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Opposing motion inhibits responses of direction-selective ganglion cells in the fish retina

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Inhibitory influences in receptive fields (RFs) of the fish retinal direction-selective ganglion cells (DS GCs) were investigated. Responses of the fast retinal DS GCs were recorded extracellularly from their axon terminals in the superficial layer of tectum opticum of immobilized fish. The data were collected from two cyprinid species — *Carassius gibelio*, a wild form of the goldfish, and the barbel fish *Labeobarbus intermedius*. Visual stimuli were presented to the fish on the monitor screen within a square area of stimulation occupying approximately $11 \times 11^\circ$ of the visual field. DS GCs were stimulated by pairs of narrow stripes moving in opposing directions. One of them entered central (responsive) area of cell receptive field (RRF) from the preferred, and the other one from the null side. Stimuli merged at center of stimulation area, and subsequently moved away from each other. It was shown that the cell response evoked by the stripe coming from the preferred side of RF was inhibited by the stimulus coming from the opposite direction. In the majority of units recorded inhibitory effect induced by the null-side stimulus was initiated in the RF periphery. As a rule, inhibitory influences sent from the RF periphery were spread across the entire central area of RF. Modifications of the inhibitory influences were investigated throughout the whole motion of paired stimuli. Evident inhibitory effects mediated from the null direction were recorded during the approach of stimuli. When stripes crossed each other and moved apart inhibition was terminated, and cell response appeared again. Null-side inhibition observed in fish DS GCs is most likely induced by starburst-like amacrine cells described in morphological studies of different fish species. Possible mechanisms underlying direction selectivity in fish DS GCs are discussed.

Keywords: Fish retina; direction-selective ganglion cells; null-side inhibition; starburst amacrine cells.

1. Introduction

Direction-selective visual neurons strongly respond to image movement in a particular (preferred) direction and are inhibited by the stimulus movement in the opposite or null direction. The basic requirement for the direction selectivity is an asymmetric nonlinear interaction between spatially separate inputs (Barlow & Levick, 1965). In

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theory, this asymmetry could arise from: (a) increased inhibition during null-direction motion or (b) increased excitation when the stimulus moves in the preferred direction. Thus, in a simplified excitatory model, the input at each position is facilitated by the prior inputs coming from the preferred side of the cell receptive field (RF). In a simplified inhibitory model, on the other hand, the excitatory input at each position is blocked by prior inputs coming from the null side. Among different types of DS units described in vertebrate retinas, mammalian ON–OFF type direction-selective ganglion cells (DS GCs) were the most thoroughly studied (see review of Borst & Euler (2011)). These GCs characterized by relatively small RF and tuned to broad range of stimulus velocities are determined as fast DS units and considered to be local motion detectors (Vaney *et al.*, 2001). They are excited by stimuli that are lighter or darker than the background and projected to the superior colliculus, comprise four physiological types with different preferred directions aligned with the horizontal and vertical ocular axes (Oyster & Barlow, 1967). The crucial role of the null-side inhibition in generation of direction selectivity was proved for mammalian fast DS GCs. The asymmetric delayed inhibition from the null side was shown experimentally to be mediated by "starburst" amacrine cells (SACs) (Yoshida *et al.*, 2001; Euler *et al.*, 2002; Lee & Zhou, 2006; Lee *et al.*, 2010).

Fish DS GCs were investigated less thoroughly. In recent studies (Maximov *et al.*, 2005a,b) it has been shown that DS GCs in the cyprinid fish *Carassius gibelio* projecting to the tectum opticum (TO) are divided into three distinct groups, characterized by their preferred movement directions: caudo–rostral, ventro–dorsal and dorso–ventral, separated by about 120°. In each group the DS GCs are represented by the ON and OFF cell subtypes in the relatively equal amount. It was shown that according to numerous physiological characteristics the fish DS GCs should be classified as "fast" DS units, which are most likely involved in the detection of small objects moving in the surrounding environment. First of all, DS GCs of *C. gibelio* characterized by relatively small responsive receptive field (RF) areas (Damjanović *et al.*, 2009) were shown to respond to a broad range of velocities of moving stimuli (Maximov *et al.*, 2005b). Central area of the cell RF, from which responses can be elicited by visual stimulation, will be referred as RRF hereafter. Furthermore, in the recent study of Maximov *et al.* (2013) it was shown that *C. gibelio* DS GCs are characterized by high spatial resolution. Extracellular recordings of responses of individual DS GCs to the motion of contrast gratings of different periods showed that the acuity of DS units is close to the limit determined by cone density.

Since starburst-like amacrine cells were described in morphological studies in the shark (Brandon, 1991) and zebrafish (Yazulla & Studholme, 2001; Arenzana *et al.*, 2005) one can suppose that inhibition coming from the null side of DS GC RRF induces direction selectivity in the fish retina, similar to the mammalian fast ON–OFF DS GCs. This supposition was proved in the recent physiological study of Damjanović *et al.* (2009), where DS GCs were stimulated by two small spots that flashed simultaneously. One of the spots always flashed in the center, while the second one flashed in various positions of the cell RF. The influence of the second spot

on the cell response evoked in the center was estimated by the difference between number of spikes in response to stimulation by paired stimuli and the number of spikes in response to central stimulus alone. If this value was negative, one could say that there was an inhibitory influence from the peripheral spot. It was shown that inhibitory signals were generated outside the DS GC RRF, on the null side of RF periphery similar to the mammalian ON–OFF DS GCs (Chiao & Masland, 2003).

Hence, one can say that the method with paired spots proved to be relevant for analysis of lateral interactions between the peripheral inhibitory zone and the RRF center. However, spread of inhibitory influences across the RRF could not be adequately studied by means of this method. The aim of the present study is to estimate more exactly the spatial properties of null-side inhibition in the RF of fish retinal DS units. DS GCs of two cyprinid fishes, *Carassius gibelio* and *Labeobarbus intermedius* were stimulated by pairs of narrow stripes moving in opposing directions. One of the stripes entered the cell RRF from the preferred, and the other one — from the null side. Stimuli merged at center of stimulation area, and subsequently moved away from each other. The method with paired moving stripes can be used for reliable estimate of the distance between approaching stimuli at which inhibitory signal sent from the null side of unit RF started to influence response elicited by the preferred-side stimulus. Accordingly, we can estimate the position in the null side of RF at which inhibitory signal was initiated and defined to which distance the influences of this signal were transmitted. Modifications of the null-side inhibitory effect are planned to be investigated throughout the whole motion of paired stimuli. Interactions between moving stripes after they crossed each other in the center of stimulation area are of particular interest. Obtained results should confirm or refuse the supposition that the inhibitory influences can be transmitted only in the null direction, i.e., during the approach of stimuli. As a whole, the data, obtained by this procedure, can give us additional information about mechanisms underlying direction selectivity in fish DS GCs.

