20 Hz membrane potential oscillations are driven by synaptic inputs in collision-detecting neurons in the frog optic tectum

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HIGHLIGHTS

* Collision-sensitive neurons were blindly patched in a frog eye-tectum preparation.
* The majority (22/26) of tectal layer 6 neurons were η class collision-neurons.
* 20 Hz membrane-potential oscillations were detected in many these neurons (15/22).
* Thus, ‘collision’ responses may be generated by OFF retinal ganglion cells.

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ABSTRACT

Although the firing patterns of collision-detecting neurons have been described in detail in several species, the mechanisms generating responses in these neurons to visual objects on a collision course remain largely unknown. This partly due to the limited number of intracellular recordings from such neurons, particularly in vertebrate species. By employing patch recordings in a novel integrated frog eye-tectum preparation we tested the hypothesis that OFF retinal ganglion cells were driving the responses to visual objects on a collision course in the frog optic tectum neurons. We found that the majority (22/26) of neurons in layer 6 responding to visual stimuli fitted the definition of η class collision-detectors; they readily responded to a looming stimulus imitating collision but not a receding stimulus (spike count difference ~10 times) and the spike firing rate peaked after the stimulus visual angle reached a threshold value of ~20–45°. In the majority of these neurons (15/22) a slow frequency oscillation (f ~ 20 Hz) of the neuronal membrane potential could be detected in the responses to a simulated collision stimulus, as well as to turning off the lights. Since OFF retinal ganglion cells could produce such oscillations, our observations are in agreement with the hypothesis that ‘collision’ responses in the frog optic tectum neurons are driven by synaptic inputs from OFF retinal ganglion cells.

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1. Introduction

Collision detection is vital for the survival of many species including humans, and is often performed by specialised collision-sensitive neurons [6,10,21], which can detect objects approaching on a collision course and estimate the time remaining to collision, which is vital to trigger an appropriate escape response [5].

Although the firing patterns of collision-sensitive neurons are well studied [6,13], little is known about the underlying synaptic and biophysical mechanisms [15]. Most data on collision-sensitive neurons were obtained with extracellular recording methods that do not permit the investigation of synaptic and voltage-gated currents generating these responses.
We visually identified layer 7 of the frog optic tectum as a well-defined white strip near the middle of the cross-section of the tectum [16,20]; the recorded neurons were located slightly below (<~100 μm) this layer towards the centre of the tectum (i.e. layer 6). Most recordings were a few hundred micrometres from the rostral–caudal midline around its central section, corresponding to the upper lateral visual field–zones 13–111 of the optic tectum [4]. Data were obtained from 14 frog preparations.

All visual stimuli were dark images on a bright background because collision neurons of the frog optic tectum respond very weakly to bright images on the dark background [13]. All images were presented on a LED backlit LCD monitor (frame rate 75 Hz, 35 cm × 17 cm). Tests showed that dropping one frame from the looming image did not affect the neuronal response shape suggesting the employed frame rate was sufficient to obtain an illusion of a continuous visual stimulus in the frog eye. The monitor was located 10 cm from the left eye of the frog (Fig. 1A) resulting in a very wide visual field (the full screen subtended >120° at the eye). At the centre of the screen, 1 cm corresponded to a visual angle of approximately 5.7°. The monitor had 1024 × 768 image pixels or >30 pixels/cm corresponding to a minimal stimulus size of <0.3°, which is below the reported retinal sensitivity of ~1.5° in frogs [1]. Images were generated by employing an open source software package PsychoPy [14] controlled by a program written in the Labview environment (National Instruments, Austin, TX, USA).

Most of the frog vision is monocular [7] and they cannot use parallax to estimate the distance to an object of a visual scene. A monocular representation of an approaching at a constant speed object, a looming stimulus, can be described by a single parameter, the ratio between the object’s half-size, L, and velocity of the object, \( v \) [6]. The corresponding image radius \( R \) on the computer monitor was determined by the following equation:

\[
R = \frac{(l/v) \times (D_0/E)}{(T-t)}
\]

where \( T \) is the collision time, \( t \) is the time measured from stimulus onset, \( D_0 \) is the distance from the frog eye to the computer monitor and \( E \) is the half width of the computer monitor. \( T \) was set to 5 or 10 s corresponding to an initial image size of <4° for \( l/v \) ratios used in our experiments. For the receding stimulus \( T \) was set to 0 and \( t \), the time, was negative in Eq. (1). The stimulus centre was set in the receptive field (RF) of the recorded neuron that was determined by scanning the entire screen with a dark square (5° × 5°) moving diagonally at a constant angular velocity of 24°/s (Fig. 1Ab). The responses, detected as inward synaptic currents from the baseline current, were recorded in voltage clamp mode at ~70 mV (asterisks in Fig. 1Ba). For our recorded neurons all RFs were located in the lower left part of the monitor corresponding to the caudal upper part of the frog eye visual field (Fig. 1Bb). The typical field size was 15–25° although it reached ~40° in some neurons, but never exceeded 45°.

