

Color properties of the motion detectors projecting to the goldfish tectum: III. Color-opponent interactions in the receptive field

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Interactions between color channels (long-wave (L), middle-wave (M) and short-wave (S)) in the receptive field of direction-selective (DS) and orientation-selective (OS) ganglion cells (GCs) were investigated with combined selective stimulation of pairs of cone types (L and M, L and S, M and S). In the experiments with DS GCs of both ON and OFF types, it was shown that: (1) M and S channels were synergistic relative to each other and opponent to L channel. (2) Three-parameter signal (from L, M and S cones) is transformed to one-parameter signal at the output of DS GC, thus illustrating the principle of univariance. (3) In the experiments with OS GCs, it was shown that L and M channels were synergistic in the OFF-pathway, while the S channel was opponent to them. Our results suggested that photoreceptor synaptic connectivity of the bipolar cells hypothetically involved in the goldfish OS circuitry substantially differs from connectivity of bipolar cells presumably targeting DS GC. (4) To sum up, the results obtained on DS GCs confirmed the plausibility of proposed DS GC wiring diagrams; as to the OS circuitry of fish retina it still remains unclear and needs further investigation.

Keywords: Goldfish; retina; tectum opticum; direction-selective ganglion cells; orientation-selective ganglion cells; color channels; principle of univariance.

1. Introduction

Six types of direction-selective ganglion cells (DS GCs) projecting in TO are divided into two groups according to their preference to the sign of achromatic stimulus contrast relative to background (Maximov *et al.*, 2005a, 2005b). DS GCs of the first group respond when the stimuli are lighter than the background (ON units), and those of the second — when the stimuli are relatively darker (OFF units). DS GCs are characterized by high temporal and spatial resolution and apparently are designated to the detection of moving objects in their receptive fields (Maximov *et al.*, 2013). At the same time, they are not involved in color discrimination. Color matching experiments have shown that they are color-blind (Maximova *et al.*, 2005;

Maximov *et al.*, 2007, 2014). Spectral sensitivity of any type of DS GCs was confirmed to be determined mainly by the long-wave (L) cones with a weak negative (opponent) contribution from the middle-wave and short-wave cones (M and S cones, respectively). In other words, the results of color matching experiments indicate that the color blindness of the DS GCs does not signify that they are connected exclusively with the L cones. Electrophysiological evidence that movement detectors projecting to the fish TO receive signals from all three chromatic types of cones and possess color-opponent properties was demonstrated in our recent work (Maximov *et al.*, 2015). It was shown that the ON-type DS GCs respond to the selective excitation of the L cones just as they do to white stimuli — by ON spike discharge, whereas responses to selective excitation of the M or S cones are of the opposite sign — OFF spike discharge. Completely symmetric pattern was observed in the OFF-type DS GCs — OFF spike discharges were evoked by the selective stimulations of the L cones, and ON type responses — when M or S cones were stimulated. Hence, the opponency of cone inputs (L/(M, S)) was demonstrated by the responses of both ON and OFF types of DS GCs. As a rule, responses to the selective stimulation of M and S cones were much weaker than when the long-wave cones were selectively stimulated. Accordingly, weak opponent contributions of M and S cones observed formerly in color matching experiments (Maximov *et al.*, 2007, 2014) have been confirmed here.

In the present study, we have investigated the plausibility of the model of wiring in the goldfish DS circuitry described in the previous paper (Maximov *et al.*, 2015). The suggested model was based on the new physiological and morphological data testifying that both sign-inverting and sign-conserving glutamate receptors can colocalize on the same bipolar cell. Each bipolar cell targeting DS GC (ON or OFF) is presumably connected with all three types of cones by diverse types of synapses, sign-conserving or sign-inverting synapses using the iGLU or the mGLU R6 postsynaptic receptors correspondingly (Wong & Dowling, 2005; Li *et al.*, 2012). We suggest that the convergence and interaction of the signals from different cone types takes place as early as in the outer plexiform layer (OPL) on the dendrites of bipolar cells. Hypothetically, a bipolar cell targeting ON-type DS GC receives signals from the L cones through sign-inverting synapse, while input signals from M and S cones are transmitted through the sign-conserving synapses (Fig. 1(a)). On the contrary, a bipolar cell connected with the OFF-type DS GC is supposed to form sign-conserving synapse with the L and sign-inverting synapses with the M and S cones (Fig. 2(a)). The model explains the fact that three-parameter signal about color of the stimulus is transformed to one-parameter signal — change of membrane potential of the bipolar cell — the input neuron of the DS GC. Thus, the suggested scheme of wiring in the OPL illustrates the principle of univariance (Naka & Rushton, 1966; Bilotta & Abramov, 1989). The main goal of the present study was to test the principle of univariance predicted by the model and demonstrate possible interactions of the color channels.

