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Electroretinographic evaluation of spectral sensitivity in yellow and silver eels (*Anguilla anguilla)*

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Abstract

Although differences in visual pigments between developmental stages of the European eel are well known, the expected differences in spectral sensitivity have not been demonstrated at the electrophysiological level. In fact, one past electroretinographic study led to the conclusion that in eels there is no change in scotopic sensitivity, with increasing sexual maturity. In the present experiments, electroretinograms (ERGs) were recorded from *in situ* eyecups of immobilized eels *Anguilla anguilla* (L.) caught in coastal running waters. It was shown that the ERG *b*-wave is as good an indicator of spectral sensitivity as the unmasked late receptor potential (LRP) which directly reflects the responsiveness of photoreceptors. Complete spectral-sensitivity curves, based on *b*-wave thresholds and on thresholds of LRP subsequently isolated by means of sodium iodate, have been obtained in the same eel. Using fitted amplitude-log intensity functions for threshold calculation, and two models for computer-assisted fitting of spectral-sensitivity curves, significant differences in λ_{max} were found between yellow and silver developmental stages of the eel, identified by ocular index measurements.

Keywords: Electroretinograms, European eel, Spectral sensitivity

Introduction

In their classical work on eel's visual pigments, Carlisle and Denton (1959) concluded that when an immature "yellow" eel assumes the "silver" livery of approaching maturity, and even before the fish leaves fresh water, a "metamorphosis" of its visual pigments occurs by chromophore exchange. It results in a shift of the wavelength of maximum absorption (λ_{max}) of the retinal pigment by about 33 nm towards the shorter wavelength end of the spectrum. Thereafter, a transition occurs from the rhodopsin of the yellow eel and early stages of the silver eel to a different rhodopsin. A new opsin, forming an A1-based visual pigment ($P482_1$), is inserted into retinal rods. It confers increased short-wave retinal sensitivity typical of deep-sea fish, and presumably represents a unique surface to deep-sea shift in visual pigment system occurring in one and the same species (Beatty, 1975; Partridge et al., 1989; Wood & Partridge, 1993). The gene sequence for the two opsins has been worked out, and putative amino acids involved in spectral tuning of the λ_{max} identified (Archer et al., 1995). The shift from long-wave to short-wave sensitive rhodopsin is generally interpreted as being adaptive, due to sensitivity matching between visual pigment absorptance spectra and the spectral distribution of light in different water types. Freshwater usually has a relative excess of long-wave photons, the deep sea has a preponderance of short-wave photons, and coastal waters are intermediate in spectral characteristics (Wood & Partridge, 1993).

During seaward migration, eels may possess a mixture of pigments in their retinas. According to Beatty (1975), the less mature silver eels probably possess three pigments, $P482_1$, $P501_1$, and $P523_2$ in variable amounts. In more mature silver eels, however, the visual system is based solely on rhodopsins, with $P482_1$ dominating. It is only when the fish has spent some time in the ocean that $P501_1$ finally disappears and $P482_1$ becomes the only pigment left (Beatty, 1975). This certainly highlights the difficulties that may be encountered when attempting to establish differences between eels in different stages of metamorphosis, but caught in fresh or brackish coastal waters, before they actually enter the ocean. The presence of different pigments in variable amounts may then seriously interfere with spectral-sensitivity differences measured electrophysiologically. Indeed, differences in spectral sensitivity, expected in the electroretinogram (ERG), have never been demonstrated. On the contrary, a comparative ERG study of eels in different phases of sexual maturity led to the conclusion that there is no change in scotopic sensitivities, with increasing sexual maturity (Pankhurst & Lythgoe, 1983). Unconvinced by this essentially negative finding, we decided to reexamine the possibility of demonstrating, by way of quantitative electroretinography, differences in spectral sensitivity between yellow and silver eels caught in running coastal waters, the developmental stage being identified by eye index measurements according to Pankhurst (1982).

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Previous ERG studies of eel's spectral sensitivity were based exclusively on *b*-wave measurements (Gordon et al., 1978; Pankhurst & Lythgoe, 1983). The electroretinographic *b*-wave reflects, however, the activity of photoreceptors only indirectly (Newman & Odette, 1984). Therefore, our reevaluation of the spectral sensitivity in eels included, in addition to *b*-wave recordings, measurements of the chemically isolated late receptor potential (LRP) as a direct reflection of receptor activity (Brown, 1968; Rodieck, 1973). The *in situ* eyecup preparation of the immobilized eel (see Methods) was robust enough to allow both *b*-wave and LRP measurements to be made sequentially using the same preparation.

To enable a computer-aided search for the best-fitting λ_{max}, two sets of analytical methods were applied to the presently obtained ERG data. The first set was based on the widely known polynomial of Dawis (1981), which has the advantage of satisfying the requirements of Dartnall's fundamental hypothesis as the basis of his nomogram (Dartnall, 1953). Both rhodopsin (vitamin A_1-based) and porphyropsin (vitamin A_2-based) templates were used, since the presence of both pigments, in an unknown ratio, was expected in the metamorphosing eels. The second set of fitting procedures was based on empirical equations for fitting bell-shaped curves, offered by a commercially available computer program (TableCurve 2D, Jandel Scientific AISN Software, Corte Madera, CA), and used for the first time, to our knowledge, in ERG spectral-sensitivity studies. The two sets of fitting procedures were applied to both the *b*-wave and the LRP-derived spectral-sensitivity data. It was hoped that such an approach would help in revealing significant differences in spectral sensitivity between yellow and silver stages of the eel, which might be obscured by the presence, in both, of pigment mixtures of an intermediate composition.

