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HORIZONTAL CELLS OF THE FROG RETINA AS COMPARED WITH SUCH CELLS OF FISH AND OTHER VERTEBRATES

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Since amphibians belong to the intermediate group between fishes and reptiles, some kind of continuity in the organization of retinal networks is expected. Retinal horizontal cells in lower vertebrates reflect partially such continuity. However, amphibian retinas provide some specific features uncommon to both fishes and reptiles. Both *Rana temporaria* and *R. ridibunda* (like *Xenopus, Ambystoma* and *Necturus*) possess axon-bearing and axonless horizontal cells. Unlike in many fishes, axons do not leave the outer margin of the inner nuclear layer and have no well developed axon terminal swellings and branching typical of horizontal cells in other vertebrates studied. Along their length, which varies considerably (from 100 to 800 µm), axons give short branchlets which contact photoreceptor terminals. Physiolog-

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ically, the receptive field size of horizontal cells in *R. ridibunda* retina is quite variable: the length constants measured with a light bar vary from less than 0.1 to 1.3 mm, suggesting a syncytial organization of the horizontal cell network. However, no correlation was found between receptive field size and horizontal cell morphology revealed by means of Lucifer yellow staining. No signs were found of a morphological syncytium of axons or cell bodies, except for the staining of two, occasionally three, adjacent horizontal cell bodies, all of them bearing axons. In both species of *Rana* studied mixed rod-cone inputs were found in many horizontal cells. This is known to occur in urodelae but not in fishes, most of the fishes having either pure rod or pure cone inputs. Horizontal cells of the chromatic (C) type are very uncommon to most of the amphibians studied, and only one such cell was found in *R. ridibunda*. This may indicate that, at least in *Rana*, C-type horizontal cells are not a necessary link in the retinal color processing system.

Key words: cone inputs, frog, fish, horizontal cells, outer retina, rod inputs, spatial properties, syncytium

INTRODUCTION

The horizontal cells (HC) in retinas of different vertebrates have many features in common. Being connected with numerous photoreceptors, they play an important role in the interaction of signals from photoreceptors of various morphological and spectral types, located in different retinal areas. Horizontal cells take part in the spatial processing of signals, as well as in color-coding.

Amphibians belong to a kind of an intermediate group of species between the fishes and the reptiles, so that some kind of continuity could be expected in the horizontal cell organization. Nevertheless, amphibian horizontal cells show some specific features that are not common to fishes and reptiles. Among such features is the spatial organization of the horizontal cell networks.

In all vertebrates studied so far, horizontal cells of the same type are electrically coupled in a syncytial network capable of spreading potentials to long distances (Raviola, 1976; Lasansky, 1980). Horizontal cells of each type form their own independent network. The axon-bearing horizontal cells usually form two separate networks of electrically coupled cell bodies and coupled axon terminals. However, both of the networks are electrically disconnected from each other, in spite of the existence of thin connecting axons. This has been shown in the retinas of the turtle (Byzov, 1975; Lamb, 1976), the tiger salamander (Lasansky and Vallerga, 1975; Lasansky, 1976; 1980) and the cat (Nelson *et al.*, 1975). Each network of cell bodies and of axon terminals has its independent photoreceptor input. In the turtle retina, the axon terminal network has stronger rod inputs than that of cell bodies (Leeper and Copenhagen 1979; Ohtsuka and Kouyama, 1982), while in the cat axon terminals have exclusively rod inputs (Nelson *et al.*, 1975). Both networks also differ in respect to their spatial properties. In the turtle retina for instance, the length constant (λ) of the cell body syncytium is almost three-fold shorter than that of axon terminals (Byzov and Shura-Bura, 1983). Almost all afore said is also true for the fish retina, which only differs by having horizontal cell axons ascending proximally and forming a syncytium of axon terminals in the inner nuclear layer (INL) instead of spreading strictly tangentially. Morphologically, this network has no separate receptor input (Stell, 1975; Parthe, 1982)

and is supposed to receive electrical signals from cell bodies *via* thin axons in contact with both syncytia (Yagi and Kaneko, 1988; Yagi, 1989). In amphibians, especially in anurans, the organization of the horizontal cell network was not studied in such detail. Some years ago, when the present study was started, there was practically no relevant published data, with the exception of the classical morphological work of Cajal (1892). He described horizontal cells of two types in the frog retina: axonless and axon-bearing. Stephen and Weiler (1981) discovered in *R. esculenta* small (3 µm) axon terminals and short axon branches ascending toward photoreceptors. In our physiological experiments (Byzov, 1966), the receptive fields of horizontal cells in the retina of *R. temporaria* were estimated as very small: less than 200 µm in diameter.

