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Lecture 6: How Do Photoreceptors Work?

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CSC 259/459, Fall 2024 Computer Imaging & Graphics

Logistics

Programming assignment 1 is due 9/25, 11:30 AM

• Can work in groups of 2.

Project proposal due 10/16 11:30 AM.

• I've put up a list of ideas. In the past, many choose to implement some concepts

- discussed in the class.
- Can work in groups of 2.

Digital Camera Imaging

Color in Nature, Arts, & Tech (a.k.a., the birth, life, and death of light)

The Roadmap

Theoretical Preliminaries

Human Visual Systems

Photoreceptors Introduction and Overview Light and Dark Adaptation Retinal Representations Color Vision and Colorimetry

Modeling and Rendering

Applications

Why Study Photoreceptors?

- First step in seeing; sets the limit of our vision.
- A great example of how different levels of abstraction are connected.
	- Why and why don't certain physiological results match behavioral results?

Recall: Photoreceptors on the Retina *500* **C.A. CURCIO ET AL.**

state of all profiles in a crucio et al. 1990 state in B and profiles in B and \sim 5 brane is the discontinuous dark line passing through the letters A and *C.* Tissue shown in **A** and **C** is from 2-fim-thick glycol methacrylate sections 10 um

Anatomical Structure

 (a) Rod Rod photpigment Outer segment **411** Inner segment Synaptic ending Light imaged from

cornea and lens

Light imaged from cornea and lens

* dimensions for a fovea cone; from Polyak 1941

Wandell, 1995 6

Counting Photons (Principle of Univariance)

A Simplified Functional View of Photoreceptors

- Each photoreceptor is a bucket that collects photons.
-
- Many photons that enter the eye don't hit any bucket (absorbed by lens and macular pigments)
- Any photon that hits a bucket has a probability of being absorbed.
	- The probability $p(\lambda)$ is wavelength-specific and varies between photoreceptor types

A Simplified Functional View of Photoreceptors

- Once a photon is absorbed, it has a probability of exciting the photoreceptor
	- Excitation means generating an electrical response, also called isomerization or pigment bleach
	- This probability is called the Quantum Efficiency of the photoreceptor (Qe), and is generally constant in the visible spectrum

- Excitation probability of a single photon: $p(\lambda)$ Qe
- - Usually we ignore Qe since it's a constant and just talk about absorption

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Principle of Univariance

• For each photoreceptor type:

• The electrical response caused by a pigment excitation is constant regardless of the photon's wavelength.

• Each photon generating an electrical response has the same effect as any other photon that does so.

• The only effect that wavelength has is to impact the probability a photon gets absorbed; after absorption the wavelength information is lost.

Principle of Univariance

- As if each photoreceptor is counting excitations
-
- If two photoreceptors (of the same type) have the same "counter value", their electrical responses are the same.
	- It does not mean the electrical response is proportional to the number of excitations; more later.
		-

Measuring Spectral Absorption

- Single photon absorption probability is the percentage of photons absorbed in a flux, which we can measure
- Two general methods, one direct and the other indirect
	- Direct: Microspectrophotometry
	- Indirect: Measuring electrical responses (using suction electrode)

Modeling Pure Absorption (Ignore Back Scattering)

13 Optical Models for Direct Volume Rendering, Nelson Max 1995

Concentration (# of pigments / unit volume) Consider a very thin slice of a photoreceptor (so thin that no pigments are covering each other)

Total area covered by the pigments

Percentage of area occluded by pigments

Modeling Pure Absorption

14 Optical Models for Direct Volume Rendering, Nelson Max 1995

Percentage of absorbed photons

Consider a very thin slice of a photoreceptor (so thin that no pigments are covering each other)

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Beer-Lambert Law

$$
\frac{dI}{dl} = -cI\epsilon
$$
\n
$$
\int_{T} = Ie^{-cI\epsilon}
$$
\nIf density is uniform

 $I_T = Ie^{-\int}$ *l* If density is non-uniform

"*The person credited with a scientific law is almost certainly not the one who discovered it.*" [Bouguer](https://en.wikipedia.org/wiki/Pierre_Bouguer) was the first to discover this law.

As *s* increases (light travel farther), the intensity decreases exponentially!

Beer-Lambert Law

16 https://en.wikipedia.org/wiki/Beer%E2%80%93Lambert_law

Exponential decay happens when a quantity decreases at a rate proportional to its current value.

• Opposite of exponential growth.

Exponential decay means lights are hard to be completely absorbed.

• 10% of light @ 70 m deep under ocean, but you can still see lights at 850 m deep.

