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Research report

Putative targets of direction-selective retinal ganglion cells in the tectum opticum of cyprinid fish

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HIGHLIGHTS

- DS GCs use separate ON- and OFF-channels prefer three directions of stimulus movement.
- DS tectal units posses large size of their receptive field (RF) and ON-OFF character of the responses.
- Rostro-caudal preference in the 4 type of DS TNs should vary from that in other types.

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ABSTRACT

Responses of direction selective (DS) units of retinal and tectal origin were recorded extracellularly from the tectum opticum (TO) of immobilized fish. The data were collected from three cyprinid species – goldfish, carp and roach. Responses of the retinal DS ganglion cells (GCs) were recorded from their axon terminals in the superficial layers of TO. According to their preferred directions DS GCs, characterized by small receptive fields ($3-8^{\circ}$), can be divided in three distinct groups, each group containing ON and OFF subtypes approximately in equal quantity. Conversely, direction-selective tectal neurons (DS TNs), recorded at two different tectal levels deeper than the zone of retinal DS afferents, are characterized by large receptive fields (up to 60°) and are indifferent to any sign of contrast i.e. can be considered as ON-OFF type units. Fish DS TNs unlike the retinal DS GCs, select four preferred directions. Three types of tectal DS units prefer practically the same directions as those already selected on the retinal level – caudo-rostral, dorso-ventral and ventro-dorsal. The fact that three preferred directions of DS GCs and DS TNs coincide allows us to assume that three types of DS GCs are input neurons for corresponding types of DS TNs. The fourth group of DS TNs has the emergent rostro-caudal preference not explicitly present in any of the DS GC inputs. These units are recorded in deep TO layers exclusively. Receptive fields of these DS neurons could be entirely formed on the tectal level. Possible interrelations between retinal and tectal DS units are discussed.

1. Introduction

Most fish species inhabit a visually rich environment. Accordingly, they possess highly developed vision, which plays an important role in different forms of visually guided behavior: in object detection and recognition, in orientation and navigation, in foraging and avoiding predators, in social behavior, including the territorial or schooling behaviors, etc. Processing of visual information already starts at the retinal level. Retinal output neurons, ganglion cells (GCs), transmit partially processed visual information to primary visual centers settled in different brain structures. Axons of specialized GCs (detectors) transfer information about the different properties of the retinal image. The tectum opticum (TO) in fish is the principal visual center that plays a crucial role in the information processing and control of visually guided behavior. Detailed information on fish retinotectal system was provided on the base of microelectrode recordings. The properties of the GCs terminating in the fish tectum were first investigated more than 50 years ago in the early works of Jacobson and Gaze (1964) and Cronly-Dillon (1964). Since then different types of retinal detectors projecting to TO (direction selective GCs, orientation selective GCs, spot

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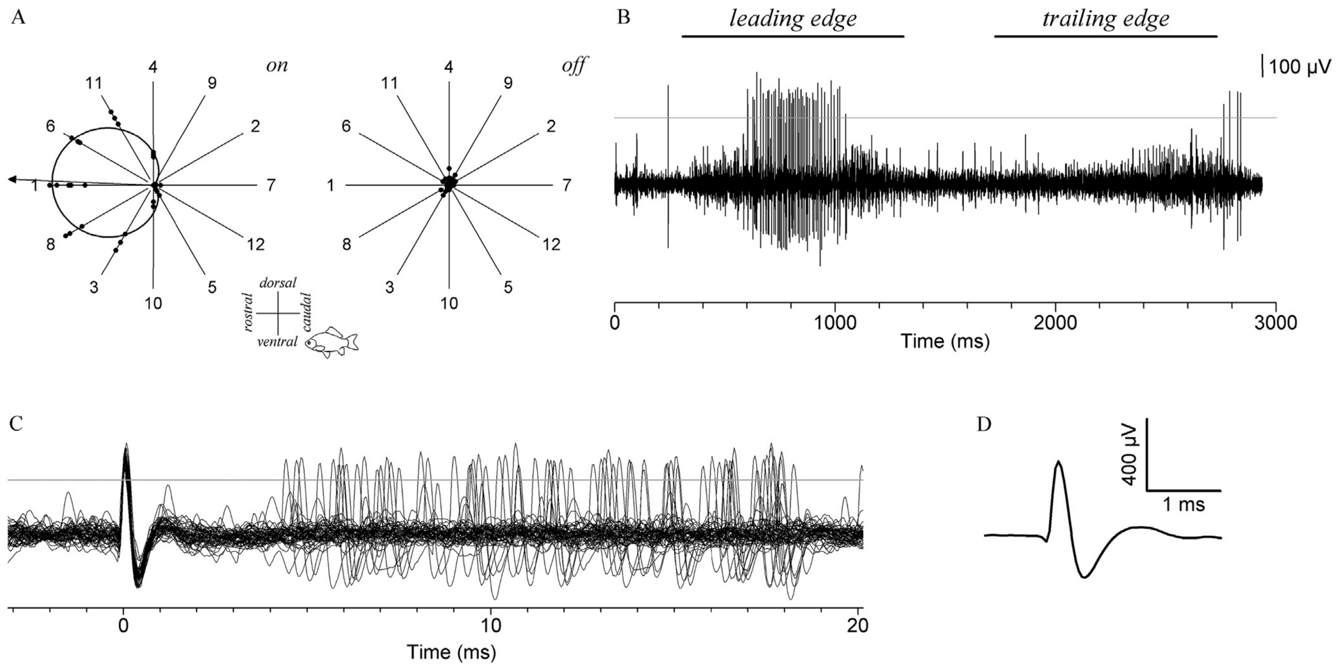

