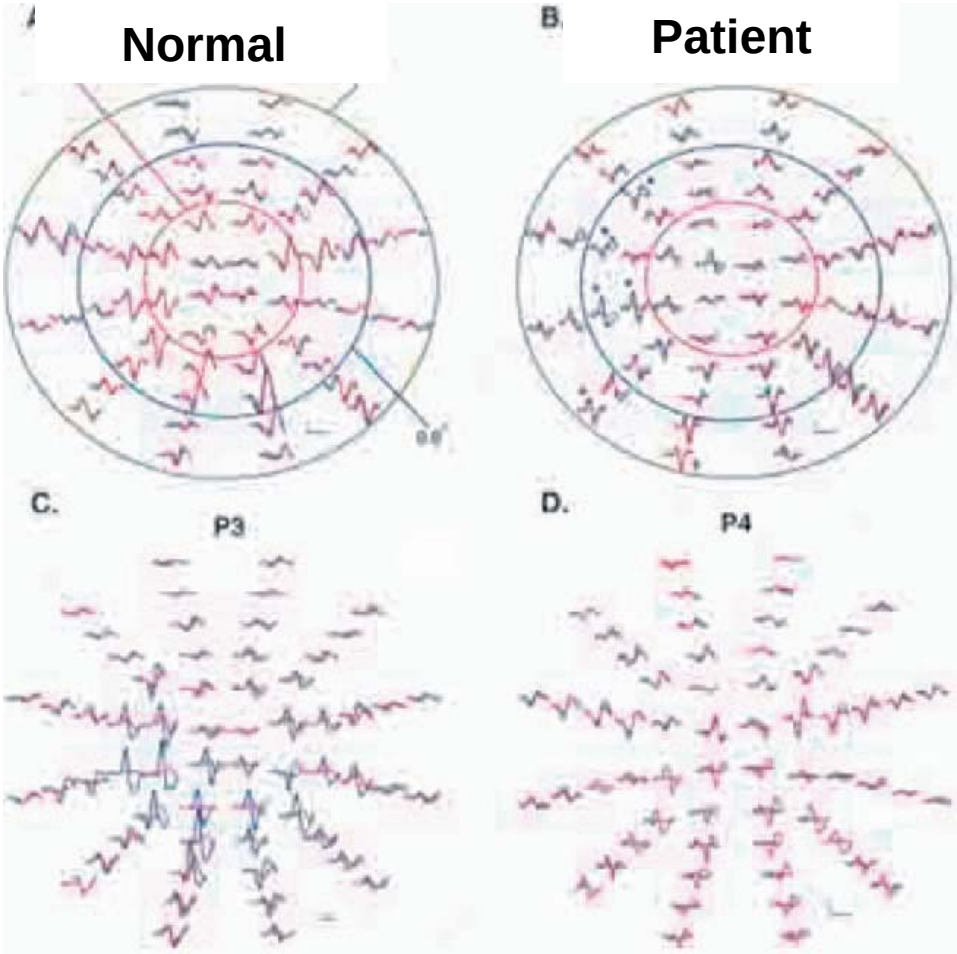


mfVEP

- To assess local defects in the visual field
- The display covers about the same retinal area as the 24-2 Humphrey visual field (HVF).
- To detect small abnormalities in visual signal transmission from centric and eccentric field and to provide a topographical display of these deficits.
- Ruling out non-organic visual loss, diagnosing and following patients with optic neuritis/multiple sclerosis, evaluating patients with unreliable or questionable HVF, and following disease progression
- When combined with the mfERG, diseases of the outer retina (before the retinal ganglion cells) can be distinguished from diseases of ganglion cells and/or optic nerve.

mfVEP response

- mfVEP response bears a superficial resemblance to the pattern-reversal VEP
- in the responses from the lower field, there is an initial negative component (**C1**) around 65 ms followed by a prominent positive component (**C2**) around 95 ms, analogous to the N75 and P100 of pattern VEP.

