

Presynaptic and postsynaptic single-unit responses in the goldfish tectum as revealed by a reversible synaptic transmission blocker

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A variety of visually evoked responses are recorded in the fish optic tectum using single-cell recording technique. Based on indirect criteria (frequency power spectrum of spikes, spike waveform, receptive field size), they may be divided into two groups: those presumably recorded from axon terminals of retinal ganglion cells projecting to the tectum (presynaptic recording), and those recorded from tectal neurons (postsynaptic recording). In the present study, we used cobalt, a reversible blocker of synaptic transmission, as a more crucial criterion to identify the source of these responses. After cobalt application, some units (such as ON- and OFF-types of direction-selective units, orientation-selective and spontaneously active units) were visually responsive, while others (including ON-OFF direction-selective units with large receptive fields) ceased firing. Discrimination of the units by the use of cobalt has been found to coincide with that by the indirect physiological criteria. Thus, the differences in frequency power spectrum of spikes, spike waveform, and receptive field size may be used for efficient and reliable discrimination between pre- and post-synaptic recordings in the fish tectum.

Keywords: Retinal ganglion cells; optic tectum; extracellular recordings; direction-selective units; detectors of oriented lines; spontaneously active units; goldfish.

1. Introduction

The optic tectum is the principal visual center playing a crucial role in the visual behavior of the lower vertebrates. Visual processing in the fish optic tectum has been investigated by extracellular recording of spike activity evoked by visual stimuli for almost 50 years (Jacobson & Gaze, 1964; Cronly-Dillon, 1964; Zenkin & Pigarev, 1969; Maximova *et al.*, 1971; Wartzok & Marks, 1973; O'Benar, 1976; Guthrie &

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Banks, 1978; Kawasaki & Aoki, 1983; Maximov *et al.*, 2005a; Damjanović *et al.*, 2009a,b). Various types of retinal ganglion cells form an ordered retinotopic projection to the optic tectum. Their axons terminate at a different depth in the optic tectum to form lamina-specific synaptic connections with tectal neurons (Vanegas & Ito, 1983; Nevin *et al.*, 2010). This arrangement is reflected in ordered sequences of responses recorded in single-unit electrophysiological studies (Jacobson & Gaze, 1964; Zenkin & Pigarev, 1969; Maximova *et al.*, 1971; Galand & Liège, 1975; O'Benar, 1976; Kawasaki & Aoki, 1983). The visually evoked activity in the optic tectum was attributed either to axonal terminals of the retinal ganglion cells, whose axons arborize in different retinorecipient laminae, or to the tectal neurons that integrate inputs from the ganglion cells.

Up to a dozen of feature detectors coding perceptually significant stimuli have been described in the optic tectum of various fish species. Those usually attributed to the retinal ganglion cells include direction-selective (DS) and orientation-selective (OS) units, spot detectors, color-coded units, and units with light and dark spontaneous activity. Direction-selective units of six subtypes (Maximov *et al.*, 2005a) produce the strongest response to a stimulus moving in one (preferred) direction and a much weaker response, if any, to a stimulus moving in the opposite (null) direction. They are recorded in the most superficial layers of the visually responsive zone (about 50 μm below the tectal surface in goldfish). DS units comprise three groups preferring either caudo-rostral, ventro-dorsal, or dorso-ventral directions. Each group, in turn, comprises units of ON and OFF types sensitive to different signs of contrast. Orientation-selective units of two subtypes or detectors of horizontal and vertical lines (Maximova, 1999; Maximov *et al.*, 2009) are excited by either vertical or horizontal edges or stripes, both stationary or moving, regardless of sign of contrast. Their recording sites were deeper than those of DS units (about 100 μm below the tectal surface). Spot detectors and color coding units (Maximova, 1977) are recorded at almost the same depth. They were not regularly encountered in the present experiments and will not be considered further. Spontaneously active (SA) units of two subtypes display spontaneous activity either in darkness or in light (dimming and lightening detectors, respectively). SA units are recorded deeper than all other units attributed to the retinal ganglion cells (about 200 μm below the tectal surface). All these units have small receptive fields (RFs) of the order of $3 - 7^\circ$ (Damjanović *et al.*, 2009a,b).