2. Methods

2.1. *Experimental animals*

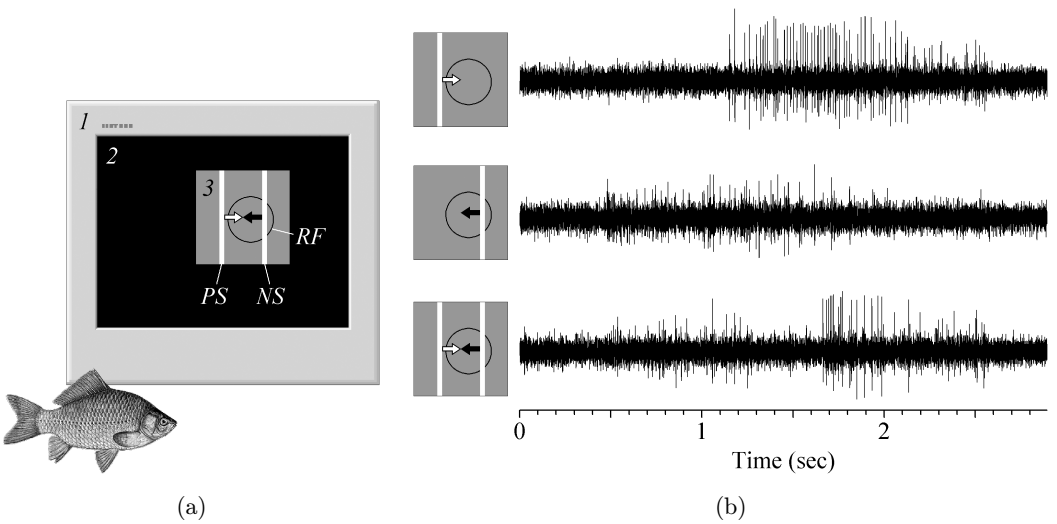
The data were collected from two cyprinid species: *Carassius gibelio*, a wild form of the goldfish (36 fishes; body weight varied from 40 g to 100 g), and the barbel fish *Labeobarbus intermedius* (1 fish; body weight 150 g). *C. gibelio* were acquired from local suppliers (Moscow region) and maintained for several months in aerated fresh water aquaria at room temperature and natural daylight regime. The only experiment on *L. intermedius* was performed in order to elucidate whether the properties of null-side inhibition revealed in DS GCs of *C. gibelio* are relevant for other fish species.

For electrophysiological experiments an immobilized fish (d-tubocurarine, i.m.) was placed in a Plexiglas tank and fixed in natural position with perfusion of aerated water through its gills. The constant water level in the tank was maintained such

that the eyes of the fish were under water but water was not poured into the brain. The fish looked on the monitor screen through the transparent tank wall. Visual responses were recorded from a contralateral lobe of the TO. 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. *Visual stimulation*

Visual stimuli were presented to the fish on the computer-controlled 17" CRT monitor LG Flatron 775FT from a distance of about 30 cm. From this distance, the screen occupied $43 \times 32^\circ$ of the fish visual field. To stimulate the motion detectors, moving contrast edges or narrow stripes were presented on the screen within a gray square area of stimulation with angular dimensions of $11 \times 11^\circ$, exceeding RRF of the recorded DS unit approximately 2–3 times (see Fig. 1(a)). In the main experimental procedure DS units were stimulated by single and paired narrow stripes, moving in preferred or null directions. Paired stripes moved simultaneously in



(a) (b)

Fig. 1. Stimulation procedure with paired stripes moving in opposing directions. (a) A schematic view of the experimental paradigm. 1 — stimulating monitor; 2 — monitor screen; 3 — gray stimulation area (angular dimensions of $11 \times 11^\circ$); RF — rough estimation of RRF of the recorded unit with regard to the stimulation area; PS — preferred-side stripe; NS — null-side stripe. Paired stripes (PS and NS) moved simultaneously in opposing directions — one stripe entered the cell RRF from the preferred direction and the other one — from the null (marked by white and black arrow, respectively). (b) Stimulation procedure and results obtained in one ON DS GC of *C. gibelio*. ON DS unit was stimulated by white stripes, moving in either preferred or null directions across gray stimulation area, which occupies approximately $11 \times 11^\circ$ of the fish visual field. Stimuli: top panel — single stripe moving in the preferred direction; middle panel — single stripe moving in the null direction; bottom panel — paired stripes moving simultaneously in opposing directions. Width of stimuli was $30'$; velocity of stimulus movements was $5.5^\circ/\text{s}$. Stimulation areas are schematically presented by gray square regions. Rounded area in the square represents the unit RRF. Responses of the unit are shown near corresponding stimuli.

opposing directions — one of them crossing stimulation area in the preferred, and the other one in the null direction (Fig. 1(a)). The stimulation area could be placed at arbitrary locations of the screen. It was usually placed so that the RF of the recorded unit was located in its center. In order to fixate the beginning of null-side inhibition, in some experiments the position of the stimulation area was shifted relative to the unit RRF.

2.3. *Data acquisition*

Single-unit responses of DS 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 (Gesteland *et al.*, 1959). Experimental setup, used for amplifying, digitizing, storing and processing of the DS GC records, containing AC preamplifier (band pass 100–3.5 kHz), 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.*, 2005; Damjanović *et al.*, 2009; Maximov & Maximov, 2010).

2.4. *Experimental procedures*

When a good DS unit was isolated and an approximate position of its RRF was found the first step was always to measure its directional tuning curve (polar diagram) with contrast edges moving in different directions across the RRF. This allowed to confirm the type of the recorded unit and to determine the size and location of its RRF. The next step was to measure excitatory/inhibitory interactions in the RFs of fish DS unit by means of specially designed experimental procedure, which included stimulation with paired stripes moving in opposing directions. The standard experimental procedures (polar diagrams, stimulation with paired stripes etc) were designed in the form of program tools.

2.4.1. *Polar diagram measurements*

A typical procedure for the measurement of a polar diagram by moving contrast edges was as follows. The monitor and the stimulation area on its screen were placed in the visual field of the fish in such a way as to cover the estimated RF, and the values of the following stimulation parameters were specified: the speed of movement of the edge, the brightness of the background and of the edge, as well as the brightness of the surroundings outside the stimulation area, the initial direction of movement, the total number of different directions of movement (usually 12), and the number of repetitive trials in each direction (usually 3). Mean number of spikes was calculated over repeated trials for each direction. At the end of the procedure a measurement for the first direction was repeated in order to check the unit response level. Preferred directions of the stimulus movement were determined according to phase of the first harmonic of Fourier transform of polar diagram.

