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Jeremy Nathans' Lecture Part 1A:
The Vertebrate Retina

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Contents

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1. Keywords and Terms

eye, vision, retina, rhodopsin, 11-cis retinal isomerization, threshold sensitivity of human vision, single photon response, electrophysiology, phototransduction, signal amplification, enzyme cascade

2. Lecture Notes

Photoreceptors and Image Processing:

Similarities between the vertebrate eye and a camera:

- The retina is analogous to the film in a camera: both can adjust to different levels of illumination, and both can focus on objects at different distances.
- Vertebrate and insect eyes are very different anatomically, but they use related transcriptional control genes, suggesting a common evolutionary origin.

The vertebrate retina has three layers of neurons, with photoreceptors occupying the outermost layer.

Vertebrate photoreceptors (rods and cones) are highly specialized cells, with an outer segment that is filled with membranes.

Visual pigments are integral membrane G-protein coupled receptors.

Cis to trans photoisomerization of the 11-cis retinal chromophore is the fundamental event in phototransduction.

A recently discovered intrinsically photo-sensitive retinal ganglion cell controls non-image forming vision (circadian rhythms and pupil construction).

Selig Hecht and his colleagues determined the absolute threshold for human vision and found it to be remarkably low: 5-7 absorbed photons are sufficient to register a response.

The light response of single photoreceptors can be measured with a suction electrode. Under dim light conditions, single photon responses can be recorded, and in the absence of light there are rare single-photon like responses (due to thermal isomerization of 11-cis retinal).

The retinal ganglion cell solves the problem of reliably detecting a weak signal in the presence of thermal noise by acting as a coincidence detector: under conditions of complete dark adaptation, 5-7 single photon responses must arrive within a few milliseconds to be considered a real light flash and not just background thermal noise.

Phototransduction uses an enzymatic amplification cascade to convert a small stimulus (one absorbed photon) into a large membrane voltage signal.

3. Recommended Reading

1. Gardner, E.P, and Martin, J.H.. (2001) Coding of Sensory Information. (chapter 21), in Principles of Neural Science (fourth edition). Kandel, E.R., Schwartz, J.H., Jessell, T.M., editors. New York: Elsevier.
2. Purves, D. et al. (2001) Vision: The Eye (chapter 11), in Neuroscience. Sunderland, MA: Sinauer.
3. Available online at http://www.ncbi.nlm.nih.gov/books/NBK10799/
4. Rodieck, R.W. (1998) The First Steps of Seeing. Sunderland, MA: Sinauer.
5. Schnapf, JL, and Baylor, D.A. (1987) How Photoreceptor Cells Respond to Light. Scientific American (April) 40-49.
6. Stryer, L. (1987) The Molecules of Visual Excitation. Scientific American (July) 42-50.
7. Tessier-Lavigne, M. (2001) Visual Processing by the Retina (chapter 26), in Principles of Neural Science (fourth edition). Kandel, E.R., Schwartz, J.H., Jessell, T.M., editors. New York: Elsevier.

4. Review Questions

1. In what ways does the eye resemble a camera?
2. What are the principal types of light sensing cells in the vertebrate retina?
3. What is the general structure of rhodopsin?
4. What is the effect on rhodopsin of absorbing a photon?
5. Under fully dark-adapted conditions, how many photons must be absorbed by the human retina for an observer to reliably detect a flash of light?
6. Why can humans not reliably detect the absorption of a single photon?

7. Signal transduction in the photoreceptor outer segment uses a series of coupled enzyme reactions. What advantage or disadvantage is conferred by this arrangement?

5. Answers to Review Questions

1. Both have a lens that focuses and inverts the image onto a 2-dimensional light-detecting layer.

2. Rods, cones, and intrinsically photosensitive retinal ganglion cells.

3. Rhodopsin consists of an integral membrane protein (opsin) covalently linked to a chromophore, 11-cis retinal.

4. Photon capture produces an isomerization of the 11-12 double bond in 11-cis retinal, converting it from 11-cis to all-trans retinal. This isomerization induces a conformational change in the attached apoprotein that causes it to catalyze that activation of the photoreceptor G-protein, transducin.

5. 5-7

6. Rods exhibit a low background of electrical activity due to rare thermal activations of rhodopsin, and these thermal events are indistinguishable from the signal that is produced by the absorption of a single photon. Therefore, for a flash of light to be accurately recognized, it must produce a signal that rises above this background noise at the level of the retinal ganglion cell (which integrates input from about 500 rods).

7. A cascade of enzymatic reactions has the advantage of greatly amplifying a small signal. It has the disadvantage that the amplification process takes time, thereby delaying the response.

6. Discussion Questions

1. Each mammalian rod outer segment has approximately 1,000 discs, each of which consists of a flattened lipid vesicle. What is the functional utility of this arrangement?

2. How does the focusing mechanism differ between a camera and the vertebrate eye?

3. Although not discussed in the lecture, it is interesting to note that photoreceptor outer segments are continuously synthesized throughout life, with about 10% of each rod outer segment assembled at its base and 10% discarded from its tip each day. Given that the discarded 10% of the outer segment is engulfed and digested by a specialized epithelial cell (which makes up a sheet of cells referred to as the retinal pigment epithelium, or RPE) and that each of these large epithelial cells performs this function for about 50 rods, how much biomass is an RPE cell digesting each day?

