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Electrophysiological and spectral properties of second-order retinal neurons in the eel

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Abstract

Several classes of second-order neurons have been electrophysiologically explored in immature European eels (*Anguilla anguilla*) from two distant and ecologically different localities (in Russia and Yugoslavia). The majority of L-horizontal cells (58 explored) had both rod and cone inputs, an uncommon phenomenon among teleosts. Spectral sensitivity characteristics of a number of horizontal and bipolar cells indicated that yellow-sensitive and green-sensitive cones coexist in the retina of the European eel, and that rods and green-sensitive cones contain similar visual pigments. Pronounced color-opponent properties, often taken as the capacity of color vision, were identified in one amacrine cell, apparently of the B/Y (or B/G) type. Differences in retinal structure and responsiveness between eels from the two localities, presumably due to differences in local conditions for growth, were less important than between eels of the yellow and silver stage.

Keywords: Amacrine cells; Bipolar cells; Color vision; European eel; Horizontal cells; Retinal electrophysiology; Retinal neurons; Spectral sensitivity

1. Introduction

The visual system of eels (Anguillidae) is renowned for a number of peculiarities linked to the specific features of their life history and migratory behavior. This applies in particular to the spectral sensitivity of the eel's visual system under scotopic conditions, involving rods as photoreceptors. It was first found by spectral sensitivity studies that freshwater eels differ from many other freshwater teleosts by possessing, in addition to a vitamin A_2-based pigment (porphyropsin), the vitamin A_1-based rhodopsin, otherwise characteristic of ocean-living fishes (for a survey of older literature see Ref. [14]). It soon became evident that eels may actually possess various pigments and pigment mixtures in their retinas, depending on the stage of their development. When an immature 'yellow' eel assumes the 'silver' livery of approaching maturity, a 'metamorphosis' of its visual pigments occurs, resulting in a shift of the maximum of absorption (λ_{max}) of its rod pigments towards shorter wavelengths. Details of the differences in scotopic spectral sensitivity between different developmental stages of the eel have been well documented. This was achieved by means of spectrophotometry of pigment extracts and by single-cell microspectrophotometry in a number of laboratories [1,8,33,36]. Finally, differences between developmental stages of the eel have been confirmed by means of electroretinographic analyses in one of our recent studies (unpublished). It became evident that, in general, in catadromous species such as the eel, a shift occurs during the spawning

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migration from mostly porphyropsin to mostly rhodopsin, while the converse happens in anadromous species, such as the salmonids [2]. Following the shift of λ_{max} of retinal rods by chromophore exchange from 523 to 501 nm, a transition occurs in the eel to a different opsin: a new opsin, forming a new A_1-based rhodopsin of shorter λ_{max} (482 nm), is inserted into the rods. The latter pigment becomes the dominant, if not exclusive, rod pigment of the prespawning eels. This presumably represents a unique surface to deep-sea shift in visual pigments occurring in one and the same species. Therefore, in addition to possessing paired visual pigments based on the same opsin, a feature not uncommon in teleosts [35], the eel has 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. The shift from long-wave- to short-wave-sensitive rhodopsin is generally interpreted as an adaptive change. It leads to sensitivity matching between visual pigment spectra and the spectral distribution of light in different water types, freshwater usually having a relative excess of long-wave photons, the deep sea having a preponderance of short-wave photons, and coastal waters being intermediate in spectral characteristics [36].

In great contrast to the fairly accurately evaluated scotopic (rod) spectral sensitivity of eels, details of the pigment content of their cones and of its eventual change with maturation have not been determined. The photopic spectral sensitivity and the capacity of wavelength discrimination (color vision) in the eel remain largely unknown. In our preliminary ERG study of European eels [9] we did not succeed in determining spectral characteristics of the photopic units, and the Purkinje shift in sensitivity could not be observed. In the American eel (*Anguilla rostrata*), however, Gordon et al. [12] succeeded in obtaining a complete photopic, ERG-based spectral sensitivity curve by exposure to a bright white, 20-Hz flickering light. Working with an isolated and bleached retina and exploiting the fact that cone pigments regenerate after bleaching, while rod pigments do not, indications were obtained that in the absence of rod responses, two cone-like mechanisms were discernible: one with λ_{max} of 550 nm, and the other with λ_{max} below 450 nm. Unfortunately, more precise information about the cone spectral mechanisms was not offered.

Difficulties associated with the electroretinographic approach in cone spectral analysis [9,12] indicated that the elucidation of the photopic sensitivity mechanism in eels requires the application of the more precise techniques of intracellular exploration. By means of such techniques, complemented with electron-microscopic studies, spectral properties have been revealed of photoreceptors and of horizontal cells in the retinas of a number of teleosts [20,28]. In the European eel, however, interactions between the photoreceptors and the second-order neurons have never been thoroughly examined, neither histologically nor electrophysiologically. The only relevant intracellular investigations were performed on a few horizontal cells of American [12] and Japanese eels [22]. We decided, therefore, to examine the possibility of intracellular recording from different second-order neurons of the European eel retina, in order to analyse indirectly the spectral properties of cones and define some of the pathways within the outer plexiform layer. We compared two experimental groups of eels differing by their geographic origin (Lake Seliger in Russia, and coastal running waters along the South Adriatic in Montenegro). Several classes of second-order neurons were successfully explored and the functional characteristics of a number of photopic units defined, including color opponency.

2. Materials and methods

2.1. Animals

Two groups of European eels (*Anguilla anguilla*) were used. One group consisted of eight yellow stage eels (4^+–5^+, $L < 40$ cm) electrofished during winter months in the mouth regions of creeks inflowing into the Boka Kotorska Bay (Southern Adriatic, Montenegro; designated as 'Adriatic eels' in the text). They were transported by air to the Moscow laboratory and kept for at least 15 days in cold-room fresh-water aquaria, at 10–15°C. The second group was represented by nine considerably larger eels ($L > 50$ cm) captured with hoop nets, at the end of October, in the fresh-water Lake Seliger (Russia, Tver region; designated as 'Seliger eels' in the text). They were also kept for at least 7 days prior to the experiments in fresh-water aquaria (water temperature 12–15°C) without feeding.

2.2. Histology

For histological analysis eyecups were prepared from eyeballs excised after rapid decapitation of the fish. Cornea, lens and most of the vitreous were removed surgically. The eyecups were fixed in Bouin solution, embedded in paraffin, sectioned into 8-μm thick slices, and finally stained with hematoxylin. Radial sections of the retina were studied under a light microscope.