2.4.2. *Determination of the RRF center*

Position of the RRF in the stimulation area was outlined from cell responses to the contrast edge moving in 12 different directions. It was calculated online using the same experimental data, obtained during polar diagram measurements. The RRF center was evaluated from the sequences of moments of spike appearances in all trials for all 12 directions of movement. The automatic procedure that determines the position of the RRF center is as follows. The deviation of the moment of spike appearance from the moment a stimulus passes through the assumed RRF center is calculated for each of the applied directions. The center of the RRF was determined as a point in the visual field, where the mean square deviation calculated for all directions was minimal. As a rule, after the measurement of the polar diagram and determination of the position of the RRF, the position of the area of stimulation on the monitor screen was centered with respect to the RRF. Subsequent procedures were usually conducted with the centered RRF. As mentioned above, in some experiments the position of stimulation area was arbitrarily shifted relative to the unit RRF.

2.4.3. *Stimulation procedure with paired stripes moving in opposing directions*

Excitatory/inhibitory interactions in the RFs of fish DS units were investigated by means of specially designed experimental procedure shown in Fig. 1(b). Units were stimulated by narrow stripes, moving across gray stimulation area in preferred or null directions in the procedure composed of 3 steps. In the first two steps, single stripe moved in the preferred and null directions, respectively (top and middle panel of Fig. 1(b)). In the final step of the procedure DS units were stimulated by paired stripes that moved simultaneously in opposing directions, one of them passing stimulation area in the preferred, and the other one in the null direction (bottom panel of Fig. 1(b)). Responses of one ON DS unit of *C. gibelio* to presented stimuli are shown.

The experimental procedure presented in Fig. 1(b) was implemented by means of specially designed program tools. Program set performed several operations. It controlled stimulation in 3 steps shown in Fig. 1(b) and stored recorded data. It was allowed to regulate number of presentations of stimuli. In different experiments stimulation was repeated nine or eighteen times at each step.

3. Results

Results on excitatory/inhibitory interactions in the RFs obtained in one ON DS GC of *C. gibelio* by means of two stripes moving in opposing directions are presented in Fig. 1(b). Cell response to the stripe moving in the preferred direction is shown on the right of the corresponding stimulus on the top panel of figure. In the following step, the stimulus moved in the opposite direction (middle panel of Fig. 1(b)) and, as one can see, no response was recorded. In the final step of the procedure paired stripes

moved in opposing directions across stimulation area, one of them entering cell RRF from the preferred, and the other one — from the null side (bottom panel of Fig. 1(b); rough estimation of unit RRF is symbolized by a rounded area inside the area of stimulation). In the first half of record, while stimuli were approaching, the cell was silent. This means that the cell response, evoked by the stripe coming from the preferred side of RF was inhibited by the stimulus coming from the opposite direction. The inhibition lasted till the moment when stimuli coalesced. When stripes moved away from each other, cell response appeared again. In other words, inhibitory effect mediated from the null direction was recorded while stimuli were approaching, and it ceased after stripes crossed each other in the center of the stimulation area.

As mentioned above, stimulation could be repeated nine or eighteen times at each step. Figure 2 demonstrates averaged peristimulus data calculated over all 3 steps of stimulation for responses of three different DS GCs of *C. gibelio*. Averaged peristimulus histograms are plotted against the spatial coordinates of stimulation area. Polar diagrams and preferred directions of shown units are presented above corresponding histograms on the top of figure. The histograms on diagrams were plotted for stimulations with (from top to bottom): single stripe moving in the preferred direction, single stripe coming from the null direction, paired stimuli. One can notice certain pattern in the cell responses evoked by the paired stimuli. Weak responses were recorded, considerably reduced in comparison with those evoked by the preferred-side single stripes. At a certain point before the stimuli coalesced in the center, responses had fallen to zero. When stimuli began to move away from each other unit responses appeared again. As a matter of fact, responses to paired stimuli shown in Fig. 2 repeated the tendency revealed in Fig. 1(b) — while stimuli approaching the inhibition mediated from the null side was recorded. All in all, 52 DS GCs were subjected to this experimental procedure, and in all of them null-side inhibitory effect was observed.

In order to estimate the point in the null-side of RF at which inhibition was initiated, in a number of experiments the position of stimulation area was shifted in relation to the RRF. As a consequence cell RRF was located: (a) closer to the preferred-side stripe, (b) closer to the null-side stripe or (c) juxtaposed at the center, i.e., located approximately in the center of the stimulation area. Results obtained in one ON caudo–rostral DS GC are presented in Fig. 3. Spatial coordinates of squared stimulation area are represented on the bottom of all graphs. Rounded areas on the bottom of all diagrams represent rough estimations of cell RF in relation to the center of squared area. On the diagram represented in Fig. 3(a) the cell RRF was shifted in the square closer to the null-side stripe. As a consequence, when paired stripes were presented the inhibitory stimulus coming from the null side entered the RRF before the excitatory one. Response to paired stimuli was similar to that shown in Fig. 1(b) — the cell was silent while stripes were approaching, and when stripes moved away from each other, response arose. In Fig. 3(b) the cell RRF was located almost precisely in the center of the stimulation area and paired stripes entered the RF simultaneously. As a result, a weak response, which at certain point decayed to

