DOI 10.1007/s11055-021-01138-7
Neuroscience and Behavioral Physiology, Vol. 51, No. 6, July, 2021

Fine Structure of the Receptive Fields of Orientation-Selective Ganglion Cells in the Fish Retina

A. T. Aliper, I. Damjanovic, A. A. Zaichikova, E. M. Maximova, and P. V. Maximov

UDC 591.185.63

Translated from Sensornye Sistemy, Vol. 34, No. 1, pp. 19–24, January–March, 2020. Original article submitted September 9, 2019. Revised version received October 24, 2019. Accepted November 6, 2019.

Orientation-selective ganglion cells (OS GC) were discovered in the fish retina some decades ago, though the mechanisms of orientational selectivity remain unstudied. OS GC in fish can be divided into two classes, differing in terms of presumptive orientation – close to the vertical or close to the horizontal. There are no differences in the other characteristics of these two classes. They are not selective for contrast sign, i.e., they are on-off in nature. We recorded extracellular activity from retinal tectum opticum GC axon terminals in living immobilized fish, using goldfish as the study system. Stimulus parameters and experimental series were specified using specially developed software. The experiments used a "checkerboard" method with single- and double-point stimulation. OS GC detecting horizontal and vertical edges were able to respond to single flashing points, allowing their excitatory receptive fields to be measured. Responses to this type of stimulus were markedly weaker than those to the preferred stimulus, i.e., the correspondingly oriented lines or edges. However, when stimulation was at two points simultaneously, to approximate a segment in the preferred orientation, OS GC responded with prolonged spike discharges. We also observed inhibition, when points were oriented orthogonally to the preferred orientation. Thus, pairs of points served as an adequate approximation of the preferred or orthogonal direction, allowing the local properties of the receptive fields of OS GC to be studied.

Keywords: retina, ganglion cells, receptive fields.

Introduction. Ganglion cells (GC) carry out the final step in visual information processing in the retina in vertebrates. The entire visual scene is encoded by a set of GC whose receptive fields (RF) are distributed across the retinal surface. The RF of GC are generally functionally separated into central and peripheral parts. Presentation of an appropriate stimulus in the central part of the RF induces a cell response to this stimulus. If the same stimulus is presented only in the peripheral part of the RF, no response is produced. The excitatory central part of the RF (ERF) corresponds to the area covered by the cell's dendritic tree, which collects the visual signal from a relatively large area. The ERF and the area covered by GC dendritic trees in the rabbit retina [Yang and Masland, 1992, 1994; Devries and Baylor, 1997] have been shown to be of essentially identical size.

Information on the different properties of visible objects, their sizes, directions of movement, shapes, colors, etc., are processed by different types of specialized GC, i.e., detectors. Detectors have been described in, among other places, the retinas of fish [Cronly-Dillon, 1964; Gaze, 1964; Zenkin and Pigarev, 1969; Liege and Garland, 1971; Maximova et al., 1971; Wartzok and Marks, 1973; Kawasake and Aoki, 1983; Billota and Abramov, 1989]. Information processed by specialized GC is transmitted to the primary visual centers of the brain in fish, mainly one of the midbrain structures – the tectum opticum (TO). Vertical movement of the electrode though the layers of the TO reveals responses from axon terminals of different types of GC. The superficial layers mainly display the responses of directionally selective GC (DS GC), while the terminals of various elements such as

Kharkevich Institute of Information Transmission Problems, Russian Academy of Sciences, Moscow, Russia;
e-mail: outtaget@gmail.com.