In addition to the above-described units of a putative retinal origin, there is another kind of units recorded in the tectum. Some of their features suggest that they originate from tectal neurons. Their spikes are usually greater in amplitude and longer in duration compared to putative retinal units. Their responses can be recorded at a different depth in the optic tectum, from the surface to the level of SA units and even deeper. Sometimes when immersing the microelectrode through the thickness of the tectum, a distinctive damage discharge can appear that is an indicator of tectal origin of the recorded responses. Some of these putative tectal neurons are direction selective. As distinct from the retinal DS units, they are indifferent to

the sign of stimulus contrast (i.e., they are of ON-OFF type) and have large RFs (up to 60°).

Several studies proposed physiological criteria to classify a unit either as retinal or tectal (Zenkin & Pigarev, 1969; Sutterlin & Prosser, 1970; Wartzok & Marks, 1973; O'Benar, 1976; Maximov *et al.*, 2005b). Thus, the fact that DS and OS units with the same properties (as in the tectum) have been recorded in the fish retina (Daw, 1967; Bilotta & Abramov, 1989; Tsvilling *et al.*, 2012) and the optic chiasma (Jacobson & Gaze, 1964) favors their retinal origin. On the contrary, a large RF, pronounced spontaneous activity of a unit and plasticity of its responses suggest its tectal origin (O'Benar, 1976). The depth of recording and the form of spikes and spike trains can also serve as discriminating criteria. Thus, in extracellular recordings, arriving spikes usually have triphasic waveform with a positive deflection before the main negative wave; whereas the spikes that are recorded in the vicinity of the cell body are biphasic and lack such a deflection (Wartzok & Marks, 1973). As a rule, biphasic spikes have a longer duration and, consequently, a low-frequency power spectrum easily recognizable by ear (Maximov *et al.*, 2005b). As to features of the spike train in extracellular recordings, arriving spikes are approximately equal in amplitude, whereas the amplitude of the spikes generated in neurons near the microelectrode decreases substantially as spike rate increases (Maximov *et al.*, 2005b).

The difference in putative tectal and retinal responses is illustrated in Fig. 1. Two DS units, one of presumably retinal, and the other of tectal origin, were recorded simultaneously. The units were selective to the opposite directions of motion, enabling one to easily distinguish their responses. A putative retinal DS ON-type unit preferred ventro-dorsal direction and responded markedly to the white edge moving

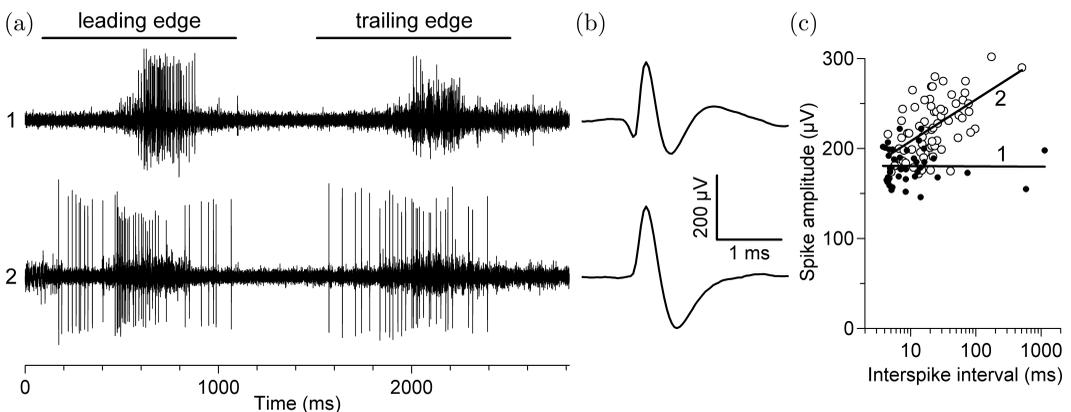


Fig. 1. Responses of two simultaneously recorded DS units with different preferred directions. The first unit (1) is a putative retinal DS GC stimulated by upward movement of a broad white stripe on a black background with a speed of $11^\circ/\text{s}$, and the second (2), a tectal DS neuron stimulated by downward movement of the same stripe with the same speed. (a) Firing patterns in response to movement of leading and trailing edges of the stripe. (b) Averaged spike forms ($N = 42$ for 1, and $N = 61$ for 2) shown in expanded time scale (negativity upwards). Plot of the spike amplitude vs. interval between the current and the previous spikes in discharges marked with closed and open circles for the first and second units, respectively. Straight lines show corresponding regression functions for the point sets.

on black background within the RF and weakly, if at all, to the black edge moving on white background (Fig. 1(a) (1)). A putative tectal DS unit responded to the moving edges of any sign of contrast in dorso-ventral direction. It had a large RF, as can be judged by the duration of the spike train (Fig. 1(a) (2)). Figure 1(b) shows the differences between the different units in the form of individual spikes. Figure 1(c) demonstrates the different dependence of the spike amplitude on the interspike interval in the discharges.

