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Color properties of the motion detectors projecting to the goldfish tectum: I. A color matching study

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Responses of direction-selective and orientation-selective motion detectors were recorded extracellularly from the axon terminals of ganglion cells in the superficial layers of the tectum opticum of immobilized goldfish, *Carassius gibelio* (Bloch, 1782). Color stripes or edges moving on some color background (presented on the CRT monitor with known emission spectra of its phosphors) served as stimuli. It was shown that stimuli of any color can be more or less matched with the background by varying their intensities what is indicative of color blindness of the motion detectors. Sets of stimuli which matched the background proved to represent planes in the three-dimensional color space of the goldfish. A relative contribution of different types of cones to the spectral sensitivity was estimated according to orientation of the plane of color matches. The spectral sensitivity of any motion detector was shown to be determined mainly by long-wave cones with a weak negative (opponent) contributions of middle-wave and/or short-wave ones. This resulted in reduced sensitivity in the blue–green end of the spectrum, what may be considered as an adaptation to the aquatic environment where, because of the substantial light scattering of a blue–green light, acute vision is possible only in a red region of the spectrum.

Keywords: Goldfish; color vision; retina; ganglion cells; tectum opticum; motion detectors; direction selectivity; orientation selectivity.

1. Introduction

The optic tectum or superior colliculus processes image motion and takes part in orienting responses revealed in rapid eye, head and/or body movements. This visual function can be accomplished without taking into account information about color, and the superior colliculus in mammals has been shown to be color blind, whereas color-opponent retinal projections use geniculostriate pathway (Michael, 1973; Schiller & Malpeli, 1977). In the frog, the system carrying chromatic information also originates as a channel separate from the tectal pathway that provides the main information needed for frog's reactions to moving objects (Muntz, 1962a,b; Maximov *et al.*, 1985). The first experiments with color stimuli in many fish species (Maximova

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et al., 1971) have also shown that a good dozen of different types of ganglion cells (GCs) projecting to the tectum does not have marked color-coding properties.

Retinal GCs that detect perceptually significant features of the retinal image form an ordered retinotopic projection in the fish tectum opticum. Their axons terminate at different depths in the tectum, what is reflected in ordered sequences of responses recorded in single-unit electrophysiological studies (Jacobson & Gaze, 1964; Zenkin & Pigarev, 1969; Maximova *et al.*, 1971). Those units that are recorded in the most superficial layers of the visually responsive zone have the property of the directional selectivity. These detectors respond to the stimuli moving in a particular (preferred) direction and give no response to the stimuli moving in the opposite or null direction. On the grounds that the application of a synaptic transmission blocker to the tectal surface does not eliminate responses of these units, it was concluded that these responses originate from the terminals of optic nerve fibers (Maximova *et al.*, 2012). Unlike similar GCs in the rabbit retina, which comprise four physiological subtypes with different preferred directions aligned with the horizontal and vertical ocular axes (Oyster & Barlow, 1967), direction-selective (DS) GCs in the fish retina are divided into three distinct groups preferring either caudo-rostral, ventro-dorsal or dorso-ventral directions that are separated by about 120° (Maximov *et al.*, 2005a,b). Each group, in turn, is represented by the ON and OFF subtypes in relatively equal amount, thus giving in total six subtypes of DS GCs in the fish retina. The ON-type GCs respond to the movement of a light edge upon a dark background, and the OFF-type GCs respond to the movement of a dark edge upon a light background. Thus, according to selectivity to the sign of stimulus contrast, the DS GCs of fish are also different from the rabbit DS GCs, which comprise only a single type of ON–OFF cells. This difference was confirmed in a morphological study (Maximova *et al.*, 2006). In the fish retina, there are different DS GCs with unistratified dendritic trees ramifying separately either in ON or in OFF sublaminas of the inner plexiform layer. The existence of three groups of preferred directions in the direction-selective retinal inputs to the tectum was confirmed for larval zebrafish, *Danio rerio* with the use of different experimental methods (Gabriel *et al.*, 2012; Nikolaou *et al.*, 2012).

Orientation-selective (OS) units of two subtypes, so called detectors of horizontal and vertical lines (Maximova & Maximov, 1981; Maximova, 1999; Maximov *et al.*, 2009), are excited by edges or stripes of one of the two orientations close to vertical or horizontal, both stationary and moving, regardless of the sign of contrast (ON–OFF-types). The latter property distinguishes the fish OS GCs from those of rabbit, which comprise separate ON and OFF subtypes. Recording sites of these units in the fish tectum were deeper than those of DS units. The existence of two subtypes of OS GCs projecting to the tectum was also confirmed for the larval zebrafish (Nikolaou *et al.*, 2012). In addition to the detectors of oriented lines, responses of a number of other, less investigated detectors were recorded in the second retinorecipient layer. Besides, there was a third, still deeper retinorecipient layer of units responding by sustained discharges in darkness or in light. This paper deals with the DS and OS units, as these

types of motion detectors are likely involved in the organization of movement, and they should be expected to be color blind.

The system is regarded as color blind, if any two physically different light radiations can be made indistinguishable (matched), by changing the intensity of one of them, but leaving unchanged its relative spectral distribution. Over the years, we have tried different color-matching methods to investigate the ability of these motion detectors to distinguish between colors. Unfortunately, the use of conventional substitution colorimeter (Bongard, 1955) was complicated by the fact that these units do not or poorly respond to diffuse illumination. The method of "paper colorimetry" (Maximov *et al.*, 1985), that used colored papers of known reflectances moved (manually or mechanically) over a colored background, allows adequate stimulation with various moving stimuli. In this case, in the absence of responses of a unit to the movement of one paper against another, one can say that these stimuli are indistinguishable for this unit under this type of stimulation. We succeeded to find a few pairs of colored pieces of paper of different colors (out of nearly thousand specially painted samples) that are indistinguishable for detectors of oriented lines (Maximova, 1999) and for DS units (Maximova *et al.*, 2003), so this was the evidence in favor of color blindness of the motion detectors. From the comparison of the spectra of the indistinguishable pieces of paper, it was concluded that the sensitivity of these detectors lie in the long-wavelength end of the spectrum. However, both types of motion detectors have rather high contrast sensitivity (Maximov *et al.*, 2005b, 2009). Thus, for example, they do not respond to movement of achromatic stimuli, only if the brightness of the stimulus and background differs by less than 3%. For this reason, a more detailed investigation of color properties of motion detectors by the method of "paper colorimetry" was completely hopeless. In contrast, more than 16 million colors produced by a computer-controlled display screen cover the color space quite tight to make it possible to pick color matches. Moreover, the design of a monitor on the cathode-ray tube (where the radiation of the screen is generated by three independent sources, controlled by three guns of the monitor) allows to construct with its help a high-grade additive three-dimensional colorimeter.

