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Review article

ELECTROPHYSIOLOGICAL AND SPECTRAL PROPERTIES OF RETINAL HORIZONTAL AND BIPOLAR CELLS IN THE EEL

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Several classes of second order neurons have been electrophysiologically explored in immature European eels 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 several 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. 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.

Key words: bipolar cells; European eel; horizontal cells; retinal

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electrophysiology; retinal neurons; spectral sensitivity.

INTRODUCTION

The visual system of eels (*Anguillidae*) is renown for a number of peculiarities linked to the specific features of their life history and migratory behavior, and details of the differences in scotopic spectral sensitivity between different developmental stages of the eel have been well documented. Spectral sensitivity studies (spectrophotometry of pigment extracts and single cell microspectrophotometry) revealed that freshwater eels differ from other freshwater teleosts by possessing, in addition to a vitamin A_2-based pigment (porphiropsin), the vitamin A_1-based rhodopsin, otherwise characteristic of ocean living fishes (Beatty, 1975; Bridges, 1972; Pankhurst, 1982; Wood and Partridge, 1993). In great contrast, however, 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, either spectrophotometrically or electrophysiologically. The photopic spectral sensitivity and the capacity of wavelength discrimination (color vision) in the eel remained largely unknown. Difficulties associated with the electroretinographic approach in cone spectral analysis (Damjanović, 1990; Damjanović, *et al*. 1991; Gordon *et al*. 1978) 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 (Mitarai, 1982; Podugolnikova and Maksimov, 1984; Stell *et al*. 1982). 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 (Gordon *et al*. 1978) and Japanese eels (Niwa, 1979).

MATERIALS AND METHODS

Two groups of European eels (*Anguilla anguilla*) were used and compared in the present experiments. One of the groups consisted of eight yellow stage eels (4^+ - 5^+, L < 40cm) electrofished during winter months in the mouth regions of creeks inflowing into the Boka Kotorska Bay (Southern Adriatic, Montenegro, Yugoslavia; designated as "Adriatic eels" in the text). The second group was represented by seven considerably larger eels (L > 50 cm) captured by means of hoop nets, at the end of October, in the fresh-water Lake Seliger (Russia, Tver region; designated as "Seliger eels" in the text).

For histological analysis eyecups were prepared from eyeballs excised after rapid decapitation of the fish. The preparations were surgically deprived of cornea, lens and most of the vitreous. The eyecups were fixed in the Bouin solution, embedded in paraffin, sectioned into 8 µm thick slices, and stained finally with hematoxylin.

Eyecups were cut into two approximately equal fragments. Each time, the eyecup fragment was stretched, scleral side down, over a plastic ball, fixed in a small platform inside a lightproof Faradey cage. Since residues of the vitreous are known to block the microelectrode tip, the vitreous was expelled by means of forced perfusion with teleost

Ringer of constant temperature (10-12 °C). Microelectrodes (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 fragment and soaked with teleost Ringer. Electrodes were connected to the input stage of a microelectrode preamplifier. The amplified signals were recorded using an X-Y plotter.

For photostimulation, a halogen lamp was used with a series of 8 interference filters (427, 470, 490, 543, 587, 620, 648 and 664 nm), their 50% bandwidth being of the order 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 $\cdot$ mm^{-1} $\cdot$ sec^{-1} . The duration of the photostimuli was 1. 3 ms.

In order to identify cell types, impaled cells were tested using light spot and superimposed annular stimuli. The same test was used to estimate the approximate size of receptive fields of HCs, using light spots of different diameters (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 (1975).

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. In all our figures, S_q values are accompanied with data on r500 and p523 absorption spectra, calculated according to Maksimov (1988).

Experimental eels were characterized by their eye index, according to Pankhurst (1982).

