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The collision-sensitive neurons in the optic tectum of the bullfrog, Rana catesbeiana

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Running head: collision-sensitive neurons of the frog

Abstract

In this study, we examined the neuronal correlates of frog collision avoidance behavior. Single unit recordings in the optic tectum showed that eleven neurons gave selective responses to objects approaching on a direct collision course. The collision-sensitive neurons exhibited extremely tight tuning for collision bound trajectories with mean half-width at half height values of 0.8° and 0.9° (n=4) for horizontal and vertical deviations, respectively. The response of frog collision-sensitive neurons can be fitted by a function that simply multiplies the size dependence of its response, $e^{-\alpha\theta(t)}$,by the image's instantaneous angular velocity $\theta'(t)$. Using fitting analysis, we showed that the peak firing rate always occurred after the approaching object had reached a constant visual angle of 24.2° (SD, 2.6) (n=8), regardless of the approaching velocity. Moreover, a linear relationship was demonstrated between parameters l/v (l: object's half-size, v: approach velocity) and time-to-collision (time difference between peak neuronal activity and the predicted collision) in the eleven collision-sensitive neurons. In addition, linear regression analysis was used to show that peak firing rate always occurred after the object had reached a constant angular size of 21.1 ° on the retina. The angular thresholds revealed by both theoretical analyses were comparable and showed a good agreement with that revealed by our

previous behavioral experiments. This strongly suggests that the collision-sensitive neurons of the frog comprise a threshold detector, which triggers collision avoidance behavior.

Introduction

For many animal species, an image of an approaching object typically elicits avoidance behavior, presumably because encounter with a predator or collision with a stationary object is potentially lethal. This strong selection pressure has led to the evolution of visually guided collision avoidance behavior in many kinds of animals, including humans (Ball and Tronick 1971; Bower et al. 1970; Hayes and Saiff 1967; Schiff et al. 1962). Therefore, it is expected that common behavioral strategies and neuronal mechanisms might be at work in different animals. At the behavioral level, two different strategies are typically used to perform such an important task. The first is to use estimated time-to-collision as a sensory cue for avoidance. The second is to use a threshold value for the size of the retinal image subtended by an approaching object as the cue for avoidance (Lee et al. 1981, 1993; Schiff 1965; Wagner 1982).

Time-to-collision, τ (t), is the ratio of retinal expansion velocity to retinal size under constant closing velocity (Lee, 1976). The following behavioral examples are elicited when τ (t) reaches a critical value: onset of streamlining in the gannet during the plunge dive when attacking fish schools (Lee and Reddish 1981), onset of pre-landing deceleration in the fly, and onset of pre-landing foot extension in the pigeon (Lee et al. 1993; Wagner 1982). Humans also use this parameter, when playing ball games or

driving a car (Lee et al. 1976, 1985), by means of a visual pathway that also is sensitive to time-to-collision (Regan and Hamstra 1993).

The size of the retinal image subtended by an approaching object is the cue used in the fiddler crab and in chicks. When the visual angle of an approaching object reaches 30 – 35°, an avoidance response appears in both species (Schiff 1965). Also, collision avoidance in flying locusts is initiated when the target subtends more than 10° in the animal's field of view, whereas a constant time to collision is not used (Robertson and Johnson 1993a,b).

To understand the neuronal mechanisms underlying a particular behavior, both behavioral and neurophysiological studies in the same animal species are required to demonstrate an inter-relationship. While behavioral strategies for collision avoidance have been established in many animal species, the corresponding neuronal mechanisms in these species essentially have been ignored (Holmqvist and Srinivasan 1991; Lee and Reddish 1981; Schiff 1965; Wagner 1982). On the other hand, putative collision-sensitive neurons which could mediate the above behaviors have been reported in several animal species including monkey, cat, hawk moth, crab and even humans, but in many of these cases, the corresponding quantitative behavioral study has not been performed (Oliva et al. 2007; Regan and Cynader 1979; Wicklein and Strausfeld 2000;

Wunderlich et al. 2002; Zeki 1974).

Only in the locust have intensive behavioral and neurophysiological studies been carried out at the same time. The neurophysiological results center on two collision-sensitive neurons in the locust known as the lobula giant movement detector (LGMD) and the descending contralateral movement detector (DCMD) (Hatsopoulos et al. 1995; Judge and Rind 1997; Rind and Simmons, 1992, 1997, 1999; Schlotterer 1977; Simmons and Rind 1992). The DCMD exhibits extremely tight tuning to an object approaching on a direct collision course (Judge and Rind 1997). Peak DCMD activity occurs with a fixed delay (15 – 35 ms) after the approaching object has reached a specific angular threshold on the retina (15 – 40°) (Gabbiani et al. 1999, 2001). Thus, neurophysiological studies also have shown the importance of retinal threshold size in eliciting collision avoidance behavior (Gabbiani et al. 2002, 2004). The following observations further support the hypothesis that DCMD plays a major role in the locust collision avoidance behavior. The DCMD axon descends into the thoracic ganglia and makes connections to flight interneurons and motoneurons (Burrows and Rowell 1973; O'Shea et al. 1974; Simmons 1980), and thus seemed to be a good candidate for mediating visually guided steering or predator evasion during flight (Gray et al. 2001; Robertson and Johnson 1993a,b).

