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Photoreceptor fine structure in the southern fiddler ray *(Trygonorhina fasciata)*

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Summary. The fine structure of the retinal photoreceptors has been studied by light and electron microscopy in the southern fiddler ray or guitarfish *(Trygonorhina fasciata)*. The duplex retina of this species contains only rods and single cones in a ratio of about 40:1. No multiple receptors (double cones), no repeating pattern or mosaic of photoreceptors and no retinomotor movements of these photoreceptors were noted. The rods are cylindrical cells with inner and outer segments of the same diameter. Cones are shorter, stouter cells with a conical outer segment and a wider inner segment. Rod outer segment discs display several irregular incisures to give a scalloped outline to the discs while cone outer segment discs have only a single incisure. In all photoreceptors a non-motile cilium joins the inner and outer segments. The inner segment is the synthetic centre of photoreceptors and in this compartment is located an accumulation of mitochondria (the ellipsoid), profiles of both rough and smooth endoplasmic reticulum, prominent Golgi zones and frequent autophagic vacuoles. The nuclei of rods and cones have much the same chromatin pattern but cone nuclei are invariably located against or particularly through the external limiting membrane (ELM). Numerous Landolt's clubs which are ciliated dendrites of bipolar cells as well as Müller cell processes project through the ELM, which is composed of a series of zonulae adherentes between these cells and the photoreceptors. The synaptic region of both rods (spherules) and cones (pedicles) display both invaginated (ribbon) synapses and superficial (conventional) synapses with cones showing more sites than the rods.

Key words: Photoreceptors, Electron microscopy, Elasmobranch, *Trygonorhina fasciata*

Introduction

Vertebrate retinal photoreceptors are highly polarized and greatly specialized cells in both structure and function and as the first neuron in the visual pathway have been studied in a variety of animals (Walls, 1942; Polyak, 1957; Cohen, 1972; Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1983, 1987, 1989, 1990). Morphological studies have confirmed that all vertebrate photoreceptors are constructed on a similar plan with an outer segment (light-sensitive region) joined to an inner segment (synthetic region) by a non-motile connecting cilium, a nuclear region and a synaptic ending (Cohen, 1963, 1972; Crescitelli, 1972; Rodieck, 1973).

Traditionally retinal photoreceptors have been classified as either rods or cones on the basis of morphological criteria (Walls, 1942; Cohen, 1972). With the advent of electron microscopy it was felt by some workers that a classification based solely on morphology could however be somewhat ambiguous (Sjöstrand, 1958, 1959; Pedler, 1965, 1969). Despite attempts to introduce other criteria for classifying photoreceptors, for most species the terms of rods and cones still adequately and quite accurately describe and differentiate these cells (Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1984, 1985, 1988, 1990).

As part of a comparative morphological study of vertebrate photoreceptors, this report describes the fine structure of the rods and cones in the rod-dominant duplex retina of an elasmobrach, the southern fiddler ray or guitarfish *(Trygonorhina fasciata)*.

Materials and methods

For this study the eyes of four adult southern fiddler rays or guitarfish *(Trygonorhina fasciata)* were investigated by light and electron microscopy. Eyes from both sexes of both light- and dark-adapted specimens were examined.

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The animals were killed by severing the spinal cord and the eyeballs were quickly removed, opened at the equator and fixed for 5 h. in 5% glutaraldehyde buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer at 4° C. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorensen's buffer (pH 7.3), divided into tapetal and non-tapetal areas and cut into pieces less than 1 mm^2, taking care not to detach the retina. The tissue was then post-fixed for 2 h. in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and then 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 (50 - 60 nm) of selected areas were then cut and collected on copper grids. These sections were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Phillips EM 201 transmission electron microscope.

Results

The duplex retina of the southern fiddler ray or guitarfish *(Trygonorhina fasciata)* is rod dominant with a rod:cone ratio of about 40:1. All photoreceptors are single with no multiple receptors present in this species and no repeating pattern or mosaic of rods and cones was noted (Figs. 1, 2, 6, 7). The distribution of rods and cones appeared to be fairly uniform throughout the retina with no obvious changes in arrangement dictated by the presence of a choroidally located tapetum lucidum in the superior fundus (Braekevelt, 1991a). Finally although both light- and dark-adapted specimens were examined, photomechanical or retinomotor responses of the rods and cones in the southern fiddler ray were felt to be minimal or non-existent.

