A behavioral measure of dark adaptation in the American red squirrel*

ROBERT DIPPNER and JOHN ARMINGTON
Northeastern University, Boston, Mass. 02115

A behavioral tracking technique was used to investigate dark adaptation in two American red squirrels (*Tamiosciurus hudsonicus*). Increase of sensitivity was followed over a range of about 4 log units. A break typical of duplex retinas appeared in the dark-adaptation curve about 18 min after the beginning of adaptation. More than 30 min was required for complete adaptation, with the final threshold of the squirrel about 1.5 log units above that of the human measured on the same apparatus.

According to duplicity theory, the visual systems of some species are made up of two such systems, which take their origin with two types of receptors, the rods and the cones. The rod and cone systems in duplex retinas typically mediate vision at different levels of illumination. Both systems increase their sensitivity in darkness, but not to the same degree. The cone system adapts more rapidly and over a smaller range than does the rod system. As a result, one obtains discontinuous dark-adaptation curves with two sectors, each reflecting one of the receptor activities, from organisms whose retinas possess both systems. Such a curve was demonstrated long ago in humans (Kohlrusch, 1922; Hecht, 1937). The general aim of the present study was to obtain dark adaptation data for the American red squirrel, using a psychophysical tracking procedure, and to see if evidence for duplex function could be found.

Although studies using various methods have established duplex functioning in a variety of animal species, the issue has not been completely resolved for squirrels (Arden & Tansley, 1955; Tansley, 1957). A number of anatomical, electrophysiological, and behavioral studies exist, but their results appear to be inconsistent with one another. Some seem to give evidence for duplex vision, while others give evidence for cone vision only.

At first most investigators believed that squirrels of all species possessed pure-cone retinas. It was recognized that there were two general types of squirrels, those that live in trees and those that live on the ground. In addition, the retinæ are known to be not identical, tree squirrels having a double layer and ground squirrels a single layer of receptor cells. This difference was believed unimportant for visual function. After completing a

detailed anatomical study of both tree and ground squirrel retinas, Arden & Tansley (1955a, b) found that all of the receptors in both layers of the tree squirrel are morphologically similar to those of the single-layered retina of the ground squirrels. Weale (1955) measured a pigment from a tree squirrel species which had a spectral sensitivity curve typical of cones. In the first electroretinal studies, both tree and ground squirrels were shown to have rapid dark adaptation, with a lower limit slightly above the human photopic threshold. Electrophysiological studies of squirrel seemed to have the spectral properties which would be expected of pure-cone retinas (Tansley, Copenhaver, & Gunkel, 1961).

The advent of the electron microscope has now made it possible to see differences among the squirrel receptors. The ground squirrel, *Citellus*, possesses a retina with a single type of receptor with synaptic

connections typical of cone systems (Dowling, 1964). At least one tree squirrel, *Sciurus*, possesses a two-receptor retina with one type of receptor that may be the basis for a cone system and another type that may be the basis for a rod system (Cohen, 1964).

Several recent behavioral and electrophysiological studies (Arden & Silver, 1962; Dodt, 1962; Gouras, 1964; and Silver, 1966) also indicate that many tree squirrels have duplex retinas. A shift in peak sensitivity (Purkinje shift) after dark adaptation and also a dark-adapted threshold about 1 log unit above the human scotopic threshold have both been reported for *Sciurus*, using behavioral procedures (Silver, 1966). This threshold is much lower than one could expect for a pure-cone retina. Finally, Dartnall (1960) has shown that there are, in fact, two pigments in the tree squirrel retina. One of these is a rhodopsin.

Information dealing with duplex vision in red tree squirrels is incomplete. One investigator (Dodt, 1962), using the electroretinogram to follow dark adaptation in the European red squirrel (*Sciurus vulgaris*), found an increase in sensitivity ranging up to 5 log units. Such a range of sensitivity is evidence for two systems; the increment is too great for one. A discontinuity appeared just before 15 min after the start of dark adaptation and was followed by 2 log units' further increase in sensitivity during the next 15 min. Later, Liu, Yang, & Li (1965) reported an overall increase in sensitivity of less than 4 log units in the Oriental red squirrel (*Callosciurus*

Fig. 1. Diagram of the apparatus. Lamp is tungsten light source, FH is fixed value neutral density filter holder, S is shutter, W is neutral density circular wedge, SP is stimulus plate. The lower left corner shows how the stimulus appeared to the S.