Santer et al (2005) showed that there is a close match between the visual stimuli that elicit evasive gliding behavior and those that excite the DCMD neurons. They also showed that wing elevation leading into the gliding posture occurs during a maintained burst of high frequency DCMD spikes. Furthermore, the different rates of habituation of the DCMD were shown to be correlated with a change in predator escape strategies of solitary and gregarious locusts (Matheson et al. 2004). The DCMD also mediates visually guided escape jumps. Recently, by comparing electrophysiological and high-speed video recordings, Fotowat and Gabbiani (2007) examined the correlation between distinct phases of escape jumps and distinct phases of firing patterns of the DCMD.

There has not been any comparable study in the vertebrates. Collision-sensitive neurons in the brain have been investigated most intensively in the pigeon, where three types of such neurons have been found in the nucleus rotundus. One group signals the ratio of retinal expansion velocity to retinal size (τ), the second group signals absolute rate of expansion (ρ), and the third group signals yet another optical variable which encodes retinal threshold size (η) (Sun and Frost 1998). However, very little is known about collision avoidance behavior of the pigeon. It was shown that, when approaching a perch, the pigeon initiates foot extension when time-to-collision reaches a threshold

value of approximately 150ms (Lee et al. 1993). However, the properties of collision-sensitive neurons in the nucleus rotundus which signal the time-to-collision of an approaching object (τ neurons) are not necessarily consistent with visual information processing during pigeon landing (Frost and Sun 2004; Sun and Frost 1998; Wang and Frost, 1992).

To investigate the neuronal correlates of collision avoidance behavior in vertebrates, our previous behavioral experiments (Yamamoto et al. 2003) and the present study introduce a new experimental model species, the bullfrog *Rana catesbeiana*, which readily allows investigation of collision avoidance behavior at both the behavioral and neuronal levels. The frog is especially useful for understanding underlying neuronal mechanisms for the following reasons. First, because visually guided avoidance behavior is conservative in the animal kingdom, results can be generalized. Second, in the frog this behavior is a relatively stereotyped all-or-nothing event that can be detected easily and for which behavioral parameters can be measured with considerable precision (Yamamoto et al. 2003). Third, the optic tectum is easily accessible and a precise retinotopic projection from the eye to the contralateral tectal surface can be easily recognized (Gaze 1958; Potter 1969; Székely and Lázár 1976).

Our previous behavioral experiments showed that the frog displays collision

avoidance behavior when the visual angle of a looming object reaches a threshold value of about 20° (Yamamoto et al. 2003). Many authors have electrophysiologically examined the response properties of neurons in the optic tectum and the thalamus. Putative collision-sensitive neurons have been found in the optic tectum and the caudal thalamus (Ewert 1971; Grüsser and Grüsser-Cornehls 1976). However, no quantitative study of the neurons has been carried out to allow comparison with the corresponding behavioral strategy. We carried out the present series of experiments by using single electrode recordings to probe for collision-sensitive neurons in the optic tectum which respond selectively to an approaching object on a collision course, and to clarify what parameter of the collision stimulus is coded by such neurons. Here we show that collision-sensitive neurons exist in the frog optic tectum and that they signal the retinal threshold size of an approaching object, which shows good agreement with the results from our previous behavioral experiments.

Materials and Methods

Animals

Adult bullfrogs, *Rana catesbeiana,* of either sex and 12-14 cm body length, were used exclusively. Animals were obtained commercially and kept in laboratory tanks under a 12h light / 12h dark cycle before use. They were fed on chicken liver twice a week.

Preparations

All experimental procedures were approved by the Kyushu Institute of Technology Animal Institutional Review Board and were in accordance with Kyushu Institute of Technology Animal Care Use Regulations and NIH Guidelines on the Care and Use of Animals in Research. Conventional procedures for exposing the optic tectum of the frog were carried out. The animal was anesthetized by a subdermal injection of 0.6ml of tricaine methanesulfonate (MS222, Sigma.) and immobilized by injecting 0.2 ml of succinylcholine (SUCCIN, Yamanouchi). The animal was then placed on a metal frame. After infiltration of a local anesthetic (XYLOCAINE, Fujisawa) the skin of the operative area on the head was cut, the skull was opened with a drill to expose the left optic tectum, and the dura mater and the arachnoidea were locally retracted with fine forceps and scissors. The dorsal brain surface of the experimental animal was exposed in the mesencephalic region. The cranial cavity was filled with 10 % warm, liquid gelatin (DIFCO) which immobilized the brain. The ground and reference electrode was placed

in the left leg. After surgery, the frog was carefully positioned with its right eye surface parallel to a computer monitor that was placed 20 cm from the animal. Several visual stimuli were presented to the right eye. The left, non-stimulated eye was covered by an opaque occluder. During the experiments, the frog's body skin was kept moist with thin wet gauze to facilitate cutaneous respiration. All experiments were carried out at room temperature (18-22 ℃).