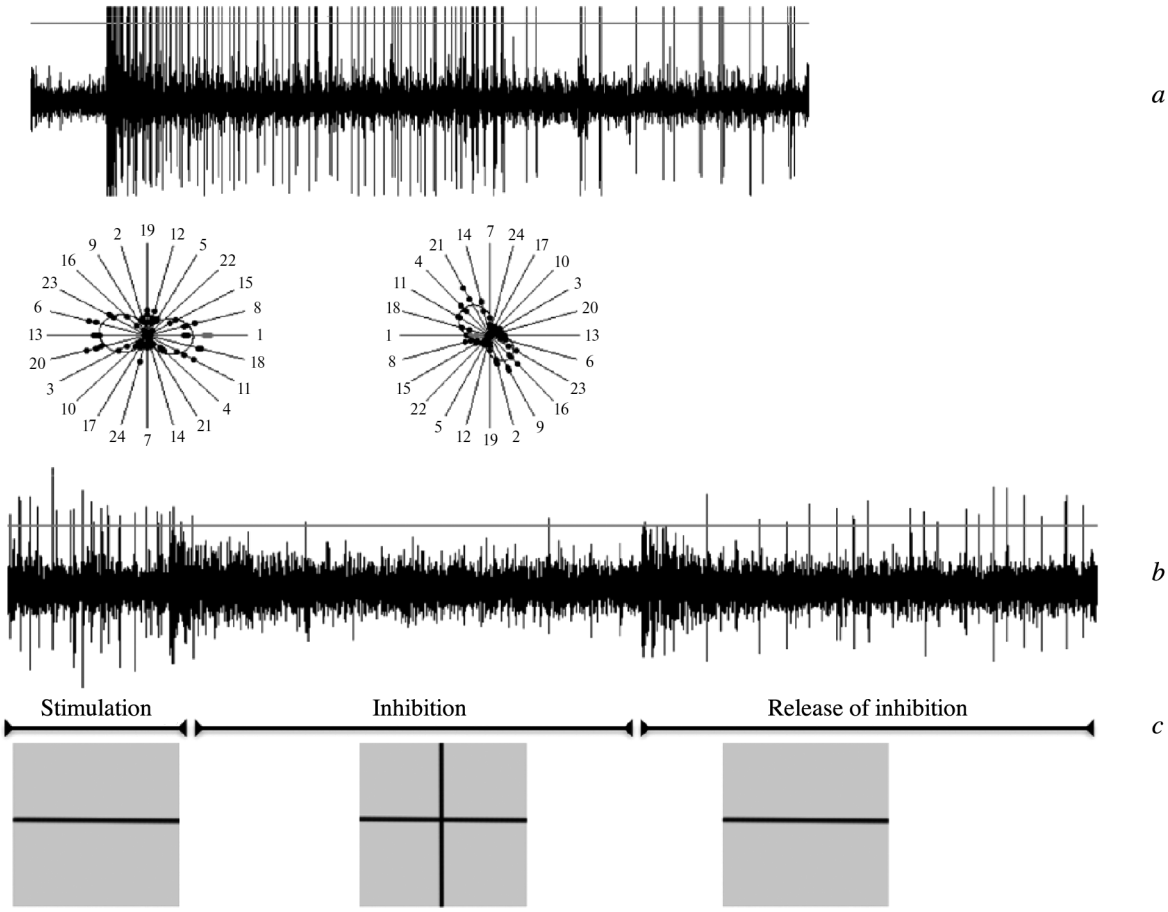


Fig. 1. Properties of OS GC in fish. Responses of OS GC to stimulation with a stationary line in the preferred orientation (*a*). Direction plots for vertical (left) and horizontal (right) edge detectors (*b*). Inhibition of the response to a stationary line in the preferred orientation by an orthogonal stimulus (*c*).

orientation-selective GC (OS GC), spot detectors, and color opponence cells, are located somewhat deeper, and the projections of long-response contrast detectors (spontaneously active GC) are even deeper [Maximov, 2009]. OS GC in fish are currently of particular interest, as the mechanism of formation of orientational selectivity thus far remains unstudied. Fish have two classes of OS GC – with preference for horizontal and vertical stimuli, respectively. These can mount long-lasting responses to stimulation with standard lines in the preferred orientation, which can be inhibited by simultaneous introduction of a stimulus in the orthogonal orientation into the RF (Fig. 1). Lines on spike activity traces show cutoff thresholds for the spikes recorded.

The aim of the present work was to study the receptive fields of OS GC in the retina of fish, detecting zones inhibiting and potentiating excitation in the central and peripheral parts of the RF.

Methods. All experiments were carried out using the goldfish *Carassius auratus gibelio* of size 10–15 cm and weight 35–100 g from pond farms in the Moscow District. Animals were kept in 60-liter laboratory aquaria with water aeration and filtration for several months at room temperature with a natural light regime.