An exception among anurans is the *Xenopus*, the retinal horizontal cells of which were extensively studied both physiologically and morphologically. Using Lucifer yellow injections, Hassin and Witkovsky (1983) have found that almost all horizontal cells have long (up to 800 µm) axons with short branchlets ascending to photoreceptors. No dye coupling was observed between horizontal cells in spite of the fact that the length constant of the horizontal cell syncytium, measured by means of a light bar, varied within a wide range and up to 800 µm. Later on, two horizontal cell networks were described (Stone and Witkovsky, 1987), similar to those in other vertebrates: of cell bodies and of axons (170 and 450 µm in diameter, respectively). Using electron microscopy after HRP labeling (Witkowsky *et al.*, 1988b), small gap junctions have been observed between distal parts of axons, as well as between horizontal cell processes invaginating the photoreceptor terminals. It was also observed that axonal and dendritic branchlets approaching photoreceptors may have both rod and cone inputs. The morphology of horizontal cells was also studied in detail in the retina of *R. pipiens*. Using light- and electron microscopy of HRP labeled cells, Ogden *et al.* (1985) found horizontal cells of three types: the inner, axon-bearing horizontal cells with inputs from all four photoreceptor types (the 433 and 502 nm rods, and the 590 and 502 nm cones); the outer axonless giant horizontal cells with dendritic fields of up to 500 µm in diameter; and the outer axonless small horizontal cells with dendritic fields of up to 100 µm in diameter. The outer horizontal cells were more selective in their receptor inputs than the inner horizontal cells. The receptive fields of giant horizontal cells (300 µm) have been found to be smaller than their dendritic fields, so that the problem of a horizontal cell syncytium simply did not exist. As to the horizontal cells of the chromatic (C) type, they are widespread among the fishes studied, being the most variable in the Siberian sturgeon where at least 6 response types were identified (Govardovskii *et al.*, 1991). Two types of C-responses have been found in the turtle retina (Fuortes and Simon, 1974). On the other hand, in most amphibians the C-cells are relatively rare. Until now they have been described in the bullfrog (Naka *et al.* 1960), only a single cell in *Ambystoma* (Byzov and Hanitzsch 1966), in *Necturus* (Fain, 1975), and more often in the frog *R. pipiens* (Ogden *et al.*, 1985). Finally, R/G-type horizontal cells have been found in the *Xenopus* retina (Stone *et al.*, 1990). C-cells of an unusual type have been recently described in *Necturus* (Kim and Miller, 1992). The retinas of two species of anurans, *R. temporaria* and *R. ridibunda*, were presently studied. The main goal was to specify spatial properties of the horizontal cell network by electrophysiological and morphological methods and to reveal their photoreceptor inputs. The results were partially published in an abstract form (Byzov *et al.*, 1994).

MATERIAL AND METHODS

Experiments were carried out with frogs *Rana temporaria* and *R. ridibunda*, caught near Moscow in different seasons. Prior to the experiments, the animals were kept for 1-3 weeks in the refrigerator at 10ºC. After decapitation, the animals were double pitched and the eyes were removed. The eyecup was prepared by removing cornea, lens and vitreous. Due to the intact pigment epithelium, the retina was able to strongly dark adapt during the experiment. All preparations were performed under dim white light. In some of the experiments, the eyecup was superfused with a cooled (14°C) Ringer solution with bicarbonate buffer (in mM: NaCl - 115, KCl -2.6, $NaHCO_3$ - 12, $CaCl_2$ - 2, $MgCl_2$ - 1, glucose - 5; pH 7.6 adjusted by HCl). During experiments lasting 2-3 hours, pH usually increased by no more than 0.2 units. In other experiments, after careful removal of the vitreous the eyecup was placed in a cold (14 -16°C) moist chamber.

Intracellular responses were recorded with micropipettes filled with potassium acetate (1 M) or with 4% solution of Lucifer yellow (LY-CH, Sigma). In the last case, the resistance of microelectrodes was usually more than 100 MΩ. The dye was ejected from the microelectrode by brief negative current pulses of 0.5-5 nA, applied during 5-10 min. For the identification of photoreceptor and horizontal cell responses during the experiment, we used the depth of the microelectrode tip and the responses to a small white spot (0.21 mm in diameter) and a superimposed annulus (0.24 and 2.0 mm in inner and outer diameter, respectively). We were thus able to distinguish horizontal cells from photoreceptors and the bipolar cells (in the last case, the annulus evoked a response of opposite polarity), and also to estimate the approximate size of the receptive field. Spatial properties were measured with more precision by means of a long light bar (2 mm in length and 0.1 or 0.2 mm in width) displaced in steps of 0.1 or 0.2 mm in the direction orthogonal to its length. This procedure, applied before in studies of the turtle rod syncytium (Detwiler *et al.*, 1978) and the horizontal cells of the turtle retina (Byzov and Shura-Bura, 1983), gives an estimate of the length constant (λ) of the syncytium. The response to the bar decreased exponentially with the distance from the microelectrode (see Fig. 3a), being the distance at which the response decreased *e*-times.

The results of such measurements are strongly influenced by stray light. Light scattering is not only due to optical defects, but also to the reflecting particles on the retinal surface, to the fact that the borders of the eyecup reflect the light bar, to the microelectrode itself, etc. To reduce the effect of scattered light, the light bar was presented against a constant diffuse background light, strong enough to induce a hyper-polarization not smaller than the maximal response induced by the light bar. With weaker backgrounds the estimated length constant increased (see Fig. 3b), probably because of the stray light effect (see later in more detail).

Spectral characteristics of horizontal cells and photoreceptors were measured as in our previous experiments with sturgeons (Govardovskii *et al.*, 1991). The retina was stimulated with flashes of different wavelength (using a set of interference filters, from 427 to 648 nm) equalized with neutral density filters by quantum content. Based on the principle of univariance, the photoreceptor spectral response curves were recalculated to spectral sensitivity curves, using the *R-logI* relationship measured separately. The same procedure applied to horizontal cells with mixed photoreceptor inputs is not entirely correct, because the relative inputs from photoreceptors of different type can vary with light intensity. However, it is precisely these variations in spectral characteris-

tics that show the existence of mixed rod-cone inputs to horizontal cells. The maximal white-light intensity corresponded to $3.2x10^3$ quanta mm^{-2} sec^{-1} (taking *log I=0.0*). In all figures the light intensities are expressed in neutral density filter units.

