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Spectral sensitivity of the dogfish shark (*Scyliorhinus canicula*)

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Abstract The action spectrum of the electroretinographic (ERG) b-wave and the unmasked late receptor potential (LRP) were measured under a variety of conditions (isolated eyecup, detached retina, b-wave recording in fresh preparations, LRP measurements after low-temperature aging, dark and light adaptation). It was shown that in the dogfish, *Scyliorhinus canicula* (L.), eyecup spectral sensitivity matched closely the pigment 502 spectral curve like other rhodopsin-possessing marine species. The ERG b-wave is as good an indicator of spectral sensitivity as the unmasked LRP that directly reflects the responsiveness of photoreceptors. Differences in spectral sensitivity were not revealed between dogfish preparations studied under a variety of conditions (isolated eyecup vs. detached retina; b-wave recording in fresh preparations vs. LRP measurements after low-temperature aging; dark vs. light adaptation). We developed a new model for spectral sensitivity data.

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Introduction

The small-spotted dogfish shark is a bottom-dweller up to 500 m in the Mediterranean and north-western Atlantic (Wheeler 1969) and one of the most abundant elasmobranch fishes in the South Adriatic. It has a relatively large but primitive brain, its greatest forebrain region being involved in olfaction. It also has relatively large eyes, with the pupillary slit resembling that of the cat (hence it is called ''sea cat'' in Serbian). Dogfish sharks are easily kept in captivity, in cold-water recirculation systems of the Institute for Marine Biology in Kotor.

Our electroretinographic studies of this fish were preceded by studies of its brain energetics (Andjus et al. 1998). The dogfish shark is extremely tolerant to anoxia and this remarkable tolerance is linked to specific features of its brain energetics. It was of no surprise to us, therefore, to find subsequently that the isolated dogfish retina, which can be regarded as an outgrowth of the brain, also shows remarkable tolerance and represents a particularly robust preparation, enabling long-lasting electroretinographic explorations in the absence of perfusion. The dogfish (*S. canicula*) is a rhodopsin-possessing marine species with a pure-rod retina with a single layer of photoreceptors.

To enable a computer-aided search for the best-fitting peak value of the spectral sensitivity curve (λ_{max}), two procedures have been applied to the presently obtained electroretinographic (ERG) data. The first was based on the widely used formula of Lamb (1995) with parameters proposed by Govardovskii et al. (2000) for the α- and β-band of A1 pigments (rhodopsin-based visual pigments), which uses Mansfield normalization, absorbance spectra plotted on normalized frequency (Mansfield 1985), to provide Dartnall's fundamental hypothesis that the absorbance spectra had a very similar shape when plotted on a frequency scale (c/λ) at the basis of his nomogram (Dartnall 1953). Bearing in mind that Lamb's formula has the advantage of providing a good fit for absorbencies between 1 and 0.5 maximum and gradually falling to 0 at short wavelengths, it is easy to decompose the entire spectrum to α- and β-bands (Stavenga et al. 1993) by subtraction of the α-band from the complete experimental spectrum. The second fitting procedure employed empirical equations with 3 parameters for fitting the α- and β-bands of A1 pigment development in our laboratory. This approach was applied to both the b-wave and the late receptor potential (LRP)-derived spectral sensitivity data.

Materials and methods

Animals

Small-spotted dogfish sharks (*S. canicula*; 150–250 g body mass) were caught by trawler nets in the South Adriatic, at a depth of about 100 m. They were maintained at 15°C for at least 1 month prior to the experiments in a sea-water recirculation system for experimental aquaculture, located in a dark and temperature controlled room. At no time were the dogfishes exposed to light for long, as this is known to be damaging to the elasmobranch photoreceptors (Hamasaki et al. 1967).

