

Fine structure of the retinal photoreceptors of the tiger salamander (*Ambystoma tigrinum*)

C.R. Braekevelt

Department of Anatomy, The University of Manitoba, Winnipeg, Manitoba, Canada

Summary. The retinal photoreceptors of the tiger salamander (*Ambystoma tigrinum*) have been studied by light and electron microscopy in both light- and dark-adaptation. Rods and cones are present in this duplex retina in a ratio of about 20:1. As in other urodele species these photoreceptors are very large cells. The rod outer segment is composed of bimembranous discs of uniform diameter displaying several very deep incisures. The rod inner segment displays an ellipsoid of mitochondria and a myoid region which changes in diameter during the lighting cycle indicating that rods undergo photomechanical movements. Rod nuclei are located at all levels of the outer nuclear layer and rod spherules are large and display several invaginated and superficial synaptic sites. Cone photoreceptors while large cells are smaller than the rods. They show a smaller tapering outer segment, a large distal ellipsoid of mitochondria and a prominent paraboloid of glycogen but no oil droplet within the inner segment. Judging by the width of the myoid region which remains similar throughout the lighting cycle, cones in this species show no retinomotor responses. Cone nuclei are less electron dense than rods and are also located at all levels of the outer nuclear layer. The cone synaptic pedicle is larger than that of the rods and also shows several invaginated and superficial synaptic sites.

Key words: Photoreceptors, Electron microscopy, Amphibian, Tiger salamander, *Ambystoma tigrinum*

Introduction

Vertebrate retinal photoreceptors are highly polarized and extremely differentiated cells which are incapable of mitosis. They form the first neuron (receptor) in the visual pathway and while generic modifications are noted, morphologically they are constructed on a

basically similar plan throughout the vertebrate kingdom. This common structural design consists of an outer segment (the light capture area) joined to an inner segment (the synthetic area) by a non motile connecting cilium, a nuclear region and a synaptic end piece (Cohen, 1963, 1972; Crescitelli, 1972; Rodieck, 1973).

Traditionally retinal photoreceptors have been classified as either rods or cones based on morphological criteria at the light microscopic level (Walls, 1942; Duke-Elder, 1958). Rods and cones also differed in various physiological parameters such as the photopigments present and the presence of photomechanical movements in response to environmental lighting (Walls, 1942; Crescitelli, 1972). With the better resolution of electron microscopy and the comparative examination of more species it was felt by some workers that the simplistic classification of photoreceptors as either rods or cones was inadequate and advance other more complex methods of categorization for photoreceptors (Sjostrand, 1958, 1959; Pedler, 1965, 1969). While these methods may indeed be more accurate they are not widely used and for the vast majority of species the terms rod and cone quite accurately and adequately differentiate these cells (Cohen, 1972; Crescitelli, 1972; Braekevelt, 1983, 1985, 1989, 1990).

As part of an ongoing comparative morphological study of vertebrate retinal photoreceptors, this report describes the fine structure of the rods and cones in the duplex retina of a tailed amphibian (urodele), the tiger salamander (*Ambystoma tigrinum*).

Materials and methods

For this study, the eyes from six adult tiger salamanders (*Ambystoma tigrinum*) were examined by light and electron microscopy. The specimens were maintained on a 12 hr light-dark cycle with the lights on at 6 a.m. and off at 6 p.m. Three animals were sampled at noon (light-adapted) and midnight (dark-adapted) when the specimens were decapitated and the eyes quickly removed. The eyeballs were opened at the

Offprint requests to: Dr. C.R. Braekevelt, Department of Anatomy, The University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, R3E 0W3 Canada

equator and immersion fixed for 5 h at 4 °C in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer. The posterior halves of the eyeballs were then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm² taking care not to detach the retina. This tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer, dehydrated up through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently reoriented to desired angles by means of a wax mount and thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (60-70 nm) were then cut of selected areas and collected on copper grids. These sections were stained with aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

Results

The retinal photoreceptors of the tiger salamander (*Ambystoma tigrinum*) are very large cells and consequently the total number of both rods and cones is reduced in this urodelean retina. The retina is duplex and rod-dominant with a rod:cone ratio of about 20:1. All photoreceptors are single with no double cones and no obvious photoreceptor mosaic.

In the light-adapted state, rod photoreceptors measure about 5.0 µm in diameter in the inner segment region while in dark-adaptation the ellipsoid portion of the inner segment measures up to 12.0 µm in width with the rest of the inner segment (the myoid) measuring about 7.0 µm in width (Figs. 1-3). The rod outer segment measures about 7.5 µm in width throughout the light cycle (Figs. 3, 7, 8). The melanosomes within the retinal pigment epithelium (RPE) also undergo photo-mechanical movements and in the light-adapted state reach almost to the external limiting membrane (ELM) (Figs. 1, 2). In dark-adaptation the RPE melanosomes retreat to the cell body region and are only present around the extreme distal ends of the rod outer segments (Figs. 7, 8). Rod outer segments consist of a stack of bimembranous discs which show several irregular but very deep peripheral incisures which give the outer segment a very scalloped profile (Figs. 7-9). The rod inner segment consists of a distal accumulation of mitochondria (the ellipsoid) and a more proximal myoid region which is rich in profiles of rough endoplasmic reticulum, polysomes, Golgi zones and presumed autophagic vacuoles (Figs. 1-4). The ELM is composed of a series of zonulae adherentes between both

