

## Rod Photoreceptors and Scotopic Vision in Ground Squirrels

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**ABSTRACT** Ground squirrel retinas contain a relatively small complement of rods (5–10% of all photoreceptors) which are thought to provide the basis for a weak scotopic visual capacity. In a previous investigation of the California ground squirrel (*Spermophilus beecheyi*) involving the recording of a retinal gross potential, the electroretinogram (ERG), electrophysiological evidence for a viable scotopic signal could be obtained from some, but not all of the ground squirrels examined. To further pursue the possibility that there is a structural/functional discrepancy in the relationship between rod photoreceptors and scotopic vision in the ground squirrel, several experiments involving electrophysiological, behavioral, and anatomical observations have been conducted. We found that although about one-third of the ERGs recorded from a large sample of California ground squirrels lack those characteristics which would indicate the presence of a viable scotopic signal, the retinas of all the squirrels appear to contain the same small population of rod photoreceptors. Additional experiments on the golden-mantled ground squirrel (*Spermophilus lateralis*), including behavioral as well as ERG measurements and anatomical observations, lead to this same conclusion.

Although for years it was believed that the retinas of the ground-dwelling sciurids contained only cones, it has recently been shown that a number of rods are also present in these retinas. As judged according to a variety of morphological criteria, the proportion of rods in several different species has been found to fall somewhere in the range of 4–10% of the total number of photoreceptors (West and Dowling, '75; Fisher et al., '76; Jacobs et al., '76). It has further been shown that these rods seem to provide the basis for a functional scotopic visual system, primarily in the appearance of a shift in the spectral sensitivity of the electroretinogram (ERG) when the adaptation state of the eye is altered (Green and Dowling, '75; Fisher et al., '76; Jacobs et al., '76). However, a puzzling aspect of our previous investigation of the California ground squirrel (Jacobs et al., '76) was that it proved impossible to find such electrophysiological evidence in all of the animals examined. Thus, of ten animals studied three gave no ERG evidence for a functional scotopic system even though they were carefully tested under conditions identical to those which did yield such evidence in the majority of animals tested. There are a variety of possible reasons for this discrepancy, including either

inadequacies or insensitivities in the measures employed. An alternative and more interesting possibility, however, is that there might actually be significant within-species variations in either the presence of retinal rods, or of a functional scotopic system, or both. We have examined this issue further by making behavioral, electrophysiological, and anatomical observations on ground squirrels.

### METHODS

Details of the behavioral, electrophysiological, and anatomical techniques used have all recently been described elsewhere. Briefly, the methods were as follows.

#### *Electrophysiological methods*

The animals were anesthetized with a mixture of sodium pentobarbital (45 mg/kg) and chloral hydrate (250 mg/kg). Supplemental anesthesia was given during the course of the experiment as required. Core temperature was maintained at 37°C through the use of a rectal thermometer and a circulating hot water heater. ERGs were recorded using tungsten electrodes which were inserted transclerally into the posterior chamber (Jacobs et al., '76). The ERG signals were recorded differentially

through an amplifier having a bandpass set at 0.2–1,000 Hz. These signals were averaged in an Ortec 4623 Signal Averager and then written out on an X-Y plotter. The ERG responses were elicited by 500-msec light flashes obtained from a double-beam Maxwellian-view optical system. One beam originated from a tungsten-filament lamp, the other from a high-intensity grating monochromator. These two beams were optically combined at a point prior to the final lens so that their combination illuminated a circular patch of the central retina 40° in extent. One of these beams could be used to provide an adaptation light while the other provided the test flashes. Further procedural details of the ERG experiments are given below.

#### *Anatomical methods*

The methods were basically the same as those described previously (Jacobs et al., '76). The eyes were fixed by intracardiac perfusion of approximately 400 ml of a fixative consisting of 1% glutaraldehyde, 1% paraformaldehyde, and 0.04% picric acid buffered with 0.067 M sodium cacodylate with 0.05% CaCl<sub>2</sub> added (pH 7.4). The eyes were then removed and the anterior chamber was cut away. The posterior chamber was immersed in the fixative for an additional 1.5 hours at 4°C. Next, the retinas were rinsed in an isotonic buffer and then postfixed in OsO<sub>4</sub> (2% solution) in veronal acetate buffer (pH 7.4) for one hour. The tissue was then rinsed in distilled water, dehydrated in a graded ethanol-water series, transferred through propylene oxide, and embedded in Araldite.

Sections were cut on ultramicrotome (Porter-Blum MT 2B). Thin sections (1 μm) for light microscopy were stained with toluidine blue. Thin sections for examination in the electron microscope were placed on mesh or bar grids, or on formvar-coated slot grids for serial examination. These sections were stained with a 1% solution of uranyl acetate for 20 minutes, followed by lead citrate for ten minutes.

#### *Behavioral methods*

Measurements of visual sensitivity were made in a three-choice discrimination apparatus described elsewhere (Jacobs, '78). The squirrel viewed three small translucent panels each of which had an adjacent response lever and a cup into which food reinforcement was dispensed. The panels were transilluminated from light sources mounted outside of the test chamber. Through a training procedure the squirrels were instructed to select that stimulus panel which was illuminated differ-

ently from the other two, which were identical in appearance. A correct choice resulted in the delivery of a 97-mg peanut-flavored food pellet. Over test trials the intensity of the test light was varied (in steps of 0.2 or 0.3 log units) so as to establish threshold sensitivity, i.e., that value of the test light which was just discriminable from the other two lights. In the experiments reported here, all three panels were steadily illuminated with achromatic lights which could be set at various luminance levels. Trial sequencing and timing, control of reinforcement delivery, control of test stimulus parameters, and the recording of subject responses were all done automatically. A number of procedures were also employed to insure that no inappropriate cues were available to influence the animal's discrimination (Jacobs, '78). Typically, each animal received about 200 test trials in each daily test session.

