Scotopic and Photopic Vision in the California Ground Squirrel: Physiological and Anatomical Evidence

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ABSTRACT The California ground squirrel is a highly diurnal species previously thought to have an all-cone retina. This issue was re-examined in physiological and anatomical experiments. The electroretinogram (ERG) was used to measure the spectral sensitivity of the eye under different conditions of adaptation. The occurrence of a Purkinje shift could be demonstrated, although there was some indication that not all members of this species show such a shift. Spectral sensitivity of the dark-adapted eye of this squirrel is close to that predicted by a typical mammalian rhodopsin. Light adaptation produces a shift in spectral sensitivity to a peak location of about 525 nm. It was shown that two mechanisms having different spectral sensitivities contribute to the photopically recorded ERG. The degree to which these two mechanisms contribute to the ERG was found to be strikingly different from the degree to which the two contribute to visual behavior.

Our anatomical results indicate that the retina of the California ground squirrel has two structurally distinct photoreceptors which, on the basis of various criteria, can be classified as cone and rod-like. The rod-like receptors comprise about 6–7% of the total. The two photoreceptor types differ in placement of their inner segments, size of their outer segments, outer segment ultrastructure, and terminal structure and organization.

Unlike the arboreal squirrels whose retinas have been shown to contain both rod-like and cone-like photoreceptors (Cohen, '64), and whose vision has clearly demonstrable scotopic and photopic components (e.g, Arden and Silver, '62; Gouras, '64; Dippner, '74; Jacobs, '74), the ground-dwelling sciurids have conventionally been assumed to have all-cone retinas and to display only those visual capacities traditionally associated with photopic vision. In addition to a number of anatomical (Vaidya, '64; Hollenberg and Bernstein, '65), physiological (Tansley et al., '61; Dodt, '62; Michael, '68), and photopigment studies (Dowling, '64) consistent with this latter conclusion, several behavioral studies have also yielded data in accord with the notion of an all-cone retina in the ground-dwelling sciurids (Jacobs and Yolton, '71; Crescitelli and Pollack, '73; Jacobs and Pulliam, '73). Recently, however, West and Dowling ('75) have reported some interesting new observations on the structure of the photoreceptors in three species of ground-dwelling

sciurids. They find evidence for two anatomically distinct types of photoreceptors in these species — one type appears to be a cone while the second type has many rod-like characteristics. The rod-like photoreceptor is scarce in these retinas, amounting to about 4% of the total number of receptors in two species of ground squirrels, and to about 10% of the total in the retina of the prairie dog. In an accompanying paper Green and Dowling ('75) produced electroretinographic (ERG) evidence for a viable scotopic system in these three species — the dark-adapted spectral sensitivity functions had the shape and the spectral peak of a typical mammalian rhodopsin while increment-threshold functions derived from the ERG gave evidence for the occurrence of a Purkinje shift.

A few years ago we carried out a number of psychophysical studies of vision in the California ground squirrel (Anderson and Jacobs, '72). In addition to observations on the characteristics of photopic vision in this species, increment-threshold functions were

measured for two test wavelengths on a variety of different achromatic backgrounds. This experiment appeared to give no evidence for the occurrence of a Purkinje shift and thus seemed consistent with the notion that the California ground squirrel has an all-cone retina. In view of the recent results showing a rod contribution to vision in some of the ground-dwelling sciurids (Green and Dowling, '75), it seemed worthwhile to re-examine the question in the California ground squirrel. Some observations resulting from an analysis of the ERG and an examination of the photoreceptors in this species are reported here.

I. ERG EXPERIMENTS

Methods

Adult California ground squirrels (*Spermophilus beecheyi*) were used. These animals were trapped locally and both sexes were used.

A two-beam optical system was used to elicit ERGs. One beam of this system originated from a tungsten-filament lamp. This beam was used as an adaptation source. The other beam was taken from a Bausch and Lomb high-intensity grating monochromator with slits adjusted so as to yield a monochromatic light having a half-energy passband of 10 nm. The outputs from these two light sources were converged at a beam splitter and the mixture was focused through lenses to illuminate a region of the retina having an angular subtense of 40°. The intensities of these two beams were attenuated with neutral-density step filters. Electromagnetic shutters were positioned in the optical system to make it possible to shutter either of the beams separately or to shutter the mixture of the two beams.

ERGs were recorded intravitreally with insulated stainless steel electrodes. An indifferent electrode was sewn into the skin above the eye. ERG signals were differentially recorded through an amplifier having a bandpass of 0.2 to 1,000 Hz. The amplified signals were averaged with an Ortec 4623 Signal Averager. This averaged signal was read out on an X-Y plotter and ERG measurements were made from these records.

Ground squirrels were anesthetized intraperitoneally with a mixture of sodium pentobarbital (45 mg/kg) and chroral hydrate (250 mg/kg). Normal body temperature was maintained throughout the experiment by the use of a heating pad. Following initial anesthesia, the squirrels were placed in a modified stereotaxic instrument. The eyelids were retracted and a cut was made in the nasal canthus of the test eye so as to expose it completely. This eye was sutured to a wire ring and the cornea and lens were carefully removed. The other eye was covered with an opaque mask.