photoreceptor types and the Müller (glial) cells of the retina (Figs. 1, 3, 4). A series of microvillar processes also project through the ELM to surround the base of all photoreceptor cells (Figs. 1-4). These processes arise from the Müller cells and average about 5.0 µm in length but only about 0.5 µm in width (Figs. 3, 4). These do not appear to vary in length or width during the light-dark cycle.

Cone outer segments also consist of a stacked series of bimembranous discs which show a much smaller diameter distally than proximally to give the cone outer segment its typical conical appearance (Figs. 7, 9). Cone outer segment discs have no incisures and hence show a smooth profile (Figs. 7, 9). Below the connecting cilium which joins the inner and outer segment of all photoreceptors, the cone inner segment also shows a distal ellipsoid of mitochondria and a more proximal myoid region rich in rough endoplasmic reticulum, polysomes, and Golgi zones as well as a large and centrally located accumulation of glycogen (the paraboloid) (Figs. 2, 5, 6). Oil droplets were not present within the inner segment region of either rods or cones nor did any photoreceptors display vertically oriented cytoplasmic fens in the inner segment region (Figs. 1, 2, 6, 9). From the apex of the cone inner segment a series of microvillar processes arise to surround the cone outer segment of its base (Fig. 9).

In both light- and dark-adaptation, cones measure about 9.0 µm in diameter at the level of the paraboloid indicating that they do not undergo retinomotor movements (Figs. 2, 5). Cone nuclei are found at all levels of the relatively thin outer nuclear layer (due to the reduced number of photoreceptors) and normally display a less dense chromatin pattern than the rod nuclei, especially during dark-adaptation (Figs. 4, 5).

