Response properties and receptive field organization of collision-sensitive neurons in the optic tectum of bullfrog, *Rana catesbeiana*

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Abstract: Objective Many studies have reported that animals will display collision avoidance behavior when the size of retinal image of an object reaches a threshold. The present study aimed to investigate the neural correlates underlying the frog collision avoidance behavior. **Methods** Different types of visual stimuli simulating the retinal image of an approaching or a receding object were generated by a computer and presented to the right eye of frog. A multielectrode array was used to examine the activity of collision-sensitive neurons, and single electrode recordings were employed to quantify visual parameter (s) of the frog collision-sensitive neurons. **Results** The multielectrode array revealed that 40 neurons in the optic tectum showed selective responsiveness to objects approaching on a direct collision course. The response profiles of these collision-sensitive neurons were similar to those of lobula giantmovement detector (LGMD) in the locust or to those of η neurons in the pigeon. However, the receptive field (RF) size of the frog neurons [$(18.5 \pm 3.8)^\circ$, $n=33$] was smaller than those of collision-sensitive neurons of the locust and the pigeon. Multielectrode recordings also showed that the collision-sensitive neurons were activated only when the focus of expansion of a looming retinal image was located within the center of its RF. There was a linear relationship between the parameter l/v (l denotes half-size of the object, v denotes approaching velocity) and time-to-collision (time difference between the peak of the neuronal activity and the predictive collision) in 16 collision-sensitive neurons. Theoretical consideration showed that the peak firing rate always occurred at a fixed delay of (60.1 ± 39.5) ms ($n=16$) after the object had reached a constant angular size of $(14.8 \pm 3.4)^\circ$ ($n=16$) on the retina. **Conclusion** The results may help clarify the mechanisms underlying the collision avoidance behavior in bullfrog.

Keywords: frog; optic tectum; collision-sensitive neurons

1 Introduction

For many species of animals, the image of an approaching object typically elicits the escape or avoidance behavior. Encounter with a predator or collision with a stationary ob-

ject is potentially lethal. Thus, visually guided collision avoidance behavior is generally an adaptive behavior that has evolved in many kinds of animals including the human, under strong selective pressure^[1-3]. Therefore, it is expected that a common mechanism underlying this activity may exist in different animals. Indeed, it has been clear that, at the behavioral level, 2 different strategies are involved in such an important task. The first strategy is to use estimated time-to-collision as a sensory cue for avoidance, and the second

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is to use a threshold value for the size of the retinal image subtended by an approaching object as a cue for avoidance^[4-6]. On the other hand, collision-sensitive neurons that perform common neuronal computation have been found in different animals, such as locusts and pigeons^[7-14]. Therefore, investigating the neuronal mechanisms underlying this important adaptive behavior may help reveal the general principles of information processing in brain.

To understand the neuronal mechanisms underlying a particular behavioral property, both behavioral and neurophysiological studies are required, and the relationship between behavior and neurophysiology should be clarified. Behavioral strategy for collision avoidance has been established in many animal species. However, in many of these animals, the essential neuronal mechanisms have been ignored^[4-6,16]. On the other hand, for some species, although putative collision-sensitive neurons have been found, the corresponding quantitative behavioral study has not been performed^[17,18]. Only in the locust have both behavioral and neurophysiological studies been carried out, and both studies have shown the importance of retinal threshold size in eliciting collision avoidance behavior^[7,8,19-23]. Unfortunately, there has not been any comparable study in vertebrate species. Very little is known about collision avoidance behavior of the pigeon in which collision-sensitive neurons in the brain have been most intensively investigated. A previous study has shown that when approaching a perch, the pigeon initiates foot extension when time-to-collision reaches a threshold of approximately 150 ms^[24]. Moreover, collision-sensitive neurons (τ neurons) that signal the time-to-collision of approaching objects are found in the nucleus rotundus in pigeon. However, the response properties of the neurons are not necessarily consistent with visual information processing during pigeon landing^[12,13,25].

We have investigated collision avoidance behavior of frog that shows stereotyped action patterns and has a rather simple central nervous system. Results have shown that frog displays collision avoidance behavior when the visual angle of a looming object reaches a threshold of about 20°^[26]. Although the possible collision-sensitive neurons have been reported, a quantitative study of the neurons is also required,

to allow comparison with the corresponding behavioral strategy. Putative collision-sensitive neurons of the frog are found in the optic tectum and the caudal thalamus^[27,28]. The collision-sensitive neurons were not found in the pigeon optic tectum until recently. In the study of Wu LQ *et al.*, 3 classes of collision-sensitive neurons were found in the pigeon optic tectum, just like those in the nucleus rotundus^[14]. The present study was aimed to identify the tectal neurons that respond selectively to an approaching object on a collision course, to clarify the tectal neuron-coded parameter of the collision stimulus, and to find out the relationship between the result and the corresponding behavioral strategy based upon retinal threshold size.