Here we present a study of color properties of the DS and OS motion detectors projecting to the goldfish tectum with the help of such colorimeter. First, it was shown that stimuli of any color can be more or less matched with the color background by varying their intensities what is indicative of color blindness of DS GCs. Moreover, the law of additivity of color matches was found to hold for DS GCs, and so sets of stimuli which matched fixed backgrounds proved to be represented by planes in the three-dimensional color space of the goldfish. A relative contribution of different types of cones to the spectral sensitivity was estimated according to orientation of the planes of color matches. The spectral sensitivity of any DS detector was confirmed to be determined mainly by long-wave cones with a weak negative (opponent) contributions of middle-wave and short-wave ones. This opponency resulted in a reduced sensitivity in the blue–green end of the spectrum, what was considered as an adaptation to the aquatic environment where, because of the

substantial light scattering of a blue–green light, acute vision is possible only in a red region of the spectrum. Next, we used the same method for OS GCs and compared them with DS GCs. During the course of the study, portions of this work were published in abstract form (Maximova, 1999; Maximova *et al.*, 2003, 2005) or as a short report in Russian (Maximov *et al.*, 2007).

2. Materials and Methods

2.1. *Experimental animals*

The experiments were performed with the *Carassius gibelio* (Bloch, 1782), a closest wild relative of the goldfish. The fish were acquired from local suppliers (Moscow region), kept in aerated fresh water aquaria at room temperature and natural daylight regime for several months prior to experiments and fed by live food and fish pellets (Tetra GmbH, Melle, Germany). The fish were treated in accordance with the European Communities Council Directive of 24 November, 1986. The experimental procedures were approved by the local ethical committee of the Institute for Information Transmission Problems.

2.2. *Preparation*

For the electrophysiological experiment, a fish of 10–15 cm standard body length with exposed optic tectum was immobilized with tubocurarine (0.3 mg per 100 g of body weight), placed in a Plexiglas tank and fixed in natural position with perfusion of aerated water through its gills. The water level in the tank was maintained so that the eyes of the fish were under water but the water was not poured into the brain. The fish looked on the monitor screen with its right eye through the transparent tank wall. Visual responses were recorded from a contralateral lobe of the tectum opticum. The surgical procedure was described in detail elsewhere (Damjanović *et al.*, 2009).

2.3. *Visual stimulation*

Visual stimuli were presented to the fish on the computer-controlled 17 inch CRT monitor LG Flatron 775FT from the distance of about 30 cm. From this distance, the screen occupied $43^\circ \times 32^\circ$ of the fish visual field. To stimulate the DS or OS GCs, color stripes or edges moving over a color background were presented on the screen within a square area of stimulation with angular dimensions of $11^\circ \times 11^\circ$. The stimulation area could be placed at arbitrary locations of the screen and was usually placed so that the receptive field (RF) of the recorded unit was located in its center. Color of the rest of the monitor screen outside the stimulation area usually was the same as the background.

2.4. *Color specification*

Values of the correlated color temperature, brightness and contrast of the stimulating display were set so as to ensure the smoothest changes of colors. The emission

spectra of the screen were measured at these settings using a Carl Zeiss Jena MCS 500 Modular Spectrometer. The red, green and blue guns of the monitor were shown to be independent and any emission spectrum $I(\lambda)$ for monitor values of R, G and B, specified in the range from 0 to 255, was well approximated by the formula:

$$I(\lambda) = \left(\frac{R}{255}\right)^{\gamma_R} \cdot r(\lambda) + \left(\frac{G}{255}\right)^{\gamma_G} \cdot g(\lambda) + \left(\frac{B}{255}\right)^{\gamma_B} \cdot b(\lambda) + c(\lambda),$$

where exponents of powers γ_R, γ_G and γ_B for different guns have different values, a little larger than 2.

The emission spectra of three phosphors of the monitor $r(\lambda)$, $g(\lambda)$ and $b(\lambda)$, used in this formula, are shown in Fig. 1. Light radiation of a dark screen, described by the spectrum $c(\lambda)$, was about 100 times weaker than maximum radiation emitted by each phosphor. The maximum radiance of a white screen (when $R = G = B = 255$) was 155 mW sr^{-1} m^{-2}.

Monitor colors of the stimuli, background and surround will be further described, first, in instrumental coordinates — the digital values of R, G and B, specifying these colors. Second, they can be described in physical terms — the total emission (radiance) of each phosphor of the monitor. These values (R_e, G_e, B_e) can be measured directly, or calculated, knowing the RGB values. Since color mixing on the screen with a good approximation can be considered as additive, coordinate system R_e, G_e, B_e has the advantage that it forms a linear space, where vector operations are applicable, in particular, operation of color addition. Third, colors, set on the stimulating monitor, can be characterized in effective radiances "from the point of view of any photosensor". If we use a set of all types of cones of the investigated animal as photosensors, it gives a physiological color coordinate system. In particular, in case of goldfish, we have three-dimensional color space with coordinates L, M and S (corresponding to long-, middle- and short-wavelength sensitive cones, respectively), whose values can be calculated using the following equations:

$$L = \int_0^\infty I(\lambda) \cdot l(\lambda) \cdot d\lambda, \quad M = \int_0^\infty I(\lambda) \cdot m(\lambda) \cdot d\lambda, \quad S = \int_0^\infty I(\lambda) \cdot s(\lambda) \cdot d\lambda,$$

if the cone spectral sensitivity functions $l(\lambda)$, $m(\lambda)$ and $s(\lambda)$ are known.

According to the microspectrophotometric measurements made by Govardovskii on different batches of *C. gibelio* from our experiments (Maximova *et al.*, 2005), their retinas contain three types of cones with absorption maxima at 622–623, 535 and 454 nm. No UV-sensitive cones were encountered in the retina of fish of this age. Functions of relative spectral sensitivity of cones were calculated using a standard template (Govardovskii *et al.*, 2000) for the visual pigment of vitamin A2 and taking into account the spectral transmission of the goldfish ocular media (Douglas, 1989). The corresponding curves are shown in Fig. 1.