Rods are the largest photoreceptors and project through the external limiting membrane (ELM) for up to 40 µm with the inner segment measuring about 25 µm of this length (Figs. 1 - 3). Rod inner and outer segments are of much the same diameter at about 4.0 µm (Figs. 1, 5, 6). Fine apical processes of the retinal epithelium (RPE) interdigitate with the rod outer segments but do not normally reach down to their inner segments (Figs. 1, 3, 5). Rod outer segments discs display several incisures in their periphery to give these discs an irregularly scalloped appearance enface (Fig. 5). Joining the rod inner and outer segment is an eccentrically located connecting cilum (Fig. 3). Below the connecting cilium in the apex of the inner segment, rods display an accumulation of mitochondria (the ellipsoid) (Figs. 1, 3, 5, 6). Beneath the ellipsoid, rod inner segments are rich in both rough and smooth endoplasmic reticulum, polysomes and Golgi zones (Figs. 1, 2, 9, 10). Autophagic vacuoles are also a common finding in this region of the rod inner segment (Figs. 1, 2, 7). Laterally projecting, vertically oriented fins emanating from the inner segment of photoreceptors were not noted in this elasmobrach and the inner segments of both rods and cones presented fairly smooth profiles at this level (Figs. 5 - 7).

Cone photoreceptors are much shorter and stouter cells and only project through the ELM for about 20 µm with the inner segment being about 12 µm of this length (Figs. 1, 4). The cone outer segment is short (8.0 - 10 µm) and tapers distally (Figs. 1, 4). Cone outer segment discs only display a single incisure and are surrounded by a palisade of about 10 fine calycal processes which arise from the inner segment at the level of the connecting cilium (Fig. 6). Immediately below the connecting cilium cone photoreceptors also display an ellipsoid which is more extensive and the mitochondria of which show a more electron dense matrix than that seen in the rods (Figs. 2, 4, 7). Cones are at their widest in the ellipsoid region where they measure up to 8.0 µm in diameter (Figs. 4, 7). Below this prominent ellipsoid, cones are also rich in rough and smooth enddoplasmic reticulum, Golgi zones, polysomes and autophagic vacuoles (Figs. 1, 2, 4).

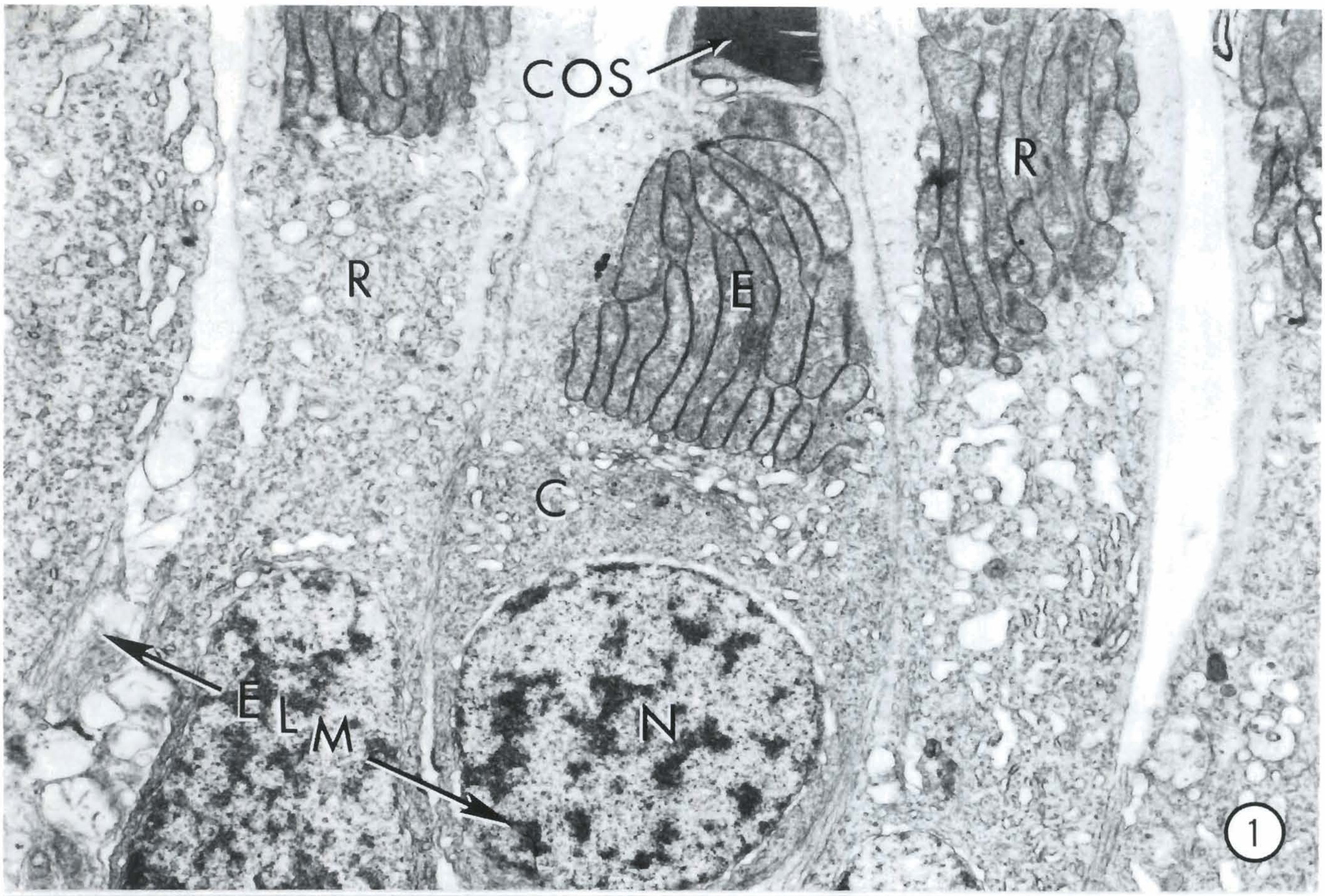
Rod and cone nuclei tend to display a fairly similar chromatin pattern in this species but can usually be differentiated by their location. As in many other species, cone nuclei are invariably located close to the ELM and indeed very often are wholly or partially projected through the ELM (Figs. 1, 2). Cone nuclei also tend to be more spherical while rod nuclei are more oval or oblong in shape (Figs. 1, 2). Rod nuclei are located at all levels within the outer nuclear layer and may in some cases occur very close to the synaptic spherule of the cell (Fig. 8).