2.3. Electrophysiology

Eyecups were cut into two approximately equal fragments. While experimenting with one eyecup fragment, the remaining fragments from the two eyes of an eel were maintained in a refrigerator at 4°C. Four experiments were thus performed on one fish. In each case,

the eyecup fragment was stretched, scleral side down, over a plastic ball, fixed in a small platform inside a lightproof Faraday cage. Since residues of the vitreous are known to prevent the oxygenation of the retina, the vitreous was expelled by means of forced perfusion. The perfusion was continued during the entire experiment, using teleost Ringer at 10–12°C.

Microelectrodes with 100–400 MΩ resistance, filled with 2 M potassium acetate, were used for intracellular recording. The reference electrode was positioned on a strand of filter paper connected with the eyecup and soaked with teleost Ringer. To minimize vibrations, the Faraday cage was hung by nylon ropes to a metal support. Electrodes were connected to the input stage of a microelectrode preamplifier. During some procedures (microelectrode cleaning) the voltage at the input stage of the preamplifier highly exceeded levels permitted for field effect transistors (FETs). An EM-8 penthode was therefore installed instead a FET on the input stage of the preamplifier. The amplified signals were recorded using an X–Y plotter.

For photostimulation, a halogen lamp was used with a series of eight interference filters (427, 470, 490, 543, 587, 620, 648 and 664 nm), with 50% bandwidths of 10–12 nm. Quantum intensities of non-attenuated stimuli of different wavelengths were made equal using a calibrated selenium photocell. In all our figures, light intensity is expressed in units of the neutral density filters (NDF) used for attenuation, $\log I = 0.0$ corresponding to 3.2×10^3 quanta $\mu m^{-1}\ s^{-1}$. The duration of the photostimuli was usually around 1.3 s.

In some of our electrophysiological tests, we used bright white light flashes of undetermined spectral characteristics and of 1.3 s duration. In order to estimate retinal sensitivity to such stimuli, $V - \log I$ profiles, obtained with a number of horizontal cells (HCs), were compared with such profiles obtained with monochromatic flashes. 'Physiological calibration' (Fig. 1) showed that responses to monochromatic photostimuli with NDF = 0.0 and to white light flashes with NDF = − 2.1 were of similar amplitude, while $V - \log I$ profiles were of practically identical slopes.

In order to identify cell types, impaled cells were tested using light spot and superimposed annular stimuli. It is known that both horizontal and off-bipolar cells (off-BCs) respond to light spot stimuli by sustained hyperpolarization, while in HCs and off-BCs the annulus stimulus evokes, respectively, additional hyperpolarization [7] and depolarization [15,34]. In on-bipolar cells (on-BCs) the response to the light spot is a sustained depolarization, while the annulus evokes a hyperpolarizing reaction [15,34]. The same test (light spot and annulus stimulation) was used to estimate the approximate size of receptive fields of HCs, using light spots of different diameters (30, 70, 115, 210, 270, 450, 620 and 700 μm) in combination with annular stimuli of always the same inner and outer diameter (0. 25 and 2 mm, respectively), following the procedures of Byzov [7].

2.4. Action spectra

The relative quantum spectral sensitivity (S_q), varying within the range [0,1], was calculated for different stimulus wavelengths (λ), λ_{max} representing the stimulus wavelength at which the maximal S_q value is observed. S_q was defined as the reciprocal of quanta necessary to evoke the response of a given (standard) amplitude and was calculated from spectral response curves using $V - \log I$ profiles for monochromatic or white light stimuli. It is well known that eel rods possess rhodopsin-500 (r500) and porphyropsin-523 (p523), the ratio between them changing during sexual maturation [8,33,36]. In all our figures (Fig. 7A–C), therefore, S_q values are accompanied with data on r500 or p523 absorption spectra, calculated according to Maksimov [17]. For spectra concerning retinal-based pigments, we used the Maksimov's approximation

$$S_q = \exp(-\xi^2 \cdot \psi^2), \quad (1)$$

where:

$$\psi = \lambda^{0.25} - \lambda_{max}^{0.25}, \quad (1a)$$

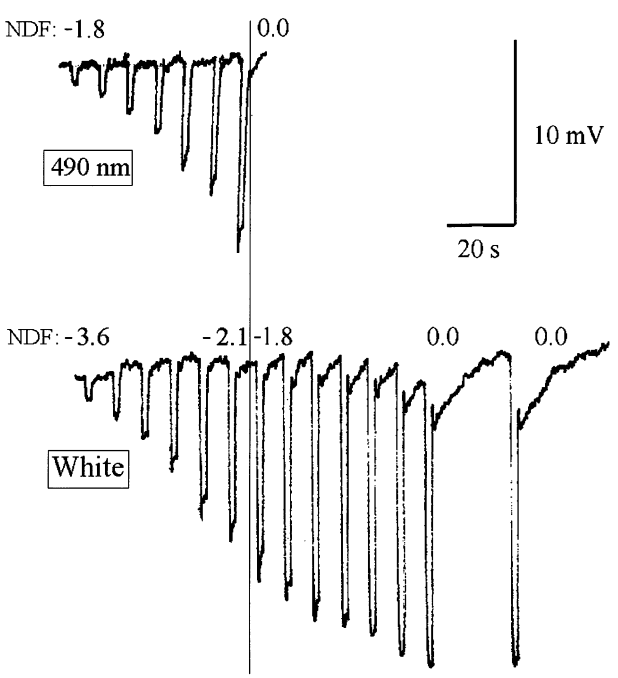

Fig. 1. Physiological calibration of the responsiveness of horizontal cells to white light flashes. NDF, attenuating neutral density filters, applied in decreasing order (0.3 $\log I$ units apart). Note that the response to a white light stimulus attenuated by NDF = − 2.1 (lower panel) corresponds to the response of the same cell to a non-attenuated (NDF = 0.0) monochromatic (490 nm) photostimulus (upper panel), as indicated by the thin vertical.

and

$$\xi = 6 + 6\psi + \frac{2}{3}\cdot \mathrm{arctg}(21\psi + 6) \quad (1b)$$

In the case, however, of 3-dehydroretinal-based pigments (porphyropsins), expression (1b) was modified, following Maksimov's recommendations, to

$$\xi = 5 + 6\psi + \frac{2}{3}\cdot \mathrm{arctg}(21\psi + 6) \quad (1c)$$

For the construction of the spectrum of iodopsin (Fig. 7C), Eq. (1b) was applied using $\lambda_{max} = 560$ nm.

