Functional significance of the taper of vertebrate cone photoreceptors

Ferenc I. Hárosi1 and Iñigo Novales Flamarique2

1Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole, MA 02543
2Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V3J 4M5, Canada

Vertebrate photoreceptors are commonly distinguished based on the shape of their outer segments: those of cones taper, whereas the ones from rods do not. The functional advantages of cone taper, a common occurrence in vertebrate retinas, remain elusive. In this study, we investigate this topic using theoretical analyses aimed at revealing structure–function relationships in photoreceptors. Geometrical optics combined with spectrophotometric and morphological data are used to support the analyses and to test predictions. Three functions are considered for correlations between taper and functionality. The first function proposes that outer segment taper serves to compensate for self-screening of the visual pigment contained within. The second function links outer segment taper to compensation for a signal-to-noise ratio decline along the longitudinal dimension. Both functions are supported by the data: real cones taper more than required for these compensatory roles. The third function relates outer segment taper to the optical properties of the inner compartment whereby the primary determinant is the inner segment’s ability to concentrate light via its ellipsoid. In support of this idea, the rod/cone ratios of primarily diurnal animals are predicted based on a principle of equal light flux gathering between photoreceptors. In addition, ellipsoid concentration factor, a measure of ellipsoid ability to concentrate light onto the outer segment, correlates positively with outer segment taper expressed as a ratio of characteristic lengths, where critical taper is the yardstick. Depending on a light-funneling property and the presence of focusing organelles such as oil droplets, cone outer segments can be reduced in size to various degrees. We conclude that outer segment taper is but one component of a miniaturization process that reduces metabolic costs while improving signal detection. Compromise solutions in the various retinas and retinal regions occur between ellipsoid size and acuity, on the one hand, and faster response time and reduced light sensitivity, on the other.

INTRODUCTION

Since the early days of vision research, pioneered by the work of Hannover (1840), Müller (1856), and Schultze (1866, 1867), vertebrate photoreceptors have been classified as rods and cones by morphological criteria. Schultze (1866) correlated the visual habits of animals with the relative preponderance of rods and cones in their retinas; this led him to formulate the concept upon which the Duplicity Theory rests. The premise of this theory is that cones are the receptors for photopic (bright light) vision, whereas rods are the receptors for scotopic (dim light) sensing. Schultze (1866, 1867) arrived at the correct conclusion that cones mediate color perception.

Subsequent to Schultze’s time, visual cells have been described with intermediate morphological, physiological, and molecular attributes that tend to blur the distinction between rods and cones (Walls, 1963; Pedler, 1965; Crescitelli, 1972; Kojima et al., 1992; Ma et al., 2001; Collin et al., 2004; Zhang et al., 2006). Nevertheless, the old classification has endured as regards to the vertebrate retina: its photoreceptors are rods and cones wherein cones typically exhibit a tapered outer segment, whereas rods do not. The functional significance of this distinguishing feature, so prevalent in nature, remains largely unexplored.

One of the potential benefits of tapering cone outer segments was introduced by Hodgkin and O’Bryan (1977) with their concept of critical taper. In their study of turtle cone electrical responses, these authors considered two limiting cases of cone geometry: the cylindrical (untapered) form and another, in which the “outer segment tapers in such a way that all molecules have an equal chance of absorbing a quantum” (Hodgkin and O’Bryan, 1977). In the latter case, the outer segment must taper at a specific, critical angle, and light must be funneled by complete internal reflection from the broad to the narrow end of cone outer segments (Hodgkin and O’Bryan, 1977). The significance of critical taper is as follows.

Rods and cones are highly specialized cells with unusual properties. First, the sensory visual pigments that they use are extremely absorbent; i.e., they possess very high extinction coefficients, corresponding to large molecular absorption cross sections (Hárosi and MacNichol, 1974). Second, visual pigment molecules are densely

Dr. Hárosi died in November 2008.

Correspondence to Iñigo Novales Flamarique: inigo@sfu.ca

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packed in lamellar membranes, which, in turn, are tightly stacked in hundreds of layers within the outer segment (the molecular packing within the membrane and the tightness of lamellar packing are probably as high as functional constraints will allow; see Wen et al., 2009). Consequently, the pigment-laden lamellae in the more proximal layers act as light filters for the more distal layers. This phenomenon is known as self-screening (Brindley, 1970). As a result of self-screening, light quanta arriving in the physiological setting have a greater probability of being absorbed near the base of an outer segment than toward the apex. Thus, in a cylindrical rod, where lamellae are of equal size, signal generation declines steadily in more distal layers with a concomitant decline in efficiency (i.e., photocurrent production per unit volume; Schnapf, 1983). One possible way to improve performance is to trim the volume slices along the length of outer segments in proportion to the fall-off of lamellar absorption caused by self-screening. A conical structure could accomplish this. Tapering is considered critical when the trimming of lamellar cross section along the taper is exactly proportional to the fall-off of absorption rate, resulting in uniform efficiency (Hodgkin and O’Bryan, 1977). This idea, which was neither generalized nor experimentally tested, is the basis for the first potential function, overcoming signal loss caused by self-screening, that we evaluate in this study.

A second function considered is that outer segments taper to enhance the signal-to-noise ratio along their lengths. Accordingly, taper would also be driven by another outer segment function: signal generation. Although the generation of signal and associated noise in photoreceptors are complex phenomena, in part because of the stochastic nature of underlying processes, such as the opening and closing of ionic channels or the binding and release of ligands at receptor sites in the enzymatic cascade of the light response, there is a consensus on the existence of thermal activation of visual pigment molecules and cGMP phosphodiesterases, both components giving rise to noise (Rieke and Baylor, 1996, 2000; Holman and Korenbrot, 2005). Based on current understanding, the receptor signal consists of a photocurrent generated through a narrow circumferential region of the outer segment membrane in response to the number of quanta absorbed in the adjacent volume containing one or a few lamellae (Baylor, 1987). Noise, on the other hand, is assumed, on the most basic level, to be proportional to the total number of visual pigment molecules or cGMP phosphodiesterases contained in the same volume (Rieke and Baylor 1996, 2000; Sampath and Baylor, 2002; Holman and Korenbrot, 2005). Either way, the signal-to-noise ratio is expected to diminish along the outer segment length (z direction) in cylindrical cells. With a tapered outer segment, however, consecutive lamellae are progressively reduced in cross section, leading to diminution of noise along the way.

The third and last function that we evaluate, efficient light collection and utilization of biomaterials, is based on the hypothesis that outer segment taper follows the optical properties of the inner segment. Rather than considering outer segment taper to be tied up with strictly outer segment functions, this idea proposes a multifaceted interdependence between inner and outer segments, as suggested by morphology.

Both cones and rods feature three distinct compartments or subcellular organelles: an outer segment (limb), specialized for trapping light; an inner segment (cone ellipsoid), concerned primarily with energy production and homeostatic functions; and a synaptic apparatus that communicates with other neurons (Fein and Szuts, 1982). Cone ellipsoids are usually the most conspicuous of the photoreceptor compartments in practically every retina, with primate foveal cones being a notable exception (Borwein et al., 1980; Packer et al., 1989; Hoang et al., 2002). Cones are almost broadest at their ellipsoid and tend to taper toward the outer segment, to which they attach closely (Fein and Szuts, 1982). In some fish retinas, the two cone compartments appear as one confluent unit, so that it is hard to discern through the light microscope where the ellipsoid ends and the base of the outer segment begins. In contrast, rods rarely have any difference in width between the two limbs. Shape and size variation notwithstanding, it is always the inner segment wherefrom light enters the outer segment in the physiological setting. For these reasons, it seems logical to consider the two compartments combined as one optical unit.

Cone ellipsoids tend to taper from the thickest proximal region toward the distal outer limb, and this, most likely, is a ploy to concentrate light (Winston 1970, 1981). And if that is so, the outer segment taper may be dependent on the light-gathering property of the inner segment. This idea is also bolstered by the observation that cones with oil droplets tend to have more tapered outer segments than those without this organelle (Nilsson, 1965; Kolb and Jones, 1982; Röhlisch and Szél, 2000; Bailes et al., 2006). In view of the high refractive index values of oil droplets (Ives et al., 1983), there is no doubt about their refractive role (Baylor and Fetipplace, 1975; Young and Martin, 1984). Given some light concentration property, cone ellipsoids could funnel parallel incident light into converging (conical) beams, which, when projected onto smaller lamellar areas, could result in equal photon catch (and signal) maintained at reduced noise. Even in the presence of light losses, increased tapering should be advantageous for the gains to be made in improved signal to noise (by lamellar shrinkage) and in savings in detector material (by volume reduction). A practical solution ought to balance the advantages against the concomitant drawbacks, such as
reduced acuity and some light loss by ellipsoid leakage. As such, a standard cone should not exist, but there should be variously tapered structures in nature that represent compromise solutions to different sets of constraints. Although the third function does not lend itself to testing via a single mathematical relationship, its validity can be ascertained by examining structure–function relationships in different species and comparing outer and inner segment taper-related variables that, from the aforementioned reasoning, should be positively correlated.

Our analysis of cone taper focuses on photoreceptor properties that have been routinely selected for during the course of evolution such as improved signal detection and metabolic savings by efficient use of biomaterials (see, for instance, the photoreceptor innovations of anchovies; Novales Flamarique, 2011). Early vertebrates, like extant hagfishes and larval ascidians, evolved ciliary photoreceptors that acted as shadow detectors, presumably conferring some of these animals an advantage in dim light environments (Collin, 2010). Natural selection acting on mutations to these ancestral designs led to a large number of novel photoreceptor features, including changes in outer segment shape (from conical to rodlike and vice versa, the transmutation hypothesis; Walls, 1963), multiple photopigments for color vision (Bowmaker, 2008), phototransduction enzymes with varying response kinetics (Hisatomi and Tokunaga, 2002), and, with a focusing eye, photoreceptor mosaics that improved overall sensitivity and/or visual acuity (van der Meer, 1992). As per other selective traits, the shape and size of photoreceptors are expected to vary, and each form may subserve multiple functions, though perhaps none optimally. Indeed, natural selection may retain a given form because it is either nondeleterious or because it confers some advantage to the individual (Bell, 2009). We therefore surmised that our analysis could reveal various advantages of taper to cone photoreceptor function.

The primary thrust of this study is theoretical. Attention is focused on morphological and biochemical properties of vertebrate photoreceptors. The aim is to gain insight into the principles governing their structure and function. In addition to the analytical approach, experimental results are used for testing theoretical predictions. The empirical data include cellular dimensions, which were derived by light and electron microscopic measurements, in situ visual pigment determinations by microspectrophotometry, in vitro visual pigment data obtained by spectrophotometry, electrophysiological determinations, and comparative anatomy. The cited empirical data are derived from either published articles in the literature or hitherto unpublished work from our laboratories. The three potential functions of cone taper that we evaluate are considered in sequence; for each, the consequences and ramifications are examined.