Some of the data were the subject of a previous preliminary communication (Damjanović et al., 1991).

Methods

Animals

European eels (*Anguilla anguilla)* were captured using an electric gear, during summer months, in coastal running waters along the Kotor Bay (Montenegro). They were kept subsequently, for at least 20 days prior to the experiments, in freshwater aquaria, located in a dark and temperature-controlled room, at 15°C.

Preparations

Eels were anesthetized (Phenobarbital sodium) and curarized (tubocurarine) by following procedures recommended by Hamasaki et al. (1967) and by adjusting the dosage to arrest respiratory movements. Artificial respiration was provided continuously by forcing aerated and temperature-controlled water through the gills. The immobilized eel was positioned laterally on a plastic platform inside a light-proof Faraday cage. The *in situ* eyecup preparations were surgically deprived of cornea, lens, and most of the vitreous, and filled with teleost Ringer. At the conclusion of experiments, eels were killed by decapitation.

In experiments with sodium iodate ($NaIO_3$), the eyecup was filled with physiological solutions in which a given amount of $NaIO_3$ was substituted for an equivalent amount of sodium chloride (NaCl). A built-in dose-dispensing and sucking device (polyethylene tubing) allowed for the replacement of solutions and intermittent washing without causing mechanical disturbances or changes in illumination. After 20 min, the iodate solution was washed out and replaced with the physiological solution.

Electroretinography

ERG potentials were detected with nonpolarizable chlorided silver ($Ag\text{-}AgCl_2$) electrodes, the active one placed in the interior of the saline filled eyecup. The reference electrode was introduced into the retro-orbital space behind the *in situ* eyecup. It was connected to the input stage of a directly coupled differential preamplifier, and responses were recorded from a storage oscilloscope display using a Polaroid camera.

Photic stimuli were delivered by a single-beam optical system using an 8-V, 50-W tungsten-halogen lamp as the light source, and providing independent control of intensity (neutral density filters), duration (electromagnetic shutter), and spectral composition (interference filters) of the test flashes. A heat filter virtually eliminated wavelengths >700 nm. The stimuli consisted of single flashes guided through a fiber optic positioned normal to the surface of the eyecup that cast a circular patch of light that covered the external surface of the preparation. Unless otherwise specified, the duration of the light stimulus was 200 ms. Intervals between test flashes were kept sufficiently long so as not to influence subsequent responses.

Light intensities were calibrated and checked by placing the active surface of the radiometer probe in the position usually occupied by the eyecup preparation. The attenuating effects of the interference filters were accounted for when comparing responses to flashes of different wavelengths. Unattenuated, the energy flux delivered by the test field was of the order of 2×10^{-2} mW/cm^2. When comparing intensity/amplitude relations in different preparations, relative intensity (I_R) scales were used, plotting ERG amplitudes (voltage) against attenuation in log units (Fig. 2).

Fitting procedures

In fitting our ERG-based spectral-sensitivity data, two sets of procedures were used in parallel. The first consisted of applying the Dawis (1981) polynomial expression of pigment nomograms

$$A(\lambda) = b_{max} + \sum_{k=1}^{8} b_k \cdot [(L_{max}/\lambda) - (L_{max}/\lambda_{max})^k], \qquad (1)$$

where $A(\lambda)$ is the logarithm of the absorption coefficient at wavelength λ. Parameters b_k ($k = 1, 2, \ldots, 8$) were used as tabulated by Dawis (1981) for three vitamin A_1-based visual pigment nomograms (characterized by L_{max} values of 432, 502, and 562 nm) and for three vitamin A_2-based pigment nomograms (L_{max} values of 438, 523 and 620 nm). Two modalities of polynomial fitting, referred to as Dawis Model 502_1 and Dawis Model 523_2, were presently used. They were based, respectively, on b_k parameter sets for the vitamin A_1-based pigment 502_1 and the vitamin A_2-based pigment 523_2 nomograms. For each pair of λ_{max} and b_{max}, eqn. (1) gives an absorption spectrum with a peak absorption coefficient of b_{max} occurring at $\lambda = \lambda_{max}$. When supplied with the tabulated parameters, the polynomial accurately represents the nomograms. It should be stressed that the polynomial is in terms of frequency ($1/\lambda$) rather than wavelength, and that the effect of changing λ_{max} is to produce a lateral shift of the absorption spectrum along the frequency scale while retaining shape. This is required by the hypothesis put forth by Dartnall (1953) to relate the shapes of

different photopigment absorption curves, which was the basis for his nomogram. Equation (1) thus represents a mathematical expression (although not a proof) of Dartnall's basic hypothesis. We choose accordingly to construct our ERG-based spectra (Figs. 3–5) by plotting relative sensitivity against the reciprocal of stimulus wavelength, $1/\lambda$, although labeling the abscissae in wavelengths. Limitations in the use of eqn. (1), specified by the author (Dawis, 1981), were duly observed. They concern the applicable λ_{max} range in the case of each nomogram, and the maximum range over which data is permissible. The latter range, between λ_1 and λ_2, was arrived at, as recommended, by using the formula

$$\lambda_i = L_i \cdot L_{max} \cdot \lambda_{max} / (L_{max} \cdot \lambda_{max} - L_i \cdot \lambda_{max} + L_i \cdot L_{max}),$$

$$i = 1,2 \quad (2)$$

along with the tabulated L_1 and L_2 values corresponding to the chosen nomogram. Data at wavelengths shorter than λ_1 or longer than λ_2 were discarded.