## RESULTS

### *1. The California ground squirrel*

In a previous experiment (Jacobs et al., '76) we were unable to find ERG evidence for the presence of a scotopic mechanism in each of the California ground squirrels was studied. In an (*beecheyi*) examined. To determine if this was a reliable finding, a new and larger sample of California ground squirrels were studied. In an attempt to rule out as many extraneous factors as possible, a rigidly prescribed protocol was followed in these experiments. The details of the procedure were as follows. After the animal had been positioned in a stereotaxic instrument, placed in the recording chamber, and the electrode had been inserted in the eye, the animal was allowed an additional 45 minutes of complete dark adaptation. After that period, lights having wavelengths of 500 and 600 nm were alternately presented, first at very low intensities and then at progressively higher levels until reliable ERGs were elicited. The responses to at least ten separate stimulus flashes were averaged at each intensity-wavelength combination. In each case the intensity needed to produce a 5-μV response to both the 500 and 600 nm stimuli was determined. The difference in threshold to the 500 and 600 nm stimuli based on the operation of a typical mammalian rod photopigment ( $\lambda_{\max} = 500$  nm) is 1.40 log units; the difference in threshold to these two wavelengths expected on the basis of this squirrel's normal photopic sensitivity (Jacobs et al., '76) is 0.71 log units. If the difference in threshold corresponded to the prediction based on a 500 nm photopigment it was concluded that the

squirrel had a "normal" scotopic system and the experiment was terminated. On the other hand, if the threshold difference did not correspond to this prediction the animal was dark adapted for an additional 45 minutes and the test procedure repeated. Again, if the second test matched the 500 nm photopigment prediction the experiment was terminated. If it did not, sufficient measurements were made to accurately determine what the difference in sensitivity to 500 and 600 nm actually was in the dark-adapted eye. All animals that failed to show the expected scotopic difference were re-tested in the same manner after an interval of two weeks, the second test made on the other eye. Several animals were tested on multiple occasions.

The essential results of the survey are shown in fig. 1. Plotted there is the threshold difference in sensitivity to 500 and 600 nm test lights for each of 30 California ground squirrels (18 males, 12 females) tested under conditions of complete dark adaptation. These values are quantally based and are corrected for preretinal absorbance in this species (Yolton et al., '74). As can be seen, these animals fall into two distinct groups. A majority of the animals (21 of 30) showed dark-adapted sensitivity differences very close to that predicted by the operation of a 500 nm photopigment. The vertical arrow on the right side of the figure shows the 500 nm photopigment prediction, while the vertical line immediately above the arrow is the computed mean difference for the group. The two figures are very close—a 500 nm photopigment prediction of 1.40 log units and an actual mean difference of 1.39 log units. The horizontal line encloses two standard deviations about the mean. On the other hand, for several animals (Xs in fig. 1) the sensitivity difference was very much less than that predicted by the operation of a 500 nm pigment. The values for these animals (9 of 30) are on the left in fig. 1. The mean for this group is very close to that expected from the photopic sensitivity of this ground squirrel species—a prediction of 0.71 log units (vertical arrow) and a mean value of 0.69 log units (vertical line). In the case of this latter group the plotted points represent the mean obtained for all recording sessions; this includes at least two such sessions for each animal.

The results shown in fig. 1 verify the outcome of our earlier experiment by demonstrating that it is not possible to find evidence for a viable (i.e., capable of generating a threshold level signal) scotopic system in the ERGs

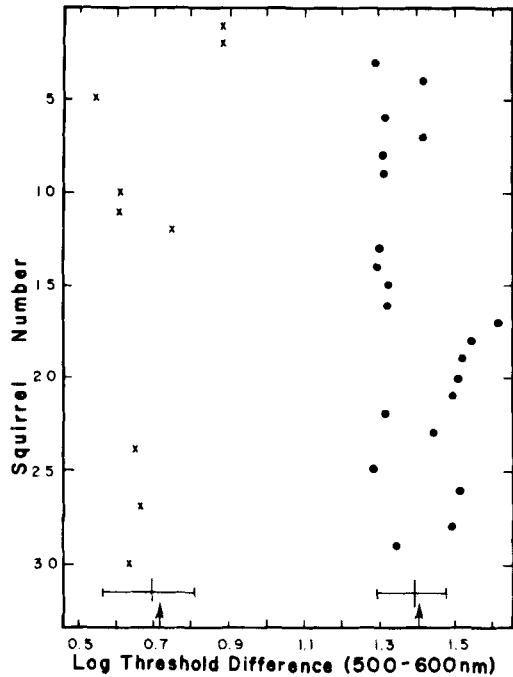


Fig. 1 Differences in ERG thresholds for 500 and 600 nm test lights determined for each of 30 California ground squirrels. Each plotted point represents the results from one animal. The vertical arrow on the right shows the threshold difference predicted by the operation of a photopigment having a  $\lambda_{max}$  at 500 nm, while the vertical arrow on the left shows an analogous prediction based on the photopic spectral sensitivity of this species. The mean threshold difference for each group of animals is given by the large vertical line. The horizontal lines drawn through these enclose two standard deviations around the mean values.

recorded from all California ground squirrels. Several other aspects of this survey are worthy of comment. First, since the same voltage criterion was used to measure sensitivity in all animals it is very unlikely that the differences found between animals could be attributed to any systematic variation in the quality of the recording. Second, no significant correlations were found between measured threshold differences and other obvious subject characteristics. Thus, the difference was not related to the sex of the animals (25% of the females tested failed to show a viable scotopic system as compared to 33% of the males). Nor did age seem critical. By using body weight and gross morphology the squirrels could be roughly divided into young-adult and fully adult groups; neither of these two was unduly represented in the two groups shown in fig. 1. Finally, to rule out the possibility that there might be some sort of a sea-

sonal variation, all of the squirrels were trapped and tested during a two-month summer period.

An examination of the actual threshold values to 500 and 600 nm stimuli reveals that the group of animals apparently lacking the usual scotopic mechanism was actually very slightly more sensitive to the 600 nm test light than those animals showing the normal scotopic mechanism (mean difference = 0.08 log units). On the other hand, at 500 nm this same group had higher thresholds than did those animals showing the normal scotopic mechanism (mean difference = 0.34 log units). This latter difference is very close to that estimate previously offered (Jacobs et al., '76) as an indication of how much of a sensitivity increase the rod-based system adds to vision in the California ground squirrel.