Statistical differences between means of individual spectral sensitivity data, and differences between λ_{max} values obtained by fitting spectral sensitivity datapoints in individual fishes by the procedure of Maksimov, were evaluated by means of the nonparametric two-tailed Mann–Whitney test, considering $P > 0.05$ as nonsignificant.

2.5. The ocular index

Experimental eels were characterized by their eye index, I, according to Pankhurst [24]:

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

where A and B are, the horizontal and the vertical diameters of the eye (in mm), respectively, while L is the total body length (in cm). All Adriatic eels, classified as yellow eels (sexually immature adults), were characterized by $L < 40$ cm and $I < 2$. Eels from Lake Seliger were longer than 50 cm, and had an eye index between 6.5 and 13. No attempt was made to further differentiate, on the basis of I and L, between males and females, and between migratory and non-migratory forms.

3. Results

3.1. Retinal morphology

Radial sections of the retina (Fig. 2) revealed a highly developed pigment epithelium. In the outer nuclear layer (ONL) two types of nuclei were distinguished: the nuclei above the external limiting membrane (ELM) belonging to cones, and several sublayers of much smaller and optically denser rod nuclei below ELM. The rod/cone ratio in Seliger eels was 40:1, twice as high as in the much smaller Adriatic yellow eels (20:1), but still much lower than the ratio known to characterize eels of the silver stage (100:1 and 250:1, according to Refs. [5,25], respectively),

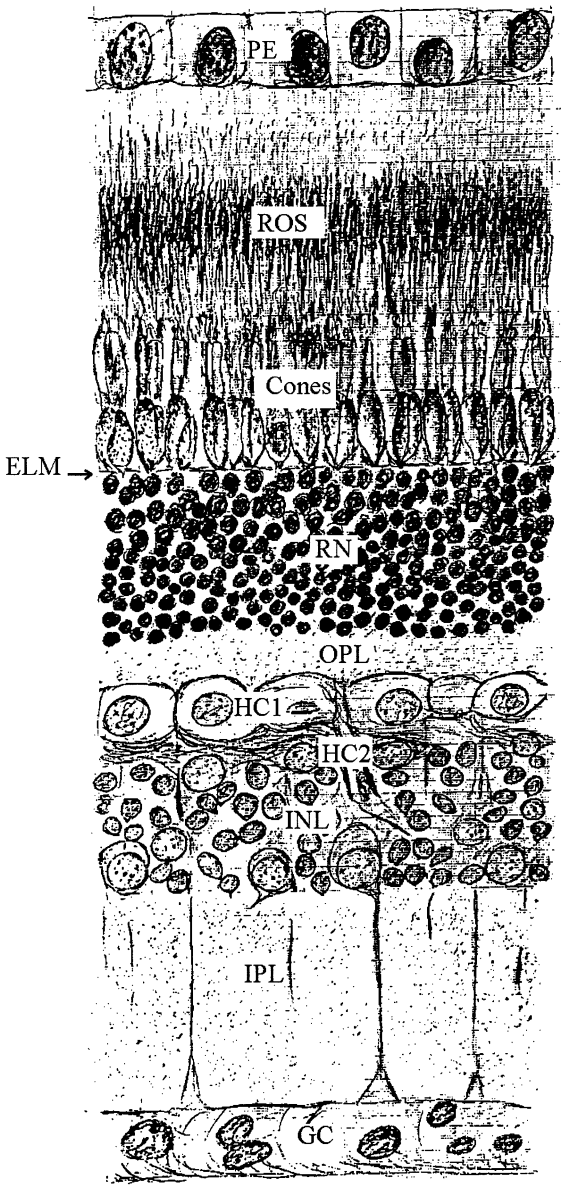

Fig. 2. Retinal morphology in European eels (semischematic drawing of a radial section). ELM, external limiting membrane; GC, ganglion cells; HC1 and HC2, horizontal cells of the first and second layer, respectively; IPL, inner plexiform layer; OPI, outer plexiform layer; PE, pigment epithelium; RN, rod nuclei; ROS, rod outer segments.

Only one compact layer of HCs was clearly distinguished, although horizontal cells of a second layer could also be observed occasionally.

In the inner nuclear layer (INL), containing bipolar and amacrine cells, the total number of cells was significantly smaller than in ONL. The rod/INL ratio in the yellow eel retinas was approximately 3:1, while in the larger Seliger eels it amounted to 6:1. The ganglion cell (GC) layer contained considerably fewer cells than INL.

The observed relation between retinal layers (numerous photoreceptors, much less abundant INL cells and

relatively scarce GCs) points to a high degree of convergence of signals from photoreceptors in the European eel.

3.2. Electrophysiology

3.2.1. Horizontal cell responses

All horizontal cells explored belonged to the luminosity-type cells (L-HCs). Among the 33 such cells explored in Seliger eels, six belonged to cone-driven cells (cone-HCs), nine to the rod-driven type (rod-HCs), and 18 to rod- and cone-driven cells (mixed HCs). In 25 HCs from yellow Adriatic eels, no rod-HCs were detected: 10 cells belonged to mixed HCs, and 15 to cone-HCs (Fig. 3A). Rod inputs to mixed HCs were less expressed in Adriatic than in Seliger eels.

In rod-HCs (Fig. 3B, upper panel), light offset was regularly followed, particularly at high stimulus intensities, by a sustained hyperpolarizing plateau (afterpotential) and the response decayed relatively slowly. At maximal intensity (white light, NDF = 0.0) the duration of the afterpotential plateau amounted up to 10–15 s. In case of stimulation with monochromatic light of $\lambda = 490$ nm, saturating levels were reached around NDF = -0.9.

In cone-HCs (Fig. 3B, lower panel) responses decayed much faster than in rod-HCs. The afterpotential was not present, even at maximal light intensities (white light, NDF = 0.0), and the saturation of the response was not reached.

In the mixed HCs, the stimulus offset was first followed by a fast cone- and then by a slow rod-component (Fig. 3C). In the majority of the mixed HCs explored, and in both experimental groups of eels, cone inputs were dominant. Only in a few cells cone- and rod-components were of approximately equal amplitude. When mixed HCs were stimulated in the presence of different monochromatic backgrounds (red or blue), the shape of the response changed: only the cone-component was observed at light offset (Fig. 3C, second and third record). Sometimes the cone-component revealed a positive off-peak, increasing with background intensity. Similar but smaller and wider off-peaks were observed in some of the rod-HCs as well (Fig. 3B, Fig. 6A).