2. Materials and Methods

2.1. *Preparation and recordings*

The studies were performed on a wild form of the goldfish, *Carassius gibelio* (Bloch, 1782). A total of 56 fish were used in the experiments. All procedures with the fish were approved by the local ethical committee of the Institute for Information Transmission Problems. The maintenance of the fish and the surgical and experimental procedures have been described in detail in the earlier papers of this series (Maximov *et al.*, 2014, 2015). Briefly, in the experiments, an immobilized fish, placed in a transparent Plexiglas tank with its eyes submerged in water, looked at the monitor screen through the transparent tank wall. Single-unit responses of the retinal DS and OS GCs were recorded extracellularly from their axonal terminals in the retinorecipient layers of the contralateral lobe of the TO. Low-impedance (200–500 K Ω) metal in glass microelectrodes tipped with a platinum cap of 2–5 μm in diameter were used in the recordings (Gesteland *et al.*, 1959). Experimental setup, used for amplifying, digitizing, storing and processing of the DS GC and OS GC records, containing an AC preamplifier (band pass 100–3500 Hz), A/D converter (25 kHz sampling rate) and a system of three mutually connected and synchronized computer modules was described in detail elsewhere (Maximov *et al.*, 2005b; Maximov & Maximov, 2010).

2.2. *Selective and simultaneous stimulation of different chromatic types of cones*

The motion detectors projecting to the fish tectum do not respond to uniform and constant illuminations, but respond only to changes occurring in their RF. In particular, they respond to moving contrast edges. To stimulate such units, it is possible to choose a pair of colored radiations for the stimulus and for the background, which would differ for only one (any) type of cones. For such selective stimulation specific monitor colors were calculated. One of them, “gray” or “neutral”, usually served as a background on which the stimuli of the remaining six colors were presented. Three of these colors (incremental colors) were 1.6 times more intense than the neutral for a given type of cones, and the intensity of the other three (decremental colors) was 1.6 times lower than the intensity of the neutral color. The incremental colors will be denoted in this paper as L+, M+ and S+, and the decremental, as L–, M– and S– for the long-wave, middle-wave and short-wave cones, respectively. The neutral color will be denoted as N. The selective stimulation of individual color channels was described in detail in the previous paper (Maximov *et al.*, 2015). In the present study, to show the interactions between color channels, the color set was extended by the colors that stimulate pairs of color channels simultaneously, each stimulated color channel being excited or suppressed. 9 of the 12 hypothetical colors for paired color channel stimulation were able to be displayed on the stimulation monitor. These colors will be denoted as L+M+, L–M–, M+S+, M+S–, M–S–, L+S+, L–S+, L+S– and L–S–.

2.3. Procedures

When a 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 us to confirm the type of the recorded unit and to determine the size and accurate location of its RF. Then the position of the area of stimulation was centered relative to the RF, and the unit was investigated by edges of various colors (from the set described above) moving over a neutral or colored background. All the procedures have been described in detail in the previous papers of this series (Maximov *et al.*, 2014, 2015). Only those features that are important for this study are noted here. When stimulating by the moving edges, the stimuli were actually wide stripes exceeding the stimulation area in width, and cell responses were recorded during the stripe movement across the stimulation area. Hence, only one edge always moved in the stimulation area. 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 of the unit.

2.3.1. Selection of DS GCs for investigation

For the DS GCs, the degree of selectivity to the sign of contrast was estimated with the contrast edges moving in the preferred direction:

$$R = \frac{N_{W/B} - N_{B/W}}{N_{W/B} + N_{B/W}},$$

where $N_{W/B}$ and $N_{B/W}$ are the numbers of spikes in response to a white edge moving over black background and to a black edge moving over white background, respectively. Earlier it was shown that most DS GCs have R values close to +1 or -1 (Maximov *et al.*, 2005a, 2005b). Only the DS GCs with $|R| > 0.5$ were selected for further processing.