3. Results

In total, we recorded 26 neurons with good responses to the changes in illumination of the computer monitor and the movement of a small bar across the whole visual field. In current clamp, 22 of the 26 neurons responded to looming but not receding stimuli with a large number of action potentials (Fig. 2A and B). A looming ‘collision’ stimulus can be determined by a single parameter, the ratio of the half-size of the object, \( l \), to its movement velocity towards the frog, \( v \) [6]. For \( l/v = 680 \text{ ms} \), the average spike count amounted to 17 ± 3 for approaching stimuli and was significantly reduced to 1.5 ± 0.7 for receding stimuli (n = 6, p < 0.05, Wilcoxon signed-rank test). Spikes were counted in a time window between 0.5 and 3 s before the approaching stimulus end or after the start
of a receding stimulus. This time interval was selected because it encompassed the peak firing rates to approaching stimuli (see below). In the same time window, the average inward current amplitude, measured in the voltage clamp mode from the baseline current before the start of the stimulus, was on average smaller by 86±7% for decreasing stimuli as compared to looming stimuli (n = 6). It is important to note that response duration and shape (the presence of a peak just before the ‘collision’) was similar in both the voltage clamp and current clamp modes (compare Fig. 2B and C). If a looming stimulus represented an object deviating from the collision course (>2°), responses disappeared when no collision occurred, even if a large fraction of the screen was covered by the looming stimulus before it veered off the screen (data not shown).

Several classes of looming/collision-sensitive neurons have been described [19,22]. Neurons of each class are believed to detect different temporal variables such as time to collision or image expansion rates. These classes can be distinguished by presenting looming stimuli of different l/v ratios. One particular class, η neurons, have a distinct peak of firing frequency before collision and respond with a shallower increase in spike frequency for larger l/v ratios [6,19]. Our recorded neurons belonged to this class because they started to respond sooner and less vigorously for larger l/v ratios and their firing rates declined before the projected collision time (Fig. 3A–C). For these neurons the average time course of firing frequency can be fit with an equation [6]:

\[ f(t) = A \times \psi(t - d) \times \exp[-\alpha \times \Theta(t - d)] \]  

where \( f(t) \) is the average firing frequency at time \( t \), \( \psi(t) \) is the angular velocity of stimulus edge, and \( \Theta(t) \) is the angle at which stimulus is observed at time \( t \). Parameters \( A \) and \( \alpha \) are used to fit the obtained spike histograms, with \( A \) determining the overall response amplitude and \( \alpha \) characterising the peak position of the fit function. The observed action potential firing patterns could be fit with Eq. (2) (Fig. 3C). For this neuron, 3–5 runs for each l/v value were performed. We used the following \( \alpha \) values in Eq. (2): 1.74 for l/v = 85 ms, 2.74 for l/v = 170 ms, 3.18 for l/v = 340 ms, and 3.08 for l/v = 510 ms. The time of peak firing frequency obtained from these fits correlated linearly with the velocity of the stimulus (r = 0.99, Fig. 3C) and the obtained slope factor 2.44 was within the range of \( \alpha \) values used for fits as expected for such collision detecting neurons [6,13]. The estimated \( \Theta_{\text{thresh}} \) or visual angle corresponding to the peak firing rate can be also calculated according to the following formula [6]:

\[ \Theta_{\text{thresh}} = 2 \times \alpha \tan \left( \frac{1}{\alpha} \right) \]  

In this neuron, \( \Theta_{\text{thresh}} \) was ~45°, while in other neurons the estimated \( \Theta_{\text{thresh}} \) values were between ~20° and 45°.

So far our data support previous claims that there are collision-detecting neurons in layer 6 of the frog optic tectum [13]. In the last results section we will describe a new feature of these neurons suggesting that OFF retinal ganglion cells provide a strong synaptic input to layer 6 neurons during the generation of ‘collision’ responses; namely, our recorded neurons have a strong tendency to fire in short bursts producing 20 Hz oscillations similar to OFF retinal ganglion cells [11].