**Materials and Methods**

**Animals**

The majority of data in this study originated from animals used in published works, either our own or those of others. However, some measurements were taken from studies that have yet to appear in the literature. These measurements originated from goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*), zebrafish (*Danio rerio*), three-spine stickleback (*Gasterosteus aculeatus*), blue gill sunfish (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), rainbow trout (*Onchorhynchus mykiss*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), African clawed frog (*Xenopus laevis*), northern leopard frog (*Rana pipiens*), American bullfrog (*Rana catesbeiana*), green-winged teal (*Anas creca carolinensis*), red-eyed slider turtle (*Trachemys scripta elegans*), and mouse (*Mus musculus*). Animals were obtained from the following locations: zebrafish, local pet shop supplier in Burnaby (British Columbia, Canada); three-spine stickleback, Swan Lake (Victoria, British Columbia, Canada); blue gill sunfish, ponds around the Woods Hole, MA area; rainbow trout, Lower Mainland Trout Hatchery (Abbotsford, British Columbia, Canada); coho salmon and chinook salmon; Capilano River hatchery (North Vancouver, British Columbia, Canada); common carp, green sunfish, African clawed frogs, and northern leopard frogs, Marine Resources Centre of the Marine Biological Laboratory (Woods Hole, MA); and bullfrogs, Aquatic Facility Centre of Simon Fraser University (Burnaby, British Columbia, Canada). The animals were kept in aerated, flow-through water tanks under a 12 h light/dark cycle while experiments were being conducted. Fixed and fresh eyes from red-eyed sliders were provided by C. Carr (University of Maryland, College Park, MD) and E. Enos (Marine Resources Centre), respectively. Fixed mouse eyes were obtained from staff at the Animal Care Facility of Simon Fraser University, and fixed eyes from Canada goose and green-winged teal (a species of northern duck) were provided by M. Juhas (Haida Gwaii Archipelago, British Columbia, Canada).

Laboratory animals were killed in a state of light or dark adaptation for histology or microspectrophotometry, respectively, and the retinas were processed as detailed in previous publications (Härosi, 1987; Cheng et al., 2006; Novales Flamarique, 2011). All experimental procedures were approved by the Animal Care Committee of Simon Fraser University or the Marine Biological Laboratory, which are in compliance with the guidelines set by the Canadian Council for Animal Care and the National Institutes of Health.

**Measurements**

We obtained cell dimensions from live retinas (microspectrophotometry experiments) and from fixed, Epon-embedded retinas cut into thin (75 nm) sections and observed using a transmission electron microscope (model 7600; Hitachi; Fig. 1). These measurements were inner segment ellipsoid diameter (d_i), outer segment diameter at the base (d_o), outer segment diameter at a distance, z, from the base (d_z), outer segment length and tip diameter when possible (Fig. 2). In addition, densities of rods and cones were obtained from thick (1 μm) histological sections.

Besides unpublished data, we obtained similar measurements from studies spanning the last 75 yr of anatomical literature. These included 116 species of fishes, covering the evolutionary spectrum from ancient groups like lampreys, elasmobranchs, and lungfishes to modern teleosts like killifishes, carps, and cichlids; 9 species of amphibians; 33 species of birds; 19 species of reptiles; 31 species of mammals; and 8 species of monotremes and marsupials. Table 1 details the species examined and the works consulted.
Each datum presented in the graphs of this study is the mean from a minimum of 15 cells for species used in live cell recordings (Table 2) and anywhere from three to thousands of cells for species data originating solely from the literature. Some publications failed to report cell numbers for the morphological data presented; in such cases, we took the numbers as averages for the entire retina. In our presentation of figures, we show parallel analyses for species for which measurements from live cells were obtained and those whose measurements originated from histological work, primarily from the published literature.

Methods to evaluate the first biophysical function
Evaluation of this function relied on a comparison of anatomically and physiologically derived measures of two parameters: characteristic length and taper, as described in Appendix 1. Two methods were used in the evaluation. The first involved the calculation of the required characteristic length, \( a_{0} \), from Eq. 6 (Appendix 1) for each cell. The results were then compared with two separate estimates of characteristic length, \( a_{0}^{-1} \) and \( a_{0}^{1/4} \), obtained with the aid of Eqs. 10 and 16 (Appendix 1). The necessary parameter values for the latter calculations were derived from video images recorded via a microscope equipped with a calibrated infrared-sensitive video system. (A) Single cone from blue gill sunfish. (B) Single cone from leopard frog. (C) Cone outer segment (left) from B and an idealized representation of that of the optically equivalent rod (right). The equivalency is based on the assumption that both cells have equal entrance aperture with diameter \( d_{o} \) and that the cone ellipsoid funnels the incident flux to the outer segment without loss. The cellular dimensions (in \( \mu \)m) for these cones were as follows: (A) for the blue gill sunfish, \( d_{i} = 8.3, d_{o} = 5.0, d_{z} = 2.9, z = 18, \) and the inner segment length, \( l_{i} = 25.2; \) (B) for the leopard frog, \( d_{i} = 7.2, d_{o} = 2.8, d_{z} = 1.3, z = 6.3, \) and \( l_{i} = 17.5. \) The parameter \( z \), in these two cases, equals the outer segment length, and \( d_{z} \) is the diameter at the tip of the outer segment. The asterisk in B depicts an oil droplet.