2 Materials and methods

2.1 Animals Adult bullfrogs, *Rana catesbeiana* (of either sex, 12-14 cm of body length), were used in the experiments. Animals were kept in laboratory tanks under a 12:12 h dark-light cycle before use. They were fed on chicken liver or crickets twice a week.

2.2 Preparations The bullfrog was anesthetized by s.c. injection of tricaine methanesulfonate (MS222, Sigma; 0.02 mg/g body weight) and immobilized by injection of succinylcholine (Succin, Yamanouchi; 0.02 mg/g body weight). Then the animal was placed on a metal frame, and conventional procedures were carried out to expose the left optic tectum. After the surgery, the frog was carefully positioned with its longitudinal body axis parallel to the computer monitor that was placed 10 cm from the animal. Several simulated looming visual stimuli were presented to the right eye, while the left, non-stimulated eye was covered by an opaque occluder. The frog's body skin was kept moist with a piece of thin wet gauze throughout the experiment, to facilitate cutaneous respiration. All the procedures were conducted at room temperature (18-22 °C).

2.3 Visual stimulation For multielectrode recording and single electrode recording, 15 and 13 kinds of visual stimuli described below were presented, respectively, in the lateral visual field of the animal. The stimulus animations were run on a PC Pentium IV 2.8 GHz equipped with NVIDIA GeForce FX5800 Graphics Controller and FlexScan L767 19-inch Multi-

Scan Color Monitor (EIZO, Japan) set to a refresh rate of 75 Hz. The background and the square (the stimulus) had an average luminance of 110 lux and 8 lux, respectively. The size of the square was $10 \times 10 \text{ cm}^2$, which corresponded to a visual angle of 53.1° . This size was much larger than the threshold angle of looming stimulus (about 20°), which was demonstrated in our previous behavioral experiments. In the multielectrode recording experiments, 15 kinds of visual stimuli were presented to the right eye of the frog, including collision (a $10 \times 10 \text{ cm}^2$ black square approaching at a velocity of 2 m/s along a path of 2 m on a direct collision course against white background), recession (a $10 \times 10 \text{ cm}^2$ black square receding at a velocity of 2 m/s along a path of 2 m on a direct collision course against white background), x-10 deviation (a $10 \times 10 \text{ cm}^2$ black square approaching at a velocity of 2 m/s along a path of 2 m on trajectories deviating from a direct collision path in the horizontal plane against white background. The final position of square was 10 cm to the left of the central axis. A 10 cm deviation represented a 3° shift of the midpoint of the square away from a direct collision path), x+10 deviation (the final position of square was 10 cm to the right of the central axis), y+10 deviation (the final position of square was 10 cm above the central axis), y-10 deviation (the final position of square was 10 cm below the central axis), anterior collision (the same as the collision, except that the initial position of the square was 5 cm to the left), posterior collision (the same as the collision, except that the initial position of the square was 5 cm to the right), upper collision (the same as the collision, except that the initial position of the square was 5 cm above), anterior recession (the same as recession except that the initial position of the square was 5 cm to the left), posterior recession (the same as recession except that the initial position of the square was 5 cm to the right), upper recession (the same as recession except that the initial position of the square was 5 cm above), reversed collision (the square and the background of collision stimulus were reversed to be white and black, respectively), reversed recession (the square and the background of recession stimulus were reversed to be white and black, respectively), and contrast (the same as collision stimulus except that the brightness of the square gradually changed from white to black).

These simulated approaching or receding objects, with 2-dimensional expansion or contraction, were presented with an interval of at least 2 min to allow recovery of the cells.

In the single electrode recording experiment, 13 kinds of visual stimuli were presented to the right eye of the frog. Also, the 2-dimensional expansion or contraction of the stimulus square was involved. These stimuli included collision, recession, x-10 deviation, x+10 deviation, y+10 deviation, y-10 deviation, reversed collision, reversed recession, contrast, and collision with a square approaching at a velocity of 0.5 m/s, 1 m/s, 3 m/s, or 4 m/s. These stimuli were presented with an interval of at least 2 min to allow recovery of the cells from any adaptation. For each kind of stimulus, 3 successive stimuli were presented with an interval of at least 30 s between trials.

