

## Photoreceptor fine structure in the bobtail goanna (*Tiliqua rugosa*)

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**Summary.** The fine structure of the retinal photoreceptors has been studied by light and electron microscopy in the bobtail goanna (*Tiliqua rugosa*) an Australian diurnal lizard. The photoreceptors in this species are readily divisible into rods or cones based on morphological criteria. Single cones are the dominate cell type with a cone:rod ratio of about 80:1. No multiple photoreceptors were present nor was a photoreceptor mosaic observed. Cones are large cells with a short, tapering outer segment. The inner segment contains a large apical oil droplet, an ellipsoid of mitochondria and a prominent paraboloid of glycogen. Rods are slender cells with a longer non-tapering outer segment. Rod inner segments display an ellipsoid but no oil droplet or paraboloid. The nuclei of cones are large and vesicular while rod nuclei are smaller and displayed more heterochromatin. The synaptic pedicle of cones is larger than the spherule of rods. Both rods and cones displayed both invaginated (ribbon) and conventional synapses with the large cones having more synaptic sites. As only light-adapted specimens were examined it is uncertain if either rods or cones are capable of retinomotor responses.

**Key words:** Photoreceptors, Electron microscopy, Lizard, *Tiliqua rugosa*

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### Introduction

Retinal photoreceptors which form the first neuron in the visual pathway have been described in a variety of vertebrate species. These cells have been extensively studied because of their extreme polarization and specialization (Walls, 1942; Detwiler, 1943; Polyak, 1957) and more recently because of their remarkable

regenerative capabilities (Young, 1971, 1974, 1976; Bok and Young, 1972; Cohen, 1972; Braekevelt, 1975, 1983a).

Traditionally retinal photoreceptors have been classified as either rods or cones on the basis of their morphological criteria (Walls, 1942; Stell, 1972). While in some species the morphology can become somewhat ambiguous (Sjostrand, 1958; 1959; Pedler, 1965, 1969) for the most part the classification of rods and cones accurately and adequately describes these cells (Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1975, 1983a,b, 1984, 1985).

As part of an ongoing comparative morphological study of vertebrate photoreceptors, this report describes the fine structure of the rods and cones in the cone-dominated duplex retina of an Australian reptile, the bobtail goanna *Tiliqua rugosa*.

### Materials and methods

For this study the eyes from ten healthy adult bobtail goannas *Tiliqua rugosa* were examined by light and electron microscopy. Specimens were 3-4 years old, of both sexes, weighed 420-550 gm and were light-adapted when sampled.

With the animals under deep anesthesia, the eyeballs were quickly removed, opened at the equator and fixed for 5 h in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer at 4°. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm<sup>2</sup> taking care not to detach the retina. The tissue was then post-fixed for 2h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reorientated to desired angles by means of a wax mount. Thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections of

selected areas (60-70 nm) were then cut, collected on copper grids and stained in aqueous uranyl acetate and lead citrate. These sections were examined and photographed in a Philips EM201 transmission electron microscope.

## Results

The duplex retina of the bobtail goanna (*Tiliqua rugosa*) is extremely cone dominant with the cone:rod ratio being approximately 80:1 (Fig. 1). All cones are single cones and no multiple photoreceptors are present (Figs. 1, 2).

The cones are large cells which project through the external limiting membrane (ELM) for up to 30  $\mu\text{m}$  in the light-adapted state (Fig. 3). Of this length the inner segment is the largest portion at 22-25  $\mu\text{m}$  with only a relatively short outer segment (Fig. 3). In the light-adapted condition, the inner segment measures about 5  $\mu\text{m}$  in diameter near the ELM and tapers slightly to 3-4  $\mu\text{m}$  near its outer end (Figs. 2, 3). At its apex all cone inner segments display a single, electron-dense, membrane-bound oil droplet (Figs. 1, 3, 5). Below the oil droplet is a very large accumulation of mitochondria (the ellipsoid) and below this a large aggregation of glycogen (the paraboloid) (Figs. 2, 3, 6). In the cytoplasm immediately surrounding both the ellipsoid and paraboloid, small moderately electron-dense granules often occur singly (Figs. 2, 3) or in small groups (Fig. 3). Below the paraboloid the inner segment (myoid region) is rich in polysomes, Golgi zones and some profiles of rough endoplasmic reticulum (Figs. 2, 3, 7). Presumed autophagic vacuoles are also noted in this region (Fig. 7).