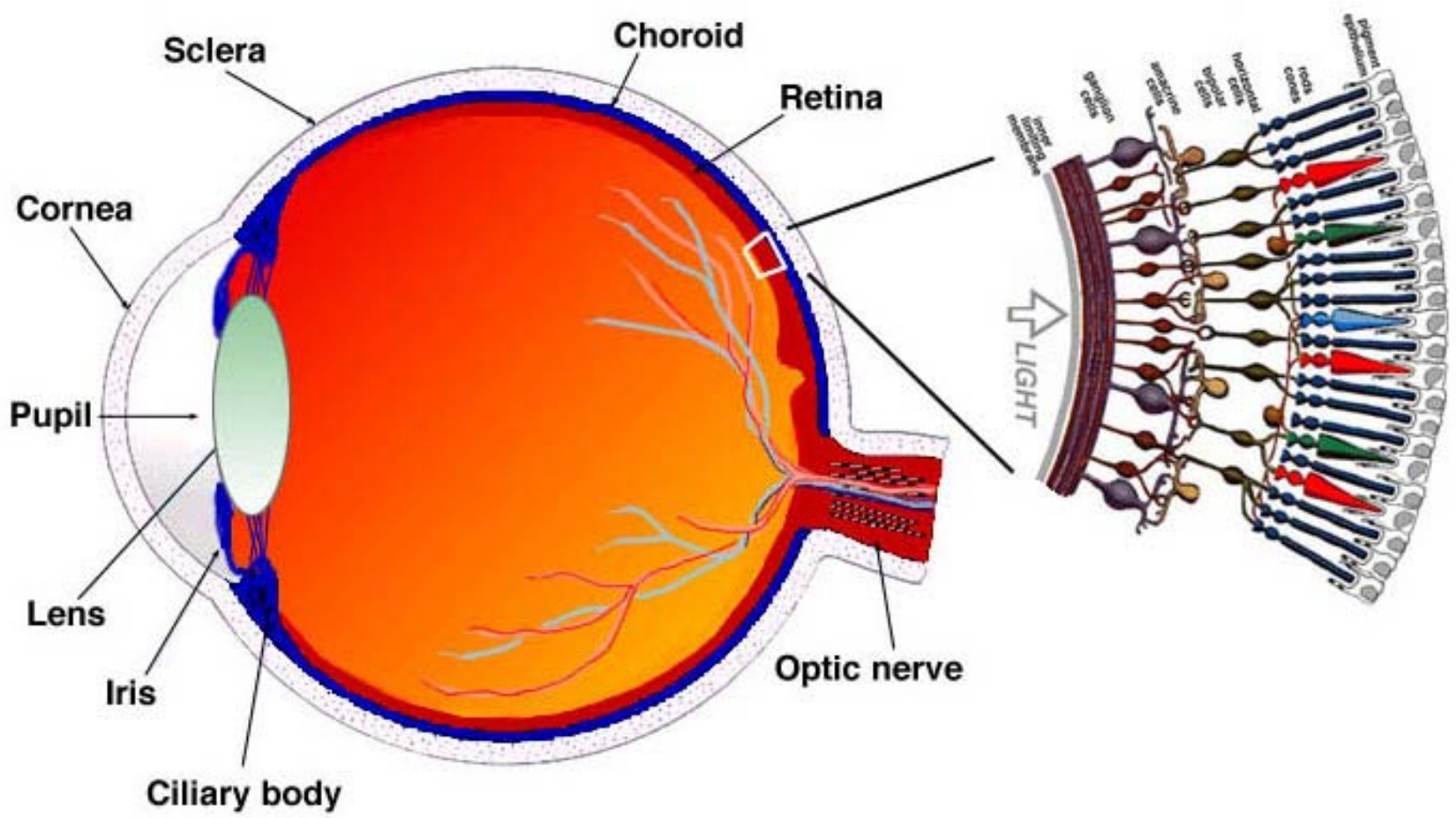


EOG

- EOG is an electrophysiological test of function of the outer retina and retinal pigment eipithelium (RPE) in which the change in the electrical potential between the cornea and the ocular fundus recorded during successive periods of dark and light adaptation.