Spatial properties of HCs were not investigated in detail. In the case of the cone-HC response shown in Fig. 3D (right), the light spot was 0.7 mm. The strong additional hyperpolarization, evoked by annulus stimulation, indicated that the receptive field of this HC was very large, larger by far than 1 mm. On the other hand, in the case of one mixed HC (Fig. 3D, left), the light spot of a considerably smaller diameter (0.27 mm) evoked an almost maximal response: the additional hyperpolarization caused by the annulus was hardly noticeable. These two HCs, shown in Fig. 3D, represent two extremes: HCs with the smallest and the largest receptive field among the presently explored horizontal cells.

3.2.2. Bipolar cell responses

Our experiments revealed the presence of both on-BCs and off-BCs in the retina of the European eel (Fig. 4). Surround illumination by an annulus light stimulus, applied after the onset of a light spot stimulus, evoked a response of an opposite sign: depolarization in off-BCs, and hyperpolarization in on-BCs. Particularly frequent and stable were our recordings from on-BCs in Adriatic eels, enabling the elucidation of their spectral properties (see later).

3.2.3. Amacrine cell responses

Amacrine cell responses (Fig. 5) were usually recorded at a retinal depth of 20–40 μm. The majority of responses were of the transient type (hyperpolarizing or depolarizing; Fig. 5a,b), with prominent on- and off-peaks, sometimes equal to, or greater than 10 mV (not shown). Some of the recorded amacrine cells responses were of the transient/sustained type (Fig. 5c), with a large positive on-wave followed by a sustained depolarization. Sometimes a negative peak appeared at stimulus offset (Fig. 5c, lower trace).

3.3. Spectral properties of retinal neurons

In all HCs (with the exception of one cell from an Adriatic eel), maximal responses were observed in the same blue–green region of the spectrum, under scotopic as well as under photopic conditions (exemplified by records in Fig. 6A). This strongly indicates that rods and green-sensitive cones contain visual pigments with similar absorption spectra. This was further supported by our results for mixed HCs (Fig. 6B). Although relatively small, the rod component increased with prolonged (> 5 min) dark adaptation (Fig. 6B, two records on the right) and no shift in the response maximum occurred when applying monochromatic stimuli of different intensities (NDF = -1.8, -1.2 and 0.0; Fig. 6B, three series of records on the left).

Records obtained with HCs of Seliger eels belonged to three types: with cone, rod and mixed inputs. The latter were studied under both photopic (high light intensities, $-0.6 \leq \text{NDF} \leq 0.0$) and scotopic conditions (low light intensities, NDF ≤ -1.2). Data were averaged together with those concerning cone and rod HCs, respectively. Fig. 7A shows averaged data from six photopic and four scotopic responses. In both cases, fitted spectral sensitivity maxima were in the same region, close to the maximum for porphyropsin (523 nm), and without a statistically significant difference between their means ($P > 0.05$).