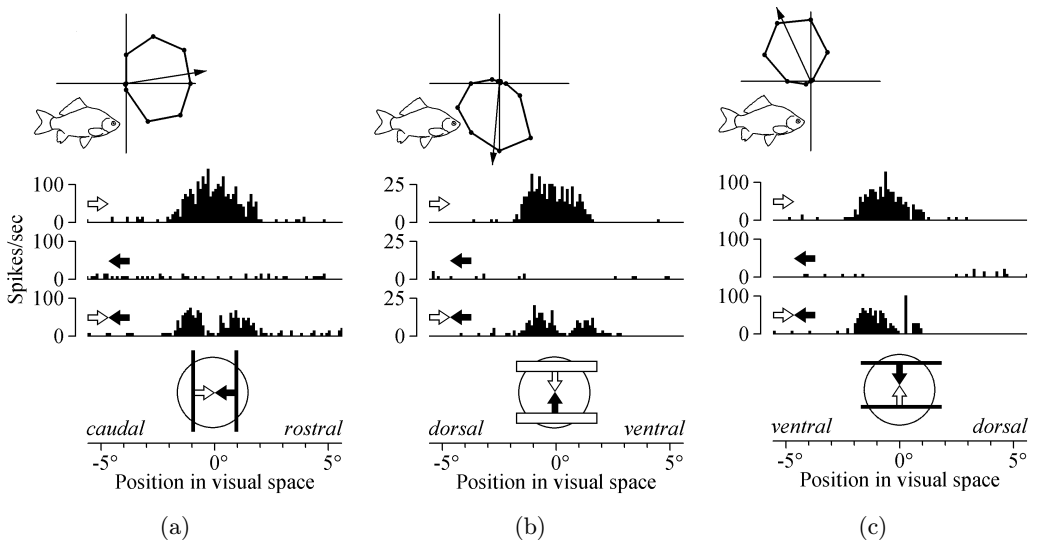


Fig. 2. Inhibitory influence of the null-side stripe on the response elicited by the stimulus coming from the preferred side of DS GC RRF. The results were obtained in three DS GCs of *C. gibelio* selective to different stimulus movements. DS units were stimulated by stripes, moving in either preferred or null directions across neutral (gray) stimulation area in the procedure consisting of three steps. The averaged peristimulus histograms on diagrams are plotted for stimulations by (from top to bottom): single stripe moving in the preferred direction, single stripe moving in the null direction, paired stimuli (preferred and null directions of stimuli movements are marked near corresponding histograms by white and black arrows, respectively). Spatial coordinates of the stimulation area are presented on the bottom of all diagrams in the coordinates of visual space (degrees; 0° marks the center of the stimulation area). Polar diagrams of investigated DS units are presented above the corresponding histograms. Below histograms are illustrated corresponding paired stimuli (white or black) moving in opposing directions — one of them in the preferred (white arrow) and the other in the null direction (black arrow). Rounded areas represent rough estimations of DS GC RRFs. (a) OFF-type DS GC selective to caudo–rostral movement. The unit was stimulated by 10′ wide black stripes, moved with the velocity of 2.75°/s along horizontal axis of fish visual field. Nine consecutive presentations of stimuli were performed at each step of stimulation. (b) ON-type DS GC selective to dorso–ventral movement. The unit was stimulated by 30′ wide white stripes, moved with the velocity of 11°/s along vertical axis of fish visual field. Nine consecutive presentations of stimuli were performed at each step of stimulation. (c) OFF-type DS GC selective to ventro-dorsal movement. The unit was stimulated by 10′ wide black stripes, moved with the velocity of 2.75°/s along vertical axis of fish visual field. Nine consecutive presentations of stimuli were performed at each step of stimulation.

zero has been recorded. It should be noted that the response disappeared before the stimuli have met in the center of RRF and arose again after stripes crossed each other in the center. Thus it is probable that the inhibition was initiated immediately after the stimuli had entered RRF. In other words, inhibitory influences were most likely spread across entire RRF. The point at which inhibition was initiated could be estimated in the case when unit RRF was located closer to the preferred-side stripe. Results obtained when the excitatory, preferred-side stimulus entered the cell RRF earlier than the inhibitory one are presented in Fig. 3(c). Peristimulus histograms calculated for two modes of stimulation, by preferred stripe and by paired stimuli, coincided in the beginning. However, at certain distance between stimuli the

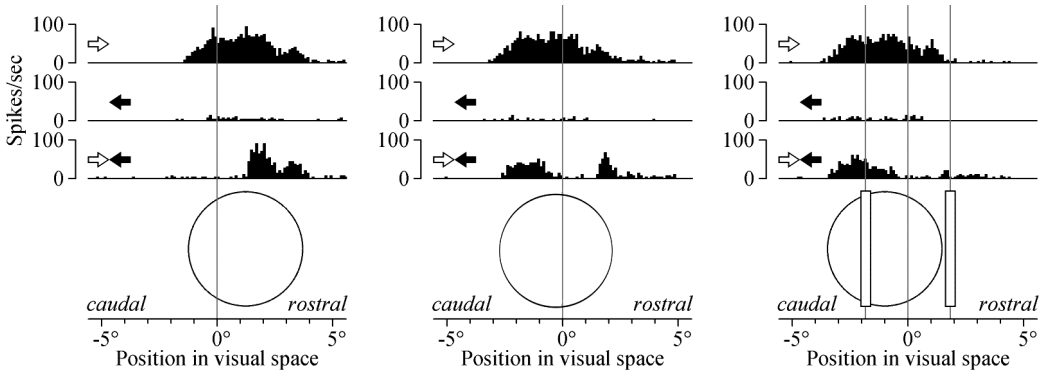


Fig. 3. Inhibitory influences of null-side stimulus on the response of an ON-type caudo–rostral DS unit recorded at different positions of its RF in the stimulation area (*C. gibelio*). The unit was stimulated by 30′ wide white stripes, moved with the velocity of 5.5°/s along horizontal axis of fish visual field in opposite directions (preferred, caudo–rostral or null, rostro–caudal direction). The stimulation procedure consisted of 3 steps (see Fig. 1). Eighteen consecutive presentations of stimuli were performed at each step of stimulation. Averaged poststimulus histograms, calculated for all modes of stimulation are presented in the same order as in Fig. 2. Cell responses were recorded at three different positions of its RRF in the stimulation area: (a) RRF of DS unit was located closer to the null-side stimulus; (b) unit RRF was located almost precisely in the center of the square; (c) unit RRF was located closer to the preferred-side stimulus. Visually approximated positions of paired stimuli at the moment when null-side inhibition began are presented (see paired narrow white stripes representing paired stimuli moving in opposing directions). Other conventions are as in Fig. 2.

histogram for paired stripes started to decay rapidly and before the stimuli merged at the center, the response disappeared. In other words, at a certain distance between paired stripes inhibitory influence from the null side was initiated, and further on the response evoked by stimulus from the preferred side was occluded. Visually approximated positions of paired stimuli at the moment when null-side inhibition began are shown in Fig. 3(c). The onset of inhibition was supposed to coincide with the moment when the response to paired stimuli started to decay abruptly. Note that a proposed position of null-side stripe at that moment was outside RRF.