Fig. 1. The response of a roach ON DS unit of retinal origin with caudo-rostral preference. (A) PDs of a roach ON DS GC to the leading and trailing edges of a broad stripe ("edge" stimulus) moving on the gray background in 12 directions in a random order across the stimulation area (a square with a side of approximately 11°) at the speed of 11°/sec; the numbers on the radial lines represent the order of movement directions; dots mark the number of spikes evoked in response to each of three runs for each applied direction (maximal response was 46 spikes); solid curve represents the approximation of the experimental data by a Fourier series with first two harmonics; preferred direction of stimulus' movement for the unit is shown by the black arrow. The diagram marked with the label "on" was built from the responses to the movements of a bright edge, that marked with label "off" was built from the responses to the movements of dark edge into the RF. The coordinate directions in the visual field of the fish are designated in the inset. (B) The unit response to the leading and trailing edges of the white broad stripe moving in the preferred, caudo-rostral direction. Stimulus moved over the gray stimulation area (a square with a side of approximately 11°) at the speed of 11°/sec. In order to select single unit response we used amplitude discrimination. Thin horizontal line represents criterion level (300 μm) used for amplitude discrimination of spikes. Only spikes exceeding the amplitude criterion were used for the PD measurement (A). (C) Superimposed individual spikes from the DS GC train, synchronized by each spike that has passed the threshold of discrimination, shown in the extended sweep. Absence of spikes in the refractory period indicates that the spikes filtered by the amplitude criterion belong to the single unit. (D) Averaged spike form shown in expanded time scale (detailed explanation in the text).

detectors) were described in several fish species (Zenkin and Pigarev, 1969; Liege and Galand, 1971; Wartzok and Marks, 1973; Kawasaki and Aoki, 1983; Nikolaou et al., 2012). Retinal GCs projecting to fish TO were extensively investigated in our laboratory as well. Responses of different types of GCs were recorded extracellularly from their axon terminals in the retinorecipient layer of the TO of intact fish. Thirteen types of GCs projecting to TO were described in several fish species (Maximova et al., 1971, 2012; Maximov et al. 2005a,b; Damjanović et al. 2009a,b). Six of these thirteen types are direction selective (DS) GCs. DS GCs are one of the most extensively studied retinal detectors. They strongly respond to stimulus movement in a particular (preferred) direction and are silent when it moves in the opposite ("null") direction (Barlow and Levick, 1965). Axon terminals of fish DS GCs are located more superficially in the tectal retinorecipient layer compared to axon terminals of other movement detectors (Maximov et al., 2005a; Grama and Engert, 2012; Gebhardt et al., 2013). It was shown that these units 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 the pure OFF units in equal proportions (Maximov et al. 2005a; Damjanović et al. 2009a). Units that respond to the caudo-rostral direction of stimulus movement are the most numerous. Small receptive fields (RFs) of all types of fish DS GCs range from 3° to 8° with averaged size of approximately 4.5° (Damjanović et al., 2009a). In the last decade retinotectal projections were investigated in *Danio rerio* (as the model visual system) during early larval developmental stage by means of Ca++ imaging and Brainbow genetic labeling (Robles et al. 2011, 2013; Nikolaou et al., 2012). Three different DS areas of excitation (GC axon terminals' activity) in the TO of the juvenill zebrafish were shown in response to gratings moving in three directions (caudo-rostral, dorso-ventral and ventro-dorsal) (Nikolaou et al., 2012; Abbas et al., 2017). The DS zone with clearly expressed caudo-rostral preference is the most superficial. Zones with the dorso-ventral and ventro-dorsal preferences to stimulus movement are located deeper and less pronounced. The relative abundance of these axonal terminals is consistent with the abovementioned data obtained in adult fish by means of microelectrode recordings (units with a preference for the caudo–rostral direction are the most numerous).

In addition to the retinal DS GCs, other types of DS units were extracellularly recorded in the fish TO. Some of their features suggest that they are tectal neurons (TNs). Contrary to DS GCs they are characterized by the RFs of a very large size (of up to 60°) and are indifferent to any sign of contrast i.e. can be considered as ON-OFF type units (Maximova et al., 1971; Maximov et al., 2005a,b). Postsynaptic nature of such DS responses was proved in special experiments: after application of cobalt chloride (a calcium channel blocker) to the tectal surface putative DS TNs discharges disappeared, whereas responses of DS afferents remained unchanged (Maximova et al., 2012).