Not all of the above-mentioned criteria are applicable to any unit. Some studies used other criteria to identify a unit recorded in the optic tectum that include additional studies, such as electrical stimulation of the optic nerve (O'Benar, 1976) or topical application of GABA to the tectal surface (Zenkin & Pigarev, 1969). However, the crucial criterion would be an application of agents that reversibly block synaptic transmission such as cobalt chloride (Kretz, 1984). This was attempted in two studies (Gallagher & Northmore, 1997; Stirling *et al.*, 2001). Neither study described physiological identity of the responses, and both were published as preliminary results in abstract form (Gallagher & Northmore, 1997; Stirling *et al.*, 2001) and were not continued. In the present study, we investigated the effect of cobalt on the responses of physiologically identified single units recorded in the goldfish tectum.

2. Materials and Methods

All experiments were performed on the cyprinid fish *Carassius gibelio* (Bloch, 1782), a wild form of the goldfish, of 10–15 cm standard body length. A fish with the exposed optic tectum was immobilized with tubocurarine (0.3 mg per 100 g of body weight) and placed in a transparent Plexiglas tank with its eyes submerged in water. Aerated water passed continuously through the gills. Visual stimuli were presented on a computer-controlled CRT monitor placed against the right eye of the fish. Responses were recorded extracellularly in the superficial layers of the optic tectum. Low impedance (200–500 K Ω) recording microelectrodes were made from micropipettes filled with Wood alloy and tipped with a platinum cap 2–5 μm in diameter (Gestesland *et al.*, 1959). Neuronal discharges were amplified, filtered, displayed on an oscilloscope, fed to an audio monitor, digitized by an A/D converter (25-kHz sampling rate), and stored in the computer. Units were isolated by adjusting the position of the microelectrode. Once a single unit recording was obtained (as can be judged from the absence of interspike intervals shorter than the period of absolute refractoriness), the unit's responses to various stimuli were recorded, and the preferable stimulus was found. Further details on the recording conditions may be found in (Maximov *et al.*, 2005a; Damjanović *et al.*, 2009a,b). About 100 units were recorded, and most of them were identified with the types described above.

To reversibly block synaptic transmission, 50–100 μl of 100 mM cobalt chloride in Ringer solution (120 mM NaCl, 3 mM KCl adjusted to pH 7.4 with NaOH) was applied to the tectal surface and allowed to diffuse to the site of recording. The effective cobalt concentration could not be determined as it depended on blood flow,

amount of cerebrospinal fluid on the tectal surface, and rate of cobalt diffusion into the brain tissue. For this reason we tried a low cobalt concentration, first increasing it progressively until a reliable effect of synaptic transmission block was achieved. The cobalt was washed out by replacing the cobalt solution with several changes of Ringer solution.

3. Results and Discussion

A typical example of the effect of cobalt chloride on a putative tectal unit is shown in Fig. 2. It was a DS neuron with a large RF responding to moving stripes and edges of any sign of contrast in the ventro-dorsal direction. The unit was recorded from a deep layer of the tectum, below the layer of SA units. Cobalt was applied at 14:47. Immediately after the application, until cobalt action became apparent, the firing pattern of the unit in response to the movement of a broad black stripe on gray background in the preferred direction was recorded. After this, the unit was continuously tested with black or white stripes moving at various speeds. Five minutes after cobalt application, the responses markedly reduced. This reduction was due to a

