

Retinal photoreceptor fine structure in the short-tailed stingray (*Dasyatis brevicaudata*)

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Summary. The fine structure of the retinal photoreceptors has been studied by light and electron microscopy in the short-tailed stingray (*Dasyatis brevicaudata*). The duplex retina of this elasmobranch contains rods and cones in a ratio of about 10:1. No multiple receptors were noted nor was a repeating or mosaic arrangement of the cones obvious. Only light-adapted specimens were studied but retinomotor movements of the photoreceptors were felt to be minimal or absent. The rods are large cylindrical cells with inner and outer segments of much the same diameter. Cones are short stout cells with a conical outer segment and a wider inner segment. Rod outer segment discs show peripheral incisures while cones do not. The inner segment of rods and cones are rich in organelles indicating much synthetic activity. The nuclei of rods and cones appear quite similar but cone nuclei are invariably at least partially protruded through the external limiting membrane which is formed by a series of zonulae adherentes between photoreceptor cells and Müller cells. The synaptic region of both rods and cones display both invaginated (ribbon) synapses and superficial (conventional) synapses.

Key words: Photoreceptors, Fine structure, Elasmobranch, Short-tailed Stingray, *Dasyatis brevicaudata*

Introduction

The photoreceptors of the vertebrate retina are extremely specialized and highly polarized and compartmentalized cells. As the first neuron in the visual pathway they have been studied in a variety of species and with a wide array of techniques (Walls, 1942; Polyak, 1957; Cohen, 1972; Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1989, 1990, 1992, 1993). Morphological studies confirm that all vertebrate

photoreceptors are constructed on a similar plan with a light-sensitive outer segment joined to an inner segment (the 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 was no longer adequate and introduced more elaborate criteria (Sjöstrand, 1958, 1959; Pedler, 1965, 1969). Despite these attempts however, 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, 1987, 1989, 1990, 1992).

As part of a continuing comparative morphological study of vertebrate photoreceptors, this report describes the fine structure of the photoreceptors in the rod-dominant duplex retina of an elasmobranch, the short-tailed stingray (*Dasyatis brevicaudata*).

Materials and methods

For this study, the eyes from two adult light-adapted short-tailed stingrays (*Dasyatis brevicaudata*) were examined by light and electron microscopy. The animals were killed by severing the spinal cord and the eyes quickly removed. The eyeballs were slit open at the 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 half of the globe was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm². This tissue was then post-fixed for 2 h in 1% O₃O₄ in the same phosphate buffer (pH 7.3), dehydrated through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were 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

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μm) 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 duplex retina of the short-tailed stingray (*Dasyatis brevicaudata*) is rod-dominant with a rod:cone ratio of about 10:1. All photoreceptors are single with no multiple receptors present in this species. In addition no repeating pattern or mosaic of rods and cones was noted (Figs. 1, 2, 4). The distribution of rods and cones appeared to be fairly uniform throughout the retina with no obvious changes in arrangement caused by the presence of a choroidally located tapetum lucidum in the superior fundus of this elasmobranch. Only light-adapted specimens were available for this study but judging from the shape and thickness of the photoreceptors, photo-mechanical or retinomotor responses of the rods and cones in the short-tailed stingray were felt to be minimal or even non-existent.

Rods are large cells in this species and project through the external limiting membrane (ELM) for about 40 μm with the inner segment being about 20 μm in length. Rod inner and outer segments are of much the same diameter at about 4-5 μm (Figs. 1, 5, 6, 9). Fine apical processes of the retinal epithelium (RPE) interdigitate with the rod outer segments but in the light-adapted state do not reach down to their inner segments (Figs. 6, 9). Rod outer segment discs display several incisures in their periphery which can even be appreciated in longitudinal section (Fig. 9). Joining the rod inner and outer segments is an eccentrically-located connecting cilium. At the apex of the inner segment, rods display an accumulation of mitochondria, the ellipsoid (Figs. 2, 6). Proximal to the ellipsoid, rod inner segments are rich in both rough (RER) and smooth (SER) endoplasmic reticulum, polysomes and Golgi zones (Figs. 1, 2, 4-6). Autophagic vacuoles are also a common feature of this region of rod inner segments (Figs. 1, 3, 6). Laterally projecting, vertically-oriented fins emanating from the inner segment of photoreceptors were not observed in this elasmobranch and the inner segments of both rods and cones present fairly smooth profiles (Figs. 3-5).