After the animal had been placed in a shielded recording chamber, aligned with the optical system, and the ERG electrode had been positioned, the animal was thoroughly dark adapted so as to insure maximal sensitivity. The dark adaptation period used ranged from a minimum of 45 minutes up to about an hour and a half. For the measurements made under the conditions of dark adaptation we always ran from the dimmest test lights to the brighter ones. A minimum of ten responses were averaged for each wavelength-intensity combination. The test flashes were 100 msec in duration presented once every ten seconds. Responses to a reference test light were frequently obtained throughout the somewhat lengthy test sessions to assure that there were no significant changes in the sensitivity of the eye. Wavelengths from 440 to 620 nm, usually in steps of 20 nm, were examined in all of the spectral sensitivity experiments. Test wavelengths were presented in an unsystematic order. With the exception of one experiment mentioned below, all ERG measurements are based on the base-to-peak amplitude of the b-wave.

Results

Purkinje shift

In order to see if a Purkinje shift could be detected in the ERG of the California ground squirrel, ERG thresholds were measured in five animals for two test wavelengths (500 and 600 nm) on white (4,800° K) adapting backgrounds covering a 4.5 log unit range from 0.003 to 103 cd/m². For each measurement the intensity of the monochromatic light was varied so as to produce a criterion response of 10 μV. Measurements were made starting from the dimmest of the adaptation levels and then moving successively to the higher levels. The mean thresholds so obtained are given in figure 1. In that figure the threshold val-
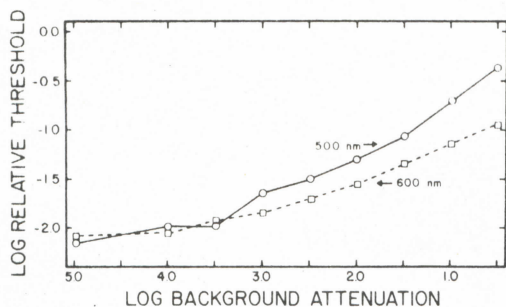
Fig. 1   Increment-threshold sensitivity for the ERG of the California ground squirrel. The data points represent mean thresholds for five animals. Other details are given in the text.
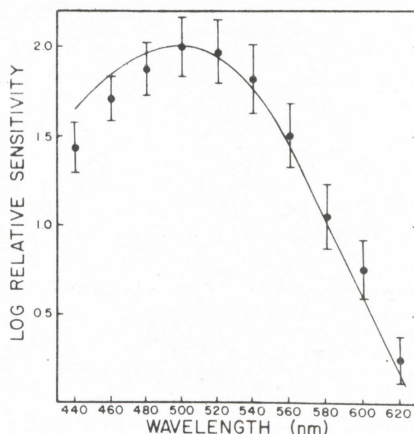

Fig. 2   ERG spectral sensitivity for the dark-adapted California ground squirrel. The solid circles are mean values for five animals, the vertical bars enclose two standard errors of the mean. The solid line represents the sensitivity curve for a nomogram photopigment having maximal absorption at 500 nm.

ues for the 500 and 600 nm test lights were equated at the least intense adaptation level (corresponding to an attenuation of 5.0 in fig. 1). As can be seen, there is no significant change in threshold for a range of 1.5 log units above this level. It is at that point that sensitivities to both test wavelengths first begin to decrease. However, above this level, threshold is consistently higher for the 500 nm test light than for the 600 nm light, i.e., spectral sensitivity has shifted.

In addition to demonstrating the occurrence of a spectral sensitivity shift dependent on adaptation state, two points about this experiment bear mention. First, the adaptation level at which this system begins to be desensitized by background light is quite high (about 0.1 cd/m²). By way of comparison, a rodent having a rod-rich retina (the flying squirrel) tested in this same apparatus showed ERG desensitization beginning at an adaptation level which is at least two and a half log units below that sufficient to desensitize the ERG of the California ground squirrel. A second point concerns the slopes of the functions shown in figure 1 — they are unusually shallow (slopes of about 0.5–0.7). Although the significance of this fact remains obscure, a similar observation appears in previous behavioral (Jacobs and Birch, '75) and electrophysiological (Green and Dowling, '75) measurements of increment thresholds in other ground-squirrel species.

Dark-adapted spectral sensitivity

Having established the presence of an adaptation-dependent spectral sensitivity shift in the ERG of the ground squirrel, we next measured spectral sensitivity under conditions of dark and light adaptation. The dark-adaptation measurements were made under those conditions indicated previously, i.e., at least 45 minutes of complete dark adaptation, dim flashes, order of stimulus wavelengths nonsystematic, etc. The intensity of each of the monochromatic lights was varied so as to produce a criterion signal of 15 μV. Complete functions were obtained for five animals. These are presented in figure 2 where the solid circles represent the mean sensitivity for five animals and the vertical bars enclose two standard errors of the means. As in all spectral sensitivity functions presented in this paper, these sensitivity values are quantally based. The sensitivity function for the dark-adapted eye is simple in form with peak sensitivity near 500 nm. The solid line in figure 2 is that for a nomogram photopigment (Dartnall, '53) having maximal absorption at 500 nm. It provides a good fit to the data except for the 440–460 nm points where the measured sensitivity is somewhat lower than that predicted by the nomogram. Despite this slight mismatch, the overall fit is sufficiently good to permit the conclusion that the ERG from the dark-adapted eye of the California ground squirrel is based on a single photopigment whose sensitivity is close to that of a typical mammalian rhodopsin.