Visual stimulation

The stimulus animations were run on a PC Pentium IV 2.8 GHz equipped with 1.0 GB of RAM, NVIDIA GeForce FX5800 Graphics Controller and EIZO model FlexScan L767 19- inch Multi-Scan Color Monitor set to a refresh rate of 75 Hz. The background and the stimulus square had an average illuminance of 110 and 8 lux (at 20 cm from the screen), respectively. Visual simulations of approaching or receding objects were produced by 2-dimensional expansion or contraction of the stimulus square, as viewed by the animal. These were the same experimental conditions as those of our behavioral experiments, except for the position of the computer monitor. Previously it was placed above the animal, whereas in the present experiments it was placed on the animal's right side. The visual stimulus was presented on the specific region of the monitor corresponding to the recording site determined from the retinotectal projection map of the optic tectum. The final size of the square was 20cm, which corresponded to a visual

angle of 53.1° . This size is much larger than the threshold angle of the looming stimulus, about 20° demonstrated in our behavioral experiments.

Four groups of visual stimuli were presented to the right eye of the frog. The first group simulated five approaching objects and involved: 1) A 35 x 35 cm black square approaching at a velocity of 2m/s along a path of 6 m on a direct collision course against a white background (collision); 2), 3), 4) and 5) A 35 x 35 cm black square approaching at the same velocity and path length on trajectories deviating from a direct collision path in the horizontal or the vertical plane against a white background. The final positions of the squares were 20 cm to the left, right, above or below the central axis (represented by x-20, x+20, y+20, y-20, respectively). A 20 cm deviation represents a 2° shift of the midpoint of the square away from the direct collision path. The second group simulated translational movement of three objects and involved: 6), 7) and 8) A 2 x 2 cm, 10 x 10 cm or 20 x 20 cm black square moving on the front-parallel plane at a velocity of 30 cm/s (corresponding to 73.7° /s) against a white background (represented by trans2, trans10 and trans20, respectively). In four trials, the velocity was changed to 9 cm/s and 1.5 cm/s corresponding to 25.4° /s and 4.3° /s, respectively. Because the black squares were moved forward, backward, upward and downward through the initial position, the response was evaluated as an average number of spikes to the four directions. Each

black square subtended a visual angle of 5.7°, 28.1°and 53.1°, respectively. The second group also contained stimulus 1) to test whether the neuron still responded to a collision stimulus. The third group simulated an object approaching on a direct collision course at four different velocities and involved: 9),10), 11) and 12) A 35 x 35 cm black square approaching at a velocity of 1, 2, 3 and 4m/s along a path of 6 m on a direct collision course against a white background. The fourth group of stimuli involved: 13) The same stimulus as 1) except that the contrast of stimulus and background was reversed (reversed contrast), 14) A 20x20 cm square with its brightness (hence, contrast) varying from white to black as the nonlinear time course given for the looming stimulus of 1) (brightness change) and 15) A 35 x 35 cm black square receding at a velocity of 2m/s along a path of 6m on a direct collision course against a white background (recession). For the four groups, the stimuli were presented randomly at an interval of at least 2 min to allow the cell to recover from any adaptation. Each stimulus type (1-15) was presented three times with an interval of at least 3 s (1-8 and 13-15) or 1 min (9-12) between trials.

To make tuning curves, in four neurons the response was also examined to the collision stimulus and the deviation stimulus in which the final position of the square was displaced 10cm or 5 cm from the central axis (represented by x-10, x+10, y-10, y+10,

x-5, x+5, y-5, y+5, respectively). The 10 cm and 5 cm deviations represent 1.0° and 0.5° shifts from the direct collision path, respectively. Again, these stimuli were presented randomly with an interval of 2 min. Each stimulus contained 3 successive trials with an interval of at least 3 s. Consequently, the response to an object approaching at a velocity of 2m/s on a direct collision course was examined up to five times in a series of experiments.

Recordings

Neuronal activity was recorded at depths of 200 - 600 μm using glass-coated tungsten electrodes with active tips 30μm in length and an impedance of 5 MΩ. 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). The extracellular signals were led to a four channel digital tape recorder (PC204Ax SONY) together with changes in image size monitored as a voltage produced by a software-controlled DA converter (PCI 3336 Interface).

Analysis

The data were analyzed by using the '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 text files. To extract single unit activity based on amplitude and to construct post stimulus time histograms (PSTH), a local laboratory VC++ program was used. The threshold level of the window to discriminate single unit activity was set to detect only the unit showing sustained response during the final stage of image expansion in all three successive presentations. Therefore, in Fig1A, both the large unit that did not respond to all successive looming stimuli (■ in Fig1A) and the small unit showing a spontaneous response before image expansion (□ in Fig1A) were eliminated. Moreover, we confirmed that both the profile and peak of the PSTH were not changed even though the window discriminator was set to detect fewer spikes. Therefore, we concluded that the extracted spikes were most likely from a single unit or possibly multiple units showing the same response profile but subtle amplitude differences.