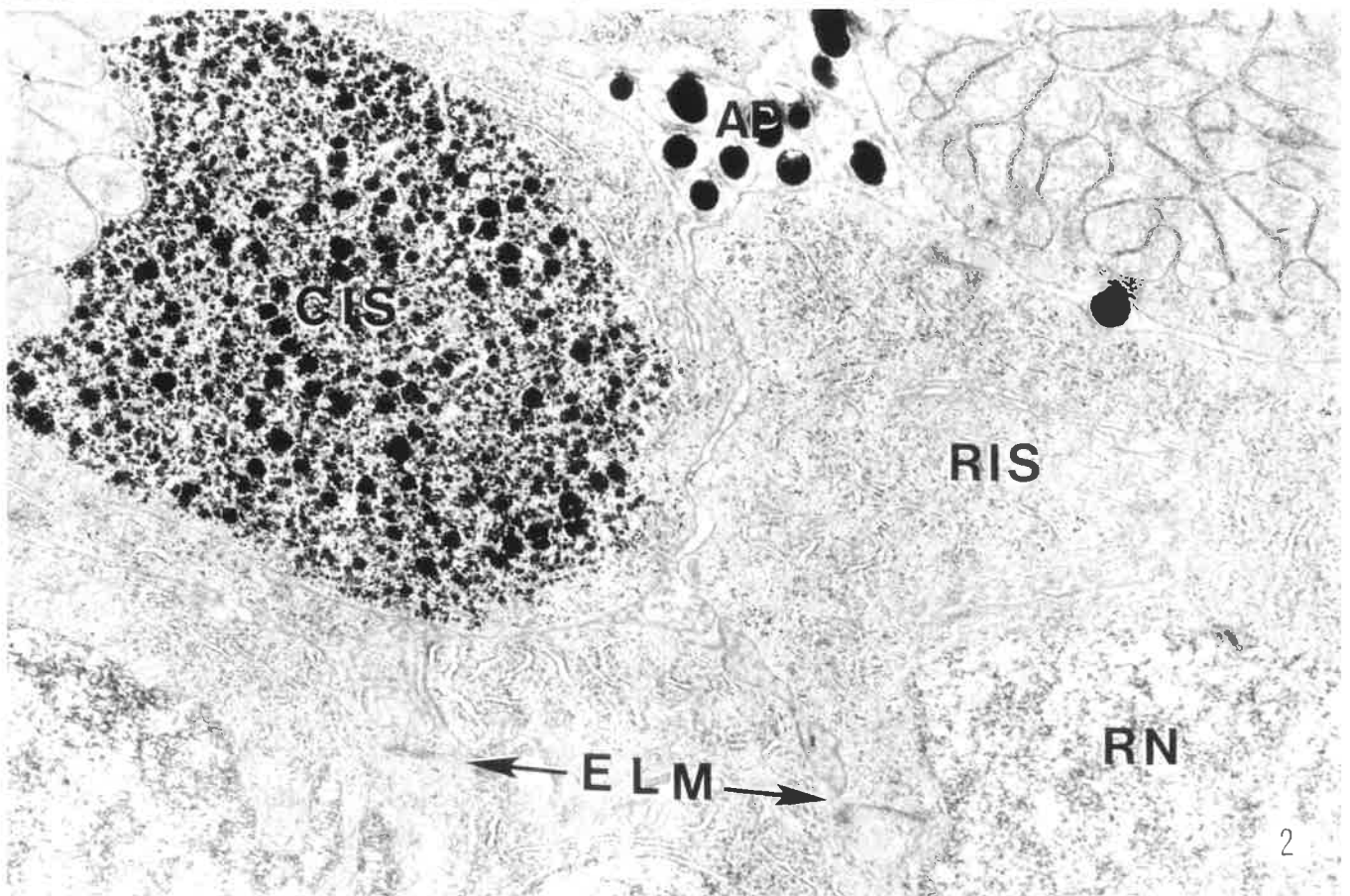
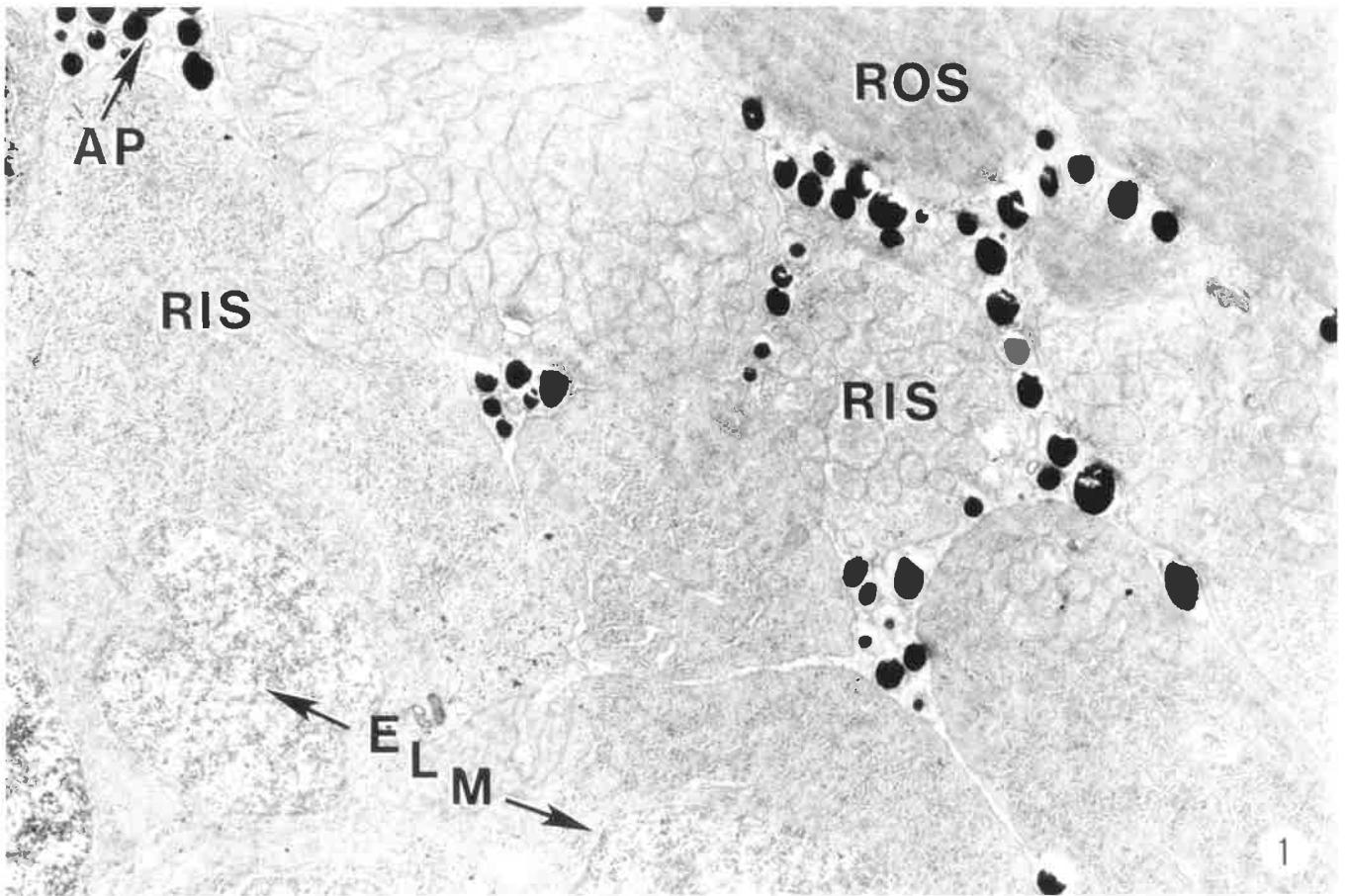
Within the outer plexiform layer, both rods and cones display both ribbon (invaginated) and superficial (conventional) synaptic sites with cones displaying more of each type presumably because of their relatively larger synaptic pedicle (Fig. 10). Both rods and cones are rich in synaptic vesicles in the synaptic region and cone photoreceptors are normally slightly more electron lucent in this region (Fig. 10).