2.3.2. Selective and paired chromatic channel stimulation sequences

Investigations of the units' responses to selective and paired chromatic channel stimulation were conducted under the general scheme. Values of the parameters of stimulation, which remained unchanged during the whole measurement, were specified in advance. In the case of stimulation with moving edges or stripes, the parameters were the direction of movement (usually coincided with the preferred direction of the unit under investigation) and the speed of movement. The main experimental sequence was as follows. The neutral (N) background was selected for all the subsequent stimulus presentations, and the measurement began with the presentation of the L+ color. Then, the remaining selective colors were presented in the following sequence: M+, S+, L-, M- and S-. After that, the colors stimulating pairs of chromatic channels were presented. At the end of the procedure, a

measurement for the L+ color was repeated in order to check the stability of the unit response. As a rule each stimulus presentation was repeated six times.

Another stimulus sequence was used to test the responses of motion detectors to selective and paired stimulation of different chromatic types of cones under both the neutral (N) and reddish (L+) backgrounds. At first, a stimulus of a certain selective color (M+, S+ or S− could be selected in advance) was presented on the neutral (N) background. Then, the color of the stimulus was changed to additionally excite L cones (L+M+, L+S+ or L+S−, correspondingly). Next, the background color was changed to L+, and the same color (L+M+, L+S+ or L+S−) was presented as a stimulus. (It is important to note that the latter colors, initially designed to stimulate pairs of chromatic channels, actually stimulate only one color channel (M or S) when presented on the L+ background.) After that, the stimulus of the initially selected color (M+, S+ or S−) was presented on the L+ background (in this case two chromatic channels, L and M or L and S are simultaneously stimulated). Finally, the first stimulus was presented on the neutral (N) background to test whether the recording remained stable throughout the experiment. Each stimulus presentation was repeated six times.

The sequences of stimulation described above were performed automatically using two program tools constructed for this purpose.

3. Results

There are three types of cones in the retina of adult goldfish: (S) short-, (M) middle- and (L) long- wave sensitive cones containing three A₂-based visual pigments absorbing maximally at 454, 535 and 622–623 nm, respectively (Maximova *et al.*, 2005). Visual receptors hyperpolarize when illuminated (Trifonov, 1968). The sign of the gradual response of postsynaptic bipolar cells (depolarization or hyperpolarization) is determined by the type (sign-conserving or sign-inverting) of their synapses with the receptors. In the wiring model tested here the DS GCs of ON- and OFF-types are considered to be unistratified and receiving their input signals from either ON- or OFF- bipolar cells in the appropriate strata of IPL. According to the model, each bipolar cell (both ON- and OFF-types) is connected with all three cone types by diverse synapses (Figs. 1(a) and 2(a)). Bipolar cells that target ON-type DS GCs receive signals from the L cones through the sign-inverting synapses, while those from the M and S cones — through the sign-conserving synapses (Maximova *et al.*, 2005) (Fig. 1(a)). On the contrary, bipolar cells connected with the OFF-type DS GCs are considered to form sign-conserving synapses with the L and sign-inverting synapses — with the M and S cones (Fig. 2(a)). In spite of this difference, both types of bipolar cells work as summators: they receive a three-parameter input signal (from the L, M and S cones), which they convert into a single one-parameter output signal, i.e., the change of their membrane potential. The input signal exciting DS GC is the gradual alteration of the level of bipolar cell membrane potential evoked by the contrast between the background illumination and the passing stimulus. It is

important to note that the DS GC thus makes a comparison between the bipolar cell membrane potentials induced by the background and by the stimulus. If the membrane potential induced by the stimulus is relatively higher, the depolarization of the GC takes place, and it discharges. If the membrane potential of the bipolar cell evoked by the stimulus does not exceed that induced by the background, the DS GC will not discharge. According to the proposed model, when the stimulus, e.g., increasing the excitation of the L cones (stimulus L+) or decreasing the excitations of M or S cones (stimulus M- or S-), comes into the ON-type DS GC RF over the gray neutral (N) background, the ON-bipolar cells depolarize and transmit their excitation to the ON-type DS GC dendrite and the DS GC discharges (IN response) (Fig. 1(a)). However, if the selected color stimuli of opposite sign of contrast are used (L-, M+, S+), the membrane potential evoked by the stimulation of the bipolar cell should become lower relative to that induced by the background, and DS GC will not discharge. But in the latter case the ON-type DS GC will discharge in response to the withdrawal of the stimulus from the RF (OUT response). The similar symmetric mechanism takes place in case of the OFF-type DS GCs excited by the OFF- bipolar cells (Fig. 2(a)). OFF DS GCs discharge when the stimulus either decreases the excitation of the L cones (L-), or increases that of the M or/and S cones (M+, S+).