DS TNs were first recorded in deep zones of the mullet tectum (see early work of Maximova et al., 1971). Later on DS TNs were regularly recorded in deep layers of the goldfish tectum (Maximova et al., 2012; Damjanović, 2015). This brief scientific report presents the latest data on the fish retino-tectal DS system collected from three cyprinids (goldfish, carp, roach) by means of microelectrode extracellular recordings. Possible interrelations between retinal and tectal DS units are discussed.

2. Results

As mentioned above, responses of the DS units of retinal origin are regularly recorded in the superficial layers of the fish TO. This pattern has been observed in all three tested species. Physiological criteria to classify a recorded unit as retinal were defined in several studies (Zenkin and Pigarev, 1969; Sutterlin and Prosser, 1970; Wartzok and Marks, 1973; O'Benar, 1976; Maximov et al., 2005b). Typical response from a retinal caudo-rostral DS unit to an "edge" stimulus recorded in a roach is shown in Fig. 1. The unit was stimulated by the white "edge" moving over the neutral gray background in the preferred, caudo-rostral direction. One can see that the leading edge of the moving stimulus evoked considerable excitation of the unit (Fig. 1B). However, the same DS GC did not respond to the trailing edge of the same stimulus, i.e. it remained silent to the RF darkening. In other words, the unit responded exclusively to the introduction of light stimulus into RF, and should be identified as an "ON" caudo-rostral DS unit. Short duration of the spike discharge evoked by the stimulus' leading edge indicates a small RF, which is characteristic for DS units of retinal origin. In the trace shown in Fig. 1B response of ON DS GC with high spike amplitudes can be easily distinguished from spikes of lower amplitudes generated by remote units. Spikes in the DS GC train are approximately equal in amplitude, differing by the noise level only. In order to select a single unit response we used amplitude discrimination. Only spikes exceeding the amplitude criterion were used for the measurement of the polar diagram (PD) (Fig. 1A). Superimposed individual spikes from the DS GC train are demonstrated in Fig. 1C. Absence of spikes in the refractory period, indicate that the spikes filtered by the amplitude criterion belong to a single unit.

Unlike DS GCs, DS units of TO were as a rule recorded from the deep tectal sublaminae located deeper than the retinorecipient layer ("retinal afferent layer")[1]. Typical response of one DS TN recorded in a carp is shown in Fig. 2. One can see that the unit responds to both, leading and trailing edges of the black stimulus moving across an neutral gray background, i.e. it is indifferent to the type of stimulus contrast (ON-OFF type unit) (Fig. 2B). Polar diagram, measured for spikes filtered by the amplitude criterion, revealed ventro-dorsal preference of the unit (Fig. 2A).

Long-duration discharges indicate a large unit RF. One can see that the amplitude of the spikes decreases substantially as spike rate increases, which is characteristic for the recordings from the cell body i. e. from the cell body of the tectal neuron in this case. Superimposed individual spikes from the DS TN train are demonstrated in Fig. 2C. Absence of spikes in the refractory period, indicate that the spikes, filtered by the amplitude criterion, belong to a single unit. Another discriminatory criterion used to distinguish responses of retinal origin from those of tectal units is the form of spikes in the discharge (see Maximova et al., 2012). Unlike spikes arriving from the retina, spikes that are recorded in the vicinity of the DS TN cell body are biphasic and lack the initial negative deflection (Figs. 1D and 2D).

Our more recent experiments revealed that responses of the TNs with DS properties can be recorded not only in deep TO layers but also more superficially, close to the DS GCs projections (hereinafter referred to as "superficial DS TNs"). The response of one goldfish's superficial DS TN that preferred the dorso-ventral direction of stimulus movement is shown in Fig. 3A. The stimulus first moved in the preferred and afterwards in the opposite ("null") direction. One can see TN prominent long-duration responses to leading and trailing edges of the stimulus in the preferred direction. Low-amplitude spike discharges recorded in the "null" (ventro-dorsal) direction belong most likely to retinal projections located in the vicinity of the TN.

Results of our numerous experiments (conducted from 2004 year) revealed that fish DS TNs contrary to retinal DS GCs select four preferred directions. Three types of DS TNs that prefer practically the same directions as those already selected on the retinal level were recorded in both, superficial and deep TO levels. The fourth population of DS TNs is with the emergent rostro-caudal preference not explicitly present in any of the DS GC inputs. These units were recorded in deep sublaminae exclusively. Response of one goldfish rostro-caudal DS TN to stimulus (black "edge") movement in preferred and "null" directions is shown in Fig. 3B. Prominent ON-OFF response of the unit to stimulation in the preferred direction was recorded. One can see that no discharges were evoked when the stimulus moved from the opposite side.