To measure the size of receptive field (RF) of collision-sensitive neurons, another kind of visual stimulus was used. A black circle expanding with a constant velocity ($72^\circ/\text{s}$) was presented to the right eye of the animal. Based on the interval between the first and the last spikes of the responses (t_0), the angular size of RF was calculated using the following equation: $\text{RF (deg)} = t_0 * 72$.

2.4 Recordings Neuronal activity was recorded at the depth of 200-800 μm using multielectrode and single electrode methods. In the multielectrode array (Bionic Technologies), electrodes (1.0 mm long, with active tips of 50 μm in length and impedances of 100 k Ω) were mounted in a 5×5 array, with electrode spacing of 400 μm . The square array plate was suspended by a flexible cable from an adjacent input circuit board next to the preparation. With a long micromanipulator probe attached to the cable, the array could be positioned carefully onto the optic tectum surface. Penetration was accomplished by bringing the flat tip of a glass tube to bear against the back of electrode array. The glass tube was attached to a second micromanipulator and driven precisely by a stepper-motor. Firstly, the glass tube was advanced to insert the electrode array into the brain and then the array cable was advanced to an equal distance manually. The depth of the array electrodes was controlled by the manipulators to an accuracy of 1-5 μm . The neuronal activity across the retino-tectal map was amplified with a 16-channel differential pream-

plifier (on channels 1-15, Bionic Technologies). The output was displayed on the screen of an oscilloscope (CS-4125 KENWOOD), and was directly recorded and saved in a computer (PC Pentium(r) II 3 GHz equipped with 128 MB of RAM) for the subsequent analysis with WinAcq Neural Data Acquisition Program.

In the single electrode recordings, a glass-coated tungsten electrode was used. Neuronal responses were amplified by a preamplifier (DPA102E DIA MEDICAL) and an extracellular amplifier (DPA-100 DIA MEDICAL), and were fed to an oscilloscope (CS-4125 KENWOOD). Data were stored on a data recorder with 4 channels (PC204Ax SONY) and were digitized and processed with the software for spike interval analysis (LaBDAQ2000).

2.5 Data analysis Data of multielectrode recordings were analyzed by using WinAcq Neural Data Acquisition Program (Bionic Technologies). It allowed selection of 16 channels of an appropriate combination of neural and stimulus signals for recording. When recordings were taken from a microelectrode array penetrated into neural tissues, many electrode channels typically show the activities of more than one neuron. Another program 'Classify' (Bionic Technologies) was used to identify the individual neuron, based on the recorded waveform shapes. Data were processed by the following procedures. Firstly, the multichannel data were saved in 'Winacq' as an '.RND' file, and action potentials were then extracted using threshold amplitude discrimination ($\pm 58 \mu\text{V}$). The extracted action potentials were saved as a '.NEV' file for further analysis. Secondly, the classification of single unit on each recording channel, using user-generated time-amplitude templates, was completed and the results were saved in 'Classify' as a '.NEV' file. Thirdly, with 'Nev2txt' program (Bionic Technologies), the '.NEV' file of single unit was transformed into the corresponding text file. Finally, the text files were used to calculate a post-stimulus time histogram (PSTH) by means of a VC++ program developed in our laboratory.

The data from single electrode recordings were analyzed by using 'LaBDAQ2000' program (Corporation of Matsuyama Advan). Data kept in DAT tapes were fed into a computer by an A/D board (ADM-682PCI, Corporation of

Matsuyama Advan), where the data were sampled at 10 kHz and saved as a text file. To extract single unit activity based on amplitude and to construct a PSTH, another local laboratory VC++ program was used. From the PSTH of single unit spikes, the time-to-collision was estimated according to the peak firing time. Based on the correlation of time-to-collision versus visual parameter l/v (l : object half size, v : approaching velocity), a threshold angular size of stimulus producing the peak response of a collision-sensitive neuron was calculated^[7].

Data were presented as mean $\pm$ SD. Statistical difference of spike number between collision stimulus and other stimuli were analyzed by Student's *t*-test.

3 Results

3.1 Multielectrode recordings

3.1.1 Directional sensitivity of collision-sensitive neurons

Multi-unit activities (MUAs) of tectal neurons which showed vigorous responses to an expanding retinal image were recorded in 59 trials from 32 different animals. Of the 59 MUAs, 39 (in 25 animals) showed a higher frequency of discharge in response to an approaching object on a direct collision course than to the deviation and recession stimuli. Classification of single unit from these 39 MUAs revealed 66 single-unit activities (SUAs), among which 40 neurons (22 animals) showed discharges to direct collision stimulus more than twice as many as to different kinds of visual stimuli. Responses of typical collision-sensitive neurons to different stimuli were shown in Fig. 1. The responses of these 40 neurons were further analyzed quantitatively. Fig. 2 illustrated the average number of spikes obtained from the 40 collision-sensitive neurons in response to various visual stimuli. The number of spikes in response to collision stimulus was statistically different from the numbers of spikes in response to other stimuli ($P < 0.01$) except the reversed collision stimulus. The results showed that these collision-sensitive neurons could selectively respond to objects approaching on a direct collision course, but not to other deviational approaching or recessing objects.