The PSTH was used to test whether the response of the collision-sensitive neuron could be fitted by a function that simply multiplies the size dependence of its response, $e^{-\alpha\theta(t)}$, by the image's instantaneous angular velocity, $\theta'(t)$, where $\theta(t)$ is image size and α is positive constant. As that was the case, the angular threshold size ($\theta_{threshold}$) characterizing the peak response of the collision-sensitive neuron was computed with the equation of Gabbiani et al. (1999):

$$\theta_{threshold}=2\cdot\tan^{-1}(1/\alpha) \quad (1)$$

This duplicates the analysis reported for the LGMD and the DCMD in the locust.

The parameter α also can be obtained by an alternate analysis. PSTHs were constructed from single unit response to an object approaching at four different velocities. The time-to-collision (time difference between the peak of the neuronal activity and the predicted collision) was estimated according to the peak firing time for each velocity. The linear relationship between the time-to-collision and visual parameter l/v (l: object half size, v: approaching velocity) was examined. The slope of the regression line should be α. Again, the angular threshold producing the peak response of the collision-sensitive neuron was calculated from equation (1) (Gabbiani et al. 1999).

Statistical analysis and linear regression analysis were performed in Excel 2003 (Microsoft). Averages reported here are given as the mean (standard deviation). Student's t test for paired data sets was used to compare the means of spike numbers between the collision stimulus and other stimuli. Model fits were performed with Deltagraph (Japan Poladigital). Tuning curves were fitted to Gaussian functions with Origin (OriginLab).

Histology

To locate an electrode recording site, a lesion was made by passing a 10 μA negative

current through the electrode for 4 to 10s. At the end of a recording session, the animal was deeply anesthetized and prepared for histological examination. The brain was removed from the skull and fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in a refrigerator overnight. The trimmed optic tectum was embedded in 25% gelatin and stored in 10% buffered formalin for 2-4 hours. The gelatin block was placed in 30% sucrose overnight at the refrigerator. Frozen sections were cut out at 40 μm and stained with cresyl violet.

Results

Directional sensitivity of collision-sensitive neurons

Single unit visual responses of eleven collision-sensitive neurons were recorded in the optic tectum of eleven different frogs. The typical responses of a collision-sensitive neuron to various visual stimuli are shown in Fig.1. This neuron showed a selective response to an object approaching on a direct collision course compared to that receding on the same trajectory or approaching on near miss trajectories (Fig.1A, C). The response to the direct collision stimulus was greater than that to translating movement with a velocity of 30 cm/s (Fig.1A, B). The translating stimuli would have been in the receptive field for a shorter period of time, which could explain why the translating stimuli produce fewer spikes than the looming stimuli. To test this possibility we also examined the responses to translating stimuli with slower velocities (Fig.1 D). The collision-sensitive neuron showed much smaller responses to the translating stimuli at velocities of 9cm/s and 1.5cm/s than of 30cm/s. Therefore, this hypothesis can not account for the difference seen between responses to looming and translating stimuli.

Moreover, this neuron showed almost no response to both a bright object approaching against a dark background and brightness change without image expansion (Fig.1C). Figure 2 shows the distribution of the foci of expansion (FOE) of looming stimuli on the

computer display for the eleven neurons. These collision-sensitive neurons showed a similar response pattern to each presented stimulus, regardless of the location of the stimulus FOE.

The responses of the eleven neurons were further analyzed quantitatively. The excitatory receptive field (ERF) sizes were mapped with a 2 cm translating black square. The mean horizontal and vertical extents of the ERF were 11.0° (SD 5.0) (n=4) and 12.8° (SD 3.5) (n=4), respectively. Figure 3 illustrates the average number of spikes (mean, SD) obtained from the eleven collision-sensitive neurons to 11 kinds of visual stimuli (collision, x-20, x+20, y+20, y-20, trans2, trans10, trans20, reversed contrast, brightness change, and recession; see Materials and Methods). The average spike numbers were compared between the collision stimulus and other stimuli with a paired t-test. A significant difference occurred between the neuronal response to the direct collision stimulus and those to other stimuli ($P<0.01$). The neurons did not show any response to brightness change, demonstrating that they did not respond to a decrement of brightness accompanied with expansion of the retinal image (Figs. 1, 3). The objects, especially the large one, produced little response when moved on a front-parallel plane (translational movement, Figs. 1, 3). The average spike number in response to a 20cm translating object was significantly smaller than that to a 10cm translating object

($P<0.05$). However, in both looming and translating stimuli, adaptation occurred when the neurons were stimulated repeatedly (Fig. 4).

