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Neurons in the Optic Tectum of Fish: Electrical Activity and Selection of Appropriate Stimulation

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In all animals the optic tectum (OT) (or superior colliculi in mammals) provides guidance for external attention; this is not the only function of the tectum but is critically important for the development of behavioral visual reactions. In fish, the OT is the main primary visual center. It receives signals from most ganglion cells (GC) of different (known) types in the retina. Knowledge of the properties – both structural and physiological – of the neurons in the OT is important for understanding the mechanisms organizing behavior. We recorded extracellular electrical activity in the OT in living adult fish (*Carassius auratus gibelio*). Simultaneous recordings were made of the responses of retinal GC (from their axon terminals) and tectal neurons (TN), probably from cell bodies. Four types of TN are described with directional selectivity (DS) (henceforth these neurons are termed DS TN) at different (defined) depths of the OT. In addition to these, rare sporadic spikes lacking DS and arising on stimulation at any locus in a large area were consistently recorded simultaneously (superficially) with the responses of caudorostral DS GC with the electrode in one position. These are presumptively the responses of superficial tectal neurons (superficial inhibitory neurons, SIN). Various different types of stimulation were applied with the aim of obtaining clear SIN responses. Comparison of the results of our electrophysiological studies with published data (most studies in this direction have used calcium imaging in transparent *Danio rerio* fry) showed that DS TN were identical to glutamatergic periventricular interneurons in the OT, while SIN were identical to GABAergic inhibitory interneurons (SIN). These latter presumptively mediate detection of the main object (pop-out) in the field of vision.

Keywords: optic tectum, fish, vision, retina, directional selectivity, tectal neurons.

Introduction. The output neurons of the retina are ganglion cells (GC), which send information on the picture of the world from the retina to neurons in the optic tectum (OT), which is the primary visual center in fish. One of the functions of the tectum in all animals is to guide external attention, which is needed for triggering behavioral programs in the ongoing situation [Northmore, 2011]. Successful selection of a behavioral reaction requires a "feature detection" mechanism.

The tectum has a layered structure and its retinoreceptive layer contains the axon terminals of GC and the bodies and terminals of tectal neurons (TN). Complete covering of the receptive field of the retina by the dendritic branches of GC of each of the 13 types ("tiling") and the retinotopic principle of information transmission from GC to OT neurons allows the significant features of images to be extracted and the ordered transmission of visual information to be preserved [Maximov et al., 2005a, 2009; Maximov et al., 2005b; Maximova et al., 2012; Damjanović et al., 2009]. Understanding the operation of the visual system requires investigation of interactions between GC and TN, i.e., how further transformation of signal from GC to OT neurons occurs.

At this time, the transmission of visual information in the retina has received more study than its subsequent processing in the primary visual centers. Tectal neurons have received less study: most information on TN is morpholog-

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ical and was obtained in *Danio rerio* fry, members of the same family as Prussian carp [Nevin, 2010; Walker et al., 2013; Barker and Baier, 2015]. However, there are also electrophysiological data obtained by a variety of methods. Patch-clamp studies using *Danio rerio* fry addressed TN when an object resembling prey entered the fry's field of vision [Preuss et al., 2014]. Behavioral experiments combined with extracellular recording from tectal neurons in archerfish addressed the mechanisms of extraction of the main stimulus (pop-out) from the overall visual picture [Ben-Tov et al., 2015; Kardamakis et al., 2015]. Several types of tectal neuron have been described containing different transmitters, and different suggestions have been made for the functions of these TN [Gabriel et al., 2012; Barker and Baier, 2013; Preuss et al., 2014].

Direction-selective GC (DS GC) in the retina respond to stimulus movement in a particular direction. Electrophysiological studies have also provided descriptions of direction-selective TN (DS TN), and the properties of DS TN are very similar to those of GC (similar contrast sensitivity and resolving ability) [Damjanović et al., 2009]. These, like DS GC, respond to movement of different stimuli: wide moving boundaries, bars, and spots, the main properties of such elements being movement of any stimulus in the preferred direction of the neuron under study. Experimental studies extracted four groups of DS TN with different preferred directions. Preferred stimulus movement directions of three groups of TN (caudorostral, dorsoventral, and ventrodorsal) coincided accurately with the directions known for DS GC. Tectal neurons of the fourth group had a rostrocaudal preferred direction, which was absent from DS GC (Fig. 1). This illustration shows data for DS GC in blue, $n = 299$ (all Prussian carp), data for DS TN are in gray, $n = 117$ (98 Prussian carp and 19 common carp). Of the 117 tectal neurons, 39 were group 1 DS TN, which in the tectum are located at a depth of about 100 μm, while 78 were group 2 DS TN, at a depth of about 300 μm (exact depths are given in Table 1).[1] In contrast to DS GC, DS TN are on/off-type elements, i.e., indifferent to the sign of contrast, while DS GC in fish are either on- or off-type.

It is now known how the depths of the axon terminals of GC and TN in the tectum correlate [Aliper et al., 2019; Damjanović et al., 2019]. Thus, the reactions of DS GC and DS TN are recorded at different depths. DS GC are located in the superficial sublayers of the retinorecipient layer of the OT. In turn, the reactions of DS TN can be recorded at several levels: in the sublayer of the retinorecipient layer which also contains line orientation detectors and spot detectors, and beneath the sublayer of GC axon terminals with dark and light background activity (mean depth = 195 μm). Data on the exact depths are shown in Table 1, as determined from results reported by Damjanović et al. [2019].