Many real-world scenarios exhibit exponential growth/decay.

Exponential Decay

 $I_T = Ie^{-cle}$

Transmittance, Absorptance, Absorbance

Concentration

Absorptance *(fraction absorbed)*

$$
T = \frac{I_T}{I} = e^{-cI\epsilon}
$$

$$
p=1-T=1-e^{-c l \epsilon}
$$

 $A = -\log(T) = cl\epsilon$

Transmittance −*clϵ* (fraction transmitted)

 $I_T = Ie^{-c l \epsilon}$ Optical length (Unit: m)

Absorbance (Optical Density)

(Unit: 1/m3) section area (Unit: m2) Proportional to cross-

(Normalized) Absorbance Spectra

L

5.0

 nm

- Absorbance is usually normalized to peak at unity
- MSP results showed three distinct cone spectra, providing the first physiological evidence for the existence of three cones
	- L(nog), M(edium), S(hort)

George Wald (Nobel prize, 1967)

Complication: Transverse vs. Axial Illumination

green exciting light single rod cell in the electrode retina explant

(b)

Wandell, 1995; Principle of Neurobiology 1ed (p. 123); <u>http://www.cvrl.org/database/text/outseg/length.htm</u> 20

Electrical Responses From Suction Electrode

 (A)

green exciting light

single rod cell in the electrode

> retina explant

Suction Electrode

- Electrical response (photovoltage) vs. flash intensity at two wavelengths.
- The responses at 644 nm and 539 nm are the same, but the light intensity (# of photons) at 539 nm is 18.6 times higher than that at 644 nm.
- It must mean the absorptance at 539 nm is 5.3% (1/18.6) of that at 644 nm — according to the Principle of Univariance.

Electrical Responses vs. Light Intensity vertical coincidence. All three responses have the same shape, as would be expected in the effect of absorbed individual responses. The effect of absorbed \sim independent of wave-length. The constants by which the intensity which the intensity were constants by which t
The intensity were constants by which the intensity were constants by which the intensity were constants by wh

Baylor et al. 1987; Baylor and Hodgkin, 1973 23

Spectral Sensitivity From Electrical Responses vertical coincidence. All three responses have the same shape, as would be expected in the effect of absorbed in the effect of absorbed ${\sf E}$ independent of wave-length. The constants by which the intensities were constants by which the intensity were c
The intensity were constants by which the intensity were constants by which the intensity were constants by wh **EIECTRICAL RESponses**

Baylor et al. 1987 24

Spectral Sensitivity From Electrical Responses

- The spectral sensitivities are relative
	- We can only tell absorptance of A nm is 5% of that of B nm, but we don't know absolute fraction absorbed at A/B.
	- So the results are equivalent to the *normalized* absorptance spectra measured from MSP, which can give us *absolute* absorptance.
	- 1-e^(-A) \sim = A, so normalized absorbance can be approximated by normalized absorptance.

Cone Fundamentals

Spectral Sensitivity Measured from Cornea

- So we have focused on absorption by just the photoreceptors
- But for a flux of photons enter the eye, they are absorbed by other media before reaching the retina
	- They are called pre-receptoral filters
- There are two main pre-receptoral filters, optics and macular pigments

Absorption by Ocular Media *Number* 6

Fig. 7. Total transmittance through entire eye.

Absorption by Macular Pigments

Wavelength (nm)

• Macular pigments

- Concentrated in a small area inside the fovea (so don't affect rod vision much, and the results at periphery will be different)
- Believe to exist to counter optical aberrations and protect retina from light damage

Cone Fundamentals (Cornea-Referred Spectral Sensitivity)

 $E(\lambda) = l(\lambda)m(\lambda)p(\lambda)$

Ocular transmittance

Macular transmittance

Photoreceptor absorptance

29 Wandell et al., 2022

Cone Fundamentals From Psychophysics

- For each photoreceptor type and frequency:
	- Adjust the intensity of the test light so that it's just noticeable from the background
	- The sensitivity at that frequency for that photoreceptor type is 1/threshold intensity
- Repeat this for each frequency and for each photoreceptor type
- Assumption is that the electrical response needed for "just noticeability" is constant.

Data from Physiology and Psychophysics Match tensity dependence of the rod after-image signals with the rod after-image signals with the rod after-image si
The rod after-image signals with the rod after-image signals with the rod after-image signals with the rod aft the Dhucialows and rods in man.

