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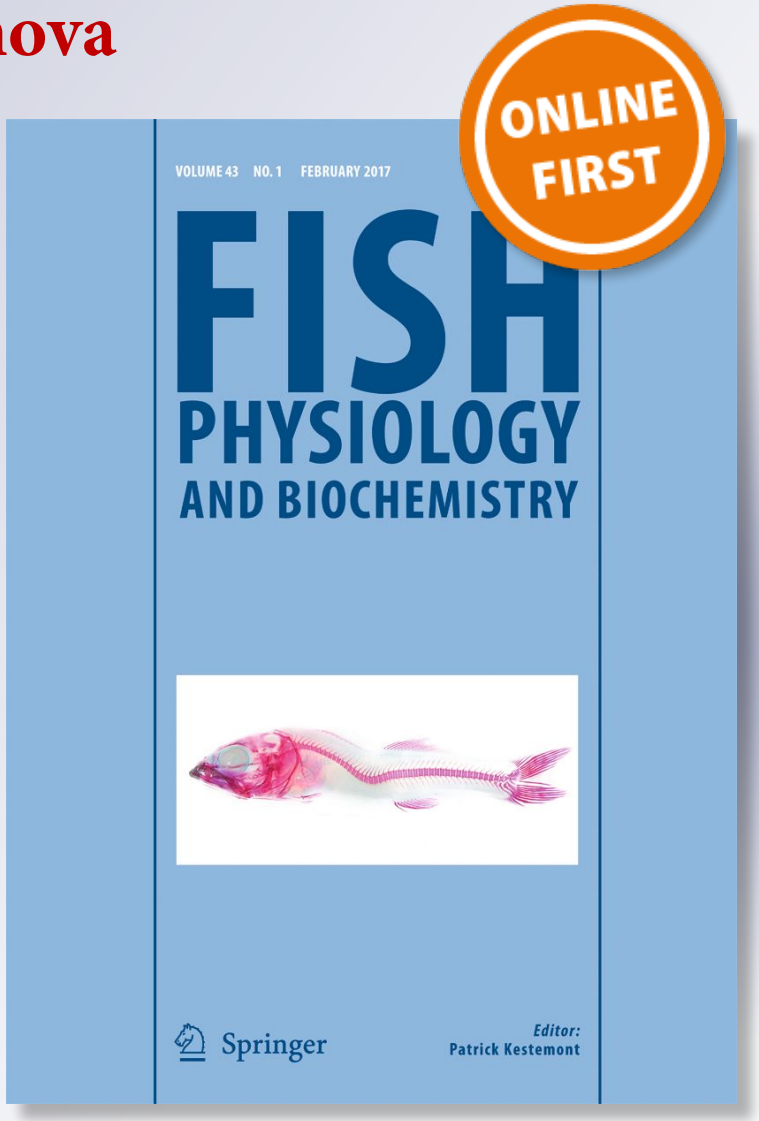

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Updated functional segregation of retinal ganglion cell projections in the tectum of a cyprinid fish—further elaboration based on microelectrode recordings

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Abstract Single-unit responses of retinal ganglion cells (GCs) were recorded extracellularly from their axonal terminals in the tectum opticum (TO) of the intact fish (goldfish, carp). The depths of retinal units consecutively recorded along the track of the microelectrode were measured. At the depth of around 50 μm, the responses of six types of direction-selective (DS) GCs were regularly recorded. Responses of two types of orientation-selective (OS) GCs and detectors of white and black spots occurred approximately 50 μm deeper. Responses of GCs with dark- and light-sustained activity were recorded deeper than all others, at about 200 μm. The receptive fields of consecutively recorded units overlap, so they analyze the same fragment of the visual scene, focused by eye optic on the photoreceptor raster. The responses of pairs of DS GCs (ON and OFF units that preferred same direction of stimulus movement) and OS GCs (detectors of vertical and horizontal lines) were often simultaneously recorded at one position of the microelectrode. (The paired recordings of certain units amounted about fourth part of all recordings.) This suggests that their axonal arborizations are located close to each other in the tectal retinorecipient layer. Electrophysiological method, thus, allows to indirectly clarify and make precise the morphology of the retino-tectal connections and to establish a morpho-physiological correspondence.

Keywords Goldfish · Carp · Extracellular recording · Ganglion cells · Retino-tectal projections · Tectum opticum · Tectal neurons

Introduction

Basic morphological types of vertebrate retinal neurons were thoroughly studied and described for the first time more than 100 years ago (Ramon y Cajal 1892). General morphological and physiological properties of vertebrate visual systems as well as characteristics specific to the visual systems of distinct groups of animals were later revealed by means of advanced experimental methods. The highest intraspecific and interspecific plasticity in the retinal structure takes place on the

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photoreceptor level: rods/cones ratio, spatial arrangement of the photoreceptors, types and number of photopigments that may reflect both the evolutionary history and the ecological requirements of the species life (Walls 1942; Lamb et al. 2007; Peichl 2005; Bowmaker 1999; Wagner and Kröger 2005).

However, the general principle of the primary image processing is universal and in many respects similar in different animals. Approximately 20 morpho-physiological types of output neurons—ganglion cells (GCs)—were identified in the retinae of various species (Robles et al. 2013; Hong et al. 2011). This estimation of the number of GC types is supported by the molecular phenotyping and genetic labelling technics (Marc and Jones 2002; Marc and Cameron 2002). The number of distinguished GC types depends on the criteria. For instance, morphometric analysis based not only on dendrites but also on GC axon targets revealed more than 50 types of GCs (Robles et al. 2014).

Dendrite mosaics of any GC type cover the entire retinal surface at their stratification level in the inner plexiform layer (IPL). As a rule, the dendritic arbors of neighboring GCs of the same type contact but do not overlap ("tiling") (Masland 2012; Roska and Meister 2014). Thus, about 20 different descriptions of the visual scene are formed as early as at the retinal level.

The number of primary visual centers of the brain (10 identified) is also consistent among vertebrates. The degree of complexity and the power of afferentation of primary visual centers vary between animal species. In particular, the retino-tectal system is superiorly developed in the lower vertebrates, while in mammals, the retino-geniculo-cortical system is the primary pathway of visual information processing (Burrill and Easter Jr 1994; Montgomery et al. 2017; see review of Vanegas and Ito 1983).

Tectum opticum (TO) in fish is the main visual center—it receives 98% of axons of retinal GCs. TO is a laminar structure with six distinct layers: stratum marginale, stratum opticum, stratum fibrosum et griseum superficiale, stratum griseum centrale, stratum album centrale, and stratum periventriculare (Northmore 2011). Stratum fibrosum et griseum superficiale (SFGS) is in turn stratified into six sublaminae (Nevin et al. 2010; Northmore 2011; Robles et al. 2013; Preuss et al. 2014). The optic nerve fibers enter the rostral region of the contralateral TO. Here, they diverge and ramify predominantly in SFGS at six distinct sublaminae, where they make synaptic contacts with dendrites of the tectal neurons proper. The cell bodies of tectal neurons locate mainly in the deep periventricular layer (Kinoshita and Ito 2006; Northmore 2011; Gabriel et al. 2012; Grama and Engert 2012; Kassing et al. 2013; Hunter et al. 2013). Axons of the retinal GCs project to each SFGS sublamina in the retinotopic order: the frontal area of the visual field is represented in the anterior (rostral) part of the TO, the posterior area in the caudal part, the dorsal area in the medial part, and the ventral area in the lateral ventral part of the TO (Jacobson and Gaze 1964). Thus, the morphological structure of the TO accurately represents the map of surrounding world (Jacobson and Gaze 1964; Schwassmann and Kruger 1965). The complete retino-tectal map is a set of six parallel maps of sublaminar layers. Further information processing takes place in the TO as well, given that the parts of tectal neurons are interneurons. After that, the information is transmitted through the axons of the distant TO neurons to the motor nuclei where it is used to form a variety of output motor reactions of the animal (turning to a prey object, turning away from a large stimulus that might pose a potential threat to the animal, the optomotor reaction, etc.) (Springer et al. 1977; Robles et al. 2011; Tsvilling et al. 2012).

By recording the single-unit responses (spike activity) of GCs to various stimuli from their axon terminals at different depths of TO, one can reach the understanding of "what the fish eye tells the fish TO" and correlate functional segregation to the morphological stratifications of retino-tectal projections. This method was developed, used for the first time in the study of the frog retino-tectal projections, and described in the classic work "What the frog's eye tells the frog's brain?" (Lettvin et al. 1959; Maturana et al. 1960). Later, it was used in investigations of visual information processing in the retino-tectal systems of various fish species (Jacobson and Gaze 1964; Cronly-Dillon 1964; Zenkin and Pigarev 1969; Liège and Galand 1971; Wartzok and Marks 1973). We have also used this method in studies of the fish retino-tectal system. In a series of works, we described 13 types of output retinal neurons—GCs (among 20 types mentioned above) projecting to TO of the goldfish, the size and structure of their receptive fields (RFs), contrast sensitivity, resolving power, and color properties (Maximov et al. 2005a, b; Maximov et al. 2009, Maximova et al. 2012; Damjanović et al. 2009b). Six of these 13 types,

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recorded in the superficial sublaminae of the tectal retinorecipient layer, are direction-selective (DS) GCs. They are divided into three distinct groups according to their preferred directions of stimulus movement—caudo-rostral, dorso-ventral, and ventro-dorsal, respectively. Each of these groups comprises both the pure ON and OFF units in equal proportions. Axons of another class of GCs identified as orientation-selective (OS) units terminate below the sublaminae of DS GC projections. There are two types of OS units sensitive to either vertically or horizontally oriented edges or stripes (both of them are ON–OFF-type cells). Moreover, responses of other retinal units, spot detectors nonselective to direction of stimulus movement, are regularly recorded in the same sublaminae as OS units. These GCs are divided into two types of cells predominantly sensitive to small moving (and stationary) contrast spots that are brighter or darker than the background (ON and OFF units, respectively). Finally, two types of sustained units which provide responses that increase to either the darkening or the lightening of their RFs were constantly recorded deeper relative all the unit types described above. Single-unit responses of all the abovementioned GC types were recorded from their axon terminals in TO of carp, pike, roach, barbel, mullet, wrasse, goatfish, and picarel as well (Maximova et al. 1971; Maximova and Maximov 1981; Damjanović et al. 2015). The responses of 13th GC type—the color-opponent one (that were only of the R/G type)—were encountered in TO of the carp and the goldfish very rarely, in spite of the fact that these cells are very numerous in fish retina (Maximova et al. 1975). The schematic representation of the types of responses recorded in fish TO is shown in Fig. 1.