Cone outer segments are relatively short (5-7  $\mu\text{m}$ ) and taper distally (Figs. 1, 3). Neither cones nor rods display incisures within the discs of the outer segment (Figs. 3, 4, 5). Both rods and cones however show a typical eccentrically located cilium joining the inner and outer segments.

Rod photoreceptors are much narrower than cones and have an inner segment measuring only about 2  $\mu\text{m}$  in diameter in the light-adapted condition (Fig. 4). The rod outer segment is of much the same diameter, does not taper and measures about 10  $\mu\text{m}$  in length (Fig. 4). A prominent ellipsoid is located at the tip of the outer segment but no oil droplet is present nor is a paraboloid (Fig. 4). Unlike the cone ellipsoid where the mitochondria are large and mainly oval in shape, the mitochondria of the rod ellipsoid are mostly long and slender and aligned with the length of the rod (Fig. 4).

Lateral fins projecting from the inner segment which are often prominent in lower vertebrates were not obvious in this reptilian species (Figs. 1, 3).

In the light-adapted condition the photoreceptor outer segments and to a great extent the inner segments are essentially isolated from one another by the pigment-laden apical processes of the retinal epithelium (RPE) (Figs. 1, 5, 6).

Cone nuclei are large and vesicular and located at all levels of the relatively thin outer nuclear layer (Figs. 3, 7) and in the light-adapted condition may even protrude

slightly through the ELM (Fig. 3). Rod nuclei are smaller and display more heterochromatin.

The external limiting membrane (ELM) is formed by a series of zonulae occludentes between Müller cells and photoreceptors. (Figs. 3, 7). Processes of the Müller cells project through the ELM for 5-10  $\mu\text{m}$  around the base of the photoreceptor inner segments (Figs. 3, 7).

The cones display large synaptic pedicles which contain 15-20 invaginated (ribbon) synapses as well as numerous superficial (conventional) synaptic sites (Fig. 8). Rod spherules were extremely difficult to locate but only displayed 2-3 invaginated sites and a few superficial synaptic contacts.

## Discussion

The traditional division of vertebrate retinal photoreceptors into either rods («Stäbchen») or cones («Zapfchen») was originally proposed by Schultze (1866). In this classical separation, typical rods have cylindrical inner and outer segments of much the same diameter, while typical cones have a shorter conical outer segment and an inner segment of greater diameter. This classification was used exclusively in numerous light microscopic studies (Walls, 1942; Polyak, 1951; Duke-Elder, 1958). With the advent of electron microscopy and the more detailed examination of these cells in more species, it was felt that not all photoreceptors were adequately described by the simplistic terms of rod or cone (Dowling, 1965). This led various workers to propose new categories of photoreceptor classification based on criteria other than just cell shape. (Sjostrand, 1958, 1959; Pedler 1965, 1969). While these more elaborate classifications are perhaps more accurate and in some non-mammalian species may even be necessary, in most cases retinal photoreceptors can be adequately

**Fig. 1.** Electron micrograph of the photoreceptors of the bobtail goanna. Several cones (c) are sectioned at various levels. The apical processes of the retinal epithelium (RPE) are indicated.  $\times 4,300$

**Fig. 2.** Electron micrograph to indicate the ellipsoid (E) and paraboloid (P) of cone cells.  $\times 6,600$

**Fig. 3.** Electron micrograph to illustrate the ellipsoid (E), paraboloid (P), oil droplet (OD) and nuclei (N) of cone cells.  $\times 4,100$

