Vision Science II - Monocular Sensory Aspects of Vision

Lecture 10 - Photochromic interval, Purkinje shift, Dark adaptation

REVIEW LECTURE 9

The eye's optical system, including its two lenses, the cornea and crystalline lens, begin the visual process by forming an optical image on the retina. The retina must then transform the optical image into neural signals that are relayed to the brain for processing and analysis. An important step in the visual process is the conversion of optical data to neural signals (electrophysiological impulses). This step, referred to as **phototransduction**, occurs in the photoreceptor outer segments, and starts when a photopigment molecule captures a photon.

In the case of the rods (scotopic system), the photopigment is **rhodopsin** (visual purple). One rhodopsin molecule can absorb one photon of visible light, but its probability of capturing a photon varies with wavelength. It captures light most efficiently if the wavelength is about 507 nm, because a photon of this wavelength has the best "fit" with a rhodopsin molecule.

Note that once a photon is absorbed, the response of the visual system is the same, regardless of the wavelength. This is the *principle of univariance*. Although, for example, 580-nm light is less likely to be absorbed, if you increase its intensity, you can cause as much of 580-nm wavelength light to be absorbed as 507-nm wavelength light. This was illustrated in Figure 5 of Lecture 9.

We discussed some properties of rhodopsin, such as its absorption spectrum and transmission spectrum. We also discussed the spectral sensitivity of the scotopic system and noted that its plot looked similar to a plot of rhodopsin absorption across wavelengths. Recall that the rhodopsin threshold spectrum is the inverse of the rhodopsin sensitivity spectrum.

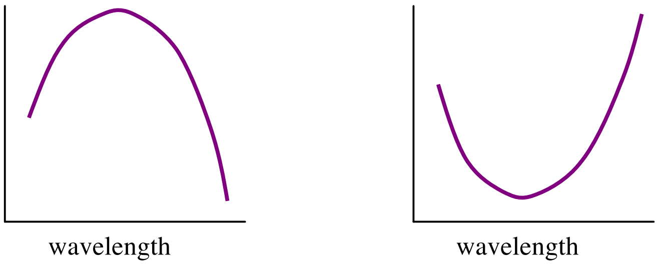


Figure 1. Plots that could represent the rhodopsin spectral functions for transmission, absorption, threshold or sensitivity. Which graph could represent which function?

CONE PHOTOPIGMENTS

There are three different kinds of cones, and each contains a different photopigment that is different from rhodopsin. They differ in their absorption spectra, as shown in Figure 2 below (like Schwartz Fig. 3-6A). Because of their characteristic absorption spectra the cones are labeled as S (short wavelength), M (middle wavelength) or L (long wavelength) cones as summarized in Table 1, below.

Compared to L and M-cones, S-cones are much more sparse, and *there are no S-cones in the fovea*.

Although the three cone types were sometimes referred to as "blue," "green" or "red" cones, this is a misnomer. The color designation simply describes the *peak* of their absorption spectra. Note that they all absorb light over a *broad range of wavelengths*. The presence of three types of cone photopigments allows us to discriminate different colors.

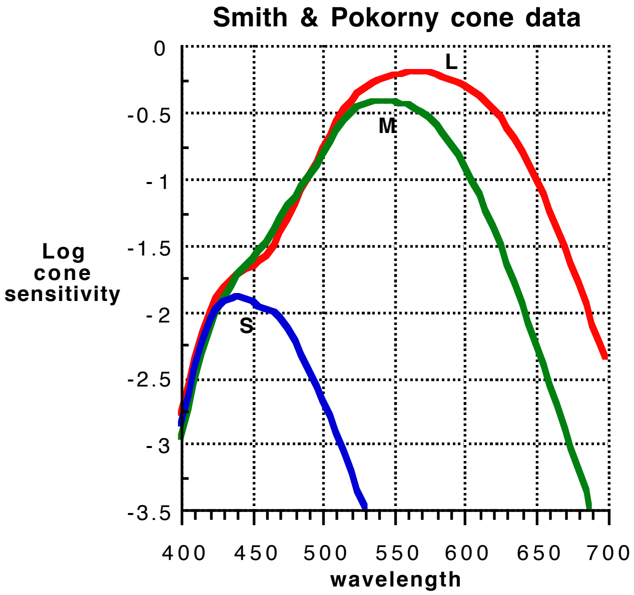


Figure 2. Absorption spectra for the photopigments in the S, M, and L cones.

