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RESEARCH NOTE

RODS IN THE ANTELOPE GROUND SQUIRREL[1]

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The ground-dwelling sciurids have conventionally been assumed to constitute one of the few groups of animals whose member species have all-cone retinas. However, over the past few months evidence has been presented to show that for at least some of the species in this group the claim for a pure-cone retina cannot be substantiated. Thus, using a variety of anatomical criteria, four species of ground-dwelling sciurids (prairie dogs, Mexican ground squirrels, 13-line ground squirrels, and California ground squirrels) have all been shown to have a small number of retinal rods in addition to a large population of cones (West and Dowling, 1975; Jacobs, Fisher, Anderson and Silverman, 1976; Anderson and Fisher, 1976). Although the number of rods in these retinas is small, less than 10% of the total number of photoreceptors, it has also been demonstrated that rod-generated signals can be clearly seen in the electroretinograms (ERGs) recorded from these animals (Green and Dowling, 1975; Jacobs *et al.*, 1976).

In addition to the four species referred to above, there is another ground-dwelling sciurid which has been the frequent subject of vision experiments. This animal, the antelope ground squirrel (*Ammospermophilus leucurus*), has been used in behavioral (Crescitelli and Pollack, 1965; 1966; 1972), anatomical (Johnston and Gardner, 1959), and electrophysiological (Crescitelli, 1961; 1962) studies of vision. All of these studies indicate a belief that the antelope ground squirrel has an all-cone retina; indeed, there are no results in any of these studies inconsistent with this supposition. To determine if the antelope ground squirrel in fact has a pure-cone retina, or whether like the other species recently studied has a small population of rods, we have examined both the anatomy of the photoreceptors in this species and the nature of the ERG recorded from the intact eye.

RESULTS AND DISCUSSION

In order to anatomically determine the types of photoreceptors present one antelope ground squirrel was prepared for both autoradiography and electron microscopy. The squirrel (200 g) was injected intraperitoneally with 30 mCi of (4,5-^{3}H)-L-leucine (sp. activity 51 Ci/mM; 389 Ci/mg; Amersham Searle) dissolved in 2.0 ml of distilled water. After 72 hr the animal was given a lethal dose of sodium pentobarbitol and fixed by intracardiac perfusion of a glutaraldehyde–picric acid fixative as described in detail elsewhere (Jacobs *et al.*, 1976; Anderson and Fisher, 1976). The retina was postfixed in 2% buffered osmium tetroxide dehydrated in ethanol, and embedded in Araldite. For autoradiography, 0.5 μm sections were placed on pre-cleaned glass slides and dipped into a 1:1 aqueous solution of Kodak Nuclear Track Emulsion (NTB-2) maintained at 40°C. Autoradiograms were developed for 2 min in Dektol (17°C) diluted 2:1 with water and placed in acid fixer for 5 min. After washing in distilled water the autoradiograms were stained with a 1:1 mixture of 1% methylene blue and 1% azure II. Light micrographs were taken on 35 mm Kodak high contrast copy film.

Sections for histological examination by light and electron microscopy were taken from the same retina as was used for autoradiography. Sections for light microscopy were stained with the methylene blue–azure II mixture or a saturated aqueous solution of *p*-phenylenediamine while thin sections for electron microscopy were placed on either bar or mesh grids, stained with 1% aqueous uranyl acetate (20 min) and lead citrate (10 min), and examined in a Siemens 101 electron microscope.

Using structural criteria, two photoreceptor types were easily identified in the electron micrographs. One type can be clearly classified as identical to rods described in other species of ground squirrels on the basis of outer segment ultrastructure (Jacobs *et al.*, 1976; Anderson and Fisher, 1976), the presence of "pale" or "lucent" cytoplasm (West and Dowling, 1975), and shape and organization of the synaptic terminal (West and Dowling, 1975; Jacobs *et al.*, 1976) (see Figs. 1–4). Using the pale cytoplasma as a criterion for recognizing rods in light microscopy, counts of photoreceptors were made in 1 μm sections taken from the central portion of the eye. Out of 975 receptors 56 (5.7%) could be classified as rods, a value close to that reported for other ground-dwelling sciurids (West and Dowling, 1975; Jacobs *et al.*, 1976).