3.1.2 Time course of response of collision-sensitive neurons

The summed number of spikes in 50-ms bin width was calculated from responses of 40 collision-sensitive neurons

Fig. 1 Responses of a typical collision-sensitive neuron to an object approaching on a direct collision course, near-miss trajectories (x-10: anterior, x+10: posterior, y+10: upper, y-10: lower) and a receding object. For each visual stimulus, the upper and the middle traces showed isolated neuronal activities and voltage changes monitoring size of approaching or receding objects, respectively, while the lower trace showed the PSTH. Scale bars: horizontal, 500 ms, vertical, 5 spikes.

Fig. 2 The number of spikes per response obtained from 40 collision-sensitive neurons in response to various visual stimuli. The number of spikes in response to the collision stimulus was statistically different from those in response to other stimuli, except for the reversed collision stimulus. These results indicated that the collision-sensitive neurons could respond selectively to approaching objects on a direct collision course, but not to a decrement of brightness during approach. Besides, the spike can not be triggered by an approaching object unless the focus of expansion of the image was located at the center of its RF. The strong response to the second collision stimulus indicated no habituation to stimuli presented with an interval of 2 min. * $P < 0.01$ vs collision, # $P < 0.01$ vs 2nd collision.

Fig. 3 The PSTH obtained from the 40 collision-sensitive neurons in response to an approaching object on a direct collision course. Summed number of spikes within a bin width of 50 ms was calculated. Time of 0 ms was identified as the time of collision. At about 100 ms before the predictive collision, the spike frequency reached a peak and then decreased.

and plotted against time-to-collision (Fig. 3). The response profile of the collision-sensitive neurons to looming objects was quite distinctive. However, it was hard to determine which parameter (retinal threshold size or time-to-collision) the collision-sensitive neurons code, based on the plotted data. The neurons started firing early during the approach phase and the firing rate gradually increased as the object became larger, as if the cells were “tracking” the object over the approach. At about 100 ms before predictive collision, the firing rate reached a peak and then rapidly decreased. That is to say, when the approaching object had reached a specific visual angle on the retina, firing rate no longer increased even if the object continued growing larger until the final cessation on the screen. The response profile was very similar to those of lobula giant movement detector (LGMD) in the locust and η neurons in the pigeon^[14,26] which code a specific angular threshold on the retina. To clarify this point, the following single electrode experiments were conducted to examine the linear relationship between visual parameter l/v and time-to-collision by changing the approaching velocity.

3.1.3 RF property and depth distribution of collision-sensitive neurons The average RF-size for collision-sensitive neurons was $(18.5 \pm 3.8)^\circ$ ($n = 33$), smaller than those of looming-sensitive neurons reported in locusts and pigeons (approximately 180° and 110° , respectively)^[29,30]. The relationship between the focus of expansion of the looming stimulus and the center of RF of the collision-sensitive neuron that

responded to the visual stimulus was examined based on the multielectrode recording data. Here, 'RF order' of each recording channel was defined as the order in which the neuronal response was evoked in the specific channel during image expansion on the retina. Fig. 4 showed that the collision-sensitive neurons which were recorded with the channel had a specific RF order. Clearly, most of the collision-sensitive neurons had a RF order of 1, indicating that the collision-sensitive neurons were activated only when the foci of the expanding images were located within the RF center of these neurons. In other words, the activated collision-sensitive neurons were mainly located close to the tectal region corresponding to the focus of expansion of retinal images in the retinotectal map. The results suggest that the collision-sensitive neurons can not respond to an approaching object unless the focus of retinal image expansion is located at the center of RF. This hypothesis was further supported by the observation that collision-sensitive neurons did not show any response to a collision stimulus coming from the anterior, posterior or upper visual field (Fig. 2).

The depth distribution of the 40 collision-sensitive neurons in the optic tectum of frogs ranged from 350 μm to 750 μm (Fig. 5). However, most of the neurons (77.5%) were located within the range of 450–600 μm below the dorsal surface of the optic tectum, corresponding to layers 7 and 6.