Table 1. Cone photopigments

Cone type	Photopigment	Range centers on	Peak wavelength (nm)
S cone ("blue cone")	cyanolabe	short λ	430
M cone ("green cone")	cholorolabe	middle λ	535
L cone ("red cone")	erythrolabe	long λ	565

If we test sensitivity of the photopic system to lights of different wavelengths, we can plot the data as a function that looks somewhat like the V(λ) curve (Schwartz Fig. 3-6B). This function is formed primarily from the combined absorption spectra of the M- and L-cones. The S cones make only a minor contribution to the photopic luminosity function and their contribution is variable, depending on test conditions.

PHOTOCHROMIC INTERVAL

When both the rod (scotopic) and cone (photopic) spectral sensitivity curves are plotted on the same scale, we get a graph like Figure 3, or Schwartz Fig. 3-7. Note that for most wavelengths the rods are more sensitive than the cones, and the rod peak is about 507 nm, while the cone peak is at about 555 nm. At long wavelengths (red region) rod sensitivity decreases, and at about 650 nm, it is essentially the same at that of the cones.

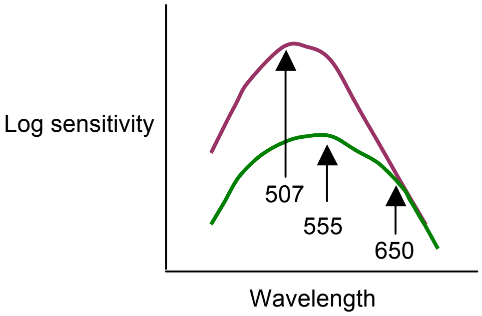


Figure 3. Comparison of scotopic and photopic sensitivity functions.

These curves can be determined by the following experimental procedure:

- Dark adapt the subject
- Rods and cones will be fully regenerated and maximally sensitive
- Start with a very dim (sub-threshold) monochromatic (one wavelength) light
- Increase radiance until the light can first be detected. This measures the scotopic (rod) threshold for that wavelength
- Continue to increase radiance until the subject can detect the light's color. At this level cones are beginning to work, so this indicates color detection and the threshold for the cone system.

The difference between the sensitivity (or threshold) for the rod and cone systems at each wavelength is known as the **photochromic interval**. This is represented on the graph as the *vertical distance between the two curves*. Note that the photochromic interval is greatest for mid wavelengths but is essentially zero for long wavelengths.

PURKINJE SHIFT

The scotopic and photopic spectral sensitivity functions are based on measurements *at threshold* light levels for the two systems. These curves also help predict the relative brightness of different wavelengths at *suprathreshold light levels*. If, for example you were looking at a yellow-green flower (555 nm) and a blue-green flower (505 nm), both of which reflected equal radiance, the yellowish flower would appear brighter during the day. In the evening, as it becomes dark and the eye transitions to scotopic vision, you would then notice that the bluish flower begins to look brighter than the yellowish flower. The relative increase in brightness for blue and green objects, as the eye adapts to low light levels, is known as the **Purkinje Shift** or **Purkinje Phenomenon**. This is caused by the shift in peak sensitivity from 555 nm to 507 as the eye changes from photopic to scotopic vision. The Purkinje shift coincides with the horizontal separation of the two peaks shown in Figure 3 above or Schwartz Fig. 3-7.

INTRODUCTION TO DARK ADAPTATION

Once bleached, rods and cones recover at different rates. The half-life of rods is about 5 minutes while that of cones is 1.5 minutes. If you completely bleach the rod and cone photopigments by exposing a subject to a very bright light (like a BIO), then test his *detection threshold* for a faint light over time, as his vision begins to recover, you will be measuring his dark adaptation function. The data can be plotted on a curve such as the one shown in Figure 4 or Schwartz Fig. 3-10. Curve A is for a large stimulus with a wavelength of 420 nm.