Preparations

Isolated eyecups were prepared under dim red light from dogfish eyeballs (about 10 mm in diameter) excised after rapid decapitation of the fish. The preparations were surgically deprived of cornea, lens, and most of the vitreous. The eyecup was filled with elasmobranch Ringer (Rybak 1973) and placed on a cotton-wool bed soaked with the same solution, in a plastic temperature-controlled chamber inside a light-proof Faraday cage. After mounting, the preparations were dark-adapted for an additional 30 min before actual ERG recording. In the case of photopic b-wave recording, the eyecup was continuously exposed to a 500 nm background illumination capable, at its onset, of evoking a b-wave response of saturating amplitude and reduce sensitivity more than 3,000-fold. The temperature within the eyecup was measured with thermistors. During low-temperature aging (Andjus et al. 1983), the isolated dogfish eyecup was maintained in the dark, at a constant temperature of 6°C.

The isolated retina preparation consisted of a circular piece of the retinal layer, cautiously detached from an eyecup slice pinned down to a paraffin bed. The detached retina was laid, receptor side up, on a piece of filter paper soaked with the elasmobranch Ringer.

Electroretinography

The ERG potentials were detected with nonpolarizable chlorided silver (Ag-AgCl) electrodes, the active one being introduced into the interior of the saline-filled eyecup. The reference electrode was in contact with the cotton-wool bed underneath the isolated preparations of the dogfish. It was connected to the input stage of a directly coupled differential preamplifier, and responses were recorded by means of a Polaroid camera from a storage oscilloscope display.

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 in a normal position with regard to the

surface of the eyecup and casting a circular patch of light, which covered the external surface of the preparation. Unless otherwise specified, the duration of the light stimulus was 200 ms, the same as in the experiments of Dowling and Ripps (1972) featuring eyecup preparation of the skate. Preliminary experiments showed that in the dogfish as well, this duration of the light stimulus was well beyond the duration-sensitive range of the b-wave amplitude. The 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 extent in log units (Fig. 1).

Fitting procedures

In fitting our ERG-based spectral sensitivity we used our 3-parameter model for the α-band of A1-based pigments. It is a 3-parameter (a–c) equation of the form:

$$S_{(\lambda)} = a \cdot (1+n)^{-(b+1)/b} \cdot n \cdot (b+1)^{-(b+1)/b}, \qquad (1)$$

with

$$n = e^{\frac{[(\lambda + c \cdot \ln(b) - \lambda \max)]}{c}}.$$

where the set of parameters in Eq. (1) that provide a good fit to the full range of our A1 data is a = 27.5749, b = 0.3809, and c = 0.35.5.

The short-wave peak remaining after subtraction of the α-band template (1) was fitted with the Gaussian equation:

$$S_\beta(\lambda) = A_\beta \cdot e^{\{-[(\lambda - \lambda_{m\beta})/d]^2\}} \qquad (2)$$

where A_β is the amplitude of the β-band relative to the α-band, $\lambda_{m\beta}$ is the position of β-maximum, and d is a bandwidth parameter. A_β was fixed at

