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The visual sensitivity of the retina of the conger eel

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[Plate 1]

We measured the visual sensitivity of the conger eel retina by means of its electroretinogram (e.g.) and whole nerve responses. The spectral sensitivity of the retina closely corresponded to a prediction based on the density spectrum of the conger visual pigment, measured in situ. The pigment density in the conger eel retina is high, perhaps as high as 1.0. Thus, the predicted spectral sensitivity would be much broader than is observed if the absorption spectrum of the pigment governed the visual sensitivity. The reason why the visual spectral sensitivity corresponds to the density spectrum and not to the absorption spectrum is that the photoreceptors in the conger eye are arranged in tiers and only the inner tier contributes to vision.

INTRODUCTION

The eye of the conger eel is peculiar and interesting in at least two ways. First, the rod photoreceptors of this fish contain chrysopsin, the yellow visual pigment usually present in the eyes of deep-sea fish (Denton & Warren 1957; Denton & Walker 1958). Secondly, the density of the visual pigment in the eye of the conger is unusually high (Denton & Walker 1958). Our research was aimed at finding the visual electrophysiological consequences of these specializations of the conger eye. We also sought to compare the eye of the conger eel with the eye of the common eel Anguilla rostrata, which we had studied previously (Gordon et al. 1978). What we found implies that the eyes of both conger and anguilla have tiers or palisades of rod photoreceptors that resemble the tiered photoreceptor layers in many deep-sea fish (cf. Munk 1966; Locket 1977). Furthermore, results on the electrophysiology of the conger eel retina lead to the unexpected conclusion that only the response of innermost tier is functionally significant.

METHODS

Conger eels were caught with a long line just outside Plymouth Harbour. The animals ranged in mass from 5 to 25 kg and in length from 1 to 3 m. They were kept in a large holding tank until 12-20 h before an experiment and then

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transferred to a smaller tank in a darkroom. The dark-adapted animals were
decapitated and pithed under dim red light. This procedure served to keep
the retina almost completely dark-adapted. The eye was dissected from the head,
often with a piece of optic nerve. Cornea, lens and excess vitreous were removed.
The eyecup preparations and whole nerve preparations were complete at this
stage, but for the isolated retina preparation the retina was peeled off the pigment
epithelium and placed on a large glass slide. The conger eye is quite large and the
dark-adapted retina peels off easily. All the dissections were performed under dim
red illumination so that dark adaptation would be maintained.

We recorded the electroretinogram (e.g.) and whole nerve potentials with
standard techniques. Silver/silver chloride electrodes were used. One electrode
made contact with the vitreal surface of the eye through a pipette filled with
conger Ringer. A second electrode made direct contact with the outside of the eye.
Whole-nerve recording was accomplished with the pipette inserted into the optic
nerve behind the eye. Recording was differential between the two electrodes.
The bandwidth of the e.g. recording was either d.c. to 100 Hz or 0.1 to 100 Hz.
The bandwidth of the whole nerve recording was 1 to 100 Hz because of worse sig-
nal-to-noise and greater problems with drift.

The visual stimulus was produced by a two-channel optical projection system
essentially similar to one that has been described in detail before (Gordon et al.
1978). The light sources were quartz–iodide lamps run off a regulated d.c. power
supply. In one channel, wavelength control was achieved with a monochromator
(Bausch and Lomb high intensity, 15 nm half amplitude bandwidth); in the other
channel it was achieved with interference filters (Optics Technology, 10–15 nm
half amplitude bandwidth). Stimulus intensity was varied with Inconel neutral
density filters. Stimulus duration was controlled with Uniblitz shutters (Vincent
Associates). Stimuli were uniform across the retina. The light sources and neutral
filters were calibrated with a radiometer (UDT Model 40A), the pickup head of
which was placed in the same plane as the biological preparations. The radi-
ometric calibration was subsequently corrected by calibration with an EGG Model
580-11A radiometer. Retinal irradiances for a particular experiment are given in
the figure captions. The maximum unattenuated irradiance of our optical system
was 1.45 μW/cm² at 440 nm, rising to 10.2 μW/cm² at 600 nm.