The general morphological structure of TO is well known (as described above). The extensive experimental database that we have acquired using the electrophysiological approach allows us to provide a more elaborated scheme of the retino-tectal connections. Here, we summarize the results of an extensive number of electrophysiological experiments focused on detailed elaboration of the functional segregation of the GC projections in the goldfish and carp tectum. The microelectrode was perpendicularly advanced through the fish TO, and responses of the abovementioned types of units were recorded at different TO depths. Here, we pay special attention to the cases of simultaneous recordings from two single units ("paired recordings"). We use the data to:

1. associate various types of single-unit responses recorded at different TO depths with the known TO morphological strata;
2. estimate the relative location of the sources of single-unit responses (axon terminals of different types of GCs) recorded at different TO strata on the basis of simultaneous recordings from two single units;
3. estimate the location of the RFs of the single units that have been recorded at various depth levels along the microelectrode track (i.e., to demonstrate the compatibility of the maps); and
4. on the basis of simultaneous recordings from the units of retinal and tectal origin, we intend to make some assumptions regarding the physiological properties of identified TO neurons.

Materials and methods

The data were collected in two species of cyprinid fish: goldfish (*Carassius gibelio*; 411 fish) and common carp (*Cyprinus carpio*; 12 specimens). The fish were acquired from local suppliers (Moscow region) and kept in aerated fresh water aquaria at room temperature and natural daylight regime. 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 of the Russian Academy of Sciences (Protocol No. 1 of April 24 2018).

Experiments were performed on the practically intact live animals with normal blood circulation and intact optics and lasted several hours which guarantees normal, stable cell responses and allows to significantly expand the range of applied visual stimuli close to natural. The criteria for a good physiological state of the fish were the velocity of the blood flow in the tectal blood vessels, background noise of remote tectal units, and the reproducibility of single-unit responses. Fish of 10–15-cm standard body length was immobilized with tubocurarine (0.3 mg/100 g of body weight i.m.), then placed in natural position in a transparent Plexiglas tank where artificial respiration was provided continuously

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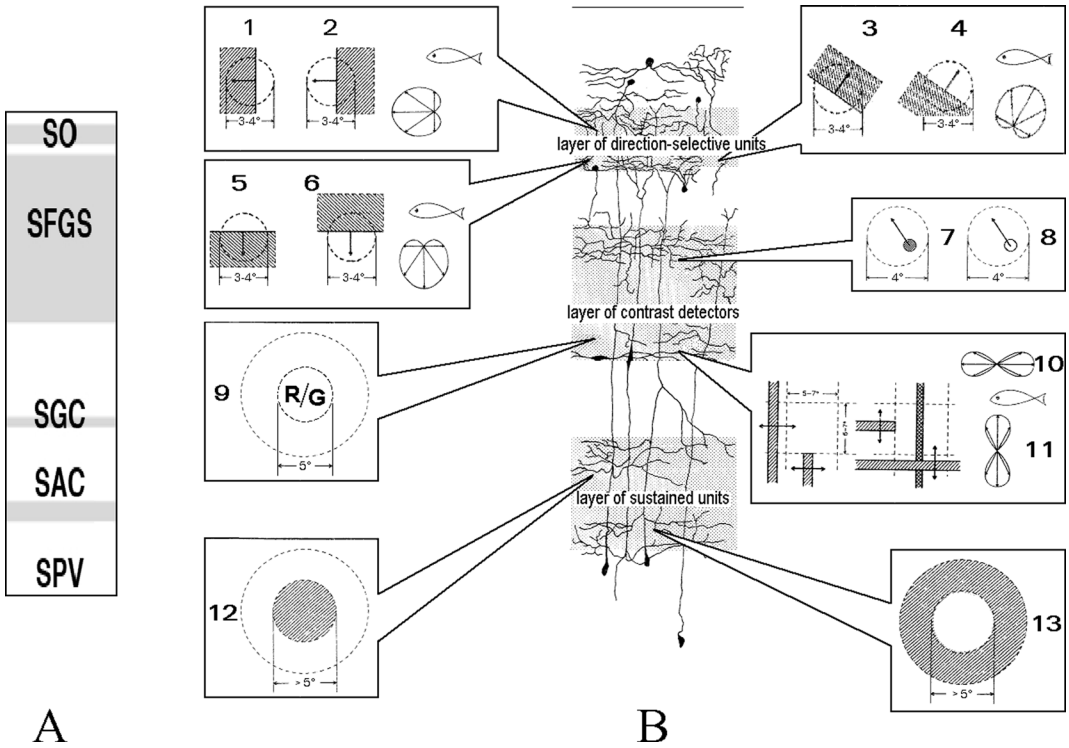

Fig. 1 Stratification of retino-tectal projections in fish. **a** Schematic transversal section through the fish tectum opticum: SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; SPV, stratum periventriculare. **b** Segregation of different types of retinal projections in adult goldfish SFGS as derived from extracellular recording (SFGS thickness 200 μm approximately): (1, 2) ON and OFF DS GCs of caudo-rostral preferred direction; (3, 4) ON and OFF DS GCs of ventro-dorsal preferred direction; (5, 6) ON and OFF DS GCs of dorso-ventral preferred direction; (7, 8) black spot and white spot detectors; (9) color-opponent units (R/G type); (10, 11) detectors of horizontal and vertical line; (12, 13) dark-sustained and light-sustained units

by forcing aerated water through the fish gills with a pump. Water temperature was 21–23 °C. The level of the water in experimental tank was kept constant, so that the fish eyes were under the water but the water did not reach the surface of the brain. In order to reveal the TO contralateral to the stimulated eye, an opening was made in the skull over the contralateral midbrain. During surgery, the preparation site of the head was anesthetized with ice. The borders of the skull opening were moistened with lidocaine.

Fish right eye was facing the monitor on which visual stimuli were usually presented at a limited square area of approximately 11° in angular values (whole screen occupied 43° × 32° of the fish visual field). The stimulation area could be placed at arbitrary locations of the screen and was usually placed so that the RF of the recorded unit was located approximately in its center. In rare cases, square area of stimulation was increased up to 22° in angular values (mainly for tectal neurons with large receptive fields). The background in the stimulation area usually had the effective radiance of 14.5 mW $m^{-2}sr^{-1}$, and the effective radiances of the light and dark stimuli were respectively 65 and 0.13 mW $m^{-2}sr^{-1}$. Constant brightness was maintained for the rest of the monitor screen outside the stimulation area, which effective radiance was usually equal to 7.0 mW $m^{-2}sr^{-1}$.

Visual responses were recorded from a contralateral (left) lobe of the TO. Low impedance (200–500 KΩ) extracellular microelectrodes made from glass micropipettes filled with Wood's metal and tipped with a platinum cap of 3– 5 μm in diameter were used (Gesteland et al. 1959). The microelectrode was visually guided under a microscope (Olympus SZ51) to the surface of TO according to the required retinotopic projection and then perpendicularly advanced through

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the TO with a micromanipulator (MP-225, Sutter Instrument).

The location depth of the electrode tip relative to the TO surface was acquired from the micromanipulator monitor during the recordings. The recordings from a single unit were equally successful when a microelectrode was guided into or out of the TO, with the recorded locations of the unit differing only by a few microns in the two cases. It should be noted that TO is an elastic and viscous structure, so one should measure the relative, not the absolute depths of the units' locations. Data on the relative depth of distinct recorded units (axon terminals of different retinal GC types ramifying in tectal SFGS and various types of neurons of the TO itself) were stored and statistically analyzed using Statistica 6.0 Software (StatSoft, Inc.). Shapiro–Wilk test was used to determine if the data on depth of the retinal projections and tectal neurons were normally distributed. Data for different units were afterwards compared by the one-way ANOVA followed by Tukey's post hoc test. The level of significance for all comparisons was set at $p < 0.05$.

Experimental setup, used for amplifying, digitizing, storing, and processing of the 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, is described in detail elsewhere (Maximov et al. 2005b; Maximov and Maximov 2010).