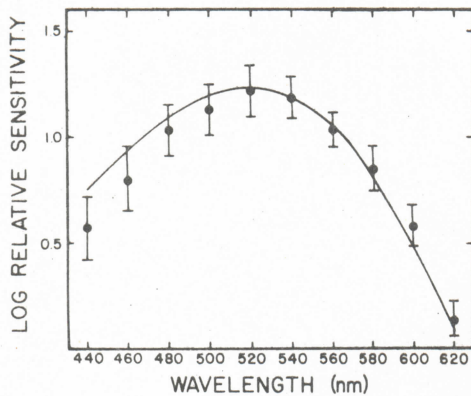JACOBS, FISHER, ANDERSON AND SILVERMAN

Fig. 3 ERG spectral sensitivity for the light-adapted California ground squirrel. The solid circles are mean values for five animals, the vertical bars enclose two standard errors of the mean. The solid line represents the sensitivity of a nomogram photopigment having maximal absorption at 525 nm. Adaptation light: achromatic, 3 cd/m².
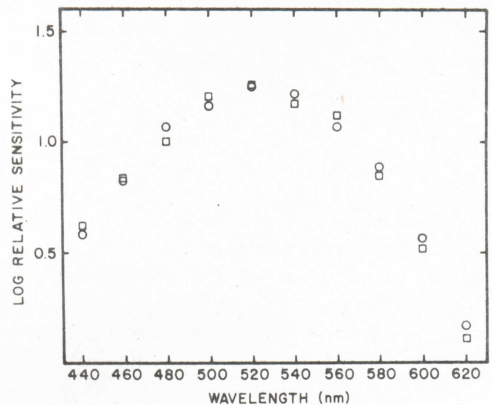

Fig. 4 Spectral sensitivity of the b-wave (circles) and d-wave (squares) components of the ERG recorded from light-adapted California ground squirrels. The data points represent mean values for four animals each.

Light-adapted spectral sensitivity

For these measurements a level of light adaptation was used that was well above that established in the first experiment as being necessary for producing the spectral sensitivity shift in the California ground squirrel. This adaptation light (3 cd/m²) was on continuously, the 100 msec test flashes were added to it. Otherwise, spectral sensitivity measurements were made in the same way as described previously. Figure 3 shows light-adapted spectral sensitivity functions for five California ground squirrels. Again, the solid circles represent mean values (criterion signals of 50 μV) and the vertical bars enclose two standard errors of the mean. Under conditions of light adaptation, peak sensitivity is at about 520 nm; again, there is no evidence for any discontinuities in the function. The solid line in figure 3 is for a nomogram photopigment having a peak at 525 nm. It provides a reasonable fit to the data, although it is somewhat too elevated at the 440 and 460 nm test wavelengths.

One of the most prominent features of the cone-dominated ERG is a large off-response, the d-wave. It has occasionally been suggested (Crescitelli, '61; Jacobs and Yolton, '72) that the spectral sensitivities for the b and d-wave ERG components may be different. Since the d-wave must be recorded from the light-exposed eye, but the b-wave need not be, it seemed conceivable that the earlier reported differences in spectral sensitivity between the two components might simply reflect differences between photopic and scotopic mechanisms. Accordingly, we checked this possibility by comparing b and d-wave spectral sensitivity when a continuous state of light adaptation was assured. Monochromatic stimuli were presented in conjunction with the same adaptation light as used in the previous experiment. To enhance the d-wave component, the duration of the test stimulus was increased to one second. Manipulation of the intensity of the monochromatic light permitted simultaneous measurements of the b and d-wave components. The spectral sensitivities so obtained are shown in figure 4. For each component the points represent mean values for four animals. As can be seen, when a condition of consistent light adaptation is assured, the spectral sensitivities for the ground squirrel b and d-wave components are almost precisely the same. Earlier reports of a difference in sensitivity for these two components very likely reflect a contribution of the scotopic mechanism to the b-wave response.

Scotopic variability

The results reported above make it clear that these ground squirrels can be shown to possess a viable scotopic system having maximal sensitivity at 500 nm. However, we were quite unable to detect the presence of

this system in a few individuals of this species. This seems worthy of note because in these cases our procedures were the same as for all other animals and, indeed, the preparations were good ones in the sense of showing normal amounts of noise and of having normal photopic sensitivity. In each of these cases special steps were taken to try and bring out the scotopic system; for example, by lengthening the period of dark adaptation and by averaging greater numbers of responses. Neither of these procedures were successful. For three animals falling in this category, spectral sensitivity was measured under the normal scotopic conditions. The functions so obtained were not significantly different from those shown in figure 3. That is, although obtained under the standard scotopic conditions, the spectral sensitivities for these animals are not different from the usual photopic functions. In fact, none of these animals showed sensitivity shifts when they were later examined under conditions of light adaptation. Since there are no obvious alternatives, we are forced to conclude that the mechanisms underlying scotopic vision in the California ground squirrel are either absent in some members of this species or are so weak as to be undetectable in the ERG. The latter alternative is probably more likely although no experimental evaluation is available at this time. How frequent is this variation? Out of ten animals examined in detail, where the recording preparation seemed otherwise normal, three gave no evidence of a scotopic mechanism. Insofar as these observations are adequate, it appears that there may be a non-trivial within-species variation in the possession of a viable scotopic system in California ground squirrels.