Landolt's clubs which are ciliated dendrites of bipolar cells which project through the ELM into the inter-photoreceptor space (optic ventricle) are not present in this species but finger-like processes of Müller cells

project through the ELM to surround the basal region of the inner segments of both rods and cones (Figs. 2, 4, 5, 7).

Cone photoreceptors in these light-adapted specimens are short stout cells which only project through the ELM for 15-20 μm with the inner segment being about 10-15 μm of this length (Figs. 3, 4). The cone outer segment is short, tapers distally and shows no longitudinally-aligned incisures (Fig. 8). Cone outer segments are surrounded proximally by a palisade of fine calycal processes which arise from the inner segment at the level of the connecting cilium (Fig. 3). Immediately proximal to the connecting cilium, cone photoreceptors also display an ellipsoid of mitochondria which is usually larger than that of rods (Figs. 3, 4, 8). Cones are at their widest just below the ellipsoid where they measure up to 8 μm in diameter (Fig. 3). Between the ellipsoid and the nucleus, cone inner segments are also rich in RER, SER, Golgi zones, polysomes and autophagic vacuoles (Figs. 3, 4, 8).

Rods and cones tend to display a fairly similar chromatin pattern in this elasmobranch species but they can usually be differentiated by their locations. As in many other species, cone nuclei are invariably located closest to the ELM and indeed are often partially or even wholly projected through the ELM (Figs. 2-4, 7). Cone nuclei also tend to be more spherical while rod nuclei are more oval or oblong in shape (Figs. 1, 4). Rod nuclei are located at all levels within the outer nuclear layer and may occur very close to the synaptic region of the cell (Fig. 10). The ELM in this species consists of a series of zonulae adherentes between photoreceptors and Müller cells (Figs. 1, 3, 4, 7).

The synaptic spherule of rod photoreceptors display 3-4 invaginated (ribbon-associated) synaptic sites as well as several of the more conventional synapses which only involve a membrane thickening (Fig. 10). The rod spherule is also rich in synaptic vesicles (Fig. 10). Cone synaptic pedicles differ from rod spherules in that they are larger, usually slightly more electron-lucent and display 7-10 ribbon (invaginated) synapses as well as more superficial (conventional) synaptic sites.