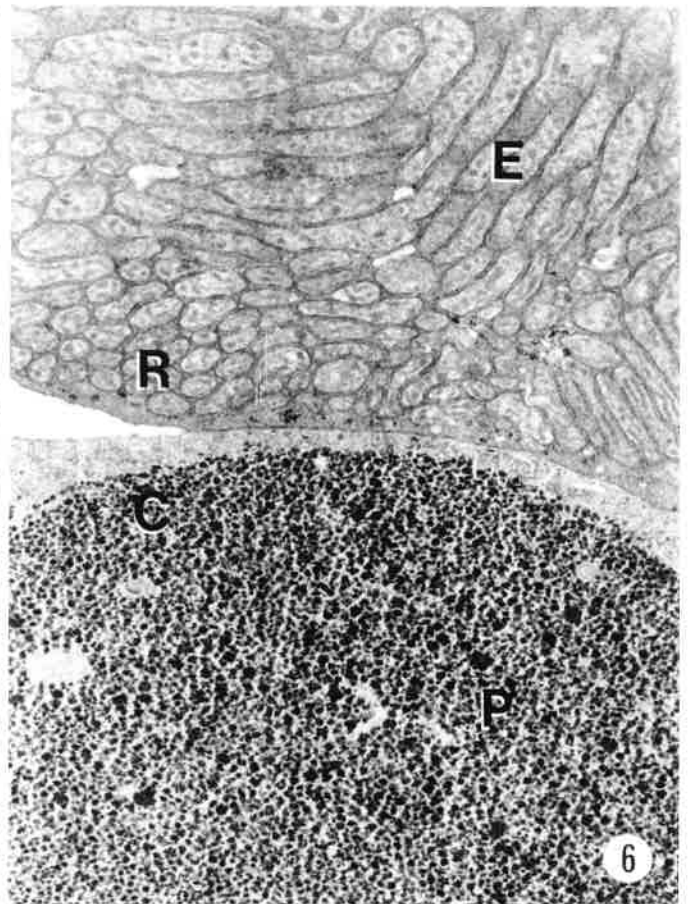
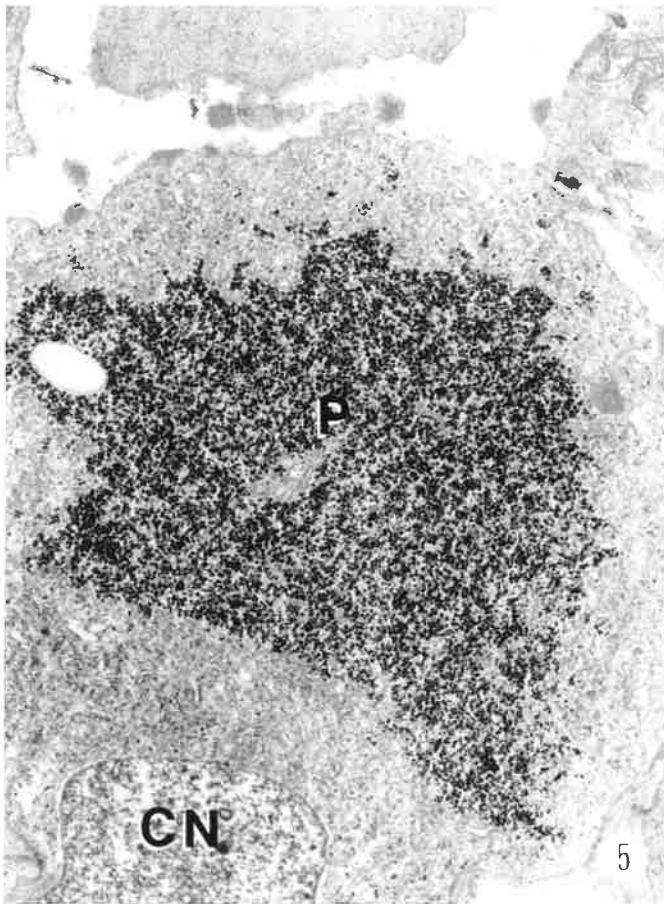
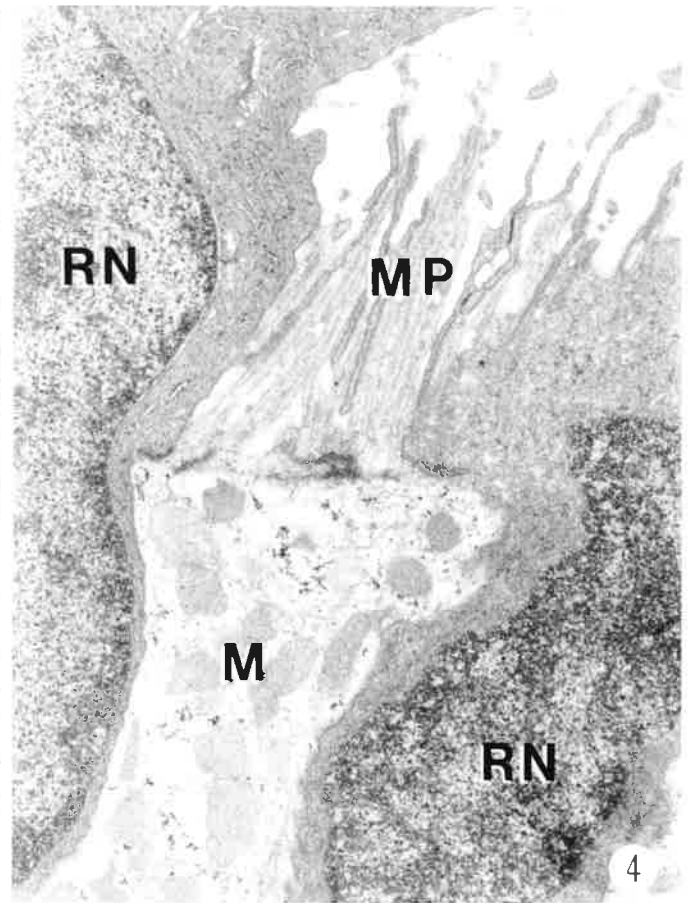
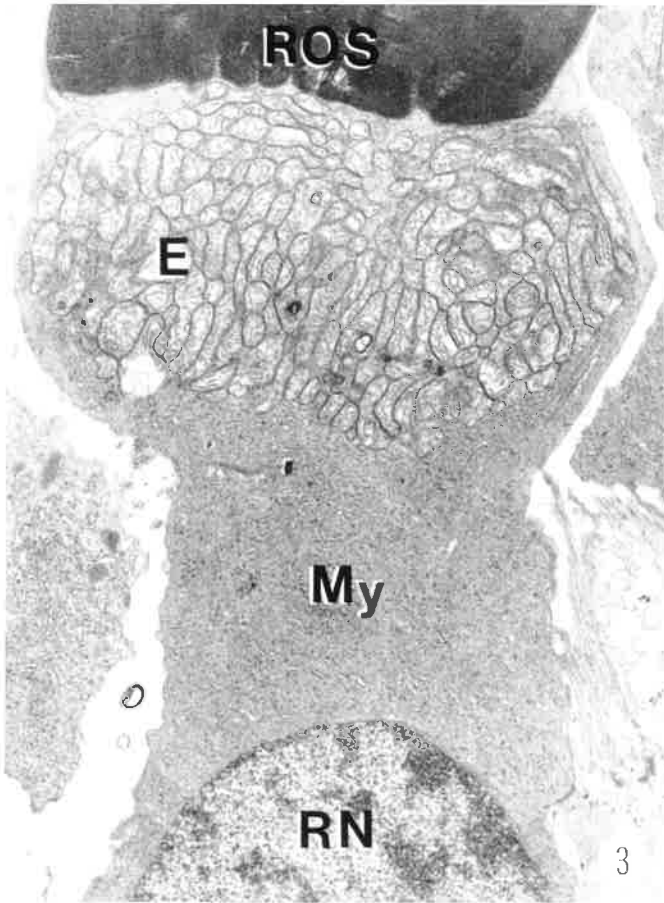
Discussion