31 Baylor et al., 1984; Baylor, 1987

Wavelength (nm)

Photoreceptor Isolation in Psychophysics

- Isolate rods vs. cones
	- Adjust light intensity to bleach rods
	- Measure from rod monochromats
- Isolate S cones by measuring from Scone monochromats
- Isolating M cones from Deuteranopes, who have only M and S cones
	- Use high spatial/temporal frequency lights, to which S cones are insensitive.
	- Isolating L cones from Protanopes

Sensitivities overlap substantially, so in psychophysics how do we measure the responses from just one type?

Beyond Photon Counting: • Phototransduction

• Deactivation and Pigment Regeneration

George Wald (Nobel prize, 1967)

Pigment = a Long Opsin + a Small Retinal

Pigment Isomerizes Upon Photon Absorption 1 Absorb pedicle Inner

Phototransduction

After a pigment is excited, it roams

cG-Gated Ion Channels Close

a single rod pigment excitation reduces about 1,400 cG channels (2% reduction in dark current)

Photocurrents are Produced

(always negative)

Steps in Phototransduction

An activated/ excited pigment

An activated pigment roams about and can activate many (~700) G proteins by replacing the GDP with GTP

Steps in Phototransduction

An activated/ excited pigment

An activated G protein roams about and binds to a phosphodiesterase (PDE) and activates it.

Steps in Phototransduction

A single excited pigment removes thousands of cG molecules

An activated/ excited pigment

An activated PDE roams about and hydrolyzes multiple cG molecules.

Beyond Photon Counting: • Phototransduction

• Deactivation and Pigment Regeneration

Phototransduction Deactivation

- There are a finite number of pigments (in fact all the materials involved in phototransduction) and cG-gated ion channels.
- When all the biomaterials are consumed or all the channels are closed, transmembrane current will be 0 and there will no further response saturation.
- Why aren't our photoreceptors constantly saturated?!

"Counteracting Forces"

• Deactivation/termination of phototransduction and re-open ion channels. • When the closed ion channels are re-opened, we have restored the dark current and

- For a photoreceptor to be able to continue responding to lights, two things must happen.
- - those open channels are available for being closing again
- Continuously regenerate pigments (retinoid cycle or visual cycle)
	- Re-generated pigments are re-available for isomerization
- - steady vision; otherwise our vision is saturated (no responses to further lights).

• Both are constantly at work to "counter" the affect of phototransduction.

• Usually the countering forces and the activation reach an equilibrium and we enjoy a

Proof that Deactivation is Constantly at Work complete suppression is ${\color{red} \bullet}$

- After a brief flash of light (i.e., a fixed amount of photons are delivered), the response first rises and then falls.
	- The response rises because more ion channels are being open, and it falls because the closed ion channels are being re-opened, i.e., deactivation.
	- Without deactivation the response would have fell.

- With a steady light (called step light), the response eventually reaches an equilibrium.
	- Steady light means new photons are constantly being delivered to the photoreceptor, so new ion channels are constantly being closed.
	- But the response reaches equilibrium, which means the ion channel closing rate matches that of the re-opening rate.

Proof that Deactivation is Constantly at Work TAAL TRANSPORTATION IS AANSTALLE

Deactivation Mechanisms

• Just like the activation of the phototransduction, deactivation also involves

• Just to give you a flavor, here is how excited pigments are deactivated. • An enzyme, rhodopsin kinase (RK) binds to and phosphorylates an activated pigment • Another protein called *arrestin* (A) then binds to phosphorylated pigment, which inhibits

- a set of biochemical reactions.
- -
	- the pigment to activate G proteins, essentially deactivating the pigment.

•

Accelerating Deactivation via Negative Feedback

- Two opposing forces:
	- Activated pigments reduce cG concentration, which closes ion channels and reduces Ca2+ concentration
	- Ca2+ inhibits guanylate cyclases (GCs), which synthesize cG.
	- So reduction in Ca²⁺ increases the cG synthesis rate.
- This is a classic negative feedback in a dynamical system, which accelerates the time to equilibrium.