Visual pigment density measurements were performed on isolated retinas, with
a method similar to that used by Denton & Walker (1958). A silicon photovoltaic
cell was placed under the preparation. The response of the photocell to a brief
(10 ms) bright pulse of light was measured as a function of wavelength, before and
after the retina was bleached for 5 min by a microscope illuminator. The photocell
response was linear over the intensity range tested. Therefore, the logarithm of
the ratio of the photocell responses after and before bleaching was taken as the density.
Thus, \( \log (R_b/R_a) \approx D \), where \( R_a \) and \( R_b \) are the photocell responses after and
before bleaching. This approximation is based on the assumption of only a small
amount of intraretinal absorption by photoproducts produced by bleaching. We
expected that this would be a good approximation for the long wavelength portion of the density function, and therefore would allow us to estimate $\lambda_{\text{max}}$ of the photopigment from this measurement.

The morphology of the conger eel retina was studied in a preliminary way by means of light microscopy. Dark-adapted eyecups or isolated retinæ were fixed in glutaraldehyde, dehydrated, embedded in plastic, sectioned at 5–10 μm, and stained with toluidine blue. The photoreceptor layers were examined at low power and also at high power with a Zeiss × 63 oil immersion objective.

\[
\lg \left[ \text{stimulus irradiance/} (\mu \text{W cm}^{-2}) \right]
\]

![Graph showing the logarithmic relationship between stimulus irradiance and response.]  

**Figure 1.** E.r.g. of the conger eel retina. The dark-adapted eyecup was exposed to 0.1 s flashes of 490 nm light at five different stimulus irradiances. The stimulus was spatially uniform over the whole retina. Bar, 100 μV. The stimulus trace below the e.r.g. also serves as a time calibration, 0.1 s for the stimulus duration. The bandwidth of the recording system was 0.1 to 100 Hz.

**Results**

**Electrophysiological responses**

Our initial experiments were designed to reveal whether there was more than one type of photoreceptor in the conger retina. One way to do this is to study the e.r.g. waveform at different stimulus intensities and at different wavelengths. If there were rods and cones in the conger retina, one would expect to see a rod-dominant waveform at short wavelengths and low intensities and a mixed rod–cone response at long wavelengths and higher intensities.

The e.r.g. waveforms from a typical dark-adapted eyecup are shown in figure 1. The stimulus wavelength for these responses was 490 nm. The stimulus duration was 0.1 s. The response waveform definitely changed with stimulus irradiance. From the lowest to the highest stimulus irradiance, a range of approximately $10^4$, the latency from stimulus onset to the peak of the response decreased from 320 to 200 ms. The halfwidth of the response varied by a corresponding amount. Nevertheless, these responses all were rod-dominated. They were slow-rising, long-lasting,
and without any bumps or shoulders that might suggest cone intrusion. Even the response to the highest intensity stimulus showed no evidence of a rapidly rising cone-driven phase at response onset. However, one might not expect to see the cone-driven e.r.g. responses to the short wavelengths at which the conger rods are most sensitive, so that the results in figure 1 do not rule out the presence of cones.

\[ \ln \left( \frac{\text{stimulus irradiance}}{\mu \text{W cm}^{-2}} \right) \]

\[ \begin{array}{c}
-1.86 \\
-3.18 \\
-0.96 \\
-2.39 \\
1.01 \\
-0.39 \\
\end{array} \]

\[ \begin{array}{c}
\mu \text{V} \\
20 \\
100 \\
500 \\
0.5 \text{ s} \\
\end{array} \]

**Figure 2.** E.r.g. at two different wavelengths (\( \lambda \)). There are three pairs of e.r.g.; each pair is at a different response level. In each pair, the lower response is to 490 and the upper to 600 nm light. Vertical calibrations are given for each pair. A stimulus irradiance of 1 \( \mu \text{W cm}^{-2} \) at 600 nm is approximately equivalent to \( 2.7 \times 10^{12} \) quanta cm\(^{-2}\) s\(^{-1}\). 1 \( \mu \text{W cm}^{-2} \) at 490 nm is approximately equivalent to \( 2.2 \times 10^{12} \) quanta cm\(^{-2}\) s\(^{-1}\). The stimulus duration was 0.5 s. The recording bandwidth was d.c. to 100 Hz.