Morphological studies were carried out in two ways. Horizontal cells of *R. ridibunda* were impregnated by the Goldgi method (details have been described by Podugolnikova, 1985). After silver impregnation, the retina was dehydrated through a graded series of alcohols, cleared in acetone and embedded in soft epon. Radial and tangential sections were cut (50-70 and 120 µm in thickness). Well-stained cells were reproduced by means of a drawing tube with a x30 objective under water immersion, the final magnification being x450. The cells stained in electrophysiological experiments with Lucifer Yellow, were examined and photographed under a luminescent microscope LUMAM-2, in flat-mount preparations. The isolated retina was placed on the glass plate receptor side up, and the layer of photoreceptor outer segments with traces of pigment epithelium was carefully removed by means of a strip

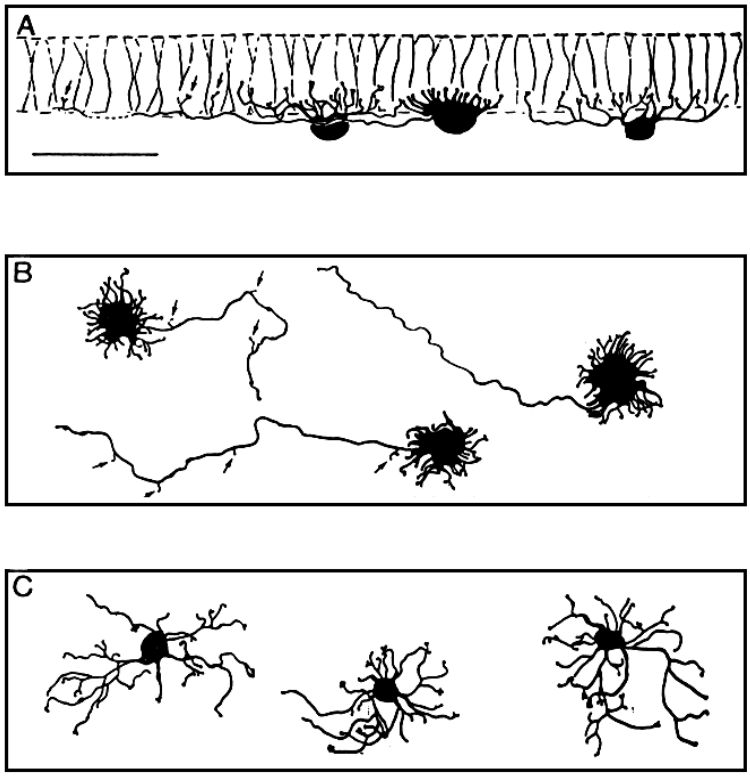

Fig.1. Horizontal cells in the retina of Rana ridibunda stained by the Goldgi method. **A.** *Camera lucida drawings from radial sections. One axon-bearing cell is shown (H1) with the axon covering horizontally some distance and giving short branches ascending to rod and cone terminals (shown by arrows), and two axon-less horizontal cells (H2) with dendrites terminating close to photoreceptor terminals. Calibration 50 µm.* **B.** *Three axon-bearing horizontal cells in tangential sections; arrows indicate branches with buttons at the ends ascending to photoreceptor terminals.* **C.** *Three axon-less cells in tangential sections.*

of thin filter paper. This procedure did not usually damage retinal layers proximal to the external limiting membrane, and did not introduce significant topographic distortions. At the same time, it strongly improved the clarity of the stained cells.

RESULTS

Morphology of horizontal cells stained by the Goldgi method.

Two types of horizontal cells were found in the retina of *R. ridibunda*, similar to H1 and H2 in *R. esculenta* (Stephen and Weiler, 1981). H1 are axon-bearing cells with soma size of 10-18 µm. We studied 186 cells of this type. Their numerous very short dendrites emerge from the distal flat surface of the soma and terminate by one or two "buttons" at the level of photoreceptor terminals in the OPL (Fig.1). These buttons represent probably the lateral elements in the photoreceptor synaptic triads. The dendrites emerging laterally from cell bodies spread tangentially and terminate at the same level in the OPL. The diameter of the dendritic field is usually no more than 50 µm. The axon emerging from one of the lateral dendrite or from the soma extends to a long distance without collaterals. Along its length it forms varicosities and gives short branches ascending to rod and cone terminals, as seen in radial sections (Fig.1a). In axonless horizontal cells (H2) the round-shaped soma is smaller (8-12 µm). Five to six first-order dendrites, after emerging from the soma, branch dichotomically once or

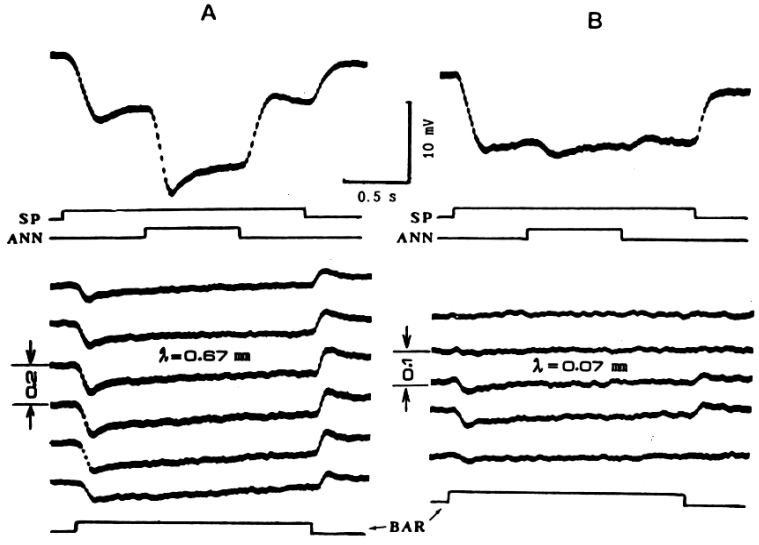

*Fig.2. Examples of two horizontal cells, one with a large (**A**) and the other with a small (**B**) receptive field. At the top are the responses to a light spot 0.21 mm in diameter and superposed light annulus (inner and outer diameters are 0.24 and 2.0 mm respectively). Below are the responses of the same horizontal cells to a light bar 0.2 mm wide in (A) or 0.1 mm wide in (B) given against a steady background. The light bar was displaced, with respect to the recording microelectrode, in steps of 0.2 mm (A) or 0.1 mm (B). Length constants (λ) of the horizontal cell syncytium, measured with the light bar (see Fig.3a), are indicated.*

twice and terminate with a button-like structure, or with clusters of 2-6 buttons 1 µm in diameter. The dendritic tree is rather wide (50-95 µm in diameter). A total of 23 H2 cells were stained. The morphology of the cells stained with Lucifer Yellow will be described later.