In four preparations, horizontal and vertical deviation of 0.5° and 1.0° from a direct collision path were simulated in different approaching trajectories to examine the tuning properties in detail. Typical responses to such stimuli are shown in Fig. 5. The most vigorous response was given to an object on a direct collision course. Total spike numbers for each trajectory were plotted against displacement from a collision trajectory (Fig. 6A and 6B for horizontal and vertical deviation, respectively). The results showed that the response declined as object trajectories moved away from a direct collision course. Fitting a Gaussian function to the results for the neuron of Fig.6 showed that the peak in the sensitivity profile deviated 0.5° horizontally and 0.3° vertically from a direct collision course (half width values at half peak were 1.1° and 1.0° ,respectively). The sensitivity profiles obtained from the four neurons to the above stimuli deviated on average 0.4° (SD 0.3) horizontally and 0.4° (SD 0.2) vertically from a direct collision course (half width values at half peak were 0.8° (SD 0.2), 0.9° (SD 0.2), respectively). The directions of horizontal and vertical deviations of the peaks of the tuning curves obtained from the four neurons are shown in Fig.2.

<u>The location of the collision-sensitive neurons in the tectal layer</u>

The responses of the collision sensitive neurons were recorded at a mean depth of 402.7 μm (SD 53.5). During penetration of microelectrodes into the tectum, the responses usually were recorded just below those of R3 and R4 retinal ganglion cells. This suggests that collision-sensitive neurons are located within layer 7, the main efferent layer of the tectum. To test the hypothesis, lesion experiments were performed to locate the electrode recording sites (N=6). All six penetrations showed that the lesion occurred in layer 7, as seen in a typical result (Fig.7). The waveshape of an action potential of a collision-sensitive neuron was biphasic (Fig.1 inset). This shows that the electrode was positioned adjacent to the site of action potential initiation and subsequent propagation (Winkowski and Gruberg 2005). Combined with the observation that the neurons showed retinotopy, it is very likely that the action potentials were recorded at or near the initial segments of the axons in layer 7.

<u>The response of the collision-sensitive neurons can be fitted with a function that multiplies the image's instantaneous angular velocity and an exponential function of the size of the object's image on the retina.</u>

The time-rate response profiles of the collision-sensitive neurons to a looming object were quite characteristic. The typical response profile to a black square of 35 x 35 cm approaching at a velocity of 2m/s through a path of 6 m is shown in Fig. 8, where the

number of spikes in 50 ms bin widths is plotted against time-to-collision (N=15 trials). The neuron started firing early during the approaching phase and the firing rate gradually increased as the object became larger, as if the cell were "tracking" the object during the approach. The firing rate peaked before the predicted collision time and then rapidly decreased. It should be noted that this neuron gave its maximum response to approaching objects with sizes ranging from 25° to 28°, which, if moving laterally, elicited few, or no, responses (Figs.1, 3 and 4). The response profile is very similar to those of the LGMD in the locust and η neurons in the pigeon (Hatsopoulos et al. 1995; Sun and Frost 1998). As in those well characterized collision-sensitive neurons, the response of the frog collision-sensitive neurons also can be fitted with a function that multiplies the size dependence of its response ($e^{-\alpha\theta(t)}$) by the image's instantaneous angular velocity ($\theta'(t)$). In this particular case, the response can be described with the following equation (Fig. 9):

$$f(t)=61.6\cdot\theta'(t)\cdot e^{-4.1\theta(t)} \quad (2)$$

From equation (1), we obtain $\theta_{threshold} = 27.5°$. This shows that the peak firing rate occurred after the approaching object had reached a visual angle of 27.5° on the frog's retina. Figure 10 shows response profiles to the same black square approaching at velocities of 1m/s, 3m/s and 4m/s through a path of 6 m. These responses also can be

fitted with a function that multiplies the size dependence of its response by the image's instantaneous angular velocity. At a velocity of 1m/s, the response can be described with the following equation (Fig. 11A):

$$f(t)=12.2\cdot \theta'(t)\cdot e^{-4.7\theta(t)} \quad (3)$$

At a velocity of 3m/s and 4m/s, the responses can be fitted with the following equations, respectively (Fig. 11B, C):

$$f(t)=27.9\cdot \theta'(t)\cdot e^{-4.4\theta(t)} \quad (4)$$

$$f(t)=34.5\cdot \theta'(t)\cdot e^{-5.0\theta(t)} \quad (5)$$

From equation (1), at a velocity of 1, 3, and 4m/s, $\theta_{threshold}$= 23.8°, 25.6°,22.8°, respectively. Thus, despite different approaching velocities, the peak firing rate always occurred after the approaching object had reached a constant visual angle of 24.9° (SD, 2.1) (n=4). This strongly suggests that the collision-sensitive neurons of the frog, like LGMD and η neurons, play the role of an angular threshold detector. Table 1 summarizes calculated angular thresholds at different approaching velocities obtained from the eight collision-sensitive neurons. The mean angular threshold obtained from the eight neurons was 24.6° (SD, 2.5, n= 8), thus further supporting the above threshold detector role.