Photopic components

The California ground squirrel is a dichromat and must, therefore, have two classes of photopigments operative at photopic light levels (Anderson and Jacobs, '72). However, photopic spectral sensitivity functions derived from the ERG of this species are simple in form with a single peak at about 525 nm and with no other discontinuities (fig. 3). The differences between these ERG results and the earlier behavioral results are very striking. Figure 5 shows a comparison of a behaviorally-determined
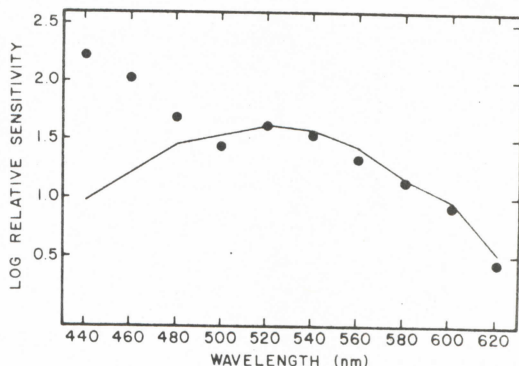

Fig. 5 A comparison of spectral sensitivity functions determined behaviorally (solid circles) and from the ERG (continuous line). Both are for conditions of light adaptation. These functions reflect sensitivity at the retinal level — the ERG data were obtained from the aphakic eye while the behavioral measurements have been corrected for preretinal absorption in the eye of this species (Yolton et al., '74).

photopic spectral sensitivity function (Anderson and Jacobs, '72) and the ERG photopic function. The two sensitivity curves match very well for test wavelengths beyond about 500 nm, but at shorter wavelengths the behavioral sensitivity is very much higher than that seen in the ERG. It is as if the short-wavelength mechanism contributes substantially to the behavioral capability, but little or not at all to the ERG. To see if a second system having a peak in the short wavelengths could be demonstrated in the ERG, some chromatic-adaptation experiments were carried out.

ERG spectral sensitivity functions were obtained when the eye was adapted to bright orange light (dominant wavelength = 605 nm; radiance = 302 μW/cm²). An additional light was employed to provide sufficient achromatic light to insure that the eye was adapted to a level above that necessary to cause the spectral sensitivity shift documented in figure 1. With these adaptation conditions spectral sensitivity functions were obtained in the usual manner. The resulting functions for two such experiments are given in figure 6. As shown there, under this chromatic-adaptation condition, sensitivity to the middle and long wavelengths is severely depressed and a peak at about 450–460 nm is revealed. Thus, the presence of a photopic mechanism peaking in the short wavelengths can be demonstrated by
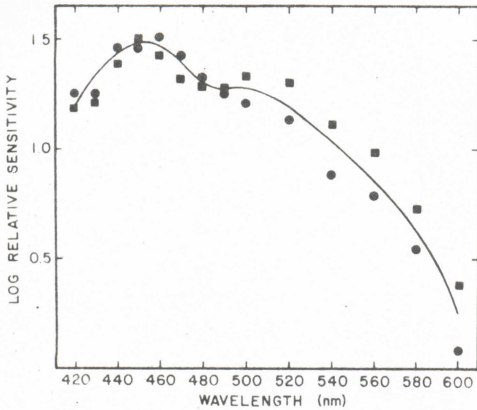
Fig. 6   Effects of chromatic adaptation on the ERG of the California ground squirrel. The plotted points show sensitivity measured for two animals during the time the eye was exposed to an orange (dominant wavelength = 605 nm) adapting light. The continuous curve was drawn freehand through the data points.

this procedure. Unfortunately, with this adaptation condition we were unable to suppress completely the contributions from the mechanism peaking in the middle wavelenths, and thus it is impossible to make any further comments about the spectral nature of the short-wavelength mechanism. At any rate, it seems clear that under conditions of neutral adaptation at photopic levels, the short-wavelength mechanism contributes little if anything to the ERG signals of the California ground squirrel, but it contributes substantially to visually guided behavior. The results of Green and Dowling ('75) suggest a similar situation exists in some other species of ground-dwelling sciurids.

II.   ELECTRON MICROSCOPY

Methods

The retinas from two adult male California ground squirrels were prepared for electron microscopy. In one squirrel the eyes were enucleated and the anterior chamber removed with a razor blade. After most of the vitreous humor had been removed, the posterior chamber was immediately immersed in a fixative consisting of 1% glutaraldehyde, 1% paraformaldehyde, and 0.04% picric acid buffered with 0.067 M sodium cacodylate with 0.5% $CaCl_2$ added (pH 7.4) (modified from Ito and Karnovsky, '68). After 1.5 hours, the retinas were rinsed

in isotonic buffer and post-fixed in a 2% solution of OsO_4 in veronal acetate buffer (pH 7.4) for one hour. After rinsing in distilled water, the tissue was dehydrated in a graded ethanol-water series, transferred through propylene oxide, and embedded in Araldite. The eyes from the second squirrel were fixed by intracardiac perfusion of the same aldehyde-picric acid fixative at a constant pressure of 90 mm Hg. When approximately 400 ml of fixative had been perfused through the circulatory system, the eyes were removed as before and immersed in the fixative for an additional 1.5 hours at 4°C; the other steps in the procedure remaining the same.

Sections were cut on a Porter-Blum MT 2B ultramicrotome. One micron thick sections for light microscopy were stained with a saturated aqueous solution of p-phenylenediamine. Thin sections for electron microscopy were placed on mesh or bar grids, or on formvar coated slot grids for serial examination, and stained with a 1% aqueous solution of uranyl acetate for 20 minutes, followed by lead citrate for ten minutes.