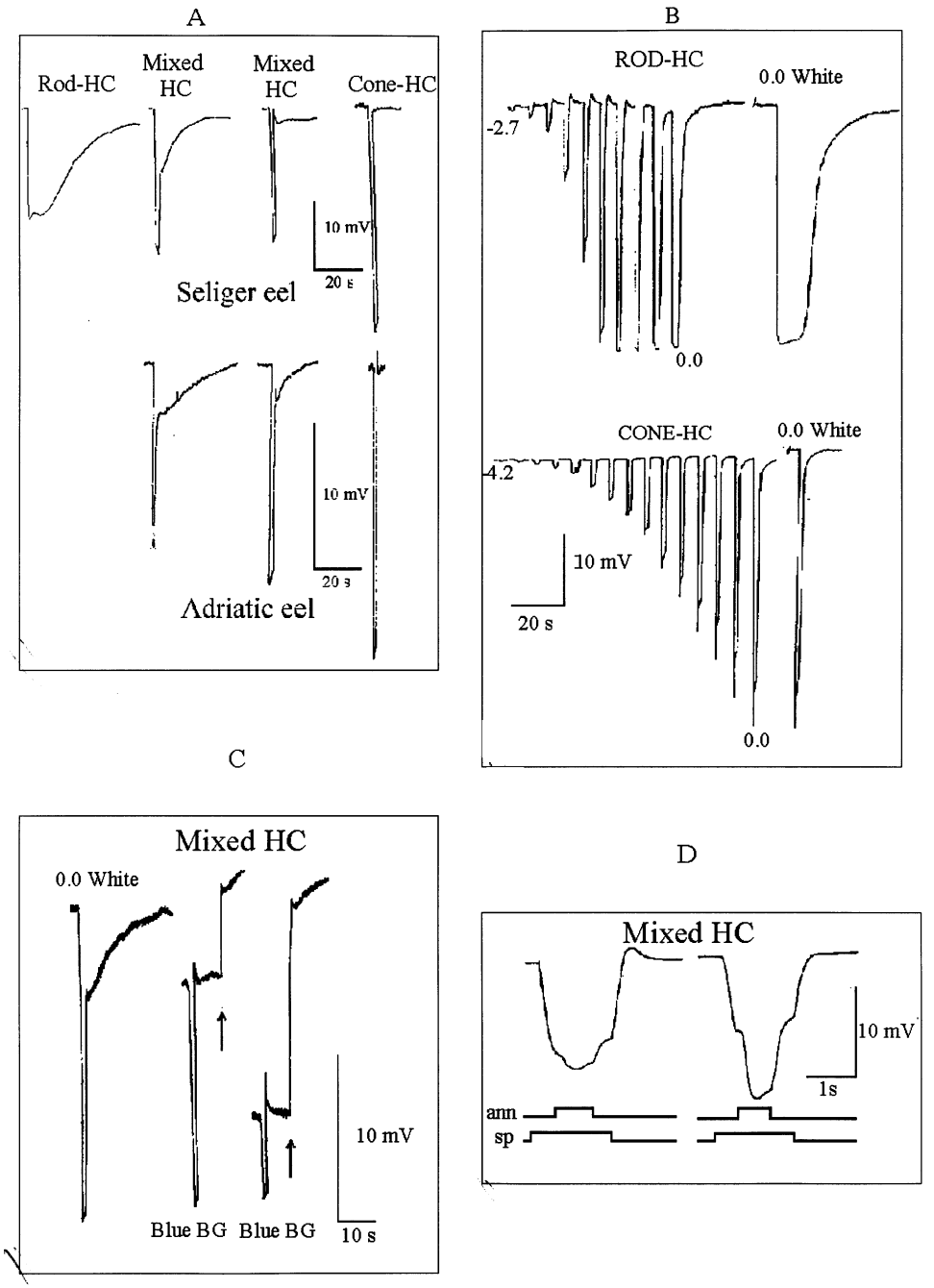

Fig. 3. Responses of horizontal cells (HCs) to various modalities of photostimulation. (A) Examples of the three types (as indicated) of intracellular records of HCs responses to white light stimuli (NDF = 0.0, 1.3 s). Note that in Adriatic eels rod-HCs are lacking and that a substantially greater amplification had to be applied. (B) Responses of cone- and rod-driven horizontal cells to incremental stimulation by 490 nm light flashes (0.3 log unit increments) and by a white light flash of maximal intensity. (C) Responses of a mixed-type HC (Seliger eel) to white light, as influenced by a blue background illumination of increasing intensity (two steps, second and third record). Note that in the presence of background illumination (ending at arrows) only the cone-component is present at light stimulus offsets (signaled by conspicuous off-peaks, greatest in the last record). (D) Spatial properties of HCs from Seliger eels as evaluated by the spot-and-annulus test (sp, spot; ann, annulus). Left: a mixed-type HC; spot diameter, 0.27 mm. Right: a cone-driven HC; spot diameter, 0.7 mm.

Since in yellow Adriatic eels the rod-components of the response of mixed HCs were too small for a detailed analysis, and since rod-HCs were not recorded, spectral sensitivity was analyzed only under photopic conditions in seven cells. The fitted λ_{max} values differed from the p523 maximum by only $+7.2$ and -3.2nm in Seliger and Adriatic eels, respectively. Although the difference between the two λ_{max} values of the green-sensitive cones was statistically significant ($P = 0.042$), they were both very close to the p523 maximum.

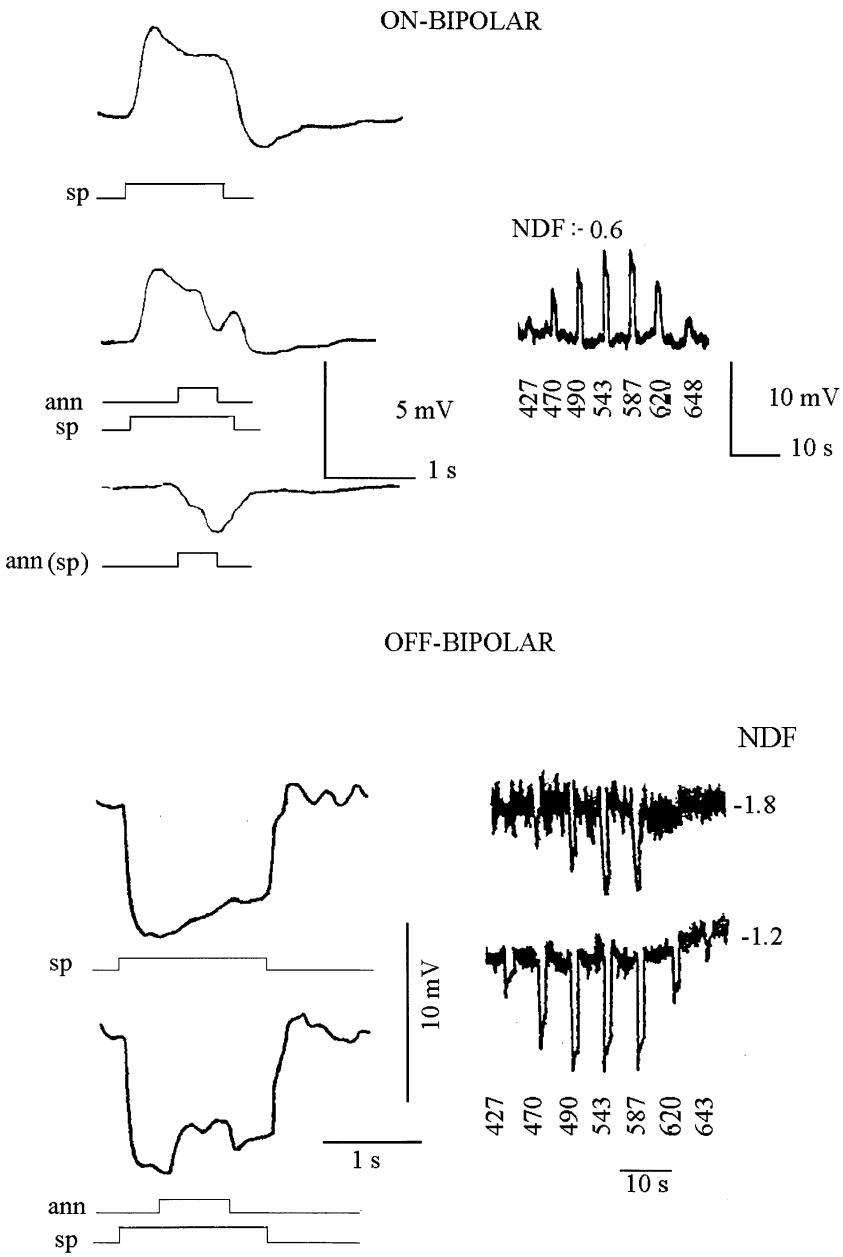

Fig. 4. Responsiveness of bipolar cells. Responses to different stimulation patterns are shown on the left sides of the two panels (sp, spot; ann, annulus; ann(sp), annulus superimposed upon a continuous spot stimulus), and responses of the same cells to serial flashes of increasing wavelength (427–648 nm) are displayed on the right sides (calibration and NDF values as indicated). Upper panel, on-bipolar cell from a yellow stage Adriatic eel; lower panel, off-bipolar cell from a Seliger eel.