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### Table 1

**List of species for which literature data were obtained and sources**

| Common name                      | Scientific name              | References                                      |
|----------------------------------|------------------------------|-------------------------------------------------|
| **Fishes**                       |                              |                                                 |
| Narrow-mouthed lamprey          | *Geotria australis*          | Collin et al., 2003                             |
| South hemisphere lamprey         | *Mordacia mordax*            | Collin et al., 1999, 2004; Collin and Pottert, 2000 |
| Sea lamprey                      | *Petromyzon marinus*         | Dickson and Graves, 1979                        |
| River lamprey                    | *Lampetra japonica*          | Ishikawa et al., 1987, 1989                     |
| Southern fiddler ray             | *Trygonorhina fasciata*      | Braekevelt, 1992a                               |
| Short tail stingray              | *Dasyatis brevicaudata*      | Braekevelt, 1994a                               |
| Blue spotted mask ray            | *Dasyatis kuhlii*            | Kohbara et al., 1987; Theiss et al., 2007       |
| Giant shovelnose ray             | *Rhinobatus typus*           | Hart et al., 2004                               |
| Eastern shovelnose ray           | *Aptychotremus rostrata*     | Hart et al., 2004; Litherland and Collin, 2008  |
| Sting ray                        | *Dasyatis sayi*              | Hamasaaki and Gruber, 1965                      |
| Electric ray                     | *Narcine brasiliensis*       | Ali and Ancil, 1974                             |
| Freshwater sting ray             | *Pantyrgyon motoro*          | Ali and Ancil, 1974                             |
| Red stingray                     | *Dasyatis aksei*             | Kohbara et al., 1987                            |
| Sepia stingray                   | *Urolophus aurantius*        | Kohbara et al., 1987                            |
| Thornback ray                    | *Platykina sinensis*         | Kohbara et al., 1987                            |
| Spiny dogfish                    | *Squalus acantias*           | Stell, 1972                                     |
| Mediterranean dogfish            | *Cephaloscyllum coelolpis*   | Bozzano, 2004                                   |
| Small spotted dogfish            | *Scyllorhinus canicula*      | Bozzano et al., 2001                            |
| Black mouth dogfish              | *Galeus longum*              | Bozzano et al., 2001                            |
| Longsnout dogfish                | *Denia eglantina*            | Kohbara et al., 1987                            |
| Nurse shark                      | *Ginglymostoma cirratum*     | Hamasaaki and Gruber, 1965                      |
| Lemon shark                      | *Negaprion brevirostris*     | Gruber et al., 1975; Gruber and Cohen, 1985     |
| Great white shark                | *Carcharodon carcharias*     | Gruber et al., 1975; Gruber and Cohen, 1985     |
| Mako shark                       | *Isurus oxyrinchus*          | Gruber et al., 1975                             |
| Thresher shark                   | *Alopaia vulpinus*           | Gruber et al., 1975                             |
| Blue shark                       | *Prionace glauca*            | Gruber et al., 1975; Kohbara et al., 1987       |
| Banded houndshark                | *Triakis scyllia*            | Kohbara et al., 1987                            |
| Silly shark                      | *Carcharhinus falciformis*   | Gruber et al., 1975                             |
| White tip shark                  | *Carcharhinus longimanus*    | Gruber et al., 1975                             |
| Sandbar shark                    | *Carcharhinus nilberti*      | Gruber et al., 1975                             |
| Brown bamboo shark               | *Chiloscyllum punctatum*     | Harashush et al., 2007                          |
| White tip reef shark             | *Trienodon obesus*           | Litherland and Collin, 2008                     |
| Epoulette shark                  | *Hemiichthyus ocellatum*     | Litherland and Collin, 2008                     |
| Ornate wobbegong                 | *Orectolobus ornatus*        | Litherland and Collin, 2008                     |
| Australian lungfish              | *Neoceratodus forsteri*      | Railes et al., 2006                             |
| South American lungfish          | *Lepidosteum paradoxa*       | Ali and Ancil, 1973                             |
| Coelacanth                       | *Latimeria chloroana*        | Locket, 1973                                    |
| Green sturgeon                   | *Acipenser medirostrum*      | Sillman et al., 2005                            |
| Pallid sturgeon                  | *Scaphirhynchus albus*       | Sillman et al., 2005                            |
| Siberian sturgeon                | *Acipenser baeri*            | Govardovskii et al., 1992                       |
| Amur sturgeon                    | *Acipenser schrenckii*       | Fang et al., 2004                               |
| Shovelnose sturgeon              | *Scaphirhynchus platorhynchus*| Sillman et al., 1999a                           |
| Paddlefish                       | *Polyodon spathula*          | Sillman et al., 1999a; Sillman and Dahlin, 2004 |
| Florida garfish                  | *Leptosomus platyrhincus*    | Collin and Collin, 1993                         |
| Bowfin                           | *Amia calva*                 | Ali and Ancil, 1976                             |
| Goldeye                          | *Hiodon alaoides*            | Braekevelt, 1982                                |
| European eel                     | *Anguilla anguilla*          | Braekevelt, 1984, 1985, 1988a,b                  |
| American eel                     | *Anguila rostrata*           | Ali and Ancil, 1976                             |
| Northern anchovy                  | *Engraulis mordax*           | Novales Flamarique, 2011                        |
| Goldfish                         | *Carassius auratus*          | Stell and Hárosi, 1976                          |
| Carp                              | *Cypripinus corpeo*          | Bunt and Klock, 1980                            |
| Japanese dace                     | *Tribolodon hakonensis*      | Hárosi and Hashimoto, 1983                      |
| Roach                            | *Leuciscus rutulus*          | Engström and Ros torso, 1963; Zanreiter et al., 1991 |
| Common name               | Scientific name             | References                                                                 |
|--------------------------|-----------------------------|-----------------------------------------------------------------------------|
| Tench                    | Tinca tinca                 | Douglas and Wagner, 1982                                                    |
| Zebrafish                | Danio rerio                 | Nawrocki et al., 1985; Forsell et al., 2001; Kim et al., 2005              |
| Cutlips minnow           | Exoglossum maculillngua     | Collin et al., 1996a                                                        |
| Silverjaw minnow         | Erimyza bucca               | Moore et al., 1950                                                          |
| Creek club               | Semotilus aromaratus        | Collin et al., 1996b                                                        |
| Gangfish                 | Longonus ladusus            | Reckel et al., 1999                                                         |
| Common white sucker      | Catostomus commersoni       | Novales Flamarique and Hario, 1997; Novales Flamarique and Hawryshyn, 1998 |
| Razorback sucker         | Xyrauchen texanus           | Novales Flamarique et al., 2007                                            |
| Channel catfish          | Ictalurus punctatus         | Sillman et al., 1993                                                        |
| White catfish            | Ictalurus eatus             | Sillman et al., 1993                                                        |
| Brown bullhead           | Ictalurus nebulosus         | Welsh and Osborn, 1937; Ali and Ancil, 1976                                |
| Coho salmon              | Oncorhynchus kisatch        | Cheng et al., 2006, 2007                                                    |
| Chum salmon              | Ongorhynchus keta           | Cheng et al., 2006                                                          |
| Pink salmon              | Ongorhynchus gorbuscha      | Cheng et al., 2006                                                          |
| Chinook salmon           | Ongorhynchus tschauyi       | Cheng et al., 2006                                                          |
| Atlantic salmon          | Salmo salar                 | Novales Flamarique, 2002, 2011; Cheng et al., 2006                          |
| Rainbow trout            | Ongorhynchus mykiss         | Kasmir and Gualtieri, 2000; Cheng and Novales Flamarique, 2007; Cheng et al., 2007, 2009 |
| Smelt                    | Osmerus eperlanus           | Reckel et al., 2003                                                         |
| Salamander fish          | Lepidogalaxias salamandroides | Collin and Collin, 1998                                                    |
| Waryfish                 | Scopelosaurus lepoda        | Munk, 1977                                                                  |
| Greenland cod            | Gadus ogue                  | Ali and Ancil, 1976                                                         |
| Antarctic cod            | Dissostichus maowoni        | Meyer-Rochow and Klyne, 1982                                               |
| Bald rock cod            | Trematomus borghoekvinkii   | Meyer-Rochow and Klyne, 1982                                               |
| Emerald rock cod         | Trematomus bernacchi        | Meyer-Rochow and Klyne, 1982                                               |
| Common cod               | Gadus callarias             | Engstrom, 1961                                                             |
| Burbot                   | Lota lota                   | Engstrom, 1961                                                             |
| Tadpole fish             | Raniceps raninus           | Engstrom, 1961                                                             |
| Silver hake              | Merluccius bilinearis       | Ali and Ancil, 1976                                                         |
| Mummichog killifish      | Fundulus heteroclitus       | Anctil and Ali, 1976; Novales Flamarique and Hario, 2000                   |
| Mangrove killifish       | Rivulus marmoratus          | Ali et al., 1989                                                           |
| Four-eyed fish           | Anablepus anableps          | Borwein and Hollenberg, 1973                                               |
| Guppy                    | Lebistes reticulatus        | Muller, 1952; Yacob et al., 1977; MacNichol et al., 1978; Kunz et al., 1983 |
| Pipefish                 | Corytholichyes paytoni      | Collin and Collin, 1999                                                    |
| Halfbeak                 | Dermogenys pusillus         | Reckel et al., 2002; Reckel and Melzer, 2003                              |
| Australian rainbowfish   | Melanotaenia macullochi     | Reckel et al., 2002; Reckel and Melzer, 2003                              |
| Celebes rainbowfish      | Marosatherina ladugesi      | Reckel et al., 2002; Reckel and Melzer, 2003                              |
| Sea needle               | Belone belone               | Reckel et al., 2001, 2002; Reckel and Melzer, 2003                        |
| Lesser weever            | Trachinus vipera            | Kunz et al., 1985                                                           |
| European perch           | Perca fluviatilis           | Ahlbert, 1973; Guma’a, 1982                                                |
| Yellow perch             | Perca flavescens            | Ali and Ancil, 1976                                                        |
| Green sunfish            | Leptomis cyanellus          | Burnsise and Arkland, 1984; Darry and Barlow, 1987                       |
| Butterfly fish           | Pantodon buchholzi          | Braekevelt, 1990a                                                           |
| Black bream              | Acropisthes piscinus       | Shand et al., 1999                                                         |
| Blackstriped cardinalfish| Apogon angustatus            | Fishelson et al., 2004                                                    |
| Iridescent cardinalfish  | Apogon kalahotters        | Fishelson et al., 2004                                                    |
| Yellowstriped cardinalfish| Apogon cyanosoma           | Fishelson et al., 2004                                                    |
| Cook’s cardinalfish      | Apogon cookie               | Fishelson et al., 2004                                                    |
| Rock bass                | Ambloplites rupestris       | Munz and McFarland, 1977                                                   |
| Large-mouth bass         | Micropterus salmoidei       | Garcia and de Juan, 1999                                                   |
| Striped bass             | Morone saxatilis            | Paillart et al., 2006                                                      |
| West Australian dhufish  | Glaucosoma hebraicum        | Shand et al., 2001                                                          |
| Black sea bass           | Centropristis striata       | Singarajah and Hario, 1992                                                 |
| Common name                  | Scientific name | References                                                                 |
|-----------------------------|-----------------|-----------------------------------------------------------------------------|
| Snake mackerel              | Gempylus serpens| Munk, 1985                                                                  |
| Walleye                     | Stizostedion vitreum vitreum | Zyznar and Ali, 1975; Januschka et al., 1987                             |
| Sauger                      | Stizostedion canadense | Ali and Amtel, 1977                                                        |
| Nile tilapia                | Oreochromis niloticus | Braekevelt et al., 1998                                                  |
| Golden dwarf cichlid        | Neocaraana anomala | Wagner, 1978; Douglas and Wagner, 1982                                        |
| Velvet cichlid              | Astronotus ocellatus | Braekevelt, 1992b                                                     |
| Burton’s haplochromis       | Haplochromis brutoni | Pietzsch-Rohrschneider, 1976                                               |
| Goldsinny wrasse            | Ctenolabrus saulius | Engström, 1963                                                            |
| Corkwing wrasse             | Ctenolabrus melops | Engström, 1963                                                            |
| Winter flounder             | Pseudopleuronectes americanus | Evans and Fernald, 1993                                                  |
| **Amphibians**              |                  |                                                                             |
| African clawed frog         | Xenopus laevis  | Kinney and Fisher, 1978a,b; Hollyfield et al., 1984; Röhlich et al., 1989; Röhlich and Szél, 2000 |
| Leopard frog                | Rana pipiens    | Nilsson, 1965                                                              |
| Bullfrog                    | Rana catesbeiana | Hisatomi et al., 1998                                                     |
| Edible frog                 | Rana esculenta  | Reichenbach and Fuchs, 1983                                                |
| Tropical toad               | Buto marinus    | Moody and Robertson, 1966; Hárosi, 1975                                      |
| Tiger salamander            | Amblystoma tigrinum | Hárosi, 1975; Mariani, 1986; Braekevelt, 1993a; Sherry et al., 1998; Ma et al., 2001 |
| Axotol                      | Amblystoma mexicanum | Custer, 1973                                                                 |
| Red-back salamander         | Pletodon cinerous | Braekevelt, 1992c                                                        |
| Newt                        | Triturus viridescens | Keefe, 1971                                                                 |
| **Birds**                   |                  |                                                                             |
| Chicken                     | Gallus domesticus | Meyer and May, 1973; Araki et al., 1984; Szél et al., 1986; Oishi et al., 1990 |
| Pigeon                      | Columba livia   | Cohen, 1963; Mariani and Leure-du-Pree, 1978; Cserháti et al., 1989       |
| Crow                        | Corvus brachyrhynchos | Braekevelt, 1994b                                                        |
| Great blue heron            | Ardea Herodias  | Rojas et al., 1999a                                                       |
| Yellow-crowned heron        | Nycticorax violaceus | Rojas et al., 1999a                                                   |
| Black-crowned heron         | Nycticorax nyticorax | Gondo and Ando, 1995                                                  |
| Cattle egret                | Babuleus ibis   | Rojas et al., 1999a                                                       |
| Tricolored egret            | Egretta tricolor | Rojas et al., 1999a                                                       |
| American white ibis         | Eudocimus riber  | Rojas et al., 1999a                                                       |
| Roseate spoonbill           | Ajasa ajasa    | Rojas et al., 1999a                                                       |
| Wilson’s plover             | Charadrius wilsonia | Rojas et al., 1999b                                                    |
| Short-billed dowitcher      | Limnodromus griseus | Rojas et al., 1999b                                                  |
| American woodcock           | Scolopax minor  | Rojas et al., 1999b                                                       |
| Black-winged stilt          | Himantopus himantopus | Rojas et al., 1999b                                                   |
| Willet                      | Catoptrophorus semipalmatus | Rojas et al., 1999b                                               |
| Red-tailed hawk             | Buteo jamaicensis | Braekevelt, 1993b                                                        |
| Great horned owl            | Bubo virginianus | Braekevelt, 1993e                                                        |
| Barred owl                  | Strix varia    | Braekevelt et al., 1996                                                    |
| Mallard duck                | Anus platyrhynchos | Braekevelt, 1990b                                                          |
| Australian galah            | Eolophus roseicapillus | Braekevelt and Richardson, 1996                                         |
| Emu                         | Dromaius novaehollandiae | Braekevelt, 1998                                                       |
| Japanese quail              | Coturnix coturnix japonica | Konishi, 1965; Oishi et al., 1990; Rojas et al., 2007                |
| Eastern tree sparrow        | Passer montanus | Gondo and Ando, 1995                                                       |
| House swallow               | Hirundo rustea | Gondo and Ando, 1995                                                       |
| Great tit                   | Parus major    | Engström, 1958                                                            |
| American robin              | Turdus migratorius | McNeil et al., 2005                                                       |
| Hermit thrush               | Catharus guttatus | McNeil et al., 2005                                                       |
| Mourning dove               | Zenaida macruana | McNeil et al., 2005                                                       |
| Common grackle              | Quiscalus quiscula | McNeil et al., 2005                                                       |
| Oil bird                    | Steatornis caripensis | Martin et al., 2004; Rojas et al., 2004                                   |
| Common pauraque             | Nyctidromus albicollis | Rojas et al., 2004                                                      |
| Common name                   | Scientific name                      | References                                                                 |
|-------------------------------|--------------------------------------|-----------------------------------------------------------------------------|
| Ring billed gull              | Larus delawarensis                   | Emond et al., 2006                                                          |
| Gray gull                     | Larus modestus                       | Emond et al., 2006                                                          |
| **Reptiles**                  |                                      |                                                                             |
| Red-eared slider              | Pseudemys scripta elegans            | Baylor and Fettiplace, 1975; Leeper, 1978; Kolb and Jones, 1982, 1987; Ohtsuka and Kawamata, 1990 |
| Snapping turtle               | Chelydra serpentina                  | Baylor and Fettiplace; 1975; Leeper, 1978                                  |
| Reeve’s turtle                | Gekkonyx reevesis                    | Ohtsuka, 1985; Ohtsuka and Kawamata, 1990                                  |
| Garter snake                  | Thamnophis sirtalis                  | Wong; 1989; Sillman et al., 1997                                           |
| Ball python                   | Python regius                        | Sillman et al., 1999                                                       |
| Common boa                    | Boa constrictor imperator            | Sillman et al., 2001                                                        |
| Mississippi alligator         | Alligator mississippiensis           | Kalberer and Pedler, 1963; Sillman et al., 1991                            |
| Caiman                        | Caiman crocodilus                    | Govardovskii et al., 1988                                                  |
| Tokay gecko                   | Gekko gecko                          | Pedler and Tilly, 1964; Crescitelli, 1972                                  |
| Blue-tailed day gecko         | Phelsuma inguiceps                   | Pedler and Tansley, 1963; Pedler and Tilly, 1964                          |
| Coastal banded gecko          | Colonia variegata                    | Dunn, 1966                                                                 |
| Mediterranean gecko           | Heneuryctes turcicus                 | Pedler and Tilly, 1964; Loew et al., 1996                                 |
| House gecko                   | Heneuryctes garnotii                 | Loew et al., 1996                                                          |
| Scinc gecko                   | Tarentoscincus scincus               | Govardovskii et al., 1984; Loew et al., 1996                               |
| Chameleon                     | Chamaleo chamaleo                    | Armengol et al., 1981                                                       |
| Western fence lizard          | Sceloporus occidentalis              | Young, 1977; Bernstein et al., 1984                                       |
| Ornate dragon lizard          | Ctenophorus ornatus                  | Barbour et al., 2002                                                        |
| Tuatara                       | Sphenodon punctatus                  | Meyer-Rochow et al., 2005                                                  |
| Bouton’s skink                | Cryptoblepharus boutonii             | Röll, 2001                                                                 |
| **Mammals**                   |                                      |                                                                             |
| Stumptail macaque             | Macaca arctoides                     | Hoang et al., 2002                                                          |
| Rhesus monkey                 | Macaca mulatta                       | Cohen, 1961; Leach, 1963; Dowling, 1965; Young, 1971; Borwein et al., 1980 |
| Crab-eating macaque           | Macaca fascicularis                  | Borwein et al., 1980                                                        |
| Pigtail macaque               | Macaca nemestrina                    | Packer et al., 1989                                                         |
| Owl monkey                    | Aotes trivirgatus                    | Jones, 1965; Murray et al., 1973; Ogden, 1975                              |
| Vervet monkey                 | Chlorocebus aethiops                 | Braekevelt, 1987                                                           |
| Human                         | Homo sapiens                         | Missotten, 1966; Dieterich and Rohen, 1970; Steinberg et al., 1977; Curcio et al., 1990; Hoang et al., 2002 |
| Dog                           | Canis lupus                          | Shively et al., 1970; Hebel, 1971                                          |
| Wolf                          | Canis lupus                          | Peichl et al., 2001                                                         |
| Cat                           | Felis domesticus                     | Steinberg et al., 1973; Braekevelt, 1990                                   |
| Ferret                        | Mustela putorius furo                | Braekevelt, 1983a                                                           |
| Mink                          | Mustela vison                       | Dubin and Turner, 1977; Braekevelt, 1990                                   |
| Spotted hyena                 | Crocuta crocuta                      | Calderone et al., 2003                                                      |
| Rabbit                        | Oryctolagus cuniculus                | Szél et al., 1988                                                           |
| Cow                           | Bos taurus                           | Szél et al., 1988                                                           |
| Pig                           | Sus scrofa domestica                 | Szél et al., 1988; Hendrickson and Hicks, 2002                              |
| Domestic sheep                | Ovis aries                           | Braekevelt, 1983b                                                           |
| Mouflon                        | Ovis musimon                         | Peichl, 2005                                                                |
| Long-finned pilot whale       | Globicephala melasoma                | Peichl et al., 2001                                                         |
| 13-lined squirrel             | Spermophilus tridecemlineatus        | West and Dowling, 1975; Anderson and Fisher, 1976                          |
| Mexican ground squirrel       | Spermophilus mexicanus               | West and Dowling, 1975; Anderson and Fisher, 1976                          |
| California ground squirrel    | Spermophilus brevica                 | Anderson and Fisher, 1976                                                  |
| Eastern gray squirrel          | Sciurus carolinensis                 | Cohen, 1964; West and Dowling, 1975; Anderson and Fisher, 1976             |
| Western gray squirrel         | Sciurus griseus                      | Anderson and Fisher, 1976                                                  |
| Prairie dog                   | Cynomys ludovicianus                 | West and Dowling, 1975                                                      |
| Mouse                         | Mus musculus                         | Carter-Dawson and LaVail, 1979                                              |
| Tree shrew                    | Tupaia belangeri                     | Kühne, 1983; Foelix et al., 1987; Müller and Peichl, 1989; Petry and Hárosi, 1990; Petry et al., 1993; Knabe et al., 1997 |
| African giant rat              | Cricetomys gambianus                 | Peichl, 2005                                                                |
for instance, the blue-absorbing single cone on line 25 of Table 2, row 1) that came close to having critical taper, the $a_{hl}^{-1}$ values for the rest of the cones are much smaller than expected. A smaller characteristic length, however, means a larger absorption coefficient. We can consider, for instance, the blue-absorbing single cone on line 25 of Table 2. For it to have critical taper, the axial extinction coefficient $a_{hl}^{-1}$ would have to be 3.8-fold higher than a realistic value.