Spatial properties of horizontal cells

In the present work, most experiments with horizontal cell receptive field measurements concerned the central region of the retina in *R. ridibunda*. Receptive fields were found to be very variable in size. Figure 2 shows two extreme examples: with a big (A) and a small (B) receptive field. The difference is clearly seen in responses to the spot and annulus: in cell A, the annulus evoked an additional strong hyperpolarization; in cell B, only a small one, *i.e*. the spot almost completely covered the receptive field. More accurate measurements of the spatial properties of the same cells are shown below. For the cell with a big receptive field, the 0.2-mm wide light bar was displaced in 0.2-mm steps. The response amplitude declined gradually with increasing distance from the microelectrode, demonstrating the wide spread of potentials. On the contrary, in the horizontal cell with a small receptive field, the spread of potential was minimal: with a narrow (0.1 mm) light bar displaced in small (0.1 mm) steps, the response declined abruptly on both sides of the receptive field center. Fig. 3a shows the logarithm of response amplitude as a function of light bar position in respect to the receptive field centre (centres are superposed for both cells). The length constants (λ) of horizontal cell syncytium were calculated from the slope of lines through the experimental points (Byzov and Shura-Bura, 1983):
where V_0 is the response amplitude at the light bar edge, V_x - the same at distance x

$$\lambda = \frac{x}{\ln\left(\frac{V_0}{V_x}\right)}$$

from the edge. The calculated values are 0.67 mm for cell A in Fig. 2, and 0.07 mm for cell B.

As indicated in the Methods, accurate measurements of the receptive field size require the elimination of the effect of stray light. This can be achieved by presenting the light bar in the presence of a constant background light. However, if the background is not bright enough, the visible size of the receptive field can be overestimated. This is shown in Fig. 3b. A 0.1-mm wide light bar was moved across the receptive field in steps of 0.1 mm, and responses to the onset were recorded for each bar position. When recording was terminated, the background light was turned off to estimate its relative intensity (vertical arrows). With a background of weak intensity, which evoked a hyperpolarization amounting to less than a half of the amplitude of the maximal response to the light bar, the calculated λ was 0.27 mm (upper trace). After increasing the background intensity, the length constant was reduced more than twice (0.1 mm; lower trace). This decrease in λ could, in principle, be interpreted as being the result of a stronger light adaptation, accompanied by receptive field "shrinkage". However, in our previous experiments with turtle horizontal cells, where the luminance level was changed more than 200 times (but in such a way that the ratio of light intensities of the bar and of the background was kept constant), the size of the receptive field was practically invariable (Byzov, 1966). Therefore, the apparent increase of λ with

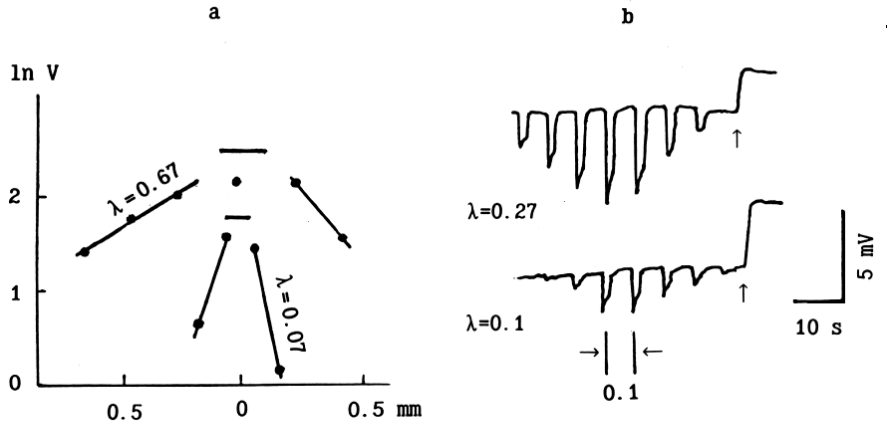

Fig.3. **a.** *The amplitude of horizontal cell responses shown in Fig.2 (in the logarithmic scale) as a function of distance of the light bar from the recording microelectrode. The length constant λ was calculated according to Byzov and Shura (1983)(see text).* **b.** *The responses and estimated length constants of horizontal cell syncytium under different backgrounds. With a background of weak intensity (upper trace) which evoked hyperpolarization less than the maximal response to the bar, the calculated λ was 0.27 mm. But with higher background intensities (lower trace) λ dropped to 0.1 mm probably due to the effect of stray light. Vertical arrows indicate the moments when the backgrounds were turned off.*

background decrease (Fig. 3b) should be related to the stray light effect rather than to adaptation.