RESULTS

Retinal morphology. In eels from lake Seliger, radial sections of the retina (Fig. 1) showed a highly developed pigment epitelium, its processes descending to the external limiting membrane (ELM). Two types of nuclei were clearly distinguishable in the outer nuclear layer (ONL), those above and below ELM belonging to cones and rods, respectively. Among the latter, electron microscopy revealed 9-10 optically dense sublayers. In Seliger eels, the rod/cone ratio was 40:1, twice as high as in the Adriatic yellow eels (20:1) (Fig. 2). The inner plexiform (IPL) and the horizontal cell layer were relatively thin. Radial sections revealed that horizontal cells (HCs) do not form a compact layer as in retinas of a number of teleosts. The inner nuclear layer (INL) consisted of bipolar (BCs) and amacrine cells (ACs) with large cell bodies (Fig. 1). The total number of cells was significantly smaller in INL than in ONL, and the rod/INL ratio was aproximately 6:1. Considerably fewer cells were present in the ganglion cell (GC) layer 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.

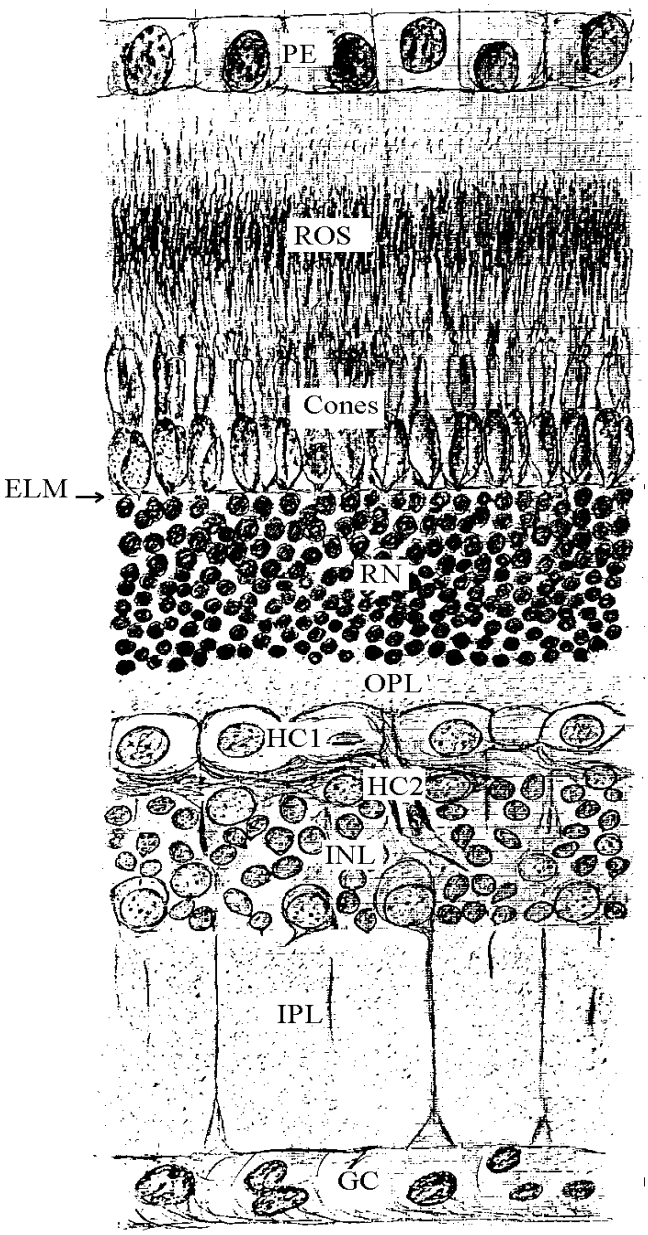

Fig. 1. Retinal morphology in European eels (semischematic drawing of a radial section). ELM: external limiting membrane; GC: ganglion cells; HC1 and HC2: horizontal cells of the first and second layer, respectively; IPL: inner plexiform layer; OPI: outer plexiform layer; PE: pigment epithelium; RN: rod nuclei; ROS: rod outer segments.