<u>Linear relationship between time-to-collision and visual parameter l/v</u>

Here, we perform an alternative analysis to reveal the angular threshold detected by the frog collision-sensitive neurons. The relationship between two essential visual parameters related to collision avoidance, l/v and time-to-collision, was examined. L/v defines the time course of expansion of the retinal image during object approach. L denotes the object's half-size, and v denotes its approach velocity. Theoretical consideration showed that if time-to-collision and l/v are linearly related, the peak firing rate always occurs at a fixed delay after the object has reached a constant angular size on the animal's retina (Gabbiani et al. 1999).

This possibility was tested by changing the velocity of an approaching object (1, 2, 3, 4m/s), and examining the relationship between time-to-collision and l/v for the same collision sensitive neuron in which response profiles for each approach velocity were fitted with equations (2) to (5). The result showed that the two parameters were linearly related (R^2=0.98) (Fig.12). The value of fitted slope yielded α in equation (1). Thus, in this case, the peak firing rate always occurred after the approaching object had reached a visual angle of 26.5° on the frog's retina. This value shows a good agreement with that obtained from the alternate analysis in the previous section (24.9°). Similar results were observed in all eleven neurons in which linear regression analysis was performed using the mean values for time-to-collision (Fig. 13). The results

again showed that the relationship between l/v and time-to-collision was close to linear (R^2= 0.99). The result also clearly showed that the peak firing rate always occurred after the object had reached a constant angular size (21.1°) on the retina. The value of $\theta_{threshold}$ showed a good agreement with those obtained from both the curve fitting analysis and earlier behavioral experiments (Yamamoto et al. 2003).

The two types of regression analysis described above revealed intercepts of 62.3 ms and 47.9 ms, respectively. This means theoretically that the threshold angle occurs after the peak response of collision-sensitive neurons rather than before it. Fotowat and Gabbiani (2007) also reported that the fit of the lines to the timing of the DCMD peak revealed a positive intercept in response to stimuli with large l/v values (40-120 ms) but not to those with small l/v values (5-50 ms). They concluded that intercepts of linear fits computed over the larger range are less reliable than those computed over the small range because of the larger extent of extrapolation and larger variability in the peak firing time for the former range. Our looming stimuli have l/v values of 43.8, 58.3, 87.5 and 175 ms corresponding to the former range. Therefore, we do not discuss the response latency further in this report.

Discussion

Collision-sensitive neurons of the frog

To successfully survive, animals should distinguish between approaching dangers and harmless objects in the natural environment. Therefore, the neuronal correlates would be expected to show response characteristics appropriate for this task. Hypothetically, the tectal neurons characterized in the present study are collision-sensitive neurons which are analogous to pigeon τ neurons and locust LGMD, DCMD neurons. To confirm this, the response properties of the putative collision-sensitive neurons of the frog were compared with those of well-characterized collision-sensitive neurons in other animals in terms of a) preference for objects approaching on collision trajectories, b) preference to movement in a frontal parallel plane, and c) response to recession and brightness change.

a) Preference for objects approaching on collision trajectories

All collision-sensitive neurons show a preferred response to approaching objects on a direct collision course rather than to objects approaching on near-miss trajectories. Thus, in the nucleus rotundus of the pigeon, the collision-sensitive τ neurons exhibited extremely tight tuning, centered precisely on 0° azimuth with mean half-width at half height values of 3.3° and mean above baseline widths of 16° (Wang and Frost 1992).

Similarly, the response tuning of the locust DCMD has a half-width at half height value of 2.4-3.0° for a deviation in the horizontal direction and 3.0° for a deviation in the vertical direction (Judge and Rind 1997). The frog tectal neurons examined in this study also showed extremely tight tuning with a half width at half peak value of 1.1°and 1.0° for a deviation in the horizontal and the vertical direction, respectively (Fig.6). This strongly suggests that they are collision-sensitive neurons.

However, there are several differences between the putative frog collision-sensitive neurons and those of other animals, which should be discussed here. First, the collision-sensitive neurons in the frog seem to have tighter tuning than those in other species. However, taking the apparent stimulus path length into consideration (1.9 m in Judge and Rind 1997; 5.8 m in this study, respectively), the half width at half height values in the locust and the frog both corresponded to about 10 cm displacement of the mid point of the stimulus away from the central axis. Second, in the frog, the peak of the tuning curve was shifted ≦0.5° from a collision trajectory, while the collision-sensitive neurons in the pigeon and the locust showed tuning curve peaks centered precisely on a direct collision path . Two possible explanations could account for this observation. First, objects approaching on near miss trajectories with displacements of 0.5° would still contact the animal. However, the tuning curve peak does not necessarily move toward

the animal (Fig.2). Second, a more likely explanation is that the location of the FOE was displaced from the center of the receptive field of the examined neuron. The retinotopy of tectal collision-sensitive neurons will be discussed later.

b) Preference to movement in a frontal parallel plane

While it is useful to make interspecies comparisons of collision-sensitive neuron responses to translational stimuli, there is some risk in referencing such responses to those for collision stimuli. Specifically, because the retinal image of an approaching object expands but that of an object moving in a tangent screen plane is constant, the response preference could change depending on the size of the translating object. Furthermore, the translating stimuli would have been in the RF for a shorter period of time than the looming stimuli. In the following discussion, we attempt to shed light on the complexities in stimulus preferences among collision-sensitive neurons in different species.