A majority of the inputs from the retina run in the SO and SFGS layers (SO – stratum opticum, SFGS – stratum fibrosum et griseum superficiale) [Robles et al., 2013]. All visual information is then further transmitted to the deeper layers of the OT, from which it is sent to the motor centers of the mid- and hindbrain. The deep layers of the OT, mainly the periventricular layer (SOV – stratum periventriculare), contain the bodies of so-called periventricular tectal neurons (PVN), some of whose dendrites enter the SFGS [Northmore, 2011]. Most PVN are glutamatergic [Kinoshita et al., 2006]. Two classes of PVN have been described: periventricular projection neurons (PVPN) and periventricular interneurons (PVIN). The dendritic branches of interneurons do not extend beyond the tectum. Some interneurons send their processes to the superficial retinorecipient layers. In turn, projection PVN form synapses with interneurons in the deep layers of the OT, while their efferent axons are sent to the premotor and motor areas of the brain [Nevin et al., 2010].

Apart from periventricular TN, studies using calcium imaging methods have described superficial GABAergic interneurons (SIN – superficial inhibitory neurons) in *Danio* fry. The bodies of SIN were indicated by morphological data to be located in the SO layer, with their processes densely branching in a single sublayer immediately beneath the cell body at the very surface of the retinorecipient layer [Bene et al., 2010]. These neurons receive inputs from both the axon terminals of GC and tectal PVN [Barker and Baier, 2013]. It has been suggested that SIN are involved in tuning perception to objects of a particular size, via inhibitory effects.

Studies using the extracellular microelectrode recording method used here provide for recording spike reactions of both GC and TN. It is important to note that this includes the possibility for simultaneous recording of the reactions of GC and tectal neurons with a single microelectrode position. This allowed us to study how visual information is transmitted and processed at different levels between different elements of the visual system. This study used adult Prussian carp and common carp. However, there are good grounds for comparing our electrophysiological data with published results obtained from *Danio* fry, as fry are essentially fully formed animals. Our data on the stratification of reactions from the axon terminals of different types of GC coincide with those obtained from *Danio* by calcium imaging [Mikolaou et al., 2012]. The electrophysiological method used in adult Prussian carp and common carp has been employed for many years and is known to be informative. The resulting data may be useful for further studies in this area, including those using other methods.

Knowing the inputs (responses of GC), it is natural for the next stage of studies to consider how these signals are transformed (used) by OT neurons. Correct description of the properties of different types of TN initially requires selection of appropriate stimulation. Without this, it is im-

[1] The present report revises the names of DS TN groups: Damjanović et al. [2009] divided DS TN into superficial and deep. Superficial DS TN are now termed group 1 DS TN, and deep DS TN are termed group 2 DS TN.

TABLE 1. Mean Depths of Responses from TN at Different Types of DS GC

Type of element	Mean depth ± standard error of the mean, μm	Median and interquartile range, μm
SIN, $n = 28$	41 ± 24	20–55 (median = 39)
DS GC, $n = 130$	54 ± 18	40–64 (median = 53)
DS TN group 1, $n = 18$	113 ± 56	75–147 (median = 106.5)
DS TN group 1, $n = 19$	282 ± 59	239–335 (median = 266)

n is the number of individual recording. Medians and interquartile ranges are also given for each set, from [Damjanović et al., 2019] (the Table also shows new data for some TN).

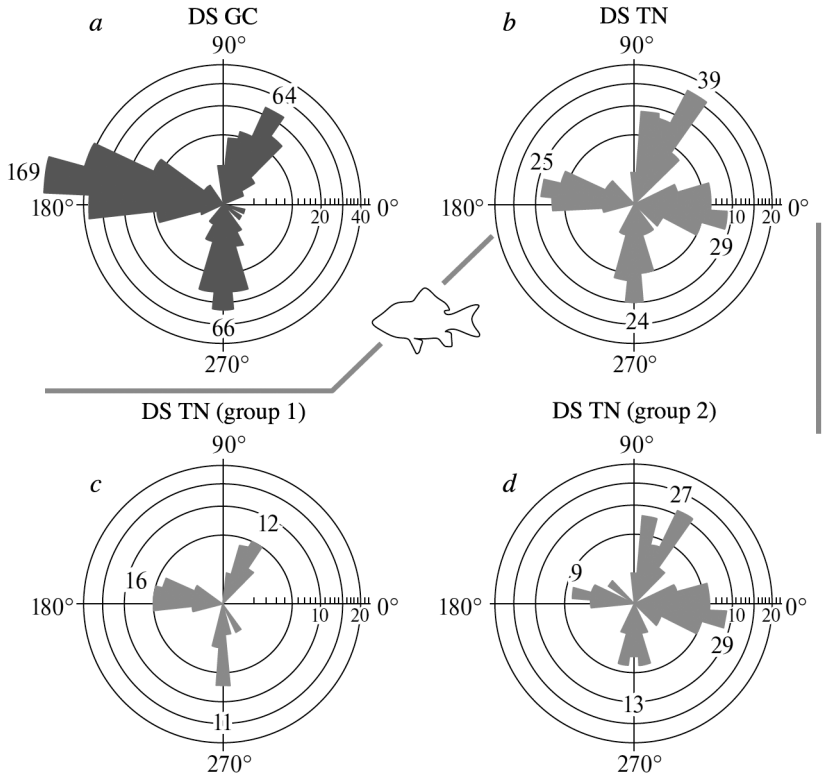

Fig. 1. Distribution histograms of types of neurons with different preferred directions in polar coordinates (modified from [Damjanović et al., 2019]). *a*) DS GC; *b*) DS TN; *c*) DS TN group 1; *d*) DS TN group 2.

possible to identify most of the parameters (for example, receptive field size and position) required for comparing the properties of TN and GC. Previously, measurements of recording depth were made in order to compare the depths of GC axon terminals and TN themselves [Aliper et al., 2019].