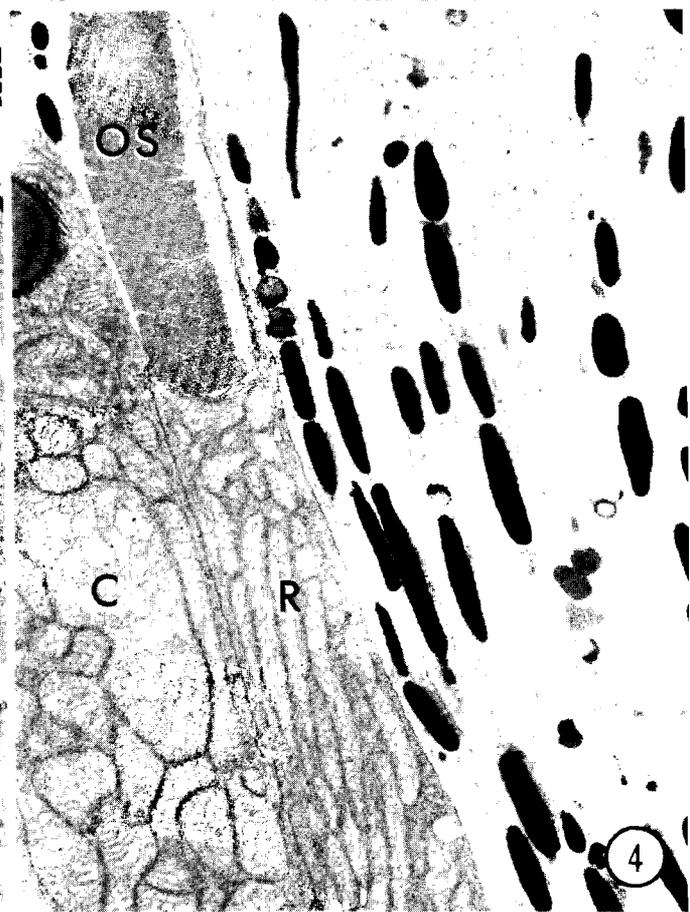
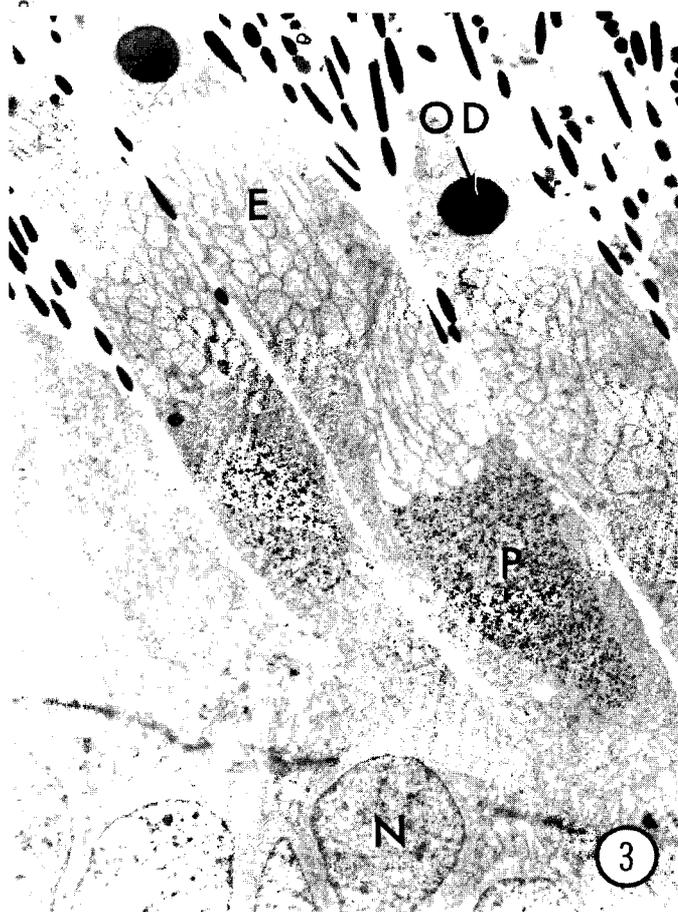
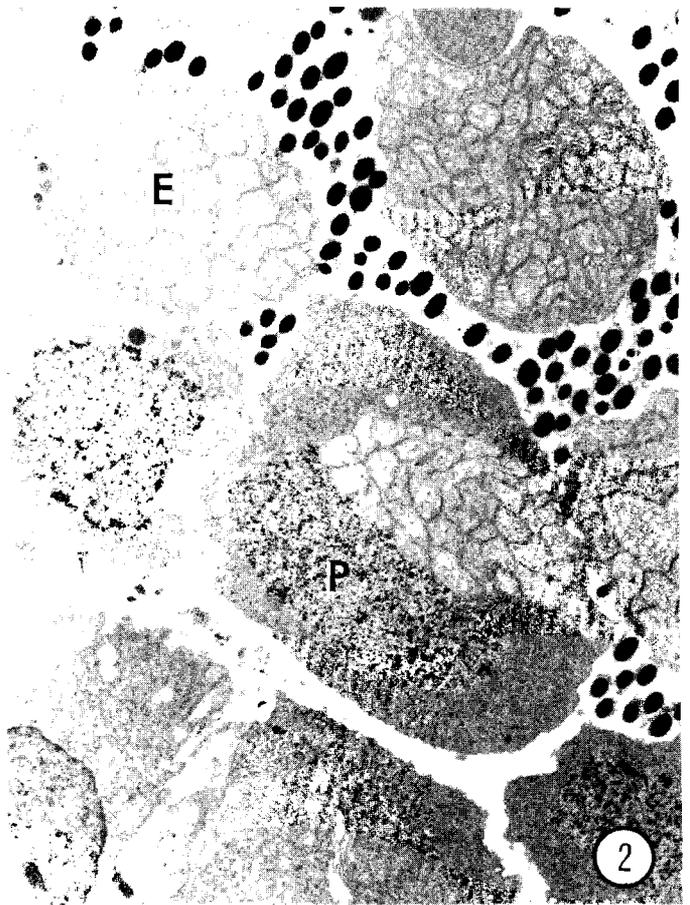
**Fig. 4.** Electron micrograph of a rod (R) and a cone (C) cell. Note the differences in ellipsoid mitochondria morphology and the cylindrical outer segment (OS) of the rod.  $\times 8,900$