A comparison of the e.r.g. waveforms at short and at long wavelengths is a more stringent test of the hypothesis that there is more than one class of photoreceptor. This was done in several experiments as illustrated in figure 2. In this figure are shown three pairs of responses at three levels of stimulus irradiance. In each pair one response was to a stimulus at 490 nm and one to a stimulus at 600 nm. The responses to the two widely separated wavelengths were similar in all respects at all three levels. Note that the stimulus duration was 0.5 s and that the recording bandwidth was d.c. to 100 Hz. Thus we could follow the very slow recovery after illumination of the rod-driven e.r.g. In these records the irradiances of the two wavelengths were not precisely balanced for their physiological effectiveness; in each pair the response to the 490 nm stimulus was slightly larger and slightly faster. But the similarity of the e.r.g. at the short and the long wavelengths
implies that the e.r.g. is dominated by a single class of photoreceptors. Below we will prove that the rods are the dominant photoreceptors.

There are other ways of eliciting cone function in the e.r.g. in a rod-dominated eye. We have previously used some of these methods in our study of the rod-dominated eye of the eel anguilla (Gordon et al. 1978). One is to use flickering

\[ \text{Ig [stimulus irradiance/(\mu W\, \text{cm}^{-2})]} \]

\[
-1.45 \\
-2.25 \\
-3.21 \\
-4.25
\]

\[
0.1s
\]

**Figure 3.** The e.r.g. of the isolated retina of the conger eel. Stimulus duration was 0.1 s and is indicated in the lower trace. Stimulus wavelength was 490 nm. The bandwidth of the recording system was 0.1 to 100 Hz. Vertical calibration bar, 20 \( \mu \text{V} \).

stimuli that flash on and off at rates of 10 Hz and above. This did not work in the conger retina because there was no measurable response to flicker at rates above 4 Hz. A different technique, which we used in our study of anguilla, is to bleach the isolated retina and then measure spectral sensitivity and also waveforms of responses to short and long wavelength stimuli. The idea behind this method is that rod pigment should not regenerate in the isolated retina while cone pigment should (Zewi 1939; Goldstein 1967, 1970; Hood & Hock 1973; Gordon et al. 1978). While this method works well in anguilla, it fails in conger for two reasons. The first is that, even after a long period of dark adaptation after the bleach, the long-wavelength sensitivity does not become measurable in conger. The second reason is that the rod visual pigment regenerates in the isolated conger retina (see evidence below). Also, the rod-driven e.r.g. recovers to a small extent. Perhaps this rod recovery may mask small cone-driven e.r.g. responses in conger. Furthermore, as demonstrated below, the spectral sensitivity of the conger eye seems to be completely determined by the rods.

In our study of the spectral sensitivity, we relied on measurements of the e.r.g. in the eyecup, on the e.r.g. of the isolated retina, and on the mass response of the optic nerve. To illustrate how these responses compared one with another, we include records of the e.r.g. of the isolated retina in figure 3 and of the mass nerve
response in figure 4. The isolated retina e.r.g. was recorded under the same conditions as was the eyecup e.r.g. in figure 1. The responses of the isolated retina had waveforms similar to those of the eyecup but the responses of the isolated retina were on the average much smaller, perhaps because the signal was partially shunted at the edge of the isolated retina. The mass nerve response was recorded with an

\[
\text{lg [stimulus irradiance/(μW cm}^{-2})]\n\]

\[
-2.01 \quad -1.06 \quad -0.06 \quad 0.94
\]

\[
10 \, \text{μV}
\]

\[
0.5 \, \text{s}
\]

**Figure 4.** The optic nerve response of the conger eel. Responses are shown for 0.5 s flashes of 560 nm light at the irradiances the logarithms of which are indicated on the figure. The bandwidth of the recording system was 1 to 100 Hz.

a.c. coupled amplifier with a shorter time constant (corner frequency 1 Hz instead of 0.1 Hz as in the e.r.g. recordings). The nerve responses were therefore somewhat differentiated and they also were somewhat smaller than the e.r.g. recorded in the eyecup.