49 Rieke and Baylor, 1998

Undershoots in Cone Kinetics 42 INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE / JANUARY 1987 VOL. 28 INVESTIGATIVE / JANUARY 1987 VOL. 28 IN
1987 Vol. 28 INVESTIGATIVE / JANUARY 1987 VOL. 28 INVESTIGATIVE / JANUARY 1987 VOL. 28 INVESTIGATIVE / JANUARY

Time (sec)

- \blacksquare of around 300 msec. The persistence \blacksquare **• Biphasic response in contribute to the rod must contribute to response in contribution and must contribute** to the slowness of human rod vision. Single photon rod vision. Single photon rod vision. Single photon re- \sim • Biphasic response in cones
- There is an undershoot in the kinetics
- \mathbf{t} of the total rods. • The negative feedback in cones is so from the rods. For example, after a dim flash, the cone strong that it causes a temporary Cone responses are also much less sensitive; about 30 photoisomerizations a rod response of the response of α rating amplitude, while the corresponding response in a cone cone requires about 2000 photoisomerizations. The contract 3000 photois about 3000 photois and 2000 pho differences in the kinetics and sensitivity of the kinetics and sensitivity of the rod and sensitivity of the r
The rod and sensitivity of the rod and sensitivity of the rod and sensitivity of the rod and sensitivity of th even in dark sensitivity and time resolution of rod and cone vision of rod and cone vision.
The resolution of rod and cone vision. The resolution of rod and cone vision of resolution of resolution of re overproduction of cG molecules, which open more ion channels than there are

Bruce Goldstein Bruce Goldstein

Retinal Falls Off from the Pigment after Deactivation $\mathbf{u} = \mathbf{u} \cdot \mathbf{v}$ the Pigment atter D ment. If you were to turn out the lights, then bleached viretina becomes detached, it has become separated from a layer that it rests on, called the *pigment epithelium,* which cours en zien en zien pigment regeneration en are necessary for pigment regeneration are necessary for pigment reg

Illustration of the three states of a single pigment

Image of a dissected frog retina; when more pigments are excited/activated/bleached, the photoreceptor becomes more transparent, since fewer pigments are absorbing lights

Pigment Regeneration in RPE

Pigment Regeneration Kinetics that the rate of rate of rate of rate of rate of \mathbf{r} **Figment Regeneration Kinetics** pigment which is still unregenerated at the moment, and is not detectably Dowling (i960) showed in the rat that there was linearity between the amount of pigment still bleached (free opsin) and the logarithm of the light flash required

Bleach all pigments using intense light, then remove lig Diamoch roganoration in conoc is foster than in rode holf time 2V foster liquene left u aveel to elicit a constant small response from the electro-retinogram. The same result α Bleach all pigments using intense light, then remove light, and measure % of pigments vs. time. Pigment regeneration in cones is faster than in rods — half time 3X faster (ignore left y-axes).

Rods vs. Cones: Absolute Sensitivity and Saturation

Rod Vision is More Sensitive than Cone Vision The Mara Sancitiva th process (see Figures 1.10). Here is a contract was able to determine the see Figures 1.10). Here is a contract \overline{a} photons reached the retina, and \overline{a} a Cana Viciar

NUC JUO $\mathbf{H} = \mathbf{H} \mathbf{H} + \mathbf{H$ that about 10 cones for h pigment molecules were isomerized by this f ash, he con**xcited in about 500 rods**

s ahout 5 nigment excitation ond the p igniture there each photons. entered a separate receptor. Thus, only one visual pigment per cone in a pool of about 10 cones for humans to signal a flash. excited in about 500 rods; in contrast, it takes about 5 pigment excitations

Rod Vision is More Sensitive than Cone Vision as normal indoor lighting or sunlight. The contributions than Cone Vicion rod recovery (Figure 11.12A).

FIGURE 11.11 The range of luminance values over which the visual system operates. At the lowest levels of illumination, only rods are activated. Cones begin to contribute to perception at about the level of starlight and are the only receptors that function under relatively bright conditions.

Why?

- A single rod has a higher signal-to-noise ratio (SNR) than a cone
	- Higher single photon response in rod
	- Lower noise in rod
- signal integration time
- Rods have a higher degree of convergence (to RGCs)

• A single rod's response kinetics is longer, which increases the effective

Signal: mean value Noise: Std. of value SNR = Signal / Noise

Signal to Noise Ratio (SNR) \mathcal{F} in \mathbf{F}

58 Essential Principles of Image Sensors, Takao Kuroda

s(*t*): real output overlapped with noise

Single Photon Response \mathbf{S}

- Single photon response
	- d • Rod is ~20x higher than cone
- Normalized single photon • ~30 pigment excitations closes half of the ion channels in a rod. It requires about 650 excitations in a cone to do **SO** (Baylor et al., 1984; Schnapf et al., 1990).
- Why? Phototransduction in rods is
- **Figure 1.4 Primate 1.4 Primate cones have already between the faster of the faster cones in the f** • So more ion channels have already been closed before counteracting forces kick in

Dark Noise in Photoreceptors

- Photocurrent should be 0 in dark, but it's not. Photocurrent in dark is called dark noise. Discrete Noise
- Two components of dark noise:
	- Discrete: spontaneous thermal excitation of pigments $\overline{1}$
	- Continuous PDE activations
- Dark noise is much higher in cones than in rods.

