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ARTICLE
Dark Adaptation and Purkinje Shift: A Laboratory Exercise in Perceptual Neuroscience

Uta Wolfe and Nasim Ali
Department of Psychology, University of St. Thomas, St. Paul, MN, 55105

The systematic measurement of luminance thresholds during dark adaptation usually requires advanced optical equipment not available in most undergraduate classes. Here we describe an easy, inexpensive alternative that uses a printed grayscale to measure visual thresholds. Adaptation curves found with this method are comparable to those found with the technologically advanced tools in the standard literature and even show the shift from cone to rod vision at around 4-8 minutes. The exercise can furthermore be easily combined with a demonstration of the Purkinje shift (the different spectral sensitivity of the rod and cone systems) and of multi-sensory integration across vision, touch and proprioception.

The lab allows students to collect, graph and analyze both qualitative and quantitative data. Student ratings of the activity are highly positive, even when compared to other visual neuroscience labs. The activity provides an effective and accessible tool for teaching several important neuroscience concepts, including retinal circuitry, spectral sensitivity, and multi-sensory integration.

Key words: dark adaptation, spectral sensitivity, cross-modality, class demonstrations

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Memorable personal experiences of applied principles can be excellent teaching tools in neuroscience. They not only help consolidate learning of complex information but also serve as a motivator for students to explore advanced material that might otherwise seem too abstract. Retinal neurophysiology can be a daunting subject for undergraduate students and can profit greatly from striking demonstrations that make material more alive and accessible. However, access to laboratory demonstrations in this area is often limited because many require expensive and technologically sophisticated equipment not available to most teaching institutions. For example, the systematic measurement of luminance thresholds during dark adaptation usually needs carefully calibrated optical equipment, such as a dark adaptometer that can moreover only be used by one person at a time (see, e.g., Peters et al., 2000). Yet dark adaptation is an important principle discussed in most standard textbooks of neuroscience and sensation/perception (e.g., Wolfe et al., 2011; Kandel et al., 2012).

Dark (and light) adaptation is a crucial ability of the eye that allows the visual system to function over light intensities that vary by many orders of magnitude. Full adaptation to darkness after being exposed to bright light takes around 30 minutes. While changes in pupil size make a small contribution, the process is accomplished mainly by retinal mechanisms: the switch from cone to rod vision, regeneration of photopigment, and horizontal cell input regulating the sensitivity of photoreceptors.

While the exact time course of dark adaptation depends on a number of factors (e.g., intensity and duration of light exposure before adaptation, part of retina used for threshold measurement, wavelength of adapting light; see http://webvision.med.utah.edu/ for a discussion), the standard course of dark adaptation is shown in Figure 1. The solid black line in the figure shows the drop in threshold intensity for light detection over time as measured for the entire visual system. Note that the curve has two distinct parts reflecting the duplex nature of the retina: During the early part of the process, vision is carried by the cone (photopic) system which adapts to darkness faster but to a lower sensitivity (see 'Cone curve' in figure). After 4-8 minutes, the rods (scotopic system) have adapted to a higher sensitivity than the cones so that the rest of the solid curve represents the rod system's dark adaptation that reaches its maximum sensitivity (which is much higher than that of the cones) at around 30 minutes. The point in time where vision shifts from cone to rod vision is called the rod-cone break.

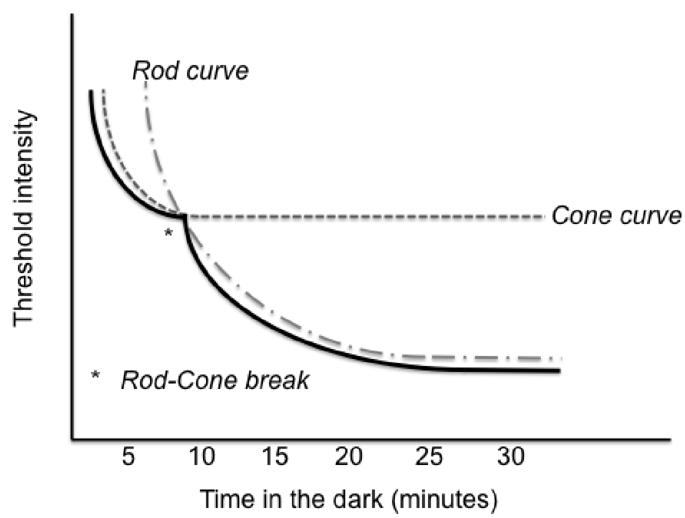

Figure 1: Standard dark adaptation curve.