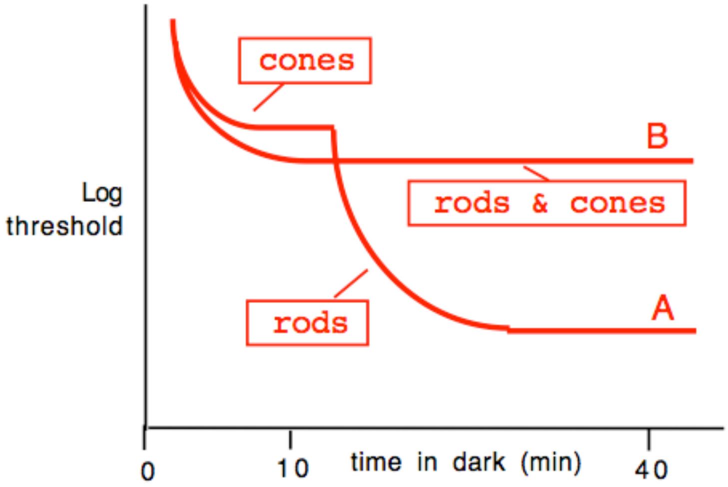


Figure 4. Dark adaptation threshold function

Important features of the dark adaptation curve (Refer to curve A in Figure 4, above.)

- Steady decrease in threshold (increased sensitivity) over ~ 40 minutes

- Overall threshold decreases (sensitivity increases) about 5 log units (100,000 fold)
- Curve reaches a plateau after about 5 minutes in the dark
- Slope abruptly drops again at about 10 minutes (the rod-cone break)
- Curve steadily falls to a second plateau at about 30-40 minutes
- The abrupt drop-off after the first plateau is called the **rod-cone break**.

The half-life for cone recovery is shorter than for rods, so the rapid improvement in sensitivity (decline in threshold) in the first 10 minutes is due to recovery of the cones. During the first 10 minutes or so, most of the rods have not yet recovered to the point that they are more sensitive than the cones. This accounts for the first section of the dark adaptation curve. This is followed by a plateau, which indicates the light level where the cone threshold stops improving significantly.

At the rod-cone break, enough time has elapsed that rod sensitivity surpasses that of the cones (their threshold goes below that of the cones). With time, the rods steadily recover more and more sensitivity and the threshold continues to decline until the rods approach their minimum threshold (maximum sensitivity), and the function reaches the final plateau.

DARK ADAPTATION WITH DIFFERENT WAVELENGTHS
On dark adaptation plots, such as those discussed above, the vertical distance between the cone and rod plateaus correlates with the *photochromic interval*. In the example shown in Schwartz Fig. 3-10, we were considering the dark adaptation function for a stimulus with a wavelength of 420 nm.

Referring back to Figure 3 (redrawn below; like Schwartz Fig. 3-7), how does the photochromic interval at a short wavelength, such as 420 nm compare to the photochromic interval at a long wavelength, such as 650 nm?

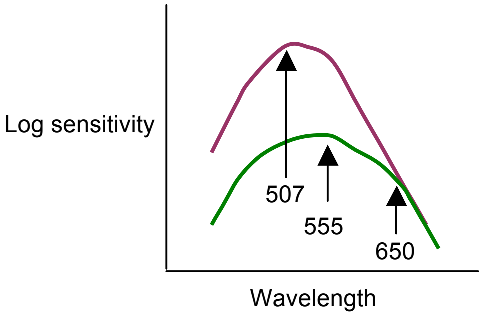


Figure 3. Comparison of scotopic and photopic sensitivity functions.

Instead of 420 nm, if the stimulus had a wavelength of 650 nm, the dark adaptation curve would look like Curve B in Figure 4 (redrawn below; like Schwartz Fig. 3-11). The first portion of the curve is similar to before, but there is no rod-cone break and no decrease in threshold below the cone plateau.