For a precise evaluation of the beginning of inhibition, processed data were subjected to further statistical analysis. Peristimulus histograms calculated for paired stripes were compared with those calculated for stimulation with preferred stimulus alone. The aim of the procedure was to estimate position of the preferred stimulus in the RF at which inhibitory signal was initiated. For this purpose numbers of spikes elicited by single preferred stripe and paired opposing stimuli were compared over sufficiently small space intervals distributed along the motion trajectory of the preferred-side stimulus. As long as responses to two kinds of stimuli coincided it was considered that there was no inhibitory influence from the null side. But, when the response to paired stripes apparently decreased and difference between responses to two types of stimuli (single and paired stripes) become statistically significant it was considered that null-side inhibitory effect was initiated at that point. For the relevant

statistical analysis nonparametric Mann–Whitney U-test was used. As mentioned above, in different experiments stimulation was repeated either 9 or 18 times for each kind of stimuli. Hence, over each small space interval we had to compare two equal samples with 9 or 18 sets of data. The length of this space interval was 20′ of the visual field. Corresponding time interval depended on the adjusted speed of movement of stripes. The data obtained for such small running window along the motion trajectory of the preferred-side stripe determined the dependence of Mann–Whitney U-values on the position of preferred stimulus in the RRF. The point at which U-value fell below the critical level was considered as the beginning of inhibition. In other words, by using Mann–Whitney test we could estimate inhibitory effects of the null-side stimulus at different positions of the preferred-side stripe in the RRF over the period of opposing motion.

Results processed by Mann–Whitney test in one ON caudo–rostral DS unit are shown in Fig. 4. White tracks at the top panel of diagrams represent trajectories of movements of white stripes against the gray background light during the period of stimulation (width of tracks corresponds to width of stimuli — 10′). The stripes could move in the preferred or in the null direction over the stimulation area. The x-axis shows the positions of stimuli in coordinates of visual space (degrees). The time of stimulation in seconds is given on the y-axis. Peristimulus histograms shown on the middle panel of diagrams represent the averaged values for nine consecutive presentations of light stimuli (stripes) on each step of stimulation. As before, histograms are shown for stimulations by (from top to bottom): single stripe moving in the preferred direction, single stripe moving in the null direction and paired stimuli. On the bottom panel of diagrams peristimulus histograms calculated for paired stripes were compared with those for stimulation with single preferred stimulus (Mann–Whitney test). Mann–Whitney U-values, were calculated for these two samples at different positions in the stimulation area, and consequently Mann–Whitney curve was constructed. The inhibitory effect from the null side was statistically valid when the U-values fell below the critical level. The solid vertical line in Fig. 4 signed as "start of inhibition" marks the position of the preferred-side stimulus at which inhibitory effect started during the opposing motion of paired stripes. The diagram also demonstrates the distance between stimuli in the moment when inhibition started (marked by the thick horizontal arrow). One can see, that in the presented ON DS GC inhibitory effect induced by the null-side stimulus was initiated outside the unit RRF (RRF diameter corresponds to the width of discharge evoked by single preferred stripe; vertical dashed line marks the location of the null-side stimulus in RF periphery at which inhibitory signal was initiated). Mann–Whitney test also provides information regarding the duration of inhibitory influences. In the present DS unit inhibitory effect mediated from the null direction was recorded while stimuli were approaching, and it ceased after stripes crossed each other in the middle of the stimulation area.

Results of this statistical analysis performed for the ON caudo–rostral DS unit illustrated in Fig. 3 are shown in Fig. 5. Two diagrams, regarding the cases when cell

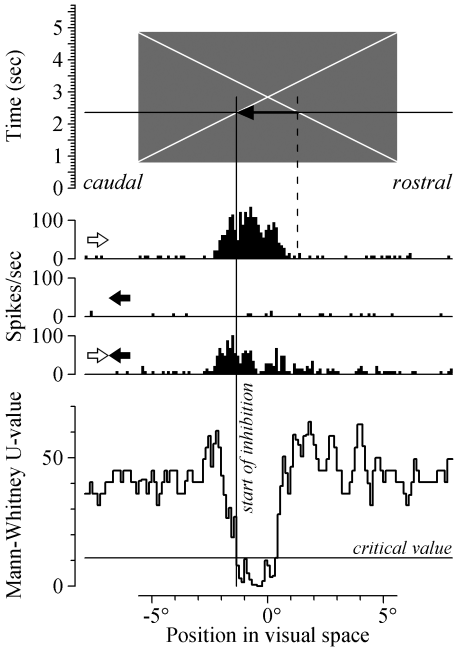


Fig. 4. Statistical analysis (Mann–Whitney test) of null-side inhibitory influences in an ON-type DS GC selective to caudo–rostral movement (*C. gibelio*). Top panel of diagram. White tracks represent the trajectories of movements of white stimuli during the period of stimulation. Stimuli (single and paired stripes) could cross the stimulation area in the preferred (caudo–rostral), or in the null (rostro–caudal) direction (caudal and rostral sides of fish visual field are marked below diagram). The width of tracks corresponds to the width of stripes (which was 10′). Velocity of stimulus movements was 2.75°/s. Paired stimuli move in opposing directions — one of them crosses stimulation area in the preferred, and the other one in the null direction. Stripes merged at center of stimulation area, and subsequently move away from each other. Central point of stimulation area where stripes meet (0° on x-axis) corresponds to the cross point of motion trajectories on the diagram. The time of stimulation in seconds is given on the y-axis. Estimated distance between stimuli at which the null-side inhibition started is marked by the thick horizontal arrow. Middle panel of diagram. Peristimulus histograms, shown on the middle panel of diagrams, represent the averaged values for 9 consecutive presentations of light stimuli (stripes) at each step of stimulation (from top to bottom): single stripe moving in the preferred direction, single stripe moving in the null direction, paired stimuli. The adjusted location of the null-side stimulus in RF periphery from which inhibitory influences were initiated is designated by the vertical dashed line on the diagram representing peristimulus histogram for single preferred-side stripe. Bottom panel of diagram. Mann–Whitney U-values presented on the bottom panel, were calculated at different positions of preferred-side stripe in the stimulation area for 2 samples with 9 sets of data (single stripe moving in the preferred direction and paired stimuli moving in opposing directions). U-values were calculated over narrow intervals occupying 20′ of fish visual field. When the U-values fell below the critical level it was considered that the inhibitory effect from the null side was initiated at that point (position of preferred-side stripe in that moment is marked by the solid vertical line signed as "start of inhibition"; criterion U-value was fixed at the $\alpha = 0.05$).