Besides this difference in their rate of adaptation and absolute sensitivity to light, the scotopic and photopic systems also differ in their spectral sensitivity. This phenomenon is known as the Purkinje shift and is another principle discussed in many undergraduate textbooks (e.g., Blake and Sekuler, 2005). The photopic system is most sensitive to longer and the scotopic to shorter wavelengths (with peaks at 555 nm and 505 nm, respectively). Perceptually, this has the consequence that a red surface will look brighter than an equi-radiant blue surface in the light, but the blue surface will look brighter in the dark (where color will no longer be discernible because the scotopic system, with only one photopigment type, is colorblind).

Both dark adaptation and the Purkinje shift are part of the classic literature in perceptual neuroscience and are described in many undergraduate textbooks. Here we describe an easy, inexpensive lab demonstration of both principles that can be used for a medium-sized undergraduate class and requires nothing more than a printed grayscale to measure luminance thresholds during dark adaptation and a color printout to demonstrate the Purkinje shift. Adaptation curves found with this method are comparable to those found with the technologically advanced tools in the standard literature and even show the shift from cone to rod vision at around 4-8 minutes.

The exercise can furthermore be easily combined with a vivid demonstration of a cross-modal illusion induced by asymmetric dark-adaptation discussed in depth elsewhere (Wolfe, 2010; Wolfe et al., 2007): When only one eye is dark-adapted, only the dark-adapted eye can see in a dim environment while the light-adapted eye is blind. This condition leads to the compelling illusion of the ‘blind’ (light-adapted) eye’s eyelid as sagging or drooping. The illusion seems to result from the brain’s ‘explaining’ the asymmetry in vision by creating a somatosensation that could make sense of it: “The eye that cannot see is closed.” (Accordingly, the illusion is decreased when covering the eye, that is, when introducing somatosensory information that is congruent with the interocular difference in vision). On a neural level, the illusion might arise from the activity in bimodal neurons that code for both vision and somatosensation of the face (Graziano and Gross, 1995; Làdavas et al., 1998).

In sum, the laboratory activity is an inexpensive, accessible way to provide even larger undergraduate classes with a memorable experience of several important neuroscience principles including multi-sensory neurons, the duplex nature of the retina, spectral sensitivity, and adaptation mechanisms. It allows for the collection of quantitative data on dark adaptation that shows the standard trends found with more sophisticated equipment.

MATERIALS AND METHODS

The following procedure and materials have been used eight times in recent years in both an Introduction to Neuroscience course with a laboratory (15-20 students) and a Sensation and Perception course with a laboratory (6-30 students). They have also been adopted (as described by Wolfe, 2010) for a shorter classroom demonstration for non-laboratory courses. Unless otherwise noted, the data shown in the results section are from the most recent time the exercise was used (in a Sensation/Perception laboratory course, Spring 2012, 28 students).

Background reading and class materials for the lab covered retinal anatomy, dark adaptation, and spectral sensitivity (e.g., Wolfe et al., 2011, Chapter 2; Blake and Sekuler, 2005, Chapter 3).

Illuminance levels below were measured with a photometer, Tektronix J16, Illuminance Probe J6511. More inexpensive light meters (e.g., Light Meter LX1010B) can be purchased online for less than $20. Alternatively, illuminance can be estimated given the chart at http://en.wikipedia.org/wiki/Lux. If desired, photometry and a discussion of the physical aspects of light can be integrated into the lab and students could perform the illuminance measures themselves.

The introduction to the lab was given in a well-lit classroom (about 750 lux) so that students were light-adapted for 20 minutes or more. Each student was given two printouts, the grayscale and the red and blue squares shown in Figure 2.