The second set of fitting procedures consisted of fitting experimental spectral-sensitivity data by means of the computer program TableCurve 2D (Jandel Scientific AISN Software), designated in the text as fitting procedure "TCWIN2". The program is capable of ranking a large number of empirical equations according to the magnitude of the fitting error they produce when applied to a given set of experimental data. In the case of each of our sets, two equations provided by the program were applied: (1) the equation producing the smallest fitting error (designated as "Rank 1 Eqn"), and (2) the 5-parameter equation identified in the program by the number 8063 ("Eqn 8063" in the text). The choice of the latter equation was based on our finding that it represented the first ranking equation when applied to the fitting of Dartnall's nomogram data (Dartnall, 1953).

Spectral curves obtained by all fitting procedures were appropriately adjusted for plotting on a quantum sensitivity scale, using a frequency rather than wavelength abscissa, so as to enable the same type of comparison between equal quantum intensity action spectra and nomogram-based spectra, originally recommended by Dartnall (1953). In the case of fitting by means of the Dawis procedure, the relative root-mean-squared error (ρ) between data points and eqn.(1) was used for estimating accuracy of the fits in the form:

$$\rho(x_{theor.}, x_{exp.}; \sigma) = \left[\sum \left(\frac{x_{exp.}}{x_{theor.}} - 1 \right)^2 \Big/ N \right]^{1/2}. \quad (3)$$

Statistical differences between λ_{max} values obtained by fitting spectral-sensitivity data in individual fish were evaluated by means of the nonparametric two-tailed Mann-Whitney test.

The ocular index

The developmental stage (yellow or silver) of eels used in the present experiments was specified on the basis of the eye index, *I*, according to Pankhurst (1982):

$$I = \left[\frac{\left(\frac{A+B}{4} \right)^2 \cdot \pi}{L} \right] \cdot 100, \quad (4)$$

where *A* and *B* are, respectively, the horizontal and the vertical diameter of the eye, while *L* stands for total body length. All of our eels classified as being in the yellow stage (sexually immature adults) were characterized by $L < 50$ cm and $2 < I < 5.5$, while our silver-stage eels (sexually maturing adults) had $L > 50$ cm and *I* between 6.5 and 13. No attempts were made at differentiating between males and females (there are no gender differences in ocular index), and between migratory and nonmigratory forms of the silver eels.

Results

Waveforms

In comparison to the "intact eye" ERG records on which Pankhurst and Lythgoe (1983) based their study of eel's spectral sensitivity, our "*in situ* eyecup" ERGs were qualitatively similar, but showed substantially greater amplitudes of their *a*-, *b*-, and *c*-waves (Fig. 1). In the absence of chemical pretreatment, electroretinograms obtained in the dark-adapted eel (body temperature 15°C, duration of light flashes 200 ms) were characterized, at saturating light intensities, by a prominent positive *b*-wave. It reached amplitudes of the order of 400–500 μV. It was preceded by a conspicuous, although much smaller (<100 μV), negative *a*-wave. Finally, it was followed by a longer lasting "late negativity" phase leading, beyond the termination of the light stimulus, to a salient, slow and positive *c*-wave which never reached amplitudes as high as those of the *b*-wave. A negative *off*-response was recorded at the termination of the light stimulus, but only when the duration of the latter was extended beyond the usual 200 ms (1.0 and 1.35 s in case of records shown in Fig. 1).

After introducing $NaIO_3$-containing solutions in the *in situ* eyecup, the ERG underwent gross changes (Fig. 1, lower sequence of records) resulting in a complete disappearance of the *b*-wave and the transformation of the ERG into a single negative deflection, the isolated late receptor potential (LRP). The duration and extent of this transformation depended, however, on the concentration of iodate. When a 25 mM solution was used, the gradual disappearance of the *b*-wave took some 25 min to accomplish. The unmasked negative wave, when stabilized, still ended in a slow positive deflection, reminiscent of the *c*-wave present in the records of the aspartate-isolated LRP of the skate (Dowling & Ripps, 1972). After higher concentrations of iodate (50 mM in Fig. 1), the unmasking effects were speedier and more complete. The rapid initial negative deflection of the resulting ERG was followed, often after a conspicuous plateau phase, by a slow recovery of the initial potential level, without any sign of overshooting. At saturating intensities of the light stimulus, the amplitude of the iodate-isolated LRP was of the order of 100 μV, considerably smaller therefore than the amplitude of the *b*-wave recorded in the absence of iodate. Nevertheless, the eel's LRP proved equally adequate for our spectral sensitivity measurements.