The external limiting membrane (ELM) in the guitarfish consists of a series of zonulae adherentes between photoreceptors, Müller cells and bipolar cells (Figs. 1, 2, 9, 10). This species is unusually rich in what are referred to as Landolt's clubs, which are ciliated dendrites of bipolar cells which project through the ELM into the interphotoreceptor space (optic ventricle). Landolt's clubs and finger-like processes of Müller cells project through the ELM to surround the photoreceptors near the ELM (Figs. 2, 9, 10).

The synaptic spherule of rod photoreceptors displays 3 - 4 invaginated (ribbon-associated) synaptic sites as well as several of the more conventional synapses which involve a membrane thickening (Fig. 8). The rod spherule is also rich in synaptic vesicles (Fig. 8). Cone synaptic pedicles differ from the rod spherules in that they are larger, usually more electron lucent and display 7 or 8 ribbon (invaginated) synapses as well as more superficial (conventional) synaptic sites.

Discussion

The traditional classification of vertebrate retinal



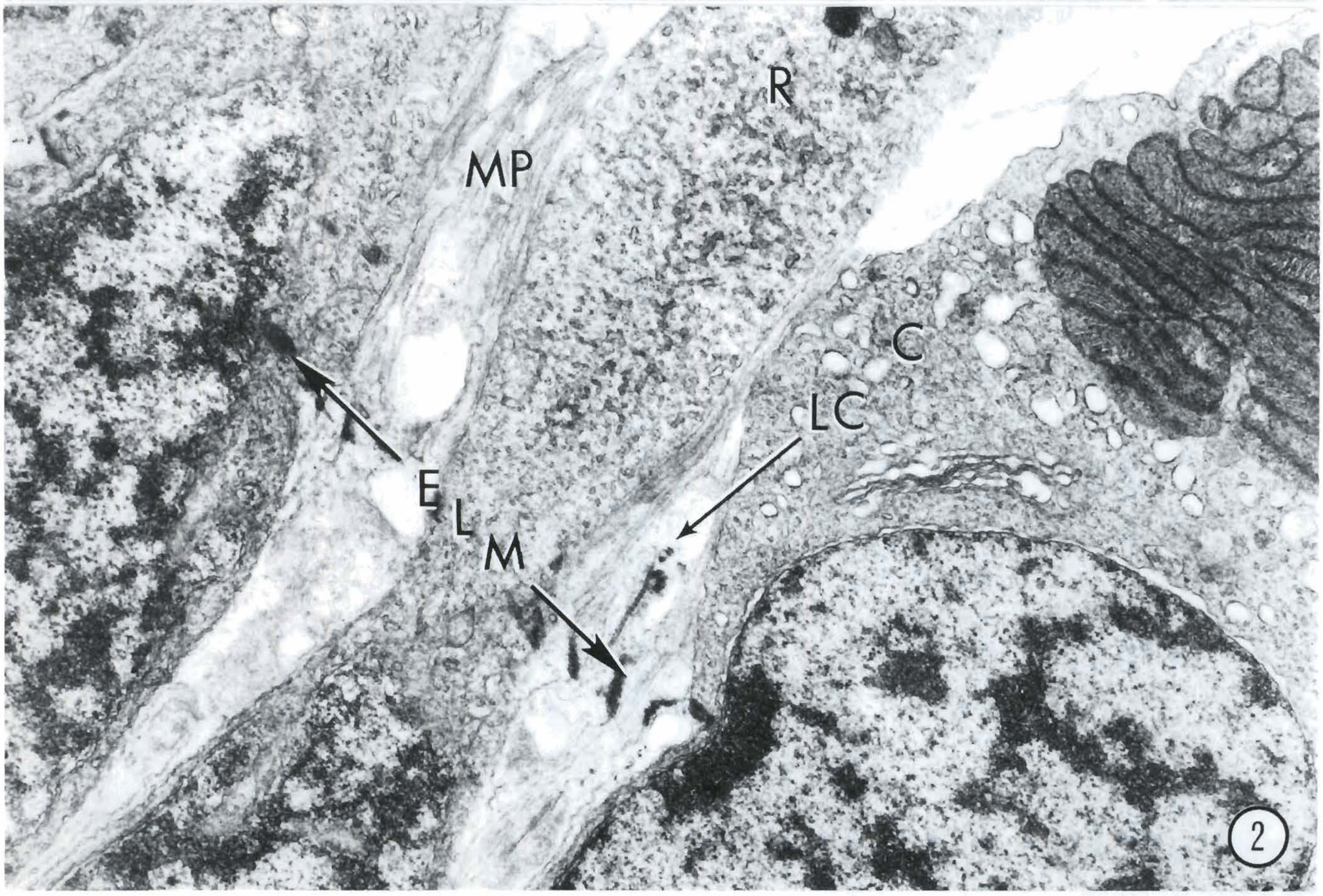
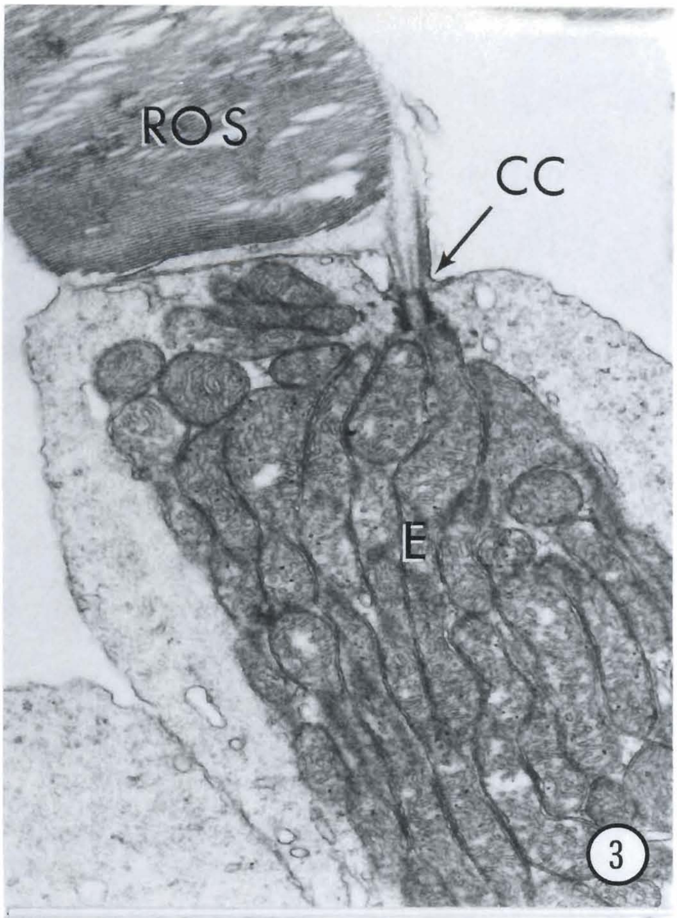
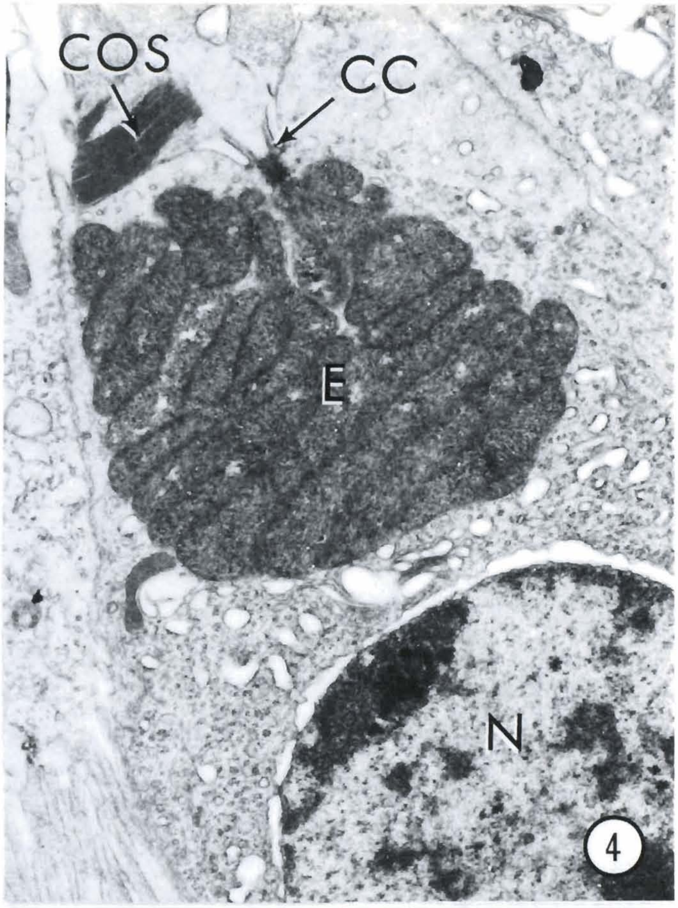
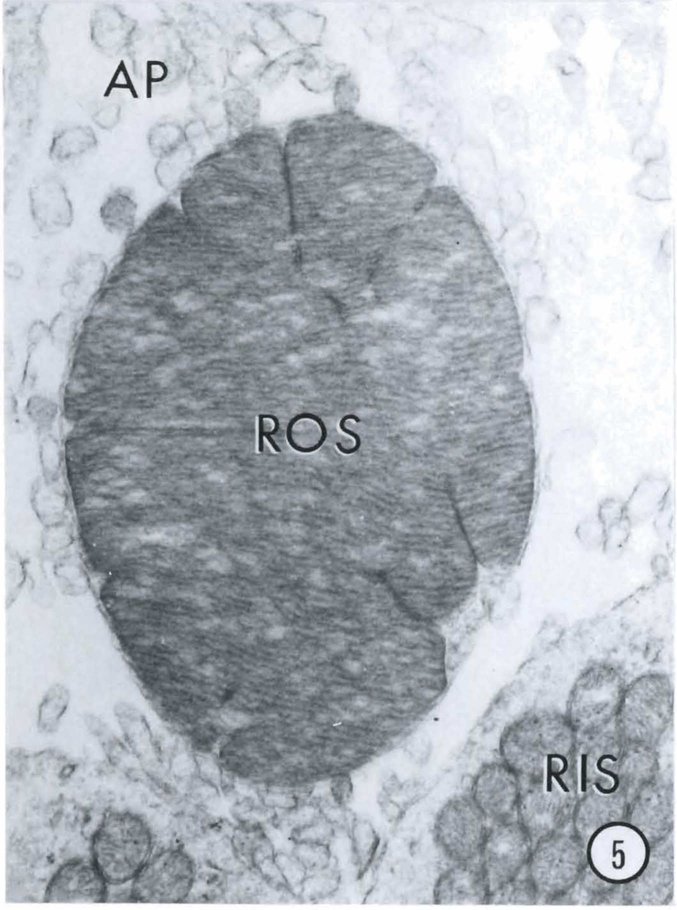