As SIN in the literature are attributed with the stimulus pop-out function on the basis of their morphological and neurotransmitter properties, and we believe that this hypothesis is logical, we sought to study the properties of the electrical responses of these neurons.

Methods. *Experimental objects and preparation*. Studies were carried out on members of two fish species of the *Cyprinidae* family: silver Prussian carp (*Carassius gibelio*) and common carp (*Cyprinus carpio*) from fish farms near Moscow. Body size was 10–15 cm and weight was 40–100 g. Experiments used 36 individuals (and 533 recordings of GC and TN responses from the database). Before the experiments started, animals were kept in aerated laboratory aquaria at room temperature with natural illumination for several months.

During experiments, animals with normal circulation and unimpaired visual systems were immobilized by i.m. injections of d-tubocurarine (0.3 mg/100 g body weight). Animals were then attached in a natural position (in which Prussian carp and common carp move in water) in a transparent Plexiglass aquarium with a forced flow of aerated water across the gills. Circulation was with a pump and thermostat. Through the transparent aquarium wall, the animal's right eye watched a monitor screen on which computer-generated stimuli were presented. The OT was accessed by removal of

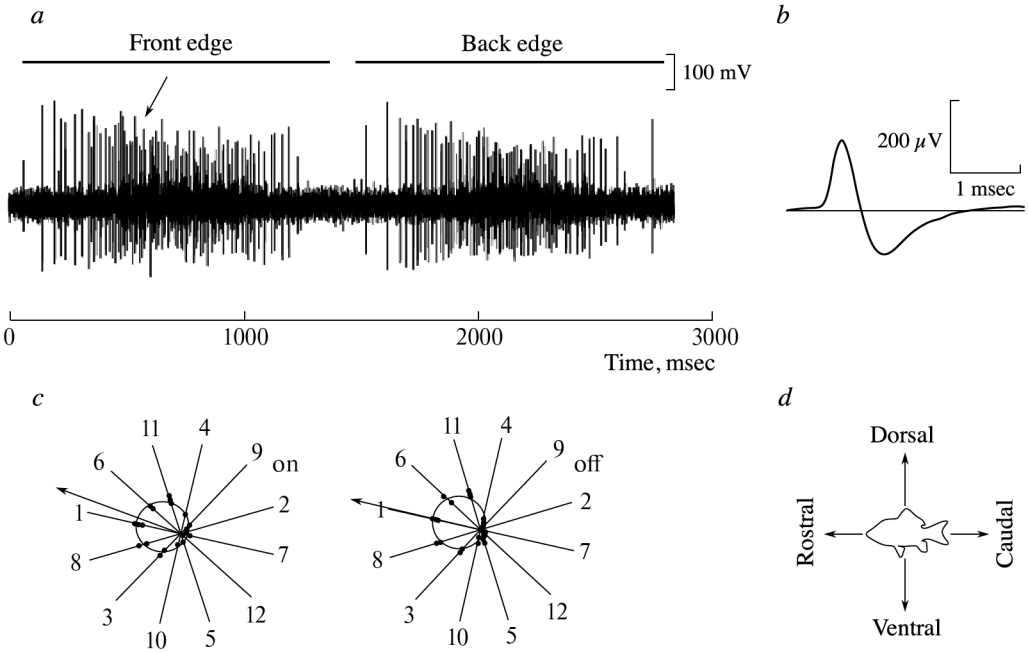

Fig. 2. Characteristics of TN spike activity using a caudorostral DS TN as example. *a*) Spike activity of TN in response to stimulation; *b*) shape of averaged TN spike on an expanded time scale; *c*) polar diagrams; *d*) position of fish.

the parietal-occipital bone from the left side of the skull of fish anesthetized with ice on the side contralateral to the right working eye. Fatty tissue was then removed, along with the dura mater and pia mater. The water level in the experimental aquarium was maintained such that water did not enter the brain but the fish's eye was beneath the surface.

Experimental apparatus and visual stimulation. The experimental apparatus consisted of three connected synchronized computer modules: a stimulation module, a recording module, and a control module. The stimulating module drove a 17″ LG Flatron 775FT RT monitor for presentation of visual stimuli. The monitor was mounted on a mobile table to allow it to be moved. The distance from the monitor to the fish's eye was 30–40 cm. Experiments were run mainly in the animals' lateral field of vision at a quite wide angle: above 60° in the vertical and 40° in the horizontal. The stimulation area on the monitor screen was restricted to a square of side 11 angular degrees; the size of the area and its position on the screen could be adjusted. The stimulation area displayed program-generated stimuli (moving boundaries, bars, flashing spots, and others), and the rest of the monitor remained unaltered, with constant brightness. This study used only "achromatic" colors (black, white, and many gray shades). The command module was designed for online graphical presentation of results with rapid processing and operative control of stimulation and recording parameters. The recording module was connected via an ADC (analog-to-digital converter, sampling frequency 25 kHz) and an amplifier to the microelectrode. This module functioned to record neuron responses and identify patterns of spike activity in the screen; reactions were listened to using a loudspeaker and experimental results were recorded in memory. An automatic protocol operated during the experiment, and offline data processing was run using a previously developed scheme. The method is described here only briefly; a detailed description can be found in [Maximov et al., 2005a].