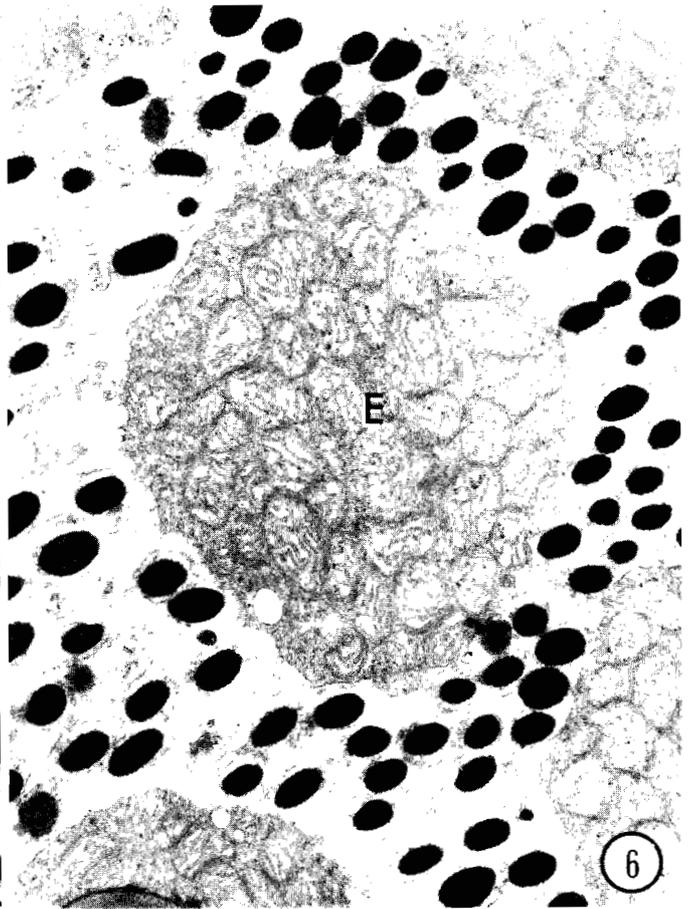
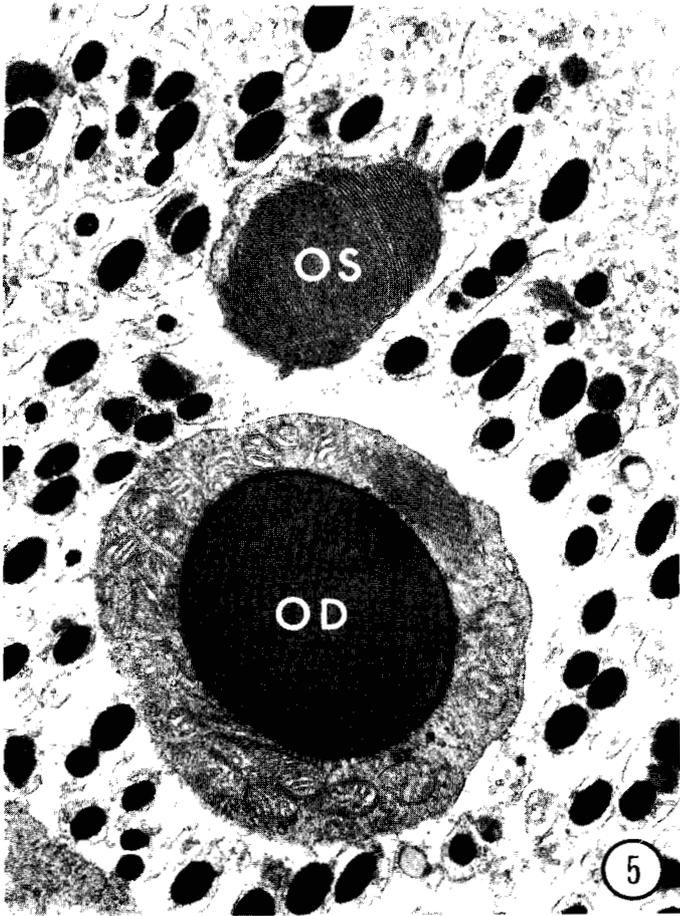
**Fig. 5.** Electron micrograph of the oil droplet (OD) and the outer segment (OS) of two cone cells surrounded by RPE apical processes.  $\times 13,200$