In the pigeon, collision-sensitive neurons show very little or no increase in the firing rate and no indication of directional preference to object motion in a tangent screen plane (Wang and Frost 1992).

In the locust, the LGMD and DCMD neurons were originally claimed to respond optimally to rapid translating movements of small objects in the visual field (Oshea and

Rowell 1975; Palka, 1967; Rowell et al. 1971a,b, 1977). However this view was overturned by a subsequent quantitative study of the DCMD responses to translational stimuli (Rind and Simmons 1992).

Rind and Simmons used a real object (a black disk of radius 25mm) controlled with an x-y plotter and compared the response to the disk translating across the eye at a uniform velocity (0.5m/s) with the response to the disk approaching or receding from the eye at the same velocity. The size of the translating disk was the same as the initial size of the approaching or receding disk. Approach of the disk toward the eye elicited 37± 1.1 spikes (mean ± SE) in the DCMD, while translation in the posterior and anterior directions elicited 14.7 ±0.9 and 11.3 ±0.7 spikes, respectively. Hatsopoulous et al (1995) also analyzed DCMD responses to constant velocity translational stimuli by using computer graphics to move squares of various sizes to the left or to the right. They confirmed the previous finding that as the stimulus size increased, the response declined (Rowell et al. 1977). They also showed that the DCMD firing rate was well fitted by an exponential function of the size of the object ($e^{-\alpha\theta}$, where θ is the size of stimulus, α is a positive constant).

Recently, in the lobula of the crab, two movement detector neurons, M1 and M2 were found to respond to an expanding image in a robust and reliable way (Oliva et al.

2007). Both neurons produced a considerable, often sustained, response to lateral displacement of a 6 cm black square (velocity: 18cm/s), but, again, the strongest response was found to a black looming stimulus. In general, the above results show that, although collision-sensitive neurons found in different animals produce a considerable response to a translating small object, the response is still smaller than that to an approaching object regardless of the size of the translating object.

The eleven putative frog collision-sensitive neurons also showed a much stronger response to objects approaching at a velocity of 2m/s (47 spikes (SD, 22)) than to translating objects with visual angles of 5.8°, 28° and 53.1° moving at a velocity of 30 cm/s (6 spikes (SD, 5), 7 spikes (SD, 6) and 2 spikes (SD, 3), respectively)(Fig.3). The mean horizontal and vertical extents of the ERF were both less than 13°. This size is much smaller than the threshold angle detected by these neurons in response to a looming stimulus. Therefore, it is unlikely that the response to approaching objects of the collision-sensitive neurons is generated artificially by the stimulus expanding into the inhibitory surround of a large off center and on surround receptive field. The inhibitory receptive field of tectal and entopallial collision-sensitive neurons in the pigeon also did not suppress their looming responses (Wu et al. 2005; Xiao et al. 2006). Thus, looming responses in both species are not determined by the receptive field

organization mapped on the front-parallel plane.

Furthermore, the collision-sensitive neurons showed a much weaker response to translating objects with velocities of 9 cm/s and 1.5 cm/s than that with a velocity of 30 cm/s (Fig.1D). This showed that the period of time during which the stimulus moved through the RF can not account for fewer spikes being elicited by the translating stimulus compared to the looming stimulus. These observations again support their identification as collision-sensitive neurons.

Differential adaptation may underlie the contrast in selective responses to a looming stimulus compared to a translating stimulus. Thus, in the locust DCMD responses to 10° objects moving tangentially on a monitor screen resulted in vigorous phasic activity which adapted drastically over successive trials. In contrast, responses to approaching objects were extremely vigorous and were much less susceptible to the effects of stimulus repetition (Schlotterer 1977). Recent pharmacological experiments demonstrated that the strong spike frequency adaptation to a translating stimulus, which is mediated by a Ca^{2+} -dependent potassium conductance, contributes to looming stimulus selectivity in the LGMD (Peron and Gabbiani 2009). Unlike the LGMD, however, the responses of the frog neuron to looming and translating objects were equally adapted (Fig.4). This different susceptibility to adaptation could be due to the

fact that receptive field sizes of frog collision-sensitive neurons are much smaller than that of the LGMD. A translating object crossing the wide receptive field of the LGMD continues to activate a constant number of photoreceptors per unit time over a long period, making the neuron susceptible to adaptation (Peron and Gabbianai 2009). On the other hand, a translating object only phasically activates frog collision-sensitive neurons because of their small receptive fields, making the neurons less susceptible to adaptation.

c) Responses to receding objects and brightness change

As in the pigeon and locust, the putative frog collision-sensitive neurons showed almost no response to a receding object, nor to any brightness change accompanied by expansion of the retinal image (Fig.3). Pigeon τ neurons show no response to both receding objects and changes in illumination (Wang and Frost, 1992). Locust DCMD neurons show much smaller responses to receding objects compared to translating ones (Rind and Simmons, 1992). Changes in overall luminance with no change in the size of the rectangular stimulus elicit much smaller and briefer responses from the DCMD than do approaching visual targets (Simmons and Rind, 1992).