The eyes from two squirrels shown to lack a scotopic mechanism were removed and prepared for electron microscopy. In the California ground squirrel, rods can be distinguished from cones by using several different morphological criteria (Jacobs et al., '76): (a) the rod inner segments are located slightly more vitread than are those of the cones, (b) the rod outer segment is slightly longer and thinner than that of the cone, (c) the terminal structures differ in that the cone terminal is larger and contains more synaptic ribbons than does the rod terminal, and (d) the outer segments of the two types of receptors show characteristically different constructions, the rod outer segment consisting of a stack of discs each independent from the plasma membrane (except at the very base of the outer segment—see fig. 9), while the cone discs appear as a continuously infolded plasma membrane in the lower half of the outer segment. Of these differences, the degree of disc-membrane continuity in the outer segment provides the most unambiguous criterion for distinguishing ground squirrel rods from cones (Anderson and Fisher, '76).

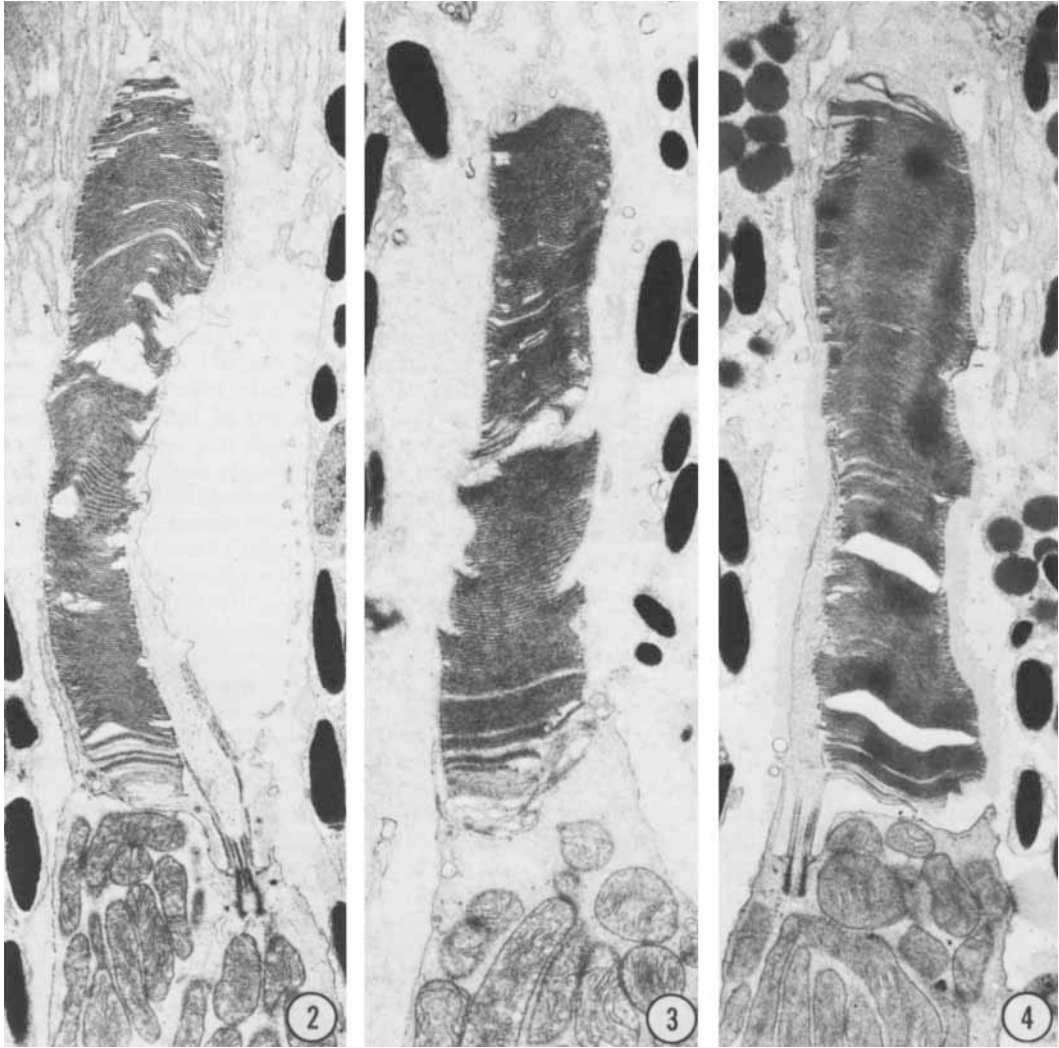
It proved to be a relatively easy task to distinguish the presence of rods in the retinas taken from each of these animals. Figure 2 is a micrograph of a rod found in the eye of one of these animals. The rods of these animals did not differ in any discernable way from abundant material already available from other squirrels previously shown to possess the usual scotopic sensitivity. Tissue from one of these animals was considerably better fixed than that from the other, so, using that animal, counts were made in the electron microscope of the number of rods and cones found in the cen-

tral retina. Of 162 photoreceptors which could be unambiguously classified one way or the other, 14 were clearly rods. This proportion (8.6%) is well within the range reported for similar surveys done on other ground squirrels (Fisher et al., '76; Jacobs et al., '76; West and Dowling, '75).

To summarize the observations on the California ground squirrel, as judged by the use of a conventional electrophysiological index (the b-wave of the ERG), there is strong evidence that not all individuals show a functional scotopic visual system. On the other hand, using the best available morphological criteria, no ground squirrel retina has ever been found to be rod-free, even those previously shown to give no ERG indication of a viable scotopic visual system. A second series of observations which lead to this same general conclusion were made on the golden-mantled ground squirrel (*Spermophilus lateralis*) and these are reported next.