Discussion

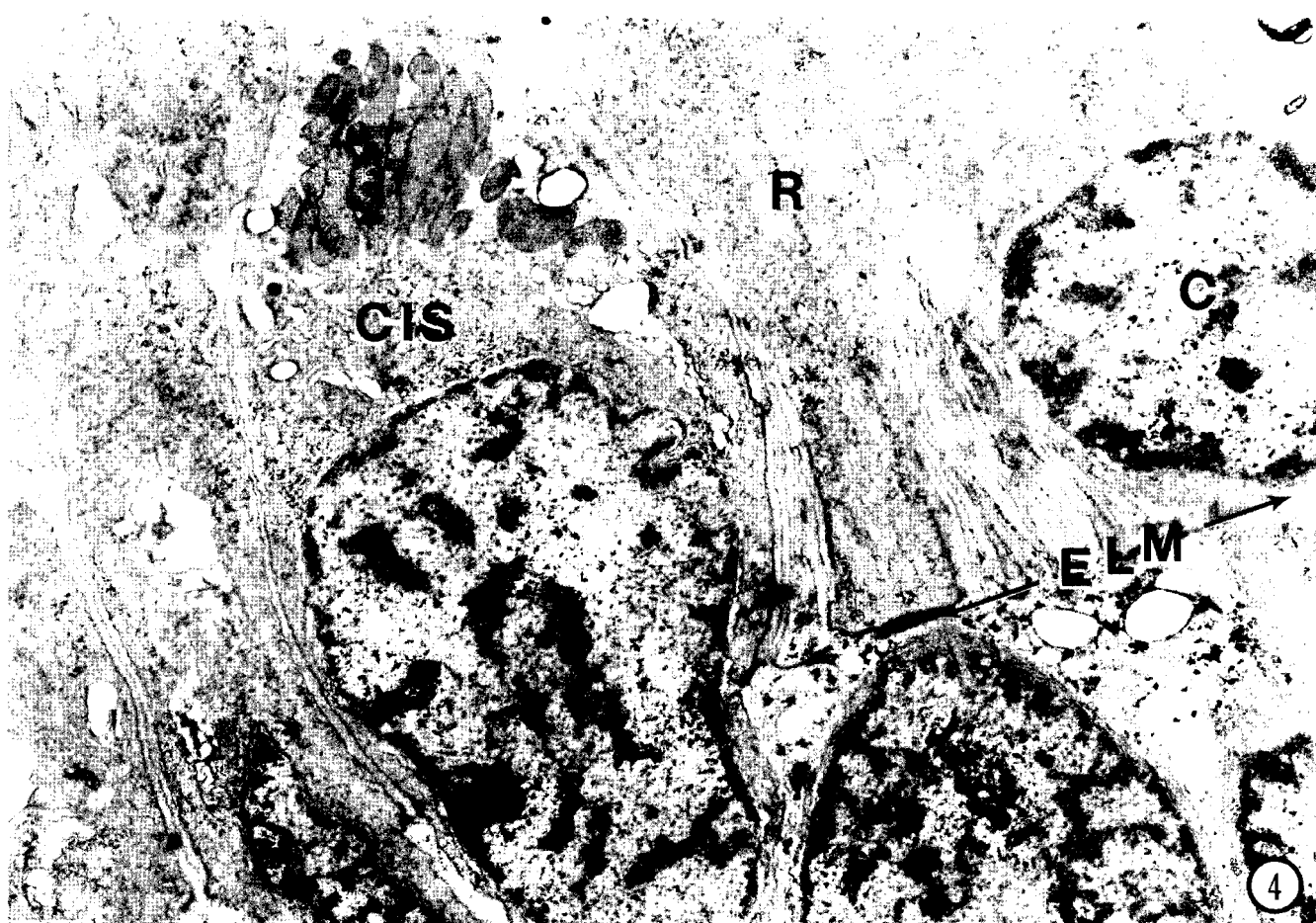
The traditional classification of vertebrate retinal photoreceptors into either rods or cones is based on morphological criteria at a light microscopic level (Schultze, 1866). In this classical division, typical rods have cylindrical inner and outer segments of much the same diameter while cones have a conical outer segment

Fig. 1. Electron micrograph of the photoreceptor types found in the retina of the short-tailed stingray. Rods (R) and cones (C) are indicated as in the external limiting membrane (ELM). X 6,500

Fig. 2. Electron micrograph to illustrate a cone nucleus (CN) wholly through the ELM. Rod photoreceptors (R) are also labelled. x 6,600

Fig. 3. Electron micrograph of a cone photoreceptor to indicate the nucleus (CN) only partially through the ELM. The ellipsoid (CE) of the cone cell is also indicated. x 6,100





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Fig. 4. Electron micrograph of rods (R) and cones (C). The inner segment of a cone (CIS) is indicated. Numerous Müller cell processes protrude through the ELM. X 8,600

Fig. 5. Electron micrograph to illustrate two cones (C) and a rod inner segment (RIS). Note the abundance of organelles in the RIS. x 9,500

Fig. 6. Electron micrograph to illustrate rod inner (RIS) and outer segments (ROS). The rod ellipsoids (RE) are also indicated. x 9,500

and an inner segment of greater diameter. This classification was felt to adequately differentiate these cells and was used in numerous light microscopic studies (Walls, 1942; Polyak, 1957; Duke-Elder, 1958). With a more detailed electron microscopic examination of the photoreceptors in a variety of species, it was felt that not all photoreceptors were properly classified by the simplistic terms of rods and 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, 1956, 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. This is particularly true in the case of species such as the short-tailed stingray (*Dasyatis brevicaudata*) that possesses only single rods and single cones.

Within the elasmobranchs, 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 short-tailed stingray both rods and cones are present in a ratio of about 10:1. While some species variation undoubtedly exists 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 electron microscopy, retinas are invariably shown to be duplex even if one photoreceptor type is in an overwhelmingly dominant majority (West and Dowling, 1975). The ratio of rods:cones reported in this species (10:1) differs from that reported for the southern fiddler ray (40:1) (Braekevelt, 1992) and presumably reflects a difference in habitat and/or feeding behaviour.