The outer and inner segments of both rods and cones occupy a single row across the retina. Both types of outer segments are cylindrical and abut the pigment epithelium at the same level (Fig. 1). Rod outer segments are slightly longer and thinner than those of the cones (averaging 8 × 1.5 μm compared

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to 6.8 × 2 μm respectively). Rod outer segments in the antelope ground squirrel are composed of a stack of independent discs surrounded by a plasma membrane (Fig. 3) whereas the cone outer segments have a continuously folded membrane forming the lower one-third of their length (Fig. 4)—characteristics shared with other ground squirrel rods and cones (Jacobs *et al.*, 1976; Anderson and Fisher, 1976). The photoreceptor terminals in this species are also very similar to those described in other ground squirrels (West and Dowling, 1975; Jacobs *et al.*, 1976) inasmuch as the cone terminals have a typical expanded pedicle shape while the rods terminate with a blunt rounded shape on the border of the outer plexiform layer (Figs. 1 and 2). As has also been previously described for other ground squirrel rod terminals (West and Dowling, 1975; Jacobs *et al.*, 1976), those in this species contain several synaptic ribbons (Fig. 2) and organization of post-synaptic processes at the ribbon synapse often appears as a triadic arrangement when examined in single thin sections.

In the autoradiograms of the antelope ground squirrel retina two distinct labeling patterns were seen in the photoreceptor outer segments (Fig. 5). These conform to the labeling patterns consistently described for a variety of vertebrate rods and cones (Young, 1967; 1971a, b; Anderson and Fisher, 1975, 1976). After 72 hr of labeling with ^{3}H-leucine a small population of outer segments show the accumulation of labeled protein into a distinct band which is displaced about 3.4 μm from the outer segment base (Fig. 5). Cells which show this pattern of labeling also have the "pale" cytoplasm characteristic of the receptors identified as rods by electron microscopy (Fig. 5). The majority of outer segments do not show the accumulation of radioactive protein into discrete bands but rather show a diffuse labeling over the entire outer segment (Fig. 5), a picture typical of vertebrate cones.

ERG evidence for rod operation was sought in the same manner as in our previous experiments (Jacobs *et al.*, 1976). In order to maximize a possible rod signal, ERGs were recorded from thoroughly dark-adapted animals using large field, low-intensity stimuli coupled with response averaging. Specifically, ERGs were recorded from anesthetized antelope ground squirrels with insulated stainless steel electrodes which were inserted through the cornea into the posterior chamber. An indifferent electrode was sewn into the skin above the eye. The ERG signals were recorded differentially through an amplifier having a bandpass of 0.2–1000 Hz. Signals from the amplifier were averaged in an Ortec 4623 Signal Averager and then printed out with an *X–Y* plotter. Photic stimuli were obtained from a double-beam Maxwellian view optical system. Spectral stimuli were produced by a Bausch & Lomb high-intensity grating monochromator (half-energy passband of 10 nm). The output from the monochromator was calibrated by recording the output from the detector head of a Hewlitt-Packard model 8330A Radiant Flux Meter placed in the test beam. Light from the monochromator, along with light from an achromatic source which could be used as an adaptation light, illuminated a region of the retina having an angular subtense of 40°.

Following the surgical preparation, the squirrels were initially dark adapted for a period of at least 45 min prior to data collection. After that, dim monochromatic test flashes (100 msec duration) were presented to the eye once every 10 sec. Responses to a minimum of 10 such flashes were averaged for each wavelength–intensity combination. Spectral locations from 440 to 620 nm, in steps of 20 nm, were explored in unsystematic order. All ERG measurements were based on the base-to-peak amplitude of the b-wave.

Spectral sensitivity functions were obtained from the ERG of the dark-adapted antelope ground squirrel by determining for each test wavelength the stimulus energy required to produce a criterion signal having an amplitude of 10 μV. The results of this procedure are shown in Fig. 6. The data shown in the top part of that figure (circles and triangles) are the sensitivity values for two antelope ground squirrels (one male, one female). Those sensitivity values are plotted from an equal quantal base and they are corrected for the preretinal absorbance of light by the lens in this species using measurements reported by Crescitelli and Pollack (1966). The data therefore represent the spectral sensitivity of the dark-adapted retina of this species. The agreement between the two animals was remarkably high—the mean difference in sensitivity for all test wavelengths was less than 0.1 log units. The solid line in the top portion of Fig. 6 represents the sensitivity curve for a nomogram photopigment (Dartnall, 1953) having a peak at 500 nm. The correspondence between the data and the nomogram is very good and it is thus apparent that the spectral sensitivity of the eye of the dark-adapted antelope ground squirrel is that expected of a retina containing a typical mammalian rhodopsin.