## 2. The golden-mantled ground squirrel

Observations on a pair of adult male golden-mantled ground squirrels were initiated as a result of our discovering that the two showed substantial differences in visual sensitivity in some behavioral experiments which were being conducted for another purpose. The behavioral experiments involved measuring sensitivity to a monochromatic test light (519 nm) when that light was added to steady achromatic backgrounds covering a wide range of luminance values. Figure 5 shows the results of this experiment for the two squirrels. Each plotted point there represents an independently measured threshold value for the test light as a function of the luminance of the background to which it was added. When the test light was added to backgrounds of moderate luminance, the measured thresholds for the two animals were very similar (right side of fig. 5). However, when the background luminance levels were below about 0.1 cd/m<sup>2</sup>, the threshold values for one squirrel ( $\Delta$ s) continued to decline while the other animal showed only slight further gain in sensitivity below this point. The result of this divergence was that although the two animals were equally sensitive when adapted to lights having a luminance greater than about 0.1 cd/m<sup>2</sup>, they were no longer so at lower adaptation levels; indeed, at a background luminance of 0.002 cd/m<sup>2</sup>, one animal was about one log unit more sensitive than the other.



**Figs. 2-4** Electron micrographs of representative rod outer segments (ROS) from three different ground squirrels. The slight disruption of outer segment structure by fixation artifact is comparable to what has been reported in other ground squirrel species (Jacobs et al., 1976; Fisher et al., 1976; Anderson and Fisher, 1976). Melanic-containing processes from the retinal pigment epithelium completely drape the outer segments.

**Fig. 2** A rod outer segment from the California ground squirrel in which there was no electrophysiological evidence for viable scotopic function.  $\times 17,600$ .

**Fig. 3** A rod outer segment from the golden-mantled ground squirrel in which there was both behavioral and physiological evidence for scotopic and photopic vision.  $\times 12,400$ .

**Fig. 4** A rod outer segment from the golden-mantled ground squirrel in which no behavioral or physiological evidence for a viable scotopic function could be found.  $\times 12,400$ .

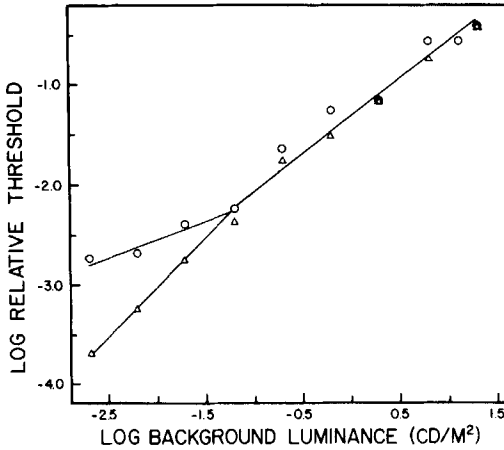


Fig. 5 Increment-threshold functions for two golden-mantled ground squirrels as obtained in behavioral discrimination tasks. Each plotted point represents an independently determined threshold for a 510 nm test light presented on an achromatic background light. Note that the thresholds for the two animals were essentially the same when the background light had a luminance of 0.1 cd/m<sup>2</sup> or higher but were considerably different at lower luminance levels.

The most reasonable interpretation of the behavioral results shown in fig. 5 is that although both squirrels had identical mechanism controlling visual sensitivity when the eye was adapted to moderate level lights one of them had an additional mechanism operative at low light levels which the other apparently lacked. In the context of the results on the California ground squirrels reported above, it appeared that these behavioral experiments had fortuitously identified a pair of individuals representative of the two groups illustrated in fig. 1. To pursue this possibility further, ERGs were recorded from the eyes of the two golden-mantled ground squirrels.

Using the procedures described previously, ERG spectral sensitivity functions were determined for both of the squirrels under conditions of complete dark adaptation. These functions are shown in the left panel of fig. 6. The symbols used there are the same as those used to identify the animals in fig. 5. Each point represents the reciprocal quantal flux required to generate an ERG having a criterion amplitude of 10  $\mu$ V. The double symbols at each test wavelength reflect the fact that each animal was tested twice, once with each eye. Two things are apparent from these experiments. First, one animal showed substantially higher sensitivity in the dark to all test wavelengths shorter than about 600 nm. Second, the spectral sensitivities for the two differed. The more sensitive animal

was maximally sensitive at 500 nm and the full function is well predicted by the absorbance curve for a 500 nm photopigment. The less sensitive animal also showed a different spectral sensitivity, one having a peak at about 520 nm.

Recordings were also made from the two squirrels under conditions of light adaptation. The right panel of fig. 6 shows ERG spectral sensitivity functions for the two animals when their eyes were continuously exposed to an achromatic light (color temperature = 4,800°K) having a luminance of 1 log cd/m<sup>2</sup>. It is apparent that under conditions of light adaptation there are no differences in spectral sensitivity for the two animals. Taken together, the ERG experiments provide strong confirmation of the behavioral results. Thus, in both cases, the two squirrels showed nearly identical visual capacity under condition of light adaptation, whereas when the eye was adapted to dim lights, or were completely dark adapted, one animal was more sensitive and showed a different spectral sensitivity. One animal clearly appeared to have a capacity based on a 500 nm photopigment that the other lacked. On this basis the most straightforward assumption would be that one animal had the usual complement of ground squirrel rods while the other did not. To test this possibility extensive anatomical observations were made on the retinas taken from both animals.

The eyes from both golden-mantled ground squirrels were removed and prepared for electron microscopy. The general structural organization of the retina of this species is illustrated in fig. 7. It appears very similar to the retinas of several other species of ground squirrels (West and Dowling, '75; Fisher et al., '76; Jacobs et al., '76). The identities of the two animals were coded so that the individuals examining the tissue did not know which eyes came from the animal showing the normal scotopic function and which did not. Such a precaution proved to be completely unnecessary as it was relatively easy to find photoreceptors fulfilling the morphological criteria described for rods in the retinas from both animals. Figures 3 and 4 are examples of rods taken from the two squirrels. No morphological differences could be seen in the electron microscope between the photoreceptors found in the two animals. Figure 9 is an electron micrograph of a rod synaptic terminal from the retina of the golden-mantled ground squirrel that lacked a viable scotopic system. It has essentially all the structural features previously described as characteristic of ground squirrel rod terminals (West and Dowling, '75; Fisher et al., '76; Jacobs et al., '76).