Electrophysiological responses of horizontal cell. All horizontal cells explored belonged to the luminosity-type cells (L-HCs). Among 33 such cells explored in Seliger eels, 6 belonged to cone-driven cells (cone- HCs), 9 to the rod-driven type (rod-HCs), and 18 to rod-and-cone-driven cells (mixed HCs). In 25 HCs from yellow Adriatic eels, on the other hand, rod-HCs were not detected at all: 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.

Typical records from the rod-HCs are presented in Fig. 2a. At high stimulus intensities, light offset was always followed by a hyperpolarizing plateau (after-potential). With monochromatic stimuli of $\lambda = 490$ nm, saturating levels were achieved around NDF 0.9 in all rod-HCs explored (Fig. 2b).

The decay of cone-HC responses was much faster than in rod-HCs (Fig. 2b). The afterpotential was not present even at maximal light intensities. Cone-HCs differed from rod-HCs in response saturating levels. In cone-HCs there was no saturation even with white light flashes of maximal intensity (Fig. 2a,b).

In the mixed-HCs, stimulus offset was followed by a fast cone, and then by a slow rod component (Fig. 2a,c). When mixed-HCs were illuminated with different monochromatic backgrounds (red or blue), the shape of the responses changed: only the cone component was observed, as illustrated by the absence of the afterpotential (Fig. 2c).

In the cone-HC response shown in Fig. 2d (right-hand tracing; light spot 0.7 mm in diameter), the strong additional hyperpolarization evoked by annulus stimulation indicated that the receptive field was much larger than 1 mm. The responses of the mixed-HC in Fig. 2c (left-hand tracing; light spot of 0.27 mm), the additional annulus-evoked hyperpolarization was hardly noticeable. These two HCs responses represented two extremes: with the smallest and largest receptive field (right and left tracings, respectively).

Bipolar cell responses. Our results indicate that both on-BCs and off-BCs exist in the retina of the European eel. Annulus illumination, applied after turning on the light spot, evoked responses of opposite sign: depolarization in off-BCs, and hyperpolarization in on-BCs (Fig. 3). Particularly frequent and stable were our recordings from on-BCs in Adriatic eels, enabling the elucidation of their spectral properties (next section).

Spectral sensitivity. In practically all HCs explored, in both Seliger and Adriatic eels, 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. 4a). This strongly indicates that rods and green-sensitive cones contain visual pigments with similar absorption spectra. This indication was further supported by our results concerning mixed HCs (Fig. 4b). Although relatively small, the rod component increased with prolonged (>5 min) dark adaptation (Fig. 4b, 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. 4b, 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,- $0.6 \leq$ NDF ≤ 0.0) and scotopic conditions (low light intensities, NDF ≤ -1.2). Data obtained in these cells were averaged together with those concerning cone and rod HCs, respectively. Fig. 5a concerns averaged data from 6 photopic and 4 scotopic responses. In both cases spectral sensitivity maxima were in the same region, close to