**Fig. 6.** Electron micrograph of the ellipsoid region (E) of a cone photoreceptor.  $\times 13,200$

**Fig. 7.** Electron micrograph of the myoid region of a cone to indicate the paraboloid (P), a Golgi zone (G) and presumed autophagic vacuoles. The external limiting membrane (ELM) is also indicated.  $\times 9,100$

**Fig. 8.** Electron micrograph of the synaptic pedicles of two cone cells (C). Both invaginated (ribbon) synapses as well as superficial (conventional) synapses are obvious  $\times 13,200$





described and differentiated by the classical terms of rods or cones.

Ironically while the lacertilian retina was felt to be one of those in which the photoreceptors did not fit the old classification of rods or cones adequately, in the bobtail goanna (*Tiliqua rugosa*) the photoreceptors can be effectively differentiated by the traditional terms. In addition where multiple cones arranged in a mosaic pattern are a common feature in diurnal lizards (Dunn, 1966) only single cones are noted in this species. Finally while a preponderance of cones is not unusual in strictly diurnal species, the diurnal lizards are reported to have «pure-cone» retinas (Crescitelli, 1972). In the bobtail goanna while cones far outnumber rods (approximately 80:1), some rod photoreceptors are present. Indeed it is doubtful if any retina is «pure-cone» or «pure-rod» as with electron microscopy, retinas are usually shown to be duplex even if one photoreceptor type is in a dominant or even overwhelming majority (West and Dowling, 1975).