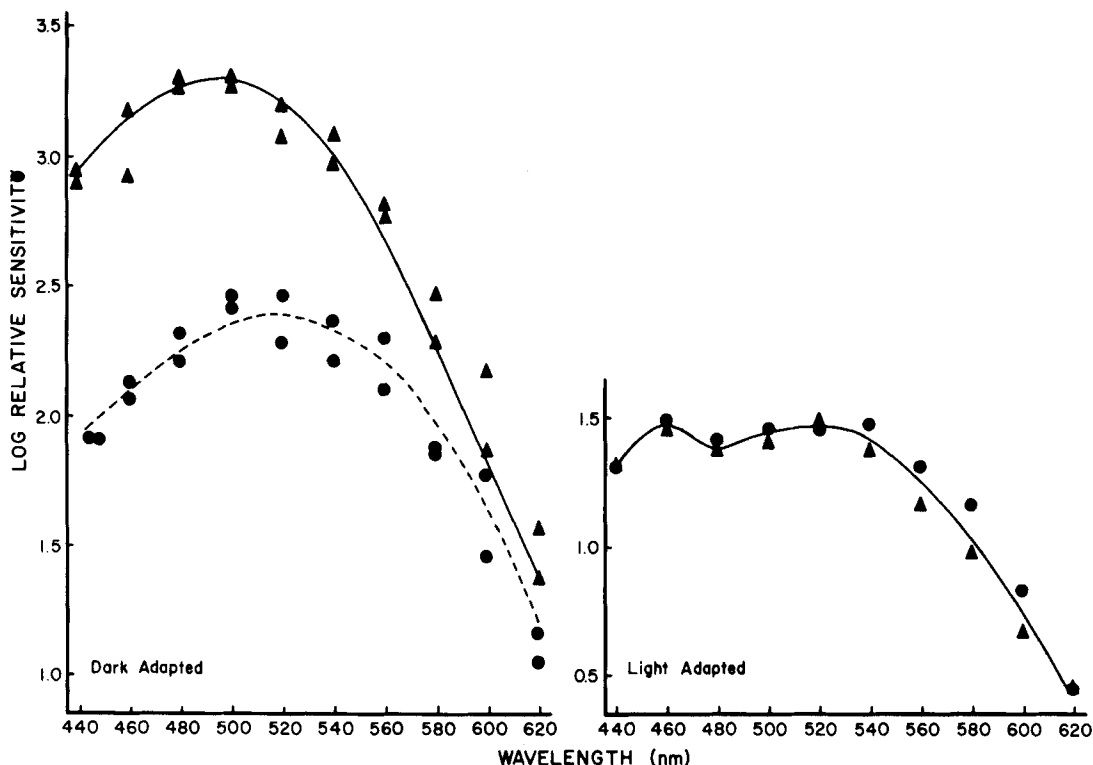


Fig. 6 ERG spectral sensitivity functions for two golden-mantled ground squirrels recorded under conditions of dark adaptation and light adaptation. The circles and triangles used to identify the two animals are consistent with their usage in fig. 3. The solid curve drawn through the data points in the left panel represents the sensitivity of a photopigment having a  $\lambda_{\max}$  at 500 nm. The other curves in the figure were fitted by eye to the data points.

To get an indication of the relative proportions of rods in the two animals, counts were made from longitudinally oriented thin sections in the electron microscope. Only one tissue section per grid was counted to minimize the possibility that the same receptor might be counted more than once. The counts were made from a tissue specimen taken from the right posterior retina of each squirrel. Those receptors which could not be positively identified were not counted. In each case the criterion used to distinguish rods from cones was the extent of the disc-membrane continuity in the outer segment (see fig. 8). The lack of such continuity over most of the length of the outer segment made the rods relatively easy to identify. For one squirrel, 22 of 446 photoreceptors (4.9%) were rods, while for the other 35 of 453 photoreceptors (7.7%) were classed as rods. Given the vagaries of sampling in this procedure we do not believe these differences are significant ones, but at any rate it was the golden-mantled squirrel that showed clear evidence for a viable scotopic system whose eye

contained the smaller proportion of identified rods.

### 3. Spectral components in the ground squirrel ERG

After the experiments reported above were completed it was discovered that two different spectral components can frequently be discerned in the b-waves of the ERGs recorded from dark-adapted ground squirrels. These observations are fully documented elsewhere (Jacobs and Tootell, '79). In essence, these experiments show that the b-wave of the ground squirrel ERG often has two waveform components that appear analogous to those seen in the ERGs recorded from a wide range of other species having duplex retinas. The result is that the positive-going b-wave appears double-humped. The first component ( $b_1$ ) has an implicit time of about 50–60 msec, while the other component ( $b_2$ ) has a considerably more extended time course. The spectral sensitivity of the  $b_1$  component is that characteristic of photopic vision in the ground squirrel, and