Electrically, TO is a volume conductor, in which visual stimulation activates multiple sources of electric potentials—GC axon terminals and TO neurons. Platinized microelectrodes efficiently record the extracellular activity from ramified axon terminals and tectal neurons as well. Spikes generated near the electrode tip are higher in amplitude than those generated by distant sources. The main criteria characterizing the single-unit response were the high and stable spike amplitude and the high signal/noise ratio. Spike amplitudes of single-unit response usually exceed the noise amplitude several times and range from 200 to 500 μV. Spikes in discharges of GCs coming into TO along the axons obey the "all-or-nothing" principle. The fact that the recording is indeed made from a single unit can be tested additionally by the analysis of refractoriness in the spike discharge (Maximov et al. 2005b). Spike discharges of retinal and tectal origin differ in a number of characteristics such as receptive field size and spike form (Maturana et al. 1960; Wartzok and Marks 1973; Maximova et al. 2012). In particular, the spikes of TO neurons recorded from their cell bodies substantially decrease in amplitude as the spike rate in discharge increases (Maximov et al. 2005b). It was demonstrated in independent experiments that spike discharges (presumably postsynaptic) attributed to tectal neurons disappeared under application on tectal surface of cobalt chloride (a reversible blocker of synaptic transmission) while spike discharges of axonal endings (presynaptic) persisted (Maximova et al. 2012).

Spike trains were monitored on the oscilloscope and simultaneously listened on the loudspeaker. The audio monitoring is of great importance as the single-unit responses of different types vary in pitch of tone, have different "voices," and can be easily differentiated by ear.

The polar diagram (PD) for each of the recorded units (the dependence of the strength of response (number of spikes) on the direction of stimulus movement) was measured in order to determine the type of the unit. A typical procedure for the measurement of a PD was as follows. Contrast edges moved across the stimulation area in different directions (usually 12, sometimes 24). Three sequential trials were performed for each of the directions and the mean number of spikes was then calculated for each direction. At the end of each experiment, an additional stimulus run was performed in the initial (first) direction in order to check for the unit response stability. The preferred direction of the stimulus movement was determined according to the phase of the first harmonic of Fourier transform of the polar diagram. The position and size of the unit's RF relative to the stimulation area were specified using the same experimental data and were evaluated from the sequences of moments of spike appearances in all trials for all directions of stimulus movement by the custom-made program tools described elsewhere (Damjanović et al. 2009a, 2015). In some cases, the RFs were mapped by the canonical method with a flickering spot ("random checkerboard"). Both methods gave consistent values of RF sizes of about 4.5°.

Depth measurements were made on 34 fish. All other results are based on our database analysis consisting of several thousand files. All recorded and processed data were stored in the specially developed database containing all necessary information (type of recorded unit, time of recording, type of applied stimulus, etc.). In the present study, the database consisting of thousands of recordings from various types of units from the

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goldfish and carp TO was used. Only those recordings that were made from two different units simultaneously were used in further analysis. Selection of the data was based on images of the recorded spike discharges. Polar diagrams and RF positions for both units could be calculated and presented simultaneously based on the dataset. In some cases, the positions and sizes of the RFs of studied units were additionally tested with the random checkerboard method. From the data on both the paired recordings and recent evidences on the relative depths of various TO units, we deduced their spatial arrangement and attributed them to particular morphological TO layers. Simultaneous recording of electrical responses provides an opportunity to clarify the relative position of the sources of these responses (i.e., axon terminals of different GC types).

Results

Simultaneous recordings of two retinal DS units—evidence on spatial arrangement of their RFs

Single-unit recordings are needed for investigation of the properties of GCs projecting to TO. Single-unit responses of two DS units to black stimulus (wide black stripe exceeding the stimulation area in width—further on referred to as an "edge") moving in the preferred direction across gray background are shown in Fig. 2a, b. The appearance of a leading black edge in the receptive field evoked the response of the first DS GC–OFF-type unit (Fig. 2a). The first unit responded to the appearance of the stimulus in its RF and did not respond to the trailing edge of the same stimulus moving out of the RF. On the contrary, the second ON-type unit (Fig. 2b) responded exclusively to the withdrawal of the black stimulus from the stimulation area, i.e., to the withdrawal of the trailing black edge. During the extracellular recordings, we often recorded responses from several DS GCs simultaneously. An example of such simultaneous ("paired") recording from two caudo-rostral DS GC units is presented in Fig. 2c. (This figure is presented in order to avoid the possible misunderstanding on the meaning of the expression "paired unit recordings".) "Paired" responses shown in Fig. 2c were evoked by visual stimulation with a wide white stripe moving in the preferred direction across neutral gray background. The first spike discharge with relatively higher amplitude was evoked by the leading edge of the white stimulus, i.e., it was the response of an ON unit. The second discharge characterized by the relatively lower spike amplitudes represents the response from another DS unit to the trailing edge of the same stimulus moving out of the stimulation area, i.e., it was the response of an OFF unit to the darkening of its RF. Similar "paired recordings" of two DS units with the preference to the same direction of stimulus movement were often met in our practice. As a rule, we could reach single (isolated) recording from one of the units by a slight vertical displacement of the microelectrode.

The paired recordings constitute about a fourth part of the dataset of 1912 files containing the PD data for various retinal DS units, the majority of which were with caudo-rostral preferred direction. As a rule, these units were located more superficially compared to the other two types of DS units. The PDs of two simultaneously recorded caudo-rostral DS units are shown on the right panels of Fig. 3a, b. Wide black stripes that exceeded in their width that of the stimulation area were used for visual stimulation of the units. The OFF unit responded exclusively to the leading edge of the stimulus, while the second—the ON unit—to its trailing edge. RFs of the recorded units were estimated based on the data on their PDs (marked with the white circles on the left panels of Fig. 3a, b). The estimates show that the RFs of these two units had an almost complete overlap (Fig. 3c). This indicates that these two DS GCs should receive their input signals from one and the same photoreceptor area, i.e., they both should be "looking" at the same area of the visual scene. We assume that the axon terminal arborizations of these two caudo-rostral ON and OFF DS units were located in close proximity one under another within the S1 sublaminae of the SFGS.

When the microelectrode is guided further into the TO, the discharges of caudo-rostral DS GC units disappear, and the responses of two other types of DS GCs can be heard over the loudspeaker: the ventro-dorsal and the dorso-ventral units. These units are encountered rarely, as compared to the caudo-rostral ones (Maximov et al. 2005a). The general pattern during the paired recordings was similar: as a rule, the simultaneously recorded DS GCs were characterized by maximal response to the same direction of stimulus movement (i.e., either ventro-dorsal or dorso-ventral) but differed in their selectivity to the sign of stimulus contrast (i.e., one of the simultaneously recorded units was the ON, another one the OFF unit) (Fig. 4). The receptive fields of the simultaneously recorded ON and OFF units had an almost

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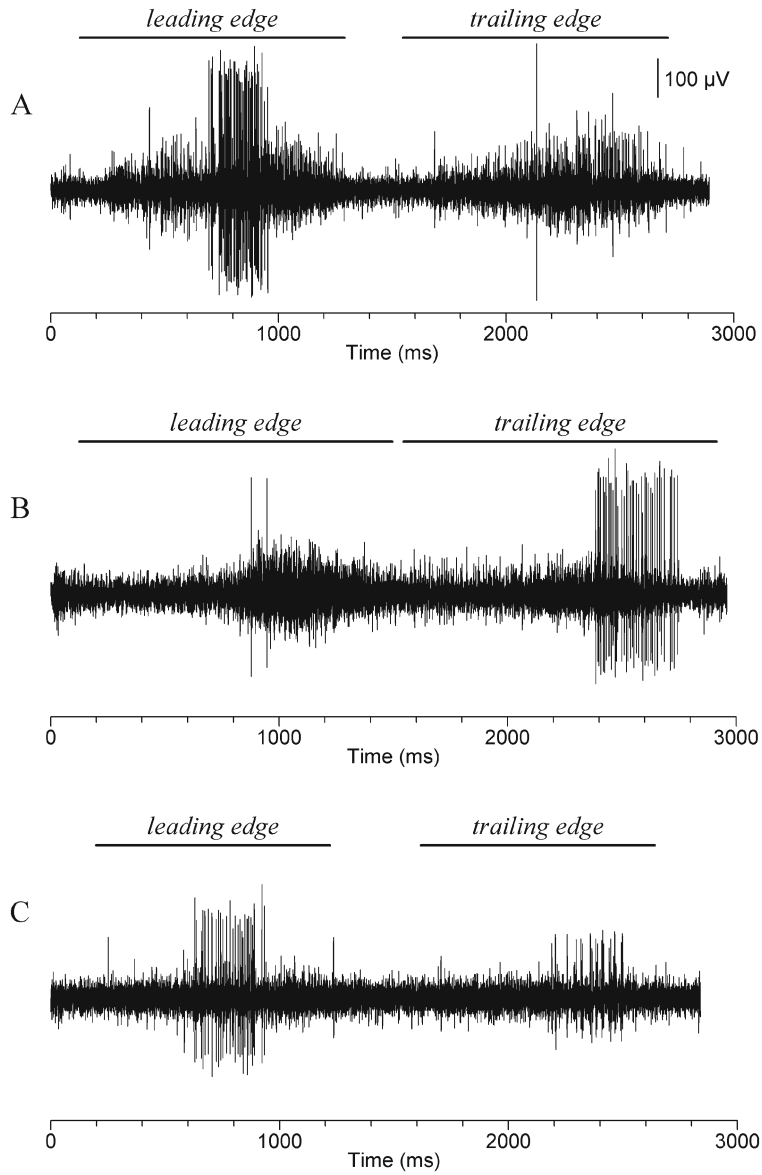

Fig. 2 Spike discharges of different single and paired retinal DS units. Spike discharges of different single (**a**, **b**) and simultaneous (paired) recording from two retinal DS units (**c**) in response to wide contrast stripes, moving in the preferred directions across the receptive field of the recorded units against gray background. Stimuli moved at the speed of 11°/s within the gray stimulation area (a square with a side of approximately 11° on the monitor screen). Responses to the leading and the trailing edges of the stimuli are shown. **a** Response of an OFF caudo-rostral DS unit to the leading edge of the black stimulus. **b** Response of an ON ventro-dorsal unit to the trailing edge of the black stimulus. **c** Responses of two caudo-rostral DS GCs to the white stimulus—spike discharge evoked by the stimulus' leading edge belongs to the ON unit, while the response to the withdrawal of the trailing edge, to the OFF unit. Detailed explanation is provided in the text

complete overlap, as in the case of the caudo-rostral GCs. On rare occasions, the responses of dorso-ventral and ventro-dorsal DS GC units were simultaneously recorded. Their RFs overlapped as well (Fig. 5).