Although our primary interest was in rods and the

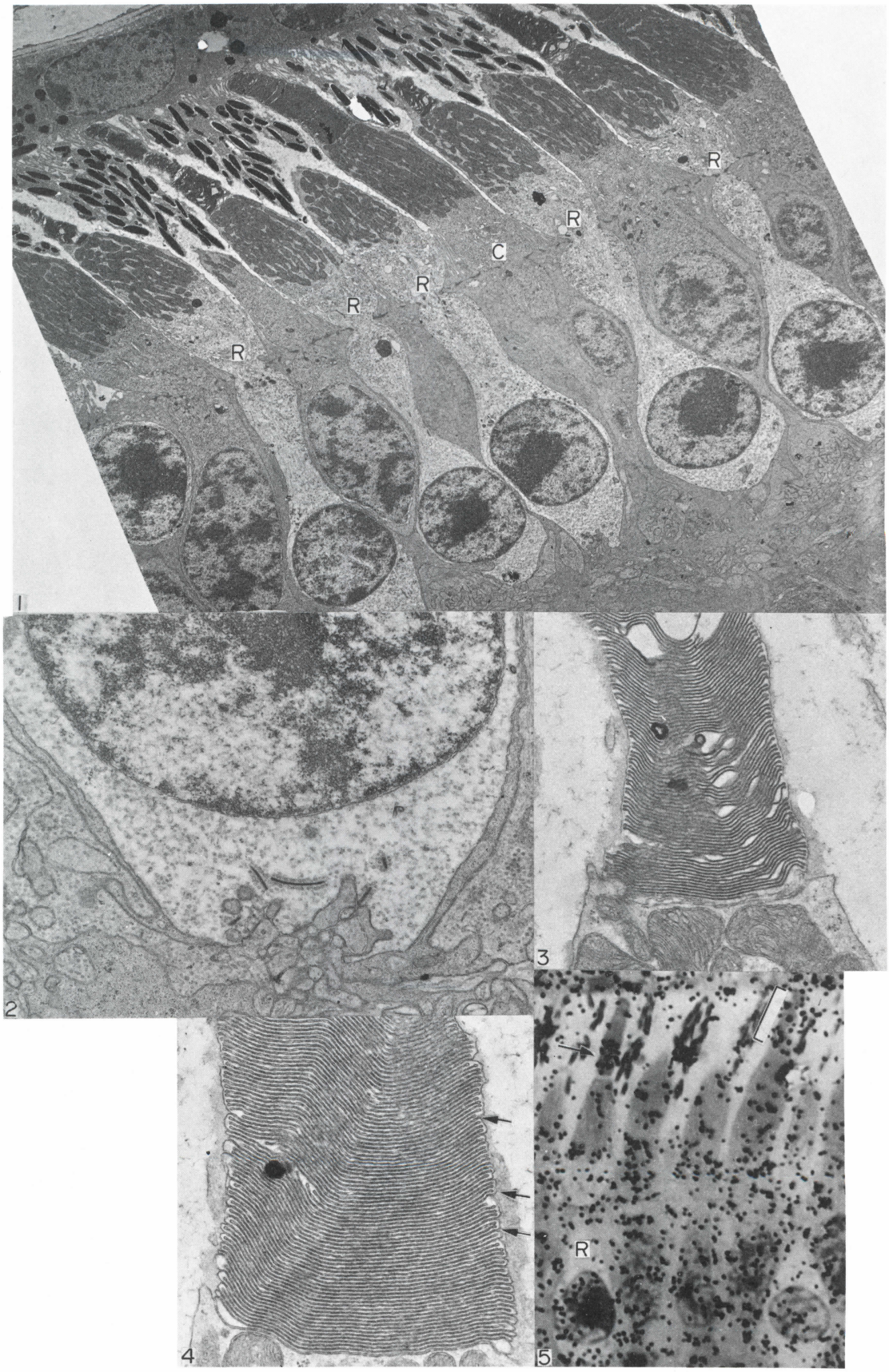
Fig. 1. A low power electron micrograph of the photoreceptor layer in the antelope ground squirrel retina. Receptors identified as rods (R) have paler cytoplasm and slightly longer and thinner outer segments than those identified as cones (C). This field of view was selected to demonstrate the presence of rods in the retina of this species; it is, however, atypical in that rods predominate. 4000 ×.