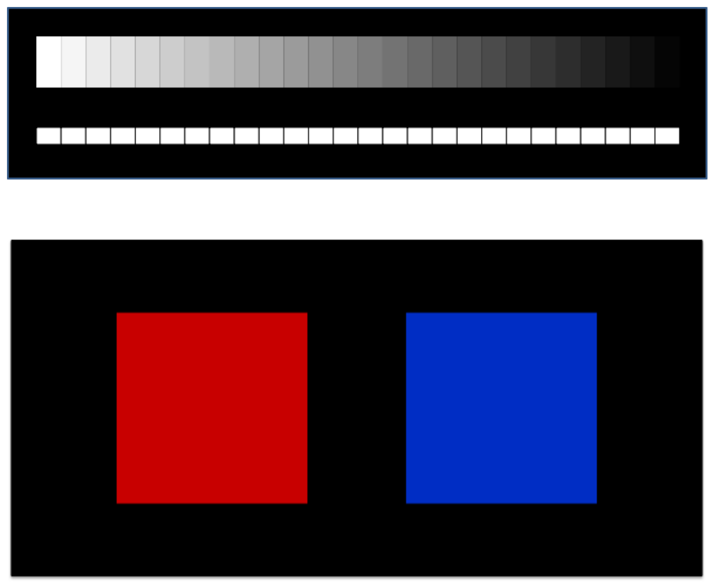

Figure 2. Top: Printed grayscale for measuring dark adaptation thresholds. The grayscale is created in MS PowerPoint with the left-most, lightest rectangle at a luminance value of 255 in PowerPoint’s units (HSL color model in custom color scheme, hue and saturation at 0) and each of the 24 succeeding rectangles decreasing in luminance by 5 units. (Note that the light amount reflected from the paper depends of course on the illuminant light and the reflectance of the paper. Thus for our methods, the PowerPoint units indicate relative, not absolute, luminance). Bottom: Iso-radiant squares used for Purkinje shift demonstration. Created in PowerPoint: Mac. Settings in HSB sliders: Hue: 359^0 (red), 240^0 (blue), Saturation: 100%, Brightness: 78%. Similar stimuli can be downloaded from http://www.yorku.ca/eye/purkink1.htm.

Students were asked to inspect the grayscale and to mark which of the rectangles they can detect against the black background while in the light (by writing “light” into the white space below the corresponding rectangle). They were also asked to record which of the two color squares looks brighter in the light. They were informed that they will repeat the following procedure as they sit in a dark room: Every two minutes, when prompted, they will mark on the grayscale the rectangle that they can just detect against the black background by writing the time in minutes (2,4,6 etc.) into the white space under the rectangle. After they are fully dark-adapted (at around 30 minutes) they will inspect the color patches and record which one looks brighter in the dark. They were reminded that they would not see color due to the colorblindness of the scotopic system. It might also be necessary to remind students that since any light exposure will interfere with dark adaptation, it is essential they do not use their mobile phones during the lab activity.

Students were then seated in a windowless room and the lights were turned off. Illuminance due to light underneath the door was about 0.5 lux, but varied slightly with distance from the door. Students were asked to keep their gaze into the same general direction throughout the experiment so that their exposure to light did not vary significantly over time. They immediately recorded which part of the grayscale they could detect (time=0 minutes). The instructor was seated immediately outside the door of the lab room and instructed students every two minutes to record their detection threshold. After marking their threshold at 30 minutes, students were asked to examine the color patches and to mark which patch appeared brighter.

After all measurements for dark adaptation and Purkinje shift were taken, students were asked to close one eye and to cover it tightly with one hand. The instructor then entered the room and turned the lights on (around 400 lux) so that students could light-adapt the open eye for two minutes, before the room was returned to the dark.

Students then opened each eye separately and marked for each eye their threshold luminance on the grayscale. Many were not able to see even the lightest part of the grayscale with the light-adapted eye. They then opened both eyes. (At this point, students often exclaim in surprise at the unusual experience, both visual and somatosensory, arising from the asymmetric dark adaptation of their two eyes). The instructor asked them to make a mental note of what sensations they are experiencing. Students were then asked specifically to take note of sensations they might have in the lid of and the skin surrounding the light-adapted ('blind') eye. (It is preferable to ask students to simply take a mental note of, rather than verbalizing, their sensations so as not to influence other students). Next, students covered one eye by hand leaving both eyes open and were asked to note any changes in the somatosensory sensations they noted earlier.

Students then returned to the regularly lit classroom. They were asked to indicate by a show of hands if they experienced the Purkinje shift so that the blue square looked brighter in the dark. They were also asked to share which if any unusual somatosensory sensations they had when the eyes were asymmetrically dark-adapted. They were also given time to plot their dark adaptation data, to compare their curves with those of other students and to discuss the reasons for any differences, such as their location relative to the light underneath the door.