Fig. 1. Electron micrograph of two rod (R) and one cone (C) photoreceptor. The cone outer segment (COS), nucleus (N) and ellipsoid (E) are all indicated. The external limiting membrane (ELM) is also labelled. × 8,600

Fig. 2. Electron micrograph of a rod (R) and cone (C) photoreceptor. The external limiting membrane (ELM), a Landolt's club (LC) and some Müller cell processes (MP) are also indicated. × 12,800







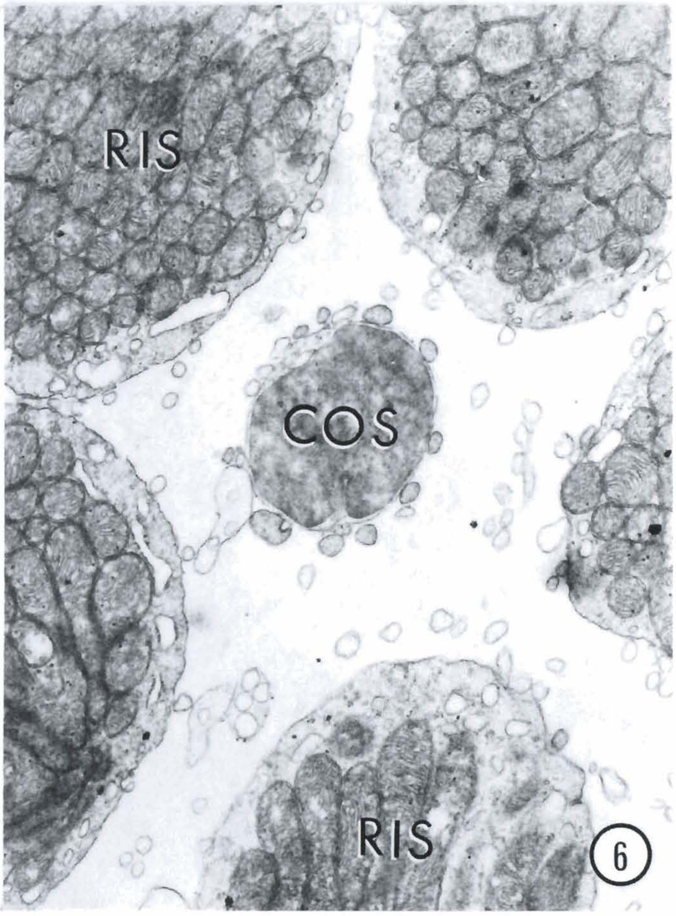
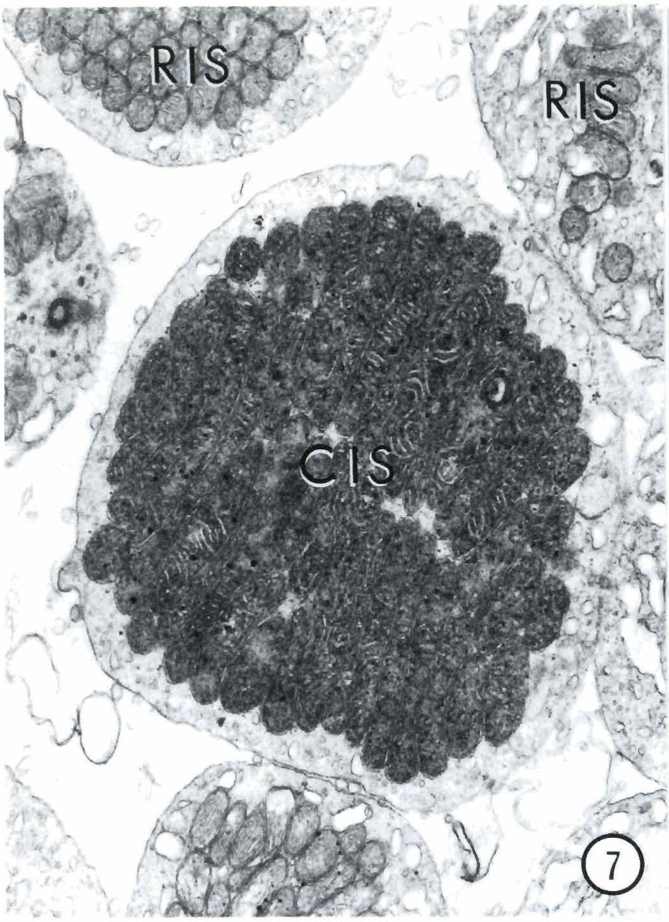