According to the model tested here, the responses of each of the DS GC types (ON or OFF) to all possible combinations of selective stimulation of the three cone types should always remain one-parameter. Consequently, the responses of the cell to all possible colors of stimuli should be aligned along the IN and OUT coordinates of an IN-OUT plot. To find out if there are any interactions between the color channels, we selectively stimulated either single cone types (L, M or S) or the combinations of two cone types (L and M, L and S, M and S). We then compared the DS GC responses, their patterns (IN and/or OUT) and relative strength.

We investigated 61 DS GCs (40 ON-type units and 21 OFF-type units). DS GCs of all three preferred directions (caudo-rostral, ventro-dorsal and dorso-ventral ones) were represented in this sample. In Fig. 1, the responses to selective and combined color stimuli of two ON-type DS GCs of dorso-ventral (b) and caudo-rostral (c) preferences are presented in IN-OUT coordinates. One can see that selective excitation of the L cones with the edges of either L+ or L- colors moving into the RF over the neutral background evoke pure IN or pure OUT responses, correspondingly. The number of spikes in IN response to L+ decreases if excitation of M (L+M+) or S cones (L+S+) is added. The additional stimulation of the M or S channels by the decremental stimuli (M-, S-) leads to the similar effect (inhibition) of the OUT response to L-. Furthermore, when an edge of the S+ color selectively exciting S cones moves into the receptive field of ON-type DS GC, it does not respond and discharges only when the stimulus leaves the RF. This pure OUT response is much weaker than the IN response to L cone stimulation. The DS GC responses to the selective M cone stimulation are similar to those when the S cones are selectively stimulated. The simultaneous excitation of the M and S cones produces a cumulative effect.

L+M+ stimulus moving over the reddish (L+) background produces an OUT response. Effect of the L+ component of combined stimulus was absent at reddish background, and actually only M channel was stimulated. L+S- stimulus is a decremental for the S channel, and as predicted by the model it should induce IN response in an ON DS unit either at reddish or at neutral background. This fact was proved for the ON DS GC shown in Fig. 1(c). As expected, the IN response to this stimulus over the reddish background (L+S-/L+) was weaker compared to the response elicited by the L+S- moving over the neutral background (L+S-/N).

One can perform a similar analysis for the responses of the OFF DS GCs in Figs. 2(b) and 2(c) in which it is shown that all responses of the OFF-type DS GCs are aligned along the IN-OUT coordinates as it was in case for the ON-type DS GCs (Figs. 1(b) and 1(c)) and follow the same principle of univariance.

Various response patterns of several DS GCs, their dynamics under selective and combined stimulation are shown in Figs. 3 and 4. In Fig. 3(a), the responses of the ON-type DS GC with dorso-ventral preferred direction to simultaneous stimulation of two types of cones (L+M+) are compared with the responses to the isolated stimulation of each type of cones separately (either L+ or M+). One can see that the movement of the L+ stimulus over the neutral background evokes a vigorous response of the cell as soon as its leading edge comes into the RF. The response increases when the edge approaches the RF's center and gradually decreases while it is leaving the RF. There is practically no response when the leading edge of M+