Recording of cell responses. Individual responses were recorded from the axon terminals of retinal GC and TN in the OT of live fish using an extracellular glass-embedded metal microelectrode (platinum cap diameter 2–3 μm, resistance no more than 300 kΩ) [Gesteland et al., 1959]. Electrodes were inserted under visual control using a micromanipulator (MP-225, Sutter Instrument), and reactions were watched and heard using an oscillograph (C1-73) and loudspeaker respectively. Input noise was significantly reduced by contact of the electrode with liquid over the tectum surface. The electrode was then accurately inserted along the sound gradient until a stable individual recording was obtained. Trace depth was evaluated using indications on the micromanipulator screen. The individuality of the recording was assessed in terms of the spike height and amplitude stability and a stable sound timbre. The magnitude of spikes from an individual element was of the order of 200–500 μV (for GC responses) and was several times greater than the noise amplitude.

Results. *General data on tectal neurons*. During the experiments, TN responses were encountered more rarely in the retinoreceptive layer of the OT than responses from GC axon terminals. Our extensive database contains thousands of recordings from GC and hundreds from TN. Individual recordings of the responses of GC and TN axon terminals differ in terms of a number of the properties of their spike

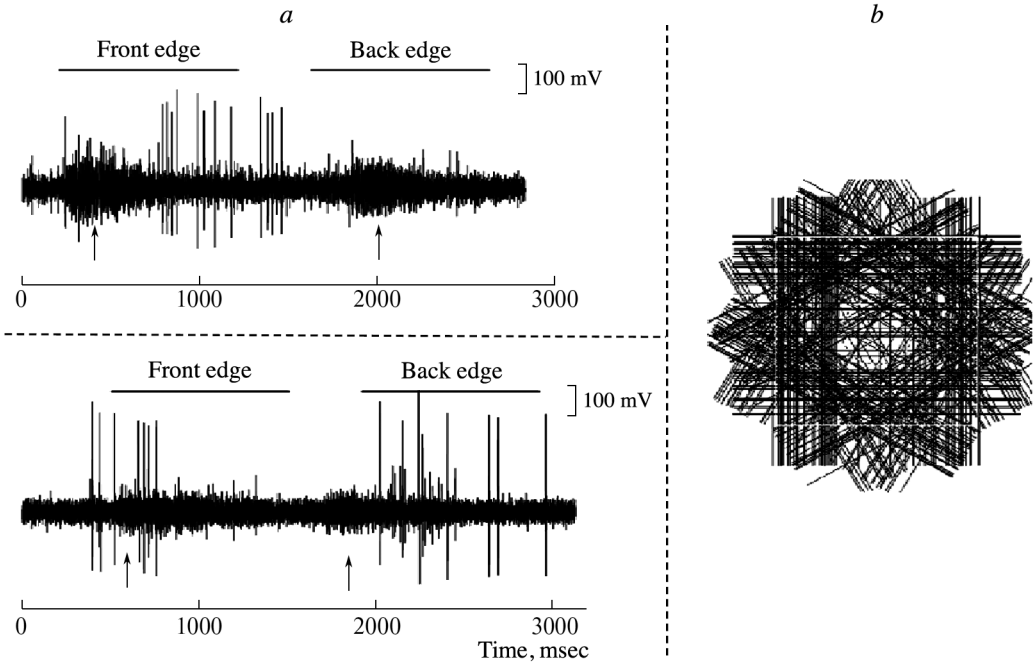

Fig. 3. Comparison of spike activity of simultaneously recorded GC and SIN axon terminals. *a*) Spike activity of SIN and DS GC in response to stimulation; *b*) trace of spike activity from SIN when the center of its RF coincided with the center of the RF of the DS GC (each black line is a spike in response to stimulus movement in the corresponding direction).

activity: pulse amplitude and shape, as well as receptive field size. A typical TN response recorded in the OT of a live fish is shown in Fig. 2, *a* as an example of a directionally selective TN with a preferred stimulus movement direction from the tail to the head (the caudorostral direction). The stimulus was a wide black bar, greater than the size of the stimulation area, moving on a light background in the stimulation area with a dark periphery. Responses were seen at both the input and the output of the contrast boundary (i.e., at the change in stimulus contrast, an increase or decrease relative to background), identifying this as an element of the on/off type. We also note that the spike activity of TN had a characteristic feature – spike amplitude decreased significantly as discharge frequency increased (Fig. 2, *a*; example shown by arrow).

Figure 2, *b* shows TN spike shape, which was biphasic. This spike shape was typical for recordings from cell bodies. GC spikes, in turn, were triphasic with an initial negative deviation [Maximova et al., 2012].

The preferred movement direction for this neuron could be assessed from polar plots (Fig. 2, *c*). Polar plots are relationships between the numbers of spikes in responses and stimulus movement direction. The left-hand panel is a plot of the responses to the dark stimulus exiting the RF of the element, i.e., the response to illumination of the RF (on response), and the right-hand panel is the response to entry of the dark stimuli to the RF of the element – the response to darkening of the RF (off response).