DS TNs were most extensively studied in goldfish and carp. We recorded from 202 DS TNs units till the present time – 47 caudo-rostral TNs, 45 rostro-caudal TNs, 39 dorso-ventral TNs and 71 ventro-dorsal TNs. Unfortunately, approximate tectal depths (superficial or deep) were not determined for all recorded units. A histogram showing the distribution of preferred directions in 117 recorded tectal DS neurons with determined TO depths is shown in Fig. 4A (98 units – goldfish; 19 units – carp). Histogram of preferred directions for 299 goldfish DS GCs is presented in Fig. 4B for comparison. Fig. 4 clearly demonstrates the abovementioned fact, that DS TNs select four preferred directions, three of which coincide with those already selected on the retinal level (caudo-rostral, ventro-dorsal, dorso-ventral). Approximately one half of these three types of DS TNs were identified in the superficial TO levels, slightly deeper than the sublaminae of DS GC projections (Fig. 5A). The fourth population of tectal DS neurons with rostro-caudal preference (29 units recorded) was identified in the deep tectal sublaminae exclusively (Fig. 5B).

2.1. Statistical analysis of tectal depths of DS recordings

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, carp and roach from our latest 38 experiments. Relative tectal depths of single-unit responses recorded from 130 DS units of retinal origin and 35 DS TNs were measured and afterwards subjected to statistical analysis. Single-unit responses of various types of DS units occurred in the following order (Fig. 6). Projections of retinal DS units were located at the depth of about 50 μm. There was a statistically significant difference between them and 16 superficial DS TNs[2] which were recorded at the depth of around 100 μm ($p = 0.00001$). Another group of tectal DS neurons (19 units)[3] was identified much deeper, from sublayers located underneath TO retinorecipient zone at the depth of approximately 300 μm from the tectal surface. There was a statistically significant difference between them and superficial DS TNs ($p = 0.000026$). Accordingly, we may say that clear stratification of DS GC projections and two groups of DS TNs was shown.

3. Discussion

Among different types of DS units described in vertebrate retinas, mammalian DS GCs projecting to midbrain superior colliculus were the most extensively studied (see review of Borst and Euler (2011)). These ON-OFF type units are divided in four physiological subtypes with

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[1] In the deepest sublaminae of the tectal retinorecipient layer, the discharges generated by the afferents of retinal sustained GCs are regularly recorded. These cells, referred to as "light-sustained" and "dark-sustained" units respond by sustained discharges to the diffuse ON and OFF flashes, respectively. DS TNs were as a rule recorded deeper than the afferents of sustained units i.e. deeper than the retinorecipient layer (see review of Damjanović, 2015).

[2] Among 16 superficial DS TNs three types of units were recorded: 8 caudo-rostral, 5 dorso-ventral and 4 ventro-dorsal.

[3] Among 19 deep tectal DS units four types were recorded: 2 caudo-rostral, 4 dorso-ventral, 5 ventro-dorsal and 8 rostro-caudal.