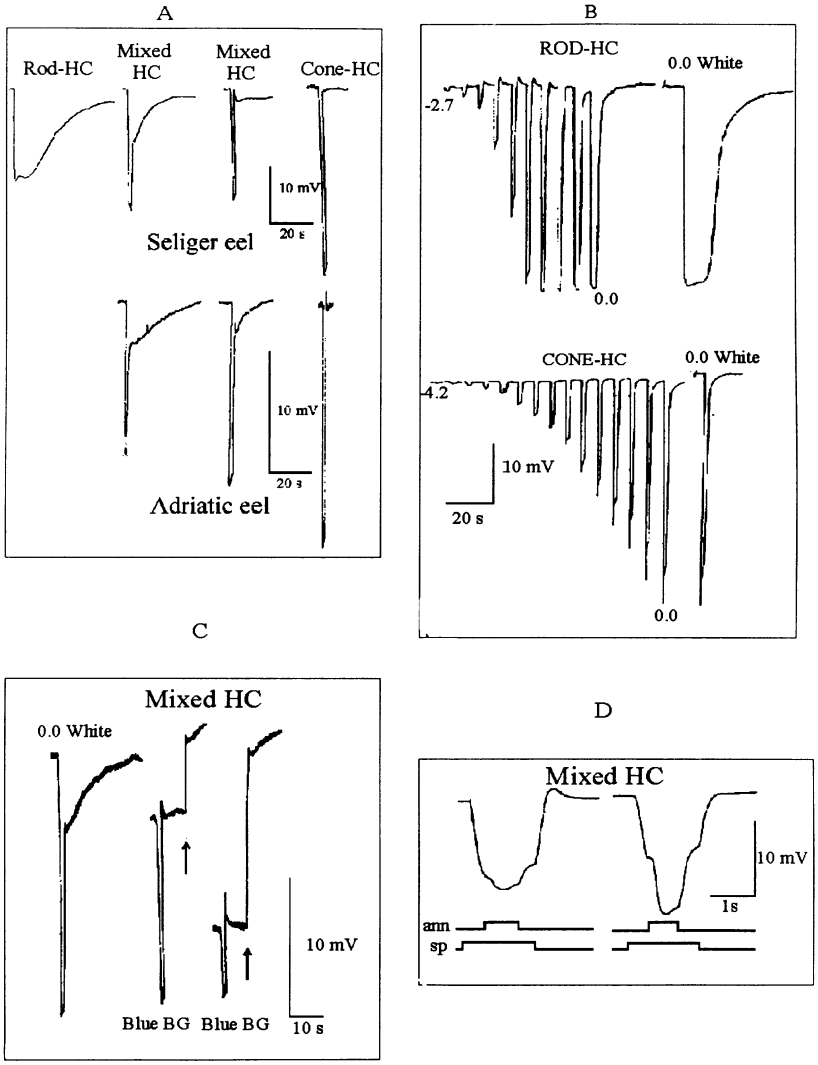

Fig. 2. Responses of horizontal cells (HCs) to various modalities of photostimulation. - (A) Examples of the three types (as indicated) of intracellular records of HCs responses to white light stimuli (NDF=0.0, 1.3 s). Note that in Adriatic eels rod-HCs are lacking and that a substantially greater amplification 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.

the porphyropsin's maximum (523 nm), but the scotopic curve was clearly narrower.

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 at all, spectral sensitivity was analyzed only under photopic conditions in 8 cells. It is obvious

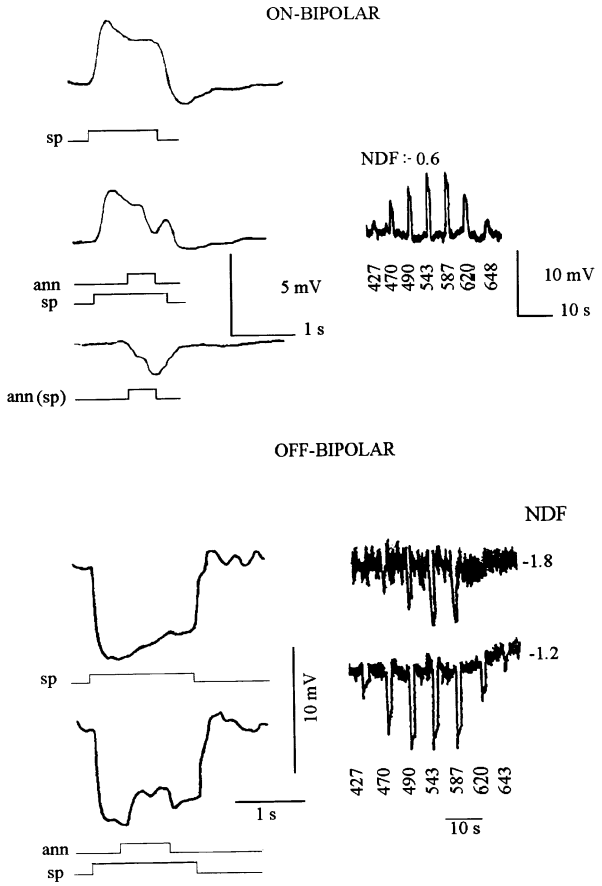

Fig. 3.Responsiveness of bipolar cells. Responses to different stimulation patterns are shown on the left sides of the two panels (sp - spot; ann - annulus; ann(sp) - annulus superimposed upon a continuous spot stimulus), and responses of the same cells to serial flashes of increasing wavelength (427-648 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.