One experimental finding was of particular interest: it concerned a clearly yellow-sensitive horizontal cell from a yellow Adriatic eel (Fig. 6C), indicating that yet another cone type, in addition to the green-sensitive cones, can be present in the retina of the European eel. This indication was strongly supported by our finding of maximal responses to yellow light (543–587 nm) in four on-BCs from Adriatic eels and in one off-BC from a Seliger eel (Fig. 4, right-hand records). In the majority of bipolar cells, responses were of a relatively small amplitude (less than 10 mV) and unstable, not allowing precise spectral sensitivity measurements. However, in one on-BC from an Adriatic eel we succeeded in constructing a complete spectral sensitivity curve for photopic conditions (NDF = −0.6; Fig. 7C). It was successfully matched by the iodopsin absorption spectrum ($\lambda_{max} = 560$ nm).

One amacrine cell from a Seliger eel exhibited pronounced color-opponent properties (Fig. 8). At two intensities (NDF values of −0.3 and 0.0), the maximal response was obtained at $\lambda = 543$ nm. The shape, however, of the response strongly depended on stimulus

wavelength. The response to $\lambda = 490$ nm and longer wavelength stimuli (543 nm in Fig. 8) consisted of an initial transient depolarization followed by hyperpolarization (Fig. 8, second trace from the bottom). Stimulation with $\lambda = 427$ and 470 nm evoked depolarizing responses (Fig. 8, last trace), indicating that the amacrine cell was of the B/Y (or B/G) type.

4. Discussion

4.1. Cone inputs to second-order neurons

Both photopic (cone) and scotopic (rod) inputs to horizontal cells have been presently identified in the European eel. Rod-HCs and cone-HCs differed greatly as to their respective saturation levels, in full agreement with earlier findings that in some fish species rod responses saturate at considerably lower stimulus intensities than cone responses [13]. In rod-HCs of the eel, saturation was reached at relatively low intensities of monochromatic stimuli, while in cone-HCs there was no saturation even when white light flashes of maximal intensity were applied. Furthermore, in some of the mixed HCs, saturation of the scotopic component occurred in the presence of background illumination of a relatively low intensity, and the rod-component completely disappeared under photopic conditions (Fig. 3C).

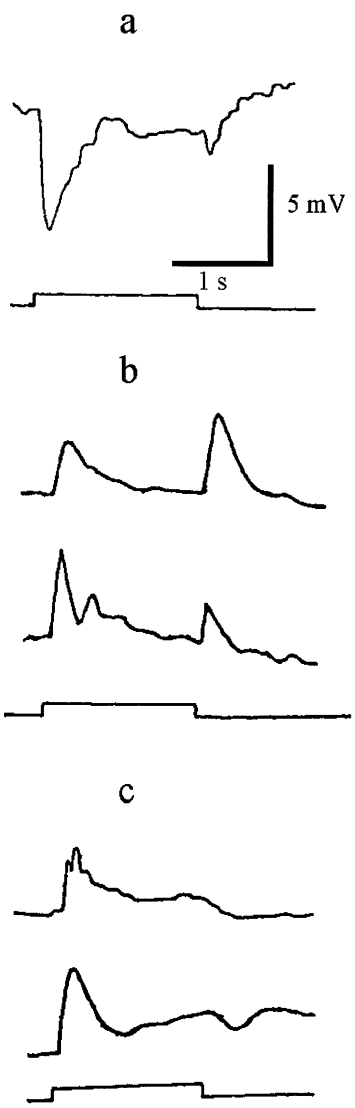

Fig. 5. Types of amacrine cell responses to white light stimuli (Seliger eels). (a) One hyperpolarizing response; (b) two depolarizing responses; (c) two transient/sustained-type responses.

Inputs of green-sensitive cones to L-horizontal cells appeared as strongly dominant. Among 49 HCs with cone inputs, only one cell was yellow-sensitive. All other cells showed a maximal photopic sensitivity in the green/blue region of the spectrum (around 520 nm).

Differences between photopic and scotopic maxima of horizontal cells were not statistically significant: both maxima were close to the maximum of porphyropsin. The scotopic spectral curve appeared, however, as somewhat broader (the differences between scotopic and photopic data-points were not statistically significant, although at seven out of eight wavelengths tested the average sensitivity values were higher under scotopic conditions; Fig. 7A). Even when statistically significant, the difference in the width of the curves does not necessarily imply that rod and cone pigments are of a different nature. It should be recalled that Carlisle and Denton [8] found that the bell-shaped curve of scotopic spectral sensitivity of the yellow eel was broader than the curve for porphyropsin constructed according to Dartnall's procedure [10]. This was ascribed to a 'self-screening effect' due to the thickness of the layer of rods, otherwise characteristic of deep-sea fishes [12]. A thick rod layer was observed in our eels as well (Fig. 2) and it could be responsible for a similar screening effect. Even, therefore, if in future experiments with a greater number of HCs, a statistically significant difference is revealed, the relative broadness of the scotopic as compared to the photopic spectral curve would not challenge the conclusion that eel rods and green-sensitive cones, having similar scotopic and photopic λ_{max} values, contain the same or very similar pigments. It should be stressed that in a number of other teleosts, rods and green-sensitive cones have been found to contain practically identical pigments [3], and that p523 was found in rods of yellow eels by a number of authors [1,8,33,36].

In comparison to findings in other fish species, our results on the strong dominance of green-sensitive HCs in the retina of the eel appears exceptional. In the goldfish, for example, the monophasic responses of L-HCs were generated predominantly by synaptic inputs from red-sensitive cones [28], while in carp G-inputs to L-HCs have been identified by means of