The historical division of vertebrate retinal photoreceptors into either rods or cones was proposed by Schultze in 1866 and was used exclusively in numerous light microscopic studies of the retina (Walls, 1942; Polyak, 1957; Duke-Elder, 1958). In this classical separation, rods have cylindrical inner and outer segments of much the same diameter and a small

Fig. 1. Electron micrograph of the photoreceptors of the tiger salamander in light-adaptation. Rod inner (RIS) and outer segments (ROS) and the external limiting membrane (ELM) are indicated as are the pigment-laden apical processes (AP) of the RPE. x 8,600

Fig. 2. Electron micrograph of the photoreceptors also during light-adaptation to illustrate a cone inner segment (CIS) and rod inner segments (RIS). RPE apical processes (AP), the external limiting membrane (ELM) and a rod nucleus (RN) are also labelled. x 12,800





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Fig. 3. Electron micrograph of a rod photoreceptor in dark-adaptation. The outer segment (ROS), ellipsoid (E), myoid (My) and nucleus (RN) of the rod are all indicated.

Fig. 4. Electron micrograph of two rod photoreceptors in dark-adaptation. A rod nucleus (RN) and Müller cell processes (MP) are indicated as in the Müller cell body (M). x 8,900

Fig. 5. Electron micrograph of the myoid region of a cone photoreceptor in dark-adaptation. The large paraboloid (P) and the cone nucleus (CN) are indicated. x 8,900

Fig. 6. Electron micrograph of an adjacent rod (R) and cone (C). The ellipsoid (E) of the rod and the paraboloid (P) of the cone are labelled. x 12,600

synaptic ending (spherule) with only a few synaptic sites. Cones have a shorter, conical outer segment, and inner segment of greater diameter and a larger synaptic region (pedicle). With the advent of the better resolution of electron microscopy and the investigation of photoreceptor morphology in more species, new methods of classifying photoreceptors were advanced (Sjöstrand, 1958, 1959; Dowling, 1965; Pedler, 1965, 1969). While these new classifications are perhaps more accurate, they have never become popular and the classical terms of rods or cones are still widely used and for the most part felt to accurately and adequately differentiate the two photoreceptor types (Rodieck, 1973; Braekevelt, 1973, 1975, 1983, 1985, 1989).