RRF was located approximately in the center (Fig. 5(a)) and closer to the preferred-side stripe (Fig. 5(b)) are demonstrated. From the left diagram (RRF centered) it is evident that the inhibition was initiated immediately after the preferred-side stimulus entered the RRF. This means that the null-side inhibitory influences sent from some point of RF periphery were spread across entire RRF. Inhibitory effect endured

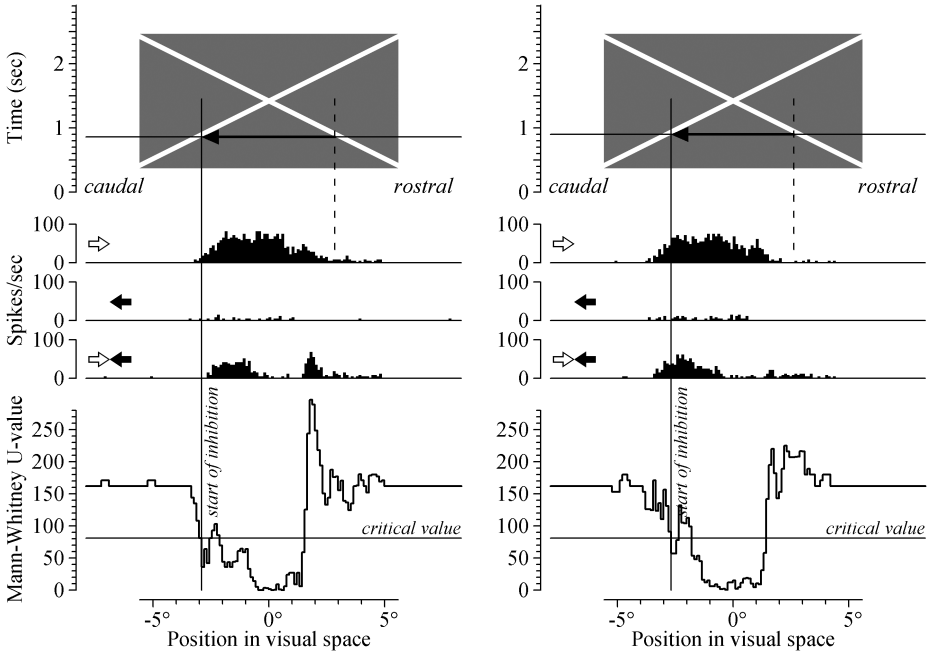


Fig. 5. Statistical analysis (Mann–Whitney test) of null-side inhibitory influences in the ON-type caudo–rostral DS GC shown in Fig. 3. Two diagrams are presented, regarding the cases when cell RRF was located (a) approximately in the center and (b) closer to the preferred-side stripe. The unit was stimulated by 30′ wide white stripes, moved with the velocity of 5.5°/s along horizontal axis of fish visual field in opposite directions (preferred, caudo–rostral or null, rostro–caudal direction). Eighteen consecutive presentations of stimuli were performed at each step of stimulation. Other conventions are as in Fig. 4.

till the moment when stripes have coalesced at the RRF center. The response, which appeared again after stripes moved away from each other, exceeded significant response to preferred stripe alone (see transient positive peak in the Mann–Whitney curve). The adjusted location of the null-side stimulus in RF periphery at which inhibition began is estimated on the right diagram (cell RRF closer to the preferred-side stripe). This position is designated by the vertical dashed line in the middle panel of the diagram. It can be assumed that the inhibitory influences in RF of the DS GC were transmitted at a distance of approximately 5.5°. In order to estimate the size of areas in μm on the retinal surface corresponding to these angle values, the geometrical parameters of the fish eye and retina were measured for one fish of 10 cm body length (Maximov *et al.*, 2005b). The diameter of the lens was 3.2 mm. From this value, according to the ratio of Matthiessen (Matthiessen, 1880) focal distance was calculated and made about 4 mm. On the basis of such focal distance, retinal area corresponding to the RRF size of 5.5° was estimated — it was an area with an approximate diameter of 500 μm.

Results of statistical analysis processed in two other ON DS units, ventro-dorsal and dorso-ventral, respectively, are presented in Fig. 6. In both cells presented

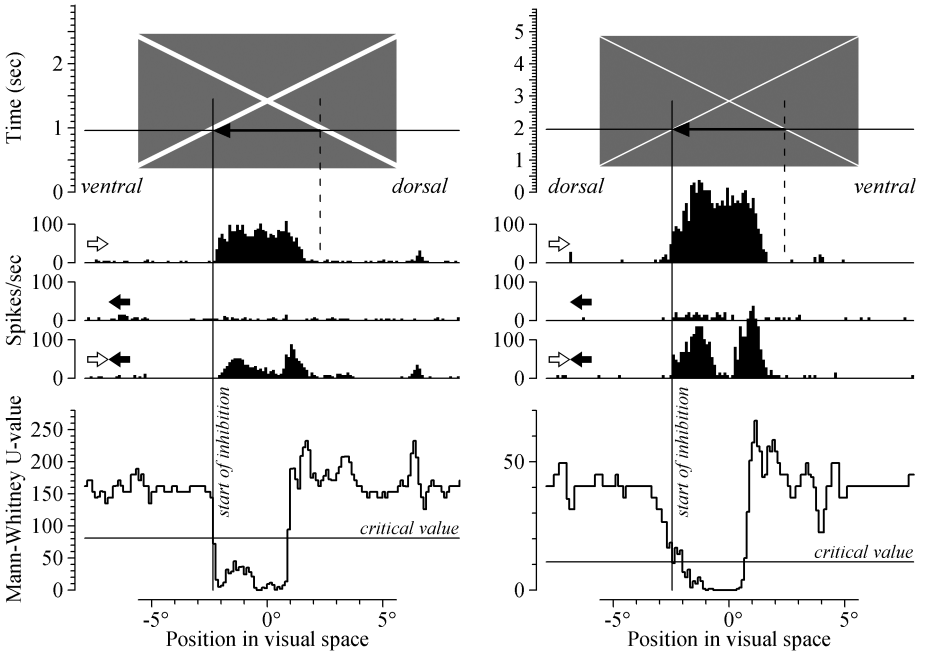


Fig. 6. Statistical analysis (Mann–Whitney test) of null-side inhibitory influences in two DS GCs of *C. gibelio* selective to different directions of stimulus movements. RRFs of both DS units were located approximately in the center of stimulation area. (a) ON-type DS GC selective to ventro-dorsal movement. The unit was stimulated by 30′ wide white stripes, moved with the velocity of 5.5°/s along vertical axis of fish visual field in opposite directions (preferred, ventro-dorsal or null, dorso-ventral direction). Eighteen consecutive presentations of stimuli were performed at each step of stimulation. (b) ON-type DS GC selective to dorso-ventral movement. The unit was stimulated by 10′ wide white stripes, moved with the velocity of 2.75°/s along vertical axis of fish visual field in opposite directions (preferred, dorso-ventral or null, ventro-dorsal direction). Nine consecutive presentations of stimuli were performed at each step of stimulation. Other conventions are as in Fig. 4.

influences of inhibitory signals induced in the RF periphery were spread across entire RRF. The same effects were recorded in OFF DS units as well. Results obtained in one OFF caudo–rostral DS GC of *L. intermedius* are presented in the Fig. 7. One can see that the influences of inhibitory signals, generated in RF periphery, were transmitted at a distance exceeding the RF diameter of the target DS unit approximately 1.5 times. Similar inhibitory effects initiated in the RF periphery were recorded in 62% of all DS GCs recorded. In remaining 38% units inhibition was initiated inside the RRF at different distances between opposing stimuli.