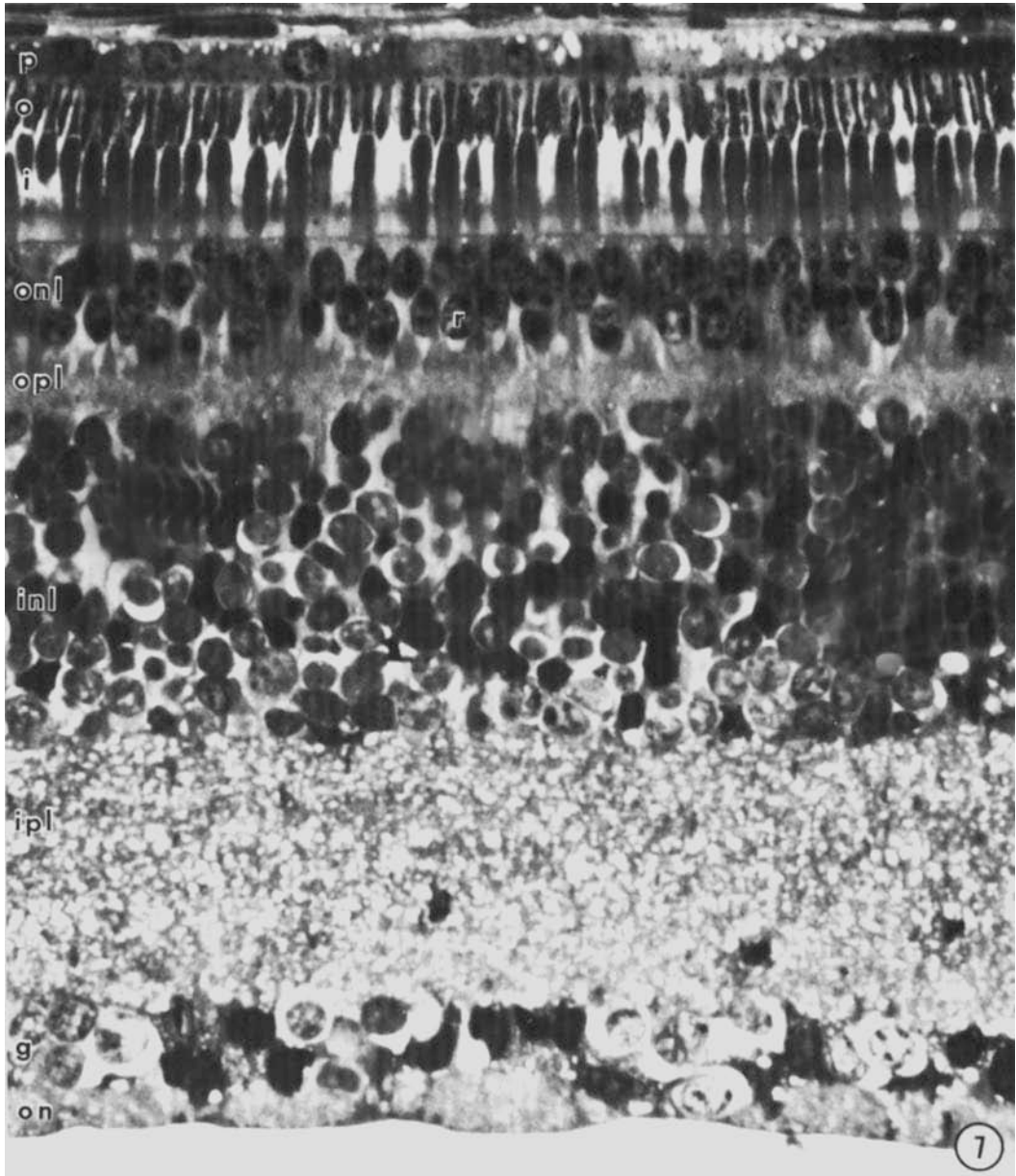


Fig. 7 A light micrograph of a 1- $\mu$ m-thick vertical section through the retina of a golden-mantled ground squirrel. The organization of the retina in this species is comparable to what has been described for other ground dwelling sciurids. The photoreceptor outer and inner segments form a single row across the retina. p, pigment epithelium; o, outer segment layer; i, inner segment layer; onl, outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer; g, ganglion cell layer; on, optic nerve layer. The "r" labels a rod cell nucleus with its light-staining synaptic terminal. Other areas of pale cytoplasm in the onl are sections through processes of Müller cells.  $\times 675$ .

Figs. 8-9 Electron micrographs from the golden-mantled ground squirrel which showed no evidence of a viable scotopic system.

Fig. 8 The base of a rod outer segment (ROS). Rod outer segments can be recognized because the disc stack consists of free-floating discs except at the very base (bracket), where newly formed discs appear as foldings of the plasma membrane and are less regularly organized than the free floating discs. The fine structure of outer segments in this squirrel was identical to that in the squirrel showing normal scotopic function. cc, connecting cilium; is, inner segment.  $\times 51,000$ .



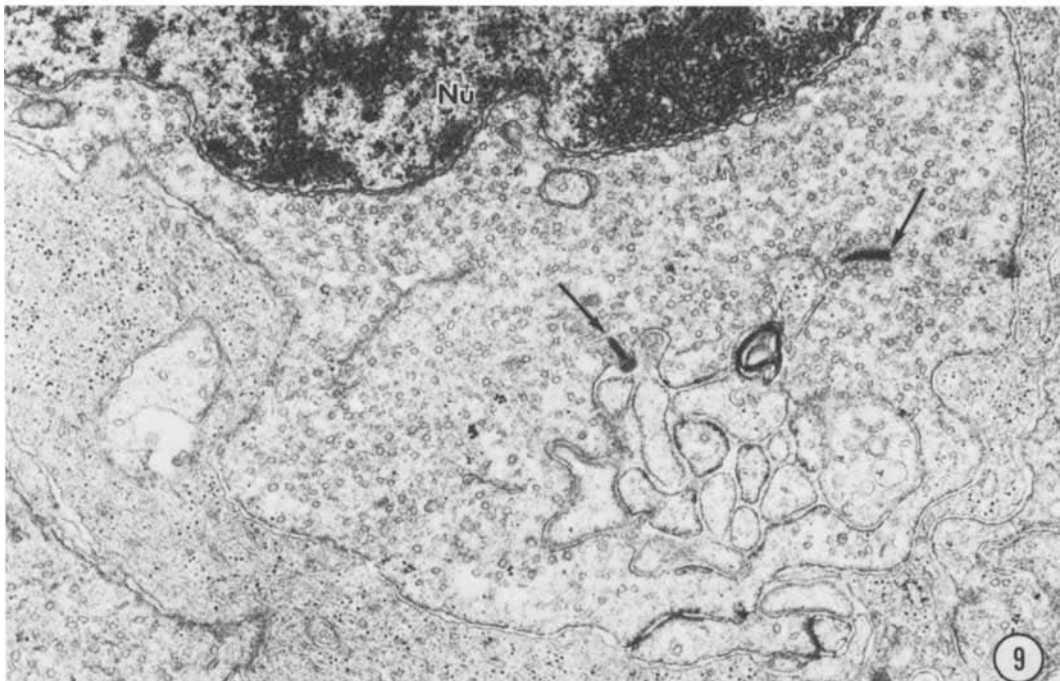


Fig. 9 A rod synaptic terminal. In single thin sections the synaptic terminals appeared identical in both golden-mantled squirrels and similar to what has been described for rod terminals in other species of ground squirrels (West and Dowling, '75; Jacobs et al., '76; Fisher et al., '76). Nu, nucleus; arrows, synaptic ribbons.  $\times 25,000$ .

thus, presumably, reflects the contribution of cone photopigments, while the  $b_2$  component has a spectral sensitivity well accounted for by the putative rod photopigment, i.e., has a  $\lambda_{\max}$  of 500 nm (Jacobs and Tootell, '79).