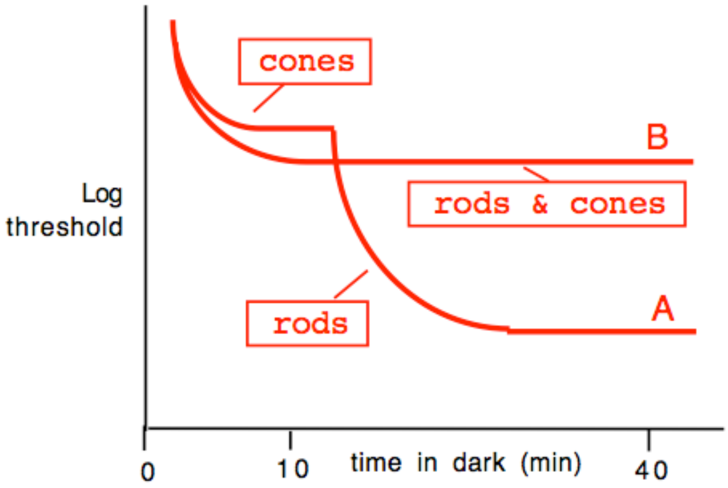


Figure 4. Dark adaptation threshold function

Q. Why?

A.

Schwartz Fig. 3-7 shows that for long wavelengths, such as 650 nm, the photochromic interval is zero and rods are not more sensitive than cones. Therefore once the cones reach their plateau, (ultimate threshold), the rods are also at their threshold. The threshold does not drop below the cone plateau since the rods are not more sensitive than cones for that wavelength, even after waiting a long time.

General principle: *On the dark adaptation curve, the rod-cone break is most pronounced, and the distance between cone and rod plateaus is greatest, for wavelengths at which the photochromic interval is greatest.*

The relationship between the rod and cone spectral threshold functions, and the dark adaptation curves, is shown in Schwartz Fig. 3-12. Refer to Schwartz' figure and draw in the dark adaptation function on Figure 5, below, for a large (i.e. 5-10° diameter spot) stimulus with a wavelength of 465 nm. You can do so using the following steps.

- Locate the cone and rod light detection thresholds for a wavelength of 465 nm on the right graph.
- This tells you the y-axis value for the cone and rod plateaus. At this wavelength the separation between the cone and rod absolute thresholds is large (large photochromic interval).
- Draw in the rod-cone break at about 10 minutes in the left curve.
- Draw in the cone portion to the left of the rod-cone break, with a rapid decline, which levels off just before the rod-cone break.
- Draw the rod plateau at about 30 minutes
- Connect the rod-cone break with the rod plateau with a curve showing a rapid drop, then a gradual transition into the rod plateau.

Follow the same steps to draw the dark adaptation curve for a large stimulus that has a wavelength of 610 nm. Note the dark adaptation curve drawn in Schwartz Fig. 3-12 for 610 nm. The dark adaptation curve for 610-nm light differs from the one for 465 nm in several ways:

- the cone plateau is lower
- the rod plateau is higher
- the rod-cone break is farther to the right, past 30 minutes
- the photochromic interval is small

You should understand why each of these characteristics of the two curves (465 versus 610) is different.

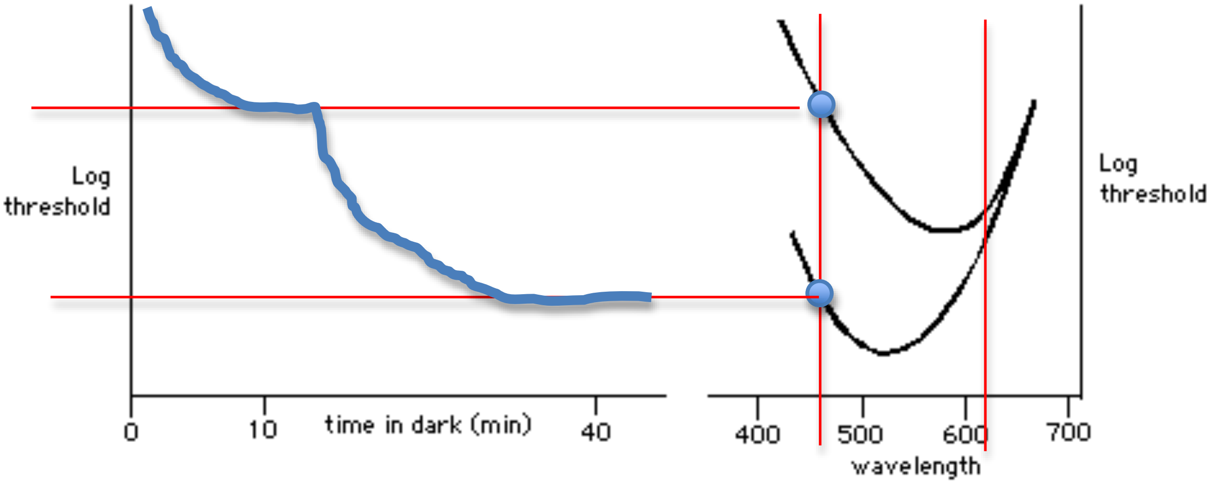


Figure 5. Complete this figure to show the dark adaptation function for a stimulus wavelength of 465, then 610 nm.