3.2 Single electrode recordings

3.2.1 Directional sensitivity and depth distribution of colli-

Fig. 4 The collision-sensitive neurons recorded with the electrode had a specific RF order. RF order indicated the order in which the neuronal responses were evoked with a particular electrode during expansion of the retinal image. Almost all the collision-sensitive neurons had a RF order of 1.

Fig. 5 Depth distribution of collision-sensitive neurons in the frog optic tectum revealed by multielectrode recordings.

sion-sensitive neurons Since the presence of collision-sensitive neurons in the frog optic tectum was confirmed, single electrode recordings were employed to examine the response characteristics of these neurons in detail. A total of 16 collision-sensitive neurons were recorded in the optic tectum in 14 different frogs. Consistent with the multielectrode results, the 16 neurons showed selective responses to a direct collision stimulus rather than to deviation and recession stimuli (Fig. 6). The neuronal response to the direct collision stimu-

Fig. 6 The number of spikes obtained from 16 collision-sensitive neurons in response to various visual stimuli. The number of spikes in response to the direct collision stimulus was significantly different from those in response to other stimuli ($P < 0.01$) except the reversed collision stimulus and the collision stimulus at various velocities. These results indicated that the collision-sensitive neurons could respond selectively to objects approaching at various velocities on a direct collision course, but not to a decrement of brightness during approach. The strong response to the second collision stimulus excluded habituation to stimuli presented with an interval of 2 min. * $P < 0.01$ vs collision, # $P < 0.01$ vs 2nd collision.

lus was significantly different from those to other stimuli ($P < 0.01$) except the reversed collision stimulus and the collision stimulus at various velocities. Also, the neurons did not show any response to contrast change, demonstrating that they did not respond to a decrement of brightness accompanied with expansion of the retinal image.

The 16 collision-sensitive neurons were mainly distributed at the depth between 450-650 μm in the tectal layers (Fig. 7), consistent with the multielectrode results (Fig. 5).

3.2.2 Linear relationship between time-to-collision and visual parameter l/v The relationship between the 2 essential visual parameters related to collision avoidance, l/v and time-to-collision, was examined. l/v is an important parameter that defines the time course of retinal image expansion during object approaching. l denotes the half size of the object, and v denotes the approaching velocity. According to the equation used for calculating kinematics of object approach^[7], theoretical consideration shows that if time-to-collision (time difference between the peak of the neuronal activity and the predictive collision) and l/v are linearly related, the peak of firing rate should occur with a fixed delay after the object has reached a constant angular size on the animal's retina.

Through changing the velocity of approaching objects (0.5, 1, 2, 3, and 4 m/s, respectively), time-to-collision and l/v were demonstrated to be linearly related ($R^2=0.9988$) (Fig. 8). Theoretical consideration showed that, in this case, the peak firing rate always occurred after the approaching object had reached a visual angle (θ_{thres}) of 15.1° on the frog's retina, with a fixed delay of 66.7 ms. Similar results were observed in

Fig. 7 Depth distribution of collision-sensitive neurons in the frog optic tectum revealed by single electrode recordings.

Fig. 8 Plot of the peak time relative to predicted collision as a function of visual parameter l/v . A strong linear relationship was found between the two parameters. Theoretical consideration showed that the peak firing rate occurred after the retinal image of an approaching object had reached a visual angle of 15.1° with a delay of 66.7 ms.

all the 16 neurons. The results showed that the linear relationship between l/v and time-to-collision was very strong. The results also clearly showed that the peak of firing rate always occurred with a fixed delay of (60.1 ± 39.5) ms after the object had reached a constant angular size of $(14.8 \pm 3.4)^\circ$ ($n=16$) on the retina, which were consistent with our previous behavioral data^[30].

4 Discussion

From the data of multi- and single-electrode recordings, we could draw 3 main conclusions. Firstly, collision-sensitive neurons existed in the frog optic tectum. Secondly, these neurons possessed a smaller RF than those reported in the pigeon and in the locust, and did not respond to a looming object unless the focus of image expansion was located at the center of its RF. Thirdly, the maximum response activity occurred when the approaching object had reached a specific visual angle of $(14.8 \pm 3.4)^\circ$ with a fixed delay of (60.1 ± 39.5) ms.

4.1 Collision avoidance behavior and the neuronal correlates in different animals The image of an approaching object typically elicits collision avoidance behavior in many kinds of animals. Infant and adult Rhesus Monkeys show

persistent avoidance responses to optical stimulus of looming^[3]. Besides, 3 different kinds of turtles all exhibit head withdrawal when a circular shadow is suddenly enlarged in front of them^[2]. Even in human, infants respond to symmetrically expanding shadows with an integrated avoidance response that consists of an initial widening of the eyes, a head withdrawal and a raising of the arms, and upset^[1,31].