Fig. 2. An electron micrograph of the rod synaptic terminal showing the general shape of these terminals as well as their pale cytoplasm, the presence of synaptic vesicles, and five synaptic ribbons. 16,000 ×.

Fig. 3. An electron micrograph of the base of a rod outer segment. The stack of discs comprising the outer segment is surrounded by the plasma membrane of the cell. 17,500 ×.

Fig. 4. An electron micrograph of the base of a cone outer segment. The lower one-third of the cone outer segment is composed of invaginations (arrows) of the plasma membrane. 20,000 ×.

Fig. 5. A light micrograph of an autoradiogram of the photoreceptors after 72 hr labeling with ^{3}H-leucine. There is accumulation of labeled protein into a distinct band in the rod (R) outer segment (arrow) while the cone outer segment shown (bracket) is evenly labeled over its entire length. 2800 ×.



Figs. 1–5.

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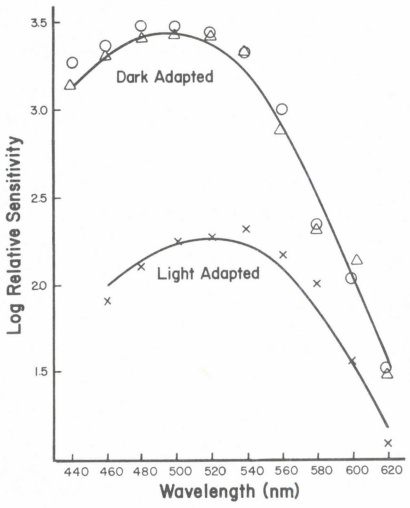

Fig. 6. Spectral sensitivity of the ERG recorded from the antelope ground squirrel. The data plotted at the top are sensitivity values for two dark-adapted animals (criterion amplitude of 10 μV). The solid line is that for a nomogram photopigment having a 500 nm peak. The data shown at the bottom were obtained from a light-adapted antelope ground squirrel (criterion amplitude of 30 μV). All sensitivity values are based on responses to stimuli having equal numbers of quanta at each test wavelength. These values are also corrected for pre-retinal absorbance in this species.

dark-adapted eye, we also measured spectral sensitivity for conditions of light adaptation for purposes of comparison. The values shown in the lower half of Fig. 6 represent the spectral sensitivity (criterion amplitude of 30 μV) of the ERG of one antelope ground squirrel whose eye was continuously exposed to an achromatic adaptation light having a luminance of 1 log cd/m^2. As noted previously (Crescitelli and Pollack, 1972), this function peaks at about 525 nm and, thus, the Pukinje shift in this species is accompanied by a shift in peak sensitivity of approx 25 nm.

Although the data shown in Fig. 6 clearly suggest the operation of rods in the retina of the antelope ground squirrel, it is worth pointing out that in at least one preparation we examined it proved impossible to find unambiguous evidence for a dark-adapted spectral sensitivity function having a 500 nm peak. This is noteworthy because it parallels our experience in much more extensive measurements made on the California ground squirrel (Jacobs *et al.*, 1976) in which it also proved impossible to find clear evidence for a function having a 500 nm peak in each of the individuals tested. Insofar as these ERG data are adequate indices, this suggests the possibility that these ground squirrel species may show some individual variation either in the number of rods present or in the effectiveness of the rod contribution to retinal signals.

In summary, in both structure and function the retina of the antelope ground squirrel appears similar to the retinas of other ground squirrels recently examined in the sense that it contains an operational rod system in addition to a much more obvious cone system. Whether or not any of the species of ground-dwelling sciurids have an all-cone retina remains an experimental question but that possibility appears increasingly unlikely.