Time (sec)

- \mathcal{L} (integration time) of around 300 msec. The persistence \mathcal{L} $\bullet\,$ Cone response decavs i the slowness of human rod vision. Single photon rod vision rod vision rod vision. Single photon rod vision. Si
Single photon redoes rod. • Cone response decays much faster than
- **the Recause** • Because deactivation force is stronger in cones
- from the rods. For example, after a dim flash, the cone \mathbf{f} • A longer duration integrates responses of Cone responses are also much less sensitive; about 30 photois more incoming photons rating amplitude, while the corresponding response in more incoming photons.
- If the time durations of two excitations overlap, differences in the kinetics and sensitivity of the rod and the total electrical response is greater than that \int rod and contribution of rod and cone visitor of rod and cone visitor of rod and cone vision. of one excitation.

Kinetics of Responses in Rods and Cones

* from macaque rod and L cone

rod

• Tens of millions of pigments in a photoreceptor, but a rod saturates after a few hundred recovers in a order times for times faster than four times faster than four times faster than four times faster br, but a rod saturate

Purves et al. Neuroscience 6ed 62

Rod Vision Saturates Much More Easily is a perception of the perception of color because in the color because in the color because in the color \blacksquare cone does not saturate at high levels of steady in the steady tion, as the rods $\mathbf r$ rods and $\mathbf r$ cones adapt to operate over a range of luminance values,

- Downside of being more sensitive. Saturation difference is unlikely affected by differences in the speed of pigment regeneration. of starlight, spatial discrimination at this light, spatial discrimination at this light level is still discrimination at the still discrimination at the still discrimination of \mathcal{L} **Jowns** \blacksquare mected by differences in the spee
	- pigment excitations. major determinant of perception under condition under condition under conditions \mathbf{r} rens or millions or pignients in a priotore

\sim This difference in adaptation is apparent in the time in the aration difference is unlikely \bullet cone, even to a bright flash that is a bright flash tha pigment regeneration.

Response vs. Intensity

amplitudes of the outward-going photon of the photon density \mathbf{r} of the flash. The saturating photocurrent, which presumably corresponds to complete suppression of the investigation of the investigation of the interpretation of the interpretation of \blacksquare half-saturating flash strength, io, by io = (in 2)/kf. Values of kf, sensitivity to dim \bullet (Peak) Response vs. Light Intensity

\star M_{O} and varied between cells (Table 1). * Macaque rods * 200 pa from recording to 18-40 pa from reco

isomerizations just saturated. Eqn. (2) describes the relation between photon density Baylor et al., 1984 64

(Peak) Response vs. Light Intensity

- Electrical response vs. light intensity is not linear!
	- ~exponential saturation
	- as if the photoreceptor becomes less sensitive when light becomes more intensity: desensitization
	- essential for adaptation; more later.
	- This doesn't contradict the Principle of Univariance!
- Why do photoreceptors desensitize? What benefit?

(Peak) Response vs. Light Intensity

- Why not linear?
	- Think of what produces the responses
- ~Linear when the light is dim
	- That's how people estimate single photon response (assuming this limited linearity)
- This is also why perceived brightness is not linear w.r.t. light power/intensity

Brightness Encoding

https://live.staticflickr.com/7431/13804978154_a5b9a45e95_z.jpg 67

Linearly increase light power/intensity

Allocate more bits to encode this range

Allocate fewer bits to encode this range

Brightness Encoding

https://live.staticflickr.com/7431/13804978154_a5b9a45e95_z.jpg 68

Key Things to Take Away

- Principle of univariance:
	- Electrical response is independent of wavelength
	- Implication: if two lights cause the same amount of pigment excitation, they are see as the same light.
- Overall photoreceptor sensitivity = ocular transmittance x macular transmittance x photoreceptor absoprtance
- Phototransduction is a cascade of biochemical reactions caused by a single pigment excitation closing ion channels and producing photocurrents.

Key Things to Take Away

• Two countering forces that prevent our vision from constant saturation

- - Pigment regeneration
	- Deactivation of phototransduction
	- Former happens in *minute*-scale and latter in *ms*-scale
- Rod vision is more sensitive than cone vision and is more easily saturated
- Photoreceptor response vs. light intensity is not linear, and that's why your perceived brightness is not linear w.r.t. light intensity