The prolonged discharge points to a large receptive field (RF) size of the tectal neuron (Fig. 2, *a*), which is a further difference between DS TN and DS GC: the RF of tectal neurons is vastly bigger than that of GC (the size of the RF of tectal elements is around 4.5°, while that of RF of tectal neurons can be up to 60°). This is of note, as the GC response volley is always located in the window of the stimulation area (a square with sides of 11°), while neuron volleys could be limited to this window as its RF is larger [Maximova et al., 2012].

Superficial tectal neurons. Apart from directionally selective tectal neurons, the OT also produced responses from another type of TN – lacking directional selectivity. During the experiment involving immersion of the electrode into the OT, their responses appeared first and reached maximal amplitude at the same depth as the responses of DS GC with caudorostral directional preference. We suggest that we are recording responses described in the literature as GABAergic SIN, so we will henceforth term these superficial tectal neurons (SIN) (presumptive SIN). In comparison with DS GC, SIN show many fewer spikes in discharges and, in addition, spikes in responses to standard test stimuli are irregular (Fig. 3, *a*). Stimuli were white (*a* – upper panel) and black (lower panel) wide bars of greater size than the stimulation area, moving on the light gray background of the stimulation area and the dark periphery. Arrows show the spike activity of DS GC through whose RF the stimuli moved.

While we were able to study DS TN using established systems of GC stimulation, sometimes the only need being to enlarge the stimulation area, appropriate stimuli had not yet been selected for these TN in electrophysiological experiments. Responses to different stimuli could appear on

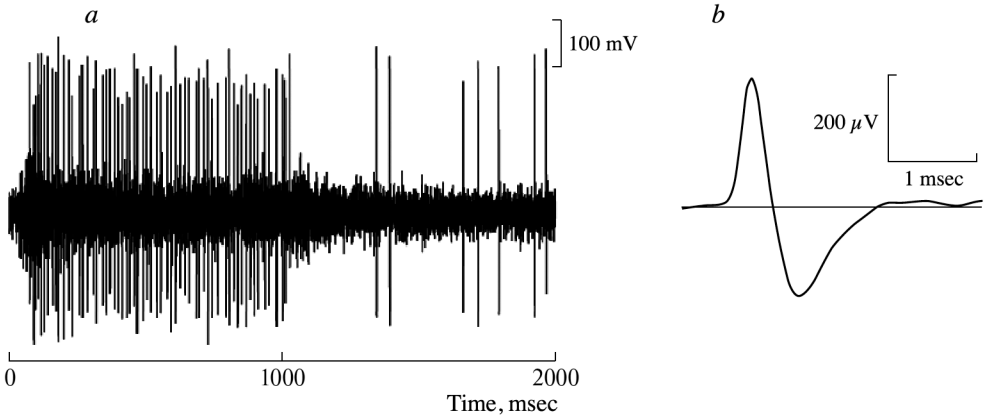

Fig. 4. Spike activity of superficial TN – SIN. *a*) "Regular" spike activity of a SIN on stimulation with a black bar (black cardboard strip) presented manually; *b*) biphasic shape of SIN spike.

visual stimulation over almost the whole area of the monitor screen, so the indications are that the RF of SIN are larger.

The reference point for mapping the RF of SIN can be the location of the RF of DS GC. These neurons (SIN) respond to exit of the stimulus from the receptive field, as shown in Fig. 3, *b*, where there were no spikes in the central part of the stimulation area. This pattern of spike activity was obtained only when the centers of the RF of DS GC and SIN coincided. In this situation, the stimulus was a light moving boundary on the dark background of the stimulation area.

The process of selecting appropriate stimulation in the absence of any other data on the neuron (GC, TN) in the general case starts with manual stimulation. Stimulation with a black bar (a black cardboard strip) was selected as the initial stimulation. The bar was placed in the periphery of the presumptive center of the neuron's field, and movement of the stimulus from the edges of the monitor screen produced regular powerful discharges of tectal neurons. Figure 4, *a* shows a regular discharge from a SIN, which was first evoked and recorded on stimulation as described above.

During the experiment, it was noted that the most clearly apparent responses arose on movement of the stimulus from stimulation areas presumptively including the center of the RF of a SIN towards the edges of the monitor screen. On this basis we created a stimulation scheme as shown in Fig. 5: the stimulation area was moved from the central position towards the perimeter.

The central position of the window of the stimulation area is shown by a blue square and was both the initial and final positions of the stimulation area. Movement from the center of the window along the coordinate system (x, y; dark-red axes) was first to the right and then clockwise around the perimeter of the central position, as shown by gray squares. Black dotted lines enclose the spike activity of TN belonging to one position of the window in the coordinate grid. During the experiment, the blue and gray areas were tightly adjacent to each other – separation on the diagram is to avoid superimposing spike activity patterns.

In each stimulation area position, neuron stimulation was applied and responses to stimulus movement to the periphery of the monitor screen were recorded, with several different directions of stimulus movement in the corner positions. The stimulus was a wide black bar larger than the size of the stimulation area moving on the light background of the stimulation area with a dark periphery. Stimulus movement direction in this position in the stimulation area is indicated by blue arrows.

Sequential movement of the stimulation window and recording of the responses of SIN yielded neuron spike activity maps – an example of this type of map is shown in Fig. 5.

Figure 5 shows responses recorded from DS GC axon terminals, apparent as widening of the background noise band with TN spikes having greater amplitude. Thus, the stimulation area was centered relative to the simultaneously recorded caudorostral DS GC; retinal activity was absent from the middle trace in the central dotted box as the stimulus moved in the rostrocaudal direction (opposite to the preferred direction of this DS GC). The regular response of the SIN is apparent in the response to illumination of the far periphery of the receptive field when there was no change in the illumination at the center (the lower-amplitude spikes come from another neuron).