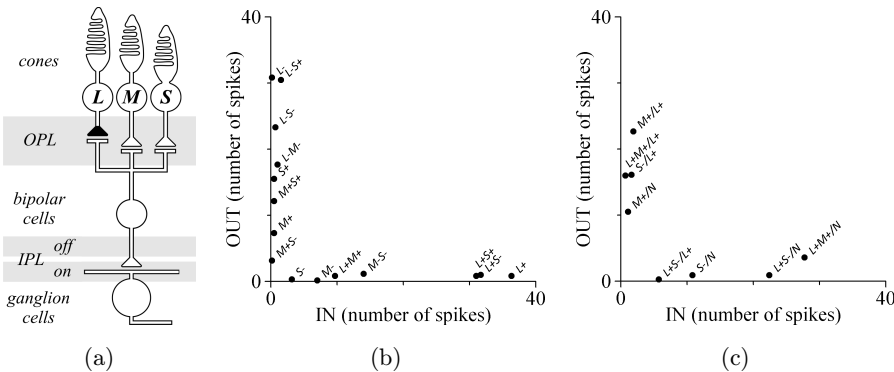


Fig. 1. Univariance of light responses in DS GCs of the ON type. (a) Putative wiring diagram of innervation of the DS unit of the ON type with three different chromatic types of cones (L, M and S) through the bipolar cells. Three layers of neurons (from top to bottom): cones, bipolar cells and ganglion cells are connected (separated) by two synaptic layers: the outer plexiform layer (OPL) and the inner plexiform layer (IPL), where these neurons transmit signals to each other. Sign-conserving synapses in the OPL are presented by white triangles whereas sign-inverting synapses are shown by black triangles. (b) Scatter plot of the OUT vs. IN responses of a DS unit of the ON type with dorso-ventral preferred direction to movement of horizontal color edges on the neutral background in top-down direction at the speed of 11 °/s. Colors of stimuli are inscribed next to the experimental points. (c) Scatter plot of responses of a DS unit of the ON type with caudo-rostral preferred direction to movement of vertical color edges on the neutral (N) or red (L+) background from tail to head at the speed of 11 °/s. Inscriptions next to the experimental points denote colors of stimuli and (after slash) background.

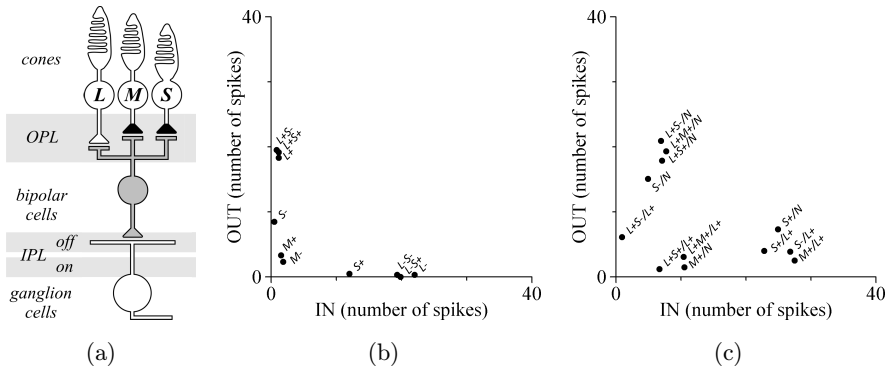


Fig. 2. Univariance of light responses in DS GCs of the OFF type. (a) Putative wiring diagram of innervation of the DS unit of the OFF type with cones through the bipolar cells. (b) Scatter plot of the OUT vs. IN responses of a DS unit of the OFF type with caudo-rostral preferred direction to movement of vertical color edges on the neutral background from tail to head at the speed of 5.5 °/s. (c) Scatter plot of responses of another DS unit of the OFF type with caudo-rostral preferred direction to movement of vertical color edges on the neutral (N) or red (L+) background from tail to head at the speed of 11 °/s. Other conventions are as in Fig. 1.

stimulus moves into the RF. Though, there is a response to simultaneous stimulation of the L and M cones by the L+M+ edge as opposed to its lack in response to the M+ edge, it is weaker than the response to the L+ edge. Hence, it is likely that the M channel inhibits the L channel.