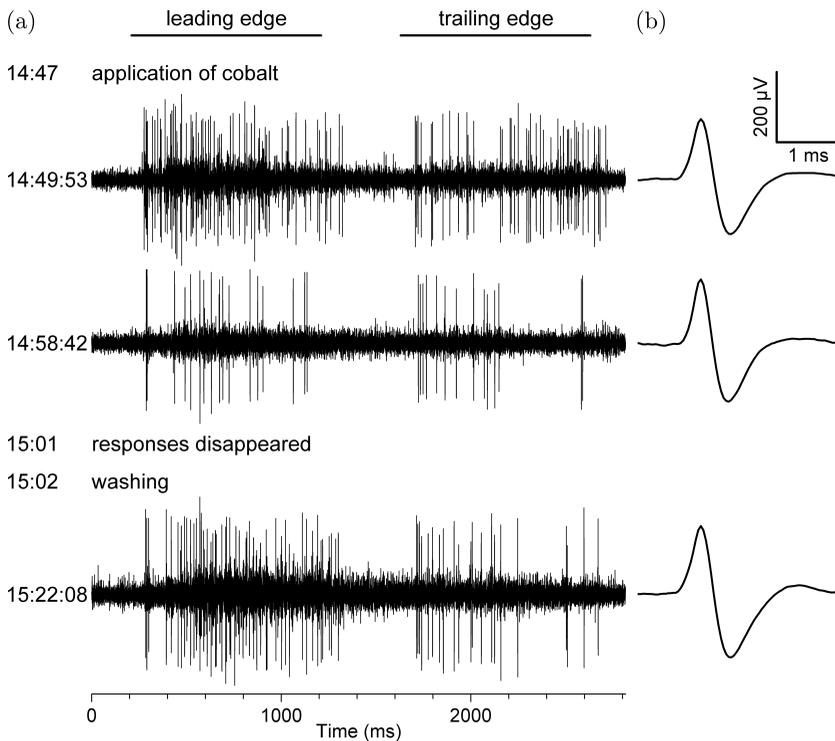


Fig. 2. A reversible change of the visual responses of a putative tectal DS neuron after cobalt application and washout. (a) Spike trains evoked by the leading and trailing edges of the wide black stripe moving in the preferred ventro-dorsal direction at a speed of $11^\circ/\text{s}$. Periods of experimental manipulations and recordings are given in the left column. (b) Averaged spike waveforms shown in expanded time scale (negativity upwards).

gradual thinning of the discharge, while the amplitude of the spikes remained practically unchanged. When tested with a black cardboard, moved by hand in front of the fish eye, it was clear that the RF became reduced and fragmented: responses could be only evoked by stimulus movement across certain parts of the RF. The reduction of responses occurred for the light and dark stimuli concurrently. It was evident that the unit first lost sensitivity to slow movement (not shown in the figure) and displayed a strong habituation to repeated visual stimulation. Fifteen minutes after cobalt application, no response of the unit could be detected even to rapidly moving edges. However, some activity in the tectum has persisted as small (distant) spikes in response to visual stimulation. In 15:02, the washout was started, and the responses gradually recovered, passing through all of the above stages in reverse order. In 15:38, cobalt administration was repeated to cease the response of the tectal unit again (not shown). The microelectrode was then lifted somewhat to see whether any response could be detected in the optic tectum. In the upper layers of the same track, a SA unit and an ON-type DS unit preferring caudo-rostral direction were recorded. Both had small RFs, and their spikes were fast and had a clear deflection before the main negative wave of the spike. To summarize the above, after cobalt administration, the surface units with mentioned properties remained responsive, when the putative tectal unit located deeper ceased firing.

As distinct from the unit described above, the firing rate or type-specific properties of DS, OS, and SA units were not noticeably affected by cobalt application. The persistence of the responses of putative presynaptic DS units to cobalt application was confirmed in experiments where they were continuously recorded during cobalt administration and washout. Figure 3 illustrates a typical experiment with two DS units preferring caudo-rostral direction. The one with the largest spikes was of ON-type and, therefore, responded only to the movement of the leading edge of the white stripe. Another one, with slightly smaller spikes, was of OFF-type and responded to the movement of the trailing edge of the same stripe. RFs of both units were about 4.5° in diameter, and were somewhat separated in space. In addition, an ON-OFF OS unit (detector of vertical lines, one with the smallest spikes in Fig. 3), was recorded. It responded to both the leading and the trailing edges. Its RF was slightly shifted in the caudal direction with respect to the DS OFF-type unit. For this reason, it responded to the moving trailing edge a bit earlier (its discharges are marked with asterisks in Fig. 3). Cobalt application had no effect on these units (Fig. 3). Not surprisingly, cobalt washout 20 min after application produced no effect (not shown).