Discussion. During these experiments, from time to time we recorded responses from tectal neurons at different levels of the OT, though we did not have the opportunity to note which neurons produced the activity recorded. Therefore, in constructing a hypothesis and suggesting the functions of the TN whose reactions were recorded we have to rely on published data obtained using different methods. These are mostly data from *Danio* fry produced by calcium imaging. However, there are also studies using genetic methods for visualizing neurons and patch-clamp recording of electrical activity from individual neurons.

Our previous data indicate that statistical analysis of results divides Prussian carp and common carp DS TN into two groups – a more superficial group, at about 100 μm, and

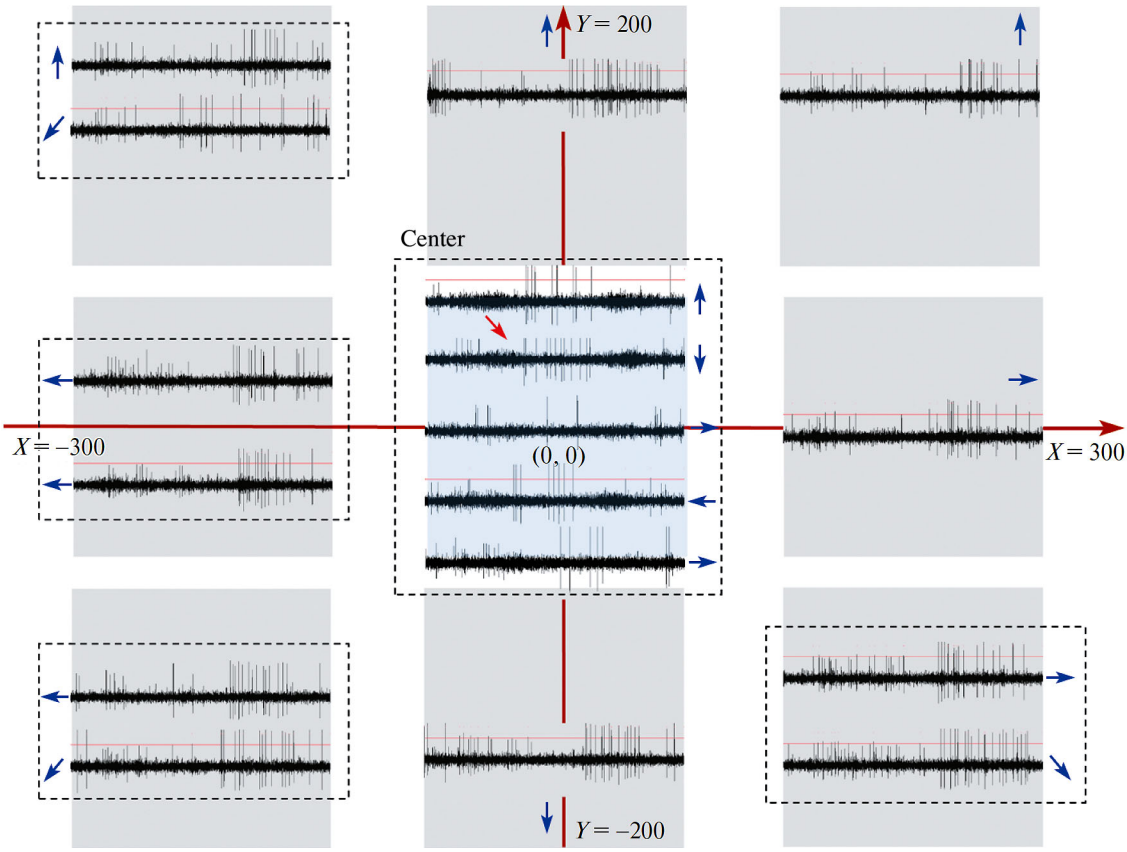

Fig. 5. Diagram showing monitor screen images with spike activity of a SIN. See text for description.

a deeper group, at about 300 μm [Aliper et al., 2019, Damjanović et al., 2019].

Biphasic spike shapes were seen, typical of recordings from neuron bodies [Maximova et al., 2012]. Thus, GC axon terminals probably form synapses on the bodies of deep DS TN and their ascending dendrites. Morphological studies have shown that neurons with bodies in the periventricular layer and dendrites ascending to the retinorecipient layer are present in the OT [Gabriel et al., 2012; Nikolaou et al., 2015]. These neurons are glutamatergic interneurons with dendritic branches in the SFGS [Robles et al., 2011].

Direction-selective tectal neurons appear to combine inputs from DS GC. DS TN of three types (in Prussian carp and common carp) with the same moving stimulus direction preferences as DS GC receive information directly from the corresponding DS GC. The fourth type of DS TN, extracting the rostrocaudal direction, probably somehow combines information arriving at them from GC.

A number of variants of how the fourth, rostrocaudal, direction arises can be proposed. First, selectivity for this direction may be formed by a combining the input signals from ventrodorsal and dorsoventral retinal DS GC. Second, it is possible that the rostrocaudal preferred direction is formed fully at the tectal level using input information from GC without DS [Grama and Engert, 2012]. This would occur with asymmetry of inhibition in the OT – Grama et al. suggested that there is a special type of inhibitory tectal interneuron. Interneurons of this type are connected asymmetrically with DS TN and respond to stimuli moving in the null direction (i.e., the direction opposite to the preferred direction).