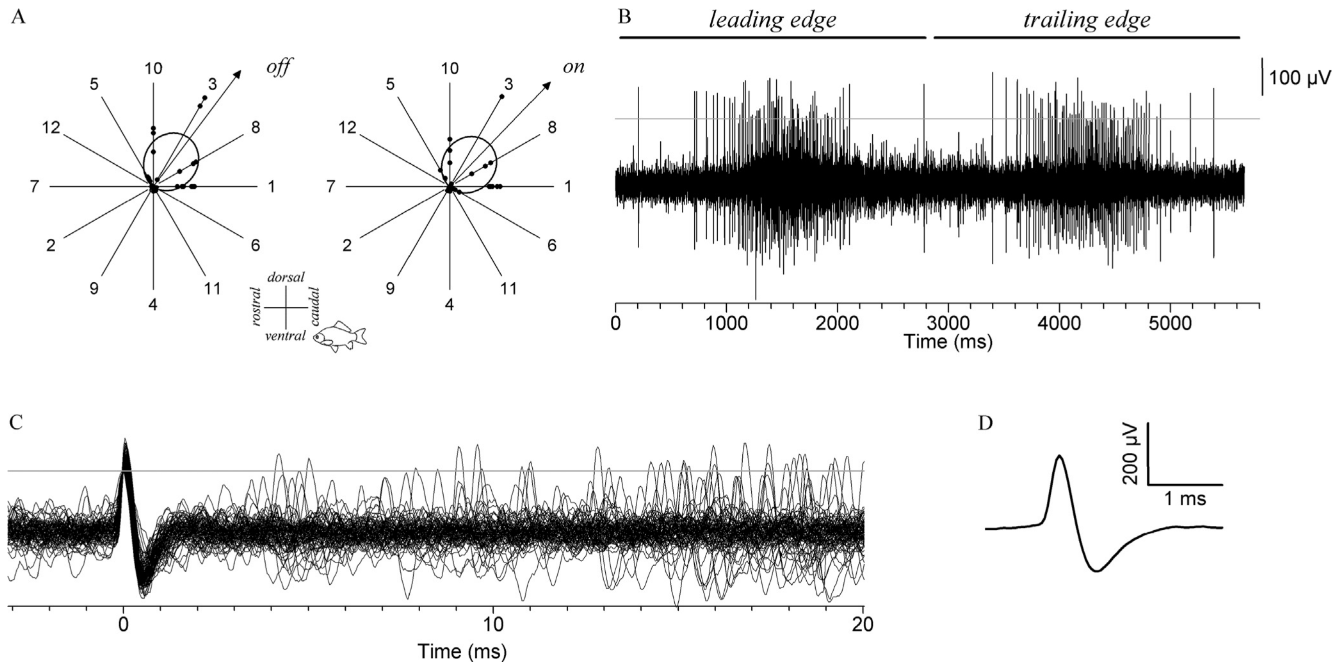

Fig. 2. The response of a carp ON-OFF DS unit of tectal origin with ventro-dorsal preference. (A) PDs of a carp ON-OFF DS TN to the leading and trailing edges of moving "edge" stimulus. Maximal response was 65 spikes (movement direction #3). Other conventions are the same as in Fig. 1. (B) Response of the unit to the leading and the trailing edges of the black broad stripe moving in the preferred, ventro-dorsal direction. Stimulus moved across the gray stimulation area (a square with a side of approximately 22°) at the speed of 11°/sec. Thin horizontal line represents criterion level (200 µm) used for amplitude discrimination of spikes. (C) Superimposed individual spikes from the DS TN train, synchronized by each spike that has passed the threshold of discrimination, shown in the extended sweep. Absence of spikes in the refractory period indicates that the spikes filtered by the amplitude criterion belong to the single unit. (D) Averaged spike form shown in expanded time scale (detailed explanation in the text).

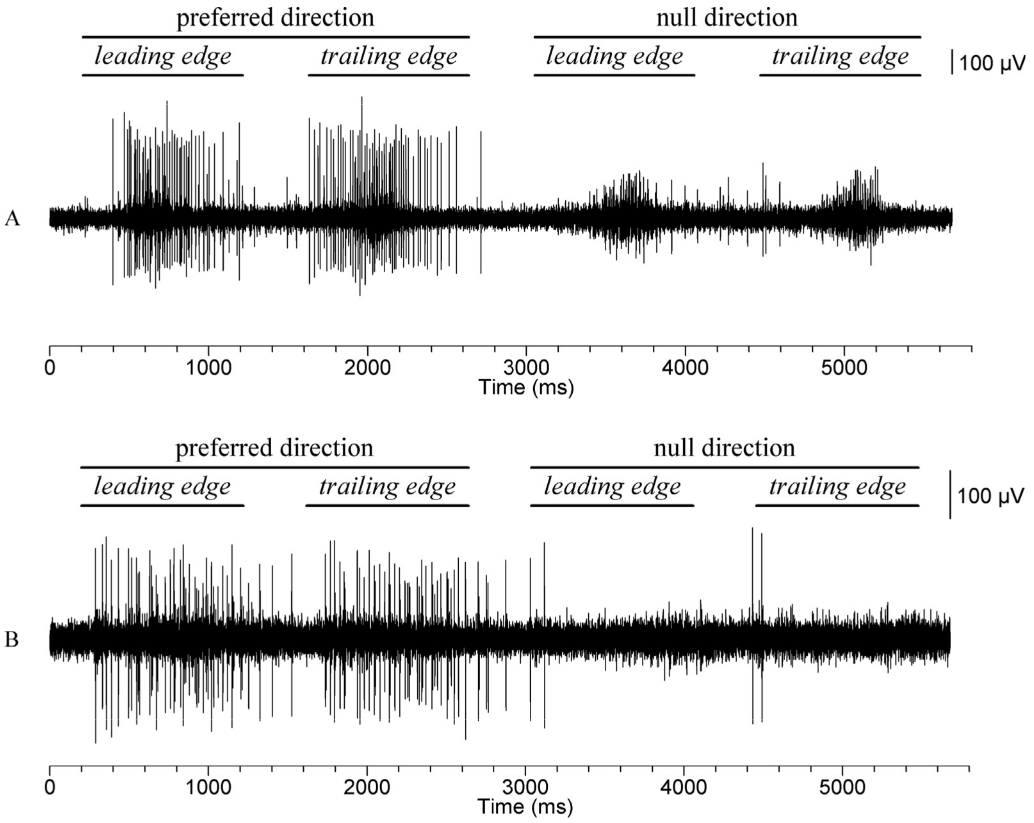

Fig. 3. Responses of two goldfish DS TNs recorded at different tectal levels. The spike discharges of two goldfish DS TNs in response to a black "edge" moving on the gray background in the preferred and afterwards in the opposite ("null") direction inside the gray stimulation area (a square with a side of approximately 11°). Periods of stimulus movements in the preferred and the "null" direction are marked on the top. (A) a superficial DS TN with dorso-ventral preference. The neuron responds to the leading as well as the trailing edge when the stimulus moves in the preferred direction; low-amplitude spike discharges recorded in the "null" (ventro-dorsal) direction belong most likely to retinal projections located in the vicinity with TN. (B) Prominent ON-OFF response of a deep DS TN with rostro-caudal preference to stimulation in the preferred direction. One can see that the responses of retinal origin are absent.