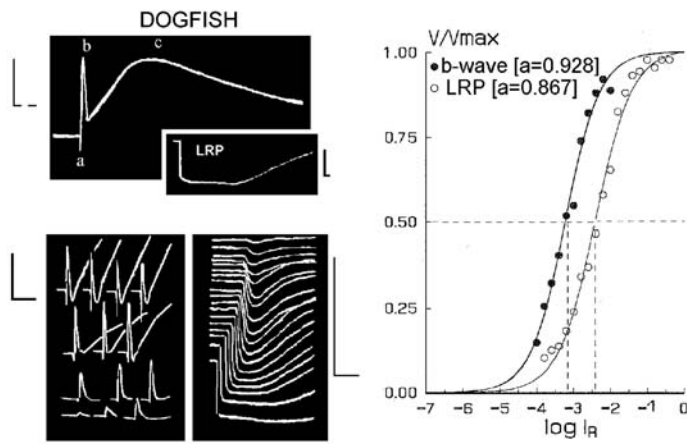

Fig. 1 Examples of electroretinographic waveforms (all calibrations: 0.1 mV, 2 s). Dogfish. *Upper left and right records* respectively: normal ERG (isolated eyecup, 14.6 mm in diameter) and LRP (detached retina, after 18 h of aging); 0.2-s test flashes of saturating intensity. *Lower left series of records*: normal ERGs in response to incremental stimulation with 0.2-s test flashes; flash intensity increasing from bottom to top and from left to right in steps of 0.3 (first 6 records) and 0.5 log units. *Lower right series of records*: LRP responses to incremental stimulation of an iodate-pretreated eyecup (flash intensity increasing from top to bottom in steps of 0.3 log units). LRP (*open circles*) and b-wave (*closed circles*) amplitude/intensity relations in a dogfish (*right*). LRP unmasked by sodium iodate. Stimulus wavelength: 500 nm. I_R: relative flash intensity. Fitting according to Eq. (5)

the value 0.26 because of the best fit with Dartnall's frog spectral sensitivity data (Dartnall 1953). The relationships between λ_{max} and the position of β-maximum ($\lambda_{m\beta}$) and between λ_{max} and d could be approximated as straight lines:

$$\lambda_{m\beta} = 170.1 + 0.339 \cdot \lambda_{max} \quad (3)$$

$$d = 41.63 + 0.0086\lambda_{max} \quad (4)$$

A complete description of the absorbance spectra of A1-based visual pigments between 400 nm and far red was provided by Eqs. 1–4.

Results

Waveforms

The ERGs of the dogfish (dark-adapted, at a temperature of 15°C, and at 200-ms light flashes of saturating intensity) were similar to those recorded from isolated pieces of the skate eyecup by Dowling and Ripps (1972). The three principal components of the rod-dominated vertebrate retina (Granit 1955) were well represented (Fig. 1, dogfish records): an initial negative deflection (a-wave), a relatively fast positive transient (b-wave), and a slow positive potential (c-wave).

After the introduction into the isolated dogfish eyecup of a solution containing 25 mM sodium iodate, and upon intermittent stimulation of the preparation with 200-ms light flashes of saturating intensity, the b-wave is seen to gradually decrease in amplitude, sink below the baseline, become reduced to a notch on the descending limb of the increasing a-wave, and finally disappear. A gradual loss of the c-wave and a concomitant enlargement of the a-wave, leading finally to complete LRP isolation, accompany this. In the end, the waveform of the iodate-isolated LRP (right-hand series of dogfish records in Fig. 1) did not differ substantially from that recorded after LRP isolation by low-temperature aging, without the use of chemical agents (Andjus et al. 1983). The ERG of the detached retina of the dogfish responded to low-temperature aging in the same way as the ERG of the eyecup preparation, but on a considerably shorter time scale, LRP unmasking becoming complete in less than 20 h (top right-hand dogfish record in Fig. 1).

Intensity-amplitude relations

Since spectral sensitivity determinations are based on threshold values derived from measurements of wave amplitude as a function of flash intensity, the regularity of the intensity-amplitude relation has been checked, in the case of both the b-wave and the iodate-unmasked LRP, by fitting experimental data to the basic model (Naka and Rushton 1966; Dowling and Ripps 1972):

$$V_o = I^a/(I_o^a + I^a) \quad (5)$$

where V_o is the normalized voltage (V/V_{max}) of the ERG signal (b-wave or LRP), I_o is the stimulating light intensity corresponding to $V_o = 1/2$, and the exponent a is a constant. As shown in Fig. 1, both the amplitude of the b-wave and of the subsequently isolated LRP responded to incremental photostimulation in the same way, in conformity with the basic model.

To calculate threshold values on which to base spectral sensitivity determinations we use fitted log-sigmoids. Criteria for sigmoids were: fitting error $\leq 0.01\ V_{max}$ and at least three data-points, 0.3 log units apart, included within the initial and terminal 10% segments of the fitted sigmoids. A signal amplitude equal to 10% of the largest response, obtained with light stimuli of the most effective wavelength, was adopted as the threshold criterion.