The retina of the tiger salamander is rod-dominant with a rod:cone ratio of about 20:1. A rod-dominant duplex retina is the most widespread type of vertebrate retina although cone-dominant retinas are not unusual, particularly in avian species (Meyer, 1977; Braekevelt, 1989, 1990). It is doubtful however if any vertebrate retina is «pure-cone» or «pure-rod» as with electron microscopy, retinas are always shown to be duplex even if one photoreceptor type is in a dominant and even overwhelming majority (West and Dowling, 1975).

In retinas which undergo photomechanical or retinomotor movements in response to a circadian cycle or changes in environmental lighting, cones are the most contracted and rods are most elongated in the light-adapted state (Walls, 1942; Rodieck, 1973; Burnside and Laties, 1979). In the tiger salamander rods show a thinner myoid region in light-adaptation (5.0 μm in diameter) than in dark-adaptation (7.0 μm in diameter) and are felt to undergo limited photomechanical changes. Cones on the other hand show an inner segment which measures about 9.0 μm in diameter throughout the light cycle and hence are felt to undergo no retinomotor

movements. Walls (1942) reports that in urodele species rods show almost no movement while cones show slight and slow movements but also reports that a pupillary response to light is present. As it is usually felt that retinomotor movements of photoreceptors are most pronounced in species with no pupillomotor response, this may account for the lessened movement of the rod photoreceptors in this species. The melanosomes of the RPE in the tiger salamander do however undergo fairly extensive movements within the RPE apical processes and this probably more than movement of the rods and cones and/or the pupillary response serves to mask and unmask the photoreceptors during light- and dark-adaptation.

The outer segments of both rods and cones consist of a stack of bimembranous discs (Cohen, 1972; Crescitelli, 1972). In rods these discs are typically all of the same diameter and also usually show one or more incisures in their periphery, presumably to increase surface area. Cones normally display outer segment discs that are smaller in diameter apically than those located basally to give the outer segment a conical shape. These cone discs seldom show any peripheral incisures (Cohen, 1972). These characteristics of rod and cone outer segment discs do not vary with the lighting cycle.

The inner segment of a photoreceptor cell is known to be its synthetic center and it is here that the materials for new outer segment discs and other cell requirements are produced (Young, 1976). In a number of lower vertebrates including amphibians an oil droplet is reported at the apex of the inner segment but this was not the case in the tiger salamander (Nilsson, 1965; Rodieck, 1973).