As is apparent from columns 6 and 7 of Table 2, the taper of real cones is excessive. For the vast majority of species studied, whether the data originated from live cell measurements (Fig. 3 A) or from fixed, histological material (Fig. 3 B), the mean cone taper ($\tau$) was at least 1.5 times the critical taper ($\tau'$). Only in the case of bats, the giant African rat, and foveal-perifoveal cones of primate retinas was cone taper statistically the same as critical taper ($P > 0.05$, paired $t$ tests). On average, the ratio $\tau'/\tau$ ± SD was 4.8 ± 2.4 for fishes, 5.1 ± 1.7 for amphibians,
Photoreceptor morphology and function and birds and some fishes and monotremes, have oil droplets (lungfishes) or have ellipsosomes (killifishes) in the ellipsoid region (Fig. 1). Among the reptiles, the ratio for the strictly diurnal garter snake (5.3) was about half that of primarily nocturnal snakes like the boa and ball python (mean of ~11). Overall, phylogenetic

5.5 ± 2.1 for birds, 5.7 ± 3.8 for reptiles, 4.6 ± 3.2 for mammals, and 4.3 for marsupials (two species). Among the fishes, the mean elasmobranch ratio (7.2 ± 2.7) was about twice that of teleosts (3.8 ± 1.4), and the highest ratios among the teleosts were for lungfishes (5.2) and killifishes (4.8), which, like most amphibians, reptiles, and birds and some fishes and monotremes, have oil droplets (lungfishes) or have ellipsosomes (killifishes) in the ellipsoid region (Fig. 1). Among the reptiles, the ratio for the strictly diurnal garter snake (5.3) was about half that of primarily nocturnal snakes like the boa and ball python (mean of ~11). Overall, phylogenetic
Signal-to-noise ratio increases beyond that predicted by critical taper

Examination of Eq. 30 (Appendix 2) reveals the following: (a) when $A_z$ is invariant in $z$ (cylindrical outer segment), the signal-to-noise ratio, $S/N$, diminishes exponentially along $z$ as a result of self-screening; (b) if $A_z$ versus $z$ diminishes by tapering, the $S/N$ will undergo proportionate increases; (c) for critical taper, when $A_z$ varies in accordance with Eq. 4 (Appendix 1), the exponential terms cancel, and the $S/N$ becomes independent of $z$. From Eq. 30 we can also deduce that, for excessive taper, i.e., when a cone cross section ($A_z$) diminishes faster than the exponential fall-off of signal, the signal-to-noise ratio may actually increase toward the apex of a cone outer segment. Eq. 30 thus reveals an important tendency: more taper means greater improvement in the signal-to-noise ratio along an outer segment. For this reason, in the absence of other requirements, cones should taper as much as possible.

Inner segment morphology as a major determinant of outer segment taper

For the lack of a compact theoretical expression (see Appendix 3), the third function linking inner segment morphology to outer segment taper could not be verified experimentally. Nevertheless, several observations were consistent with it, as demonstrated by the following analyses using examples from diverse phylogenetic groups.

Teleosts, like the goldfish, have typically large, plump inner segments and shorter outer segments compared with the rods (Fig. 1). The average goldfish cone in this study had an entrance aperture (assumed to be equal to the broadest region of the ellipsoid) of 8.7-µm diameter, wherefrom light would be funneled to the base of the outer segment with a mean diameter of 5.9 µm (Table 2). The concentration factor, $FC$, by Eq. 33 (Appendix 3) is 2.2. The outer segment volume calculated by Eq. 34 yields 277 µm³. The volume of a cylinder with equal base diameter is 399 µm³. Their ratio gives 1.4 for the geometry factor, $FG$, by Eq. 35. Additionally, the volume reduction ratio, $VR$, is obtained at 3.1 by Eq. 36. Thus, the cone outer segment uses $\frac{32}{32}$% of the volume of that of the hypothetical optically equivalent rod. The rod cells measured in these goldfish preparations were quite uniform, with an average outer segment diameter of 2.1 µm and lengths in the range of 36–44 µm. For a 40-µm-long rod outer segment, we can calculate the axial absorptance by Eqs. 3 and 22 using $a_z$ (Appendix 1). Accordingly, such a rod would absorb $\frac{69}{69}$% of the light incident at its base. By a similar calculation, the 15-µm-long cone outer segment would absorb only 35% of the flux it receives. Therefore, the rod is the better light detector of the two. However, this rod collects a mere fraction of what a single cone can gather from the retinal illumination. In fact, it would take $(8.7/2.1)^2 = 17.2$ rods
Photoreceptor morphology and function

12 ± 7.4 and 13 ± 10 (mammals), and 12 and 16 (marsupials, two species). Among the fishes, the rod/cone ratios of teleosts were better predicted than those of elasmobranchs; the mean predicted and observed ratios for these two groups were 6.2 ± 9.0 and 6.4 ± 9.1 (teleosts) and 8.5 ± 12 and 10 ± 9.1 (elasmobranchs). Overall, the highest rod/cone ratios occurred for the walleye (a teleost with “remarkably large cones”; Januschka et al., 1987) and the mink. The best predictions occurred within the teleosts and mammals, especially for primates (predicted and expected mean rod/cone ratios were 16 ± 7.3 and 17 ± 8.8). The worst predictions (i.e., ratio of expected to predicted, or vice versa, >2) occurred within the birds (owls) and reptiles (snakes) as well as for elasmobranchs and one teleost, the snake mackerel. Some of these animals (e.g., owls, several species of elasmobranchs, and large snakes) were included in the analysis because of reported crepuscular (cone driven) activity, although they may be primarily nocturnal and perhaps not very appropriate for inclusion in these regressions. Indeed, the rod/cone ratios of primarily nocturnal or dark habitat–dwelling species, which include deep ocean sharks and eels, several mammals (e.g., the mouse, African giant rat, bats, and the spotted hyena), the oil bird, and marsupials (opossums and the tammar wallaby), have large to very large rod/cone ratios (some exceeding 100:1). At the other end of the spectrum, strictly diurnal species like some ground squirrels, the prairie dog, and the tree shrew have very small rod/cone ratios (≤0.1). None of these species, located at either extreme of the rod/cone ratio range, were included in the analysis leading to Fig. 4 B.