Presently, several sensory cues have been discovered to elicit escape or collision avoidance behavior. Holmqvist *et al.* showed that darkening contrast produced by an approaching object is crucial for eliciting the visually evoked escape response in the fly^[16]. More commonly, animals use estimated time-to-collision and looming retinal image size as sensory cues for avoidance. The remaining time before the predictive collision (time-to-collision)^[32] must reach a threshold value before escape is triggered. The time-to-collision is given by the ratio of retinal expansion velocity to retinal size, the optical parameter $\tau(t)$. Even in conditions of acceleration, animals use the optical parameter $\tau(t)$ to time their actions adaptively. The gannet starts streamlining after detecting that the parameter has reached a critical value during the plunge dive^[4]. The pre-landing deceleration in flies is triggered when the parameter reaches a critical value^[6]. Humans also use this parameter when playing ball games or driving a car^[32,33], by means of a visual pathway that is sensitive to time-to-collision^[34].

The second common sensory cue that triggers escape is that the size of the retinal image subtended by an approaching object (visual angle) reaches a threshold value. In both fiddler crabs and chicks, avoidance responses will be initiated when an approaching object has reached a visual angle of 30° – 35° ^[5]. Collision avoidance of flying locusts is initiated when the targets subtended more than 10° in the insect's field of view, and not at a constant time-to-collision^[26].

Putative collision-sensitive neurons have been found in several animal species. In monkeys, cells in the cortex of the posterior bank of the superior temporal sulcus respond to two edges at a given orientation moving towards or away from each other within the RF^[20]. Single neuron or a population of neurons in area 18 of cat visual cortex are capable of signaling changing-size information^[17]. In the hawk moth, 2

classes of looming-sensitive neurons are thought to collaborate to provide information that would regulate the flight motor to maintain distance from a target during hovering flight^[19].

A recent study has found that motion towards the human observer, compared with motion away, shows, significant bilateral activations in the lateral inferior occipital cortex and in right lateral superior occipital cortex, as revealed by the functional magnetic resonance imaging^[35]. However, so far, close investigation of collision-sensitive neurons which may enable better understanding of the neuronal correlate of collision avoidance behavior has been restricted to only 2 species of animals, the pigeon^[14,29,36] and the locust^[9,10,12,37-41].

Three types of collision-sensitive neurons have been found in the nucleus rotundus of pigeons: One group of neurons signal the ratio of retinal expansion velocity to retinal size (τ neurons), the second group signal absolute rate of expansion (ρ neurons), and the third group signal yet another optical variable to encode retinal threshold size (η neurons)^[14]. The 3 classes of collision-sensitive neurons have also been found in the pigeon tectal layer 13 recently^[15]. In order to land successfully, the pigeon must regulate visually its speed of approach to a perch, and time appropriately the extension of its feet. Behavioral experiment indicates that pigeons initiate foot extension when τ reaches a threshold value of approximately 150 ms^[28]. At first sight, τ neurons appear to control the foot extension during landing. However, the following observations are inconsistent with this hypothesis. The first is that τ neurons do not respond to a simulated approach toward stationary objects as in the case of pigeon landing^[14,29]. The second is that the maximum responses of τ neurons are elicited 800 to 1400 ms, with a modal value of 1000 ms, before the stimulus would contact the bird^[29]. Thus, even in the pigeon, the neuronal correlate of collision avoidance behavior remains unclear.

Correlation between collision avoidance behavior and the collision-sensitive neurons has been well demonstrated only in the locust. The collision-sensitive neurons in the locust are known as the LGMD and the descending contralateral movement detector (DCMD)^[9,10,12,38,39,41]. Peak DCMD activity occurs with a fixed delay (15–35 ms) after the approaching object has reached a specific angular thresh-

old on the retina (15° – 40°)^[7,8]. Consistent with the role of DCMD in triggering collision-avoidance behavior, observations of tethered, flying locusts indicate that the steering maneuvers used to avoid an approaching object are triggered when the image of the object subtends more than 10° rather than at a particular time before collision^[26,42]. The DCMD axon descends into the thoracic ganglia and makes connections to flight interneurons and motoneurons^[43-45]. Thus, the DCMD is a good candidate for mediating visually guided steering during flight. However, with combined behavioral and physiological experiments, Gray *et al.* showed that the discharge characteristics of the DCMDs were not obviously related to collision avoidance steering and suggested that they may be better suited for a role in predator evasion^[46]. Santer *et al.* show that there is a close match between the visual stimuli that elicit gliding behavior and those that are effective at exciting the DCMD neurons^[23]. They also demonstrate that wing elevation leading to the gliding posture occurs during a maintained burst of high frequency DCMD spikes. Furthermore, the different rates of habituation of the DCMD are shown to be correlated with a change in predator escape strategies of solitary and gregarious locusts^[47].