**Spectral sensitivity**

**Pigment density spectrum**

Our work on spectral sensitivity consists of a comparison of the action spectra of various electrophysiological responses with the density spectrum of the rod photopigment. This pigment has been studied extensively previously (Denton & Warren 1957; Denton & Walker 1958; Denton 1959). It has been christened chryopsin because of its golden colour; in solution it absorbs maximally at 487 nm. We measured the density spectrum of the pigment *in situ* by measuring the amount of light transmitted through the isolated retina, before and after a full bleach, as a function of wavelength (see methods). As mentioned above, we found evidence that the chryopsin regenerated in the isolated retina, and so we had to measure the bleached condition immediately after the bleaching light was turned
off. This was done by bleaching, measuring at a particular wavelength, then re-bleaching and measuring at the next wavelength, until the transmission of the bleached retina was measured at all wavelengths. On one occasion we measured the density immediately after the bleach and then again 30 min later. The pigment density had recovered after 30 min to approximately 25% of its dark-adapted

\[ \text{Figure 5. Visual pigment density spectrum. Shown are four different estimates of the density spectrum of chrysopsin; (○) our measurements of the difference spectrum of an isolated retina, by the method described in the text; (◇) the difference spectrum measured by Denton & Walker (1958); (△) the density of the isolated retina compared to Ringer's solution (Denton & Walker 1958); (□) an estimate of the conger pigment density spectrum derived from the dichroic absorption spectrum (Denton 1959). The solid curve is the 492 nm Dartnall nomogram. The dashed curve is the 487 nm nomogram.} \]

value. This observation was made at several wavelengths on the long wavelength side of the peak sensitivity, to rule out the idea that the observed recovery was due to the photoproducts of bleaching. At all wavelengths the density recovered by approximately the same amount, implying that we were observing the regeneration of chrysopsin.

The pigment density spectrum that we measured immediately after the bleach is shown in figure 5. Also drawn in this graph are Dartnall nomograms for vitamin A\(_4\) pigments with wavelength maxima of 487 and 492 nm. It seems clear that the 492 nm nomogram is a better fit. This confirms the results of Denton & Walker, who found that the absorption spectrum of conger chrysopsin in situ was shifted to slightly longer wavelengths than the spectrum of the extracted pigment in solution. In fact one should expect that the difference spectrum that we measured ought to be slightly lower than the true density spectrum at short wavelengths. Some hint of this effect is shown in figure 5 which shows four different spectra: (1) the density of the conger retina compared with Ringer's solution (Denton & Walker 1958); (2) the difference spectrum (Denton & Walker 1958); (3) the density of orientated visual pigment measured by receptor dichroism (Denton 1959); (4) our measurement of the difference spectrum. The data are all in remarkably good
agreement, but they suggest that estimates of the density by the difference spectrum are a little lower at short wavelengths, below 460 nm, than the more precise estimates of Denton (1959). However, for the purposes of this paper, the tolerably good fit of the density estimates and the solid curve in figure 5 allow us to use it as an approximation to the true pigment density spectrum of chrysopsin in situ. The pigment densities at 490 nm as measured in four different conger retinæ were: 0.83, 0.88, 0.69, 0.70. These numbers are probably an underestimate of the true density because any light that reaches the photocell without passing through the photoreceptors will make the measured density smaller than the true density.

E.r.g.

The spectral sensitivity of the e.r.g. in the eyecup and in the isolated retina may be used to infer important features of photoreceptor mechanisms. The spectral sensitivity was measured by means of the following procedure. The dark-adapted retina (eyecup or isolated) was exposed to brief stimuli (0.1 s) every thirty seconds. Stimulus irradiance at 490 nm was set so that the response was small but reliably measurable. Then the retina was stimulated at or near this low irradiance at an ascending series of wavelengths from 440 nm to 620 nm. (At the extreme ends of the series (440 nm, 600 nm, 620 nm) stimulus irradiance was set to be ten times higher to achieve approximately equal responses for all stimuli.) After the ascending wavelength run, the stimuli were repeated but in descending order of wavelength. This was a control for fluctuations in sensitivity and also for the adaptive effects of stimulation. Then stimulus irradiance was increased by about a factor of ten (1 log unit) by removing filters from the stimulus beam. The wavelength series was repeated at this higher irradiance. Usually the experiment was continued until increments in stimulus irradiance produced no further increment in response (saturation). The sensitivity was defined as the reciprocal of the stimulus irradiance required to produce a peak positive deflexion of the e.r.g. of a given magnitude, usually 25 or 10 μV. The results are not critically dependent on the value chosen since the response amplitude against intensity functions are approximately parallel across wavelength.