In these examples, the photochromic interval may be seen in either the spectral threshold functions or the dark adaptation threshold functions. The spectral threshold functions show the cone and rod thresholds after full dark adaptation for each wavelength (abscissa shows wavelength), while the dark adaptation curves show the change in threshold over time (abscissa shows time) for one wavelength. The table below summarizes the relationship between the photochromic interval and dark adaptation.

Table 2. The effect of the photochromic interval on the dark adaptation function

Example λ (nm)	Photochromic interval	Cone threshold	Rod threshold	Rod-cone break	Plateau height difference
< 560	large	high	below cones	early	large
560-600	small	minimum	below cones	late	small
> 650	zero	medium	same as cones	none	none

DARK ADAPTATION AND STIMULUS SIZE/LOCATION

It is possible to adjust the stimulus size and location to include or exclude the rods. For example a large (greater than about 2° diameter), centrally fixated target would take in both the fovea and the surrounding rod-rich portion of the retina. On the other hand, a very small stimulus (2° diameter or less), centrally fixated, would limit results to a cone-only response.

Q. How would the dark adaptation curve look with this kind of stimulus? (See Schwartz Fig. 3-13.)

MECHANISM OF DARK ADAPTATION

We previously learned that spectral sensitivity of the scotopic system (Schwartz Fig. 3-5B) closely matches the spectral absorption spectrum for rhodopsin (Schwartz Fig. 3-4C). We assumed that the sensitivity of the rods for different wavelengths is largely based on the capacity of a rhodopsin molecule to capture a photon of that wavelength. Similarly it is assumed that the absorption characteristics of the L and M-cone photopigments (chlorolabe and erythrolabe) determine the photopic spectral sensitivity function (sensitivity of the cone system to different wavelengths; see Schwartz Fig. 3-6).

Continuing this logic, you might assume that dark adaptation can be explained by the regeneration of bleached photopigment to its unbleached state. This hypothesis is known as the **photochemical explanation** of dark adaptation. If this were true, we would expect that, bleaching of 50% of the rhodopsin should double the rod threshold (see Schwartz Fig. 3-14). In fact, bleaching 50% of the rhodopsin increases the threshold about 10 billion times! Therefore, although photopigment regeneration is necessary for dark adaptation, it does not fully explain the process.

Schwartz Fig. 3-15 shows experimental results, which support this observation with a *rod monochromat*. This is a person with a retinal disease that leaves him with only rods (no cones) in the retina. By testing dark adaptation with a rod monochromat, we are able to measure the rod threshold prior to where we would normally see the rod-cone break.

The figure shows two dark adaptation curves, but also note that there are two vertical scales (ordinates). The right scale shows the percentage of rhodopsin in the bleached state, and the left ordinate shows the log of the threshold. Circles show the data for the rod monochromat, and for comparison, the dark adaptation curve for a normal retina is also shown.

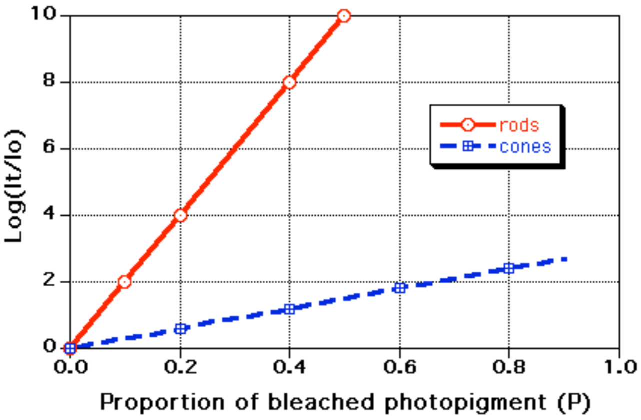
During this experiment, the person dark adapts, and as usual, we measure his light detection threshold. But in addition, we monitor the amount of bleached rhodopsin in his retina. The amount of bleached rhodopsin in the retina can be determined by a technique known as **retinal densitometry**. The retina is illuminated with a known amount of light, and the amount reflected back is measured to determine the amount absorbed.