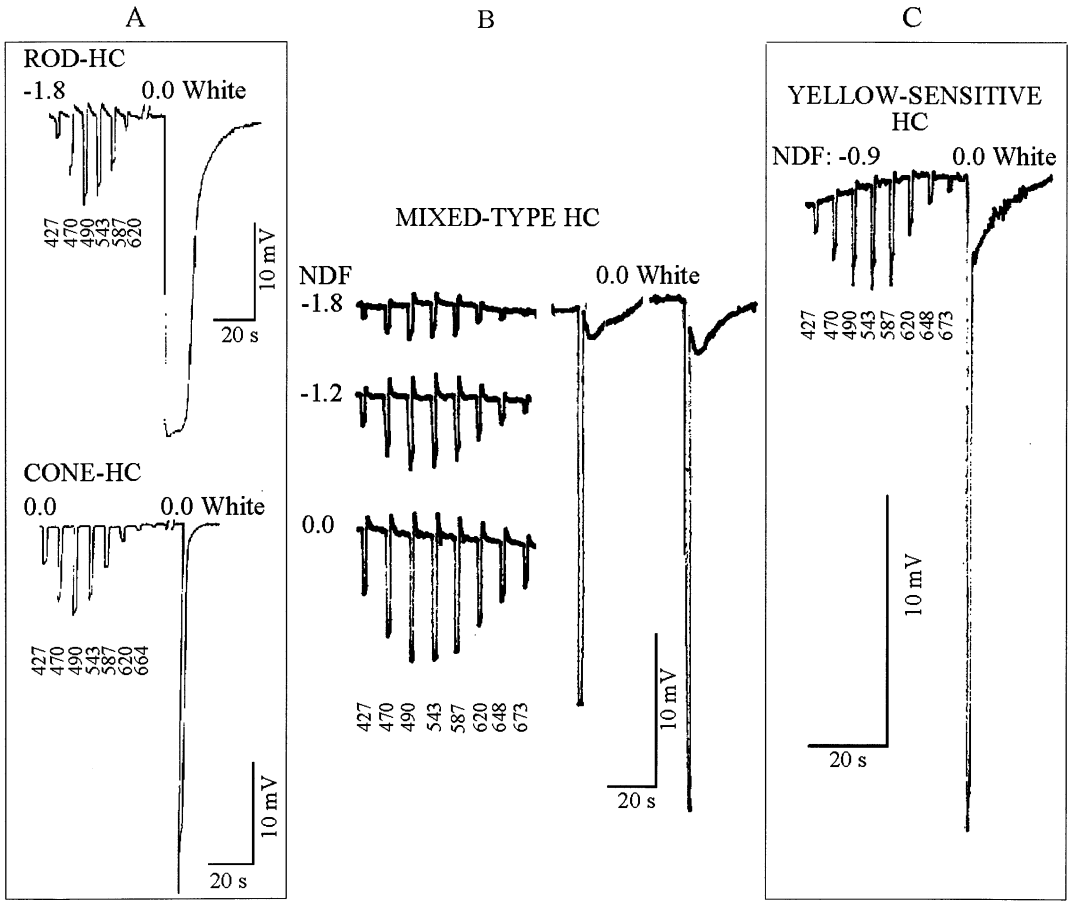

Fig. 6. Spectral responses of horizontal cells (HCs). (A) Responses of rod- and cone-driven HCs to a series of monochromatic stimuli of increasing wavelength (Seliger eel; numbers below the records refer to nanometers). In both cases maximal responses were recorded with 490-nm flashes. Responses to non-attenuated white-light flashes presented for comparison. NDF values in the case of the monochromatic stimuli indicated above each series of records (− 1.8 and 0.0). (B) Spectral responses of a mixed-type HC (Adriatic eel). Maximal responses recorded with 490-nm flashes, irrespective of flash intensity (NDF = − 1.8, − 1.2 or 0.0). Two records on the right: responses to white light flashes before and after prolonged dark adaptation. (C) Spectral responses of a yellow-sensitive HC (maximal response at 543 nm; Adriatic eel).

colorimetric measurements [23] and intracellular recordings [21]. L-green and pure L-red HCs were found in parallel in two teleosts: *Eugerres plumieri* [16] and *Mugil cephalus* [20]. Various types of color opponent HCs were also present in the two mugilids. However, among the various types of horizontal cells, the green-sensitive HCs were never dominant. In contrast, our experiments with the European eel revealed monophasic green-sensitive HCs as the only type of horizontal cells: there was no red-sensitive or color opponent HCs of any type.

Only one among the 49 horizontal cells presently explored was clearly yellow-sensitive. It should be mentioned in this connection that in the Japanese eel (*A. japonica*) the photopic L-response of a few horizontal cells was said to exhibit a spectral maximum around 548 nm [22]. Although a spectral sensitivity curve was not constructed, the author defined the spectral response as being close to the chicken iodopsin spectrum ($\lambda_{max} = 560$ nm).

However, the strongest indication that yellow-sensitive and green/blue-sensitive cone units exist side by side in the European eel has been obtained in five presently explored bipolar cells. Why the yellow-sensitive spectral reactions are mainly found in bipolar cells, remains to be elucidated. It should be recalled that in the congener American eel (*A. rostrata*), both yellow and blue cone units have been described based on electroretinographic analyses [12].

One amacrine cell with color-coding properties has been identified in our experiments, presumably of the B/Y (or B/G) type (Fig. 8). The presence of such cells is usually considered as pointing to the possibility of wavelength discrimination and color vision. Different types of color opponent HCs (R/G, G/R, Y/B, B/Y), were described in mugilids, fish species renown for a well-expressed color vision [20,30]. Let us mention finally that in the Japanese eel (*A. japonica*) the response of one HC was described as a chromatic-type (C) response of unspecified characteristics [22].