The spectral sensitivity of the conger e.r.g. corresponded very closely to the visual pigment density. This is shown in figure 6a, which shows results from a typical eyecup. The points are the measured spectral sensitivities and the solid curve is the 492 nm nomogram, which fits the visual pigment density (corrected for quantal absorption, cf. Stiles (1948) and Dartnall (1962)).

Optic nerve

It is possible that the e.r.g. may reflect the activity of only a special class of photoreceptors, and that it may not be a good indicator of the true spectral sensitivity of the conger cell. To test this hypothesis we measured the spectral sensitivity of the massed optic nerve response, as an index of the neural output of
Visual sensitivity of conger eel retina

**Figure 6.** (a) Spectral sensitivity of the e.r.g. The e.r.g. sensitivity was determined from the responses to 0.1 s flashes at several irradiances. The preparation was the dark-adapted, excised eyecup of the conger eel. The solid curve is the predicted spectral sensitivity from a 492 nm Dartnall nomogram. (b) Spectral sensitivity of the massed optic nerve response. The sensitivity was determined from nerve responses (like those in figure 4) to stimuli at several irradiances. The stimuli were 0.1 s flashes. The circles are a set of e.r.g. data. The triangles are whole nerve data. The solid curve is the predicted spectral sensitivity from a 492 nm Dartnall nomogram. (c) E.r.g. spectral sensitivity of the isolated retina in the 'normal' orientation. Light entered the isolated retina from the vitreal side. The stimulus duration was 0.1 s. The solid curve is the spectral sensitivity predicted from the 492 nm Dartnall nomogram. The dashed curve is spectral sensitivity predicted from the absorption spectrum of the 492 nm nomogram at a density of 0.9. (d) E.r.g. spectral sensitivity of the isolated retina in the inverted position. Light entered the retina from the scleral surface. Stimulus duration was 0.1 s. The solid curve is the predicted sensitivity derived from the 492 nm Dartnall nomogram, the density spectrum. The dashed curve is predicted from the model discussed in the text, which is based on the idea that the photoreceptors of the conger retina are in tiers and that only the inner tier influences the e.r.g.

the retina. Results are shown in figure 6b; e.r.g. results from one eyecup and whole nerve results from another eyecup are plotted together. Clearly, the spectral sensitivity of the optic nerve and that of the e.r.g. are identical, and both are well fitted by the density spectrum of the 492 nm pigment.
The agreement between the spectral sensitivity and the pigment density spectrum is somewhat mysterious. The pigment density is so high that one might have expected the spectral sensitivity to be a much broader function than the pigment density spectrum, by the following reasoning (Hecht et al. 1942; Dartnall 1962). The physiological response should be determined by photon absorption. Thus the physiological spectral sensitivity should be related to the absorption spectrum $A(\lambda)$ not the density spectrum $D(\lambda)$.

\[ A(\lambda) = 1 - 10^{-D(\lambda)} \]

In the limit when $D(\lambda) \ll 1$ for all $\lambda$, $A(\lambda) \approx D(\lambda)$. But when $D(\lambda) \approx 1$, then $A(\lambda)$ departs significantly from $D(\lambda)$. Therefore, agreement between the spectral sensitivity and $D(\lambda)$ means that not all the photon absorptions are contributing equally to physiological responses. At this point in the investigation we began to think that the photoreceptors in the conger retina might be in layers or tiers, as in deep-sea fish (Munk 1966; Locket 1977). We thought perhaps that the high pigment density might be due to the layering of photoreceptors, each of which was rather short and thus had only a low pigment density. If this were the case, and if the photoreceptors in different layers did not contribute equally to the e.r.g. andoptic nerve responses, one might expect a spectral sensitivity as narrow as that which we had observed. The way to test this hypothesis is to measure the spectral sensitivity of the isolated retina. If one compares the spectral sensitivity when the light enters the vitreal side of the retina with the sensitivity when the light enters the eye from the scleral surface of the retina, one may test the idea that different layers of photoreceptors do not contribute equally to neural responses.