In *Danio rerio*, DS TN can also be divided into four physiological subtypes with different preferred directions, similar to those recorded in adult Prussian carp and common carp [Hunter et al., 2013]. These include rostrocaudal DS TN, which are clearly not present in the input signals of the retina.

The SO contains not only GC axons, but also the bodies of superficial TN, i.e., superficial inhibitory neurons (SIN). These neurons are GABAergic [Bene et al., 2010]. These are also seen in preparations stained by the Golgi method [Lazarević et al., 1998] and examined using different visualization methods, such as calcium imaging [Barker and Baier, 2013; Bene et al., 2020]. The literature contains a number of hypotheses regarding the functions of SIN, mostly based on the suggestion that there is similarity between SIN and stellate amacrine retinal cells (ARC). Their morphological similarity consists of having a large central symmetrical dendritic tree located in just one layer. Some authors take the view that SIN have directional selectivity [Hunter et al., 2013; Yin et al., 2019]. However, during our electrophysiological experiments we did not see any prefer-

ence for stimulus movement direction in the superficial TN which we regard as presumptive SIN (SIN).

According to one hypothesis, which makes an analogy between SIN and ARC, SIN may provide inhibition of the visual field between two neuron subtypes tuned to large and small objects [Preuss et al., 2014]. In other words, GABAergic SIN are regarded as part of the system recognizing objects of different sizes in the field of vision, which is a key point for triggering suitable behavioral programs.

Another hypothesis suggests that SIN, functioning on the ARC principle, are involved in forming connections allowing the rostrocaudal direction to be extracted. In this case, SIN must form long-range connections with DS TN, which are located deeper in the OT. Periventricular neurons are known to send long dendrites in the superficial layers of the tectum, such as the SO and SFGS. Thus, SIN can form GABAergic synapses on the dendrites of DS TN, while ARC form synapses on DS GC dendrites.

Another hypothesis is also linked with extraction of object features, though at a different level: this suggests that SIN may take part in extracting the most significant stimulus in its field of vision (pop-out stimulus) operating by the "winner-takes-all" mechanism [Preuss et al., 2014]. This mechanism operates when the field of vision contains several competing stimuli, selection between which occurs as a result of lateral inhibition of the neighboring areas of the OT, which receive retinal projections from different parts of the field of vision.

This hypothesis seeks to explain the pattern seen in Fig. 5. This pattern of spike activity is the most successful variant of the eight produced for different SIN (a total of around 20 SIN were recorded and analyzed). The picture for other neurons is more complex, as the stimulation scheme was not fully developed, such that the positions of the stimulation window were not precisely verified and traces of reactions were partly missed.

It can be suggested that each spike from a superficial TN (SIN) is the moment at which GABA is released, i.e., at which inhibition occurs. SIN in the central part of the stimulation area at the center of the RF of off-type DS GC did not produce regular spike activity, i.e., did not exert inhibitory influences, thus subsequently missing the signal from this off-type DS GC. As shown by visualization of spike activity simultaneously with DS GC activity, TN spikes were either absent or very few in number (Fig. 5, example indicated by red arrow). However, the picture is different at the periphery: regular SIN volleys are produced, which inhibit the responses of the GC "seeing" them when this area of the field of vision of the fish contains other stimuli. Thus, SIN appear to pass information from GC feature detectors on the most important stimulus (the pop-out stimulus) in the field of vision at a given time point.

We obtained the first regular responses using one of the several stimulation methods tested. This progress was the prompt for continuing experiments in this direction. Optimization of experimental studies and obtaining larger numbers of visualizations of the spike activity of SIN evidently requires creation of a new programmable system. This instrument would allow automatic measurement of SIN responses to stimulus movement in the peripheral part of the RF. For example, we can imagine an instrument as follows: the stimulus in the form of a black ring is initially positioned in the stimulation area which presumptively contains the center of the RF of the neuron, and the ring is then enlarged in diameter, eliminating the lighter periphery. In this way, we simulate movement of the stimulus from the boundary of the stimulation area (and RF center) to the periphery, though on all sides relative to the stimulation area (in Fig. 5 from the blue central square to the periphery of the screen). This stimulation will confirm or refute our interpretation of the cause of the SIN reaction.

Tectal neurons are thus connected with at least several types of retinal elements, such as DS GC. In addition, TN respond to small stimuli (size barely greater than 1°) just like intrinsic retinal spot detectors. However, in tectal activity we found virtually no activity linked with GC orientation selectivity (line orientation detectors: horizontal, vertical), despite the fact that DS TN are located at the same level [Aliper et al., 2019]. The literature contains calcium imaging data on orientation-selective neurons in the OT [Hunter et al., 2013].

Further studies of the functions of tectal neurons on the OT at different levels are required. A better understanding of the role of SIN, which we regard as presumptive SIN, requires determination of which retinal elements and which TN of other types they form connections with in the tectum. In addition, creation of a programmed instrument for stimulation of these SIN will probably allow the appropriate stimulation for TN of this type to be identified, bringing us closer to understanding the functions of these neurons.

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REFERENCES

Aliper, A. T., Zaichikova, A. A., Damjanović, I., et al., "Updated functional segregation of retinal ganglion cell projections in the tectum of a cyprinid fish – further elaboration based on microelectrode recordings," *Fish Physiol. Biochem.*, **45**, No. 2, 773–792 (2019).