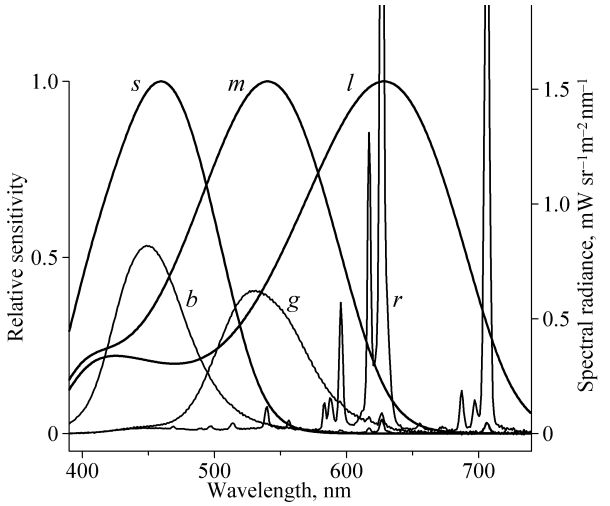

Fig. 1. Spectral sensitivities of the *C. gibelio* cones (l, m and s) and emission spectra of three phosphors of the monitor at maximum brightness (r, g and b).

2.5. *Data acquisition*

Single-unit responses of DS and OS GCs were recorded extracellularly from their axonal terminals in the superficial tectal retinorecipient layers, using low impedance (200–500 KΩ) recording microelectrodes made from metal-filled micropipette and tipped with a platinum cap of 2–10 μm in diameter (Gestesland *et al.*, 1959). The microelectrode was guided to a necessary tectal area under visual control by means of a micromanipulator (MP-225, Sutter Instruments). Neuronal discharges were amplified, filtered, displayed on an oscilloscope, fed to an audio monitor, digitized by an A/D converter (25 kHz sampling rate), and loaded into the computer. The units were isolated by adjusting the position of the microelectrode. Responses, loaded into the computer during the registration interval, were stored in the database, either without preprocessing (for subsequent analysis of the spike form), or after filtering according to the amplitude discrimination. In the latter case, they were stored as a sequence of time points of the spike appearance for further analysis.

2.6. *Procedure*

When a good DS or OS unit was isolated and an approximate position of its RF was found, the first step was always to measure its directional tuning curve (polar diagram) with contrast edges moving in different directions across the RF. This allowed to confirm the type of the recorded unit and to determine the size and accurate location of its RF. For details of automatic procedures of polar diagram measurements and determination of the RF center, see Maximov *et al.* (2013). Then the position of the area of stimulation was centered relative to the RF, and measurements of contrast sensitivity or direct color-matching investigations were initiated with the use of achromatic or colored stripes or edges moved against some background. At

first, there were specified parameters of stimulation (color of background and surround, the direction and speed of the stimulus, the number of runs in the session for which the responses were averaged) and recording parameters (the cut-off level of amplitude discrimination and the duration of the recording interval), which remained unchanged throughout the study. In case of stable recordings, the DS and OS units provide reproducible number of spikes in response to repeated stimulation. Therefore, the measurement was usually limited to three runs as a compromise between the desire to get more accurate numbers, and the desire to complete the entire measurement cycle before the cell is lost. Actually when stimulating by edges, stimuli were wide moving stripes exceeding stimulation area in width. Cell responses were recorded during the stimulus movement across the stimulation area. In this case, at first the leading edge of the stimulus gradually went across the RF of the unit, which initiated the IN-response. After some delay, the trailing edge crossed the RF resulting in a gradual substitution of the stimulus by background, which initiated the OUT-response.

3. Results

3.1. *DS GCs: Response magnitude in relation to stimulus intensity*

By definition, the system is considered as color-blind, if any two of light radiations can be made indistinguishable (matched) for it, by changing the intensity of one of them, but leaving unchanged its relative spectral composition. In this section, we show that for DS GCs of the fish retina any two radiations can actually be more or less equalized by changing their brightness.

To conduct the color matching with motion detectors, one needs to move a stimulus of one color against a background of another, and by changing the RGB values of the stimulus (leaving the background color constant) one have to look for those colors when there is no response of the unit both to moving the stimulus into the RF and to its removal. However, in practice, this can be difficult to achieve even for isochromatic colors (e.g., for different grades of gray) as the contrast sensitivity of these cells is very high (Maximov *et al.*, 2005b, 2009). As already mentioned, the DS GCs can be divided into two principal types by achromatic stimulation: the ON-type and the OFF-type. In both types, the relation between the intensity of the stimulus (moved upon the background of constant intensity) and the response magnitude is a step function: the ON-type DS GCs respond mainly to the moving in of a light stimulus (edge) against a darker background and do not respond to its removal, while the OFF-type DS GCs respond mainly to the removal of a light edge. Figure 2(a) shows such "stepped" intensity–response relationships of the OFF-type DS GC to the moving in and removal of achromatic stimuli ($R = G = B$) upon a constant background (with $R = G = B = 127$). It is seen that if the intensity of the stimulus is very different from the background, the response magnitude is nearly independent of intensity. Despite the fact that with the approach of the stimulus intensity to the background, the responses somewhat reduced, the total absence of the unit response