4. In complete darkness, mammalian rods occasionally exhibit changes in membrane current that are indistinguishable from single photon responses. These occur on average once per minute and each arises from the thermal isomerization of the 11–cis retinal chromophore within a single rhodopsin. Given that each mammalian rod has 40 million rhodopsins, calculate the average thermal stability of rhodopsin.

5. Consider the molecular mechanisms operating in different sensory systems, such as hearing, olfaction, vision, taste, and temperature sensation. Which ones use a cascade of enzyme reactions and which ones use a more direct response mechanism?

7. Answers to Discussion Questions

1. This arrangement allows a high concentration of the integral membrane protein rhodopsin, which increases the efficiency of light capture. Moreover, the plane of the flattened face of the outer segment disc membrane is perpendicular to the direction of light propagation along the long axis of the outer segment, and this produces an orientation of rhodopsin's 11-cis retinal chromophore that is optimal for photon absorption. The stack of outer segment discs also compartmentalizes the phototransduction proteins (transducin, cGMP phosphodiesterase, etc) so that different single photon absorption events produce very nearly the same response amplitude. This happens because the activation of a single rhodopsin on the face of

one disc leads to the activation of all or nearly all of the phototransduction proteins within the thin zone of cytoplasm in contact with that active rhodopsin, with minimal spill-over to adjacent inter-disc regions.

2. Both the camera and the eye focus the image by refraction through a lens, although it is interesting to note that most of the refractive power of the eye comes not from the lens but at the air/cornea interface. This is because the refractive index difference between air and tissue (the cornea) is substantially larger than the refractive index difference between the lens and the surrounding fluid (the aqueous humor). To bring objects at different distances into sharp focus, a camera moves the lens further from or closer to the light sensing layer (film or its electronic equivalent). In the eye, the lens remains at the same location, but it can be squeezed by a set of muscles to make it more round thereby producing greater bending of the light rays and allowing the images of closer objects to be focused on the retina. This phenomenon of lens deformation is called accommodation. As people age, the lens becomes less flexible and the images of close objects are focused behind the retina – i.e. they are out of focus at the retina. This far-sightedness can be easily corrected with eyeglasses. Of historical interest: Thomas Young, whose contributions to color vision are describe in lecture 2, discovered the mechanism of accommodation when he was 20 years old.

3. The mass of an outer segment is quite substantial – it is roughly the size of a small mammalian cell such as a red blood cell. Since 10% of each of 50 outer segments is digested per RPE cell per day, this is the equivalent of approximately 5 red blood cells per day. Each RPE cell does this every day for the 70+ years of the person's life, an amazing feat of cellular digestion!

4. Since 40,000,000 rhodopsins give on average only one thermal event per minute, the average stability of a rhodopsin is 40,000,000 minutes. To convert this to years, we note that there are 60 minutes per hour, 24 hours per day, and 365 days per year. Therefore there are 52,560 minutes per year, and the average thermal stability of rhodopsin is about 800 years. (Note: this is the stability with respect to thermal activation of 11-cis retinal isomerization; this calculation does not address thermal stability properties that relate to protein unfolding.)

5. As described in lecture 1, vision uses a two-stage enzyme amplification: light activated rhodopsin catalyzes the activation of many G-proteins (stage 1), and each activated G-protein activates a cGMP phosphodiesterase, which then hydrolyzes

many cGMP molecules (stage 2).   cGMP binds directly to a plasma membrane cation channel and opens it. Therefore a decrease in cGMP concentration leads to channel closure.

A very similar system operates in the olfactory system, except that the G-proteins activate an adenylyl cyclase, which increases cAMP levels. In olfactory sensory neurons, a channel that is homologous to the photoreceptor channel is opened by binding directly to cAMP.   Sweet and bitter taste also use G-protein couple receptors.

The senses of hearing and balance (both in the inner ear) are very different: these senses are at their core mechanosenory, i.e. a microscopic movement must be transduced into a molecular signal, and in both cases the mechanical stimulus directly gates plasma membrane channels. The result is extremely fast (less than milli-second) signal transduction. Thermosensation is based on a family of channels that change the ratio of open to closed conformations as a function of temperature. One set of channels, found in heat sensing neurons, opens at higher temperature; and a second set, found in cold sensing neurons, opens at lower temperature.

8. Explain or Teach These Concepts to a Friend

1. Explain how Selig Hecht and his colleagues used random fluctuations in the number of absorbed photons to determine the minimal number of photons that are required to see a dim flash of light.
2. How does the mechanism of phototransduction in rod cells resemble hormone signaling through G-protein coupled receptors? For example, compare phototransduction to the mechanism of adrenalin (=epinephrine) action in the heart and liver.

9. Research the Literature on Your Own

1. What are the structures of the eyes of octopus and squid (two invertebrates)? How do these eye structures compare to the structures seen among other invertebrates and among vertebrates? How could such structures evolve?

2. Animals are not the only living organisms that detect light. Discuss examples from the bacterial and plant kingdoms of light sensing. What are the photoreceptor molecules in these cases, and how do they change as a result of capturing a photon?
3. Retinitis pigmentosa (RP) refers to a group of inherited disorders in which rod photoreceptors are impaired and ultimately die. What are the causes of RP? What potential therapies are being investigated to treat or prevent it?
4. Signal amplification is not unique to the visual system. Discuss the way in which enzymes act as signal amplifiers in blood clotting.