different preferred directions aligned with the horizontal and vertical ocular axes. In comparison with them fish retinal DS units prefer three direction of stimulus movement and use separate ON- and OFF-channels. This fact, clearly demonstrated in our numerous electrophysiological studies in goldfish (Maximov et al., 2005a,b; Maximov et al., 2013; Damjanović et al., 2009a) now was confirmed in carp and

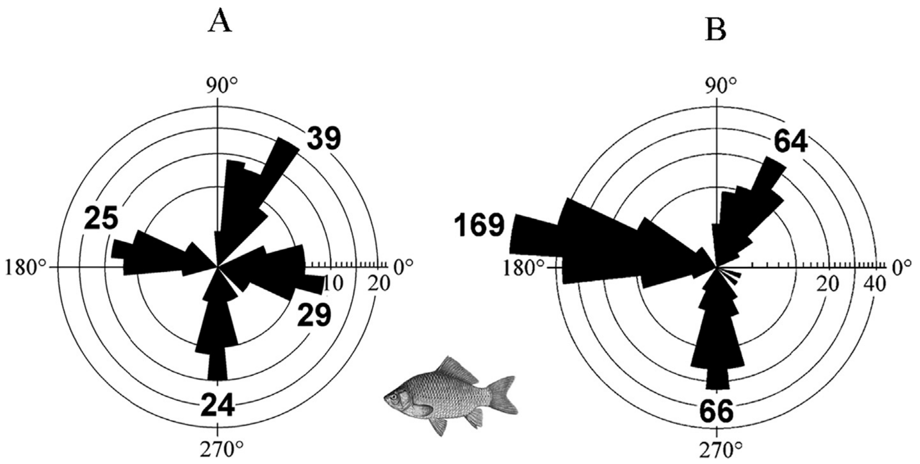

Fig. 4. Histograms of preferred directions for tectal DS neurons and retinal DS GCs plotted in polar coordinates. (A) Distribution of preferred directions calculated in 117 tectal DS neurons (98 goldfish and 19 carp units). (B) Distribution of preferred directions calculated in 299 goldfish DS GCs. One can see that the fourth, rostro-caudal preferred direction (lacking in the retina) emerged among tectal DS units. Detailed explanation in the text.

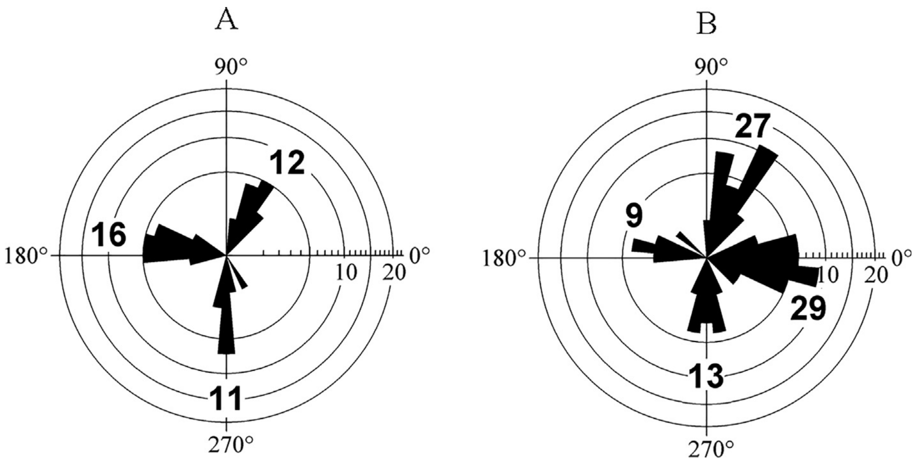

Fig. 5. Polar histograms of preferred directions for two groups of fish DS TNs recorded at diffetent tectal levels. DS TNs presented in the Fig. 4 are separated into two groups – superficial and deep DS units. (A) Distribution of preferred directions calculated in 39 superficial DS TNs. (B) Distribution of preferred directions calculated in 78 deep DS TNs. One can see that the fourth, rostro-caudal preferred direction was recorded among deep DS units exclusively. Detailed explanation in the text.