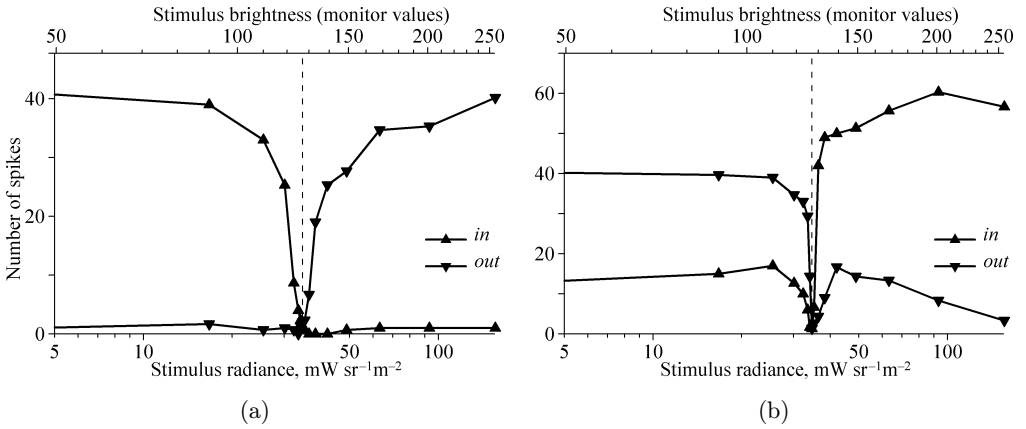

Fig. 2. Intensity–response profiles showing the responses of DS GCs as functions of the light intensity, expressed on a logarithmic scale. The ordinate indicates the number of spikes (mean of three runs) in the cell discharge in response to the movement of achromatic edges of various intensities at a fixed gray background through the RF in a preferential direction. Two branches of the curves correspond to responses to the leading (*in*) and trailing (*out*) edges of the stimulus. (a) A DS GC of the OFF-type selective to caudo-rostral movement, stimulated by vertical edges moving in the caudo-rostral direction. (b) A DS GC of the ON-type selective to dorso-ventral movement, stimulated by bottom-up movement of horizontal edges. In both cases, the background radiance was equal to 34.6 mW $sr^{-1}m^{-2}$ (marked with dashed line), and the speed of the stimuli was 11°/s.

occurred only at intensities that differ from the background by no more than one to two brightness steps (when measured in monitor values).

It should be noted, however, that it is not always the case that DS units do not respond to stimuli of inadequate contrast. About two-thirds of the ON-type DS GCs also give a weak discharge to the moving in of dark edges, and the OFF-type DS GCs respond to the moving in of light ones. But these non-specific responses are always much smaller and different in the structure of discharge from the responses to stimuli of adequate contrast. Therefore, the presence of non-specific responses does not preclude the identification of the type of the unit. Figure 2(b) is an example of the ON-type unit, which differed by very high contrast sensitivity (as evidenced by a narrow gap between the opposing steps), and the presence of non-specific responses.

Similarly two opposing steps near the background intensity can be seen in experiments with stimulation by colored stimuli when the background and stimulus do not differ in the relative spectral composition (for example, pure *R*, *G* or *B*). When the stimuli and the background were of different spectral composition, the results were not so uniform. Sometimes, there could be seen the same pair of opposing steps as in Fig. 2(a), separated by an interval of intensities, where responses are completely absent. This primarily refers to the cells without non-specific responses that have low contrast sensitivity. But in some cases, the intensity–response relationships do not possess such "silent" interval. The corresponding curves for the ON-type DS GC responses to the presentation of pure red or pure green stimuli on the same bright blue background (0, 0, 255) are shown in Fig. 3(a). It can be seen that the curves for the removal of the red and green stimuli (branches marked with triangles pointing

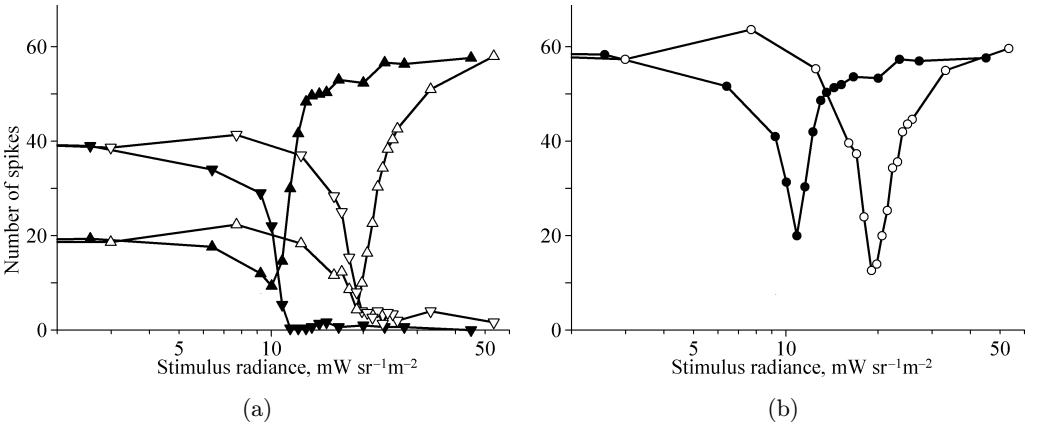

Fig. 3. Intensity–response profiles showing the responses of a DS GC as a function of the stimulus intensity in case of different colors of stimuli and background. A DS GC of the ON–type selective to dorso-ventral movement (the same unit as in Fig. 2(b)), stimulated by horizontal red (black marks) or green (open marks) edges moving in the dorso-ventral direction at the speed of 11°/s on a bright blue background. (a) Separate branches of the curves indicate responses of the unit to the leading (triangles pointing up) and trailing (triangles pointing down) edges of the stimuli. (b) Intensity–response profiles for the total number of spikes in responses to the movement of stimuli in and out of the RF (sums of the responses to their leading and trailing edges). Other conventions are as in Fig. 2.

down) have the same stepped shape characteristic for achromatic stimulation of the ON-type DS GCs. These cells respond mainly to the removal of the stimuli darker than the background and do not respond to the removal of the lighter ones. However, the opposite steps (responses to the edges moving in the RF) were less distinct. In this case, some responses (albeit very weak) to moving the red and green stimuli on the blue background were observed at any intensities (triangles pointing up in Fig. 3(a)). In other words, for this GC, pure monitor colors could not be matched in intensity absolutely (it was impossible to achieve an absence of response to red or green edges moving into the RF), but it was possible to reach some minimum responses (Fig. 3 (b)), evidencing some sort of "approximate" colorimetric matches.

Note that in Fig. 3(b), the intensity of green monitor light that approximately matches the bright blue light almost double the intensity of red light, equated with the same blue light. This means that the DS GC is more sensitive to the red monitor light than to the green one. This finding applies not only to a given cell, but to all DS GCs because such mutual arrangement of curves as shown in Fig. 3(b) have been observed in all experiments where the intensity–response profiles for the red and green stimuli on a blue background were measured. This observation confirms our findings that the DS GCs are sensitive to the long-wavelength end of the spectrum (Maximova *et al.*, 2003).