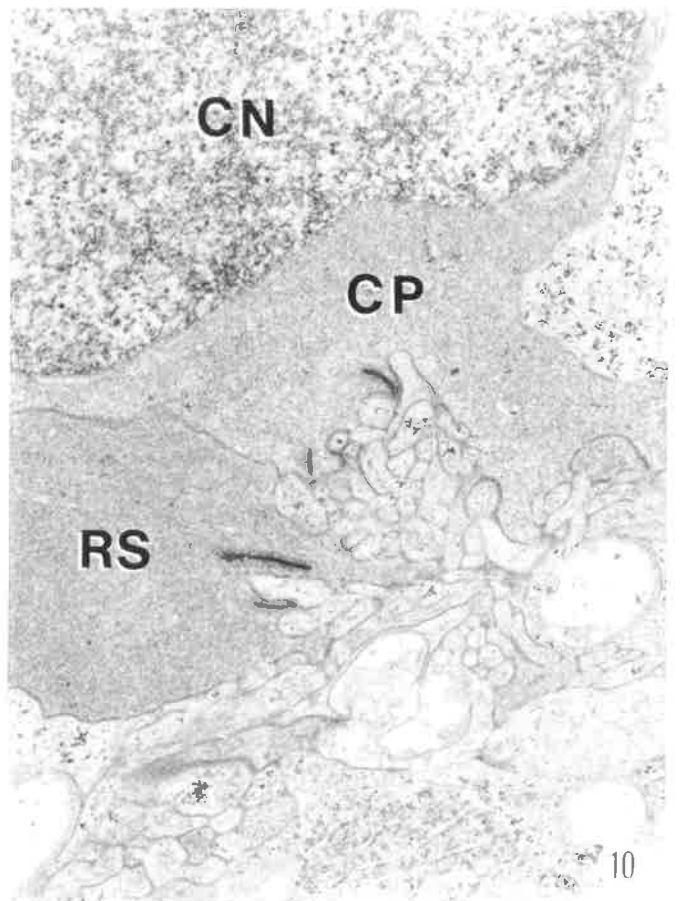
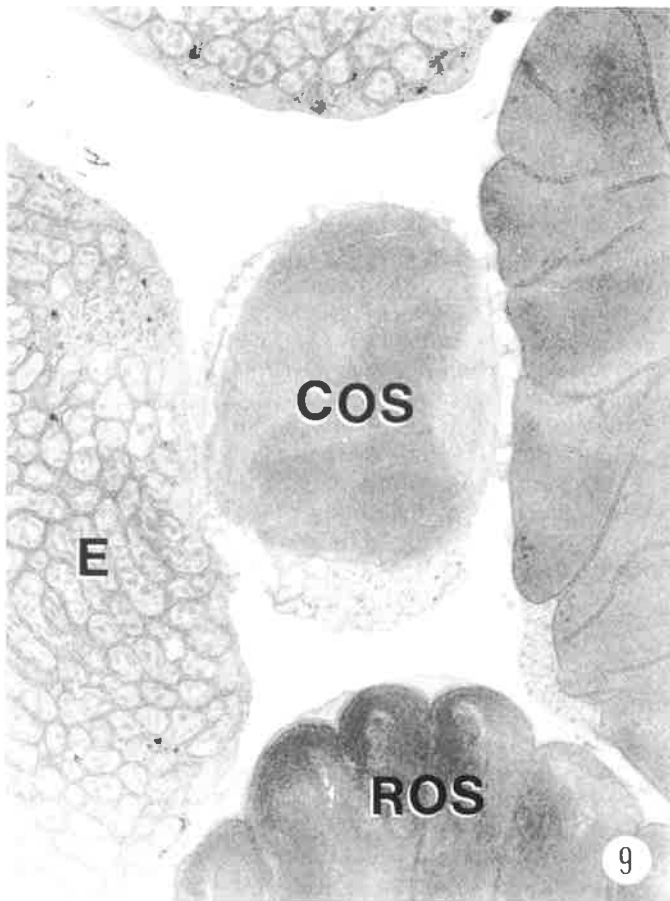
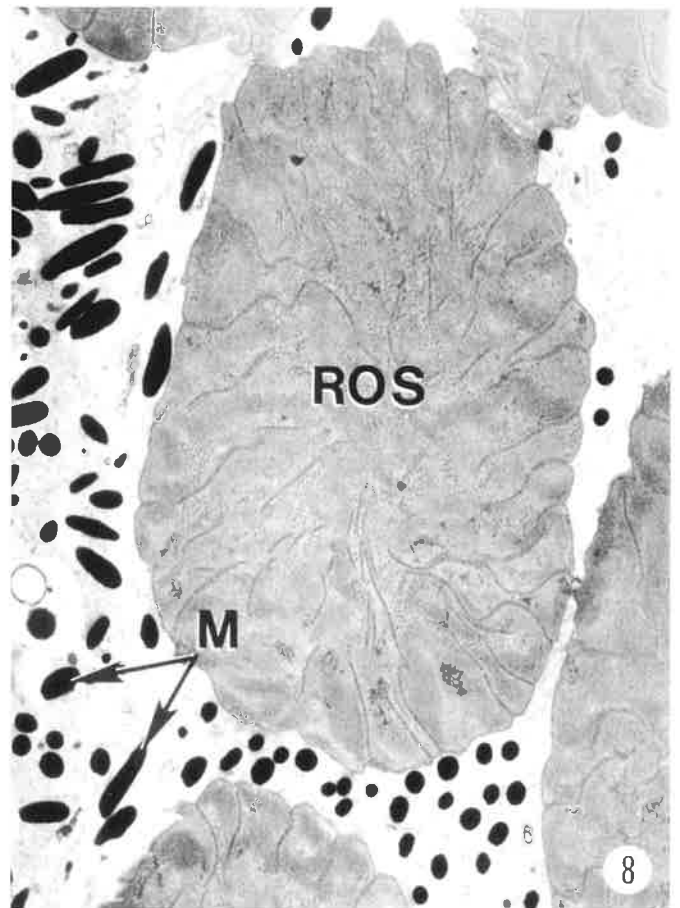
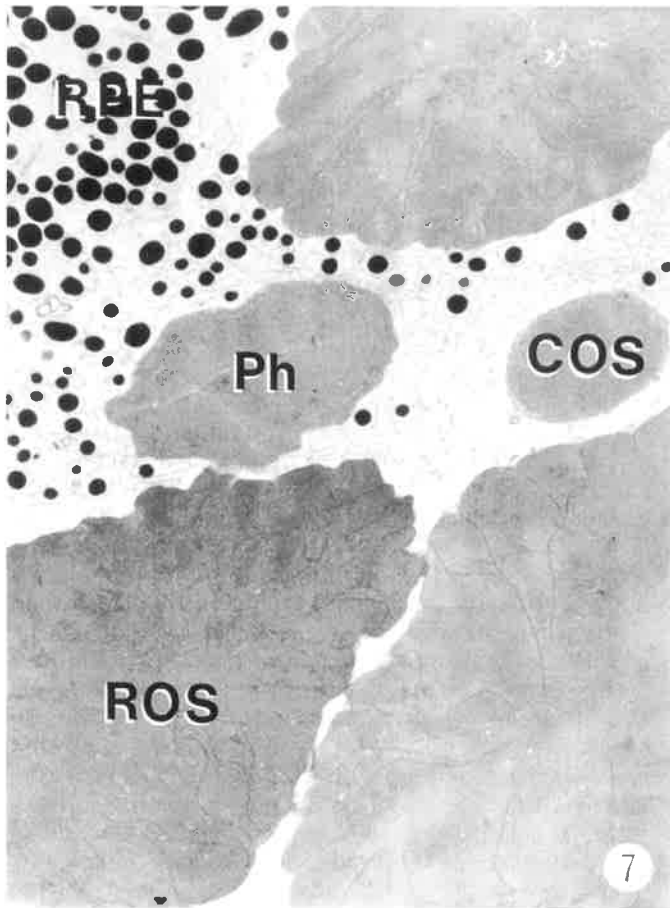
The large accumulation of mitochondria at the apex of the inner segment (the ellipsoid) is however a constant feature of both rods and cones (Cohen, 1972;