*This research was supported by Public Health Service Grant 5 ROI EY-00589.

erythmaeus), using the same technique and preexposure conditions as Dodt. A discontinuity appeared about 18 min after the start of dark adaptation, but was followed by an increase of sensitivity amounting to only 0.3 log units. The dark-adapted thresholds of the squirrels in both studies were nearly equal to about 1 log unit above the human scotopic threshold. These physiological data, although incomplete, point to a duplicity of function.

Direct behavioral procedures can best clarify the picture. There have not been many well-controlled behavioral studies and none on dark adaptation in squirrels. Dark adaptation is a useful measure because it gives us two indices that can point to duplicity of function, the "rod and cone" break due to the difference in rate of adaptation and the overall range of adaptation which may be too great for one system.

METHOD

The behavioral method used in this study was the tracking method developed by Blough (1958). Blough's technique produces sensory data from animals comparable to reports of trained human Os. The procedure is a useful way to get a nonverbal organism to describe its sensations. One advantage of such a method is that it allows tracking of continuously or rapidly changing thresholds of the animal over time. Another advantage is that the method employs two keys so that the animal always has something to do. The Ss in this experiment were provided with two response keys, one of which (Key A) decreased and the other of which (Key B) increased stimulus luminance. The two keys permitted the S to control stimulus luminance as he adapted, so that luminance was always close to threshold. One can think of the method as a control system, the stimulus luminance controlling the behavior of the organism and, in turn, the behavior of the organism controlling the stimulus intensity. Blough's method was originally developed for use with pigeons but has been used successfully with many other species of animals (Blough, 1966).

APPARATUS

A diagram of the apparatus is shown in Fig. 1. Light was produced by a tungsten filament lamp operated at a constant 6 V 16 A. A pair of lenses, L_1 and L_2 , was used to image the filament on the vanes of a shutter, S. The shutter, with a narrow vertical aperture, was under electric control and could be used to switch the stimulus off and on. After passing the shutter, the beam was collimated by Lens L_3 , and focused again by

Lens L_4 , on a 4-log-unit circular neutral density wedge. Since the beam was narrow at this point, a compensating wedge was not required. L_5 collimated the beam and distributed it uniformly over a translucent diffusing plate located on the end wall of the test cage directly between and slightly above two response keys. The keys were connected through logic circuits to a stepping motor which controlled the position of the circular wedge. Responses on Key A turned the wedge so as to decrease the luminance of the stimulus in .16-log-unit steps; responses on Key B increased the luminance in steps of similar size.

The test cage was attached to the end of the optical system, as shown in Fig. 1. The inner wall of the test cage had a 2-in. recessed opening cut in its center, about 2 in. above the floor. The two response keys were just inside, and the stimulus plate was mounted between the keys against the outer wall. To make a response, the squirrel put his head into the opening, which raised the probability of his attending to the stimulus, and pushed down on the keys with his nose. The interior of the test cage, painted flat white, was uniformly illuminated from above through a sheet of flashed glass by a 150-W light bulb. This produced a luminance of about 100 m μ L on the floor and walls, as measured by a Spectra Spot Meter.

The S was reinforced with sunflower seeds. A seed dispenser mounted above the cage dropped seeds through a chute to a tray between the response keys.

PROCEDURE

The squirrel was trained to respond on Key A until the shutter closed or

the stimulus intensity fell below his threshold. Then the S responded on Key B until the shutter opened or the stimulus intensity exceeded his threshold. Finally, the squirrel returned to Key A to begin a new cycle. The squirrel was reinforced only for pressing Key B and then only when the shutter was actually closed. The probability of reinforcement for a response on Key B when the shutter was closed was one-half. Responses on Key A did not bring reinforcements, although they had to be made in order to turn the stimulus off. A shift from pressing Key A to pressing Key B with the shutter open was taken as an indication that the stimulus was below the S's threshold.