Simultaneous recordings from two different types of OS units of the retinal origin

The responses from OS units of the retinal origin are regularly recorded approximately 50 μm deeper than those from the DS units. The units are represented by two types: detectors of horizontal and vertical lines. The PDs of two types of OS units are both of the typical figure-of-"eight" shape but are positioned in the mutually orthogonal orientations and are practically symmetrical for the leading and the trailing edges of the stimuli (see Fig. 6b). Both detectors of the horizontal and the vertical lines were on some occasions recorded simultaneously. In this case, the responses of both types of units were present in the PDs that look like a "Maltese cross" (Fig. 6a). Among the 1081 OS GC PD

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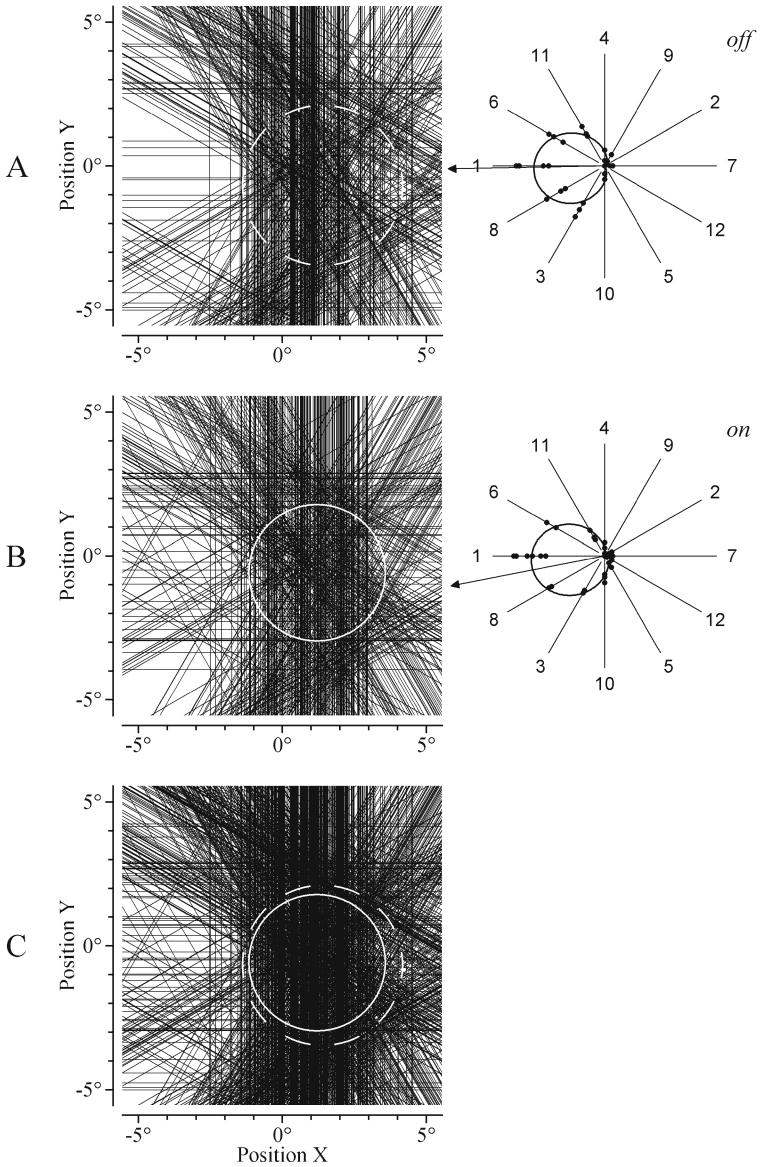

Fig. 3 The estimations of sizes and positions of RF and polar diagrams for two simultaneously recorded DS GCs of caudo-rostral preferred direction. The stimuli were wide black stripes moved in 12 directions at the speed of 11°/s inside the gray stimulation area (a square with a side of approximately 11° on the monitor screen). Left panels show responses to the leading (**a**) and the trailing (**b**) edges of the stimuli; each black line corresponds to the position of the moving edge at the moment of spike appearance; white circles represent the estimations of the units' RFs—dashed line for the OFF unit and solid line for the ON unit. The third left panel (**c**) shows all responses to the bright and dark edges and spatial relationship between estimated RFs of recorded units. Right panels show PDs of responses to the leading (**a**) and trailing (**b**) edges; numbers on the radial lines represent the order of movement direction; dots mark the number of spikes evoked in response to each of three runs for each applied direction; solid curves represent the approximations of the experimental data by a Fourier series with first two harmonics; preferred directions of stimulus' movement for each unit are shown by the black arrows. The diagram marked with the label "off" was built from the responses to the movement of dark edges into the RF; that marked with label "on" was built from the responses to the movement of bright edges

recordings (762 were denoted in database as detectors of horizontal lines and 319 as detectors of vertical lines), there were 80 paired recordings from two types of the OS units with cross-shaped PDs. With a slight vertical displacement of the microelectrode, we could often isolate one of them (Fig. 6b).

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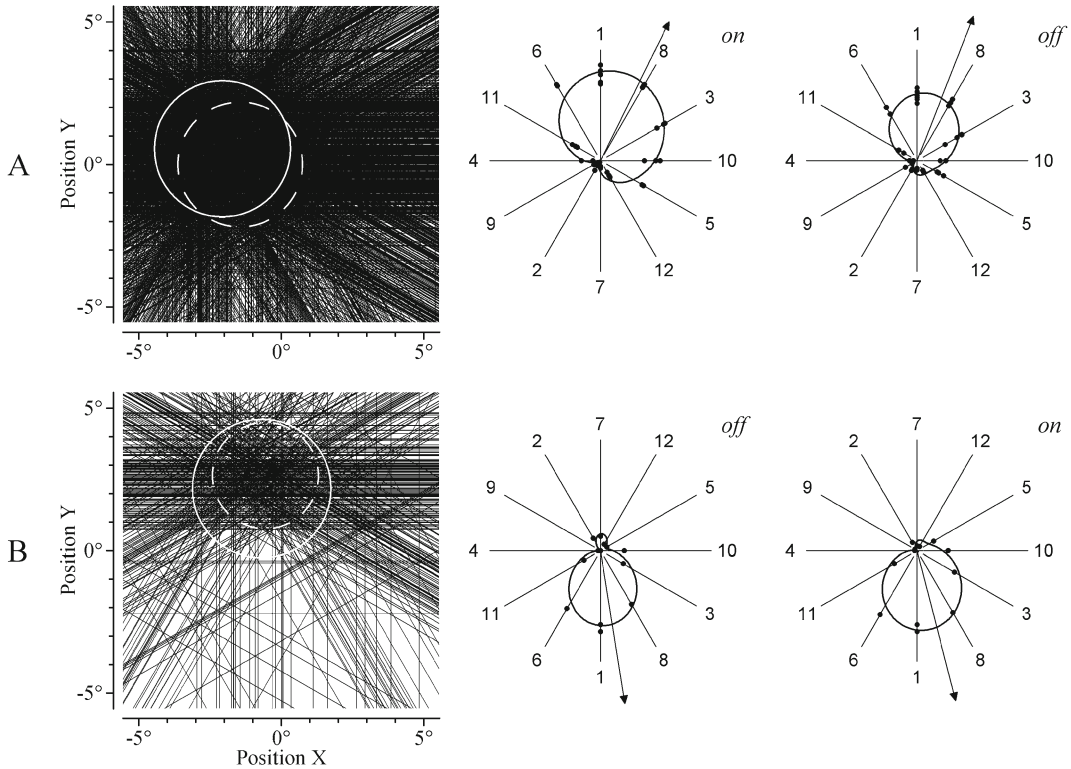

Fig. 4 The estimation of sizes and positions of RFs and polar diagrams for two simultaneously recorded DS GCs of ventro-dorsal and dorso-ventral preferred directions. **a** Responses of two ventro-dorsal units to leading and the trailing edges of wide white stripe. **b** Responses of two dorso-ventral units to leading and the trailing edges of wide black stripe. Other conventions are the same as in Fig. 3

The simultaneous recordings of pairs of the OS units that prefer the stimuli of mutually orthogonal orientations (i.e., of the detectors of horizontal and vertical lines) suggest that their axonal arborizations are located one under another in the same sublamina of SFGS.