Access to the TO in fish from the side of the skull contralateral to the eye to which stimulation was presented was obtained by removing the parietal-occipital bone and the fatty tissue and area of cerebral meninges located beneath it. During the experiment, the animal was immobilized with i.m. *d*-tubocurarine (0.3 mg/100 g). The immobilized fish was fixed in a natural position in the Plexiglass aquarium with a forced flow of water across the gills. About 10 liters of water circulated through the apparatus during the experiment with constant aeration and filtration. The circulating water was delivered with a thermostatted pump. Water was delivered to the gill apparatus of the fish under pressure of 50 cm of water. The water level in the aquarium was maintained such that the fish's eye was completely submerged but water did not enter the opened brain.

Presentation of software-generated stimuli was through the transparent wall of the aquarium on an LG Flatron 775FT monitor screen on a mobile mount, allowing it to be moved into the required position in the fish's field of vision. Mostly, the lateral visual fields were studied over a quite wide area: above 60° in the horizontal and about 40° in the vertical. The distance from the monitor to fish's eye during the experiment was 30–40 cm. The stimuli in the experiments de-

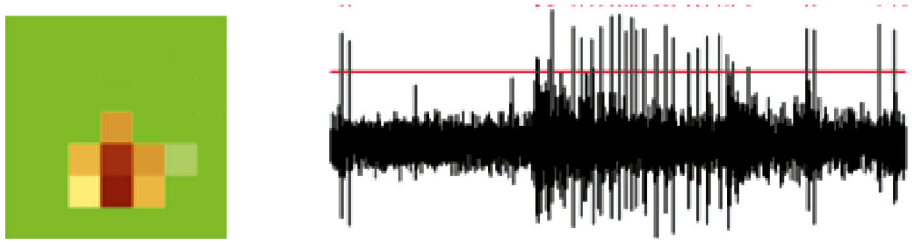
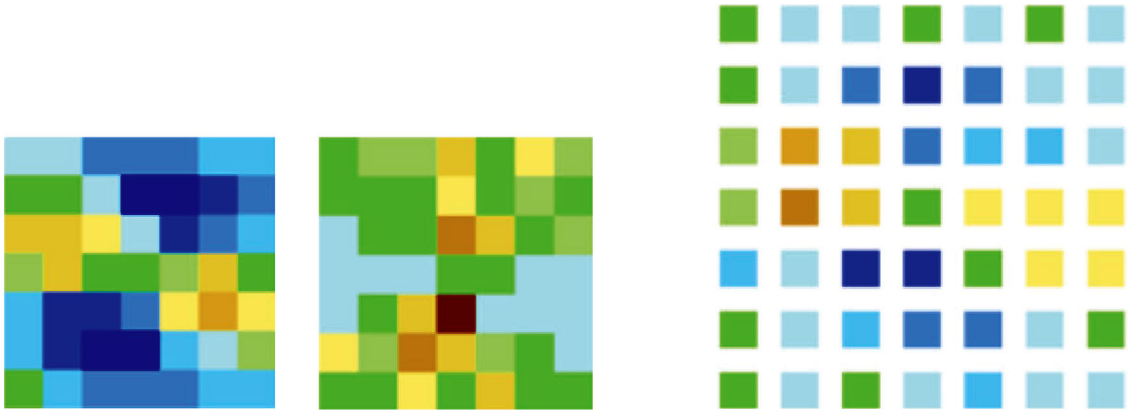


Fig. 2. Method of mapping ERF with flashing spots. Left – example of mapping the RF of a horizontal edge detector. At each point a light spot flashed three times; control stimuli were applied at the central position after each series. The number of spikes in the cell response to each stimulation is reflected as a geographical color scale. Right – the response a cell to stimulation with a flashing spot.



a *b*

Fig. 3. Results of experiments with two-point stimulation of OS GC. Horizontal (left) and vertical (right) edge detectors (*a*). Experiment with increased distance between grid cells (*b*). The distance between grid cells was 0.55°.

scribed here were gray-scale stimuli. Relative screen emission spectra were measured using an MCS 500 Modules modular spectrometer system (Carl Zeiss). Maximum screen brightness (at R = G = B = 255) was measured with a TKA-04/3 photometer and was 38 cd/m^2. In energy units, this corresponded to an effective energy brightness for photopic vision in humans, i.e., 56 mW·sr^{-1}·m^{-2}. Our data [Maximov, 2005] indicate that the photopic spectral sensitivity of ganglion cells in the goldfish retina is determined largely by its long-wavelength rods and is displaced by 75 nm toward long wavelengths. The corresponding spectral sensitivity function was computed using the Govardovskii formula [Govardovskii et al., 2000] for the visual pigment of the vitamin A2 system with λ_{max} = 622.5 nm, allowing for absorption in the anterior media of the eye [Douglas, 1989]. The effective energy brightness of this same white screen calculated in this way for goldfish retinal ganglion cells was 65 mW·sr^{-1}·m^{-2}.