3.2. *Color matching experiments with the DS GCs*

The fact that DS GCs of the fish retina are divided into ON- and OFF-types, greatly facilitates the achievement of colorimetric matches. Indeed, in the case of the

ON-type DS GCs, presence of a response to the stimulus moving into the RF means that the stimulus is brighter than the background, and to achieve a colorimetric match its intensity should be lowered. On the contrary, if the stimulus gives a response to its removal, it is necessary to increase the stimulus intensity. For the DS GCs of the OFF-type, the rule of change of the stimulus intensity will be opposite. These rules have been implemented in the form of an automated procedure of searches of colorimetric matches in a special on-line software tool. Since the RGB values that determine the radiation of the screen can take only integer values, it is possible to set the desired intensity and to maintain the same relative spectral composition only approximately while selection of colorimetric matches. Nevertheless, stimuli presented on the monitor have good color resolution — in our setup, differences in brightness between adjacent discrete values are 1%–2% in the working range. Typically, this is smaller than the values of increment and decrement thresholds of DS GCs (on average equal to $\pm 3.0\%$ of the brightness of the background), so, for most of the units in the experiments, it was possible to achieve colorimetric matches. In many cases, however, the match was not absolute (when the response is completely absent both to moving the stimulus into the RF and to its removal), but always rather low responses could be achieved by changing the intensity of the stimulus, which indicated only the approximate colorimetric match.

All six types of DS GCs were examined with this tool. The purpose of the experiments was to make sure that any two light radiations can be equalized, by varying the intensity of one of them. In order to do that, first, some fixed color background was set, against which the stimuli of various colors were moved in the preferred direction. The stimulus color (its RGB values) was first set arbitrarily, and then its brightness was automatically changed step-by-step according to the above described procedure until a minimum response value was attained. Then again, a new color of the stimulus was set by experimenters, and the search of the minimum was repeated. To avoid problems with the graphical representation of the results of the three-dimensional color space, only different colors within some two-dimensional subspace were measured in each experiment, and the dependence of the response value (number of spikes in the cell discharge) of parameters of the stimulus color was obtained.

The top row of diagrams in Fig. 4 shows the results of one of these experiments made on the OFF-type DS GC with ventro-dorsal preferred direction. In the experiment, colored stimuli of different intensities generated by the red and green guns of the monitor were moved against the dark red background (127, 0, 0). The background color is marked with a red dot in the diagrams. The unit responses were measured for 117 different colors in the color space (indicated by black dots). Three diagrams are different in that what is plotted along the coordinate axes. At the diagram *a*, the digital values of R and G (from 0 to 255) specifying monitor colors are plotted along the axes. At the diagram *b*, corresponding radiances R_e and G_e of red and green phosphors of the monitor are plotted. At the diagram *c*, effective radiances L and M of the stimuli for long- and middle-wave cones are plotted. The OFF-type

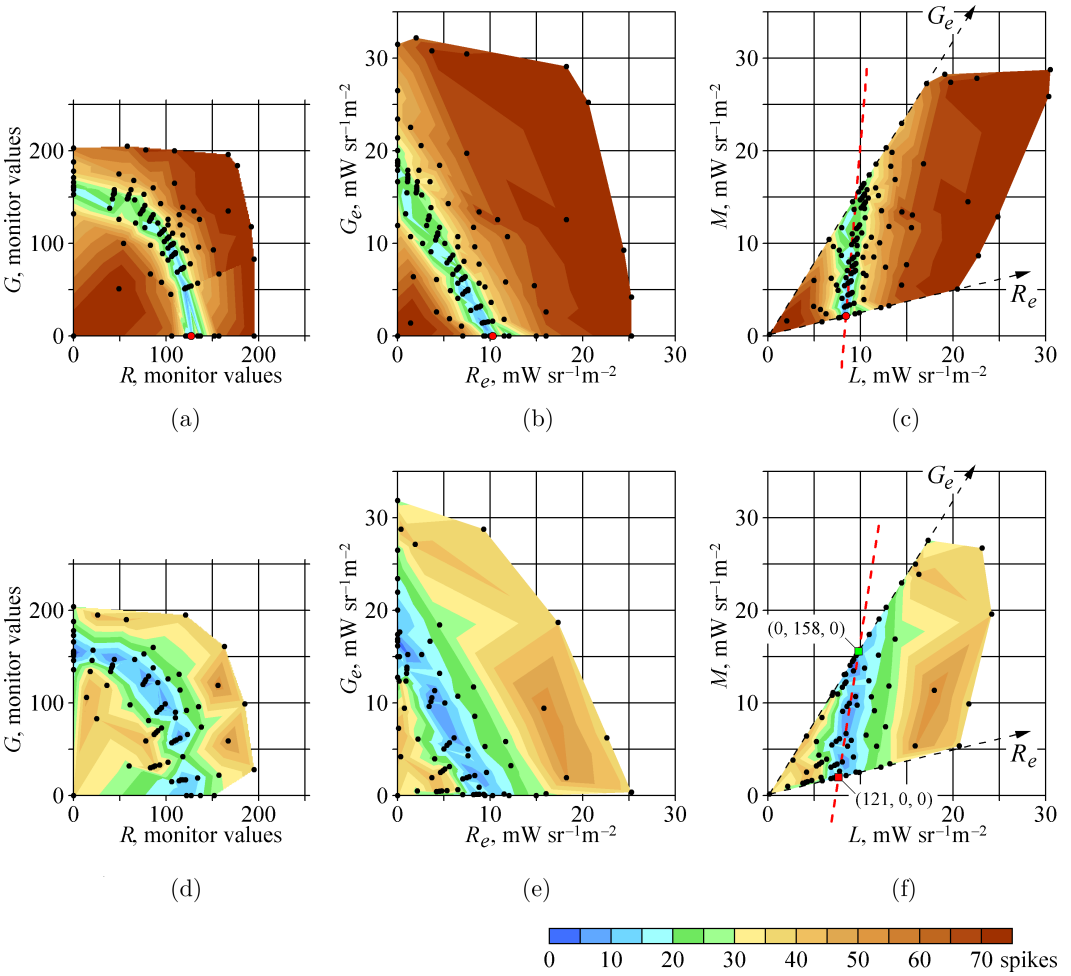

Fig. 4. Dependencies of responses of DS GCs to the movement of colored edges over the colored background on the stimulus color. Diagrams in the top row (a–c) are obtained from the OFF-type DS GC with ventro–dorsal preferred direction; response to the upward movement of the red–green stimuli on a dark red background. Diagrams in the bottom row (d–f) are obtained from the ON-type DS GC with caudo-rostral preferred direction; response to the caudo-rostral movement of the red–green stimuli on a bright blue background. Response values (average of three runs of the number of spikes in the total response to the movement of edges in and out of the RF) are color coded (see scale below).