Figure 5. Regression of expected rod/cone ratios as a function of those observed for fully diurnal and primarily nocturnal species. Data originated from the literature.

Figure 4. Regressions of expected rod/cone ratios as a function of those observed for primarily diurnal (though birhythmic) species. (A) Data for species from which live cell measurements were obtained. (B) Data for species for which measurements originated from the literature.

to intercept as much incident flux as the average cone considered here. The rod/cone ratio in goldfish was reported to be 15:1 (Stell and Hárosi, 1976). The reasonably satisfactory agreement between predicted and observed ratios suggests that, in this animal, interception of equal light flux, a property linked to inner segment aperture, appears to be the criterion driving rod and cone densities.

When this analysis was applied to the other, predominantly diurnal, species studied, the correlations between observed and expected rod/cone ratios were surprisingly good (Fig. 4 A and B). On average, the rod/cone ratio expected and observed ± SD for the various phylogenetic groups were as follows: 6.9 ± 10 and 7.6 ± 9.2 (fishes), 1.4 ± 1.6 and 1.5 ± 0.58 (amphibians), 2.0 ± 2.1 and 1.4 ± 2.0 (birds), 5.0 ± 3.4 and 6.8 ± 7.4 (reptiles), 12 ± 7.4 and 13 ± 10 (mammals), and 12 and 16 (marsupials, two species). Among the fishes, the rod/cone ratios of teleosts were better predicted than those of elasmobranchs; the mean predicted and observed ratios for these two groups were 6.2 ± 9.0 and 6.4 ± 9.1 (teleosts) and 8.5 ± 12 and 10 ± 9.1 (elasmobranchs). Overall, the highest rod/cone ratios occurred for the walleye (a teleost with "remarkably large cones"; Januschka et al., 1987) and the mink. The best predictions occurred within the teleosts and mammals, especially for primates (predicted and expected mean rod/cone ratios were 16 ± 7.3 and 17 ± 8.8). The worst predictions (i.e., ratio of expected to predicted, or vice versa, >2) occurred within the birds (owls) and reptiles (snakes) as well as for elasmobranchs and one teleost, the snake mackerel. Some of these animals (e.g., owls, several species of elasmobranchs, and large snakes) were included in the analysis because of reported crepuscular (cone driven) activity, although they may be primarily nocturnal and perhaps not very appropriate for inclusion in these regressions. Indeed, the rod/cone ratios of primarily nocturnal or fully diurnal animals are not very well predicted based on our principle of equal flux sharing between photoreceptor types (Fig. 5). Primarily nocturnal or dark habitat–dwelling species, which include deep ocean sharks and eels, several mammals (e.g., the mouse, African giant rat, bats, and the spotted hyena), the oil bird, and marsupials (opossums and the tammar wallaby), have large to very large rod/cone ratios (some exceeding 100:1). At the other end of the spectrum, strictly diurnal species like some ground squirrels, the prairie dog, and the tree shrew have very small rod/cone ratios (≤0.1). None of these species, located at either extreme of the rod/cone ratio range, were included in the analysis leading to Fig. 4 B.
Hárosi and Novales Flamarique 171

Figure 6. Regressions of the ratio of realistic to critical characteristic length \( \frac{a_{\text{nt}}}{a_{\text{h1}}} \) as a function of concentration factor \( F_c \). Recall that the characteristic length is the distance along the outer segment at which the light flux, \( \Phi \), falls to 0.368 of the incident light flux at the base, \( \Phi_0 \). The concentration factor is the square of the ratio between ellipsoid diameter at its largest cross section and outer segment base diameter, i.e., \( F_c = \left( \frac{d_i}{d_o} \right)^2 \), and represents coupling of light flux without loss from ellipsoid to outer segment. (A) Data for species from which live cell measurements were obtained. (B) Data for species for which measurements originated from the literature.

In contrast to the comparable size of photoreceptors in teleosts and mammals, the retinas of amphibians have colossal rods and minute cones (Fig. 1). The dichotomy in outer segment size is seldom as pronounced as in frog species, with the possible exception of the salamanders (Crescitelli, 1972). However, even in these animals, the important feature determining rod and cone densities appears to be the equal sampling of the plane, where, presumably, the image of the outside world is formed within the eye.

As an example, the mean cone ellipsoid diameter (entrance aperture) of the African clawed frog was found to be 7 \( \mu \text{m} \), whereas the mean diameter of the outer segment base was 2.9 \( \mu \text{m} \). Repeating the procedure used previously, we can calculate, by using Eqs. 33–36 (Appendix 3), the following: \( F_c = 5.8 \), \( V_c = 20.1 \mu \text{m}^3 \), \( V_r = 39.6 \mu \text{m}^3 \), \( F_c = 1.97 \), and \( V_k = 11.4 \). The optically equivalent cylinder, as indicated in Fig. 2 C, has a volume of \( \sim 251 \mu \text{m}^3 \). Thus, the cone outer segment volume is only \( \sim 9\% \) of that of the cylinder.

The preparations where frog single cones were located also had rhodopsin-containing red rods with an outer segment diameter equal to the widest region of the ellipsoid, with a mean diameter of 7 \( \mu \text{m} \). This finding may be interpreted in terms of the cone ellipsoid’s light-gathering property and suggests that the entrance aperture of single cones and red rods are equal in frogs. Further support for the idea of equal flux collection by these cells in the frog retina can be found in the electron microscopic studies of Nilsson (1964, 1965). He showed, among other things, that red rods and single cones have about equal areas in cross section at the inner segment level (Nilsson, 1964). These photoreceptors, therefore, may indeed gather nearly equal fluxes from a uniform retinal illumination.

The actual volume of a red rod outer segment (at a length of 66 \( \mu \text{m} \)) is \( \sim 2,500 \mu \text{m}^3 \). Using known parameters and Eqs. 3 and 22 (Appendix 1), we can calculate that these rods may catch 94\% of axially incident light, whereas the average cone catches only \( \sim 23\% \) of it. Thus, a typical red rod may be a fourfold better absorber of axial light than a single cone (at the \( \lambda_{\text{max}} \) of their respective visual pigments). However, the cone outer segment volume, \( V_c \), as calculated previously, is nearly 100-fold smaller than that of the rod. The rod/cone ratio expected, 1, is similar to that assessed from published micrographs (means of 1–1.5; Kinney and Fisher, 1978a; Hollyfield et al., 1984; Röhlich and Szél, 2000). The fact that cones absorb a smaller portion of the incident light is not a handicap under daylight conditions, when high absolute sensitivity is not required. For the same reason, the loss of light by leakage from cone ellipsoids can be tolerated when light is plentiful. Therefore, frog rods and cones can serve the retina equally well despite their large disparity in size. By making cones with large entrance aperture and an ability to concentrate light, outer segment size reduction becomes possible at some cost in detector efficiency and sacrifice in acuity.

Upon review of the preceding examples, the data suggest a positive relationship between outer segment taper and ellipsoid concentration factor, \( F_c \). The latter variable is a measure of light concentration from the ellipsoid into the outer segment. We have plotted the ratio \( \frac{a_{\text{nt}}}{a_{\text{h1}}} \), indicating how many critical characteristic lengths would equal the characteristic length considered realistic, against \( F_c \) for the various species examined (Fig. 6, A and B). The correlations are positive,
though not particularly strong, especially when all the species studied are considered (Fig. 6B). The lack of a strong correlation for the ensemble of species may in part reflect the lower accuracy of data obtained from histological material (especially from published figures) as opposed to live cell measurements. In addition, and perhaps more importantly, it may be an indication of other factors, such as the presence of oil droplets or light losses caused by ellipsoid leakage, affecting the extent of outer segment taper, none of which were considered in the calculations (for an instance of light loss estimation, see Baylor and Fettiplace, 1975). Nevertheless, the correlations, albeit approximate ones, between the cone ellipsoid concentration factor and outer segment taper expressed as a multiple of critical taper support the third function.

**DISCUSSION**

Emerging view on structure–function relations in vertebrate photoreceptors

Our analysis supports the three postulated functions for cone taper: (1) compensation for light loss resulting from self-screening, (2) increased signal-to-noise ratio along the length of the outer segment, and (3) improved light capture and material savings by shape continuity between inner and outer segment. Cone taper, as determined in this work, was found quite variable and, for the most part, beyond that predicted for critical taper (Table 2 and Fig. 3). Consequently, cones appear to achieve full compensation for the light loss caused by self-screening while improving signal-to-noise ratio along their outer segments. Support for the third function points to a dominant role of the inner segment in shaping the outer segment, leading to a process of miniaturization and, as a result, metabolic savings in biomaterials. The trade-offs in such structural modifications are in absolute sensitivity and visual acuity, with spatial resolution being inversely related to receptor cross section (Snyder and Miller, 1977; Neave, 1984; van der Meer, 1992; Haug et al., 2010). Large cone ellipsoids combined with excessively tapered outer segments could achieve miniaturization, but only in the presence of light funneling. Therefore, an ellipsoid light concentration property was suggested by logic and implied by the third function.