In view of this finding we reexamined the ERG records from a number of ground squirrels to see if any variations in the  $b_1$  and  $b_2$  components could be found that might correspond to the other indications of functional variation presented above. The most clear-cut results came from an examination of the records obtained from the two golden-mantled ground squirrels. These are illustrated in fig. 10, which shows the responses to several different monochromatic lights recorded from the dark-adapted (left column) and light-adapted (right column) eyes of the two squirrels. The re-

sponses shown in the left column were selected so that at each wavelength they are of approximately equal peak amplitudes. This mimics the situation used earlier to determine threshold, both electrophysiologically and (presumably) behaviorally. The squirrel labelled W was that animal which gave no evidence for a viable scotopic system in the ERG and behavioral experiments (data plotted as circles in figs. 5 and 6), while the records labelled L were from the other animal (data indicated by triangles in figs. 5 and 6). The vertical dashed line in fig. 10 signifies 60 msec after the onset of the stimulus flash. It is clear that the peak responses recorded from the dark-adapted eyes of these two animals represent the two different b-wave components in the ERG— $b_1$  in the case of squirrel W and  $b_2$  in the case of squirrel L. Note that

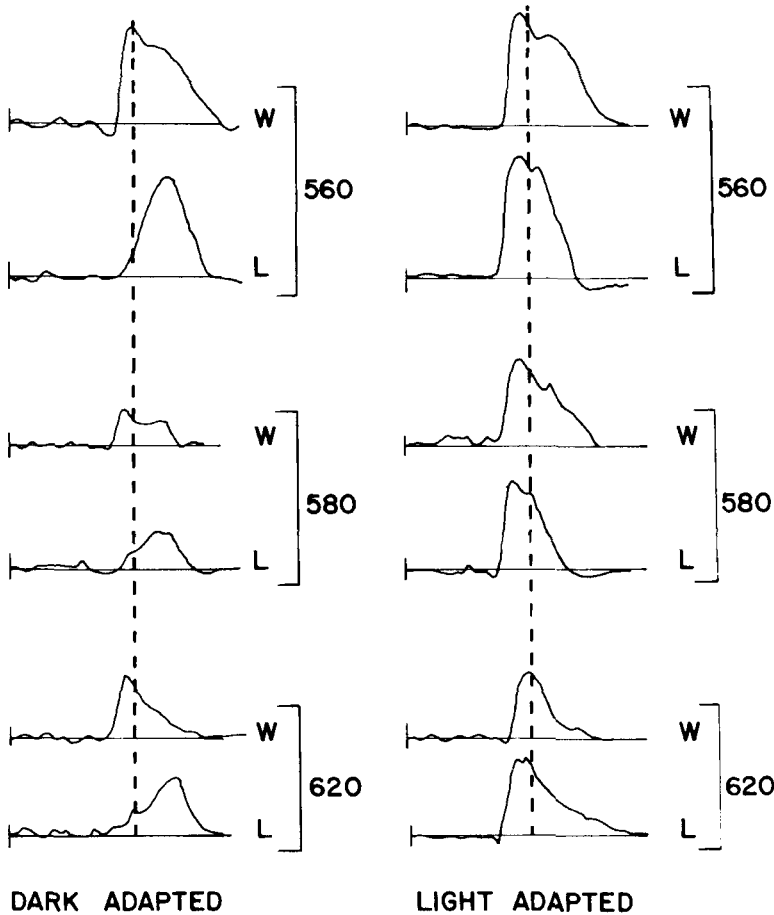


Fig. 10 ERG records taken from golden-mantled ground squirrels under conditions of dark adaptation and light adaptation. The numbers on the brackets indicate the test wavelengths. The vertical dashed lines mark 60 msec after the onset of the stimulus. For other details and related discussion see the text.

even though the peak response recorded from the dark-adapted eye of squirrel *W* represents the  $b_1$  component, there is some indication that a smaller  $b_2$  component is also present (this is most obvious in the response to the 580 nm light).

The right column of fig. 10 shows ERG responses to different spectral stimuli of equal quantal flux for the two squirrels. In this case the maximal amplitudes are the same for equally intense stimuli, reflecting the fact that the light-adapted spectral sensitivity functions for the two squirrels were identical (see fig. 5). Note also that the peak amplitude of the ERG in the light-adapted eye is generated by the  $b_1$  component with an implicit time of 60 msec or less.

The analysis of the ERG waveform in these ground squirrels verifies the previous results. That is, those animals which do not show a viable scotopic capacity under conditions of dark adaptation yield an ERG whose waveform is strongly dominated by the usual photopic ( $b_1$ ) component. This is particularly clear for the golden-mantled ground squirrels, although an examination of records from California ground squirrels leads us to believe that this conclusion is also correct for this species. At the same time, however, there is frequently some indication that even in these animals a small  $b_2$  component can be discerned although it never becomes sufficient to equal or exceed the  $b_1$  amplitude. This suggests that the mechanisms generating the scotopic signal are probably not absent entirely from any ground squirrel retina.

#### DISCUSSION

The experiments described above lead to a rather clear-cut, albeit somewhat surprising, conclusion. Although it appears from a morphological point of view that no ground squirrel retina lacks a small population of rod photoreceptors, a number of squirrels show no functional indication that these photoreceptors are capable of generating a viable scotopic visual capacity. Because this result is unusual several possible explanations need to be examined.