Results

Fixation by perfusion resulted in far better preservation of the retina than by immersion, although two types of photoreceptors could be recognized by light and electron microscopy in retinas fixed by either method. In general, these types corresponded to the cone and rod-like receptors described in other sciurids (Cohen, '64; Hollenberg and Bernstein, '66; West and Dowling, '75; Anderson and Fisher, '75). Because of the close similarity in structure of the two types, discrimination between them in light microscopy was particularly difficult, especially since neither fixation produces a "pale" cytoplasm in the rod-like photoreceptor as was described by West and Dowling ('75) in other ground squirrels. This difficulty of differentiation by light microscopy no doubt contributed to many of the early claims that these sciurids have pure-cone retinas. Out of 572 photoreceptors counted in sections from the central part of the eye, 38 (6.6%) could be classified as rods, a value only slightly higher than obtained by West and Dowling ('75) for S. tridecemlineatus and S. mexicanus.

By electron microscopy the two photore-

ceptor types were easily identificable (figs. 7, 8), based upon the relative location of outer and inner segments, and on the particular ultrastructural characteristics of each type. The cone outer and inner segments occupy a single row across the retina with the rod-like receptors located slightly more vitread (fig. 7). Both outer segment types are cylindrical and abut the pigment epithelium at the same level (figs. 7, 8). The rod-like outer segment is slightly longer and thinner than that of the cone (fig. 7) (10 × 2 μm compared to 6.5 × 3.5 μm respectively).

Differences can also be seen in the ultrastructural organization of the outer segments of the two types of photoreceptors (figs. 9, 10). The rod-like outer segment is composed of independent discs surrounded by the plasma membrane except at the very base where, typically, one or two discs are found continuous with it (fig. 10). In comparison, the cone outer segment shows a continuously infolded plasma membrane extending from its base along about one-third its length, with frequent continuities above this point to the top of the outer segment (fig. 9).

Differences also occur in the structure and organization of the synaptic terminals of the two receptor types. Both terminal types end as flattened structures with the synaptic region forming a line across the terminal base (figs. 8, 11, 14). The cone terminal, however, is much larger and has a broader pedicle (fig. 8) than the rod-like terminal which appears as a blunt termination of the photoreceptor along the border of the outer plexiform layer (figs. 11, 14). Both terminals contain many synaptic vesicles and both have the characteristic synaptic ribbons of vertebrate photoreceptors (Missotten, '65; Dowling and Boycott, '66) (figs. 8, 11, 12, 13). There are differences in the numbers of synaptic ribbons in the two types: in four serially-sectioned cone terminals there were 35, 27, 27, and 25 independent ribbons, while in three serially-sectioned terminals of rod-like cells there were 9, 8, and 8 independent ribbons. In this species all synaptic ribbons are associated with a synaptic contact. The synaptic invaginations in both terminal types appear to be organized similarly, in that each ribbon synapse has a triadic arrangement of post-synaptic processes (figs. 11, 12, 13, 14,

16, 17). Although we have not yet done extensive identification of these processes in serial sections, we have no evidence to suggest that the triad arrangement differs from what has already been extensively described in other vertebrate retinas; that is, a central bipolar dendrite flanked by two horizontal cell processes (Missotten, '65; Dowling and Boycott, '66). An important difference between rod and cone terminals in mammals is the presence of non-ribbon related contacts (basal or flat) made with dendrites of the "flat" bipolar cells (Missotten, '65; Kolb, '70). On cone terminals observed in this study, there were numerous junctions which appear to be of this type, either positioned along the terminal base or slightly invaginating into it (figs. 12, 15). Although there are also such specialized contacts on the rod-like terminal base as well it appears that many of these are from processes which also make ribbon contacts. The contacts on the rod-like base are, however, asymmetric with respect to membrane densification (figs. 11, 12), as is typical of "flat" bipolar contacts on primate cones (Dowling and Boycott, '66). We have found one example in which a bipolar dendrite contacts both a cone and rod-like terminal (figs. 14, 15), making asymmetric contacts on each.

We have also found that interreceptor contacts are made by cone terminal processes onto other cone terminals or onto rod-like terminals (fig. 12: inset). We have not seen any basal processes extending from rod-like terminals.

An unusual feature of the cone ribbon synapse in the California ground squirrel is the large number of central processes of the triads possessing ribbons in their cytoplasm (figs. 16, 17). When these processes were traced, they always arose from the inner nuclear layer edge of the outer plexiform layer and are presumably bipolar dendrites.

DISCUSSION

Basically, the results reported here verify for the California ground squirrel the contention recently advanced for three other species of ground-dwelling sciurids (Green and Dowling, '75; West and Dowling, '75). Thus, the retinas from all four of these species contain a small proportion of photoreceptors that are rod like in structure. Furthermore, the ERGs of these animals give

clear evidence for a rhodopsin presence. Thus, these species (and by analogy perhaps all of the ground-dwelling sciurids), although often assumed to have all-cone retinas, really have cone dominated but clearly mixed retinas. Although this seems contradictory to our earlier behavioral results (Anderson and Jacobs, '72), a reconsideration of those data now leads us to conclude that the increment-threshold data presented in that earlier paper also give evidence for a small adaptation-dependent shift in spectral sensitivity. Furthermore, the shift seen in those earlier data is in the same direction and is of about the same order of magnitude as that shown in figure 1 of this paper.

With a few significant exceptions our anatomical descriptions match those of Cohen ('64) on the Eastern gray squirrel, and of West and Dowling ('75) on the Eastern gray squirrel, 13-line and Mexican ground squirrels, and the prairie dog. In both of those studies the plasma membrane-disc continuity in the cone was reported to occur only part way up the outer segment. On the other hand, in the California ground squirrel these continuities occur to the top of the outer segment, although they are observed less often in the upper than in the lower half. Our results indicate that this is also true for cones in the 13-line ground squirrel (Anderson and Fisher, in preparation). The presence of independent discs in rod outer segments and of a continuously folded membrane in cones is an important identifying characteristic of these two photoreceptor types (Cohen, '72).