As has been reported in other sharks and rays, multiple cones are not a feature of the elasmobranch retina (Crescitelli, 1972; Ali and Anctil, 1976; Braekevelt, 1992) and they have not been observed in the short-tailed stingray. Such is also the case in the sturgeon (Sillman et al., 1990) but differs markedly from the teleosts where multiple cones are normally present

and often arranged in a regular repeating pattern or mosaic (Braekevelt, 1982, 1985). Also retinomotor or photomechanical responses are felt to be either absent or limited to minimal movement of the cones in elasmobranch species (Walls, 1942; Braekevelt, 1992). While only light-adapted specimens were examined in this study, judging by the morphology of the rods (relatively thick inner segments) and cones (the nuclei protruding partially or wholly through the ELM) the photoreceptors of the short-tailed stingray do not respond to environmental lighting by changing their length. This species possesses a choroidally located tapetum lucidum which is felt to be at least partially occludible and this may account for the apparent lack of movement of the photoreceptors and indeed of the melanosomes with the RPE cells (Braekevelt, 1994a,b).

The outer segments of rods and cones consist of a stack of membranous 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 or at most have one incisure (Braekevelt, 1992) while rod discs very often have a scalloped perimeter due to the presence of several peripheral incisures (Nilsson, 1965; Braekevelt, 1983). In this species rod discs have several incisures while cone discs have none. The presence of incisures is presumably a means of increasing the surface area of the light-sensitive outer segment. The connecting cilium located between inner and outer segments is a constant feature of all vertebrate photoreceptors described to date and probably reflects the phylogeny of photoreceptors from a ciliated endodermal cell (Rodieck, 1973).

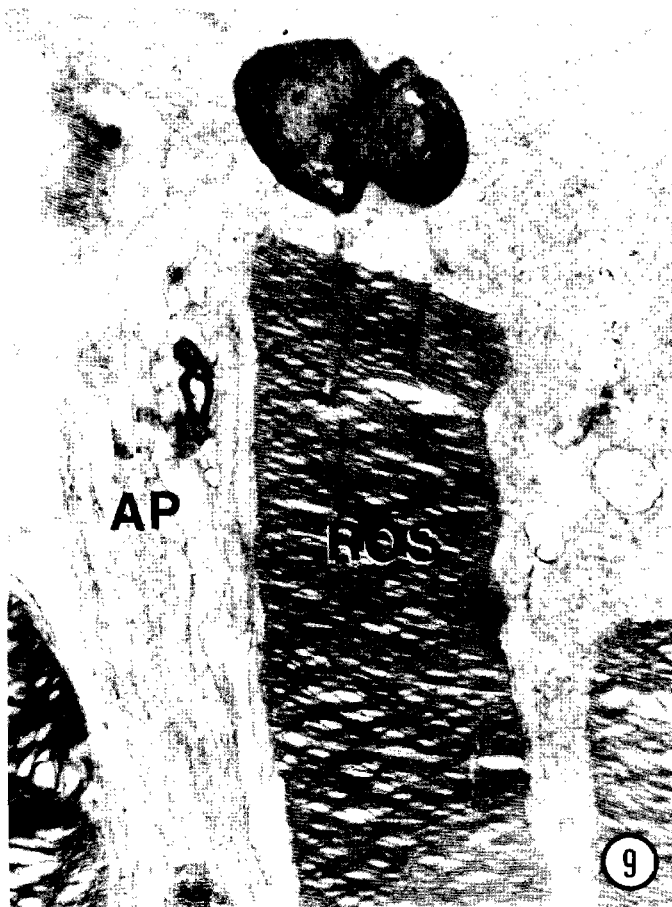
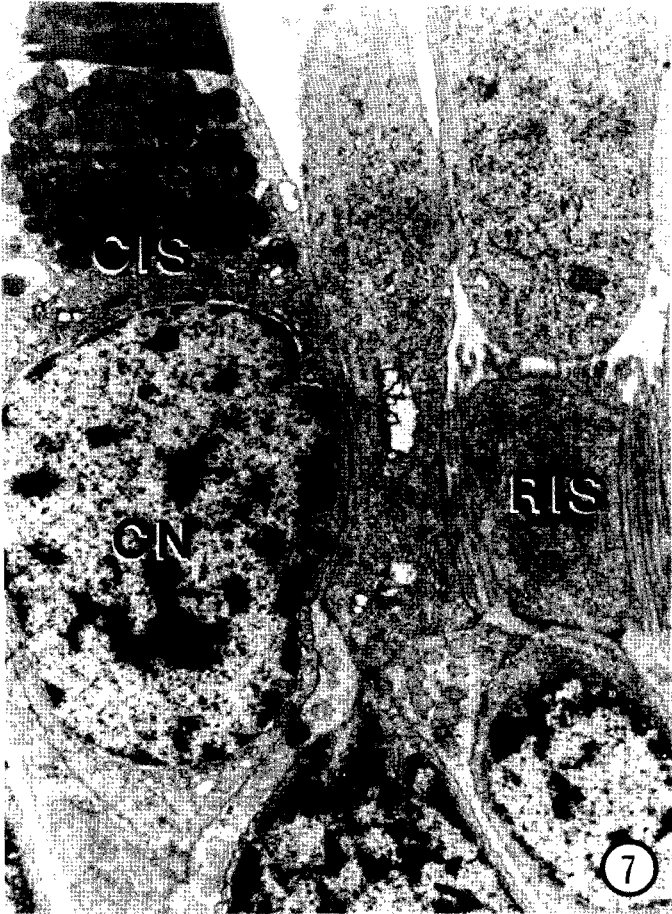
The inner segment of photoreceptor cells is known to be the synthetic centre of the cell and it is here that the material for new outer segment discs as well as for other cellular functions is produced (Young, 1976, 1978). The presence of an ellipsoid of mitochondria, numerous polysomes, both RER and SER, Golgi zones and autophagic vacuoles within the inner segment were all