Intensity–amplitude relations

The assessment of spectral sensitivity is based on threshold values derived from measurements of wave amplitude as a function of flash intensity. In the case of both the *b*-wave and the iodate-unmasked LRP, the regularity of the intensity–amplitude relation has been checked by fitting experimental data with the basic model

$$V_0 = I^a / (I_0^a + I^a), \quad (5)$$

(Naka & Rushton, 1966; Dowling & Ripps, 1972), where V_0 is the normalized voltage (V/V_{max}) of the ERG signal (*b*-wave or LRP),

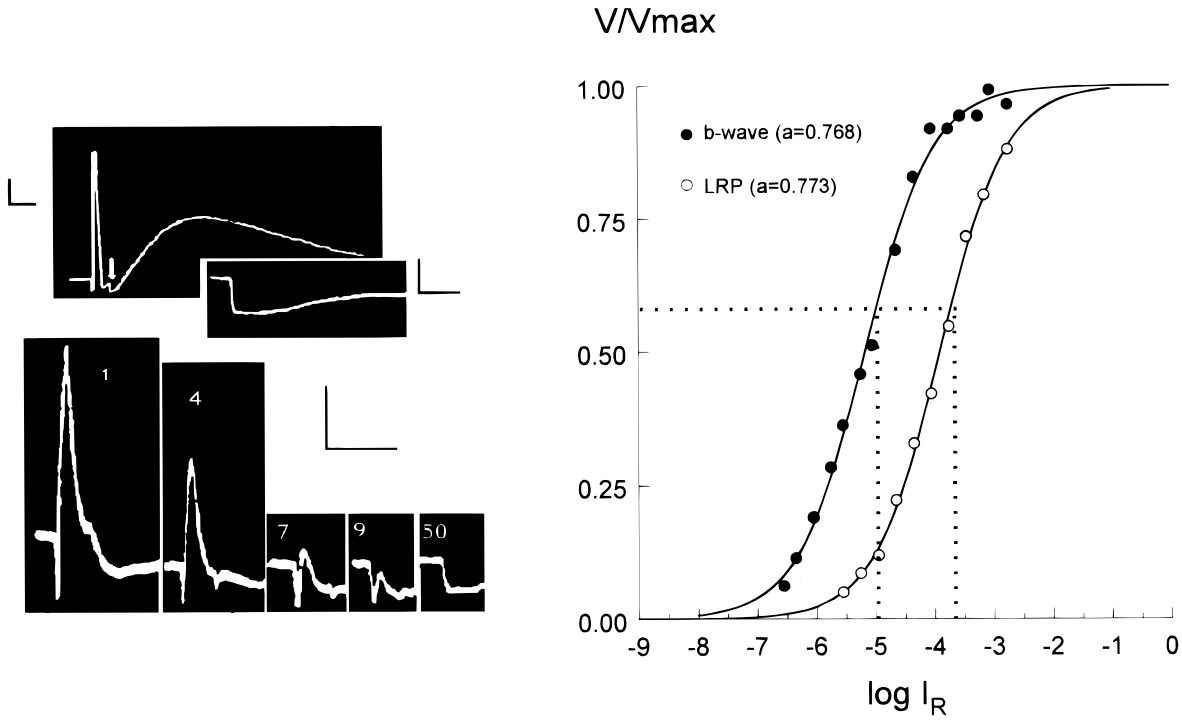

Fig. 1. Left: Examples of electroretinographic waveforms (saturating test flash intensities, white light; all calibrations: 0.1 mV, 2 s). First upper record: normal ERG (1.35-s test flash; *off*-response indicated by arrow). Next record: iodate-unmasked LRP (0.2-s test flash). Sequence of records below: five responses during LRP unmasking by 50 mM $NaIO_3$ (1.0-s test flashes; numbers indicate minutes after intraocular administration of iodate). Right: Amplitude–intensity relations in a silver eel (open circles—LRP; closed circles—*b*-wave). LRP unmasked by sodium iodate. Stimulus wavelength: 500 nm. I_R: relative flash intensity.

I_0 is the stimulating light intensity corresponding to $V_0 = 1/2$, and the exponent a is a constant. As shown by the silver eel example in Fig. 1 (right), both the amplitude of the *b*-wave and of the subsequently isolated LRP responded to incremental photostimulation in the same way, in conformance with the basic model. The slopes (parameter a values) of the normalized log-profiles were practically the same, although differing in horizontal placement. The LRP curve was shifted by 1.3 log units towards higher light intensities. In no case were there indications of the division of the amplitude–intensity curves into two (rod and cone) S-shaped branches, of the type described in the isolated retina of the frog (Zaret, 1973).