Some spatial characteristics of the null-side inhibition, observed in the majority of fish DS GCs have to be emphasized. In general, inhibitory signals induced at large distances between stimuli (early phase of inhibition) influenced the preferred-side response, but did not occlude the response completely. The early phase of inhibition in some units consisted of transient inhibitory effects (see Figs. 5 and 7). In other DS GCs early inhibition was represented by sustained effects, intensities of which could

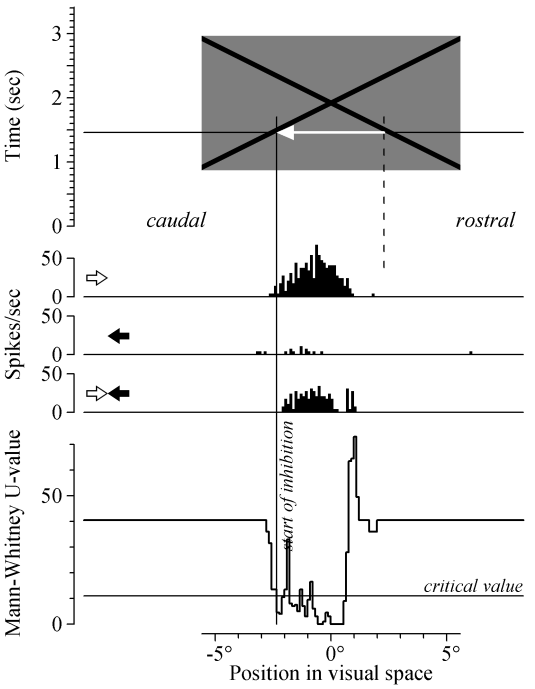


Fig. 7. Statistical analysis (Mann–Whitney test) of null-side inhibitory influences in an OFF-type DS GC of *L. intermedius* selective to caudo–rostral movement. Inhibitory influences in the presented DS unit were recorded when cell RRF was located closer to the preferred-side stripe. The unit was stimulated by 30′ wide black stripes, moved with the velocity of 5.5°/s along horizontal axis of fish visual field in opposite directions (preferred, caudo–rostral or null, rostro–caudal direction). Nine consecutive presentations of stimuli were performed at each step of stimulation. Other conventions are as in Fig. 4.

slightly vary, but remained in the zone of significance (Fig. 6). In the late phase of inhibition, when stimuli moved closer to each other, influence of the null-side stimulus significantly increased and the response of the cell was completely occluded. The onset of the late phase of inhibition was usually associated with the moment when the response to paired stimuli started to decay abruptly. On the other hand, inhibitory effects induced by faraway null-side signals as a rule were not visually accessible from the peristimulus histograms. For example, beginning of early transient inhibitory effect estimated by Mann–Whitney test in the ON DS unit shown in Fig. 5(b) could not be visually determined from the response of the same unit to paired stimuli (Fig. 3(c)). As a consequence, visually approximated beginning of null-side inhibition in Fig. 3(c) was underestimated. Approximated position of the null-side stripe at the moment of the onset of inhibition was shifted for approximately 1° closer to the unit RRF with regard to the corresponding position of inhibitory stimulus estimated by Mann–Whitney test (Fig. 5(b)). All abovementioned criteria indicate that the relevant evaluation of inhibitory interactions induced during opposing motion of stimuli requires formal statistical analysis.

4. Discussion

4.1. *Direction-selective circuitry in the fish retina*

In comparison to the mammalian fast DS GCs, few studies investigated fish DS GCs in the goldfish (Jacobson & Gaze, 1964; Cronly-Dillon, 1964; Wartzok & Marks, 1973), pike (Zenkin & Pigarev, 1969), trout (Liege & Galand, 1971) and six species of marine fish (Maximova *et al.*, 1971). These studies noted the dominance of caudo–rostral preference in the recorded DS units. However, no clear classification of these cells either by the type of response (ON, OFF or ON–OFF) or by preferred directions was provided. In our studies we have systematically investigated the responsive and directional properties of some hundreds of DS GCs in the retina of the cyprinid fish *Carassius gibelio* (Maximov *et al.*, 2005a,b). Responses from fast retinal DS GCs were recorded extracellularly from their axon terminals in the superficial layer of TO of immobilized fish. Our results indicated that *C. gibelio* has six physiologically distinct subtypes of DS GCs. Fish retinal DS units differ from those of mammals in some aspects. Unlike mammalian mixed ON–OFF type of the fast DS units, two separate types, pure ON and pure OFF units, were equally encountered in the fish retina. Both types of *C. gibelio* DS GCs were shown to be divided into three distinct groups, characterized by their preferred directions: caudo–rostral, ventro-dorsal and dorso-ventral, separated by about 120°. Units that respond to the caudo–rostral direction of stimulus movement were the most numerous. On the other hand, as mentioned above, fast mammalian DS GCs comprise four physiological subtypes with different preferred directions aligned with the horizontal and vertical ocular axes (Oyster & Barlow, 1967).

In the present study, responses from DS GCs were also recorded in another cyprinid fish *Labeobarbus intermedius*. DS GCs of *L. intermedius* were studied for the first time. Results obtained for one full-grown fish revealed the presence of ON caudo–rostral GCs, OFF caudo–rostral GCs and OFF ventro-dorsal GCs. These results indicate that physiological types of *L. intermedius* DS GCs do not essentially differ from those types of DS GCs described formerly in *C. gibelio*. Moreover, the results of our recent experiments performed in carp and roach indicated that DS GCs of these fishes also coincide with retinal DS units of *C. gibelio* (Maximova, *personal communications*). Results confirming our findings have been published by Nikolaou *et al.* (2012). Using calcium imaging techniques they recorded visually evoked activity of the retinal GC axons innervating tectum of zebrafish. Three subtypes of retinal DS units, characterized by preferred directions similar to those described in *C. gibelio* were identified. The proportion between the DS GC subtypes was practically the same as that shown in *C. gibelio* (units with the preference to caudo–rostral direction were the most numerous). All of the abovementioned facts suggest that the system of DS GCs comprising six physiologically distinct subtypes represents universal retinal DS circuitry developed during evolution of the teleost fish species.