A rather long and complicated shaping procedure is necessary to train the Ss in this sort of procedure (Blough, 1958). Certain contingencies are necessary to maintain tracking behavior. The final step, for example, included provisions for denying reinforcement for a complete cycle if the S responded to Key A after the shutter had closed. Occasionally, two reinforcements could be earned during the period when the shutter was closed to insure that the S would not immediately return to Key A after a reinforcement.

In an actual test run, the S was placed in the apparatus with the interior light on for 10 min of preadaptation. During this time, the stimulus intensity was held constant about 1 log unit above a threshold determined with the preadapting light turned on. The S made no shift from Key A to Key B unless the stimulus actually went off. Typically, the squirrel was required to make one to

Fig. 2. Dark adaptation curves for two squirrels. Each curve represents a different S and is the average of three consecutive runs. Each point on a curve represents the wedge density at which the S shifted from Key A to Key B.

five responses, number required randomly determined, on Key A to turn the stimulus off and then another one to three responses, number again randomly determined, on Key B to turn it back on again. A complete cycle took from two to eight responses and was terminated about one-half the time with a reinforcement. After 10 min with stimulus intensity held constant, the interior light went off, the stimulus intensity came under the control of the squirrel, and a fixed 1-log-unit neutral filter was placed in the light beam. The S spent the next 40 min in continuous performance, with only short pauses to consume earned sunflower seeds. Usually 120 reinforcements were earned in one session, which required over 1,200 responses.

SUBJECTS

The Ss were two feral red squirrels (*Tamiosciurus hudsonicus*). They were maintained at 85%-90% of their ad lib weight throughout the experiment.

RESULTS

The main results of this experiment are the dark-adaptation curves (Fig. 2). Each of the curves is based on three complete experimental sessions. The points on the dark-adaptation curves in Fig. 2 are the average stimulus intensities at which the S shifted from Key A to Key B when the stimulus was actually on. At first, the curves fall rather rapidly, with a large increase in sensitivity occurring in the first 10 min. An additional increase begins at about 18 to 20 min after the start of adaptation and is completed 5 to 6 min later. The first increase is about 3 log units in magnitude and the second increase is about 1 log unit. Thus, with this procedure, dark adaptation could be followed over a total range of about 4 log units.

After the interior light was turned off, the Ss halted all activity for up to 1 min. Then they pressed Bar A and, typically, did not shift from A to B unless the stimulus actually went off during the first minute or so. This suggests that their threshold was decreasing too rapidly to be tracked with the apparatus used. The remainder of the dark-adaptation period was characterized by relatively continuous responding to the keys.

As a control, a 0.6 or 1.0 neutral density filter was occasionally placed in the light beam as the S was performing. The S immediately corrected for the reduction this produced in the stimulus by moving the filter wedge to increase the intensity.

A human S was tested in the same apparatus in order to compare results. The human S viewed the stimulus

binocularly from a distance of 18 in. At this distance, the stimulus subtended a visual angle of 1.5 deg. By using switches at his fingertips, the human S was able to track his threshold down to an intensity representative of the human scotopic threshold.

DISCUSSION

The results of the present experiment provide clear evidence that the American red squirrel, like other tree squirrels, has a duplex visual system. The dark-adaptation curves found in the present study had two limbs, as did those found in two physiological studies (Dodt, 1962; Liu et al, 1965) of red squirrels. The results from the present study have shown that the range of adaptation extends over at least 4 log units, equal to the range found by Liu but short of the 5-log-unit range determined by Dodt. Although the behavioral and physiological data agree well with one another on a qualitative basis, there were clear-cut quantitative differences. This is not surprising. Even the data for the well-investigated human eye do not stand in complete agreement (Pirenne, 1962).

Dodt found a much greater change in sensitivity than did Liu, with most of the change taking place after the "rod-cone" break. Furthermore, the break occurred earlier in his study than in Liu's. Even if the physiological results were completely consistent, one still must determine dark adaptation behaviorally. Species differences may account for some of the variance, but the problem of trying to conduct behavioral and physiological experiments under similar conditions must also be taken into account. Now that a convenient way exists for following dark adaptation in red squirrels, it would be interesting to manipulate the two limbs experimentally to see if the lower could be made more distinct, as it is in Dodt's ERG curves.