Fitted log-sigmoids were used for calculating threshold values on which to base spectral-sensitivity determinations. Only those sigmoids were used which satisfied the following two criteria: (1) fitting error $\leq 0.01\ V_{max}$ and (2) at least three data points, 0.3 log units apart, included within the initial and terminal 10% segments of the fitted sigmoid (i.e. within regions of $V_0 \leq 0.1$ and $V_0 \geq 0.9$). On visual inspection, such sigmoids ran almost in parallel, but differences in parameter a values, although relatively small, did exist. In one yellow eel, for example, parameter a values ranged from 0.681 to 0.769, with a mean of 0.719 ± 0.014 for the six sigmoids; in a silver eel, parameter a ranged from 0.736 to 0.846, with an average of 0.791 ± 0.015. In no case were variations of parameter a correlated with wavelength, nor were the differences between yellow and silver eels statistically significant. An average value of 0.747 ± 0.0093 for parameter a was provided by a total of 50 *b*-wave $V_0/\log I$ sigmoids obtained at different wavelengths in eels, irrespective of their developmental stage.

The choice of the threshold criterion for sensitivity determinations is irrelevant only when $V_0/\log I$ profiles are strictly parallel. In our case, variations in parameter a were small, and therefore signal amplitude equal to 10% of the largest response, obtained with light stimuli of the most effective wavelength, was adopted as the threshold criterion.

Action spectra

The averaged action spectrum from four silver eels, based on their *b*-wave thresholds, did not differ significantly from the one obtained subsequently, in the same individuals, by measuring response thresholds for their iodate-isolated LRP. Values of λ_{max} obtained by TCWIN2 fitting amounted to 497.3 (Eqn 8063) and 501.2 nm (Rank 1 Eqn) in the case of *b*-wave, and to 501.2 (Eqn 8063) and 501.7nm (Rank 1 Eqn) in the case of LRP-based action spectra. When fitted by Dawis' Model 502_1, the same data provided λ_{max} values of 499.5 and 499.6 nm. Subjected to the same fitting procedure, the simultaneously fitted eight sets of silver eel data (*b*-wave as well as LRP) provided a common λ_{max} value of 501.4 nm (Fig. 2). The results thus showed that, in the eel, λ_{max} determinations based on *b*-wave and LRP thresholds were equally reliable. Differences between yellow and silver eels were subsequently sought using *b*-wave thresholds exclusively in constructing spectral sensitivity curves, thus following the procedure adopted by the previous workers (Gordon et al., 1978; Pankhurst & Lythgoe, 1983). The *b*-wave amplitude equal to 10% of the largest among responses obtained with the entire series of stimulus wavelengths, and chosen as the threshold criterion, was of the order of 60–70 μV.

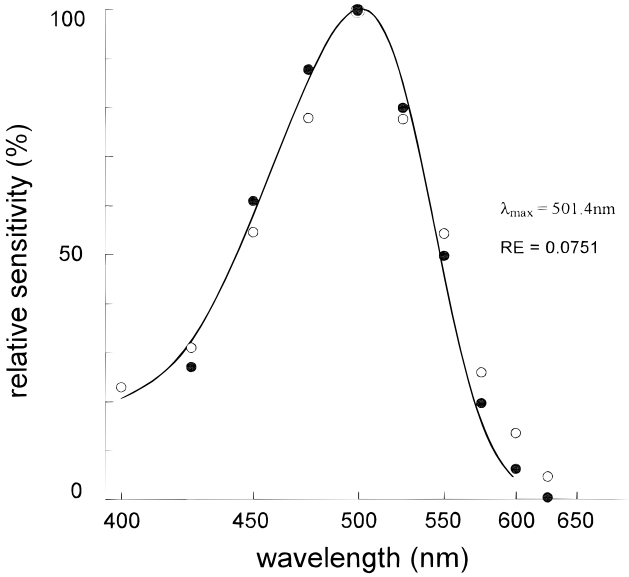

Fig. 2. Simultaneous fitting of *b*-wave (closed circles) and LRP (open circles) spectral-sensitivity data recorded in the same group of four silver eels. Data points are means from four results. Data fitted according to Dawis Model 502_1 (RE: fitting error).

Individual action spectra were constructed using *b*-wave data obtained from nine yellow and eight silver eels. In each case, fitting was achieved using the TCWIN2 program, as well as two modalities of polynomial fitting according to Dawis. Table 1 lists mean λ_{max} values resulting from the different fitting methods. Fig. 3 depicts an example of the individual spectra obtained in one and the same eel by fitting Model 502_1 and Eqn 8063. As shown in Table 1, the nine sets of data obtained in yellow eels provided individual λ_{max} values ranging from 506.9 to 518.6 nm and from 503.4 to 519.1 nm in case of fitting Model 502_1 and Model 523_2, respectively. In neither case were there overlapping with ranges occupied by the eight silver eel values (494.7–506.0 nm and 492.3–502.7 nm). In the case of both modes of fitting, the difference between group means was highly significant ($P = 0.0006$). The same was true of the difference between yellow eel spectra obtained using Model 523_2, and silver eel spectra resulting from Model 502_1, although in that case some overlapping did occur and the significance of the difference was slightly lower ($P = 0.0018$). In the case of Rank 1 Eqn and Eqn 8063 fits, values of two silver eels entered the yellow eel range, but the differences between the two developmental stages nevertheless were significant.

Irrespective, therefore, of the fitting method, the difference between yellow and silver eels was statistically significant. It pointed to a shift from relatively high λ_{max} values for the yellow eels, to relatively low values in eels in the silver stage.