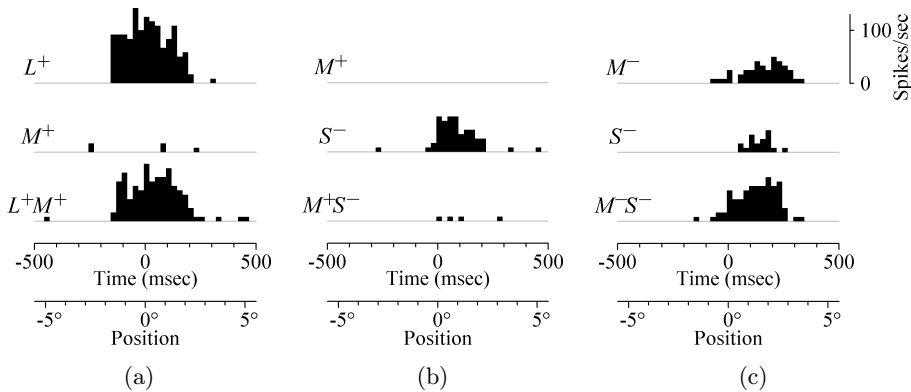


Fig. 3. Responses of the DS units of the ON type to simultaneous stimulation of two chromatic types of cones in comparison with responses to the isolated stimulation of each type of cones separately. Peri-stimulus histograms of the discharges display the density of evoked spikes along the time or position scales. The time scale: zero point corresponds to the time when the stimulus leading edge passes through the center of stimulation area. The position scale: zero point corresponds to the position of center of stimulation area. The scale enables to fix the position of stimulus leading edge in the visual space at the moment of spike appearance. Histogram with 25 ms bins was compiled from the responses to 6 presentations of the stimuli. (a) A DS GC with dorso-ventral preferred direction, responses to movement of horizontal color edges in top-down direction on the neutral background. (b) A DS GC with ventro-dorsal preferred direction, responses to movement of horizontal edges in bottom-up direction. (c) A DS GC with dorso-ventral preferred direction, responses to movement of horizontal edges in top-down direction; the same experiment as in Fig. 1(b). In all cases, the edges were moving at the speed of 11 °/s. Designations for stimuli colors are given to the left of the histograms.

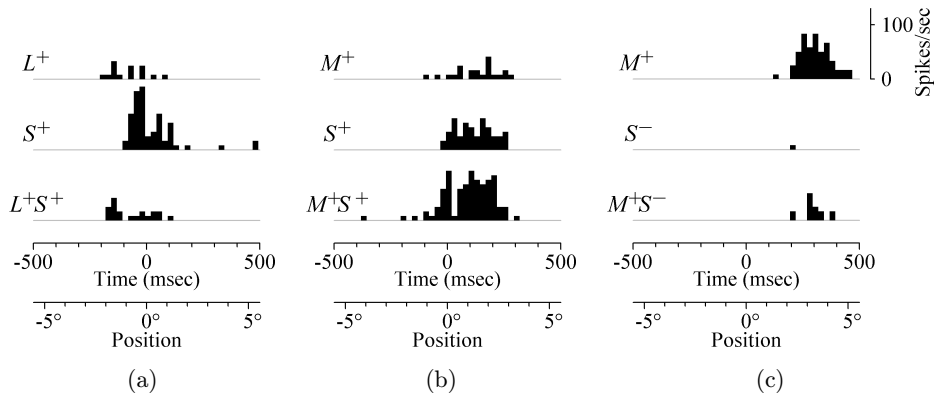


Fig. 4. Responses of the DS units of the OFF type to stimulation of two chromatic types of cones in comparison with responses to the isolated stimulation of each type of cones separately. (a) A DS GC with ventro-dorsal preferred direction, responses to movement of horizontal color edges in bottom-up direction on the neutral background. (b, c) Two DS GCs with caudo-rostral preferred direction, responses to movement of vertically oriented edges from tail to head. In all cases, the edges were moving at the speed of 11 °/s. Other conventions are as in Fig. 3.

The cumulative interactions of the M and S cones are revealed in responses of a DS GC with the ventro-dorsal preferred direction (Fig. 3(b)) and a DS GC with the dorso-ventral preferred direction (Fig. 3(c)). The incremental stimulus M+ does not excite the cell (Fig. 3(b), upper row); the decremental stimulus S- evokes a pronounced response (Fig. 3(b), central row). Combined stimulation (M+S-) evokes a very weak response. Decremental stimuli M- and S- when presented separately (Fig. 3(c), upper and central rows) evoke weak cell responses. The response to combined stimulation of the M and S cones with the decremental color (M-S-) exceeds the sum of responses to M- and S- colors.

The responses of the three OFF-type DS GCs to the separate and combined stimulation of the different color channels are shown in Fig. 4. The stimulus of the incremental color L+ moving in the ventro-dorsal direction into the RF evokes a weak response (Fig. 4(a), upper row); the incremental stimulus S+, exciting the S cones evokes a pronounced discharge. Response to the combined stimulation of the L and S cones by an L+S+ edge is weaker than the responses to either L+ or S+ color.

Cumulative interaction of the M and S channels as revealed in the responses of two DS GCs with the caudo-rostral preferred direction is illustrated in Figs. 4(b) and 4(c). The spike discharge of the cell to the M+S+ color (b, lower row) exceeds the sum of the responses to separate excitation of the M and S cones (b, upper and central rows). There is no response to the S- stimulus (middle row). The combined stimulus (M+S-) evokes a weaker DS GC response (c, lower row) as compared to the response to M+ color (c, upper row).

Figures 3 and 4 illustrate the cumulative (synergistic) interaction between the M and S channels and their opponent interaction with the L channel, manifested in the responses of both the ON and OFF DS GCs of various preferred directions. The

interactions of the color channels observed in our experiments are consistent with the model of the fish DS GCs wiring.