Readers interested in a shorter version of this demonstration could refer to Wolfe (2010). The shorter version demonstrates the Purkinje shift, cross-modal illusion and dark adaptation, without however allowing for the recording of the dark adaptation curves in Figure 1.

RESULTS and DISCUSSION

As shown in Figure 4, all 28 students experienced the difference in luminance thresholds both over time and between the eyes after light-adapting only one eye. To illustrate the quantitative dark adaptation data, representative graphs of luminance thresholds over time are shown in Figure 3 for two students. Comparing these graphs to the standard curve in Figure 1, it can be seen that they show the characteristic time course of dark adaptation including the rod-cone break. Full adaptation is complete at 30 minutes, and thresholds plateau at around six minutes and then drop more rapidly again at around 8 minutes as rod vision takes over. 80% of the student graphs showed a clear rod-cone break, while the rest of the graphs showed a more steady decline of the thresholds with no discernible plateau at 6-8 minutes.

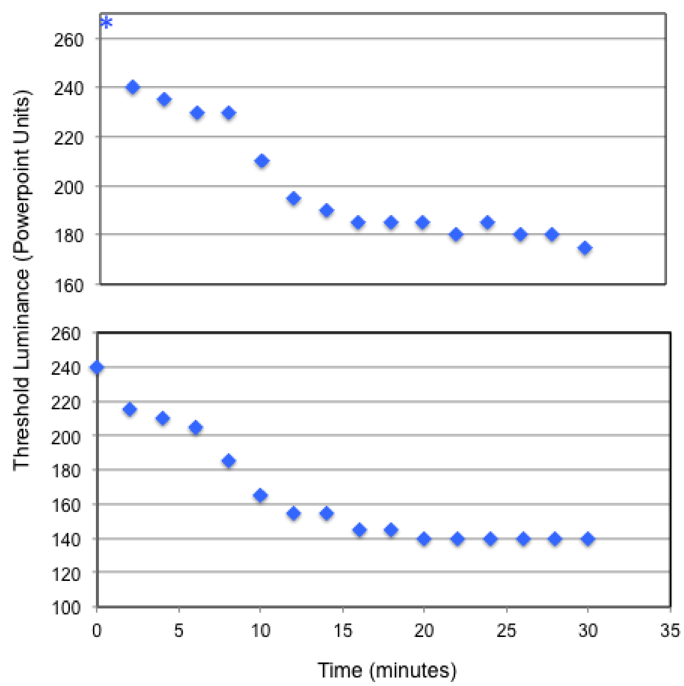

Figure 3. Two representative dark adaptation curves showing the characteristic time course of threshold reduction including the rod-cone break at 6-8 minutes. (*in the top figure at 0 minutes denotes that the threshold was above 255 PowerPoint Units, the brightest rectangle in the grayscale).

These data show that our paper grayscale is sufficiently sensitive for a quantitative measure of dark adaptation that yields the main characteristics of its time course documented in the standard literature. To our knowledge, there is currently no alternative apparatus available that would have this capability and that would at the same time be inexpensive and accessible enough for simultaneous use by all students in a mid-size undergraduate class. The more inexpensive methods developed for basic and clinical research over the years (e.g., Cheng et al., 1945; Patryas et al., 2013) still require substantial resources that few institutions could provide for an average size class.

One weakness of the measure (as mentioned in the footnote above) is that the luminance of rectangles in the grayscale depends on both the illuminant intensity and the reflectance of the paper surface. Thus the luminance values here are relative not absolute. In a room with non-uniform illuminance (such as ours where light entered from under the door) this might lead to differences in the curves with student location in the room. While we did not examine any consistent changes with location in our data, it could serve as a point of class discussion when students sitting in different locations relative to the light source have very different dark adaptation curves.

As illustrated in Figure 4, all but two students experienced the Purkinje shift such that the square that looked brighter changed from red (in the light) to blue (in the dark). Thus the paper stimuli in the bottom of Figure 2 serve as a reliable teaching tool in demonstrating the difference in spectral sensitivity of the scotopic and photopic system.

An in-depth discussion of the crossmodal illusion as a teaching tool can be found in Wolfe (2010). Here we only note that consistent with the data shown previously, all but two students reported the illusion (of the eyelid as sagging, closed, or numb) and all students experiencing it reported its reduction when covering the eye by hand.