Simultaneous recordings from white spot and black spot detectors

Approximately at the same level of SFGS, where the OS units were detected, the responses from a different group of GC units were recorded, though far less

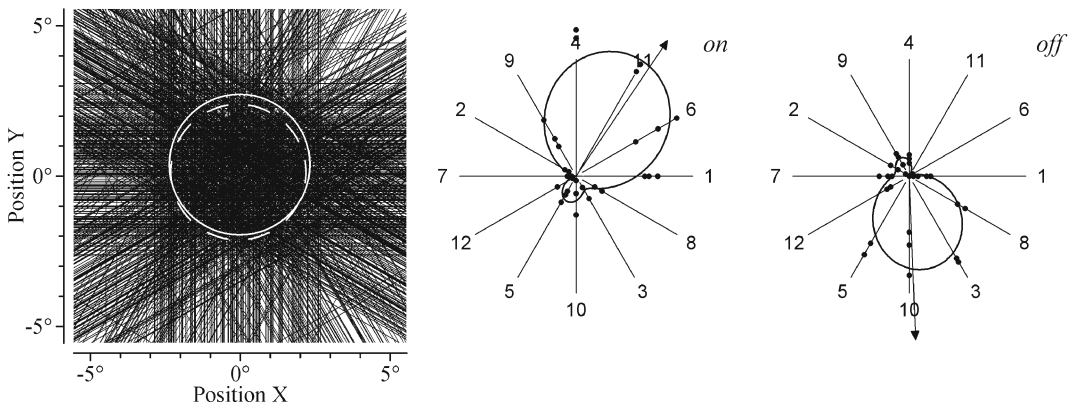

Fig. 5 Spatial relations of RFs and PDs for an ON ventro-dorsal and an OFF dorso-ventral units recorded simultaneously. Stimuli were wide white stripes. Other conventions are the same as in Fig. 3

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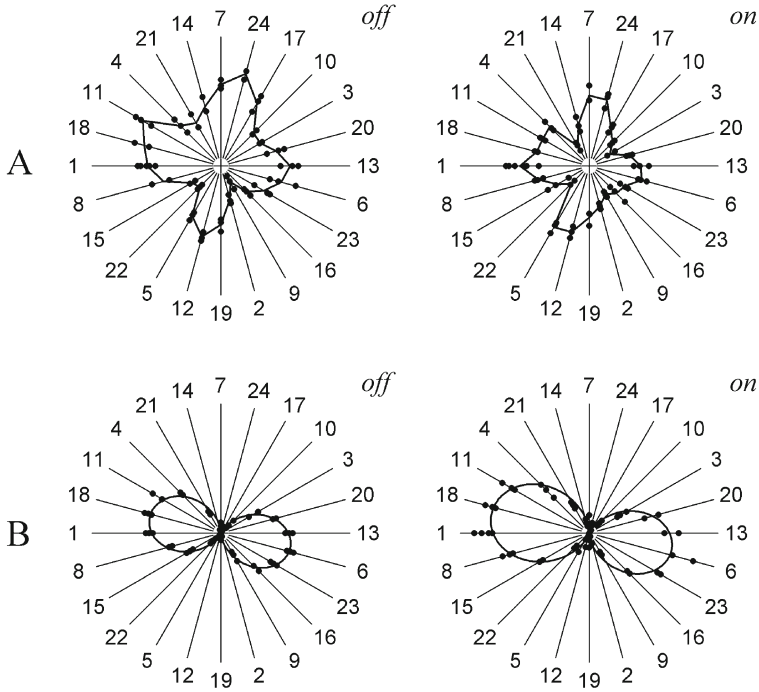

Fig. 6 The PD of two simultaneously recorded OS units with mutually orthogonal preferred orientations and repeated measurement of the PD after fine-tuning of the microelectrode depth. Two OS units preferring mutually orthogonal orientations of stimulus (horizontal and vertical ones) were recorded simultaneously, and the PD for this pair of units was measured (**a**). Then, one of the units (preferring vertical orientation of stimulus) was tuned by minute displacement of the microelectrode, and the PD was measured again (**b**). Stimulus was a wide black stripe moving in 24 directions at the speed of 16°/s against gray background. The PDs of responses to leading and trailing edges of stimulus (left and right panels) look similarly because OS units are ON–OFF type. In **a**, mean numbers of spikes in response to stimuli moving in different directions are joined by the solid line. Other conventions are the same as in Fig. 3

frequently. These units of the retinal origin are non-selective both to the direction of motion and to the orientation of stimuli and are not characterized by any spontaneous spike activity. They are characterized by a sustained response to a stationary small contrast spot that appears in their visual field. The same stimulus moving in any direction across their RF evokes an even more pronounced response from these units. It should be also noted that the responses of these detectors to small moving spots are considerably stronger than those to contrast edges and stripes moving in the same directions. Similar to DS GCs and OS GCs, they do not respond to onset or offset of ambient light.

Some of these units respond to stimuli that are brighter than the background (i.e., they are ON units), while the others respond to stimuli that are darker than the background (OFF units). By analogy with the black spot detectors of the frog (Lettvin et al. 1959; Maturana et al. 1960), which they resemble, we refer to these two types of the fish GCs as white spot and black spot detectors (SDs), respectively. Single records of 207 spot detectors were stored in our database (134 black spot detectors and 73 white spot detectors). Responses of white and black SDs were simultaneously recorded in the half of all recordings approximately. It was shown that the RFs of the simultaneously recorded white spot and black spot detectors almost completely overlap. In Fig. 7, the RFs of two spot detectors (OFF and ON unit) were mapped with a small white spot that flashed on and off against gray background at different positions of the square stimulation area in a quasi-random order ("random checkerboard"). One can see a pronounced response of a white spot detector to the stimulus turning on (left panel) and a much weaker response of a closely located black spot detector to the stimulus turning off (right panel).

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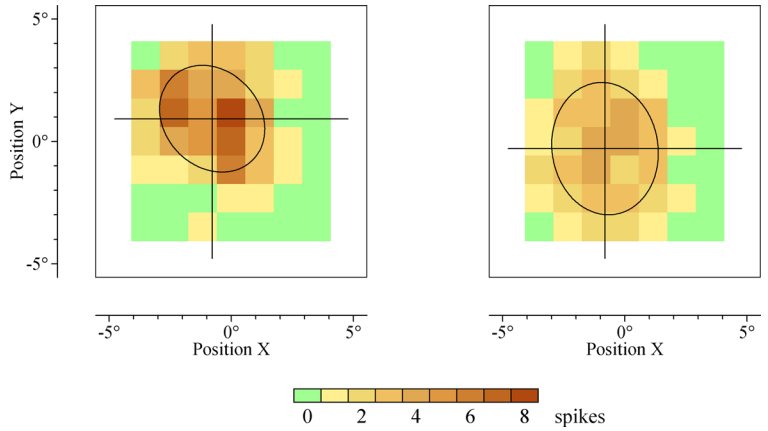

Fig. 7 The RFs of two spot detectors mapped by a "random checkerboard" method. Units' responses to the white spots (small squares with a side of 1.1°) switching *on* and *off* at different locations of the gray stimulation area were recorded and presented in the form of a topographic map (see the scale at the bottom of the graph). The flashing spots were presented three times at each location. The ellipses represent the estimates of the units' RFs. The left panel shows responses of a white spot detector to the white spot turning *on*; the right panel shows responses of a black spot detector to the white spot turning *off*

Simultaneous recordings from two different types of sustained units of the retinal origin

Responses of the abovementioned movement detectors can be evoked only by visual stimuli that are adequate for each of them. Unlike them, the responses of the sustained units can be recorded in the absence of adequate visual stimulation at the moment the electrode is guided to the relative depth of about 200 μm from the TO surface (deeper than all other retinal units). Total sustained activity recorded at this position of microelectrode increases either to the darkening or to the lightening of the stimulation area. The "voices" of units excited by the darkening (OFF-sustained) differ from those evoked by the lightening (ON units) in their "pitch of tone." Single responses from the ON- and the OFF-sustained units can be often isolated by adjustment of the microelectrode position. Single records of 149 OFF-sustained and 75 ON-sustained units were stored in our database. In two cases, pairs of units of the ON type and the OFF type were simultaneously recorded. Responses from pairs of units to presentation of wide black stripe moving across neutral gray background are shown (Fig. 8). At the gray background, the ON-type unit was activated while the OFF unit was suppressed. Accordingly, prior to the visual stimulation with the

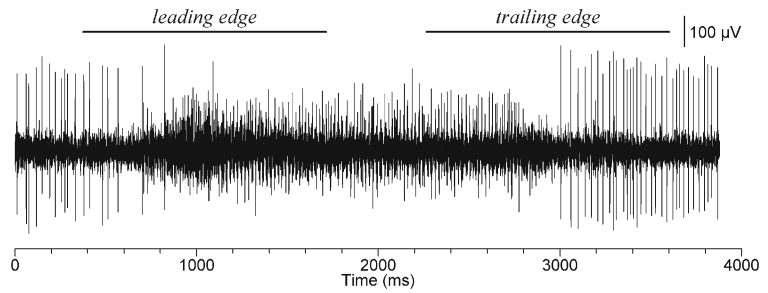

Fig. 8 The simultaneous recording from a pair of sustained units of the retinal origin. Responses of an ON-sustained and an OFF-sustained unit to wide black stripe moving against gray background. The stimulus moved at the speed of 16°/s inside the gray stimulation area (a square with a side of approximately 22° on the monitor screen). High-amplitude spikes represent response of an ON unit, while the spikes of relatively lower amplitude represent response of an OFF unit. Detailed explanation is provided in the text