Fig. 4 shows an example of averaged spectra obtained in silver and yellow eels. They were constructed by simultaneously fitting all *b*-wave spectral-sensitivity data obtained in eels of the same developmental stage, as defined by their eye indices. Dawis polynomial fits were applied using b_k parameters for the 502_1 pigment nomogram, and the nine yellow and eight silver eels provided λ_{max} values of, respectively, 513.6 and 499.4 nm. Clearly, however, data points suggested a somewhat wider spectrum than the one provided by applying the rhodopsin template. Other fitting methods provided slightly different λ_{max} values (Table 1), but the difference between yellow and silver eels remained of the same order. It was greatest (14.2 nm) in the case of simultaneous fitting with Dawis Model 502_1.

Discussion

Waveform

The *in situ* eyecup preparation of the immobilized eel proved to be as robust as the intact eye of similarly immobilized elasmobranchs studied by Hamasaki et al. (1967), enabling the recording of good ERGs over 24 h. We were able to obtain for the first time, in one and the same animal, complete spectral-sensitivity data based on

Table 1. *λ_{max} values (nm) obtained in nine yellow and eight silver eels by fitting b-wave spectral-sensitivity data separately in each individual (means ± SE, range in brackets) or by fitting simultaneously all data points obtained in eels of the same developmental stage*[a]

Fitting method	Yellow eels ($n = 9$)	Silver eels ($n = 8$)	Difference and P
Individual fitting			
Dawis Model 502_1	514.0 ± 1.7 (506.9–518.6)	500.6 ± 1.3 (494.7–506.0)	13.4 ($P = 0.0006$)
Dawis Model 523_2	511.7 ± 1.9 (503.4–519.1)	497.3 ± 1.3 (492.3–502.7)	14.4 ($P = 0.0006$) 11.1 ($P = 0.0018$)[b]
Rank 1 Eqn	508.5 ± 1.8 (502.8–516.9)	497.9 ± 1.6 (491.7–504.3)	10.5 ($P = 0.0045$)
Eqn 8063	510.4 ± 2.4 (502.7–523.7)	498.9 ± 1.5 (492.5–504.5)	11.5 ($P = 0.0045$)
Simultaneous fitting			
Dawis Model 502_1	513.6	499.4	14.2
Dawis Model 523_2	511.7	498.2	13.5 12.3[b]
Rank 1 Eqn	509.1	498.6	10.5
Eqn 8063	508.9	498.6	10.3

[a]Results obtained by means of various fitting methods (spectral curve models specified in the Table). Significance of differences according to the Mann-Whitney two-tailed test.
[b]Difference between yellow and silver eel λ_{max} values obtained by fitting with Dawis Models 523_2 and 502_1, respectively.

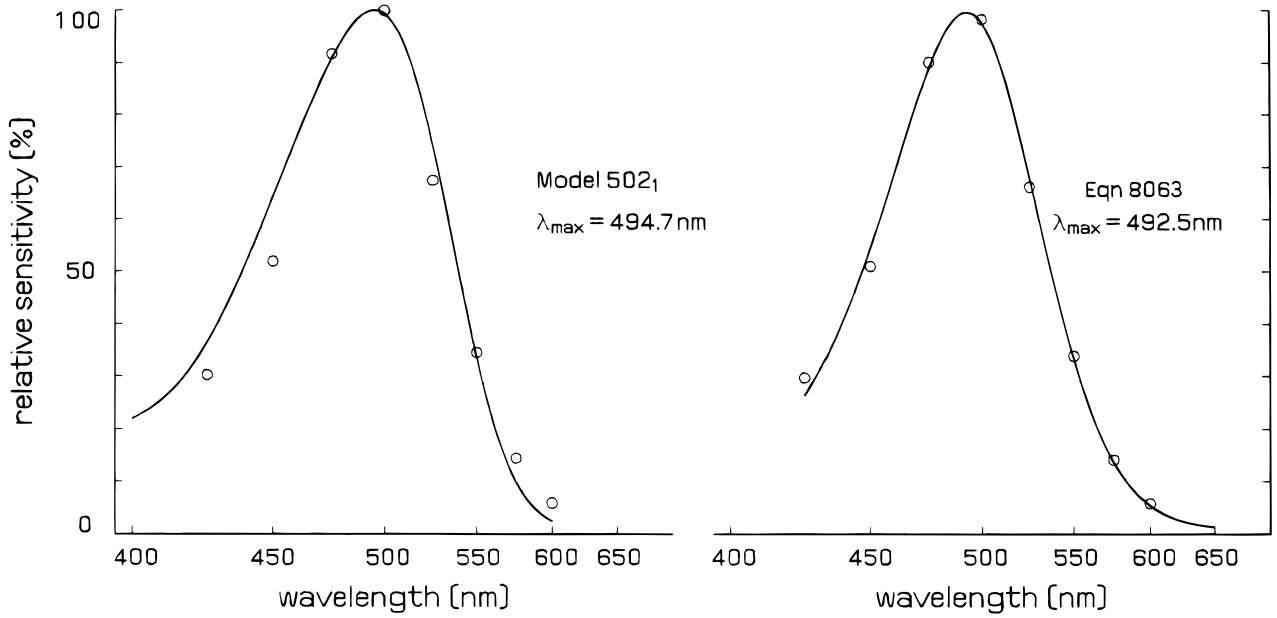

Fig. 3. Spectral sensitivity of one silver eel, based on *b*-wave data subjected to two fitting methods. Left: Data fitted according to Dawis Model 502_1. Right: Data fitted according to Eqn 8063.

the *b*-wave amplitude and on the amplitude of the subsequently unmasked LRP. To achieve this, the whole procedure of exploring ten wavelengths, each tested with a series of at least 15 properly spaced flashes of increasing intensity, had to be repeated twice, before and after the addition of iodate to the *in situ* eyecup.