Such oscillations could be detected in the majority of responses to a collision stimulus; in 15 of 22 collision-detecting neurons (Fig. 4A and B). Their shape was similar in both current clamp and voltage clamp recordings (Fig. 4B) suggesting that synaptic inputs but not intrinsic membrane properties were responsible for the oscillations. In the same neurons, 20 Hz oscillations could also be seen during responses to the ‘OFF’ stimulus (switching off of the computer monitor, Fig. 4C). Power spectra showed a clear peak around 20 Hz with a smaller peak of the second harmonic (~40 Hz; Fig. 4D, double and single asterisks, respectively).

Interestingly, ‘OFF’ responses started on average 148 ± 8 ms after the monitor was switched off (n = 4), which was similar to the

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Fig. 1. Experimental setup. (Aa) Tectal neurons were recorded with preserved direct inputs (sent via optic nerve) from the contralateral eye to the optic tectum. (Ab) Receptor fields (RFs) were detected with a moving dark bar. The typical scan density was double of that shown here. (Ba) Current traces with responses to a dark moving bar. Asterisks indicate responses while arrows point to truncated action potentials. (Bb) Representative sample of five RFs.

Fig. 2. Neurons in the frog optic tectum layer 6 responded more vigorously to looming than decreasing stimuli. (A) Screen shots of a stimulus taken at times indicated above each shot (time to collision for the looming stimulus and time after the stimulus appearance for the decreasing stimulus). (B) Membrane voltage traces of a neuron responding to a looming (upper trace) and a decreasing (lower trace) stimulus in current-clamp mode. (C) Current traces of the same neuron responding to a looming (upper trace) and a decreasing (lower trace) stimulus in voltage-clamp mode. Black arrows indicate responses to the end of the stimulus (ON response).
delay $\delta$ between $\Theta_{\text{thresh}}$ and the peak firing frequency in responses to looming stimuli (Fig. 3D). In the same cells ‘ON’ responses were usually brief and no 20-Hz oscillations could be detected in any ‘ON’ response (Fig. 4E). This notion can be expressed quantitatively as a reduction of the integral of inward current amplitude measured at $-60$ mV during the first 500 ms after the response start; in ON responses it was on average equal to $0.24 \pm 0.07$ of that of the ‘OFF’ responses ($p < 0.001, n = 5$).

4. Discussion

In this paper we report the first whole-cell recordings from collision-sensitive neurons of the frog optic tectum. We found that the majority of layer 6 neurons could be assigned to $\eta$ type collision-sensitive neurons [19]. The similarity in shape between responses recorded in current and voltage clamps indicates that neuronal responses to visual objects on a collision course are largely driven by synaptic inputs. These results could not be obtained by employing the extracellular recording methods used in a previous study [13].

We also showed that 20-Hz membrane potential oscillations could be detected in the majority of these neurons. Since similar oscillations are detected in OFF retinal ganglion cell responses [9,18], our data is agreement with the hypothesis that synaptic inputs from OFF retinal ganglion cells are generating ‘collision’ responses in these neurons, although further experiments are needed to provide direct evidence. These OFF ganglion cells have relatively large RFs and the optimal visual angle of a stimulus that activates these cells is often high, on average $16^\circ$ [9,13,18], which is close to the observed $\Theta_{\text{thresh}}$ values of $\sim 20^\circ$ to $45^\circ$. However, further work is needed to explain why the peak firing rate occurs only above $\geq 20^\circ$ ($\Theta_{\text{thresh}}$ values) and why ‘collision’ responses disappear for small deviations ($\sim 2^\circ$) from the collision course that are much less than the RF size of OFF ganglion cells. This is especially interesting because the retinal image is likely to move in some frogs because of head movements [17]. Another important issue is the role of diverse neurotransmitters in the formation of the response: retinal fibres may co-release two neurotransmitters, glutamate and acetylcholine [2].

It is quite surprising that almost all of the recorded neurons belonged to a single class of collision-sensitive neurons. In the nucleus rotundus and the tectum of pigeons [19,22] three types of collision-sensitive neurons can be found and only a fraction of them belong to the $\eta$ type. In cats, most collision-sensitive neurons in the superior colliculus, a homolog of the optic tectum, were of $\eta$ type, but only $\sim 1/3$ of all recorded neurons were collision-sensitive [10]. It is likely that by patching cells from a single layer in a small optic tectum area close to the midline and by selecting only the cells responding to visual stimuli we were able to pick up a very specific cell population.

Data suggest that in several diverse species such as frogs and mice, initial collision detection is performed in the retina [8,12]. Collision detection is a vital function in the majority of species and it is not surprising that such detection is performed at very early stages of visual processing. Our results are consistent with this notion.

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