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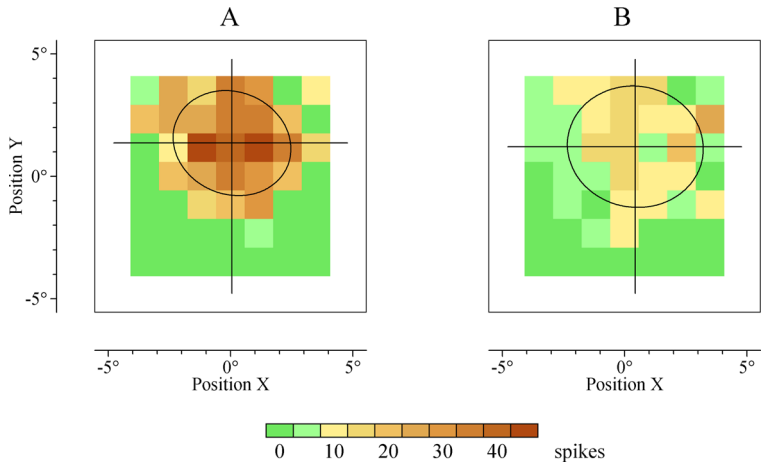

Fig. 9 The RFs of two simultaneously recorded sustained units of the retinal origin mapped by a "random checkerboard" method. **a** Responses of an ON unit to the white spot turning on. **b** Responses of an OFF unit to the black spot turning on. Other conventions are the same as in Fig. 7

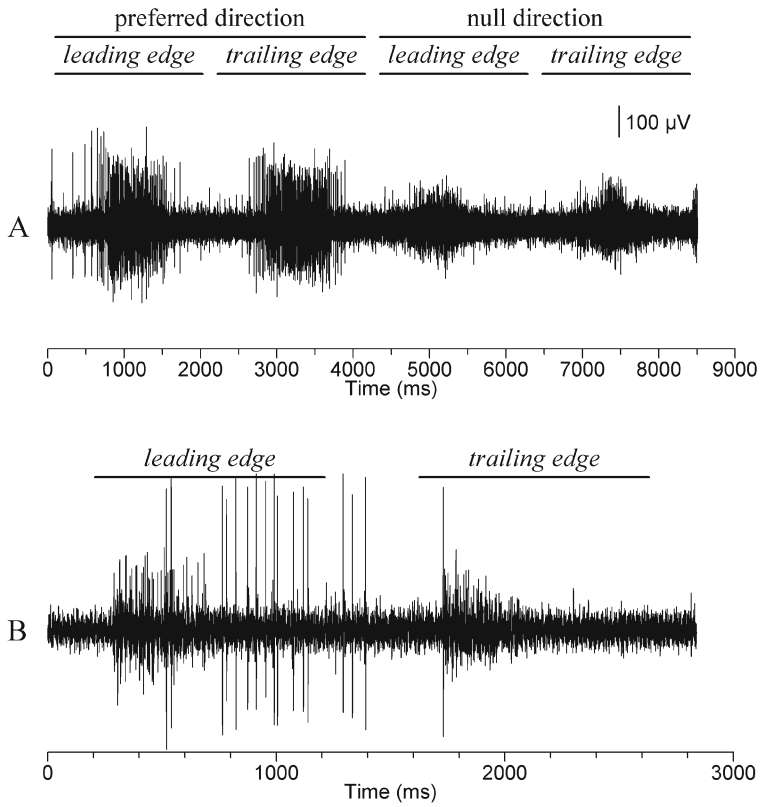

Fig. 10 The spike responses of retinal and tectal units recorded simultaneously. **a** The spike discharges of direction-selective TO neuron preferring ventro-dorsal direction in response to the wide black stripe entering in ("leading edge") and moving out of ("trailing edge") the gray stimulation area (a square with a side of approximately 16° on the monitor screen). The stimulus moved at the speed of 11°/s in the preferred and afterwards in the opposite ("null") direction. Periods of stimulus movements in the preferred and the "null" direction are marked on the top. The DS neuron responds to the leading as well as the trailing edge when the stimulus moves in the preferred direction and does not respond at all when it reverses ("null" direction). The responses recorded in the "null" (dorso-ventral) direction are of retinal origin. These discharges with lower-amplitude spikes are considerably shorter in duration (i.e., this indicates to small RFs). **b** The spike discharges of a tectal neuron of unknown specificity (high-amplitude spikes) and a pair of DS GCs of caudo-rostral preference recorded simultaneously in superficial TO layer. The stimulus was wide black stripe moving in the caudo-rostral direction against gray background. Other conventions are the same as in Fig. 2

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moving wide black stripe, sustained discharge of the ON unit is seen (high-amplitude spikes on the left part of the trace in Fig. 8). The appearance of the leading edge of the black stimulus in the RFs of two closely located sustained units resulted in the suppression of the high-amplitude spikes (the spike discharge of the ON unit) and the activation of the OFF-sustained unit (the spikes of lower amplitude in the central part of the trace). In other words, the practically simultaneous darkening of the units' RFs activated the OFF-sustained and simultaneously inhibited the ON-sustained unit. On the contrary, the subsequent withdrawal of the stimulus' trailing edge from the RFs (i.e., lightening of the RFs) suppresses the OFF unit discharge and recovers the ON unit activity (high-amplitude spikes in the right part of the trace).

The RFs of another pair of simultaneously recorded sustained units were mapped by means of the "random checkerboard" with white and black spots, respectively (Fig. 9). It is clearly seen that the RFs of the two units occupy the same positions in the stimulation area. Accordingly, one can conclude that these GCs are connected with the same photoreceptor area and their axonal arborizations are located under each other in close proximity in the deepest stratum of SFGS.

As a rule, we succeeded to record the responses from retinal units of all the abovementioned types in one microelectrode track consecutively. Considering an average size of units' RFs to be approximately 4.5° (Damjanović et al. 2009a, b), we can say that the RFs of these retinal units partly or completely overlap, i.e., they receive their input signals from the same photoreceptor area.

In a fish of 10 cm long, diameter of 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 RF size of 4.5° was estimated—it was a rounded area with an approximate diameter of 300 μm. In our separate morphological study, we traced retinal GCs by the carbocyanine dye DiI in the goldfish retina (Maximova et al. 2006). GCs identified as putative ON- and OFF-type DS GCs had rounded flat "lacy" dendritic arbors of about 200–300 μm in diameter. This diameter corresponds to the RF size of approximately 300 μm estimated for different types of fish movement detectors in our physiological experiments.

Spatial relationships between responses of retinal and tectal origin

Responses of the direction-selective tectal neurons

More than 200 TO neurons proper with direction-selective properties have been recorded in our experiments until the present time. Some properties of the spike discharges of tectal DS units indicate that we record from their cell bodies. In particular, the spikes of DS neurons recorded from their cell bodies substantially decreases in amplitude as the spike rate in discharge increases. These tectal DS neurons respond to the moving edges of any sign of contrast (they are ON–OFF-type units) and are characterized by extra-large RF sizes, reaching up to 60°. As a rule, responses from these units were recorded in the deep tectal zones, approximately 50–100 μm underneath the layer of retinal sustained activity. In rare cases, responses from the DS neurons were also encountered in the more superficial layers, approximately in the same zone where the retinal OS units and spot detectors were recorded (Fig. 10a). Sometimes, responses from these neurons were recorded even more superficially, simultaneously with the responses from the ventro-dorsal and the dorso-ventral DS units of the retinal origin, but have never been recorded simultaneously with the caudo-rostral DS GCs. It has been shown recently that the fish TO DS neurons select four preferred directions of the stimulus movement compared to three preferred directions of the retinal DS GCs (Damjanović 2015). Three of these directions selected by the DS neurons corresponded to those selected by the retinal DS units. These DS neurons were recorded in both deep and superficial population of TO DS neurons. On the other hand, the tectal DS units, preferring the fourth, rostro-caudal direction of stimulus movement (absent in the retina), were observed exclusively in the deep TO zones.

Responses of superficial TO neurons with undefined properties

Responses from the caudo-rostral DS GCs are often simultaneously recorded with the separate high-amplitude spikes of presumably tectal origin (Fig. 10b). These responses are not selective to the direction of stimulus motion. Adequate stimulation for these neurons has not been found yet. Simultaneous

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Table 1 *P* values for the comparisons between the data on the relative location depths of various unit responses recorded in the goldfish and carp tectum

Recorded units	tsN (40.786)	rDSU (52.982)	rOSU (97.771)	rSD (107.67)	tsDSU (100.00)	rSust (191.45)	tdDSU (283.41)
tsN		0.642692	*0.000026*	*0.000026*	*0.000056*	*0.000026*	*0.000026*
rDSU	0.642692		*0.000026*	*0.000029*	*0.000382*	*0.000026*	*0.000026*
rOSU	*0.000026*	*0.000026*		0.975100	0.999996	*0.000026*	*0.000026*
rSD	*0.000026*	*0.000029*	0.975100		0.998431	*0.000026*	*0.000026*
tsDSU	*0.000056*	*0.000382*	0.999996	0.998431		*0.000026*	*0.000026*
rSust	*0.000026*	*0.000026*	*0.000026*	*0.000026*	*0.000026*		*0.000026*
tdDSU	*0.000026*	*0.000026*	*0.000026*	*0.000026*	*0.000026*	*0.000026*	

One-way ANOVA followed by the Tukey's post hoc test. The level of significance for all of the comparisons was set at $p < 0.05$. Averaged depths in "micrometer" for all of the units recorded in the goldfish and carp TO are given in the brackets. Statistically significant *p* values are given in italics

tsN tectal superficial neuron (28 units recorded), *rDSU* retinal direction-selective units (109 units recorded), *rOSU* retinal orientation-selective units (48 units recorded), *rSD* retinal white and black spot detectors (12 units recorded), *tsDSU* tectal surface direction-selective units (11 units recorded), *rSust* retinal "light" and "dark" sustained units (99 units recorded), *tdDSU* tectal deep direction-selective units (16 units recorded)

recordings of their responses and the discharges of the caudo-rostral DS GCs means only the proximity of the sources generating these reactions but do not determine any functional relations.