4.2. *Spatial properties of inhibition in the fish direction-selective ganglion cells*

Irrespective of abovementioned differences between the fish DS GCs projecting to TO and the mammalian fast DS GCs projecting to the superior colliculus, according to numerous physiological characteristics fish DS GCs resemble the fast mammalian DS units and should be classified as local motion detectors (Maximov *et al.*, 2005b; Damjanović *et al.*, 2009; Maximov *et al.*, 2013). Direction selectivity in the fish DS GCs was shown to be produced by the null-side inhibition (Damjanović *et al.*, 2009), and in this respect these units also resemble the fast DS GCs of mammals (Lee *et al.*, 2010; Briggmann *et al.*, 2011).

The asymmetric delayed inhibition coming from the null side of DS GC RRF was shown to be mediated by SACs (Yoshida *et al.*, 2001). Two possible inhibitory mechanisms mediated by the SACs that are not mutually exclusive, could produce the suppression of dendritic spikes in DS GCs (Shachter *et al.*, 2010). It was previously suggested that the cardinal direction-selectivity in GCs is set up at bipolar cell terminals (Borg-Graham, 2001; Fried *et al.*, 2005). In other words, presynaptic inhibition of excitatory synaptic inputs from bipolar cells to a local dendritic region of DS GC should suppress spike initiation. The second possible inhibitory mechanism underlying direction selectivity is based on postsynaptic processing, i.e., postsynaptic inhibition from SACs within a local dendritic region of DS GC should block spike initiation (Borst, 2001; Taylor *et al.*, 2000).

In the recent work of Yonehara *et al.* (2013) concerted activity of bipolar cells, DS GCs and SACs in the DS circuitry of mouse retina was recorded using calcium imaging techniques. While the visually evoked activity of the dendritic segments of DS GCs and SACs were direction selective, direction-selective activity was absent in the axon terminals of bipolar cells. Results of that study thus confirmed that the first stage of cardinal direction selectivity is localized to dendrites of retinal GCs and so arguments supporting evidence in favor of the postsynaptic model were provided. On the other hand, no details of the inhibitory mechanism underlying direction selectivity in the fish retina have been provided yet.

Interaction between preferred-side excitation and null-side inhibition during the simultaneous presentation of the preferred-side and null-side moving stimuli, was the subject of the present study. In all DS GCs recorded cell responses, evoked by the stripe coming from the preferred side of RF were inhibited by the stimulus coming from the opposite direction. As a rule, at certain distance between stimuli unit response started to decay rapidly and before the stimuli merged at center of the stimulation area the DS GC ceased to respond. When stripes moved away from each other inhibitory influences were terminated, and cell response appeared again. This indicates that inhibitory signals were transmitted exclusively in the null direction. The inhibitory effect of the null-side signal initiated in the RF periphery was spread across the entire RRF in the majority of units recorded. Thus the influences of inhibitory signals generated in the RF periphery were transmitted to the distance

that exceeded RF diameter of the target DS unit, in certain cases even 1.5 times (see Fig. 7). If we assume that the direction selectivity in the fish retina is generated mainly by some presynaptic inhibitory mechanism, this effect could be achieved only by SACs with extra large RF diameters of nearly 1 mm. However, SACs with such large RFs are inconsistent with the data on SAC morphology reported. Some of the largest SACs were identified in the dogfish shark *Squalus acanthias* (Brandon, 1991). These cells were characterized by dendritic arbors of about 400–500 μm in diameter. Cholinergic starburst-like amacrine cells of zebrafish retina were shown to have RF diameters that were approximately twice as short (about 200 μm) (Yazulla & Studholme, 2001). Hence, spread of inhibitory signals across entire RRF shown in our studies could be hardly induced by a presynaptic mechanism, i.e., effects of the null-side stimulus induced in the early phase of inhibition are unlikely mediated by a presynaptic mechanism.

Starburst amacrines with RF sizes similar to those described in zebrafish were observed in rabbit retina (RF diameters of about 200–250 μm) (Lee & Zhou, 2006; Chen & Chiao, 2008). Their dendritic arbors were shown to be considerably overlapping (Tauchi & Masland, 1984). In their early work Masland *et al.* (1984) postulated that their apparent excessiveness of coverage was needed to create local subunits of the DS RS. Latter it was suggested that individual sectors of the starburst dendritic arbor act as independent units and that these sectors are individually direction selective, creating a directional input to the DS GC (Vaney & Young, 1988; Poznanski, 1992). A SAC dendrite is more strongly activated by motion outward from the cell body to the tip of the dendrite, than by motion in the opposite direction (Euler *et al.*, 2002). Recently it was postulated that the SAC direction selectivity might arise because different locations on the SAC dendrite are wired to bipolar cell types with different time lags (Kim *et al.*, 2014). It was shown that the proximal bipolar cells (wired near the SAC soma) lag the distal bipolar cells (wired far from the soma). Accordingly, during the outward motion signals from both bipolar cell groups will reach the SAC dendrite simultaneously, summing to produce a large depolarization. On the other hand, for inward motion bipolar cell signals will reach the SAC dendrite asynchronously, causing only small depolarizations. Therefore a SAC dendrite exhibits direction selectivity, and outward motion is proposed to be its preferred direction. The SAC direction-selective sectors were shown to be smaller than the DS GC dendritic field, thus accounting for the ganglion cell's ability to discriminate small movements within the field. RFs of mammalian DS GCs are completely covered by a great number of overlapping direction-selective sectors of different SACs (Masland, 2012). Similar circuitry acting on the basis of postsynaptic inhibitory processing could produce the spread of null-side inhibitory influences across the entire fish DS GC RF shown in the present study (Figs. 5–7). In other words, suppression of the preferred-side response by the null-side stripe during the opposing motion of stimuli could be induced by a postsynaptic inhibitory mechanism, similar to that described in the mouse retina (Yonehara *et al.*, 2013). However, results of our study cannot unambiguously

exclude involvement of a presynaptic inhibitory mechanism (Euler *et al.*, 2002; Fried *et al.*, 2002, 2005; Poznanski, 2005, 2010) in the late phase of opposing motion. Hence, from the all abovementioned one can say that the results of our study leave open the question which of two circuitries underlie null-side inhibitory effects described in fish DS GCs — the one based on the single postsynaptic mechanism or the second based on coordinated pre- and postsynaptic processing.

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