4.2 Collision avoidance behavior and collision-sensitive neurons in the frog As described above, physiological studies on collision-sensitive neurons have been mainly performed independently of behavioral experiments in different animal species. Even in the same animal, physiological properties of collision-sensitive neurons are not necessarily consistent with characteristics of collision avoidance behavior of the animal. To understand the neuronal correlates underlying collision avoidance behavior, both behavioral and electrophysiological experiments were performed on the bullfrog, *Rana catesbeiana*^[30,48]. Here the frog is selected as a model for study based on the following reasons. Firstly, collision avoidance behavior is mainly visually guided and the characteristic is very conservative among animal species. Secondly, the collision avoidance behavior of frog is a relatively stereotyped all-or-nothing event that can be easily detected and the behavioral parameters can be measured with considerable precision. Thirdly, the optic tectum (the optic

center) is easily accessible and the precise retinotopic projection from the eye to the contralateral tectal surface can be easily recognized^[49-51].

Schiff W show that the frog collision avoidance behavior can be elicited visually with a two-dimensional rapid magnification of a continuous silhouette by using a shadow-casting device^[5]. Ingle and Hoff adopted a black square with 4 sides of 8-10 cm long moving toward the frog as the visual stimulation and found that the animal produced avoidance behavior when the retinal image expanded beyond a visual angle of 30° , although the data were not shown^[48]. Our previous experiment using computer graphics has confirmed Schiff's observation^[5]. We have also shown that the frog displays avoidance behavior when the visual angle of approaching objects, not time-to-collision, reaches a critical value, the retinal image size of about 20° ^[30]. Moreover, removal of the optic tectum can abolish any response to approaching objects^[53]. Based on these behavioral results, it should be possible to find collision-sensitive neurons with thresholds in angular size of about 20° in the frog optic tectum.

Hemisection of either the posterior or the anterior portion of the optic chiasm causes a selective loss of behavioral responses to a large looming stimulus, but not to a prey stimulus anywhere in the visual field. This suggests that visual information concerning prey and threatening objects is mediated by separate optic nerve fiber systems^[54]. Furthermore, unilateral injection of tetrodotoxin or cadmium chloride into the frog optic nerve could selectively abolish the avoidance behavior to a looming object, while prey orienting behavior remains unaffected. This selective loss of behavior is correlated with the loss of activity of R4 retinal ganglion cells, the dimming detectors^[55]. A recent study has revealed a very strong correlation between synchronized oscillatory activity among retinal dimming detectors and collision avoidance behavior of the frog, using behavioral experiments and multielectrode recordings^[56]. Thus, it is very likely that the dimming detectors play an important role in eliciting collision avoidance behavior. However, the dimming detectors never exhibit major characteristic of collision-sensitive neurons, which is a directional selectivity to an approaching object on a collision course^[57,58].

Putative collision-sensitive neurons of the frog have been found in the optic tectum and the caudal thalamus. Tectal neurons T3 respond vigorously to approaching objects, contrast to their low sensitivity to receding objects or objects moving around the animal. The excitatory RFs are located predominantly in the nasal part of the visual field and extended over 20° – 30° ^[32]. Pretectal TH6 neurons are also sensitive to looming objects, especially the objects from the dorsal visual field. The excitatory RF includes the entire visual field^[31]. Unfortunately, the tightness of tuning for collision trajectories of these putative collision-sensitive neurons has not been examined quantitatively. The particular optical parameters of approaching objects have not been determined, either.

In the present study, the tuning property of frog collision-sensitive neurons was examined and results showed that the spiking response declined dramatically if the object's trajectory deviated from a direct collision course by 3° . Moreover, the peak firing rate of the collision-sensitive neurons always occurred with a fixed delay after the object had reached a threshold angular size of about 15° on the retina, which was consistent with our behavioral observation. Thus, we conclude that the frog collision-sensitive neurons found in the present study may serve as valuable candidates for elucidating the neuronal correlates underlying collision avoidance behavior of the frog.

Animals should detect imminent collision with an approaching object on a collision course, which comes from anywhere in the visual field. For this goal, the collision-sensitive neurons should have tight tuning for collision trajectories. Furthermore, the neurons should have extremely large RFs and show the same strong response over the entire RF, as long as one object is on a collision course.