DS GC gave significant responses to the movement of dark stimuli into the RF regardless of their chromaticity, and to the removal of light stimuli, what is shown by two brown colored areas at the diagrams. And at that, there is a narrow canyon-like gap between the dark and light stimuli, where the responses of the cell are minimal — the line of colorimetric matches. Diagrams in the bottom row (d – f) illustrate the results of similar experiments made on the ON-type DS GC with caudo-rostral preferred direction using red–green stimuli (85 different colors) moved against the bright blue background (0, 0, 255). Reliefs, built on the basis of the experimental points, allow to state that any red–green stimuli always can be matched with a bright

blue background by varying their intensity. The results shown in Fig. 4 are typical for all the experiments performed with different backgrounds and for all types of DS GCs. Hence it can be concluded that in general any two light radiations can be matched, and therefore, all the DS GCs are color blind under these stimulation conditions.

3.3. *Linearity of color matches*

The simplest variant of the color blind system would be a system controlled by a single type of cones. However, color blindness itself does not mean that only one type of radiation detector is effective in the system. In the retina of the goldfish, there are at least three types of cones. How, and in what combination these cones may be involved in the detection of movement is not *a priori* clear. One of the most important characteristics of a light-sensitive system is the validity of the Grassmann's law of additivity of color matches, which states that a color match between two lights persists if the same light radiation is added to both of them. In case of a one-dimensional (color blind) system the law can be formulated as follows: if some radiations, that are equal in intensity, are added to some other radiations, that are also equal to each other in intensity, the obtained mixtures will be equal in intensity. Consequence of the additivity (and this applies to any light-sensitive systems, not only to the light sensors of the eye) is the ability to completely characterize a light sensor by its spectral sensitivity curve — "the concept of spectral sensitivity is closely associated with additive light sensors" (Nyberg, 1950). On the contrary, "there are systems in which any two light radiations of different spectral composition can be matched, and yet it is meaningless to talk about the spectral sensitivity" (Bongard & Smirnov, 1961). For such systems, the law of additivity is not satisfied.

Instead of direct verification of the additivity of color matches in experiments, it is easier to explore them for linearity, which follows from the law of additivity. In the general case, linearity means that if a number of independent sources which determine the spectra of stimuli exceeds the dimension of color vision, the set of colors indistinguishable from the current color forms a hyperplane in the linear space of the sources (where intensities of each of them are plotted along the axes). In the case of a one-dimensional (color blind) system, it is sufficient to generate color stimuli using two sources (for example, two guns of the monitor). Then, under the law of additivity, all colors of colorimetric matches shall be placed on some straight line in the color space.

In fact, the necessary data for this analysis were obtained in the experiments described in the previous section. Figure 4(b) is a relief for the same experiment as in Fig. 4(a), plotted in an additive coordinate system — radiances of the red and green phosphor of the monitor. It is evident that in these coordinates "canyon" really got a straight shape, indicating the linearity of the colorimetric matches. The bulk of the colorimetric experiments was done with blue (0, 0, 255) and dark red (127, 0, 0) backgrounds. With the blue background, there was 51 colorimetric experiments

made in total on 39 DS GCs of all six types. Of these, 35 experiments were large enough to be able to verify the linearity of the colorimetric matches. Thirty experiments were carried out on different DS GCs with the dark red background. None of them showed obvious contradictions to the hypothesis of linearity. In 13 of these experiments, numbers of used colors were enough to make sure that all the colorimetric matches really fit a straight line.

3.4. *Spectral sensitivity of the DS GCs*

By definition, the spectral sensitivity curve expresses the excitation of the light sensor to its stimulation by monochromatic light of different wavelengths (Nyberg, 1950). Therefore the measurement of spectral sensitivity curve involves the use of some spectral device. However, in the specific case of the visual system of the goldfish, the response of any cell is determined by three types of its cones. Since, as has been shown above, the law of additivity of color matches is valid for the DS GCs, their spectral sensitivity curve cannot be anything other than a linear combination of the spectral sensitivity curves of the three types of cones. And since the latter are known to us, it remains only to determine the relative contribution of each cone type in the total spectral sensitivity. From previous experiments, we already know that the shape of this curve is largely determined by the long-wave cones, so we will look for the spectral sensitivity function of the DS GCs as:

$$f(\lambda) = l(\lambda) + \alpha \cdot m(\lambda) + \beta \cdot s(\lambda). \tag{1}$$

It is assumed that the coefficients α and β define some small additions to the spectral sensitivity of the long-wave cones. Accordingly excitation of a unit in response to any radiation will be described as a linear combination of the excitations of cones:

$$F = L + \alpha \cdot M + \beta \cdot S.$$

In order to determine experimentally the unknown coefficients α and β, it suffices to find three different light radiations which are matched to each other. Excitation of cones to these radiations can be calculated according to its spectra and the known spectral sensitivity curves of the cones (see Materials and Methods). Then coefficients α and β can be found by solving corresponding equations. Geometrically, this means that in the color space LMS, it is necessary to find a plane in which the colors of these three sources lay. The orientation of this plane will give us the values of α and β.

The farther the three colors are scattered in the color space, the more exact orientation of the plane can be determined. In the case of colors generated on the monitor, such furthest colors are pure colors generated by the red, green and blue guns. In the experiments, a bright blue (0, 0, 255) has been selected as one of the colors and was used as background. The colors generated by the red and green guns were experimentally equalized with the blue background. For greater accuracy, not only pure red and green were compared with the background, but also all kinds of their mixtures. In such a way, in parallel, these experiments allow to test the hypothesis of linearity of color matches.