On the basis of outer-segment structure (Cohen, '64; Anderson and Fisher, '75), protein renewal, and permeation of the dye procion yellow (Anderson and Fisher, in preparation) the classification of rods and cones in various species of sciurids seems straightforward. On the basis of terminal structure, however, such a classification is not as clear (West and Dowling, '75) because, although the two terminal types are different with respect to general shape and numbers of ribbon-related contacts, the differences are not as great as in cats and primates (Dowling and Boycott, '66; Boycott and Kolb, '73; Missotten, '65). In the California ground squirrel the terminal distinction is even more difficult than in other squirrels when examinations are made in single thin sections. The rod-like terminals do not have the "inverted tear drop" shape nor the special cytoplasmic characteristics described in other ground squirrels by West and Dowling ('75). In addition, the presence of ribbon-related synaptic triads which occur in separate invaginations along the terminal base, and the appearance of "flat" contacts on rod-like terminals often make the distinction difficult. The presence, in the California ground squirrel, of bipolar dendrites which contact both rods and cones appears to be unique among mammalian retinas. Such bipolar contacts are apparently common in the rudd (Scholes and Morris, '73), goldfish (Stell, '67), and tiger salamander (Lasansky, '73).

Having concluded that these strongly diurnal squirrels do have a viable scotopic system, it is appropriate to ask what this system contributes to vision in these animals. Although there is not sufficient information to permit any very detailed answers to this question, it appears that this scotopic system does not extend the range of vision in these species very substantially over that which they would achieve with cones alone. In addition to the present study, visual thresholds have been measured in these species in a number of different contexts, both ERG (Dodt, '62; Dowling, '64; Green and Dowling, '75) and behavioral (Jacobs and Yolton, '71; Jacobs and Pullium, '73), and it has been uniformly observed that these thresholds are quite high relative to those obtained from other known duplex visual systems. Some indication of how much sensitivity is gained by the presence of this rather feeble scotopic system is perhaps given by the observation that, in this study, the dark-adapted thresholds (at 500 nm) for those animals showing a Purkinje shift were on the average 0.34 log units lower than were the equivalent thresholds for those animals showing no Purkinje shift. The very fact that we were unable to find ERG evidence for a scotopic system in some individuals would also seem to argue that its presence cannot be critically important. It might also be worth noting that no evidence of a rod contribution appears in any of the several experiments done on the responses of single cells in the ground-squirrel visual system (Michael, '68), although both the present results and the Green and Dowling ('75) study

show that one must look rather specifically in order to see functional evidence of a scotopic system in these squirrels.

As a final point, these results would appear to add some weight to the view that there are probably no mammalian retinas constructed entirely on the basis of one type of photoreceptor. In an interesting review article, (Cohen ('72) has recently noted that all retinas examined to date contain some photoreceptors having pedicle-like terminals. Along with the current evidence showing rods in heavily cone-dominated retinas, this would seem to suggest that all contemporary mammals make do with some variation on the duplex theme. Indeed, there is little evidence that any vertebrate retinas contain only one photoreceptor type. Perhaps the retina of the skate is an exception to this rule (Dowling and Ripps, '71), although even there it is not clear if the system has all of the characteristics classically expected of a pure-rod retina (Green and Siegel, '73).