Two hypotheses have been proposed to explain the collision-specific responses of the LGMD. In the first model, the LGMD multiplies postsynaptically two inputs, angular velocity (θ) and angular size ($e^{-\alpha\theta}$). Using a logarithmic transformation, such multiplication might be accomplished by linear summation of $\log\theta$ and $-\alpha\theta$ on the dendritic tree of the LGMD. This computation is followed by exponentiation through active membrane conductances^[7,9,22]. The other hy-

pothesis is the so-called 'critical race'. The race is between the excitation over the dendritic fan of the neuron, caused by movement of the edges of the expanding image, and the lateral inhibition that occurs between the retinotopic afferent neurons from the medulla. The vigorous responses of the LGMD occur in the final stages of object approach where excitation passing down the network overtakes inhibition directed laterally^[57,58]. Invariant computation of angular threshold to changes of object approach angle was suggested to arise in part from non-linear integration of excitatory inputs within the dendritic tree of the LGMD^[8].

On the other hand, in vertebrates, the neuronal mechanisms underlying collision-sensitive responses remain largely unknown. Sun *et al.* have developed a neuronal model to explain the physiological response properties of pigeon collision-sensitive neurons^[13]. As explained by their model, the RF of a rotundal collision-sensitive neuron is composed of radial arrangement of concentric arrays of RFs of tectal neurons. The tectal neurons respond to movements oriented radially from the center of the concentric array and converge onto the rotundal neurons. Thus, the rotundal neurons would selectively respond to a collision stimulus with the center of expansion overlapping the center of RF radial layout. This arrangement is shown schematically in Fig. 9A. However, in this study, we demonstrated that some tectal neurons of the frog showed vigorous responses only to a collision stimulus, but not to a straight front parallel movement in a particular direction. We also showed that the collision-sensitive neurons had rather small RFs and were excited by the looming object only when the expansion focus of the image was located at the center of the RF. Even in the pigeon, the 3 classes of collision-sensitive neurons in the optic tectum use the same computations found in rotundal τ , ρ and η cells, respectively^[15]. Based on these experimental results, we propose an alternative neuronal model to explain the physiological response properties of frog collision-sensitive neurons. In our model, the RF of a thalamic collision-sensitive neuron is composed of a radial arrangement of concentric arrays of RFs of tectal neurons. The tectal neurons respond to looming stimuli that are expanded from the center of RF and converge onto the thalamic neurons. Thus, the thalamic colli-

Fig. 9 Two neuronal models to explain how tectal inputs integrate and build up the wide RF of thalamic collision-sensitive neurons. An outer large circle represents the RF of a thalamic collision-sensitive neuron. A series of concentric circles represent RFs of tectal neurons. **A:** The model proposed by Frost and Sun suggests that the tectal neurons selectively respond to movements that are oriented radially from the center of the concentric array. **B:** Our model based on the present results suggests that the tectal neurons respond selectively to looming stimuli that are expanded from the center of their RFs independent of the locations.

sion-sensitive neurons could detect objects approaching on a collision course independent of the approaching angle. This arrangement is shown schematically in Fig. 9B. Frost's model and ours correspond to a direction mosaic hypothesis and a vector field hypothesis, respectively, which are well-known hypotheses for optic flow selectivity^[59,60]. Based on the experimental results obtained from the pigeon and the frog, we conclude that the RF organization of a collision-sensitive neuron in the midbrain is more consistent with the vector field hypothesis.

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青蛙视顶盖碰撞敏感细胞的反应特性和受容野结构的研究

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摘要: **目的** 许多研究显示, 当一个靠近的物体在视网膜的成像达到一定阈值时, 动物即表现出逃避行为。本研究旨在探讨青蛙的这种复杂的神经活动机理。**方法** 用计算机模拟不同类型的视觉刺激, 并用多电极和单电极记录方法来记录碰撞刺激引起的视顶盖细胞的神经活动。**结果** 多电极记录显示视顶盖神经细胞对碰撞刺激的反应具有选择性, 且反应特性相似于蝗虫的小叶大运动侦察器神经元(lobula giant-movement detector, LGMD)和鸽子的 η 碰撞神经细胞。但与这些细胞相比, 视顶盖神经细胞受容野的大小相对较小。单电极记录研究了这些碰撞敏感细胞的视觉参数。其视觉参数 $1/v$ 和即将碰撞时间(time-to-collision)之间存在线性关系。**结论** 这些结果进一步证实了青蛙视顶盖的碰撞敏感细胞的神经活性与一定的视网膜成像大小有关, 有助于进一步阐明青蛙逃避行为的内在机制。

关键词: 青蛙; 视顶盖; 碰撞敏感细胞