Figure 4 presents an experiment on cobalt impact on the visual responses of an OS unit. Both putative retinal and tectal units were recorded in the same track. First, a putative tectal unit was recorded. After cobalt application at 16:54, it ceased firing. At 17:05, cobalt was applied for the second time. At 17:09, the microelectrode was shifted and a putative retinal OS unit (detector of vertical lines) was recorded. At 17:16, immediately after washout onset, its response to the wide white stripe against gray background was recorded. During washout, the recording conditions changed slightly. However, this unit could still be recorded. After washout, a unit with a

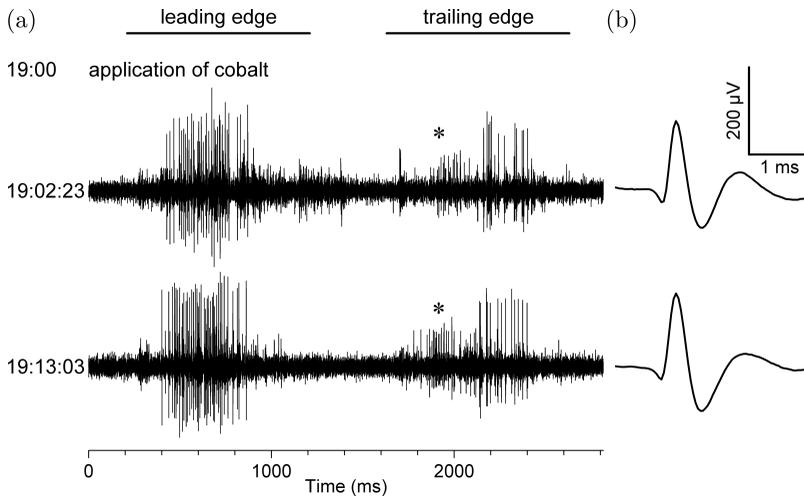


Fig. 3. Visual responses of putative retinal DS units after cobalt application. (a) Spike trains evoked by the leading and trailing edges of the wide white stripe moving in preferred caudo-rostral direction at a speed of $11^\circ/\text{s}$. The ON-type DS unit responded to the leading edge and the OFF-type DS unit responded to the trailing edge of the stripe. Asterisks indicate the position of the OFF discharges of the OS unit. One can see that cobalt had no apparent affect on the responses. (b) Averaged spike waveforms of the ON-type unit in expanded time scale.

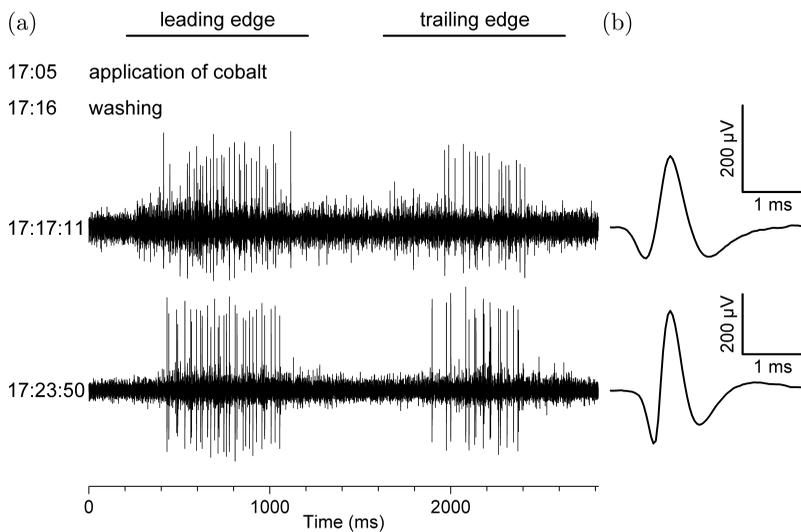


Fig. 4. Visual response of a putative retinal detector of vertical lines after cobalt application. (a) Spike trains evoked by the leading and trailing edges of the wide white stripe moving in caudo-rostral direction at a speed of $11^\circ/\text{s}$. One can see that cobalt had no apparent affect on the responses. (b) Averaged spike waveforms of the unit in expanded time scale.

low-frequency spike spectrum lacking initial positive potential deflection was recorded again suggesting a recovery of firing of tectal units.

It should be noted that in all experiments responses of different types of units were recorded within the same electrode track and often in the same site of a track. In most cases, responses of DS, OS, and SA units were recorded after neighboring putative tectal units ceased firing. This implies that units of either origin were exposed to the same effective concentration of cobalt.

The present results indicate that DS, OS, and SA units are of retinal origin, while units with large RFs and biphasic responses are of tectal origin. This finding is supported by the fact that DS, OS and SA units similar to the presently studied ones were recorded from the optic chiasm or retina in several fish species including goldfish (Jacobson & Gaze, 1964; Zenkin & Pigarev, 1969; Bilotta & Abramov, 1989; Tsvilling *et al.*, 2012). Thus, the present findings indicate that receptive field size, spike waveform and frequency power spectrum of spikes can be used as efficient and reliable criteria for identifying the origin of units recorded in the optic tectum.

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