SUMMARY

(1) Blough's behavioral technique for measuring dark adaptation in pigeons can be successfully employed with squirrels.

(2) The threshold of the American red squirrel was found to change at least 4 log units, going from a light-adapted state to a dark-adapted state. The final threshold, as measured with this technique, is about 1.5 log units above the human scotopic threshold.

(3) A discontinuity appears in the dark-adaptation curve after about 18 min. Following this, there is an additional increase in sensitivity amounting to about 1 log unit.

(4) Points 2 and 3 point to duplex functioning of the American red (tree) squirrel visual system.

REFERENCES

- ARDEN, G. B., & SILVER, P. H. Visual thresholds and spectral sensitivities of the grey squirrel (*Sciurus carolinensis leucotis*). *Journal of Physiology*, London, 1962, 163, 540-557.
- ARDEN, G. B., & TANSLEY, K. The spectral sensitivity of the pure-cone retina of the souslik (*Citellus citellus*). *Journal of Physiology*, London, 1955a, 127, 225-232.
- ARDEN, G. B., & TANSLEY, K. The spectral sensitivity of the pure-cone retina of the grey squirrel (*Sciurus carolinensis leucotis*). *Journal of Physiology*, London, 1955b, 127, 592-602.
- BLOUGH, D. S. A method for obtaining psychophysical thresholds from the pigeon. *Journal of the Experimental Analysis of Behavior*, 1958, 1, 31-43.
- BLOUGH, D. S. The study of animal sensory processes by operant methods. In W. K. Honig (Ed.), *Operant behavior: Areas of research and application*. New York: Appleton-Century-Crofts, 1966. Pp. 357-383.
- COHEN, A. I. Some observations on the fine structure of the retinal receptors of the American gray squirrel. *Investigative Ophthalmology*, 1964, 3, 198-216.
- DARTNALL, H. J. A. Visual pigment from a pure-cone retina. *Nature*, London, 1960, 188, 475-479.
- DODT, E. Vergleichende Untersuchungen über das adaptive Verhalten reiner Zapfennetzhaute (*Citellus citellus*, *Sciurus vulgaris*). *Pflügers Archiv*, 1962, 275, 561-573.
- DOWLING, J. E. Structure and function in the all-cone retina of the ground squirrel. In: *The physiological basis for form discrimination*, a symposium sponsored by the Visual Sciences Study Section, NIH, at Brown University, Providence, R.I., January 23-24, 1964. Pp. 17-23.
- GOURAS, P. Duplex function in the grey squirrel's electroretinogram. *Nature*, London, 1964, 203, 767-768.
- HECHT, S. Rods, cones, and the chemical basis of vision. *Physiological Review*, 1937, 17, 239-290.
- KOHLRAUSCH, A. Untersuchungen mit farbigen Schwellenprüflichtern über den Dunkeladaptation verlauf des normalen Auges. *Pflügers Archiv d. Gesamte Physiologie*, 1922, 196, 113-117.
- LIU, Y., YANG, C., & LI, J. Some observations on the electroretinogram of the pure-cone eye of a species of tree squirrel. *Acta Physiologica Sinica*, Peking, 1965, 28, 243-262.
- PIRENNE, M. H. Dark adaptation and night vision. In H. Davson (Ed.), *The eye*. Vol. II. New York: Academic Press, 1962. Pp. 93-119.
- SILVER, P. H. A Purkinje shift in the spectral sensitivity of grey squirrels. *Journal of Physiology*, London, 1966, 186, 439-450.
- TANSLEY, K. Some observations on mammalian cone electroretinograms. *Bibliotheca Ophthalmologica*, 1957, 48, 7-14.
- TANSLEY, K., COPENHAVER, R. M., GUNKEL, R. D. Spectral sensitivity curves of diurnal squirrels. *Vision Research*, 1961, 1, 154-165.
- WEALE, R. A. Bleaching experiments on the eyes of living gray squirrels (*Sciurus carolinensis leucotis*). *Journal of Physiology*, London, 1955, 127, 487-491.