Fig. 7. Electron micrograph to indicate rod (RIS) and cone (CIS) inner segments. Again the cone nucleus (CN) is partially through the ELM. x 6,400

Fig. 8. Electron micrograph of a cone photoreceptor to illustrate the outer segment (COS), ellipsoid (CE) and inner segment (CIS). x 9,900

Fig. 9. Electron micrograph of a rod outer segment (ROS) surrounded by retinal pigment epithelial apical processes (AP). x 15,000

Fig. 10. Electron micrograph of the synaptic spherule of a rod (RS). Note both invaginated and superficial synaptic sites. A presumed cone pedicle (CP) is also indicated. x 15,000



indicative of metabolically very active cells (Cohen, 1972; Remé and Sulser, 1977; Alberts et al., 1989). Neither an oil droplet nor a paraboloid (an accumulation of glycogen) were present within the inner segment of the photoreceptors of the short-tailed stingray.

As is the case in other vertebrate species, the ELM in this species is formed by a series of zonulae adherentes between photoreceptors and Müller cells (Uga and Smelser, 1973). Landolt's club which is a ciliated dendrite of bipolar cell that projects through the ELM has been described in a variety of species (Cohen, 1963; Hendrickson, 1966). These clubs are of unknown function and were particularly numerous in another elasmobranch, the southern fiddler ray (*Trygonorhina fasciata*) (Braekevelt, 1992) but were not observed in the short-tailed stingray. Also projecting through the ELM of many species including the short-tailed stingray are numerous short finger-like processes of the Müller cells. These surround the photoreceptor inner segments at their base and although of uncertain function, 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 is quite similar in both rods and cones in this species, cone nuclei are invariably located closer to and often protrude through the ELM whereas rod nuclei are located at all levels of the outer nuclear layer. This arrangement of rod and cone nuclei is a constant feature of duplex retinas and possibly reflects the order of appearance and differentiation of photoreceptors (Walls, 1942; Braekevelt, 1982, 1988, 1990).

Within the outer plexiform layer, the synaptic pedicle of cones is typically larger, often more electron-lucent and displays more synaptic sites than does the spherule of rods (Cohen, 1972; Crescitelli, 1972). Synaptic sites of retinal photoreceptors are either invaginated and associated with a synaptic ribbon (Missotten, 1965) or are of the more conventional superficial type involving a surface membrane densification (Dowling, 1968; Cohen, 1972). While both bipolar and horizontal cells are 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 short-tailed stingray displays both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rod spherules and cone pedicles.

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