from Fig. 5b that in Adriatic and Seliger eels spectral sensitivity maxima of green-sensitive cones do not differ from each other, being in both cases close to the p523

maximum.

Of particular interest was the finding of a clearly yellow-sensitive horizontal cell in a yellow Adriatic eel (Fig. 5c). It indicated that yet another cone type, in addition to the green-sensitive cones, can be present in the retina of the European eel. This

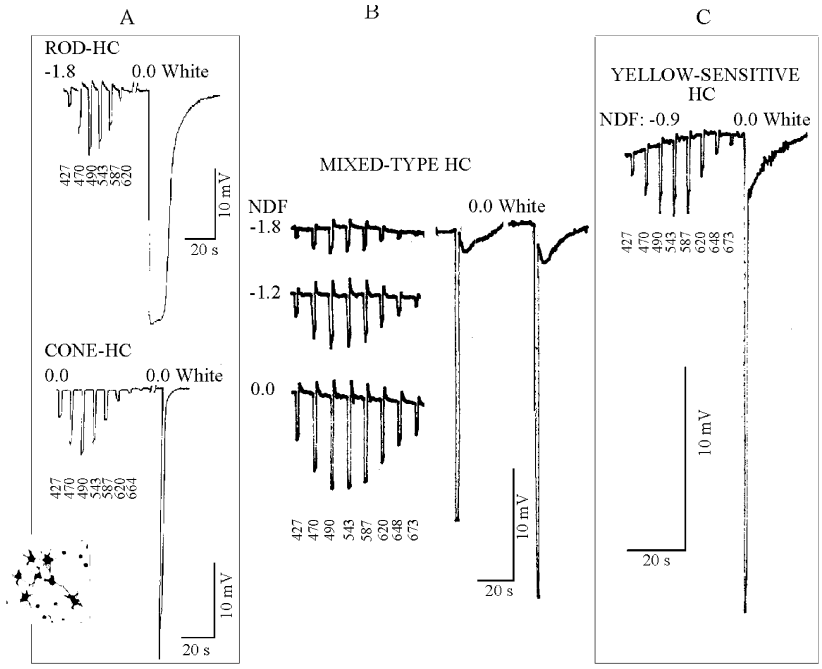

Fig. 4. Spectral responses of horizontal cells (HCs). - (A) Responses of rod- and cone-driven HCs to a series of monochromatic stimuli of increasing wavelength (Seliger eel; numbers below the records refer to nanometers). In both cases maximal responses were recorded with 490 nm flashes. Responses to nonattenuated 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), inset: Lucipher yellow marked sintitium of cone HCs. - (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).

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. 3, right-hand records). In the majority of bipolar cells, responses were of a relatively small amplitude (less than 10 mV) and unstable, not allowing for 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. 5c). It was successfully matched by the iodopsine absorption spectrum (λ_{max}=560 nm).