ACKNOWLEDGMENTS

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PLATE 1

EXPLANATION OF FIGURE

7   An electron micrograph of a cone (C) and a rod-like (R) photoreceptor from the California ground squirrel. Inner segments of the two cell types are located at slightly different levels in the photoreceptor layer and the outer segment of the rod-like receptor is slightly longer and narrower than that of the cone. × 12,500.
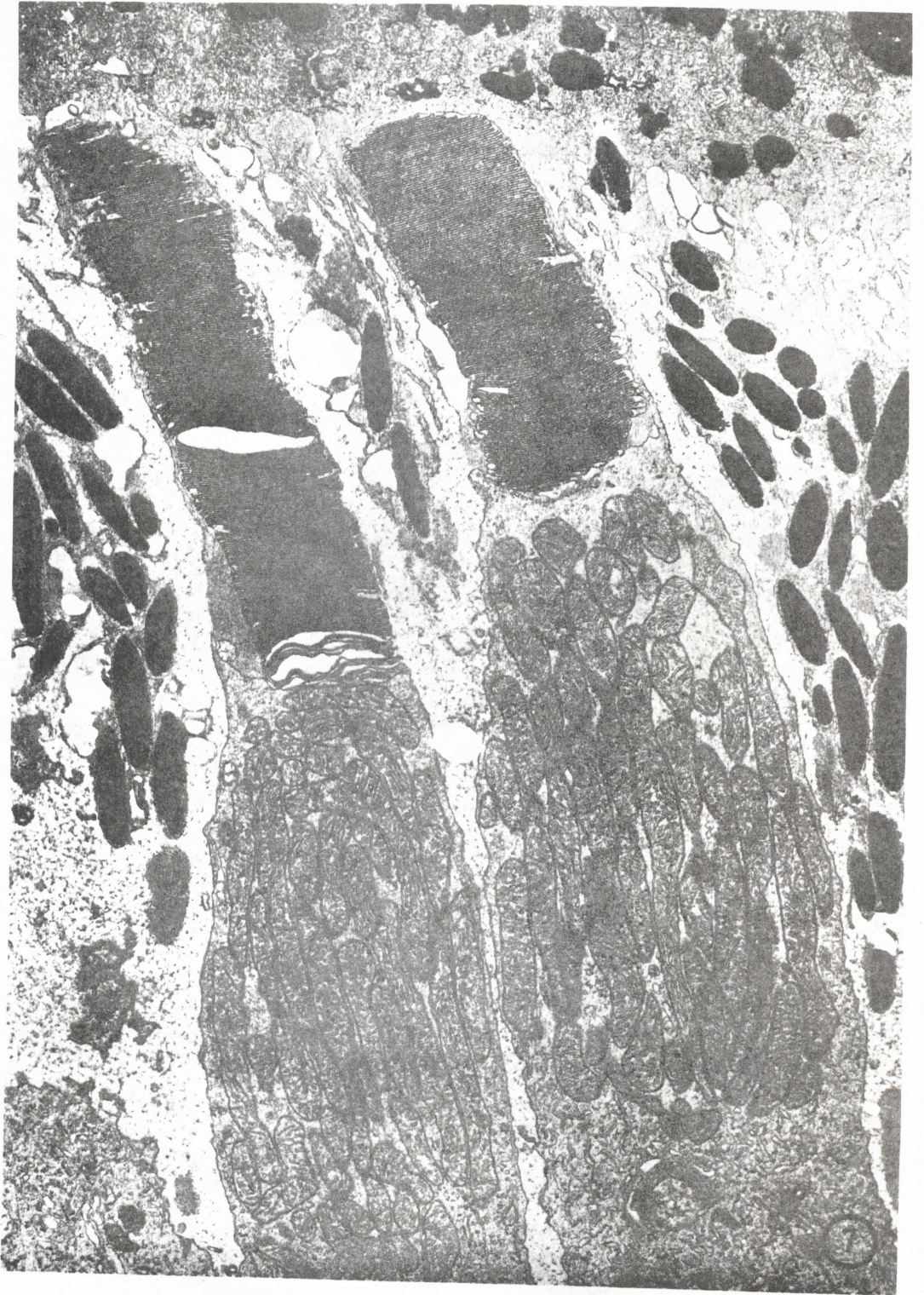
PLATE 2

.8 An electron micrograph of a cone photoreceptor from the California ground squirrel. The cones end in a pedicle shaped terminal on the border of the outer plexiform layer. Although the cytoplasm of this particular cone appears to be slightly lighter than that of its neighbors this is not a defining characteristic of either receptor type in this species (see text). × 4,500.

9 An electron micrograph of a cone photoreceptor outer segment from the California ground squirrel. A continuously folded plasma membrane forms the basal one-third of the outer segment while above this level continuities between the discs and membrane still occur (arrow) but are much less frequently seen in single sections. Small dense particles are numerous in the connecting cilium cytoplasm as it extends along the outer segment but do not seem to occur in the stalk of the connecting cilium. × 24,000.

PLATE 3

10   An electron micrograph of the base of a rod-like photoreceptor outer segment from the California ground squirrel. The outer segment discs occur independently of the plasma membrane except at the very base (arrow) where a few continuities between the plasma membrane and the most basal discs occur. These continuities are seen as inward invaginations of the membrane and probably signify the assembly of new rod discs. × 5,000.

11   An electron micrograph of the nucleus and terminal of a rod-like photoreceptor from the California ground squirrel. Three ribbon-related synapses are shown, two of which have a triad arrangement of post-synaptic processes. The arrow indicates an apparent basal or flat contact with asymmetric membrane densification. × 15,000.

12   An electron micrograph of the terminal from a rod-like photoreceptor from the California ground squirrel. A triad of post-synaptic processes occurs at the synaptic ribbon and an asymmetric basal or flat contact at the arrow. × 48,000. Upper inset shows interreceptor junction between laterally extending processes of three cones. Tangential plane of the section taken just above the receptor terminals. × 60,000. Lower inset shows an interreceptor junction between a cone process (C) and the terminal of a rod-like receptor (R). × 48,000. The series of "dots" in the intercellular space of the junctions in the upper inset is a characteristic of gap junctions in tissue not stained *en bloc* with uranyl acetate.