The distribution of length constants in all horizontal cells tested (n=46) is shown in Fig. 4. Most measurements concerned the *R. ridibunda* retina. In different horizontal cells the spatial summation varied strongly, and it was hardly possible to divide them into compact groups. The hatched bars (n=9) show the horizontal cells where Lucifer Yellow staining was successful. In Fig.5, drawings of 6 cells (out of 9) are superimposed. The stained cells had axons of different length, sometimes bifurcating (two of them are shown in Fig.5). The axons bear short branchlets terminating with small "knobs" (arrowheads). There are also many knobs along the length of the axon. The

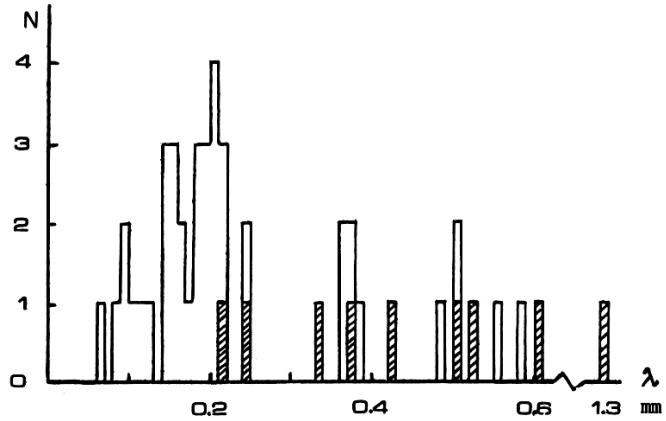

Fig.4. Histogram of length constant distributions in all horizontal cells studied in R. ridibunda. Hatched bars (N = 9) show horizontal cells where Lucifer yellow staining was observed.

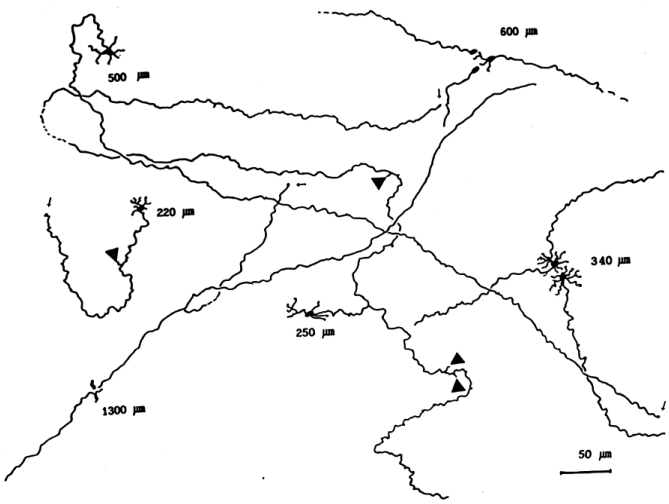

Fig.5. Combined drawing of six horizontal cells stained with Lucifer yellow in flat-mount retinal preparations of R. ridibunda. Figures adjoining the cell bodies give the length constants measured electrophysiologically in the same cells. Arrowheads show short axon branches ascending to photoreceptor terminals; thin arrows show terminal swellings of axons.

axons terminate (when it was possible to trace them) by small expansions (thin arrows). Often, two or even three cell bodies were stained with one injection (examples are shown in Fig.5), their axons usually diverging in opposite directions. In many respects similar observations have been made in retinas of *R. esculenta* (Stephen and Weiler, 1981) and *Xenopus* (Witkovsky *et al.*, 1988).

By studying the spatial properties of horizontal cells we attempted to understand how their receptive fields are organized, since they often strongly exceed their dendritic fields. This attempt was unsuccessful. Although we labeled two or three horizontal cell bodies with short dendrites, we could never observe the diffusion of the dye to the neighboring horizontal cells. Such an observation is not an argument against electrical coupling between horizontal cells: Lucifer Yellow does not diffuse through gap junctions between photoreceptors, although the latter are syncytially organized (Baylor *et al.*, 1971; Detwiler *et al.*, 1978).

All the successfully stained horizontal cells were axon-bearing. As seen in Fig. 5, the length and the course of axons are quite variable in different horizontal cells. No correlation could be found between axon size, its course and the length constants (figures adjoining the cells) measured electrophysiologically.

Photoreceptor inputs to horizontal cells

Rods. Among the three well-known spectral types of photoreceptors in the frog retina (the common or "red" rods, 502 nm; the cones, 561 nm; and the "green rods", 440 nm), we succeeded in recording the intracellular light responses only from the common rods. Rods were identified according to physiological criteria as well as with Lucifer Yellow. In total, 10 rods were recorded in *R. ridibunda* and the fluorescent label was found in three of them.

Figure 6a shows rod responses to the light of three intensities. The response to the highest available intensity shows a long-lasting afterpotential. This property of the rod response is important for the identification of rod inputs to horizontal cells. The duration of the afterpotential in the dark-adapted rod, following the brightest white flash (ND = 0.0), was up to 20-30 seconds (Fig. 6b). Fig. 6c illustrates the response of