Thus, the procedure for finding the values of the coefficients α and β was as follows. In the course of the experiment, a variety of red–green mixtures were presented on a constant blue background, and responses of the unit were measured. Then, colorimetric matches, characterized by the absence or very low level of the unit response, were achieved by changing the intensity of the stimulus. And by the points accumulated in the experiment, an appropriate relief of responses was being built on the coordinate plane LM of the color space during offline processing. A straight line of colorimetric matches was drawn on the relief by hand or by an automatic procedure (see Maximov *et al.*, 2007, for details). The points of intersection of this line with the axes R_e and G_e determined the coordinates of the furthest monitor colors, which provide the equality with the blue background. Figure 4(f) illustrates this procedure. Points of intersection of dashed line, drawn through the "canyon", with the coordinate axes R_e and G_e are shown in small red and green squares, next to which their RGB values are written in brackets.

As it has been mentioned above, each of 35 colorimetric experiments made on DS GCs was large enough to verify the linearity of the colorimetric matches (and therefore to get sufficiently accurate estimates of α and β). At least three units per each of the six DS GC types were examined. At that, no systematic difference in the values of the coefficients for different types of DS GCs was detected. The results are summarized in Fig. 5. Values of the coefficients α and β were determined for each unit, and a corresponding curve of spectral sensitivity was constructed according to Eq. (1). All these curves are plotted in gray in the figure. Since, as a rule, both coefficients were negative, all the units possess reduced sensitivity in the blue–green end of the spectrum (as compared to the spectral sensitivity of the long-wave cones). The figure showed quite considerable scatter in the curves. This variability cannot be attributed to measurement or calculation uncertainties, but reflects individual differences in the spectral sensitivity of the DS GCs (in different cells and in different fish). This was evidenced by the reproducibility of values in repeated experiments on

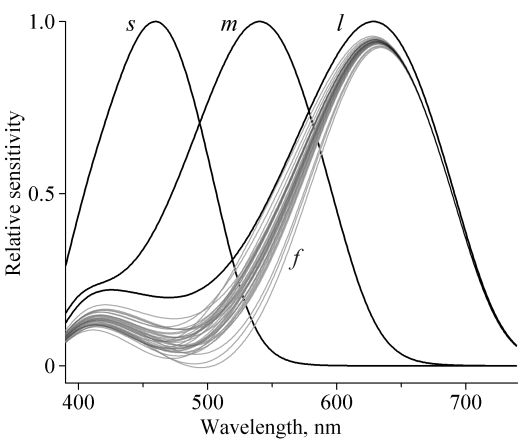

Fig. 5. Spectral sensitivity curves obtained for 35 DS GCs (f) and spectral sensitivity curves of the goldfish cones (l, m and s).

the same unit, as well as the fact that increased or decreased sensitivity (compared to usual) in the blue–green end of the spectrum was typically inherent in all units recorded in a particular fish.

Negative values of the coefficients α and β mean that in the fish retina, there is an opponent interaction between signals from long-wave cones and signals from cones of the two other types. However the opponency in DS GCs was quite weak, causing the resulting spectral sensitivity curves to have always non-negative values. Such color opponency sometimes is called "hidden opponency".

3.5. *OS GCs: Response magnitude in relation to stimulus intensity*

Unlike DS GCs, represented by ON and OFF types, OS GCs respond both to increment of stimulus intensity, relative to the background, and to its decrement. Accordingly, the intensity–response curves of the OS GCs have the form of two opposing steps with a dip at intensity of the background. As that of DS GCs, responses to stimuli that differ greatly in intensity from the background are equal in magnitude, and at intensities close to the background there is a depression. Examples of such dependencies are shown in Fig. 6(a). Typically, the detectors of oriented lines almost equally respond to moving in of an edge and to its removal – *in* and *out* branches almost repeat each other. Therefore, in Fig. 6, we did not draw them apart but point out the total number of spikes in *in* and *out* responses for clarity.

Both in DS GCs, and in OS GCs, relative values of increment and decrement thresholds were practically independent of the background intensity. For the detector of horizontal edge shown in Fig. 6, the intensity–response curves were measured for two achromatic backgrounds, differing in intensity by half. It is seen that a

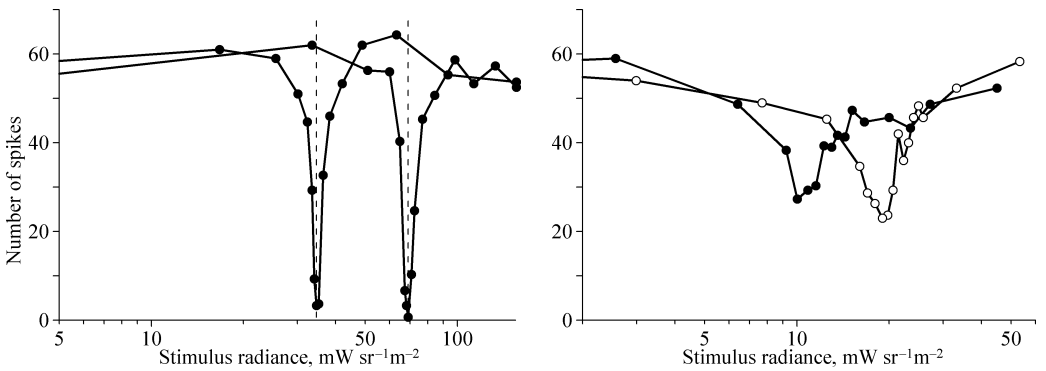

Fig. 6. Intensity–response profiles showing the responses of a detector of horizontal edge as a function of the light intensity. The unit was stimulated by moving horizontal edges of various intensities at fixed backgrounds in ventro–dorsal direction at the speed of 16.5°/s. The experimental points indicate the total number of spikes in response to the movement of edges in and out of the RF. (a) Movement of achromatic stimuli against two backgrounds (dark-gray or light-gray, whose intensities are marked with dashed lines). (b) Stimulation by red (black circles) or green (open circles) edges moving on a bright blue background. Other conventions are as in Fig. 3.

change of background intensity shifts the curve along the abscissa, not changing their shape in a logarithmic scale. In other words, the response of the cell is determined not by absolute but by relative change in intensity of the stimulus, which means the validity of the Weber–Fechner law. Increment and decrement thresholds of the detector calculated on the basis of the results of this experiment equaled about 1.8% and 1.3%, respectively, for both backgrounds.