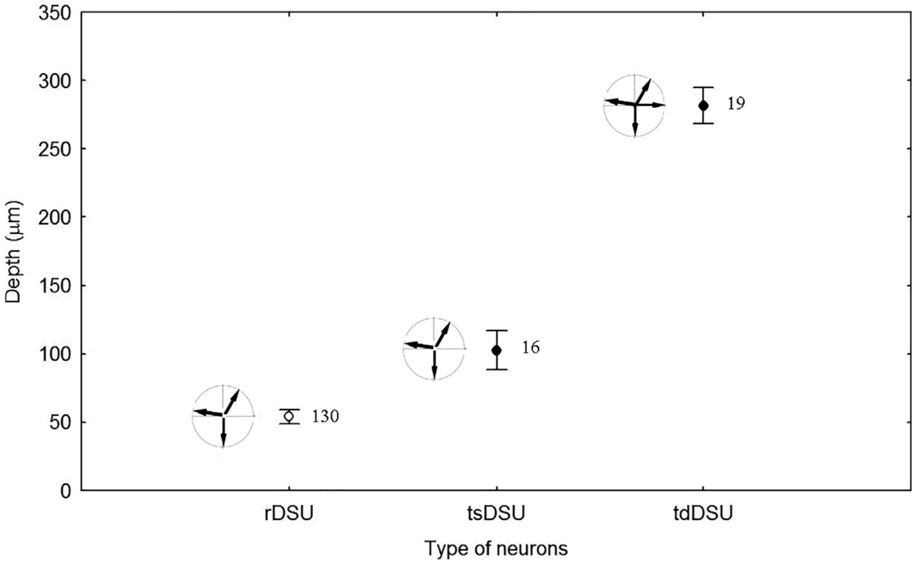

Fig. 6. Relative depth distribution of three groups of DS units recorded in fish TO. Clear segregation of DS GC projections ("rDSU") and two groups of DS TNs (superficial - "tsDSU" and deep - "tsDSU") was statistically proved. Averaged depths in "µm" for all of the recorded units are shown. "Open circles" - retinal DS GC projections to TO; "closed circles" - tectal DS neurons. Numbers of the analyzed units are given near the corresponding plotted data. Vertical bars denote 0.95 confidence intervals. Direction preferences related to different subtypes of retinal and tectal DS units are shown in polar coordinates on the left of the corresponding plotted data. DS GCs proved to comprise three distinct types which prefer caudo-rostral, dorso-ventral and ventro-dorsal direction of stimulus movement, respectively. The fourth rostro-caudal preference emerges in deep group of tectal DS neurons exclusively. The data were collected from 38 experiments conducted on: 32 goldfish, 4 carps and 2 roaches. Abscissa - various groups of DS units; Ordinate - recording depths in "µm".

roach.

Nevertheless, the data presented in our recent studies indicated that the fourth, rostro-caudal preferred direction do exist in the fish visual system, though not at the level of the retinal afferents, but among the DS neurons of the TO (Damjanović, 2015). In the present study we investigated relative locations of DS TNs of different types more thoroughly. The data obtained in goldfish and carp unequivocally demonstrated that the responses of ON-OFF type tectal DS neurons can be recorded at two different levels (Fig. 5). The responses of first cluster of DS TNs are recorded more superficially, approximately 50 µm deeper the sublayer of DS GCs projections. The coincidence of three preferred directions of ON- and OFF DS GCs and superficial ON-OFF DS TNs allows us to suppose that GCs with caudo-rostral, ventro-dorsal and dorso-ventral preferences are input neurons for these three types of DS TNs. Statistically significant difference between location depths of DS GCs and DS TNs responses does not contradict this conclusion. Most likely we record from the cell bodies of superficial DS TNs which are located in the retinorecipient layer slightly deeper than the retinal DS afferents.

The second DS TN cluster is recorded much deeper than the TO sublaminae, at the tectal depth of approximately 300 µm, i.e. 100 µm deeper than the retinorecepient layer located from 50 to 200 µm from the TO surface (Zaichikova et al., 2017). All four DS TNs types are regularly recorded in deep TO layers. Physiological properties of their responses allow us to assume that we record from their cell bodies that are located at deep tectal levels (Maximova et al., 2012). We may assume that the dendrites of at least three types of these DS neurons, which prefer similar directions as those already selected on the retinal level, arborize more superficially in the retinorecipient zone, where they receive direct inputs from corresponding retinal DS units. Tectal DS neurons with similar morphology were recently labeled in zebrafish larvae (Gabriel et al., 2012).

The rostro-caudal preference in the fourth type of DS TNs is an emergent property of the tectum. It remains to be clarified, if the direction selectivity in this population of tectal DS neurons is computed from non-DS retinal inputs using tectal asymmetric inhibition as suggested by Grama and Engert (2012). The authors allow for the existence of a special type of inhibitory tectal interneuron, which, similar to the retinal “starburst” amacrine cells responds to moving stimuli in the null-direction and is asymmetrically connected to the DS tectal output neurons.