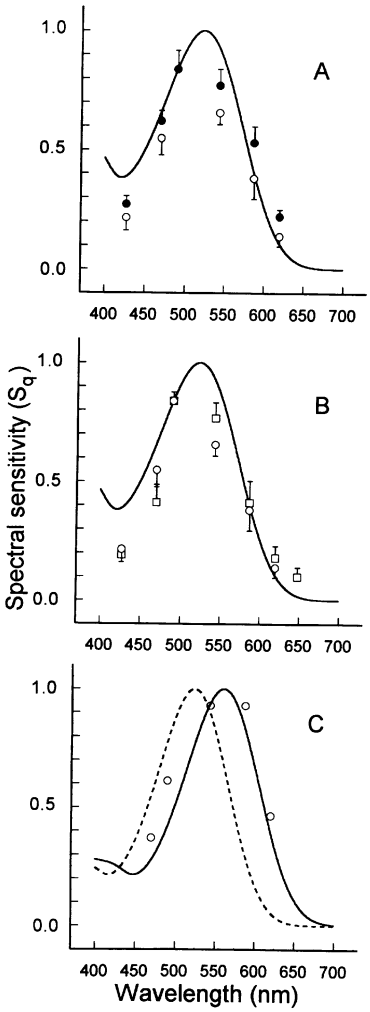

Fig. 5. Action spectra of second order neurons. - (A) Comparison between responses of HCs under photopic (open circles, n=6) and scotopic conditions (closed circles, n=4); means (SE; Seliger eels. - (B) Comparison between HCs of Seliger (open circles, n=6) and Adriatic eels (open rectangles, n=7) stimulated under photopic conditions. Continuous curves in A and B: absorption spectrum of porphyropsin-523 (derived according to (17); see Materials and Methods). - (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 (18)).

DISCUSSION

Inputs of green-sensitive cones to L-horizontal cells appeared as strongly dominant in the European eel. 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). The photopic and scotopic maxima of horizontal cells were both close to the maximum of porphyropsin. The similarity of scotopic and photopic (max values would indicate that eel rods and green sensitive cones 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 (Boettius and Boettius, 1967).

In comparison to findings in other species, our results on the strong dominance of green-sensitive HCs in the retina of eel appears exceptional. In the goldfish, for example, numerous horizontal cells were found to make contacts predominantly with dendrites of red-sensitive cones (Stell *et al*. 1982). In the carp, G-inputs to L-HCs have been identified by means of colorimetric measurements (Orlov and Maksimova, 1965), and by means of intracellular recordings (Naka and Rushtton, 1966). Pure green L-HCs in parallel with red-sensitive HCs were found in two teleosts only: *Eugerres plumieri* (Laufer and Millan, 1970) and *Mugil cephalus* (Mitarai, 1982).

The strong indication, however, 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.

In the majority of teleosts mixed HC-types were never observed: all horizontal cells belonged either to the rod- or the cone-driven type. 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.

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. The differences were considerably less important, however, than those known to exist between silver and yellow stage eels. Our electrophysiological data testified also against large differences in developmental stage between our two experimental groups of eels. Seliger eels were closer to the yellow than to the silver stage characteristics, although according to the eye index (Pankhurst, 1982) they should be classified as mature silver eels.

The peculiarities of Seliger eels 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 (Tesch, 1991). Conversely, it was found that eels from the relatively warm southern regions, the Adriatic in particular, do not show, contrary to expectations, an exceptionally rapid growth (Deelder, 1970).

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ЕЛЕКТРОФИЗИОЛОШКА И СПЕТРАЛНА СВОЈСТВА ХОРИЗОНТАЛНИХ И БИПОЛАРНИХ ЋЕЛИЈА РЕТИНЕ КОД ЈЕГУЉА

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С а ж е т а к

Извршена су електрофизиолошка испитивања код неколико класа неурона другог реда код европских јегуља *Anguilla anguilla* из два удаљена и еколошки различита локалитета (у Русији и Југославији). Највећи број *L*-хоризонталних ћелија (58 испитаних) имало је улазе и штапића и чепића, што је неуобичајен феномен код коштуриба. Карактеристике спектралне осетљивости некиолико хоризонталних и биполарних ћелија указује на присуство и коегзистенцију у ретини европске јегуље чепића осетљивих на жуту и зелену свестлост, као и да штапићи и чепићи осетљиви на зелено садрже сличне пигменте. Уочене разлике у грађи и надражљивости мрежњаче међу јегуљама из два испитивана локалитета, вероватно су последица локалних услова раста и били су мање значајне него разлике између јегуља мрког и сребрног стадијума развића.

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