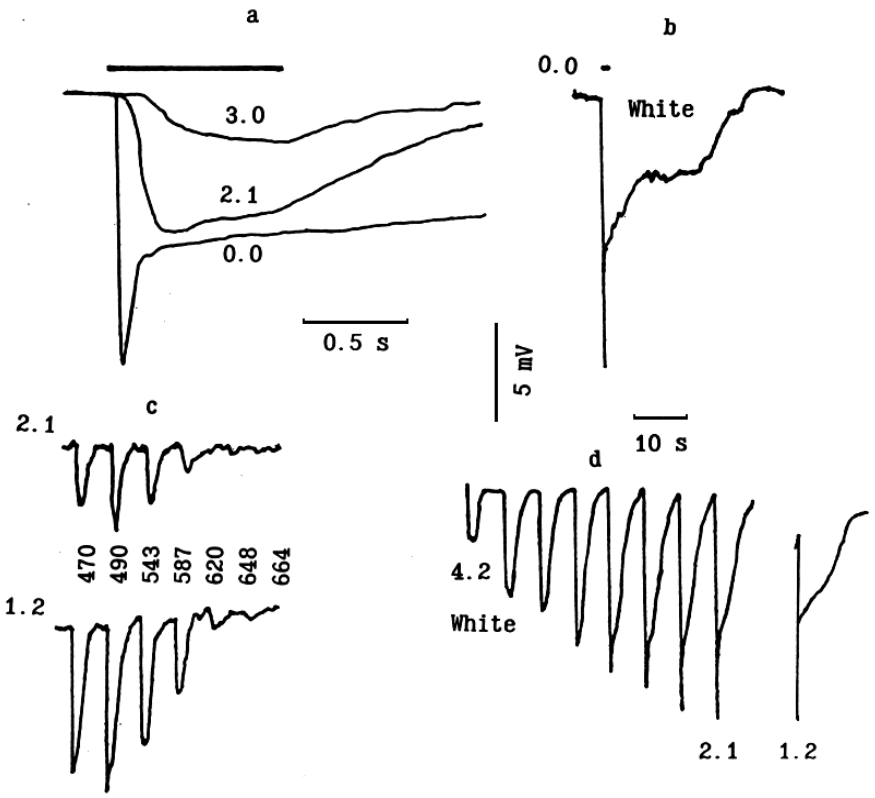

Fig.6. Properties of rod responses in Rana ridibunda retina. ***a.*** *Rod responses to the light of three intensities; prolonged after-hyper-polarization is seen after the brightest light flash.* **b.** *Response to the brightest flash in a different rod; note that the time scale is strongly compressed.* **c.** *Responses to equal quantum stimuli of different wavelengths with two different light intensities.* **d.** *Response R-log I relationship of the same rod as in b - c: the intensity of white light was increased, from flash to flash, in 0.3 log steps.*

the same rod to equal-quantum stimuli of different wavelength at two light intensities (ND = 2.1 and 1.2), and Fig. 6d displays the *R-logI* curve (for the white light) necessary for the calculation of the spectral sensitivity curves. The rod response, particularly its slow component, saturates with high light intensities. It is seen from the spectral response curves (Fig. 6c) that, in accordance with the univarians principle, the increase of light intensity did not alter the position of the response maximum. The same was confirmed by calculating the spectral sensitivity curves in all the rods tested. In their long- wave part (Fig.7), they satisfy the nomogram P502 calculated by the procedure

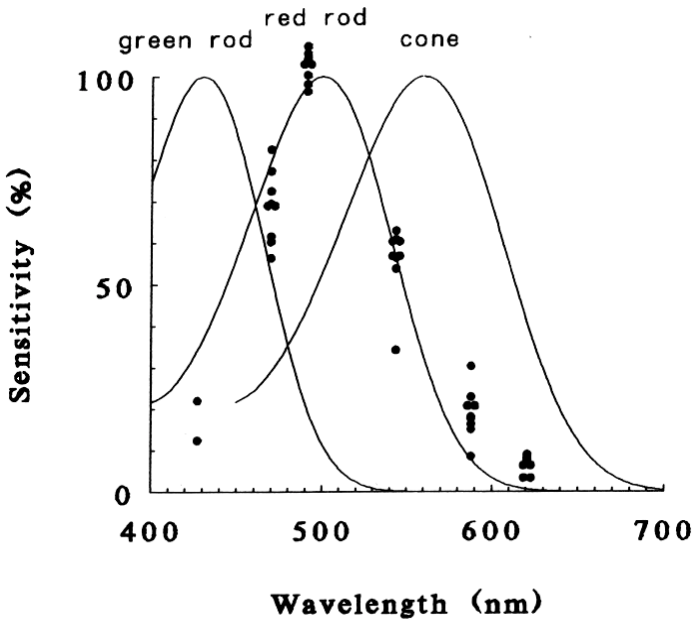

Fig.7. Spectral sensitivity of rods in the retina of R. ridibunda. The curves "red rod", "cone" and "green rod" are nomograms of pigments 502, 560 and 460 nm calculated according to Maximov (1988). Points represent experimentally determined spectral sensitivity of rods calculated from equal quantum spectral responses and R-log I measurements.

of Maximov (1988), based on a modification of the Dawis formula (Dawis, 1981). However, in the short-wave part, the experimental points are systematically below the absorption curve P502. A similar disagreement between photoreceptor absorption spectrum and spectral sensitivity measured electrophysiologically, has been observed previously in the isolated carp retina (Kaneko and Tachibana, 1985) and the sturgeon eyecup preparation (Govardovskii *et al.*, 1991). The reason for this remains unknown, and we were never successful in obtaining experimental records from red cones or green rods.

Horizontal cells. In the first experimental series we paid no special attention to the adaptational state of the preparation, as all horizontal cells studied seemed to be connected with cones only. This misinterpretation was supported by the fact that even in the light-adapted state we succeeded in recording normal responses from the rods with an on peak and a well expressed afterpotential. However, it was observed later that many horizontal cells do have rod inputs after prolonged (tens of minutes) dark adaptation. Fig. 8 shows spectral responses of a cone-dominated horizontal cell at three adaptational levels: ND = 1.2, 0.6 and 0.0. The dominance of the cone inputs is demonstrated by the independence of the position of the spectral response maximum (543 nm) from the light intensity level and by the absence of the prolonged after-potential following the brightest white flash. An example of a horizontal cell with mixed cone-rod inputs is shown in Fig. 9. The Purkinje-shift can be clearly seen: with the increase of the light intensity from 1.2 to 0.0, the maximum of the spectral response

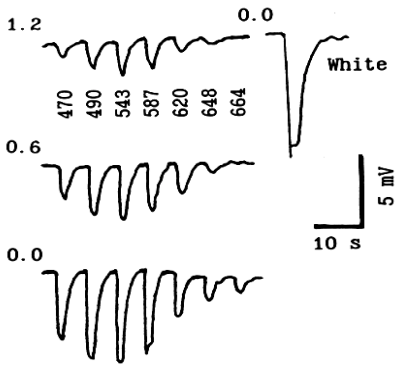

Fig.8. Spectral response curves of a cone horizontal cell observed at three light intensities. The response of the same cell to a white flash of maximal intensity is shown in the upper right corner, demonstrating that there is no prolonged afterpotential typical for rod-mediated response.