Identical spectral sensitivity curves were obtained when threshold measurements concerned the ERG b-wave and LRP unmasked by aging of the eyecup or isolated retina preparations. Action spectra matched closely the pigment 502 spectral curves. There was no Purkinje shift of the spectra upon light adaptation (as preponderantly in rod retinas). Figure 2 shows spectral sensitivity curves obtained in the small-spotted dogfish shark. They concern both b-wave and LRP data, obtained in three isolated eyecups. All eyecups provided scotopic b-wave data, while one of them was used, in addition, for photopic b-wave measurements and another one after 24-h aging, for LRP

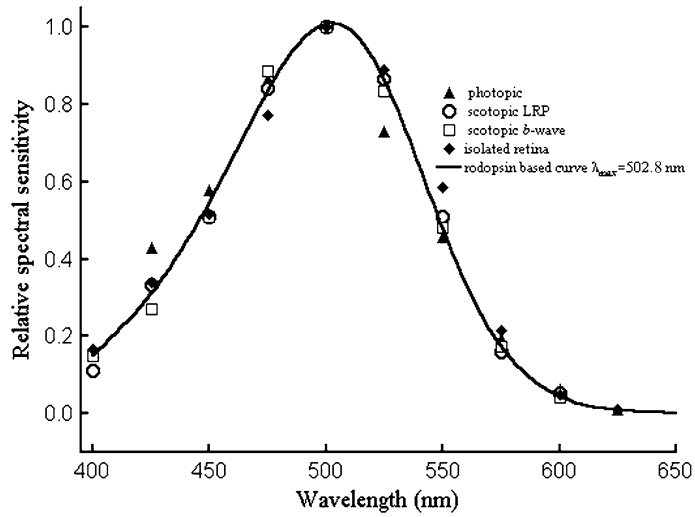

Fig. 2 Dogfish spectral sensitivity curve. Simultaneously fitted are five sets of experimental scotopic b-wave data (*open rectangles*), scotopic LRP (*open circles*), photopic b-wave data (*closed triangles*), and data from isolated retina (*closed diamonds*)

determinations under scotopic conditions. All five sets of spectral sensitivity data obtained in the three preparations were simultaneously fitted using our 3-parameter model for A1-based pigments (Fig. 2, λ_{max} = 502.8 nm). As can be seen, fitting testified to relatively small differences between spectral sensitivity data obtained under widely differing experimental conditions. λ_{max} values were very close, as expected, to those of vitamin A_1-based pigments 500 and 502 found in rats and frogs respectively (Dartnall 1953).

When separately fitted, the five sets of data obtained in dogfish eyecup preparations provided individual λ_{max} values ranging from 495.9 to 503.2 nm (average 500.5 ± 3 nm), and from 496.9 to 503.2 nm (average 500.8 ± 2.5 nm) in the case of fitting by the 502_1 and Lamb-Govardovskii models respectively (Table 1, columns of λ_{max} data). When obtained in the same isolated eyecup, the separately fitted b-wave and LRP data, subjected to fitting by means of the model 502_1, provided very similar λ_{max} values (502 and 503.2 nm respectively). LRP data provided by the detached (aged) retina resulted in a slightly greater λ_{max} (506.9 –507 nm).

Discussion

Waveform

Our ERG data on *Scyliorhinus canicula*, first reported by Andjus et al. (1983), were in general agreement with those obtained previously in other elasmobranchs (Hamasaki et al. 1967), particularly skates (Dowling and Ripps 1972; Pepperberg et al. 1978; Ripps and Dowling 1990). The late negativity, interposed in our dogfish records between the positive b- and c-waves, and increasing as flash intensity increased, was not given particular attention in earlier elasmobranch studies, although some of the published ERG records testify undoubtedly to its appearance at sufficiently high flash intensities

Table 1 Dogfish λ_{max} (in *nm*) as dependent on the type of preparation, its treatment, and the fitting