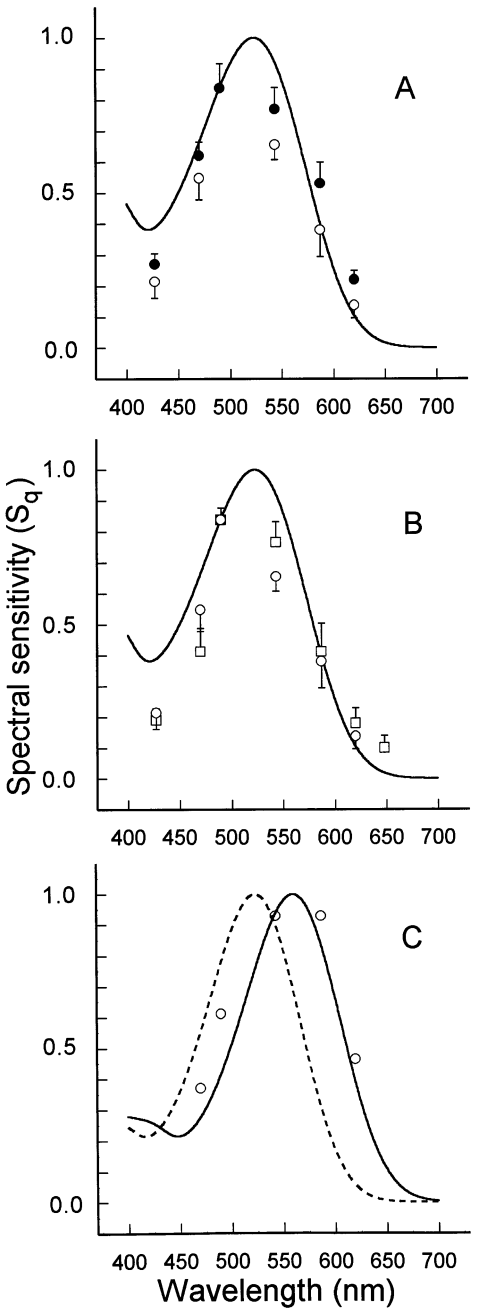

Fig. 7. Action spectra of second-order neurons. (A) Comparison between responses of HCs under photopic (open circles, $n = 6$) and scotopic conditions (closed circles, $n = 4$); means ± SE; Seliger eels. (B) Comparison between HCs of Seliger (open circles, $n = 6$) and Adriatic eels (open rectangles, $n = 7$) stimulated under photopic conditions. Continuous curves in (A) and (B): absorption spectrum of porphyropsin-523 (derived according to Ref. [17]; see Section 2). (C) Responses of an on-bipolar cell (Adriatic eel). Serial stimulation by light flashes of increasing wavelength under photopic conditions (NDF = −0.6). Continuous and dotted curves: absorption spectra of iodopsin and rhodopsin, respectively (according to Ref. [17]).

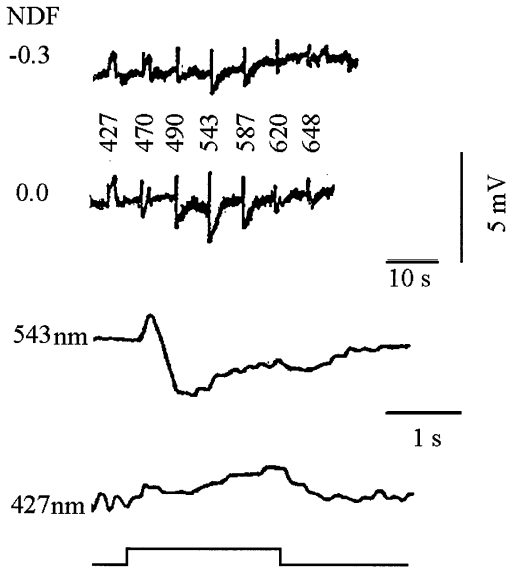

Fig. 8. Color-opponency in an amacrine cell from a Seliger eel. Above: responses to serial stimulation by light flashes of increasing wavelength (427–648 nm), and of two stimulus intensities (NDF = −0.3 and 0.0); maximal response amplitude at 543 nm. Below: different-type responses to different wavelength flashes (depolarization followed by hyperpolarization in response to a 543-nm light flash; depolarizing response to 427 nm).

4.2. Mixed HCs in the European eel—an exception among teleosts?

In the majority of teleosts, mixed HC-types were never observed: all horizontal cells belonged either to the rod- or the cone-driven type in cyprinids [15,18,19,26,28,30], in percids [6,18,19,30], in carangids [27,30], in pike [18,19], in catfish [26], and in mullets [20]. In contrast, horizontal cells with both rod and cone inputs were described in one elasmobranch (the stingray *Dasyatis akajei*) [32] and one chondrostean fish (the Siberian sturgeon *Acipenser baeri* Brandt) [13]. Among teleosts, mixed HCs were observed with certainty only in *Eugerres plumieri* [16].

Although not overly convincing, a few experiments indicate the existence of mixed-HCs in the retina of anguillids. However, a detailed study of their electrophysiological and spectral properties was never realized. In the American eel (*A. rostrata*) responses of one horizontal cell to photostimuli of different wavelengths showed two time constants of decay, the relative contributions of the 'fast' and 'slow' inputs varying with wavelength [12]. At a fixed response amplitude, the change in response waveform with wavelength was taken by the authors as strong evidence for inputs from at least two separate spectral mechanisms, probably rods and long wavelength cones. In other words, authors concluded that the HC they observed was of the mixed type. However, the actual spectral properties of different rod and cone inputs to the HC were not described with more precision.

We can conclude, therefore, that the presently described presence of numerous mixed horizontal cells in the eel represents a rare and exceptional phenomenon among teleosts.

4.3. Peculiarities of the Seliger eels

Eels were first introduced into Lake Seliger around 1960 [31]. Since Lake Seliger is connected to the river Volga, and the Volga is connected to the Baltic Sea through a system of artificial channels, the pathway to the Sargasso Sea, where European eels are supposed to spawn, seems to be open. Whether Seliger eels use this opportunity and undertake a seaward migration remains unsettled.

Eels from Lake Seliger differed from the considerably smaller yellow eels caught in Adriatic coastal waters by a number of features characterizing the structure of their retina. Rod-driven HCs were conspicuous in Seliger eels, while in Adriatic eels they appeared to be missing, and rod inputs to mixed-HCs were far better expressed in the former. The rod/cone ratio was 40:1 in Seliger eels, as compared to the ratio of 20:1 in the yellow Adriatic eels, and the rod/INL ratio amounted to 6:1 as compared to the ratio of approximately 3:1 in the Adriatic eel retina. These differences were considerably less important than those known to exist between silver and yellow stage eels. In the silver stage, for instance, eyes enlarge dramatically [24,29], new rods are added to the retina [22,23], and the rod/cone ratio increases to 100:1 [5] and even 250:1 [25], becoming thus much higher than in yellow stage eels (35:1 and 79:1, as reported in Refs. [4,25], respectively).

Our electrophysiological and spectral sensitivity data also testify against large differences in developmental stage between our two experimental groups of eels. The fitted spectral sensitivity maxima of individual Seliger eels, ranging from 524.9 to 533.1 nm (mean 530.2 $\pm$ 3.1nm), appeared as even more distant from the p500 maximum in the direction of which λ_{max} is expected to shift during the transition from the yellow to the silver developmental stage.

Therefore, according to all the enumerated criteria our Seliger eels were closer to the yellow (immature) than to the silver stage characteristics, although according to the eye index [24] they should be classified as mature silver eels. The peculiarities of eels from Lake Seliger as compared to eels from the Adriatic region may simply be a consequence of the differences in local conditions for growth. It was emphasized, for instance, in connection with the ichthyological features of Lake Seliger, that the so-called virgin lakes provide conditions for an unusually rapid growth of introduced fish [31]. Conversely, it was found that contrary to expectations eels from the relatively warm southern regions, the Adriatic in particular, do not show an exceptionally rapid growth [11].

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