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RESEARCH NOTE

RADIOAUTOGRAPHIC LOCALIZATION OF ^{125}I α-BUNGAROTOXIN BINDING SITES IN THE RETINAS OF GOLDFISH AND TURTLE[1]

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There is substantial evidence for the action of acetylcholine (ACh) as a neurotransmitter in the vertebrate retina. Cholinergic mimics and antagonists alter the response properties of optic nerve fibers in rabbit (Ames and Pollen, 1969), cat (Straschill, 1968) and the ERG of frog (Val'tsev, 1966). The hydrolytic enzyme acetylcholinesterase (AChE) is found in the retinas of a wide variety of vertebrates (Francis, 1953; Nichols and Koelle, 1968; Dickson, Flumerfelt, Hollenberg and Gwyn, 1971). In mammalian and avian retinas AChE is confined largely to the inner plexiform layer (IPL) and amacrine cells (Nichols and Koelle, 1968). However, in the newt, an amphibian, AChE was found in the outer plexiform layer (OPL) where it was associated with horizontal and bipolar cell processes (Dickson *et al.*, 1971). Since the mere presence of AChE is not conclusive proof that a neuron utilizes ACh as a transmitter, additional histological markers are needed if cholinergic transmission is to be demonstrated more convincingly and localized more accurately in the retina.

Such an approach has become possible with the discovery that α-bungarotoxin (α-BTX), the principal component of the venom from the banded krait, *Bungarus multicinctus*, binds to nicotinic receptors with high affinity (Chang and Lee, 1963). With radioactively-labeled α-BTX and the related α-toxin from *Naja nigricollis*, the ACh receptor sites at neuroeffector junctions of electric organ (Bourgeois, Ryter, Menez, Fromageot, Boquet and Changeux, 1972) and muscle (Porter, Chiu, Wieckowski and Barnard, 1973; Fertuck and Salpeter, 1974) were localized and quantified. Also, toxin binding sites were described in the chick optic tectum, rat hippocampus, and other structures of the central nervous system (Polz-Tejera, Schmidt and Karten, 1975). It seems reasonable to expect that if ACh is a retinal transmitter, the location of nicotinic receptors sites could be found by radioautography utilizing labeled α-BTX.

Retinas of goldfish (*Carassius auratus*) and red-ear turtles (*Pseudemys scripta*) were used in this study. Alpha-bungarotoxin was isolated from *B. multicinctus* venom and labeled with ^{125}iodine as described previously (Lowy, McGregor, Rosenstone and Schmidt, 1976); the specific activity was 50 μCi/μg at the start of the experiments. The specificity and extent of ^{125}I α-BTX binding to retina were analyzed biochemically. Retinas were excised and homogenized in 1.5 ml of 10 mM sodium phosphate, 1.0% Triton X-100, 0.02% sodium azide with a glass tissue grinder. Homogenates were gently agitated at 4°C for 1.5 hr and then centrifuged at 30,000 *g* for 30 min. Supernates were divided and experimental samples were adjusted to 0.875 retina/ml by addition of 10 mM sodium phosphate (pH 7.4). Control extracts were adjusted to the same concentration with unlabeled α-BTX (final concentration 7×10^{-6} M) in phosphate buffer. Aliquots of 0.04 ml of the extracts (1.47% of one retina) were assayed for ^{125}I α-BTX binding using the DEAE cellulose disk procedure (Schmidt and Raftery, 1973). The results (Fig. 1) show that both retinal extracts contain ^{125}I α-BTX binding activity. Extracts pretreated with unlabeled α-BTX nonspecifically take

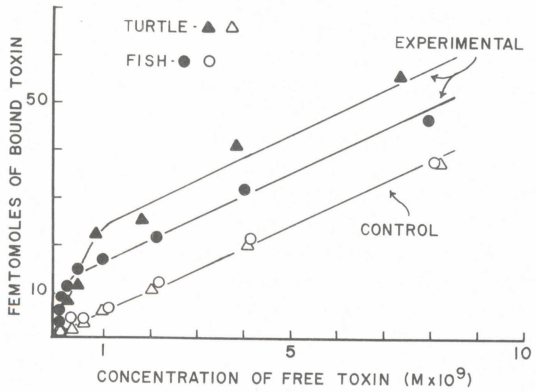

Fig. 1. Binding of ^{125}I α-BTX to retinal extracts. Triton X-100 extracts of retinas from turtle and goldfish were incubated with a range of ^{125}I α-BTX concentrations (abscissa) and the amount of bound toxin was determined (filled symbols). In the control experiment (open symbols) extracts were treated with native toxin prior to incubation with radiotoxin. For details see text.

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