When the stimuli and the background were of different spectral composition, as a rule, it was impossible to achieve an absence of response to the movement by changes of the stimulus brightness. In case of pure monitor colors, it was possible to reach only some rather broad and not very deep gaps (Fig. 6(b)). In all cases, these "approximate colorimetric matches" of pure monitor colors were significantly worse than that in DS GCs — compare Figs. 3(b) and 6(b). Again, as it was in DS GCs, in Fig. 6(b), the intensity of green edge that gives a minimum response to its movement on the bright blue background (approximately matches the background) is almost double the intensity of red light, equated with the same blue light, what means that the OS GCs are also more sensitive to the red monitor light than to the green one. This observation is consistent with the results of "paper colorimetry" which have proved that the OS GCs are also sensitive to the long-wavelength end of the spectrum (Maximova, 1999).

3.6. *Color matching experiments with the OS GC*

Because the OS GCs responded to both ON and OFF, we could not use the automatic procedure of finding stimulus intensity that gave the minimum response, which we used in experiments with DS GCs. This greatly slowed down to conduct experiments and did not always give satisfactory results. Nevertheless, the general pattern was the same as in case of the DS GCs, what is illustrated in Fig. 7. In the additive

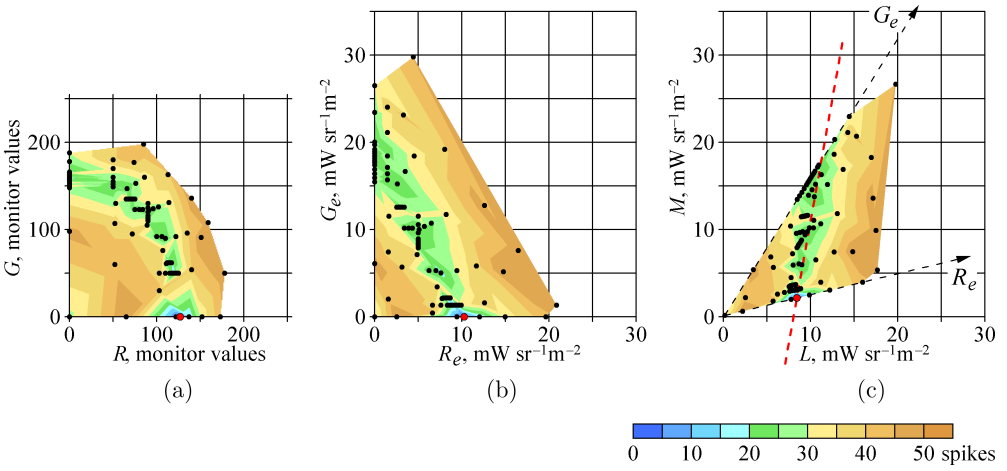

Fig. 7. Dependencies of responses of a detector of horizontal edge to the up-down movement of horizontal colored stripes of width 2.2° at the speed of 11°/s over a dark red background (127, 0, 0) on the stimulus color. Other conventions are as in Fig. 4.

coordinates, canyon passed relatively straightforward. As in the case of the DS GCs, a tilt of the canyon corresponds to opponent contribution of the middle-wave and/or short-wave cones. However, due to the lower depth and, as a rule, the greater width of the canyon in the case of OS GCs, it was not always possible to determine the contributions of the middle-wave and short-wave cones (coefficients alpha and beta) with the same accuracy as for the DS GCs. The scatter in the values of the coefficients could be quite large, and sometimes the contributions of the middle-wave and short-wave cones could be even opposite.

4. Discussion

4.1. *Color opponent nature of the motion detectors*

Color properties of the direction-selective retinal units (not to mention the detectors of oriented lines) in vertebrates were so far not investigated in detail. Most early research indicated in favor of the fact that the motion detectors projecting to the fish tectum are not color-coding (Maximova *et al.*, 1971). Current color-matching studies with color edges, moving on colored background, confirmed approximate color blindness of these units. Their responses are mainly determined by the signals of the long-wave cones. However, other types of cones also give a weak contribution to the responses, the signals of different types of cones being summed with different signs, which indicates the presence of color opponent interaction. Due to the weak contribution of the other two types of cones, it is impossible to recognize the color of presented stimulus by responses of these units. By that, the responses of the motion detectors differ from responses of the color-opponent GCs which are usually recorded from the isolated retina (Daw, 1968), and from a single type of color-opponent units recorded from their axon terminals in the tectum (Maximova *et al.*, 1971; Maximova, 1977).

4.2. *Possible functional role of the discovered hidden opponency*

It is known that the aquatic environment possesses a considerable scattering in the blue–green end of the spectrum, which gives low-contrast blurred retinal images. Therefore, on the one hand, an image formed by short-wavelength and middle-wavelength cones will be of little use for the directional selectivity. In such a situation without significant losses in efficiency the image processing can be limited to one long-wavelength channel. On the other hand, the appreciable sensitivity of the long-wavelength cones themselves to the blue–green end of the spectrum also reduces the contrast of the image formed by these cones, and nature has taken the way of reducing this sensitivity by means of neural signal processing in the retina. As a result of the subtraction of signals of short-wave and middle-wave cones from that of long-wave ones, the sensitivity in the blue–green region of the spectrum is suppressed (Fig. 5). Corresponding spectral sensitivity curve can be considered as an adaptation to vision under water, where, because of the substantial light scattering in the

blue–green region of the spectrum acute vision is possible only in the red end of the spectrum (Easter, 1975).

4.3. *Comparison with behavior*

Retinal detectors of the direction of movement are usually associated with the optokinetic (tracing eye movements in a rotating striped drum) and the optomotor (movement of the animal in the same drum) reactions. At that, the DS GCs projecting to the fish tectum seem to serve only optomotor reactions since the latter disappear when removing the tectum, while optokinetic responses remain intact (Springer *et al.*, 1977). The study of the color properties of the optomotor reactions using optomotor drums with colored stripes showed that it is possible to "equalize" the colors of these stripes in intensity — when the fish no longer follows the rotation of the drum. This color blindness of the optomotor response has been demonstrated for goldfish, *Carassius auratus* (Schaerer & Neumeyer, 1996), and zebrafish, *Danio rerio* (Krauss & Neumeyer, 2003). In both cases, spectral sensitivity curves of the optomotor response were similar to those of the long-wave cones, while in the shortwave end of the spectrum sensitivities dropped markedly steeper, indicating the negative contribution of medium-wave and/or short-wave cones. All this is consistent with our data on the color blindness of the DS GCs and their spectral sensitivity curves.

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