Similar experiments with separate and combined stimulation of L, M and S cones have been performed on the OS GCs (27 units). OS GCs were proved to divide into two subtypes according to their preferred stimulus orientation (approximately horizontal or vertical) and were characterized by ON-OFF type responses (Maximov *et al.*, 2009; Damjanović *et al.*, 2009). It was shown that the L and M inputs to OS GCs were synergic while the S channel was opponent to the L and M inputs. The response of an OS GC to a combined decremental stimulation (L–M–) of the L and M inputs was stronger than the sum of its separate L– and M– responses (Fig. 5(b)). The responses to a combined stimulation of the L and S cones (L–S–) or M and S cones (M+S+) were smaller than their corresponding sums (Figs. 5(a) and 5(c)). It is important to note that the excitation of the L cones evokes an ON-OFF response of the OS GCs (Maximov *et al.*, 2015). Therefore, in the wiring of the OS GCs having the same cone inputs (L, M, S) as the DS GCs, different bipolar cell types should be involved. A putative scheme of the OS GC wiring was previously suggested in the paper of Maximov *et al.* (2015). According to that scheme, OS GCs are considered to be bistratified (presented in Fig. 6 here). Interactions between color channels (L, M, S) in the putative OFF pathway (via OFF bipolar cells) shown in Figs. 5(a) and 5(b) are consistent with the model. Meanwhile, some of the color interactions observed in the ON pathway (via ON bipolar cells) differed from those predicted by the model. While interactions between the L and S channels, within the model are supposed to be synergistic, in some experiments they were found to be opponent. Thus, the results of the present study do not confirm the model unequivocally.

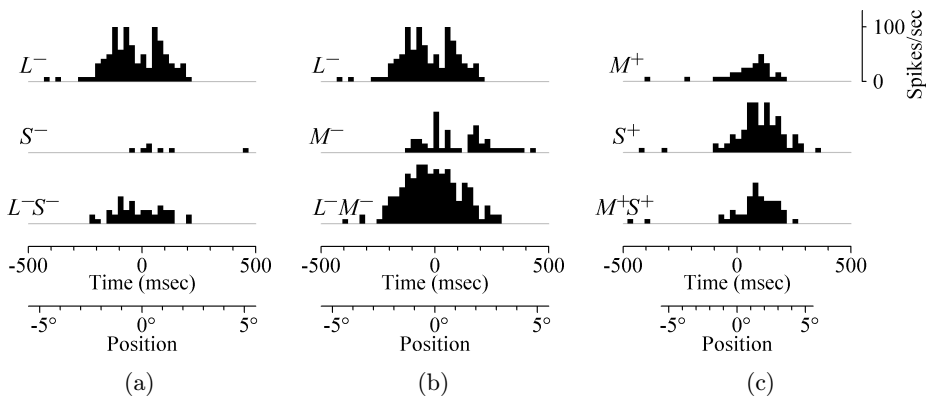


Fig. 5. Responses of the orientation-selective units to stimulation of two chromatic types of cones in comparison with responses to the isolated stimulation of each type of cones separately. (a, b) A detector of horizontal line, responses to movement of horizontally oriented color edges in bottom-up direction on the neutral background at the speed of $11^\circ/\text{s}$. (c) detector of horizontal line, responses to movement of horizontal edges in bottom-up direction at the speed of $16.5^\circ/\text{s}$. Other conventions are as in Fig. 3.

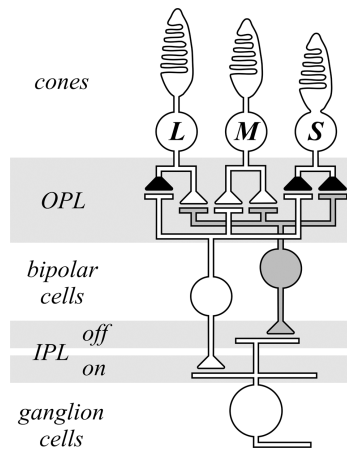


Fig. 6. Putative wiring diagram of innervation of the orientation-selective unit with different chromatic types of cones through the bipolar cells. The structure of the OS GCs should be somewhat more complicated than in DS GCs because of the ON–OFF nature of their responses to the long-wave selective colors. It looks like it will require separate types of ON and OFF bipolar cells. Hypothetic scheme of OS circuitry is suggested. All conventions are same as in Fig. 1(a).