In every retinal region, a trade-off is expected between acuity and receptor size. The typical example is the retina of some diurnal teleosts where the highest visual acuity, found in the mid to upper frontal field, is subserved by smaller, densely packed cones in the centro- and ventro-temporal areas of the retina (Beaudet et al., 1997; Novales Flamarique, 2005, 2011; Cheng and Novales Flamarique, 2007). Other more extreme examples are the specialized foveas of diurnal lizards (Röll, 2001; Barbour et al., 2002) and primates (Borwein, 1981). In primates, high visual acuity is the product of tightly packed, long, rodlike foveal cone outer segments, each having a diameter of ~1 µm; these are connected by cone fibers to the rest of their compartments that are laterally displaced and squeezed out to the slopes of the foveal pit (Borwein, 1981; Packer et al., 1989). Foveal cones have no ellipsoids, and, therefore, they must collect light without the aid of any other structure. However, parafoveal cones, and other cones in general, are built with joint outer and inner compartments, where the latter is the thicker of the two to intercept a larger area of retinal illumination at the cost of reduced regional acuity (Packer et al., 1989; Hoang et al., 2002). As flux is funneled from the entrance aperture to a smaller exit aperture at the distal end of ellipsoids, structural modifications become feasible in outer segment tapering and size. Cone miniaturization has several advantages, including improved signal-to-noise ratio, faster visual pigment regeneration (i.e., recovery from bleaching), and material savings as a result of reduced volume. The drawbacks are reduction in absolute sensitivity vis-à-vis rods and some light losses from detection as a result of ellipsoid leakage.

Rods, on the other hand, are specialized for high absolute sensitivity and not for rapid response (Burns and Lamb, 2003). The eyes of deep-sea fishes provide exquisite examples for such sensitive detector structures (Locket, 1977). The cylindrical form appears well suited for on-demand tailoring of outer segment length, which may reach hundreds of micrometers in fish species living at great depths. The use of multiple banks and assemblages of rods, in addition to long ones, also appears to be aimed at catching scarce photons in the darkness of the deep ocean (Locket, 1977; Munk, 1977; Collin et al., 1998). Rods do not jeopardize detection efficiency by the use of light-concentrating schemes at the ellipsoid level; they have wider acceptance angles for oblique rays than cones do. Other mechanisms, however, may operate to concentrate light onto the outer segment. For instance, some nocturnal mammals, like the mouse, show an inverted chromatin nuclear pattern that, together with a quasi-columnar organization of rod nuclei, serves to channel light from the outer nuclear layer toward the outer segments (Solovei et al., 2009). Signal pooling from multiple rods (at a sacrifice of spatial resolution) and coincidence detection should further improve scotopic performance as well as signal-to-noise ratio (Peichl, 2005).

**Improved light funneling by oil droplets**

Oil droplets are optical devices used by most cones in avian, amphibian, and reptilian eyes. Oil droplets are highly refractive spherical globules (Ives et al., 1983); they may be clear or colored (Kolb and Jones, 1987; Hart et al., 2006). Because of their location at the distal
end of inner segments anterior to the base of outer segments, they may serve as light filters as well as focusing devices (Young and Martin, 1984; Vorobyev, 2003). A spherical body with high refractive index immersed in a lower index medium will behave as a positive lens with a short focal length. Thus, an oil droplet (or a concentrated group of microdroplets, as in diurnal snakes; Wong, 1989) is expected to further concentrate the light funneled by the ellipsoid. As such, increased outer segment tapering and size reduction become feasible. And indeed, observational evidence bears this out: the most extreme cases of outer segment volume reduction are found in oil droplet-containing cones (Fig. 1). Oil droplet focusing might be advantageous not only in allowing further reductions in detector cross section, but also in quickening recovery from blinding exposures, as when experiencing glare.

For an elaboration of the last point, note the following: whereas scotopic sensitivity in primate and teleost vision may take tens of minutes to regain dark-adapted levels after a bright “bleaching” exposure, photopic sensitivity returns to former levels in a few minutes (Rushton, 1965; Thomas and Lamb, 1999; Kenkre et al., 2005; and unpublished data for teleosts). This indicates that cones recover their sensitivity in vivo faster than rods do. Experiments in vitro also reveal the same tendency in chemical regeneration of visual pigment: in the presence of copious amounts of exogenous 11-cis retinal, whereas rods regenerate slowly, cones recover rapidly and repeatedly after several bleaching exposures, and, in each case, they regain most of their visual pigment in a few seconds (Hárosi, 1984). Consider now the oil droplet-equipped cones in a fishing bird’s eye. Given the short focal lengths of spherical lenses, an oil droplet will focus light in a specific region of an outer segment lamella, which would receive intense illumination. Let’s assume, for the sake of the argument, that only 10% of a lamella is illuminated. When the bird skims over a body of water, reflected sunlight could be a blinding experience. In case of a human observer, extensive bleaching in retinal receptors would occur, resulting in temporary blindness. However, in cones with oil droplet focusing, only a fraction of the visual pigment complement would get bleached, and, in the assumed lamella, 90% of the visual pigment would remain unexposed. Because lateral and rotational diffusion drives visual pigment molecules rapidly in the receptor membranes (Wang et al., 2008), the bleached molecules would quickly be exchanged with unbleached ones from the adjacent membrane area. Therefore, most of the cone’s sensitivity could recover in milliseconds, at least three orders of magnitude faster than a recovery based on normal biochemical regeneration (Kenkre et al., 2005).

The aforementioned mechanism could improve the foraging performance of birds like the black phoebe, a sit-and-wait predator whose visual searching increases significantly under bright light conditions, likely as a result of the negative effects of glare (Gall and Fernández-Juricic, 2009). Other bird species like herons and osprey routinely hunt in shady habitat or at crepuscular periods (unpublished data), reducing their exposure to glare and other blinding factors such as the light flickers produced by waves near the water surface. In fact, such flicker may be used by fish for camouflage, as multiple species have developed body markings that resemble the light patterns (McFarland and Loew, 1983). Fast-moving predators, like some insects and birds, have critical fusion frequencies that surpass the predominant flicker occurring in surface waters (in the range of 1–5 Hz), improving visual contrast of underwater targets (McFarland and Loew, 1983). Given a mean diffusion coefficient of 0.4 μm² s⁻¹ for activated opsins (Wang et al., 2008), between ~3 and 13% of the (bleached) visual pigment molecules in a 1-μm-diameter lamella would get replaced by intact ones between flickers, contributing to fast recovery from bleaching.

On light collection mechanisms

The idea that cone inner segments concentrate light has been around for a long time. For instance, O’Brien (1946) proposed it in his theory explaining the Stiles-Crawford effect (O’Brien, 1951; Johnson and Tansley, 1956; Enoch, 1963). Concerning vertebrate photoreceptors, turtle cone ellipsoids have been compared with ideal light collectors (Baylor and Fettiplace, 1975; Winston, 1981). The name refers to nonimaging optical devices that concentrate light by internal reflection onto the smallest possible exit aperture (Winston, 1970). In the case of an invertebrate eye, Levi-Setti et al. (1975) suggested that the crystalline cone in an ommatidium of Limulus is an ideal light collector. Subsequent work by Land (1979), however, showed that the crystalline cones can form images and that they pass light through a refractive index variation scheme. Similar refractive index gradients have not been uncovered in vertebrate photoreceptors, and the mechanism of importance remains to be sorted out. Internal reflection, refraction, and even diffraction may play some role. Cone ellipsoids are not truly homogeneous, as they contain abundant mitochondria that might function akin to a Fresnel zone plate. The one conclusion that is certain at present is that an oil droplet must have a major contribution to the refractive power of the system. Another open question is whether investigations of waveguide modal patterns in vertebrate visual cells would facilitate the understanding of structure–function relationships between photoreceptor compartments (Snyder and Menzel, 1975).

Oblique incidence and the Stiles-Crawford effect

Stiles and Crawford (1933) discovered that the visual sensitivity of the human eye depends on the direction from which light enters the pupil (known as the Stiles-Crawford
effect of the first kind \([\text{SCE}_1]\)). O’Brien (1946) was the first to advance a theory to explain it, and there have been others (e.g., Snyder and Pask, 1973; reviewed by Enoch and Bedell, 1981). The third hypothesis is consistent with the \(\text{SCE}_1\). Accordingly, cone ellipsoids are imperfect concentrators; they lose light by leakage. The lower refractive indices of cone inner and outer segments provide reduced critical angles as compared with those of rods (Appendix 1). But rods, with more uniform and denser distal compartments, have larger critical angles and thus can tolerate a wider range of off-axis rays. The combination of these properties may be sufficient to account, at least qualitatively, for the larger photopic and smaller scotopic \(\text{SCE}_1\) (Enoch and Bedell, 1981). Further support for the third function comes from a finding by Westheimer (1967) that foveal cones of the human eye have a reduced (i.e., rodlike) Stiles-Crawford effect when compared with parafoveal cones. Also supportive is the small directional sensitivity displayed by the human achromat, an abnormal condition in which vision depends entirely on rod function (Nordby and Sharpe, 1988).

Summary
We have provided evidence to support the notion that cone outer segment taper follows from the shape of the ellipsoid, a structure that serves to concentrate light onto the outer segment. The advantages of taper and cone miniaturization include compensation for light loss caused by self-screening, metabolic savings in structural components, higher signal-to-noise ratio, and accelerated regeneration of visual pigment. The trade-offs are in absolute sensitivity and visual acuity. As suggested by the different sizes of photoreceptors and their relative densities across phylogenetic groups, species have evolved visual cells that presumably optimize these trade-offs for life in particular environments. As such, there is no model cone or rod but a range of sizes and shapes dictated by the ecological constraints guiding the evolution of each species.

APPENDIX 1

Derivation of equations to test the first biophysical function: Cone outer segments taper to compensate for light flux diminution by absorption (self-screening) so that flux density remains invariant or increases with axial distance along the outer segment

The list of symbols and definitions used in the testing of this function are shown in Table 3. The following sections present geometric and spectrophotometric definitions of key variables (e.g., absorption coefficient and taper) used to evaluate whether a cone taper is critical, i.e., whether it compensates exactly for light flux diminution by self-screening (Hodgkin and O’Brien, 1977). As a consequence, taper that is equal to or exceeds the critical value indicates compensation for self-screening.