DS neurons were also identified in the zebrafish tectum in a number of studies (Grama and Engert, 2012; Gabriel et al., 2012; Kassing et al., 2013). As a rule, cell bodies of DS neurons recorded with the calcium imaging techniques were located in the deep periventricular layer of tectum. However, superficial DS TNs were later on identified in the zebrafish larvae along with deep DS TNs (Hunter et al., 2013; Abbas et al., 2017). It was shown that zebrafish DS neurons comprise four physiological subtypes with different preferred directions similar to those recorded in adult goldfish and carp DS TNs. The DS TNs with rostro-caudal preference not explicitly present in any of the retinal DS inputs were identified in zebrafish as well. The major part of DS TNs were recorded in the deep, periventricular zone of TO, while less frequently they were identified in the tectal superficial layers. All four types of DS neurons were identified in deep TO layers exclusively, as well as in goldfish and carp.

However, the superficial DS TNs recorded in zebrafish differed from those recorded in our studies in some aspects. First of all, they were recorded in the zone located above the sublaminae of DS GC projections, while in goldfish and carp their responses were recorded slightly deeper than the DS retinal afferents. Secondly, zebrafish superficial DS TNs comprised two subtypes preferring dorso-ventral and ventro-dorsal direction of stimulus movement, respectively, while superficial DS TNs recorded in our study preferred three different directions similar to those already selected on the retinal level. Irrespective of mentioned differences, one can say that the results of Hunter et al. (2013) were consistent with our findings. Taking into account similar retinal DS circuits revealed at the adult and larval stages of different cyprinids (Maximov et al., 2005a,b; Nikolaou et al., 2012), one can say that retino-tectal DS systems of the adult and larval fish do not essentially differ from each other.

4. Conclusions

- Fish retinal DS GCs use separate ON- and OFF-channels and prefer three directions of stimulus movement (caudo-rostral, dorso-ventral and ventro-dorsal, respectively).
- DS tectal units are characterised by the large size of their RF and ON-OFF character of the responses. These features may indicate to the convergence of DS GCs of both types (ON and OFF) to DS tectal neurons with the same direction preferences.
- The rostro-caudal preference in the fourth type of DS TNs is an emergent property of the tectum. It remains to be clarified, if the direction selectivity in this population of tectal DS neurons is computed from non-DS retinal inputs using tectal asymmetric inhibition as suggested by Grama and Engert (2012).
- Results of the present study together with the data collected on the visual system of larval zebrafish (Nikolaou et al., 2012; Hunter et al., 2013) indicate that the retino-tectal DS systems of the adult and larval fish coincide with each other.

5. Experimental procedure

The data were collected from three cyprinid species: *Carassius gibelio*, a wild form of the goldfish (411 fish), carp (*Cyprinus carpio*; 12 fish) and roach (*Rutilus rutilus*; 4 fish). Body weight of experimental fish varied from 40 g to 100 g. They 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 intact animals with normal blood circulation and intact optics and lasted several hours which guarantees normal, stable cell responses and allows a significantly expanded range of applied visual stimuli that is close to natural. Fish of 10–15 cm standard body length was immobilized with tubocurarine (0.3 mg per 100 g of body weight i.m.), then placed in a natural position in a transparent Plexiglas tank where artificial respiration was provided continuously by forcing aerated water through the fish gills with a thermostatic pump. The level of the water in the 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.

Visual stimuli were presented to the fish right eye on the computer-controlled 17″ CRT monitor LG Flatron 775FT from a distance of about 30 cm. From this distance, the screen occupied 43 × 32° of the fish visual field. To stimulate retinal DS units, moving contrast edges were presented on the screen within a gray square area of stimulation with angular dimensions of 11 × 11° (DS GCs are characterized by small RFs of approximately 4.5° in angular values). 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 cases when tectal DS neurons were recorded the square area of stimulation was often increased up to 22° in angular values (DS TNs are characterized by large RFs, sometimes exceeding the whole monitor screen).

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

Platinized microelectrodes efficiently record the extracellular activity from ramified DS GCs axon terminals and from the cell bodies of DS TNs 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 was 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. Spike discharges of retinal and tectal origin differ in a number of characteristics such as receptive field size, spike form (Maturana et al., 1960; Wartzok and Marks, 1973; Maximova et al., 2012). In particular, the spikes of TO neurons recorded in the vicinity of their cell bodies substantially decrease in amplitude as the spike rate in discharge increases (Maximov et al., 2005b).

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, carp and roach from our latest 38 experiments (see Fig. 6). The location depth of the electrode tip relative to the TO surface was acquired from the micromanipulator monitor during the recordings. 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, from which the relative location depths for various units of both the retinal and the tectal origin were measured. 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 DS GCs ramifying in superficial sublaminae of the tectal retinorecipient zone and tectal DS neurons located at two different TO levels) 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 Turkey's post-hoc test. The level of significance for all comparisons was set at $p < 0.05$.

Spike trains were monitored on the oscilloscope and simultaneously listened on the loudspeaker. 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).

The polar diagram (PD) for each of 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 wide stripes that exceed the size of the stimulation area (hereinafter referred to as "edge" stimuli) moved across the neutral gray stimulation area in 12 different directions. Three sequential trials were performed for each of the directions and the mean numbers of spikes evoked by leading and trailing edges of the stimuli were 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.

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