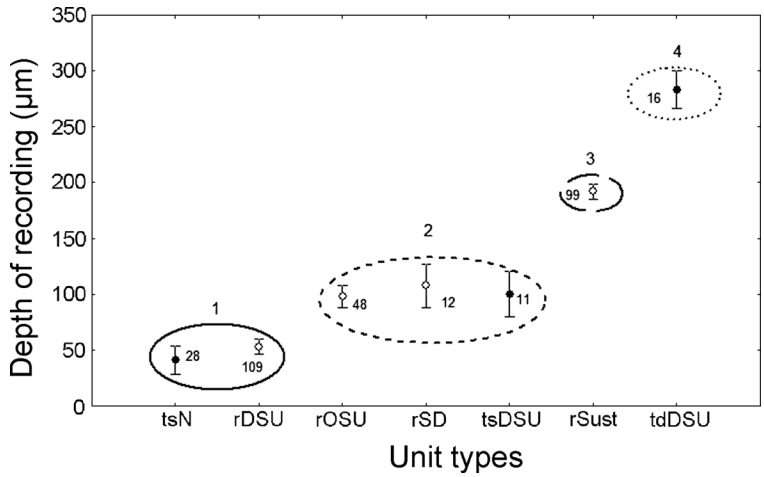

Fig. 11 Relative depth distribution of various units in the fish TO. Recorded units: "tsN"—tectal superficial neuron; "rDSU"—retinal direction-selective units (divided into three distinct groups according to their preferred directions of stimulus movement—caudo-rostral, dorso-ventral and ventro-dorsal, respectively; each of these groups comprises both, the pure ON and OFF units in equal proportions); "rOSU"—retinal orientation-selective units (two types, sensitive to either vertically or horizontally oriented edges or stripes; both of them are ON–OFF-type cells); "rSD"—retinal white and black spot detectors (divided into two types of cells predominantly sensitive to small moving (and stationary) contrast spots that are brighter or darker than the background (ON and OFF units, respectively)); "tsDSU"—tectal surface direction-selective units (select three preferred directions, that correspond to those selected by the retinal DS units; ON–OFF-type neurons); "rSust"—retinal "light" (ON) and "dark" (OFF) sustained units (two types, which provide responses that increase either to the darkening or the lightening of their RFs); "tdDSU"—tectal deep direction-selective units (select four preferred directions; three of them correspond to those already selected on the retinal level; the fourth, rostro-caudal direction of stimulus movement is absent in the retina; ON–OFF-type neurons). Abscissa—various groups of units; Ordinate—recording depths in "micrometer." "Open circles"—retinal GC projections to TO; "closed circles"—tectal neurons. Number of the analyzed units is given near the corresponding plotted data. Vertical bars denote 0.95 confidence intervals. Responses of various types recorded at the depths that did not differ statistically are surrounded by elliptical curves (1–4)

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Stratification of various types of retinal GC projections in the fish TO

Clustering of retinal projections at different levels of the tectal retinorecipient zone was already reported for various fish species (Maximova et al. 1971; Maximov et al. 2005a). In the present study, we attempted to localize more precisely the depth positions for various units (both of the retinal and the tectal origin). Thanks to the new, modern equipment, in the last few years, we got the opportunity to perform more accurate measurements of the depth positions for various units recorded in the fish tectum. Here, we present the data of these measurements collected in goldfish and carp from our latest 34 experiments. When the microelectrode is perpendicularly advanced through TO, the following general pattern is observed: initial contact with the liquid covering the TO is always followed by an abrupt decrease of the electrode noise, while the subsequent advance of the tip into the superficial TO layers results in noise increase of up to 50 μV and at that instance the first weak neuronal activity can be recorded. This marks the TO surface level ("0" point), from which the relative location depths for various units of both the retinal and the tectal origin are measured. Single-unit responses of various types of GC projections and TO neurons occurred in the following order (Table 1 and Fig. 11). Tectal neurons nonselective to direction of stimulus movement and retinal DS units were located at the depth of about 50 μm (there was no statistically significant difference between the two groups, but there is a statistically significant difference between each of them and the rest of the tectal neurons and tectal GC projections). Retinal OS units, retinal spot detectors, and one group of tectal DS neurons were all located at the depth of about 100 μm (there is no statistically significant difference for the three location depths, but there is a statistically significant difference between them and the rest of the tectal neurons and tectal GC projections). Retinal sustained units were located at the depth of 200 μm (statistically significant difference from all other units) and the tectal deep DS neurons were around 300 μm (statistically significant difference from the rest of the dataset). Despite the relatively high variation of the relative depth values in the case of both the spot detectors and the tectal neurons (due to the relatively small sample sizes and also the fact that the absolute depth of electrode location is difficult to calculate due to the viscosity and elasticity of TO), a clear stratification of tectal neurons and retinal GC projections was shown. It should be noted that the recorded distance between the more superficially located projections of the retinal DS units and the deep projections of the sustained retinal GCs that is about 150 μm (from 50 to 200 μm of the TO depth) in our view corresponds to the width of the tectal retinorecipient layer (SFGS).

Discussion

The fish retino-tectal system is currently studied by means of three different methods: the classical microelectrode recording of the single responses from the GC terminals and tectal neurons in the TO of an adult living individual, as well as with the relatively new methods of Ca++ imaging and genetic markers of certain neurons in the transparent Danio larvae (in particular, Brainbow genetic labeling). Each of these three methods has both certain constraints and advantages, and when combined, they may provide a sufficiently meaningful representation of the organization of the retino-tectal system.

We have studied the individual properties of the 13 types of GCs projecting into TO and those of the four types of direction-selective tectal neurons using the method of extracellular microelectrode recording of single-unit responses to different visual stimuli. The main focus of this paper is the attempt to refine relative localizations of identified units on the basis of simultaneous paired recordings. We have shown that such approach, just on the results of electrophysiological experiments, is possible in the absence of morphological data. Moreover, similar fine lamination of GC axonal endings has been demonstrated in zebrafish SFGS by Brainbow' genetic technique (Nevin et al. 2010; Robles et al. 2011; Robles et al. 2013).

Segregation of axonal endings of different physiological ganglion cell types in the fish tectum identified on the basis of microelectrode recordings

Direction-selective units

The sufficiently regular pattern of simultaneous paired recording (given the same microelectrode position) from two caudo-rostral direction-selective GCs of ON and OFF types (Figs. 2c and 10b) points to the high proximity of their axon terminals. The full or partial overlap of their RFs (Fig. 3) shows that two such GCs "look at

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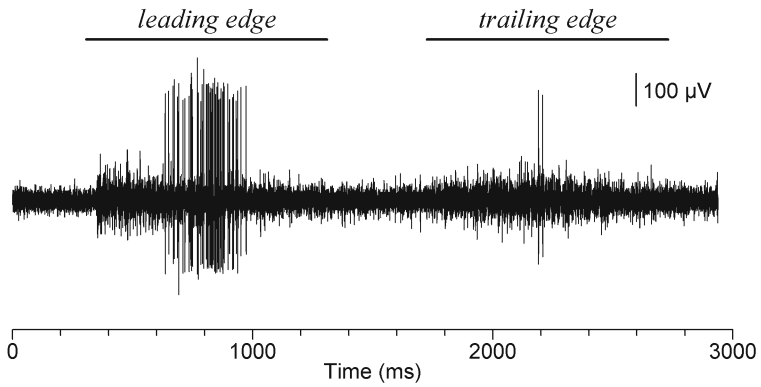

Fig. 12 The spike discharges of two caudo-rostral retinal ON DS units recorded simultaneously. The discharge of high-amplitude spikes from the ON caudo-rostral DS unit (which is closer to the electrode) is preceded by the lower-amplitude discharge of another ON caudo-rostral unit (that is far from microelectrode). Stimulus was wide white stripe, moving against gray background in the preferred direction. Detailed explanation is in the text. Other conventions are the same as in Fig. 2

the world" with the same group of photoreceptors, being connected with them via separate systems of ON and OFF bipolar cells at the retinal inner plexiform layer.

The same rule is relevant for the responses of the direction-selective GCs that prefer dorso-ventral or ventro-dorsal directions of stimulus movement: the ON and OFF responses of a particular type of direction selectivity are recorded simultaneously (Fig. 4a, b). On rare occasions, we have recorded the simultaneous responses from two direction-selective GCs with differing preference to the direction of stimulus movement and in that case with differing preference to the sign of stimulus contrast relative to the background. In these cases, the position of the units' RF coincided (Fig. 5).