The outer segments of both rods and cones consist of a stack of bitembranous discs (Cohen, 1972). In rods these outer segment discs are usually all of the same diameter while in the cones the more apical discs are smaller than those of the basal region, giving the outer segment a tapering or conical shape (Cohen, 1972). In most species studied, cone discs display a circular outline while rod discs very often have a scalloped or lobulated perimeter due to the presence of one or more incisures (Cohen, 1972; Nilsson, 1965; Braekevelt, 1983a,b). In this species neither rods nor cones show incisures in the outer segment discs.

In retinas which undergo photomechanical or retinomotor movements in response to changes in environmental lighting, cones are most contracted and rods are most elongated in the light-adapted condition (Walls, 1942; Rodieck, 1973; Burnside and Laties, 1979). In the light-adapted specimens examined in this study, cones are greatly elongated but the few rods observed were of much the same length. This might mean that rods show retinomotor movements and that cones do not or perhaps neither photoreceptor type moves appreciably. Walls (1942) reports that no rods are present in diurnal lizards and that cone movement and pigment migration (in the RPE) is very slight and slow if indeed it occurs at all.

The connecting cilium located between inner and outer segments is a constant feature of all vertebrate photoreceptors described. In this species the cilium is typical of that found in other studies in that it is eccentrically located and displays the peripheral arrangement of nine pairs of microtubules and lacks the central pair found in motile cilia (Dowling and Gibbons, 1961; Cohen, 1963).

The inner segment of a photoreceptor cell is known to be the synthetic center of the cell and it is here that the materials for new outer segment discs are produced (Young, 1976). At the apex of the cone inner segment in the bobtail goanna is a single large oil droplet. Such oil droplets have been reported in amphibians, reptiles, birds and non-placental mammals (Rodieck, 1973; Braekevelt,

1973; Meyer, 1977; Kolb and Jones, 1982). Oil droplets apparently selectively filter light and in so doing enhance contrast, reduce glare and lessen chromatic aberration (Meyer, 1977).

The large accumulation of mitochondria at the apex of the inner segment (the ellipsoid) is a constant feature of all photoreceptors (Cohen, 1972; Rodieck, 1973). The paraboloid which is an accumulation of glycogen is found in the inner segment of some fish, amphibians, birds and reptiles (Cohen, 1972). The presence of an ellipsoid, paraboloid, numerous polysomes and some RER, Golgi zones and autophagic vacuoles as noted in this species is indicative of metabolically very active cells (Cohen, 1972; Reme and Sulser, 1977).

The region between the ellipsoid and the cell nucleus is often referred to as the myoid as it is this region which changes in length during retinomotor movements (Cohen, 1972). As only light-adapted specimens were studied, it is uncertain if the myoid of either the rods or cones in the bobtail goanna will change in size and shape in response to changes in environmental lighting.

As is the case in most other species, the external limiting membrane (ELM) in the bobtail goanna is formed by a series of zonulae adherentes (Uga and Smelser, 1973).

Within the outer plexiform layer, the synaptic pedicle of the cone cell is typically larger and displays more synaptic sites than the rod spherule (Cohen, 1972; Crescitelli, 1972). Synaptic sites on retinal photoreceptors are either invaginated and associated with a synaptic ribbon (Missotten, 1965) or are the more conventional superficial type involving only membrane densifications (Dowling, 1968; Cohen, 1972). While bipolar and horizontal cells are both involved at invaginated synapses (Kolb, 1970), superficial synapses may be between photoreceptors and bipolar cells or between photoreceptors themselves (Cohen, 1964; Missotten, 1965; Kolb, 1970). In a few lower vertebrates only superficial synapses are reported (Dowling and Werblin, 1969). The bobtail goanna displays both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rods and cones.

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