Geometric derivation of the absorption coefficient
Perfect compensation for light absorption caused by self-screening imposes two requirements. The first is invariance of flux density with respect to \(z\), so that \(J_\text{z} = J_\text{o}\); in other words, the light flux impinging on the base \((\Phi_\text{o})\) divided by the cross-sectional area \((A_\text{o})\) is equal to the transmitted flux \((\Phi_\text{z})\) divided by the corresponding cross section \((A_\text{z})\) along the entire length of the outer segment. Accordingly,

\[\frac{\Phi_\text{o}}{A_\text{o}} = \frac{\Phi_\text{z}}{A_\text{z}}. \quad (1)\]

The second requirement is that visual pigment absorption be the only reason for the diminution of flux (i.e., light leakage is absent). This means that the incident light rays interact with the cell boundary at angles \((\Theta)\) below the critical angle \((\Theta_c)\) so that total internal reflection prevents light from escaping (see the section Critical angle estimations for data confirming this). Thus,

\[\Theta \leq \Theta_c. \quad (2)\]

The exponential law of absorption,

\[\Phi_\text{z} = \Phi_\text{o} \exp(-az), \quad (3)\]

sets forth the variation of flux in an absorbing medium along the \(z\) direction \((\Phi_\text{z})\) in terms of the incident flux \((\Phi_\text{o})\) multiplied by an exponential function of variable \((z)\) and a characteristic constant of the medium, called the absorption coefficient \((a)\). The latter is inversely proportional to the attenuation, or characteristic, length, at which \(\Phi\) falls to \(\exp(-1) = 0.368\) of \(\Phi_\text{o}\). Upon combining Eqs. 1 and 3, we obtain

\[\frac{\Phi_\text{z}}{\Phi_\text{o}} = \frac{A_\text{z}}{A_\text{o}} = \exp(-az). \quad (4)\]

The circular cross section of a cone with diameter \(d\) is \(A = d^2 \pi / 4\), and thus, Eq. 4 may be written as

\[d_\text{z} = d_\text{o} \exp(-az/2), \quad (5)\]

wherefrom the absorption coefficient for testing the first function is expressible as

\[a_{11} = \frac{(2/z) \ln(d_\text{o}/d_\text{z})}. \quad (6)\]

The surprising outcome gleaned from Eq. 6 is that the absorption coefficient, a purely spectroscopic quantity, may be determined from measurements of distance involving the base diameter, \(d_\text{o}\), a second diameter, \(d_\text{z}\), and their separation along the \(z\) coordinate.
Angle of incidence with respect to cell axis
Taper, as defined by Eq. 17
Absorption coefficient required for invariance of light flux
Axial distance within outer segments (µm), with z = 0 at the base

| Symbol  | Definition                                                  |
|---------|-------------------------------------------------------------|
| Φ       | Total light flux (photons s⁻¹)                              |
| Φ₀, Φᵣ  | Value of Φ at z = 0 and at distance z from base             |
| A       | Cross-sectional area of outer segment, assumed to be equal with the surface area of a transverse membrane, referred to as a lamella (Eckmüller, 1987). One rod disk, also called flattened sacculus, has the surface of two lamellae |
| Aᵣ, Aᵣ’ | Value of A at the base and at distance z from it             |
| Aᵢ, Aᵢ’ | Outer segment absorbance for transversely and axially polarized light |
| Jᵢ      | Incident light density (photons s⁻¹µm⁻²); Jᵢ = Φ₀/Aᵣ   |
| Jₒ      | Transmitted light density through level z from base; Jₒ = Φᵣ/Aᵣ |
| R       | Dichroic ratio: absorbance ratio at two orthogonal polarizations; R = Aᵣ/Aᵢ’ |
| Sₒ      | Specific absorbance at transverse polarization; Sₒ = Aₒ/d   |
| dₒ, dᵣ  | Outer segment diameter at base and at a distance z from the base |
| c       | Concentration of visual pigment in appropriate units        |
| α       | Molecular extinction coefficient (µm² per molecule; Eqs. 7 and 9) |
| ε       | Molar extinction coefficient (liter mole⁻¹ cm⁻¹; Eqs. 8 and 9) |
| λₒₘₐₓ  | Wavelength of peak extinction                               |
| k       | Anisotropy factor: ratio of anisotropic to isotropic absorbance |
| a       | Absorption coefficient (usually in cm⁻¹; Eq. 3)            |
| a⁻¹     | Characteristic length (reciprocal of a) expressed in µm (signifies the distance at which Φᵣ reduces to 36.8% of Φₒ) |
| aₒ₁     | Absorption coefficient required for invariance of light flux along the outer segment, as by Eq. 6 |
| aₒ₁⁻¹   | Characteristic length, based on invariance of light flux along an outer segment (µm) |
| aₒ’, aₒ’’ | Absorption coefficient obtained by Eqs. 10 and 16 |
| aₒ’⁻¹, aₒ’’⁻¹ | Characteristic lengths (µm) obtained from aₒ’ and aₒ’’ |
| τ       | Taper, as defined by Eq. 17                                 |
| Θ       | Angle of incidence with respect to cell axis                |
| Θᵣ      | Critical angle of Θ, defined as Θᵣ = 90° – φᵣ              |
| φᵣ, φₒ  | Critical angle with respect to normal of boundary (Eq. 19)   |
| nₒ, nᵣ  | Refractive indices of two optical media forming a boundary |
| z       | Axial distance within outer segments (µm), with z = 0 at the base |
If a cylindrical vessel is homogeneously filled with \( N \) absorbing molecules, each possessing a transition moment \( M \), the total extinction (\( E = NM \)) may be written as \( E = E_x + E_y + E_z \). The fractions expressed in the three spatial coordinates are equal for a three-dimensional random array so that \( E_x/E = E_y/E = E_z/E = 1/3 \). The measurable fraction of extinction in the \( z \) direction is half of the two orthogonal components:

\[
0.5 \left( E_x + E_y \right)/E = 1/3. \tag{11}
\]

In a similar model, a cylindrical photoreceptor may be regarded, in its simplest form, as an imperfect two-dimensional random array for which \( E_x = E_y \) and \( E_x/E = R \). With these, the \( z \) fraction is expressible as

\[
E_z/E = E_z/(2E_x + E_y) = 1/(1 + 2R). \tag{12}
\]

Viewed from the \( z \) direction (i.e., end-on), the measurable fraction is

\[
0.5 \left( E_x + E_y \right)/E = 0.5 \left( E - E_z \right)/E = 0.5 \left[ 1 - 1/(1 + 2R) \right] = R/(1 + 2R). \tag{13}
\]

The anisotropy factor \( (k) \) in the \( z \) direction is obtainable from the above as the ratio of end-on extinctions (the two-dimensional versus the three-dimensional fractions) and is given by (see Hárosi, 1975, 1982)

\[
k = 3R/(1 + 2R). \tag{14}
\]

Thus, \( e \), \( c \), and \( R \) yield the axial absorption coefficient of a photoreceptor with the aid of Eqs. 10 and 14.

The second formula for the calculation of the axial absorption coefficient makes use of the microspectrophotometrically determined transverse specific absorbance, \( S_\lambda = A_\lambda /d \), in which \( A_\lambda \) is the peak absorbance (of the \( \lambda \) band) for transversely polarized light of the contour line, which, upon precession, describes the conical surface:

\[
\tau = \tan^{-1}(d_o - d_z)/2z. \tag{17}
\]

The axial distance, \( z \), is the separation between the base of a right cone, with diameter \( d_o \), and a parallel slice, with diameter \( d_z \). For a cone cell, \( \tau \) describes an average inclination of the contour line between two slices (corresponding to \( d_o \) and \( d_z \)), and it cannot account for a point-by-point variation that Eq. 5 describes.

**Critical angle estimations**

The refractive indices for rod and cone inner and outer segments have been measured only for a few animal species in a handful of studies. The obtained values are as follows: the refractive index for rod ellipsoid and outer segment, 1.40 and 1.41, respectively; for cone ellipsoid and outer segment, 1.39 and 1.385, respectively; and for the extracellular matrix (mucopolysaccharide), 1.34 (see Borwein, 1981 for primary citations).

At a boundary between two transparent media, one denser with refractive index \( n_1 \) (inside), the other rarer with refractive index \( n_2 \) (outside), light rays will refract according to Snell’s law:

\[
n_1 \sin \varphi_n = n_2 \sin \varphi_o, \tag{18}
\]

in which \( \varphi_n \) and \( \varphi_o \) are the corresponding angles of incidence and refraction with respect to the normal. Beyond a certain angle of incidence in the denser medium, no refraction occurs, and there is total internal reflection. The critical angle (for which \( \sin \varphi_o = 1 \) ) can be expressed from Eq. 18 as

\[
\varphi_c = \sin^{-1}(n_2/n_1). \tag{19}
\]

From this, \( \Theta_c \) of Eq. 2 can now be obtained as

\[
\Theta_c = 90^\circ - \varphi_c. \tag{20}
\]

Substitution of the numerical values cited above in Eqs. 19 and 20 leads to the following critical angles in \( \Theta_c \): 15.4° and 14.6° for cone ellipsoid and outer segment and 16.8° and 18.1° for rod ellipsoid and outer segment, respectively. In these calculations, we assumed that the relevant optical parameters used in critical angle calculations are those that have been determined (Borwein, 1981), notwithstanding the fact that photoreceptors and their surroundings are neither homogeneous nor isotropic.
Table 4

Additional list of symbols and definitions pertaining to the second biophysical function

| Symbol  | Definition |
|---------|------------|
| $\Phi_a$, $\Phi_o$ | Absorbed flux in one layer at the base and at depth $z$ |
| $J_a$ | Surface density of pigment (molecules $\mu m^{-2}$); $J_a = n/A_o$ |
| $\delta$ | In situ path length in visual cells corresponding to a single pigment-laden lipid bilayer (lamella); $\delta = 15$ nm is taken as its equivalent thickness |
| $A_o$ | Total lamellar surface area of an outer segment |
| $c$ | Concentration of visual pigment; $c = n/A_o$ |
| $n$ | Total number of visual pigment molecules contained in an outer segment |
| $n_o$, $n_z$ | Number of visual pigment molecules in a lamella at the base and at $z$ |
| $p_z$ | Probability of absorption of a quantum by a single layer at $z$ |
| $\gamma$ | Quantum efficiency of bleaching; number of photons absorbed per number of photoactivations that result in bleaching |
| $S_z$ | Signal from light activation (ionic current) at level $z$ |
| $N_z$ | Noise produced in the dark at level $z$ (ionic current) |

What constitutes the surrounding medium is especially questionable. This, sometimes referred to as interstitial matrix, is commonly equated with mucopolysaccharide, as done here. However, microvilli of the pigmented epithelial cells and the calycal processes that come in contact with at least parts of inner and outer segments may play a role in setting the refractive properties of these cells.

Outer segment taper, as defined by Eq. 17 and determined from our observations and published photomicrographs (see Results), indicate angles <14°. These values are within the requirement of Eq. 2, and thus, they justify the original assumption concerning the funneling of axial rays by total internal reflection.

Appendix 2

Derivation of equations to test the second biophysical function: Cone outer segments taper to improve signal-to-noise ratio along their lengths

The list of symbols and definitions used to evaluate this function are presented in Table 4. We make the following five assumptions. (1) Incident light consists of an axial flux of parallel, uniform, and steady illumination at the base of outer segments with negligible reflection and scattering losses. (2) Visual pigment molecules are packed uniformly in transverse membranes of all rods and cones with constant surface density; $J_o = n/A_o = n_o/A_o$. (3) Activation of visual pigment molecules requires not only light absorption but also chromophore isomerization, commonly referred to as bleaching. To take this into account, the quantum efficiency of bleaching, $\gamma$, must be factored in (Dartnall et al., 1936). (4) Signal is constituted by a photocurrent generated through the cell envelope at each lamella, independent of other lamellae. Although there clearly is an observable spreading of excitation, for the sake of simplicity, all messengers for signal are assumed to originate in one lamella. (5) Noise is generated like signal in lamellae under the conditions set forth in the preceding assumption. Whether thermal isomerization of the chromophore or subsequent biochemical steps are the cause (Rieke and Baylor 1996, 2000; Sampath and Baylor, 2002; Holcman and Korenbrot, 2005), it is assumed here that noise is proportional to the number of visual pigment molecules contained within each layer.