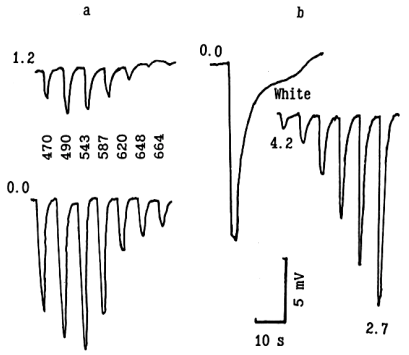

Fig.9. Example of a horizontal cell with mixed rod-cone inputs. The spectral response curves (a) reveal a shift of spectral maximum to longer wavelengths under high light intensity (0.0) in comparison with lower intensities (1.2). The afterpotential in response to the brightest white flash (b) comprises two components: a fast cone and a slow rod part. (c) R-logI relationship of the same cell to white flashes.

moved from 490 nm to 543 nm (Fig. 9a). Moreover, the response to the strong white light has a prolonged after-potential component (Fig. 9b).

In frogs, we have never observed pure rod horizontal cells similar to those of the carp (Kaneko and Yamada, 1972) or sturgeon (Govardovskii *et al.*, 1991). Also, we did not observe presently responses of the color-opponent type. A horizontal cell of the R/G type was found just once in our previous experiments on the *Rana ridibunda* eyecup (Fig.10). Evidently, its depolarizing response was mediated by cones 562 (there are no other long-wave receptors in the frog retina), and the hyperpolarizing response by red and/or green rods.

Fig.10. The response of chromatic (R/G) type recorded only in one instance out of many hundreds of horizontal cell recordings in the retina of Rana ridibunda.

DISCUSSION

The results of the present work did not provide adequate answers concerning the organization of the horizontal cell syncytium in the frog retina. On one hand, the wide receptive fields of many horizontal cells, appreciably exceeding their dendritic fields, imply an electrical coupling between horizontal cells. Electrical (gap) junctions have been found in *Xenopus* between distal parts of axons and processes inside the invaginations of horizontal cell to photoreceptor synaptic terminals (Witkovsky *et al.*, 1988). Unlike in fishes and turtles, however, Lucifer Yellow did not diffuse across these junctions both in *Xenopus* (Hassin and Witkovsky, 1988) and in frog retinas (if such junctions exist in the species studied). In all the vertebrates studied so far the axon-bearing horizontal cells of the L-type form two syncytia: of cell bodies and of axon terminals. In turtle and cat retinas, each syncytium has its own synaptic input from photoreceptors. In the frog retina there is no well-expressed axonal arborization, but unlike in other animals, the axons produce short branches along their length that make contacts with photoreceptors, presumably inside the ribbon invaginated synapses (Fig.1; see also Winslow *et al.*, 1989). This makes the existence of an axon terminal syncytium in the retina of *Rana* species improbable. Two syncytia with mean length constants of 450 µm (axon terminals) and 170 µm (cell bodies) were described physiologically and by Lucifer Yellow staining in *Xenopus* (Stone and Witkovsky, 1987; Hassin and Witkovsky, 1983). However, in the histogram of horizontal cell length constants shown by Stone and Witkovsky (1987) one could hardly see two compact groups like in the turtle retina (Byzov and Shura-Bura, 1983). The distribution of horizontal cell length constants similar to the one in *Xenopus* was observed also in *Rana* (Fig.4), but we have found it impossible to divide them into two separate groups. Most likely, some form of continuum exists, from very small to broad receptive fields. Thus, although the existence of horizontal cell syncytium in the frog retina seems obvious, its specific organization remains obscure in many respects.

In this study, started more that 15 years ago, we had no opportunity to use the recently developed tracer biocytin (Vaney, 1993). This could help in clarifying the problem of the syncytial organization of horizontal cells. One should remember, however, that the dye coupling does not necessarily correlate with electrical coupling. For instance, Lucifer Yellow coupling between cell bodies and axon terminals of the same horizontal cells in the turtle retina does not correlate with the absence of electrical coupling between them (Byzov, 1975; Byzov and Shura-Bura, 1983). It is of some interest to compare the differences in receptive field sizes of horizontal cells within anurans. As already mentioned, in *Xenopus*, according to Hassin and Witkovsky (1983), variations of horizontal cell length constants are similar to those reported for *R. ridibunda* in the present study. This can be compared with our early measurements in *R. temporaria* (Byzov, 1966). Under experimental conditions, which exclude the effect of stray light, the receptive field sizes in 15 out of 19 horizontal cells were less than 0.2 mm, and in only one horizontal cell it was 0.4 mm. The length constants should be 2-3 fold less than receptive field diameters (Lamb, 1976). Therefore, in *R. ridibunda* the receptive field sizes are probably much broader than in *R. temporaria*. According to Ogden *et al.*, (1984; 1985), *R. pipiens* has the most abundant set of horizontal cells: inner cells and two types of outer cells. The morphology of these cells,

including synaptic contacts with photoreceptors of different type, was studied in detail. However, physiological measurements of receptive fields were performed only on the outer giant horizontal cells of the chromatic type (Ogden *et al.*, 1985). The receptive field size, about 0.3 mm in diameter (length constant about 0.1 mm), was found to be even smaller than the dendritic field sizes. Therefore, the problem of electrical coupling between horizontal cells did not arise at all.