Among approximately 2000 of the responses recorded from the GC axonal terminals, we have never encountered the simultaneous recording of the caudo-rostral and any of the two remaining direction-selective types of units. Based on the subjective judgment (from the experience accumulated during many years of research on the subject), the caudo-rostral direction-selective responses of the retinal origin are recorded in TO more superficially relative to dorso-ventral and ventro-dorsal direction-selective units (Maximov et al. 2005a). This spatial segregation is particularly obvious when listening to the units' responses. The same pattern has been also demonstrated for the Danio juveniles by the Ca++ imaging method (Nikolaou et al. 2012). In the experiments on the transparent juvenile individuals of the Danio fish, visually evoked activity of the retinal GC axons innervating tectum of zebrafish was recorded. Three different areas of excitation were recorded in the TO in response to contrast gratings moving in three different directions, respectively. The most proximate to the TO surface is the zone that shows excitation in response to the stripes moving in the caudo-rostral direction. Deeper, the less pronounced zones with the dorso-ventral and ventro-dorsal preference to stimulus movement are located. The relative abundance and stratification of these axonal terminals is consistent with our data. Statistical analysis performed for the data from our latest experiments with the specially performed measurements of the relative tectal depth of signal recordings shows that the responses from all of three types of DS GCs should be grouped in a single cluster, what can be explained by the limited amount of the dataset (data from 34 experiments) (Fig. 11).

Direction-selective GCs of the fish retina are clearly segregated into the ON and OFF types, according to our data. As it is shown in the present study, the possibility of the simultaneous recording of two ON and OFF units with the same preference to a direction of stimulus movement can itself mark the proximity (within the 5-μm limit) of the structures that generate these responses. The Ca++ imaging method failed to segregate the DS GCs into these two types, most likely due to the methods' constraints. In this study, moving gratings were used for the stimulation, and it was impossible to distinguish the responses to the leading and trailing edge of each of the narrow stripes making the grids, i.e., responses to the lightening and the darkening of the visual field could not be distinguished. However, the Ca++ imaging method demonstrated the activity of

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various types of axon terminals in the whole structure of TO at once, what cannot be performed with the microelectrode method. Using the latter method, one can only demonstrate the rough retinotopic correspondence and layer by layer segregation of the various types of responses.

Besides simultaneous recordings of two single reactions of the ON and OFF types of units with the same preference to a direction of stimulus movement, we have often encountered the following recordings: the spike discharges of high amplitude of the direction-selective units that are frequently accompanied by the lower-amplitude (though significantly exceeding the noise threshold) discharges of the same direction preference as well as the same sign of contrast relative to the background. An example of such simultaneous recording from two caudo-rostral ON direction-selective units is shown in Fig. 12. The spike discharge characterized by low spike amplitude starts earlier and terminates at the moment of the onset of the second, high-amplitude spike discharge. The duration of the spike discharges is practically the same. This might indicate that the receptive fields of the two units are similar in size and are located close to each other, i.e., this is the electrophysiological illustration of the dendrite tiling of these GCs in the retinal inner plexiform layer. The notable difference in the spike amplitude of these two discharges indicates the horizontal distance from the electrode of the signal source (axon terminal) that corresponds to the first discharge.

Orientation-selective units

The responses of the orientation-selective units that are recorded deeper in TO relative to the direction-selective units have been studied in our group for many years (Maximova and Maximov 1981; Maximov et al. 2009, 2013; Damjanović et al. 2009b; Maximov 2010). In the present study, we have analyzed the data on the simultaneous recording of responses of the detectors of the horizontal and vertical lines, what marks the close proximity of their axonal terminals. The zones of axonal terminals that respond to the stimulation by the horizontally and vertically oriented edges have been also detected with the Ca++ imaging method in the Danio TO, and what is important in this study, they were also located deeper than the axon terminals of the direction-selective GCs (Nikolaou et al. 2012). According to these authors, the "Orientation-selective inputs tuned to bars moving along the vertical and horizontal axes are concentrated in posterior and anterior tectum, respectively." Our experimental dataset does not show such spatial segregation. We recorded the responses of both types of detectors from the anterior, as well as from the central and caudal areas of the TO in goldfish and carp (Maximova and Maximov 1981). The simultaneous registration of both types of responses at different areas of the fish visual field also marks the uniform distribution of the OS GCs' axonal terminals. One of the possible explanations for this inconsistency in the data acquired by the two method approaches can be the fact that the experiments with the Ca++ visualization were performed on the *Danio* larvae (from day 4 to day 12 after the fertilization), when, probably, the morphogenesis of the retino-tectal connections had not completed yet. Another explanation for the lack of the topographic segregation of the detectors of the horizontal and vertical edges in TO in the adult goldfish and carp might be the lack of the specialized areas in their retina (e.g., areas of acute vision) (Marc and Sperling 1976; Stell and Kock 1984).

Spot detectors

The responses from the detectors of small contrast spots, both white and black, were recorded approximately at the same depth as the OS detectors. The properties of these detectors resemble those of the frog spot detectors (Maturana et al. 1960) and local edge detectors (LEDs) of the mammalian retina (van Wyk et al. 2006). As different from the frog spot detectors which constitute a whole layer in the frog TO, in the goldfish TO, they are relatively rarely encountered. According to our dataset, the overall amount of the recorded spot detectors is roughly ten times less compared to the DS units and five times less than the OS units. As a rule, the receptive fields of black and white spot detectors were overlapping (Fig. 7).

Sustained units

The responses from the sustained units were recorded from the most deep strata in the TO, compared to all of the abovementioned types of responses. Some of them are stimulated by the lightening and inhibited by the darkening of the visual field (ON units), while the others, on the contrary, are triggered by the darkening and inhibited by the relative lightening of the visual field

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(OFF units). According to our subjective judgement, the sources of the sustained activity (the respective axonal terminals) that increases with the lightening are located somewhat deeper relative to the sustained units that respond to the darkening of the visual field. Statistical analysis, however, did not support our audio feeling (Fig. 11). The simultaneous recording of ON- and OFF-sustained units testifies to the proximity of the axonal terminals of these GC types in TO (Fig. 8). The units characterized by sustained activity have been described in TO of several fish species (Zenkin and Pigarev 1969; Maximova et al. 1971). Similar responses to the darkening of the visual field have been described for the *Rana pipiens* TO (Maturana et al. 1960). The sustained activity recorded in the deeper layers of the frog TO resembles to a high extent the sustained activity that we record in the corresponding layers in fish TO. ON-sustained units as well as the simultaneous recording of both types (ON- and OFF-sustained units) are provided here for the first time. The predominance of OFF inputs in the deepest sublaminae of SFGS in *Danio* larvae tectum has been demonstrated by the method of Ca++ visualization (Robles et al. 2013).

Lamination of GC axonal endings in the tectum of zebrafish larvae demonstrated by Ca++ imaging and Brainbow genetic technique—analogies with our data

The results of the outstanding beauty have been obtained with the Brainbow genetic cell-labeling technique (Robles et al. 2011, 2013). The dendrites as well as the axonal pathways to their targets and the types of GC axonal terminals in the TO of Danio larvae were visualized. The study has revealed that the retino-tectal projection of larval zebrafish is anatomically and functionally divided into fine sublaminae. It was demonstrated that lamination serves to spatially segregate inputs from retinal GC projections based on the type of information they transmit. The overlapping of axon terminals is a main feature of tectal afferentation. The axonal arborizations of GCs are equal in sizes that permits to keep retinotopic correspondence of plates (maps) formed by axon terminals of GCs of diverse types. The fact that such organization of the retino-tectal projections appears as early as at the larval developmental stage has been also shown by Ca++ imaging (Nikolaou et al. 2012). Abovementioned findings on larval zebrafish are directly related to our electrophysiological unit recordings in adult fish.

Segregation of two groups of tectal DS neurons

DS units of tectal origin were already described in a few studies of fish retino-tectal system (zebrafish—Gabriel et al. 2012; Hunter et al. 2013; Kassing et al. 2013; goldfish and carp—Maximova et al. 2012; Damjanović 2015). As a rule, cell bodies of DS neurons are located in deep tectal layers (periventricular zone) while their dendrites arborize more superficially in the retinorecipient layer (SFGS) (Gabriel et al. 2012). However, as shown in the present study, responses from rare tectal DS units can be also recorded from more superficial layers, approximately in the same zone where retinal OSUs and spot detectors were identified (Fig. 11). Superficial population of DS neurons was also identified in zebrafish by means of calcium imaging techniques (Hunter et al. 2013). It was recently shown that goldfish DS neurons of tectal origin contrary to retinal DS GCs select four preferred directions (Damjanović 2015). Three types of DS TO neurons that prefer practically the same directions as those already selected on the retinal level could be considered putative targets of retinal DS GCs. Tectal DS units, preferring the fourth, rostro-caudal direction of stimulus movement nonexisting in the retina, could be entirely formed on the tectal level.

Conclusions

1. Receptive fields of retinal units of different types which have been recorded in one track of microelectrode penetration partly or completely overlap. This fact signifies that they receive inputs and process information in parallel from the same photoreceptor area.
2. Functional segregation of the axon terminals of different types of GCs, as well as the correspondence between retinotopic maps, has been shown in the TO of adult fish electrophysiologically. The possibility to perform simultaneous recordings from certain pairs of units (such as pairs of ON-и OFF-DS GCs that prefer the same direction of stimulus movement, pairs of the OS units that prefer mutually orthogonal stimulus orientations, the black and white spot detectors, the dark- and light-sustained

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units) demonstrates an even more fine functional segregation.

3. The simultaneous recordings of high-amplitude unit responses from pairs of direction-selective ON- and OFF-caudo-rostral, ventro-dorsal, and dorso-ventral units may suggest that they converge onto dendrites of appropriate direction-selective ON–OFF tectal neurons. Thus, ON and OFF channels in fish combine only on the tectal level, whereas in mammals, this happens just in the retina on the dendrites of ON–OFF GCs.

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