Ganglion cell responses were recorded extracellularly from their axon terminals in the upper layers of the tectum using metal platinized microelectrodes [Gaesteland et al., 1959] with a platinized cap of diameter 3–5 μm and impedance 200–500 kΩ at a frequency of 1 kHz. The microelectrode was approximated to the region of interest on the tectal surface under visual control with a Suttor MP-285 micromanipulator (on the basis of the retinotopic projection oriented on a blood vessel map) and was then inserted carefully, brought to a stable single lead, as assessed in terms of the magnitude of spike activity and in terms of the signal:noise ratio. Spikes from the alternating current amplifier output with a bandpass of 0.1–3.5 kHz [Vinogradov, 1986] were monitored on a loudspeaker and oscilloscope screen and recorded in computer memory using an ADC with a sampling frequency of 25 kHz.

Results and Discussion. Within the framework of this study a total of 37 OS GC RF were investigated. Despite the fact that OS GC prefer stimulation with lines in the corresponding orientation, these cells could give some response to other stimuli, for example, small black or white spots. An example of this type of response is given in Fig. 2. This allows the size and shape of their ERF to be determined and the centers of ERF to be located using flashes of light. Spot flashes were presented in the cells of a square grid in quasirandom order (checkerboard method), after which the software counted the number of spikes produced in response to stimulation. Stimuli were presented three times in each cell of the grid. Series of stimuli for spike activity always started with presentation of a stimulus in the central

cell. Stimulation was again presented in the central position at the end of the series to monitor the cell's response level. The results of experiments on horizontal edge detectors are shown in Fig. 2. The area of stimulation was divided into 49 small squares (spots) of size marginally greater than 1°. Cellular responses over the whole of the area of stimulation were represented geographically as a topographic map. On the basis of the results of this procedure the stimulation area was displaced such that its center coincided with the presumptive (based on measurements) center of the ERF of the cell being studied. The shapes of the study cell ERF were mostly uniform in nature, without any tendency to be more extensive in any particular direction.

The fine structure of the RF of the OS GC studied here was investigated by two-point stimulation, which allows interactions within different parts of the RF to be followed. The experimental scheme was analogous to measurement of RF by the "checkerboard" method, though in our case the stimulus in the central cell flashed each time, with parallel presentation, in pseudorandom order, of one additional flash in another cell of the grid. Examples of the results of this experiment are shown in Fig. 3, *a*. Each of these experiments gave the same picture at the output – orthogonally oriented extended "figure-of-eight" activation and inhibition zones running far beyond the boundaries of previously measured ERF. Increases in the distance between cells allowed the boundaries of these zones to be identified (Fig. 3, *b*).

The data obtained in this study contradict the findings of other studies of orientation selectivity in the fish retina. The current model of orientation selectivity proposed by a British group [Antinucci et al., 2018] suggests that the shape of the dendritic tree of an OS GC is extended in the direction of the preferred orientation and that inhibition is mediated by tenm3+ amacrine cells, whose dendritic trees are orthogonal to the preferred orientation. Our measurements indicate that the dendritic trees of OS GC are probably round, not extended in any direction. This scheme is realized in vertical edge detectors in the mouse retina, where the dendritic tree of the GC itself is round in shape and the dendrite of the inhibitory amacrine cell is extended orthogonally to the preferred orientation [Nath and Schwartz, 2016]. It should be noted, however, that in this case the analogy is arbitrary because in contrast to orientation-selective cells in the fish retina, these cells in mammals are not on-off cells but show selectivity, as in the case of the sign of contrast. In addition, "recognition" of a point which, along with the central approximation of the segment, forms the preferred orientation also occurs far beyond the boundaries of the proposed ERF. Thus, there is the probability that apart from inhibitory tenm3+ amacrine cells, formation of the mechanism of orientation selectivity also involves a type of amacrine cell enhancing responses and itself forming an orientational preference. It can be suggested that some amacrine cells may form inhibitory synapses on some OS GC as well as excitatory synapses on OS GC with a preference for the orthogonal orientation. However, orientation selectivity can also be formed by different types of amacrine cells.

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