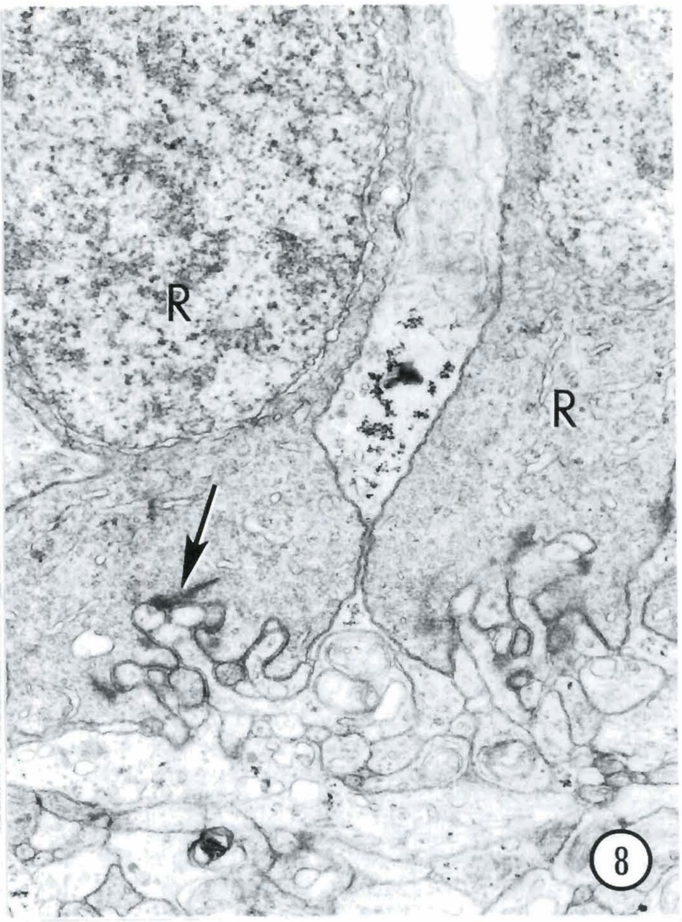
Fig. 3. Electron micrograph of a rod photoreceptor to illustrate the outer segment (ROS), ellipsoid (E) and connecting cilium (CC). × 16,700

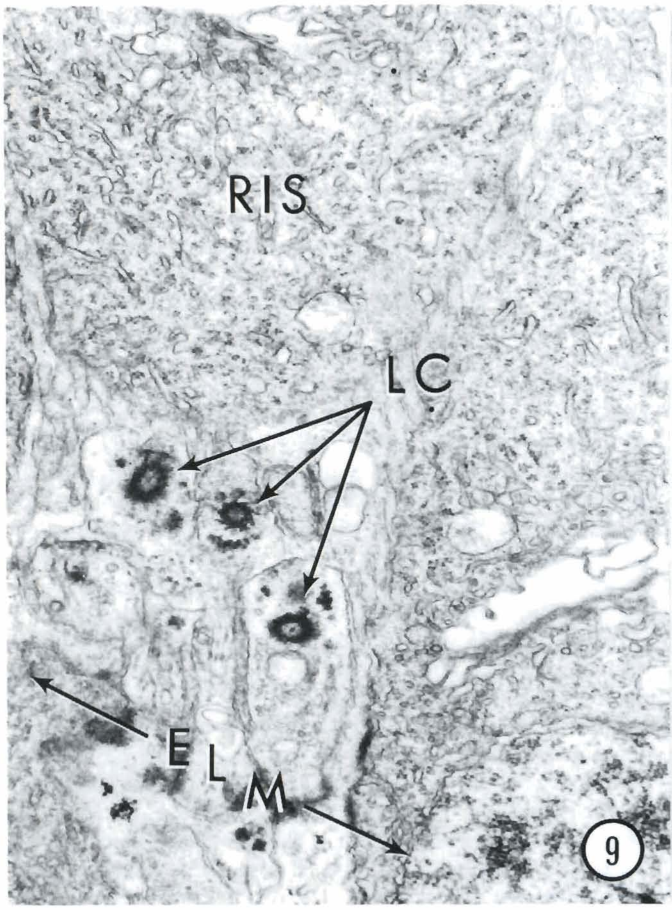
Fig. 4. Electron micrograph of a cone photoreceptor to indicate the outer segment (COS), ellipsoid (E), connecting cilium (CC) and nuclus (N). × 11,500