**Spectral sensitivity of the isolated retina: normal and inverted**

The spectral sensitivity of the e.r.g. of the isolated conger retina was first measured with the retina in the ‘normal’ orientation with respect to the incident light, that is, vitreal surface towards the light. In this orientation, the spectral sensitivity was identical to that of the e.r.g. of the eyecup, as shown in figure 6c. In other words, the isolated retina in the ‘normal’ orientation had a spectral sensitivity that corresponded to the visual pigment density. Figure 6c also shows the sensitivity that would be expected if all photon absorptions contributed equally to the response. The dashed curve is $S(\lambda) = (\lambda/\lambda_{\text{max}})^{\lambda} A(\lambda)$, where $A(\lambda)$ is the absorption spectrum, $1 - 10^{-D(\lambda)}$, and $S(\lambda)$ is the sensitivity corrected for quantal absorption.

Clearly, the e.r.g. of the isolated retina in the ‘normal’ orientation has a spectral sensitivity like that of the e.r.g. of the eyecup and that of the optic nerve. All three physiological action spectra correspond to the density of the 492 nm pigment, and are markedly narrower than the prediction based on the absorption spectrum (dashed curve in figure 6c).

When the isolated retina was inverted, so that light entered from the receptor side, the spectral sensitivity of the e.r.g. was markedly altered, as shown in
figure 6d. The sensitivity at 490 nm, which was near the peak sensitivity in the normal orientation, was actually a local minimum. The maximum sensitivity was shifted to longer wavelengths than the peak of the pigment spectrum, and there was also a local maximum in sensitivity at wavelengths shorter than the peak in the pigment spectrum. This experiment was repeated on three retinae. The results were all qualitatively the same. However, there was a general deterioration of the e.r.g. in the isolated, inverted retinae. Figure 6d is a graph of the data that were best in the sense that the retina remained viable long enough to obtain reproducible responses over three decades of stimulus irradiance.

The unusual spectral sensitivity of the isolated, inverted retina can be explained by a model of the conger retina as a tiered retina. If there are several tiers of photoreceptors, but only the innermost (most vitreal) tier of photoreceptors drives the e.r.g., then one would get qualitatively the results shown in figure 6d. The outer tiers would screen out light at the wavelengths near the peak of the pigment density spectrum, and would let through light of wavelengths near the sloping portions of the density spectrum. The dashed curve in figure 6d is the predicted spectral sensitivity in the isolated retina for a five-tiered retina with a total density of 1.1 and with only the inner tier driving the e.r.g. The fit of the curve with the data is mainly sensitive to the assumed total density. The number of tiers must be greater than three for a good fit.

The model can be formulated as follows. Suppose that there are \( n \) layers of photoreceptors and that the total density of photopigment is \( D(\lambda) \). Also suppose that each layer has exactly the same density, \( D(\lambda)/n \). Then if the innermost layer determines the response of the eye, the spectral sensitivity for light coming from the vitreal surface will be:

\[
S(\lambda) = \left( \frac{\lambda}{\lambda_{\text{max}}} \right) (1 - 10^{-D(\lambda)/n}).
\] (1)

The factor \( (\lambda/\lambda_{\text{max}}) \) is the correction for quantal absorption (Stiles 1948). The term \( (1 - 10^{-D(\lambda)/n}) \) is the absorption of the inner layer. For \( D(\lambda)/n \ll 1 \),

\[
S(\lambda) \approx \left( \frac{\lambda}{\lambda_{\text{max}}} \right) \left( \frac{D(\lambda)}{n} \right).
\] (2)