As to photoreceptor inputs to horizontal cells in the frog retina, our results are not unexpected. Only few horizontal cells had predominantly cone inputs after prolonged dark adaptation (Fig. 8). In most other cells dark adaptation revealed also rod inputs. This corresponds to our morphological finding that axon-bearing horizontal cells have synaptic contacts with both cones and rods. Surprisingly, we failed to reveal green rod inputs to horizontal cells, as well as the green rods themselves, although at the level of ganglion cells the participation of green rods is clearly discernible (Stell, 1975).

Another result is noteworthy. In the light adapted state, all horizontal cells in the *Rana* retina have only cone inputs. The same is true for *Xenopus* retina (Stone and Witkovsky, 1987). On the other hand, in the sturgeon retina pure rod horizontal cells could be found regardless of the state of adaptation (Govardovskii, *et al.*, 1991). We observed the same in the eel retina (Byzov *et al.* in preparation). Finally, typical responses of rods in the frog retina were recorded not only in the dark-adapted state, but also under moderate light adaptation. This suggests that in horizontal cells with mixed rod-cone inputs the weight of the rod inputs (but not the light responses in rods themselves) can be regulated. In particular, they can be depressed by light adaptation. Data on the *Xenopus* retina even suggest the mechanism of such a regulation, mediated by dopamine (Witkovsky *et al.*, 1988, 1989) and/or GABA and glycine (Witkovsky and Stone, 1987).

The problem of the rod/cone input ratio in the mixed visual pathways (at some level of the visual system they are inevitably mixed) is very important for all vertebrates including humans. On one hand, photophobia in rod monochromats indicates the lack of the normal strong inhibitory influences on the rod system from the cone system. On the other hand, it is known that even in daylight the rod signals are not cut off completely, but take part, together with cone signals, in color vision (Bongard and Smirnov, 1956; Bongard *et al.*, 1957; see also Brindley, 1970). Probably, the balance between rod and cone inputs under different adaptational states is realized at several levels of the visual system. At least two of them are localized in the retina: in the outer plexiform layer at the level of horizontal cells, and in the inner plexiform layer where the axons of pure rod bipolars are connected with cone bipolar cells *via* amacrine cells AII (cat and rabbit retinas; Kolb and Famiglietti, 1974; Strettoi *et al.*, 1992, 1994).

Of great interest are the horizontal cells of the chromatic type. The detection of such cells in *Rana ridibunda* (Fig.10), albeit a single one, similar to those in the larval *Ambistoma* (Byzov and Hanitzsch, 1966) and *Necturus* (Fain, 1975), makes these amphibians more similar to other lower vertebrates. The fact that these cells are rarely found can hardly be ascribed to the deficiencies of the method: the same technique applied by the same authors to fish and turtle retinas readily revealed the chromatic horizontal cells of different types (Byzov and Hanitzsch, 1966; Govardovskii *et al.*, 1991; and many others). At the same time, frogs and toads show some features of color vision especially evident in their reproductive behavior (Kondrashev *et al.*, 1976). This can indicate that chromatic type horizontal cells are not an obligatory link in color signal processing. According to the conception of Stell (1975) and Fuortes and Simon (1974), it is the L-type horizontal cells, not the C-type, that plays the key role in color

opponency. The absence of chromatic horizontal cells in mammals, which are characterized by excellent color vision, can support this notion. This problem deserves special studies.

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ХОРИЗОНТАЛНЕ ЋЕЛИЈЕ ЖАБЉЕ МРЕЖЊАЧЕ У ПОРЕЂЕЊУ СА ТАКВИМ ЋЕЛИЈАМА РИБА И ДРУГИХ КИЧМЕЊАКА

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Пошто водоземци припадају прелазној групи од риба ка гмизавцима, треба очекивати неку врсту континуитета у организацији ретиналних мрежа. Хоризонталне ћелије мрежњаче код нижих кичмењака делимично одражавају такав континуитет. Па ипак, мрежњаче водоземаца показују извесне специфичности које нису присутне ни код риба ни код гмизаваца. *Rana temporaria* и *R. ridibunda* (као и *Xenopus*, *Ambystoma* и *Necturus*) поседују хоризонталне ћелије без и са аксоном. За разлику од многих риба, аксони не напуштају спољашњу ивицу унутрашњег нуклеарног слоја и немају добро развијена завршна аксонска задебљања, нити гранања типична за хоризонталне ћелије других изучаваних кичмењака. Дуж аксона, чија дужина јако варира (од 100 до 800 *mm*), одвајају се кратки огранци који ступају у контакт са завршецима фоторецептора. У физиолошком погледу, величина рецептивног поља хоризонталних ћелија код *R. ridibunda* јако варира. Дужинске константе, мерене техником "светлосне шипке", варирају од <0.1 до 1.3 *mm*, указујући на синцицијалну организацију хоризонталних ћелија мреже. Није, међутим, констатована корелација између величине рецептивног поља и морфологије хоризонталних ћелија утврђене бојењем Луцифер жутим. Нису нађени знаци морфолошког синцицијума аксона или ћелијских тела, изузев бојења два, понекад три, међусобно додирна тела хоризонталних ћелија, која сва поседују аксоне. Мешани штапићно-чепићни улази нађени су у многим хоризонталних ћелија код обе изучаване врсте *Рана*. То је иначе познато код репатих водоземаца, али не и код риба, код којих су у већини случајева присутни било чисто штапићни, било чисто чепићни улази. Хоризонталне ћелије хроматског (*C*) типа врло су неуобичајене код већине изучаваних водоземаца, а само је у једном случају таква ћелија констатована код *Р. ридибунда*. То вероватно указује да *C*-тип хоризонталних ћелија не представљају неопходну компоненту ретиналног система за колорну обраду, бар у случају мрежњаче *Rana*.

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