Barker, A. J. and Baier, H., "Sensorimotor decision making in the zebrafish tectum," *Curr. Biol.*, **25**, No. 21, 2804–2814 (2015).

Barker, A. J. and Baier, H., "SINs and SOMs: neural microcircuits for size tuning in the zebrafish and mouse visual pathway," *Front. Neural Circ.*, **7**, 89 (2013).

Bene, F. Del, Wyart, C., et al., "Filtering of visual information in the tectum by an identified neural circuit," *Science*, **330**, No. 6004, 669–673 (2010).

Ben-Tov, M., Donchin, O., Ben-Shahar, O., and Segev, R., "Pop-out in visual search of moving targets in the archer fish," *Nat. Commun.*, **6**, No. 1, 1–11 (2015).

Damjanović, I., Maximov, P., Aliper, A., et al., "Putative targets of direction-selective retinal ganglion cells in the tectum opticum of cyprinid fish," *Brain Res.*, **1708**, 20–26 (2019).

Damjanović, I., Maximova, E. M., and Maximov, V. V., "On the organization of receptive fields of orientation-selective units recorded in the fish tectum," *J. Integr. Neurosci.*, **8**, 323–344 (2009).

Gabriel, J. P., Trivedi, C. A., Maurer, C. M., et al., "Layer-specific targeting of direction-selective neurons in the zebrafish optic tectum," *Neuron*, **76**, No. 6, 1147–1160 (2012).

Gesteland, R. C., Lettvin, J. Y., Howland, B., et al., "Comments on microelectrodes," *Proc. IRE*, **47**, No. 11, 1856–1862 (1959).

Grama, A. and Engert, F., "Direction selectivity in the larval zebrafish tectum is mediated by asymmetric inhibition," *Front. Neural Circ.*, **4**, No. 6, 59 (2012).

Hunter, P. R., Lowe, A. S., Thompson, I. D., and Meyer, M. P., "Emergent properties of the optic tectum revealed by population analysis of direction and orientation selectivity," *J. Neurosci.*, **33**, No. 35, 13940–13945 (2013a).

Kardamakis, A. A., Saitoh, K., and Grillner, S., "Tectal microcircuit generating visual selection commands on gaze-controlling neurons," *Proc. Natl. Acad. Sci. USA*, **112**, No. 15, E1956–E1965 (2015).

Kinoshita, M. and Ito, E., "Roles of periventricular neurons in retinotectal transmission in the optic tectum," *Prog. Neurobiol.*, **79**, No. 2, 112–121 (2006).

Lazarević, L., Rogač, L., and Rakić, L., "Cytoarchitectonic analysis of tectum opticum in *Serranus scriba*," *Iugosl. Physiol. Pharmacol. Acta*, **34**, No. 2, 335–341 (1998).

Maximov, V. V., Maximova, E. M., and Maximov, P. V., " Classification of directionally selective elements projecting to the tectum in Prussian carp," *Sens. Sistemy*, **23**, No. 1, 13–23 (2009).

Maximov, V. V., Maximova, E. M., and Maximov, P. V., "Classification of directionally selective elements recorded in the Prussian carp tectum," *Sens. Sistemy*, **19**, No. 4, 322–335 (2005a).

Maximov, V. V., Maximova, E. M., and Maximov, P. V., "Direction selectivity in the goldfish tectum revisited," *Ann. N.Y. Acad. Sci.*, **1048**, 198–205 (2005b).

Maximova, E., Pushchin, I., Maximov, P., and Maximov, V., "Presynaptic and postsynaptic single-unit responses in the goldfish tectum as revealed by a reversible synaptic transmission blocker," *J. Integr. Neurosci.*, **11**, No. 2, 183–191 (2012).

Nevin, L. M., Robles, E., Baier, H., and Scott, E. K., "Focusing on optic tectum circuitry through the lens of genetics," *BMC Biol.*, **8**, No. 1, 126 (2010).

Nikolaou, N. and Meyer, M. P., "Lamination speeds the functional development of visual circuits," *Neuron*, **88**, No. 5, 999–1013 (2015).

Nikolaou, N., Lowe, A. S., Walker, A. S., et al., "Parametric functional maps of visual inputs to the tectum," *Neuron*, **76**, No. 2, 317–324 (2012).

Northmore, D. P. M., "The optic tectum," in: *Encyclopedia of Fish Physiology: From Genome to Environment*, Farrell, A. P. (eds.), Elsevier (2011), pp. 131–142.

Preuss, S. J., Trivedi, C. A., Vom Berg-Maurer, C. M., et al., "Classification of object size in retinotectal microcircuits," *Curr. Biol.*, **24**, No. 20, 2376–2385 (2014).

Robles, E., Filosa, A., and Baier, H., "Precise lamination of retinal axons generates multiple parallel input pathways in the tectum," *J. Neurosci.*, **33**, No. 11, 5027–5039 (2013).

Robles, E., Smith, S. J., and Baier, H., "Characterization of genetically targeted neuron types in the zebrafish optic tectum," *Front. Neural Circ.*, **5**, No. 1 (2011).

Walker, A. S., Burrone, J., and Meyer, M. P., "Functional imaging in the zebrafish retinotectal system using RGECO," *Front. Neural Circ.*, **7**, 34 (2013).

Yin, C., Li, X., and Du, J., "Optic tectal superficial interneurons detect motion in larval zebrafish," *Protein Cell*, **10**, 238–248 (2019).