Fig. 5. Electron micrograph of rod inner (RIS) and outer segments (ROS). Non-pigmental apical processes (AP) of the retinal epithelium are also labelled. × 16,700

Fig. 6. Electron micrograph of rod inner segments (RIS) and a cone outer segment (COS). × 16,100







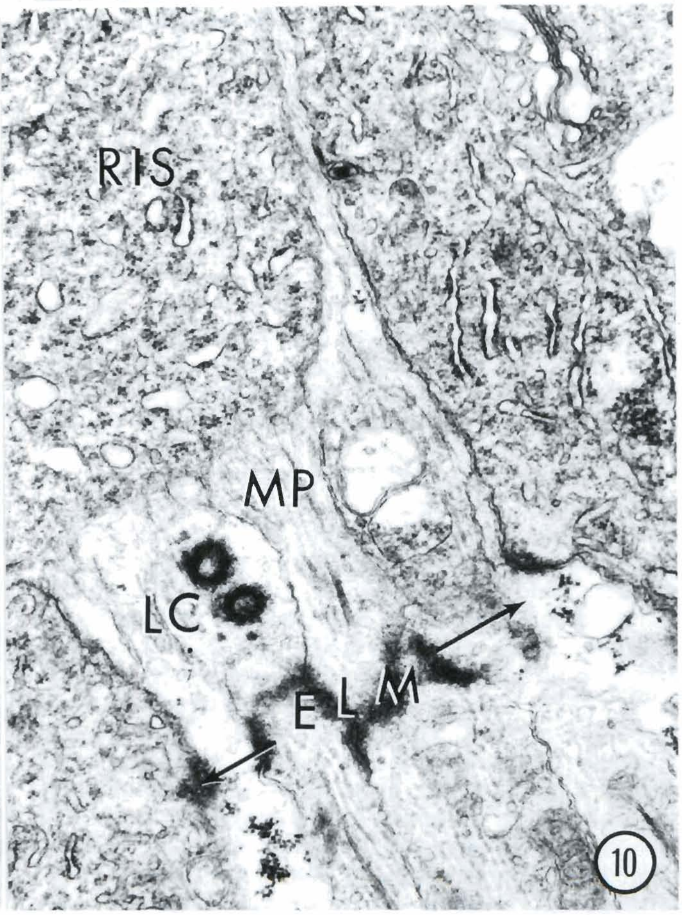


Fig. 7. Electron micrograph of a cone inner segment (CIS) with a large ellipsoid and two rod inner segments (RIS). × 13,800

Fig. 8. Electron micrograph of two rod (R) photoreceptors to illustrate the synaptic spherules. Both ribbon (arrow) and conventional synaptic sites are obvious. × 16,700

Fig. 9. Electron micrograph to illustrate three Landolt's clubs (LC), a rod inner segment (RIS) and the external limiting membrane (ELM). × 18,400

Fig. 10. Electron micrograph of the external limiting membrane (ELM) region. A Landolt's club (LC) a rod inner segment (RIS) and Müller cell processes (MP) are all indicated. × 22,700

photoreceptors into either rods or cones was based on morphological criteria at a light microscopic level (Schultze, 1866). In this classical division, typical rods have cylindral inner and outer segments of much the same diameter while typical cones have a conical outer segment and an inner segment of greater diameter. This classification was felt to adequately describe these cells and was used in numerous light microscopic studies (Walls, 1942; Polyak, 1957; 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 properly classified by the simplistic terms of rods or cones (Dowling, 1965). This led various workers to propose new categories of photoreceptor classification based on criteria other than just cell shape (Sjöstrand, 1958, 1959; Pedler, 1965, 1969). While these more elaborate classifications are perhaps more accurate and in some non-mammalian species may even be preferable, in the vast majority of cases, retinal photoreceptors can be adequately described and differentiated by the classical terms of rods or cones, particularly in the case of species such as the southern fiddler ray or guitarfish *(Trygonorhina fasciata)* that possess only single rods and single cones with no multiple receptors in the retina.