EOG

Pigment epithelium

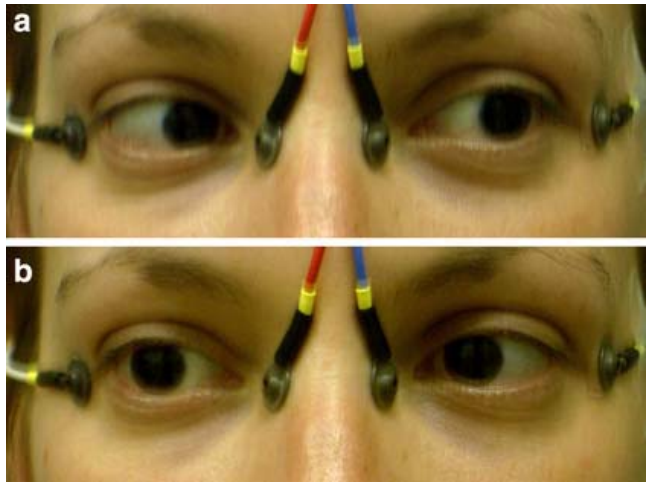


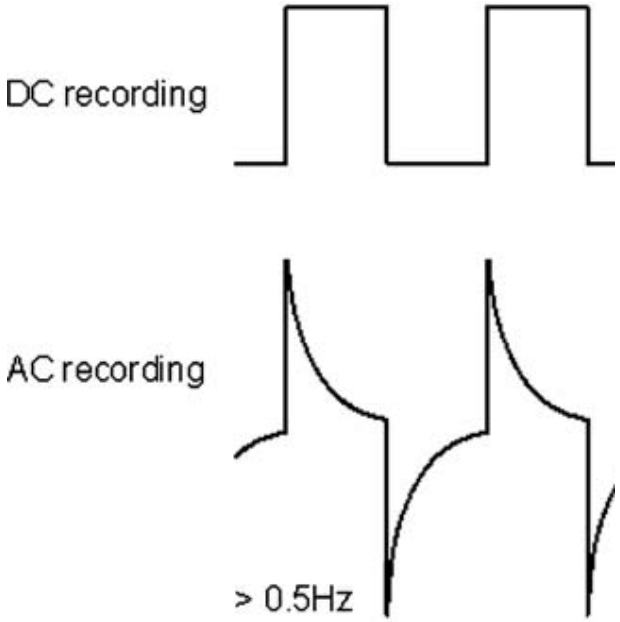
EOG Origins

- The eye has a standing electrical potential between front and back (comeo-fundal potential).
- The potential is mainly derived from RPE, and it changes in response to retinal illumination.
- The potential produces a dipole field with the cornea approximately 5 millivolts positive compared to the back of the eye, in a normally illuminated room.
- The potential decreases for 8-10 min in darkness. Subsequent retinal illumination causes an initial fall in the standing potential over 60-75 seconds (fast oscillation).
- These phenomena arise from ion permeability changes across the basal RPE membrane.

EOG Measurement

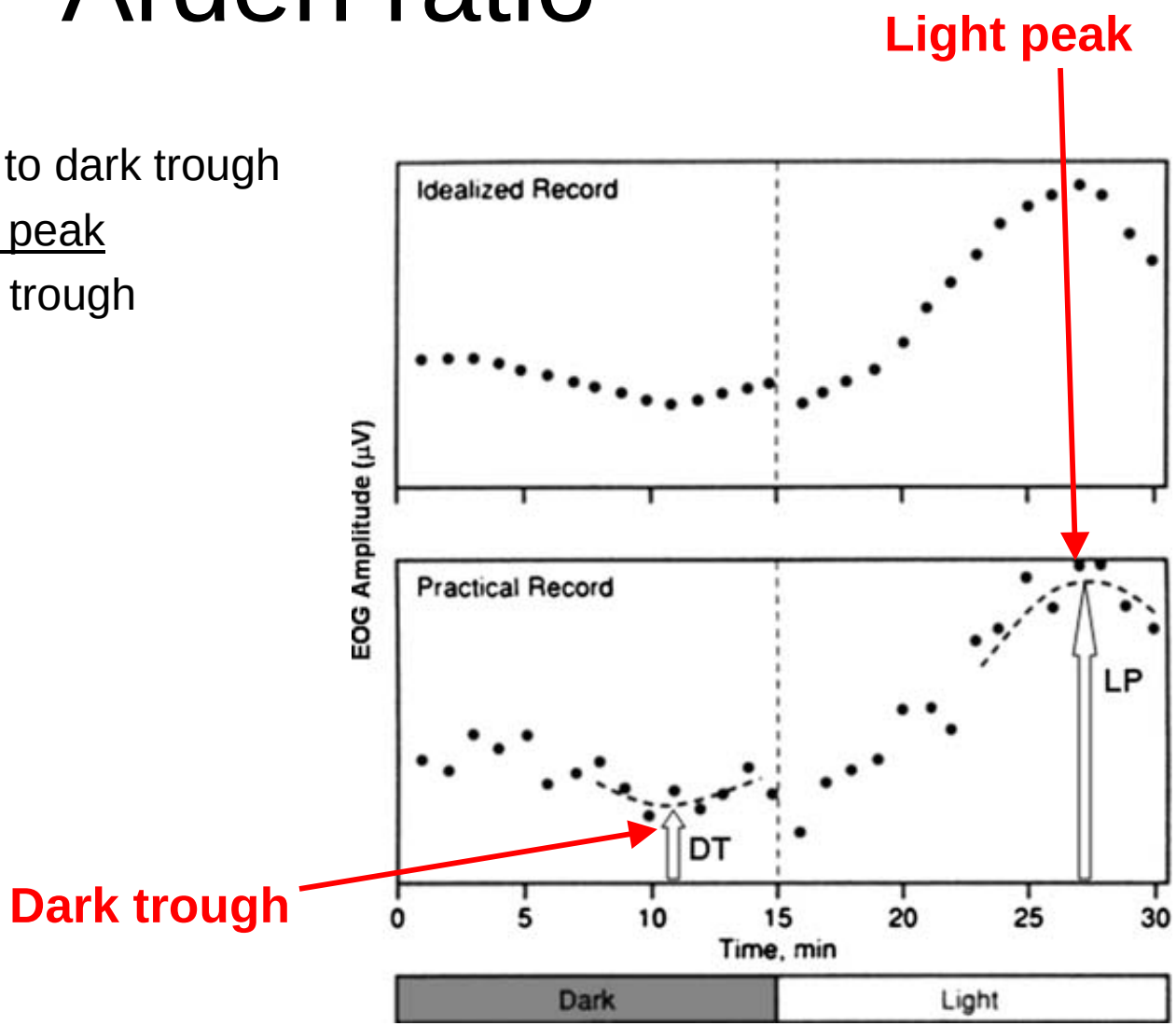
- The potential across the RPE causes the front of the eye to be electrically positive compared to the back.
- Movement of the eye produces a shift of this electrical potential approximately 5 millivolts between the electrodes on each side of the eye,
- By attaching skin electrodes on both sides of an eye the potential can be measured by having the subject move his or her eyes horizontally.
- The potential recorded from the skin electrodes resembles a square wave whose amplitude will be a fixed proportion of the corneo-fundal potential.
- During a light/dark cycle, this indirectly measured potentials will change in the same way as the source potentials, so the Arden ratios will be a close approximation to the average changes occurring across the RPE.