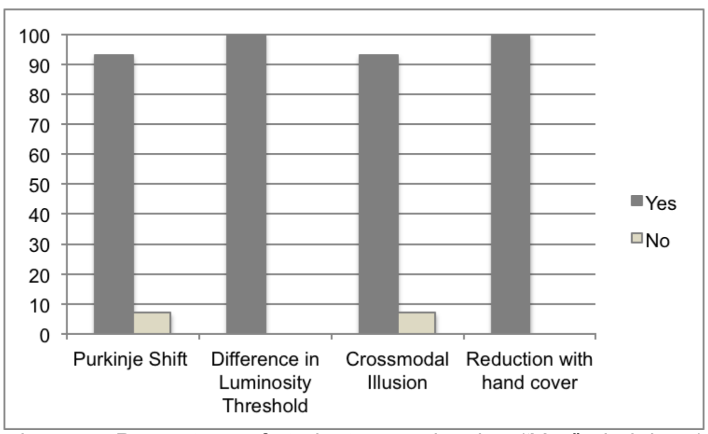

Figure 4. Percentage of students experiencing ('Yes"; dark bars) or not experiencing ('No'; light bars) each of the predicted effects.

In the mid-term evaluations of one recent lab class (Spring 2009), students (n=13) were asked to rate four lab activities on visual perception: the current dark adaptation lab, and labs on Weber's law, the contrast sensitivity function and ganglion cell activity. Labs were rated on a six-point scale (6= most positive) on four aspects: Ease of use, relevance to class, contribution to neuroscientific understanding, and level of interest. Overall, the dark adaptation lab was rated significantly higher than all other three labs individually and combined. (Overall mean rating: 5.3 (s=0.87) for dark adaptation and 4.9 (s=1.00) for the other three visual labs; independent $t(206)=2.89$, $p<0.01$). Figure 5 shows the means for all labs and questions. While all labs were rated as relevant to the course, the dark adaptation lab was rated higher on the other measures than most if not all other labs. This is particularly true for the rating of "interesting", perhaps due to the strength of the perceptual effects and the highly experiential nature of this lab.

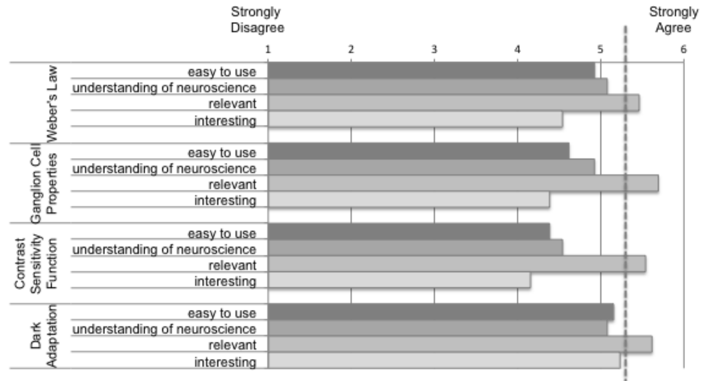

Figure 5. Student ratings (n=13) of the present dark adaptation lab and of three other visual neuroscience labs. The vertical dashed line denotes the mean of all ratings for the dark adaptation lab.

Students' positive response to the lab is also reflected in the ease with which discussion of quite complex principles (e.g., spectral sensitivity, photopigment regeneration, dark current) is generated subsequent to the activity.

While the ultimate evidence for the effectiveness of a teaching tool is of course an objective measure of performance, we do not at this point have data on this. The activity has been used in all relevant lab courses taught by the instructor so that there is no meaningful control group at this time.

Despite this limitation, the demonstration outlined here has been shown to be a valuable and accessible teaching tool. The perceptual phenomena can be reliably produced in the majority of students and provide a compelling first-hand experience of complex neuroscience principles. The lab allows students to collect, graph and analyze qualitative as well as quantitative data. The quantitative data on dark adaptation follow those found in the standard literature with much more sophisticated tools. Students rate the activity as highly positive, even when compared to other visual neuroscience labs. It can be used to teach several important neuroscience concepts, and easily leads into a discussion of topics, such as spectral sensitivity, multi-sensory integration and retinal circuitry, that could otherwise seem dry and overly technical.

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Address correspondence to: Dr. Uta Wolfe, Department of Psychology, University of St. Thomas, St. Paul, MN 55105. Email: uta.wolfe@stthomas.edu