13   An electron micrograph of synaptic contacts found with the cone terminal in the California ground squirrel. A triad of post-synaptic processes occurs at the synaptic ribbon and a basal or flat contact at the arrow. In this species about 10% of the total photoreceptor population have the very dense cytoplasm shown in this figure and in figures 14 and 17. × 48,000.
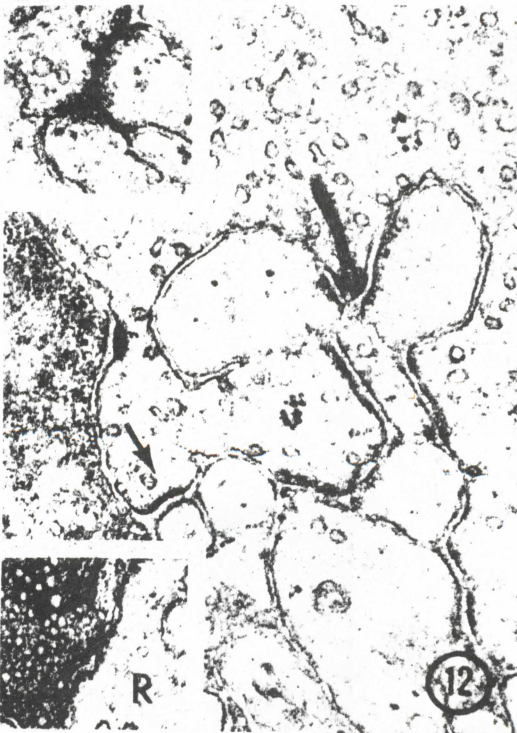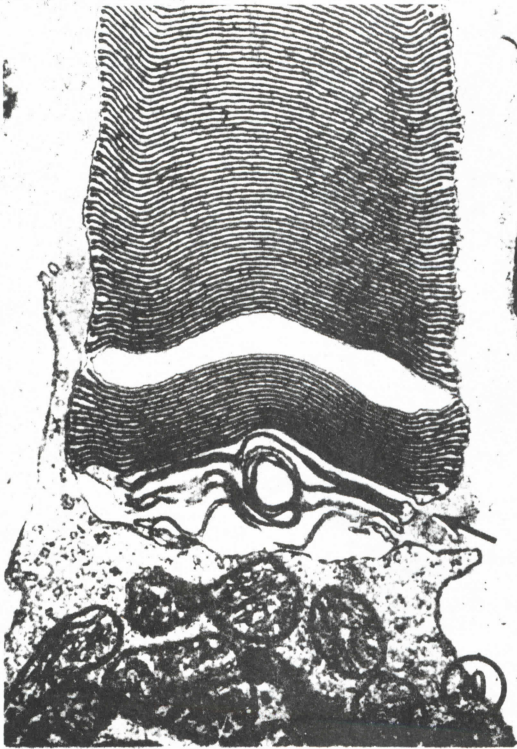
PLATE 3

14 One of a series of electron micrographs of cone (C) and rod-like (R) photoreceptor terminals from the California ground squirrel. This figure illustrates the general shape of the rod-like terminal and shows a triad arrangement of post-synaptic processes at a synaptic ribbon (arrow) in that terminal. The thick arrow indicates a process which appears to be contacting both terminal types. × 30,000.

15 An electron micrograph serial to that in figure 14. The process indicated by the thick arrow is contacting both the cone (C) and rod-like (R) terminal at the thin arrows. This process was traced in serial sections to a cell body in the middle of the inner nuclear layer and is presumably a bipolar cell dendrite. × 60,000.

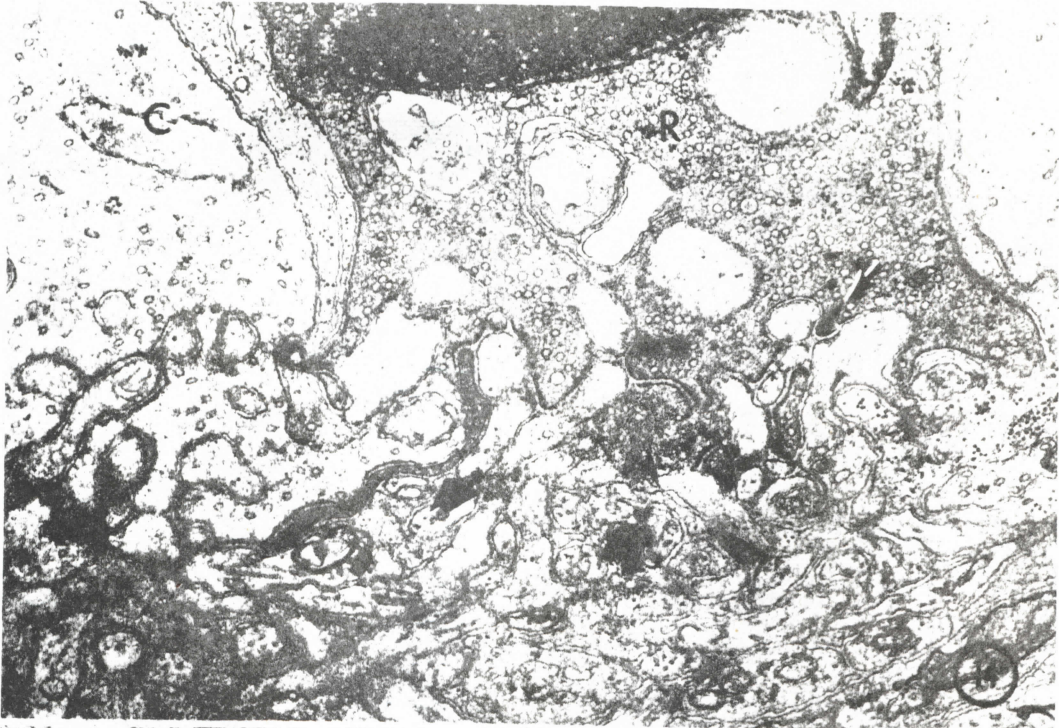16 An electron micrograph of a cone terminal from the California ground
squirrel. The central process of the triad at one synaptic ribbon (arrow)
contains synaptic ribbons and vesicles in addition to microtubules and
a small amount of endoplasmic reticulum. × 39,000.

17 An electron micrograph of a cone terminal from the California ground
squirrel. One of the post-synaptic processes has synaptic ribbons and
vesicles in addition to microtubules and a small amount of endoplasmic
reticulum in its cytoplasm. The central process of the triad indicated by
the arrow has a similar ultrastructure to those with ribbons shown here
and in figure 16. From their similar ultrastructure and their origin deep
in the outer plexiform layer these are assumed to be bipolar dendrites.
Note the apparent membrane specialization which occurs between the
bipolar dendrites and the photoreceptor terminal at the thick arrows.
× 36,000.

PLATE 5