The objective of this appendix is to relate the generation of signal and noise to outer segment taper. Given an absorbing medium, an incident flux will either be absorbed or transmitted, provided that assumption 1 holds. In general terms,

$$\Phi_o = \Phi_a + \Phi_z.$$  (21)

When normalized to the incident flux, Eq. 21 may be rearranged as

$$\Phi_z/\Phi_o = 1 - \Phi_z/\Phi_o.$$  (22)

The left-hand side in Eq. 22 is usually referred to as absorptance, $A_o = \Phi_a/\Phi_o$, whereas the fraction on the right-hand side is called transmittance, $T = \Phi_z/\Phi_o$. Optical density (or absorbance), as defined previously in Eq. 7, is the logarithm of $T^{-1}$. After substitution of Eq. 3 in Eq. 22 and rearrangement, the rate of photon absorption by a monolayer of thickness $\delta$, with incident flux $\Phi_o$, is

$$\Phi_z = \Phi_o[1 - \exp(-a\delta)].$$  (23)

Because visual pigments are hydrophobic chromoproteins, they must be membrane bound. For this reason, the shortest meaningful axial path length of the visual pigment in a vertebrate photoreceptor is a single layer. Although the exact numerical value of $\delta$ for the present is immaterial, it is assumed to be 15 nm. This is half the repeat distance of the 30 nm obtained for rod disks by electron microscopic and x-ray crystallographic determinations (for references, see Fein and Szuts, 1982). The value of $\delta = 1.5 \times 10^{-6}$ cm permits estimation of the magnitude of the exponent in Eq. 23. Based on Eqs. 10 and 14, the absorption coefficient for a “typical” rhodopsin-containing cone is expected to be near 420 cm$^{-1}$ ($a^{-1} = 24$ µm), which would make the value of the exponent $\sim 6.3 \times 10^{-4}$.

In view of the probable magnitude of $\delta$ and assumption 2, and also considering pigment anisotropy according to Eq. 10 and expressing the concentration as

$$c = n/A_o \delta = J_o/\delta = n_z/A_o \delta.$$  (24)

we can derive the following formula from Eq. 23:

$$\Phi_z = \Phi_o[1 - (1 - k_a \alpha \delta)] = \Phi_o k_a n_z / A_z,$$  (25)
in which the exponential term in Eq. 23 was represented by the first two terms of its series expansion (the second and higher order terms being negligibly small). Thus, the rate of absorption in a pigment layer is proportional to the incident light flux times the products of anisotropy factor \(k\), pigment type \(\alpha\), and surface density of the pigment \(J_n = n_x/A_x\). By extending Eq. 25 to describe a multilayered system with self-screening, the rate of absorption by one layer at depth \(z\) is

\[
\Phi_{az} = k\alpha(n_x/A_x)\Phi_o \exp(-az).
\]  

(26)

A second method of obtaining the rate of quantum absorption by a single layer of visual pigment is based on the interpretation of the chromophoric absorption coefficient \(\alpha\) as the absorption cross section of one molecule in a random array (Dartnall, 1972). The probability of a quantum at \(\lambda_{max}\) to be caught by a single layer of visual pigment, with anisotropy factor \(k\), is expressible as a ratio of the sum of all the molecular cross sections to that of the available total area:

\[
p_{z} = k\alpha n_x/A_x.
\]  

(27)

When we scale up from one photon to an incident photon flux of \(\Phi_o\), Eq. 27 will reproduce the preceding relationship described by Eq. 25.

The rate of signal generation in a lamella at \(z\) is expected to be proportional to the rate of photon absorption in that layer (assumptions 3 and 4). Thus, the signal should be formally similar to Eq. 26:

\[
S_z = C_sh\alpha\gamma(n_x/A_x)\Phi_o \exp(-az),
\]  

(28)

with \(C_s\) being a conversion factor between photon absorption and corresponding photocurrent.

The noise produced at layer \(z\) may be given (assumption 5) as

\[
N_z = C_n n_z,
\]  

(29)

in which \(C_n\) is again a conversion factor. In taking the ratio between Eqs. 28 and 29, \(n_z\) will cancel out, and the signal-to-noise ratio becomes (with \(C'\) as a new constant)

\[
S_z/N_z = C'k\alpha\gamma(\Phi_o/A_x)\exp(-az).
\]  

(30)

**APPENDIX 3**

Derivation of equations to test the third biophysical function: Cone outer segments taper in accordance with the optical properties of their inner segments, facilitating light capture and reducing use of biomaterials

The list of symbols and definitions used to evaluate this function are presented in Table 5. The following sections introduce definitions of key variables (e.g., concentration factor) for its evaluation.

Assuming a perfect two-dimensional random array of absorbers, the angle of incidence with respect to the optic axis (\(\Theta\)) will reduce the absorption probability of unpolarized light by a factor (Winston, 1981):

\[
f = 0.5(1 + \cos^2\Theta).
\]  

(31)

Evaluated for \(\Theta = 15^\circ\), Eq. 31 yields \(f = 0.97\). Thus, oblique incidence for angles up to \(15^\circ\) causes only a small drop (3%) in absorption efficiency. In the following treatment, we assume, therefore, that oblique incidence does not significantly affect the in situ absorption efficiency of the visual pigment molecules embedded in the transverse lamellae.

**Flux concentration in a tapered outer segment**

In the absence of absorption, the flux density of a converging (conical) beam increases in the direction of convergence. The conceptually simplest case occurs when the incident cone of light matches exactly the outer segment taper. This, however, leads to the same analysis covered previously, where an axially parallel beam was assumed to be incident on the base of a tapered outer segment, in which total internal reflection prevailed. In terms of flux densities, Eq. 26 may also be written as

\[
J_{az} = J_o(A_o/A_x)k\alpha(n_x/A_x)\exp(-az)
\]  

(32)

to indicate a rate of absorption density increase by the factor \(A_o/A_x\). For obtaining the signal generation in a lamella at \(z\), however, the total absorbed flux is needed, not the flux density. Multiplying Eq. 32 by \(A_o\), though, takes us back to Eq. 26. Therefore, the preceding analysis leading to Eq. 30 is also valid for this case.

Besides the matching case, the convergence of the beam incident upon the outer segment may also be lesser or greater than that of the structure. The slightly convergent case is the simplest one, and it may be handled as the axially parallel beam was above (see previous paragraph), necessitating no new analysis. The greater beam convergence, however, warrants further considerations. Some aspects of the latter case are discussed in the manuscript in connection with the effect of oil droplet focusing.

**Flux concentration in a cone ellipsoid**

The third function presupposes the existence of a mechanism whereby cone ellipsoids funnel light from a broader, proximal portion toward a narrower, distal end and that this property makes a significant impact on the structure and function of outer segments. Consider a flux \(\Phi_i\) incident at the entrance aperture (largest cross section) of an ellipsoid to produce a flux density \(\Phi_i\). If this flux is coupled without loss to a smaller exit aperture,
where the flux density is $J_o$, the following relationships hold (provided the cross sections are circular with respective areas and diameters of $A_i$, $d_i$ and $A_o$, $d_o$):

$$J_o/J_i = A_i/A_o = \left(\frac{d_i}{d_o}\right)^2 = F_{G}. \tag{33}$$

The significance of $F_G$, named here concentration factor, is that it shows the proportion by which the base of a photoreceptor can be reduced in area while still capable of detecting all of the incident flux $\Phi_i$. Although the issue of light losses by leakage remains to be considered, this property reveals the feasibility of detector miniaturization.

**Volume reduction of a cone outer segment**

Whereas the outer limb of rods approaches the cylindrical form in nearly all instances, cone outer segments usually appear truncated, not pointed. Therefore, the frustum of a cone is a more realistic representation of a cone outer segment. The volume of the frustum of a cone is defined with a diameter of base $d_{o}$, tip $d_{i}$, and altitude $h$ as

$$V_c = \frac{\pi}{4}(h/3)\left[ d_o^2 + d_i d_o + d_i^2 \right]. \tag{34}$$

Compared with the volume of a cylindrical rod, $V_r = \frac{\pi}{4}h d_i^2$, their ratio is defined as

$$\frac{V_r}{V_c} = F_G, \tag{35}$$

in which $F_G$ is named the geometry factor. Experience shows that the value of $F_G$ is variable and tends to fall between 1.5 and 3, the latter being the largest for a right cone ($d_i = 0$). With these two factors combined, the volume reduction ratio is defined as

$$V_R = \frac{V_{req}}{V_c} = F_G F_C. \tag{36}$$

$V_R$ is an indicator of proportion between the outer limb volumes of a cone and an equivalent rod, when both have equal inner segment entrance aperture and incident flux. Discounting light losses, these two cells could produce the excitation of an equal number of visual pigment molecules to equal illumination. Clearly, the cone is the more efficient receptor of the two because it uses only a fraction of the rod’s detector apparatus. This means reduced amounts in lipid membrane, visual pigment, and all the other components of the enzymatic cascade required for generating signals in terms of photocurrent modulation.

Dr. Ferenc I. Hárosi passed away in November 2008. During his career, he was the leading innovator in the field of microspectrophotometry, starting with the making of the first computerized dichroic microspectrophotometer in the 1970s. His insights into photoreceptor physiology and visual pigment properties opened new fields of investigation that are actively pursued by many a prominent scientist today. He was my friend and mentor and an inspiration to vision scientists of all ages. Dr. Hárosi is, and will always be, profoundly missed; thank you for everything, friend.

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**TABLE 5**

Additional list of symbols and definitions pertaining to the third biophysical function

| Symbol | Definition |
|--------|------------|
| $f$    | Absorption efficiency factor due to oblique incidence (Eq. 31) |
| $A_i$  | Collection area (circular); largest cross section of inner segment |
| $d_i$  | Diameter of $A_i$ |
| $\Phi_i$ | Total flux at entrance aperture of an inner segment |
| $J_i$  | Flux density at entrance aperture of an inner segment |
| $J_o$  | Rate of flux density absorption |
| $F_G$  | Geometry factor (Eq. 35) |
| $F_C$  | Concentration factor (Eq. 33) |
| $d_o$  | Diameter at the tip of a frustum |
| $h$    | Altitude or height of a cone or cylinder |
| $V_r$  | Volume of a rod outer segment |
| $V_c$  | Volume of a cone outer segment |
| $V_{req}$ | Volume of an optically equivalent rod outer segment |
| $V_R$  | Volume reduction ratio as defined by Eq. 36 |

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