Fig. 7. Electron micrograph taken at the retinal epithelium-photoreceptor interface in dark-adaptation. Note that the RPE melanosomes are mostly within the RPE cell body. A rod outer segment (ROS), cone outer segment (COS) and a phagosomes (Ph) of shed outer segment material are all indicated. x 6,000

Fig. 8. Electron micrograph of a rod outer segment (ROS) to indicate the numerous deep incisures. In the dark-adapted state the rod outer segment is only lightly shielded by melanosomes (M). x 6,000

Fig. 9. Electron micrograph of a cone outer segment (COS), rod outer segment (ROS) and rod inner segment at the level of the ellipsoid (E) in dark-adaptation. x 13,000

Fig. 10. Electron micrograph of the synaptic pedicle of a cone (CP) and synaptic spherule of a rod (RS). Note both superficial and invaginated synaptic sites as well as plentiful synaptic vesicles in both photoreceptors. The cone nucleus (CN) is also indicated. x 13,200



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Rodieck, 1973). The paraboloid which is an accumulation of glycogen found in the inner segment of the cones of some fish, amphibians, birds and reptiles (Cohen, 1972) is present within the cones of this species. A hyperboloid which is a similar accumulation of glycogen within rod inner segments is not present within the rods of the tiger salamander. Early workers felt that these glycogen bodies (paraboloids in cones; hyperboloids in rods) were refractile bodies (like the oil droplet) but it is now felt that these glycogen concentrations are energy sources for visual cell metabolism (Meyer, 1977).

The region between the ellipsoid and the photoreceptor nucleus is referred to as the myoid region as it is this part which becomes shorter and wider as photoreceptors shorten and becomes longer and thinner as these cells elongate in response to environmental lighting. In this species the myoid region of rods does change in diameter while that of the cones does not indicating that rods move to some extent while cones apparently show no photomechanical movements. The myoid region does however show an abundance of rough endoplasmic reticulum, numerous polysomes, Golgi zones and autophagic vacuoles; all organelles indicative of metabolically very active cells (Cohen, 1972; Reme and Sulser, 1977; Alberts et al., 1989).

As in the case in most other species the ELM in the tiger salamander is formed by a series of zonulae adherentes between photoreceptors (both rods and cones) and Müller cells (Uga and Smelser, 1973). Also as is noted in many lower vertebrates the Müller cells project through the ELM to produce a series of fine microvillar processes that surround the basal region of photoreceptors inner segments.

The positioning of the rod and cone nuclei is a bit unusual in this species in that the cone nuclei are not exclusively found closest to the ELM which is normally the case (Cohen, 1972; Braekevelt, 1983, 1989, 1990). In the tiger salamander many rod nuclei are situated immediately adjacent to or even partially through the ELM while cone nuclei can be found at all levels of the outer nuclear layer. This unusual arrangement of nuclei is noted in other urodele species and may be due to the large size of the rods in these tailed amphibians (Braekevelt, 1992).

Within the outer plexiform layer the synaptic pedicle of the cone cell is typically larger, more electron lucent and displays more synaptic sites than the rod spherules (Cohen, 1972; Crescitelli, 1972). As the rods in this species are quite large the spherule of these cells is also quite large and displays more synaptic sites to that the difference between rod spherules and cone pedicles is not as pronounced.

Synaptic sites in retinal photoreceptors are either invaginated and associated with a synaptic ribbon (Missotten, 1965; Cohen, 1972) or are of 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 bipolars or between photoreceptors themselves (Cohen, 1964; Missotten, 1965; Kolb, 1970). The tiger salamander displays both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rods and cones.

Acknowledgements. The excellent technical assistance of D.M. Love and R.M. Simpson is gratefully acknowledged. This work was supported in part by funds from the Medical Research Council (MRC) and the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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Accepted November 6, 1992