We start (time = 0) by flooding the retina with a bright light, and bleach 100% of the rhodopsin (right ordinate). The left ordinate shows that the threshold is very high (eye is insensitive). With time, the rhodopsin regenerates to the unbleached state. With regeneration, the percentage of bleached pigment decreases and the threshold also decreases. (In other words, the percent of unbleached pigment increases and the sensitivity increases.) In this experiment, we are interested in how the threshold changes in comparison to the percentage of bleached pigment (compare right and left ordinates).

Note how rapidly the values for the threshold decrease. When rhodopsin recovers 50% (50% unbleached pigment), the threshold has decreased ~10 log units (10,000,000,000 times). Since half the rhodopsin does not result in only one-half the threshold, dark adaptation cannot be explained solely by the photochemical hypothesis. Other mechanisms must be at work to allow the eye to improve it sensitivity much more than can be explained by photopigment regeneration.

It is also interesting to note that after about 15 minutes of recovery (time that a normal person takes to reach the rod-cone break) about 90% of the rhodopsin has regenerated, and the threshold has decrease about 16 log units.

A very small amount of rhodopsin bleaching causes a very large increase in threshold (reduction in sensitivity). This is also shown in Figure 6 below. Note the ordinate and abscissa used here. The linear relationship between percent of bleached photopigment and the log of the threshold is described by the **Dowling-Rushton equation**, beside the figure.



$$\log \frac{I_t}{I_0} = kP$$

Figure 6. The relationship between threshold and percent of bleached photopigment is described by the Dowling-Rushton equation, in which I_t represents the threshold intensity, I_0 is the threshold intensity after complete dark adaptation, P is the percent of bleached pigment and k is a constant (20 for rods, 3 for cones).

In summary, during dark adaptation the eye's threshold recovers much more quickly than can be explained by simple photopigment regeneration. Other complex factors, such as neural processing, must be involved.

CLINICAL DARK ADAPTATION TESTING – THE PHOTOSTRESS TEST

Dark adaptation is the process by which vision recovers sensitivity when changing from a very bright to a darker environment. Bleaching out the retina with a bright light, then testing the light detection threshold over 30-40 minutes can measure the complete dark adaptation process. The **photostress test** may not seem like a “dark” adaptation test, but it does test the first part of the process—that is, recovery immediately after complete bleaching of the photoreceptors. Cone photopigments regenerate quickly, so foveal vision recovers rapidly after the bright light is turned off.

Q. Why do you think a prolonged photostress recovery time indicates a macular rather than optic nerve disease?

A. A delayed brightness recovery, (i.e., the first part of dark adaptation) is probably due to inadequate regeneration of photopigments, which are contained in the photoreceptors, in this case the cones. A long photostress recovery time therefore indicates a problem with the cones, which are in the retina, rather than the optic nerve. Therefore it is a diagnostic test for macular dysfunction.

The following summarizes the procedure for the photostress test:

- Measure the best corrected VA for each eye.
- Have your patient stare monocularly at the BIO light for 10 seconds, from a distance of about 10 cm. This will bleach his o her foveal photopigments. Turn off the light, and begin timing with a stopwatch.
- Let the subject re-fixate the acuity chart and have him or her look at the second-from-the-best VA line.
- Record the time it takes for him or her to read the entire line. Be sure that he or she reads it with his or her central foveal vision (through the bleached spot) rather than with perifoveal vision. Repeat for the other eye.
- Compare the recovery time for the two eyes; they should be nearly the same.

Both eyes should recover well within two minutes. Many patients with normal vision will recover in less than 30 seconds. Any significant asymmetry could indicate some anomaly in the macula of the eye with the slower recovery time.

Q. Sometimes you need to recheck the patient’s refraction, under cyclopleged conditions, after you finished the BIO examination. How long do you need to wait after the BIO before you can do a subjective refraction?

Q. How long do you need to wait to do retinoscopy, after the BIO?