In the study of Gordon et al. (1978) of photoreceptor mechanisms in the eel retina, the first of the kind according to the authors, the waveform of ERG obtained from isolated eyecup preparations was described as being similar to waveforms produced by rod-driven response mechanisms in other retinas. They were characterized by such features as the large, sustained *b*-wave, the absence of a marked positive *off*-response, the long latency-to-peak of the *b*-wave, and the prolongation of the ERG beyond stimulus offset at high intensities. All these features were equally characteristic of our ERG records from the *in situ* eyecup of both the silver and yellow eel.

Scotopic ERGs from the *in situ* eyecup of the eel also were similar to those recorded, with an intravitreally placed reference electrode, from the intact eye of the same fish by Pankhurst and Lythgoe (1983). Quantitatively, however, our records differed by significantly higher amplitudes of the *a*-, *b*-, and *c*-waves. After adaptation to background illumination the small scotopic *off*-

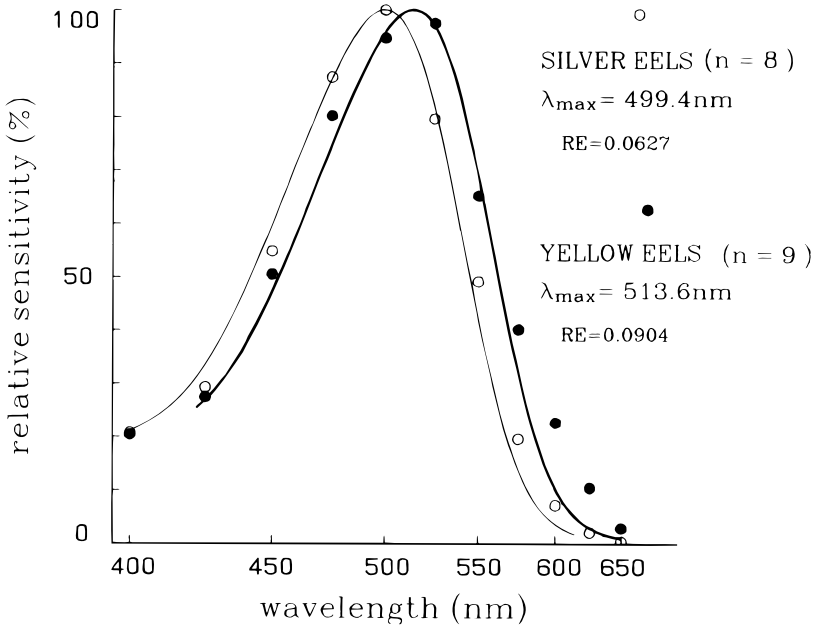

Fig. 4. Averaged action spectra of eight silver (open circles, left curve) and nine yellow eels (closed circles, right curve), obtained by fitting simultaneously all *b*-wave spectral-sensitivity data recorded in eels of the same group. Data points are means from eight and nine results obtained in silver and yellow eels, respectively. Fitting according to Dawis Model 502_1 (RE: fitting error). For data obtained by other fitting methods see Table 1.

response of the eel was replaced by a conspicuous positive *d*-wave, its amplitude markedly increasing with flash duration (not shown).

Amplitude-intensity relations

In eel preparations, the slope of the amplitude–intensity log-sigmoids, which determines the dynamic range of responses, was characterized by parameter *a* values between 0.7 and 0.8, of the same order as those reported for other animals and other electrophysiological signals (Naka & Rushton, 1966; Baylor & Fuortes, 1970; Dowling & Ripps, 1971). They were, however, definitely lower than the value of 1 found by Dowling and Ripps (1972) in aspartate-treated eyecup pieces of the skate, and testified to a substantially broader dynamic range of responses in our preparations. It invariably covered some 3–4 log units of intensity, being thus similar to the range found in intracellularly explored rods of the toad (Lipton et al., 1977) or horizontal cells of the skate (Dowling & Ripps, 1971). The continuity of the fitted log-sigmoid curves testified to the absence of a rod–cone transition of the type described in the frog (Zaret, 1973).

Spectral sensitivity

Paired visual pigment systems consisting of rhodopsin and porphyropsin are not uncommon in teleosts, and shifts in spectral sensitivity associated with A1/A2 substitution have been demonstrated in some of them (Whitmore & Bowmaker, 1989). The European eel has, however, the additional feature of a third pigment, another rhodopsin based on a different opsin, which appears during the transition from the yellow to the silver stage. A sequential replacement of one opsin by the other within one and the same rod outer segment has been envisaged. The discs of the apical ends of the outer segments are thought to undergo continual removal with renewal taking place by the balanced growth of discs from the inner segments (Bridges, 1972; Beatty, 1975). This hypothesis is supported by the finding that the deep-sea opsin is produced *de novo* by cells previously expressing only the freshwater opsin. It has been demonstrated, by means of single-cell microspectrophotometry, that shifts in the wavelength of rod peak sensitivity, induced by hormonal injection, are accompanied by a rapid synthesis of new opsin in existing rod outer segments (Wood & Partridge, 1993). Different opsins would thus be present in different parts of the outer segment, and the switch in opsin incorporation might never be complete (Wood & Partridge, 1993).