Arden ratio

- A ratio of light peak to dark trough
- Arden index = $\frac{\text{Light peak}}{\text{Dark trough}}$



Who are the Clients of an Electrophysiological Service?

- Patients whose eyes have:
  - symptoms suggestive of known neurological or ophthalmological disease
  - unexplained visual loss
    - medico-legal problems
    - workman's compensation
    - defects associated with psychiatric disturbance, mental or physical handicap
  - pediatric neuro-ophthalmic practice
    - metabolic, hereditary or neurological disease with visual impairment
    - non-seeing child

Who are the Clients of an Electrodiagnostic Service?

- Patients with media opacities
  - prior to corneal grafting or cataract surgery
  - vitreous hemorrhage (in DR or following trauma, RD)
- Patients with uveitis or inflammatory eye disease
- Patients with suspected disease or carrier status of inherited visual disorders
- Patients requiring quantitative assessment of disease progression
- Assessment of retinal and optic nerve function following trauma

Indications for ERG

- aid to diagnosis in patients suspected of having stationary or progressive inherited retinal degenerations
  - *to rule out clinically unaffected family members*
  - *to evaluate possible female carriers of x-linked disease*
- to assess patients with retinal toxicity due to metallosis (siderosis)
- retinotoxic or neurotoxic medications
  - chloroquine, Hydroxychloroquine, ethambutol, phenothiazine
  - Desferrioxamine, Tamoxifen
  - Vigabatrin toxicity?
- patients with OHT/glaucoma
- to identify eyes that are likely to develop neovascularization in diseases such as CRVO and DR
- documenting therapeutic effects of surgery or medication

Indications for VEP

- **Non-organic visual loss**:
  - Hyperopia, myopia, myopic retinal degeneration, amblyopia
  - Cataract and media opacities
  - Retinal detachment
  - Pigment dispersion syndrome

- **Optic neuropathies**:
  - Glaucoma
  - Optic neuritis/multiple sclerosis
  - Ischemic optic neuropathy
  - Papilledema
  - Compression of optic nerve or chiasm
  - Traumatic optic neruopathy
  - Optic nerve head drusen
  - Herediatory optic neuropathies

- **Systemic disorders**:
  - Albinism

- **Central nervous system disorders**:
  - Cortical blindness
  - Friedreich ataxia
  - Alzheimer Disease
  - Parkinson Disease

Indicators for EOG

- Pigment dispersion syndrome
- Best's vitelliform macular dystrophy and variants of this disease