4. Discussion

Within the established concept of organization of the retina based on the data of electrophysiology, electron-microscopy and neuron marking technique of mammalian (especially primate), retinal bipolar cells were divided into two classes: OFF-type hyperpolarizing cells, with the iGLUR sign-conserving synaptic receptors, and their axon terminals in *a* sublamina of IPL and ON-type depolarizing cells with the mGLUR6 sign-inverting synaptic receptors, and their axon terminals in *b* sublamina of IPL (Boycott & Kolb, 1973; Dacheux & Raviola, 1986; Famiglietti, 1992; Famiglietti & Kolb, 1976; Kaneko, 1973; Kolb, 1979; Kolb, 1970; Kolb & Famiglietti, 1974; Nelson & Kolb, 1983; Slaughter & Miller, 1981, 1983). However, bipolar cells combining two types of glutamate receptors on their dendrites have been found in the fish retinas in several experimental works recently (Sakai & Naka, 1983; Saito *et al.*, 1985; Wong & Dowling, 2005; Li *et al.*, 2012). The first evidence that some ON bipolar cells can make sign-conserving (non-ribbon) synapses with cones, and some OFF bipolar cells can make sign-inverting (ribbon) synapses with cones were demonstrated in the retina of carp (Saito *et al.*, 1985) and catfish (Sakai & Naka, 1983). If a bipolar cell has synapses with two or more types of photoreceptors (the type of the cell, ON or OFF, is determined with white light), it is possible that a cell classified e.g., as an ON cell could make predominantly ribbon synapses with one or two cone types, but fewer non-ribbon synapses with other cone types (Sakai & Naka, 1983). The fact that different chromatic types of cones can make different types of synapses with one bipolar cell has been also shown for the turtle retina (Haverkamp *et al.*, 1999). In the zebrafish retina, bipolar cells of 18 types were reconstructed with their dendritic morphology, cone connectivity and axonal stratification. It was shown that

bipolar cells with the same photoreceptor connectivity can have heterogeneous axonal stratification patterns and dendritic tree morphologies and vice versa — different connectivity subtypes could have the same axonal stratification pattern (Li *et al.*, 2012). As a bipolar cell's photoreceptor connectivity indicates its input and axonal stratification its response sign (ON or OFF), the authors suggested that for comprehensive definition of bipolar cell both, its photoreceptor connectivity and its axonal stratification should be described.

The above-mentioned data served as a basis for our model. As a result of coexistence of the two different types of synapses with all three types of cones on the dendrite of bipolar cell, three-parameter input signal generated on the level of OPL should be transformed to the one-dimensional signal at the output of a bipolar cell and hence at the output of its target unit, DS GC (principle of univariance; Naka & Rushton, 1966; Bilotta & Abramov, 1989). Accordingly, in the IN–OUT plot the amplitudes of all responses of the DS unit should be aligned along IN and OUT coordinates, what was clearly demonstrated in the present study, and the principle of univariance was thus confirmed (Figs. 1(b) and 1(c), Figs. 2(b) and 2(c)). Moreover, the results proved the main contribution of the L channel to DS GC response and manifested the opponent influences of the M and S cones. Opponent effects of the M cones, as a rule, were stronger than those of the S cones. Responses of the DS units to simultaneous excitation of the M and S channels were, as a rule, significantly stronger than the sum of their responses to separate stimulation of the middle-wave and short-wave cones (Figs. 3(c) and 4(b)). The synergistic interaction between the M and S channels predicted by the model was thus confirmed. On the contrary, in responses of the OS GCs M and S channels were opponent to each other (Fig. 5(c)). However, color interactions predicted by the model of OS GC wiring (Fig. 6) were experimentally proved only for the OFF pathway. Moreover, there exists another model of the OS GCs based on extensive data on the observed features (receptive field size, contrast sensitivity, spatial resolution, color properties) which was computer simulated (Maximov, 2010). The OS GCs were mentioned there as being unistratified in the OFF (*b*) sublamina of IPL, despite them being ON-OFF units. The ON-OFF responses of the cells were explained within the framework of the model by the inclusion of lateral interactions. Further research is required to provide more detail on the mechanism involved in order to assess which of two models of OS GCs is more realistic.

In summary, main conclusions have to be emphasized. Experimental results on the interactions of the color channels as reflected in the DS GC responses are consistent with the proposed model of the cell wiring. As to the OS circuitry of fish retina it still remains unclear and needs further investigation.

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