A replacement of $P501_1$-containing photoreceptor cells by entirely new cells containing $P482_1$ was considered as another possible mechanism responsible for the presence of different rhodopsin mixtures in the retina of the metamorphosing eel. As there is an increase in the number of receptors with maturation, it is not improbable that replacement of the opsin occurs *via* the recruitment of a new population of photoreceptors (Pankhurst, 1982). Obviously, the difference at the electrophysiological level between individuals of a different stage of maturity will depend on the extent to which the replacement of pigment sets has been accomplished.

The difference in λ_{max} values between our ERG-based spectra of yellow and silver eels was considerably smaller than the 33 nm originally reported by Carlisle and Denton (1959) as the difference between λ_{max} values of the respective visual pigments. Smaller differences could, however, be expected on the basis of pigment data by other authors. Differences spreading from a minimum of 2 nm to a maximum of 23 nm were reported by Wald (1960), and also ranged from 15 to 25 nm in the experiments of Beatty (1975). The substantially greater difference reported earlier by Carlisle and Denton (1959) was presumably due to a more advanced migratory stage of their eels, while those of Beatty (1975) were nonmigratory individuals as presumably were the silver eels from coastal waters in our experiments.

After hormonally induced metamorphosis λ_{max} values of rod visual pigments shifted from 495–516 nm towards shorter wavelengths (Wood & Partridge, 1993). It took, however, 50 days for λ_{max} to reach values in the vicinity of 482 nm and finally to stabilize at the new level. In the meantime, the whole range of λ_{max} values between 503 and 482 nm could have been recorded. This certainly highlights the difficulties that may be encountered when attempting to establish differences between eels in different stages of metamorphosis, in the presence of a mixture of pigments.

The difference in average λ_{max} values, obtained by electrophysiological means (ERG) between the two age groups of our eels, was statistically significant irrespective of the fitting procedure applied. It varied, depending on the procedure, between 10.5 and 14.4 nm (Table 1). Similarly, the two action spectra obtained by simultaneously fitting all sets of data recorded in each of the two age groups differed by 14.2 nm (Fig. 4). On the other hand, considering all the fitting procedures applied, the minimal difference in λ_{max} between individual values of our nine yellow and eight silver eels ranged from 0.7 to 1.8 nm, whereas the maximal difference spread from 23.9 to 31.2 nm. This compares favorably with the already mentioned results of Wald (1960) obtained by spectral analysis of pigment extracts.

Thus, the differences between our ERG-based spectra of yellow and silver eels was significant and of the same order as some of the differences obtained previously by spectral analysis of pigment extracts (Wald, 1960; Beatty, 1975). This strongly opposes the conclusion of Pankhurst and Lythgoe (1983) that in eels, according to ERG criteria, there is no change in scotopic sensitivity, with increasing sexual maturity. The latter conclusion was drawn, however, from an ERG study in which thresholds were explored at two stimulus wavelengths only, 480 and 520 nm, without actually attempting to identify the relevant λ_{max} values. On the other hand, at these two wavelengths our spectral curves for yellow and silver eels (Fig. 4) differed from each other by not more than 12.7 and 12.4%, respectively. Besides, the cross-point of the two overlapping curves in Fig. 4 occurred at a high level on the sensitivity scale (98.75%). Our yellow and silver eels from Fig. 4 therefore had the same spectral sensitivity at a wavelength differing from λ_{max} by not more than 6.6 and 7.6 nm, respectively. The same figure also shows that pure pigment templates do not strictly match the actual width of the action spectrum, which is to be expected when dealing with a mixture of pigments. These facts illustrate again the difficulties encountered when, in the presence of a mixture of pigments, one attempts to reveal, on the ground of whole retina responses, changes in spectral sensitivity which accompany the maturation process in eels. Responses to a sufficiently large range of properly spaced stimulus wavelengths must be employed and the results robustly examined.

As to the absolute λ_{max} values, our ERG-based figures agreed basically with those concerning eel's pigment mixtures, irrespective of the fitting procedure applied. If the results obtained by both fitting procedures are taken into account, λ_{max} values varied between 502.7 and 523.7 nm in yellow eels, and between 491.7 and 506.0 nm in eels in the silver stage. The old finding of Granit (1941) that the electrophysiologically estimated λ_{max} of the eel (of an unspecified developmental stage) amounts to about 500 nm is, therefore, in agreement with our ERG data on silver eels. On the

other hand, our results on yellow European eels are not rigorously at variance with those obtained in a presumably yellow American eel, *Anguilla rostrata* (Lesueur), by Gordon et al. (1978). This is not surprising, since according to Beatty (1975) the American eel should possess visual pigments spectrophotometrically indistinguishable from those of the European eel, at least in the yellow stage.

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