Fitting method		
Preparation	Model 502_1	Lamb-Govardovskii 502_1
1 Eyecup, scotopic b-wave	502.3	502.2
2 Eyecup, scotopic b-wave	502	501.8
Eyecup, photopic b-wave	498.9	499.9
3 Eyecup, scotopic b-wave	495.9	496.9
Eyecup, scotopic LRP	503.2	503.2
Mean:	500.5 ± 3 (n = 5)	500.8 ± 2.5 (n = 5)
4 Detached retina, scotopic LRP	506.9	507
Average spectrum[a]	502.8	502.9

[a] Simultaneous fitting of the 5 sets of spectral sensitivity data from preparations 1 to 3

(Hamasaki et al. 1967). After the addition of sodium iodate, or during the earlier phases of low-temperature aging, the late negativity accompanying the progressively disappearing b-wave notch is seen to enlarge and finally fuse with the concomitantly increasing a-wave into a single negative deflection, the completely unmasked LRP.

Our experiments showed that in dogfish preparations a complete LRP unmasking can be achieved either by chemical ($NaIO_3$) or by physical means (low-temperature aging, Andjus et al. 1983). The latter method proved advantageous in many respects. It avoids the use of poisonous chemicals with possibly unwanted damaging effects, which, in the case of sodium iodate, proved to be strongly species-dependent (Wioland et al. 1988).

Amplitude-intensity relations

In dogfish preparations the slope of the amplitude-intensity log-sigmoids, which determines the dynamic range of responses, was characterized by similar parameter a values (usually between 0.7 and 0.9), of the same order as those reported for other animals and other electrophysiological signals (Naka and Rushton 1966; Baylor and Fuortes 1970; Dowling and 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

Deep-sea sharks were shown to have ''chrysopsis-like'' photopigments with spectral maxima at 472–484 nm (Denton and Shaw 1963); similarly. therefore to a great number of deep-sea fish species that all possess a single rhodopsin with a wavelength of maximum absorbance in the range 470–495 nm (Partridge et al. 1989). Among deep-sea fish species, however, 1 elasmobranch, as well as 14 teleosts has more than one visual pigment in their retinas (Widder 1984). On the other hand, elasmobranchs inhabiting shallow water possess a more common rhodopsin: in two of the latter (*Mustelus californicus* and *Rhinobatus productus*) retinal extracts revealed the presence of a single $P497_1$ photopigment (Crescitelli 1969) like *S. canicula* retinal extracts (Bozzano et al. 2001). It seems reasonable, therefore, to expect such a ''shallow-water'' visual pigment to underlie the ERG-expressed spectral sensitivity in the electrophysiologically studied elasmobranchs, which all belonged to the shallow water group. ERG spectral sensitivity data in elasmobranchs are scarce. Those obtained in the lemon shark (*Negaprion brevirostris*, O'Gower and Mathewson 1967), were not convincing, considering the low degree of accuracy with which the seemingly ''bimodal'', ''night-adapted'', action spectrum was plotted and the two maxima estimated (around 533 and 435 nm) in a single animal. An action spectrum was obtained by Dowling and Ripps (1972) in one preparation of the skate retina. It was derived from LRP thresholds recorded in an aspartate-pretreated and dark-adapted preparation (the authors did not specify to which of the two species used in their experiments, *Raja erinacea* or *R. ocellata*, the preparation actually belonged). The 7 data-points, although not fitted, were said to be in good agreement with a nomogram-derived curve for $P500_1$. When redrawn from the original graph and subjected to the presently applied fitting procedure, λ_{max} values of 501.9 nm were obtained. Therefore, they closely agreed with the presently obtained sensitivity spectra of the entire eyecup of the dogfish shark, irrespective of the type of threshold measurements (b-wave thresholds in fresh preparations, or LRP thresholds in aged or iodate-treated eyecups).

The λ_{max} values of the rod visual pigments in dogfish shark correlate well with habitat depths. The small-spotted dogfish shark lives in shallow to moderately deep water and the λ_{max} value of 501 nm is quite usual for fishes living at this depth range (Bozzano et al. 2001).

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