Perhaps the most obvious possible explanation for these results is that the functional indices used were simply not sensitive enough. It is important to remember that, even at best, the scotopic system found in the ground squirrels is a feeble one, quite unlike that scotopic system one usually associates with duplex vision in other mammals (Green and Dowling, '75; Jacobs et al., '76). The best indication of this

fact is that even the presence of a scotopic system in the ground squirrels was undetected in a large number of studies conducted prior to a few years ago. Given that the system is not a robust one, might it not be possible that the functional indices were not sensitive enough to register its contribution in all of the individuals tested? We believe this unlikely on two counts. First, the conditions employed in the ERG experiments were such as to maximize the possibility of seeing a scotopic signal. They included such features as prolonged periods of dark adaptation, large stimulus fields, and response averaging. Furthermore, all animals in which a scotopic signal was not detected were tested on more than one occasion. The most persuasive aspect of this repetitive testing was that *no* animal initially found to lack a threshold-level scotopic signal was found to show one in subsequent testing. If the measure were simply insensitive, or unreliable, or dependent on extraneous variables (for example, the level of anesthesia), one might expect to see some changes in outcome with repeated testing. A second indication that an explanation of the results cannot be found in an appeal to an inadequacy of the functional indices is that in the case of the golden-mantled ground squirrels both behavioral and electrophysiological tests were employed. The results from these two approaches corresponded almost perfectly. We believe that all of these facts make it unlikely that the separation of the ground squirrels into two groups based on functional evidence for the presence or lack of a viable scotopic signal is due simply to insensitivity of the functional measures.

A second possible explanation for the failure to find consistent evidence for a scotopic capacity (even though all ground squirrel retinas contain rods) might be that there is some individual variation in either the relative proportion of rods or in the retinal location of the rods. That is, perhaps those animals which did not show scotopic capacities simply had insufficient numbers of rods to generate a criterion-sized signal, or their rods were retinally distributed such that their contribution to function was minimized. The possibility that there might be significant within-species variations in the relative proportions of rods and cones does not appear likely, although it would require much more extensive receptor counts than have been made to rule out the possibility conclusively. At any rate, the counts that have been done indicate no obvious differences between the proportion of rods found in retinas of California ground squirrels previously shown

to generate scotopic signals (Jacobs et al., '76) and those which do not (this study). Even more convincing on this score are the counts made on the retinas taken from the golden-mantled ground squirrels where the result was in the direction opposite to that required if one were to explain the functional difference on the basis of differences in proportions of rods. In sum, there is no evidence that those animals which did not show a scotopic capacity have fewer rods.

It is considerably harder to reject the possibility that there might be some systematic differences in the retinal distributions of rods accounting for the functional difference, particularly because the "normal" topographic distribution of rods in the ground squirrel retina is not known. However, it is important to remember that the within-species variations appeared in the functional measures, and those measures were such as to minimize any selective sampling of specific retinal areas. Thus, the ERG experiments used a large (40°) stimulus field. Furthermore, the within-species differences were also apparent in the behavioral measures. These were also done with large stimulus fields but, more importantly, in the behavioral experiments the animal was unrestrained and as such could view the stimuli in any way he chose. The reinforcement contingency in force in these experiments would be expected to cause these well-trained animals to maximize their performance, i.e., to use that portion of the retina which yielded the highest sensitivity. Thus, to the extent that there is any evidence on a possible within-species variation in the retinal distribution of rods, it is counter the possibility.

Two comments can be offered on the possibility that the morphological criteria used to distinguish the presence of rods in ground squirrel retinas are not valid ones and it might be this fact that accounts for the results. First, as noted previously, multiple criteria were used to distinguish rods from cones. They include those structural aspects traditionally used to distinguish rods from cones (Cohen, '72), as well as features which are peculiar to the sciurid retina (Anderson and Fisher, '76). Second, the same structural criteria were found to identify rods in all of the retinas examined. Since only some of these animals had displayed a scotopic capacity, then, if the structural criteria were inadequate ones, it would have to be supposed that all of the rod identifications were inaccurate. This possibility seems unlikely. Although there may be some difficulties in distinguishing ground squirrel rods and cones, that

would not seem to be a factor contributing to the main results of these experiments.

Since none of the alternative explanations appear persuasive, the results of these experiments lead us to the conclusion that although all ground squirrel retinas contain a small population of rods, not all ground squirrels have a fully functional scotopic system. How frequently this condition occurs is not known, but it is apparently not rare since in our two experiments on the California ground squirrel 12 of 40 animals (30%) were found to lack the "normal" scotopic system. This discrepancy between photoreceptor complement and functional capacity is not unprecedented. The most persuasive analogous instances can be found in the substantial literature on human monochromacy. The human monochromats, the truly color-blind, form a heterogeneous group, but in at least some members of this group the absence of a functional capacity is not paralleled by the absence of a photoreceptor class (see Alpern, '74, for a recent review and discussion).

Much along the lines of explanation offered for human monochromacy, two possible explanations exist to account for our findings on ground squirrels. If we assume that all ground squirrels' retinas contain rods, then to explain the variation in function one might suppose that in a minority of animals some of the rods contain the ground squirrel cone pigment rather than the 500 nm photopigment. Alternatively, these results could also be understood if all ground squirrel retinas contain rods and all of these rods contained the normal 500 nm photopigment but in some animals the neural connections were such that the rod signal was made ineffective; for example, by being swamped by a much stronger cone signal. We note in this regard the presence in the retina of the California ground squirrel of bipolar cell dendrites which contact both rods and cones (Jacobs et al., '76), an arrangement that has been seen in other sciurids (West, '78) but not so far in other mammals. As in the case of the human monochromats, there are a number of experiments that could be tried to separate these various explanations (Alpern, '74). However, such procedures run into the serious practical difficulty that even in the "normal" ground squirrel the rod-generated signal is a relatively feeble one. Nevertheless, this within-species variation is an intriguing one. The fact that a not insignificant proportion of ground squirrels are affected suggests its occurrence may have some significance for

ground squirrel vision and, perhaps, some general significance for understanding structural and functional interrelationships in the retina.

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