This explains why the spectral sensitivity is approximately fitted by the density spectrum for light incident on a retina in the 'normal' orientation. The spectral sensitivity for the inverted retina will be

\[
S(\lambda) = \left( \frac{\lambda}{\lambda_{\text{max}}} \right) (1 - 10^{-D(\lambda)/n}) (10^{-D(\lambda)/(n-1)}).
\] (3)

The term \( 10^{-D(\lambda)/(n-1)} \) is due to the screening of the innermost layer by the \( n-1 \) outer layers. The dashed curve of figure 6d was calculated from (3) with a density of 1.1. and the number of layers \( n = 5 \).
Anatomy

Our main interest in the anatomy of the conger retina was the structure of the photoreceptor layer. As shown in figure 7, plate 1, the conger photoreceptor layer is quite thick. It is composed of several tiers of photoreceptors, just as our model predicted. The innermost tier and the next tier out are quite regularly arranged in parallel rows, but the photoreceptors in the outermost layers are not lined up so neatly. As in other tiered retinae, the photoreceptors near the outermost part of the photoreceptor layer are somewhat larger in diameter than those in the inner tiers. On morphological grounds, approximately 99.99% of all the photoreceptors in the conger retina are rods; an occasional cone is seen in this sea of rods, as indicated in figure 7.

Discussion

This work on the retina of conger raises one outstanding question: what are most of the rods doing if they are not contributing to the visual sensitivity of the animal? One speculation suggested by Professor E. J. Denton is that they may confer wavelength discrimination. If the outer rods are functionally connected to retinal interneurons, they might make the interneurons sensitive to wavelengths longer and shorter than 492 nm. It is conceivable that these interneurons could be the basis of a colour-opponent system, like those that occur in many vertebrate retinae (DeValois & DeValois 1975; Gordon et al. 1978). However, it still must be true that this system would have to be much less sensitive than the retinal neurons driven by the inner layer of rods; in our experiments both the e.r.g. and the optic nerve reflected only the activity of the innermost layer of rods.

There is another possibility, namely that the outer rods are not important for vision but are reservoirs of visual pigment. It seems probable that there is not the same close functional connection between the pigment epithelium and the photoreceptors in conger eels as there is in terrestrial vertebrates. In the latter animals, pigment regeneration, and also rod disk shedding, are dependent on an active pigment epithelium in close contact with the photoreceptors (Goldstein 1967; Young & Bok 1969). In the conger eel retina, visual pigment can regenerate without any pigment epithelium. Also, the tiered arrangement of photoreceptors in conger would seem to preclude the conventional terrestrial-vertebrate process of photoreceptor disk shedding, because the outer segments of the rods in the inner layers are over 100 μm away from the pigment epithelium. Therefore, one may speculate that the retina of these animals has evolved so as to be more or less independent of the pigment epithelium. The photoreceptors in the outer tiers may serve as a reservoir of retinal, the visual chromophore, in the event of exposure of the retina to a bleaching light.

The conger eel retina may be a useful model system in which to study the visual physiology of deep-sea fish. Like the retinae of deep-sea fish, the conger retina has the following specializations: chrysopsin as a visual pigment, an ex-
Figure 7. Light micrograph of the photoreceptor layer of the conger retina. The calibration indicates 5 μm. Cones are labelled c. All other receptors are rods (r.).
ceptually high pigment density, and tiers of photoreceptors. These specializations have traditionally been interpreted as conferring enhanced sensitivity to light on the organisms that develop them (Denton & Walker 1958; Munk 1966; Locket 1977). The need for this enhanced sensitivity in deep-sea fish is obvious. Light from the sun is greatly attenuated in the depths of the sea and is concentrated in wavelengths around 480 nm. Nevertheless, our work on the conger eel suggests that while the wavelength maximum of the visual pigment conforms to the traditional explanation, the high pigment density and tiers of receptors do not confer greater sensitivity. In conger, 80% of the pigment (and the same percentage of the photoreceptors) does nothing to increase sensitivity. We speculate that in deep-sea fish, as in the conger eel, the tiers of photoreceptors may be present as a consequence of the diminished importance of receptor-pigment epithelium interaction. Furthermore, another implication of our work is that a fish with a tiered retina should have a spectral sensitivity identical to that of the density spectrum of the visual pigment, even if the pigment density is high.

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