Within elasmobranch species, sharks (Selachii) and rays (Batoidea) are variously reported to be pure rod (Walls, 1942; Dowling and Ripps, 1970) or more commonly possessing some cones (Gruber et al., 1963; Hamasaki and Gruber, 1965; Ali and Anctil, 1976). In the guitarfish both rods and cones are present in a ratio of about 40:1. While some species variations undoubtedly exist correlating with such things as habitat and diurnal/nocturnal activity, it is unlikely that any retina is «pure rod» or indeed «pure cone» as with the electron microscope, retinas are usually shown to be duplex even if one photoreceptor type is in an overwhelmingly dominant majority (West and Dowling, 1975).

As has been reported in a variety of sharks and rays, multiple (double) cones are not a feature of the elasmobranch retina (Crescitelli, 1972; Ali and Anctil, 1976) and they have not been recognized in the southern fiddler ray. Such is also the case in the sturgeon but differs markedly from the teleosts where multiple cones are normally present and often arranged in a regular mosaic pattern (Braekevelt, 1982, 1985). Also retinomotor or photomechanical responses are felt to be either absent or limited to minimal movement of the cones in elasmobrach species (Walls, 1942). Both light- and dark-adapted specimens were examined in this study and little or no movement was detected in either rods or cones or indeed of the pigment in the retinal epithelium (RPE) (Braekevelt, 1991b). This species does however possess a choroidally-located occlusible tapetum lucidum and the extensive pigment migration within the melanocytes of the choroid which either «masks» or «unmasks» the reflective material of the tapetal cells may account for the lack of movement of pigment within the RPE or of the photoreceptor cells (Braekevelt, 1991a).

The outer segments of both rods and cones consist of a stack of bimembranous discs which incorporate the photopigments (Cohen, 1972; Crescitelli, 1972). In rods these outer segment discs are usually all of the same diameter while in cones the more apical discs are smaller than those of the basal region giving the outer segment its characteristic tapering or conical shape (Cohen, 1972). In most species studied, cone discs display a circular outline while rod discs very often have a scalloped perimeter due to the presence of incisures in the discs (Nilsson, 1965; Braekevelt, 1983). In the guitarfish, rod discs have several unequal incisures while the cone discs display a single incisure. The presence of incisures is most probably a means of increasing the surface area of the light-sensitive outer segment. The connecting cilium locate between inner and outer segments is a constant feature of all vertebrate photoreceptors described to date and possibly reflects the ontogeny of photoreceptors from a ciliated ependymal cell (Rodieck, 1973). In this species, the cilium is typical in that it is eccentrically located and displays the peripheral arrangement of nine pairs of microtubules while lacking 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 centre of the cell and it is here that the material for new outer segment discs as well as for other cellular functions are produced (Young, 1976, 1977). The large accumulation of mitochondria at the apex of the inner segment (the ellipsoid) is a constant feature of all vertebrate photoreceptors (Cohen, 1972; Rodieck, 1979). The presence of an ellipsoid, numerous polysomes, both rough and smooth endoplasmic reticulum, Golgi zones and autophagic vacuoles within the inner segment are all indicative of metabolically very active cells (Cohen, 1972; Remé and Sulser, 1977; Alberts et al., 1983). Neither an oil droplet nor a paraboloid (an accumulation of glycogen) were present within the inner segment of either the rods or cones of the southern fiddler ray.

As is the case in most vertebrate species, the external limiting membrane (ELM) in the guitarfish is formed by a series of zonulae adherentes between photoreceptors and Müller cells and in this species bipolar cells (Uga and Smelser, 1973). Landolt's club which is a ciliated dendrite of a bipolar cell that projects through the ELM to be situated in the optic ventricle beside photoreceptor inner segments has been described in a variety of species but is of uncertain function (Cohen, 1963; Hendrickson, 1966). Landolt's clubs are particularly numerous in the guitarfish but the significance of this is obscure. Also projecting through the ELM are numerous short finger-like processes of the Müller cells which surround photoreceptor inner segments at their base. The function of these processes is also uncertain but they are speculated to be of importance in exchange functions as they are normally more numerous in avascular retinas (Uga and Smelser, 1973). While the nuclear chromatin pattern in both rods and cones are quite similar in this species, cone nuclei are invariably located close to or even protruding through the ELM whereas rod nuclei are located at all levels of the outer nuclear layer (ONL). This arrangement of rod and cone nuclei is a constant feature of duplex retinas and may reflect the order of appearance and differentiation of the rods and cones (Walls, 1942; Braekevelt, 1982, 1988, 1989).

Within the outer plexiform layer, the synaptic pedicle of the cones is typically larger, often more electron-lucent, and displays more synaptic sites than does the rod spherules (Cohen, 1972; Crecitelli, 1972). Synaptic sites on retinal photoreceptors are either invaginated and associated with a synaptic ribbon (Missotten, 1965) or are of the more conventional superficial type involving a membrane densification (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). The southern fiddler ray displays both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rod spherules and the slightly larger cone pedicles.

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