However, the frog collision-sensitive neurons differed from others in terms of the effects of image contrast on responses to approaching objects. The response selectivity of

τ neuron for a looming stimulus was unaffected by contrast reversal (Wang and Frost, 1992). The DCMD could distinguish approach from recession not only of objects that were darker than the background but also of objects that were lighter than the background (Rind and Simmons 1992). On the other hand, the frog collision-sensitive neurons showed almost no response to a bright object approaching on a dark background (Fig.3). Our physiological observations are in agreement with the finding that no jumping occurred in response to magnification of the reversed-contrast silhouette (Schiff 1965).

Thus, although there is a subtle difference in response characteristics, it is concluded that the collision-sensitive neurons in the frog optic tectum are analogous to pigeon neurons and locust LGMD, DCMD neurons.

Frog collision avoidance behavior and underlying neuronal correlates

Effective visual stimuli and threshold parameters have been demonstrated for frog collision avoidance behavior. Schiff (1965) showed that collision avoidance behavior can be elicited with a two dimensional rapid magnification of a continuous silhouette by using a shadow-casting device. Ingle and Hoff (1990) adopted a black 8 to 10 cm square moving toward the frog as the visual stimulus and found that it produced avoidance behavior when the retinal image expanded beyond a visual angle of 30° (although the

data were not shown). In the frog, previous experiments using computer graphics confirmed Schiff's observation and also showed that the frog displays avoidance behavior to an approaching object when the retinal image size, not time-to-collision, reaches a critical angle of about 20° (Yamamoto et al. 2003).

The neuronal mechanisms underlying frog collision avoidance behavior have been examined by many authors. Hemisection of either the posterior or anterior portion of the optic chiasm produces 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 (Waldeck and Gruberg 1995). Furthermore, unilateral injection of tetrodotoxin or cadmium chloride into the frog optic nerve selectively abolishes avoidance behavior to looming objects, but not prey orienting behavior. This selective loss of behavior was correlated with a loss of activity of R4 retinal ganglion cells, the dimming detectors (King et al. 1999). A recent study combining behavioral experiments and multielectrode recordings from isolated retinas showed a very strong correlation between synchronized oscillatory activity among retinal dimming detectors and collision avoidance behavior of the frog (Ishikane et al. 2005). Thus, it is very likely that the dimming detectors have an important role in eliciting of collision avoidance

behavior. However, the dimming detectors lack the major characteristic of collision-sensitive neurons: a directional selectivity to an approaching object on a collision course (Hartline 1938; Lettvin et al. 1959). Combined with these results, the finding that removal of the optic tectum abolishes any response to approaching objects (Ingle 1973, 1977) strongly suggests that collision-sensitive neurons with angular thresholds of about 20° should be in the frog optic tectum.

Indeed, putative collision-sensitive T3 neurons in the frog optic tectum have been reported to respond vigorously to an approaching object, in contrast to their low sensitivity to a receding object or an object moving around the animal. The excitatory receptive fields were located predominantly in the nasal part of the visual field and extended over 20 – 30° (Grüsser and Grüsser-Cornhels 1976). Thus, the collision-sensitive neurons examined in the present study could be T3 neurons. However, the tightness of tuning to a collision trajectory was not examined quantitatively by Grüsser and Grüsser-Cornhels (1976), nor was any sensitivity to particular optical parameters of an approaching object determined for these neurons. On the other hand, we have shown in this study that the tuning of tectal collision-sensitive neurons declines dramatically if the object's trajectory deviates from a direct collision course by about 1°. Moreover, we showed that the peak firing rate of the collision-sensitive

neurons always occurred at a fixed delay after the object had reached a threshold angular size of 20 - 25 ° on the retina.

The threshold angular size for collision avoidance behavior of about 20° (Yamamoto et al. 2003) is smaller than that for the collision-sensitive neurons reported here. However, in the behavioral experiments the height of the eye from the experimental stage varied 2.5 to 5 cm depending on the attitude of the animal. Since the threshold value was computed based upon the distance between the computer monitor and the experimental stage, variation in the position of the eye would yield angular size threshold values of 25.2° to 29.2° . Thus, the behavioral and neuronal threshold angular sizes from our studies showed good agreement. We conclude that frog collision-sensitive neurons found in this study should be good candidates for elucidating the neuronal correlates of collision avoidance behavior of the frog.

Finally, we should consider why collision-sensitive neurons signaling time-to-collision of approaching objects, like τ neurons of the pigeon, were not found in the frog optic tectum. It is useful to consider what kind of behavior is controlled by time-to-collision and retinal threshold size, respectively. Time-to- collision is used for approach behaviors, such as control of the plunge dive of the gannet (Lee and Reddish 1981), pre-landing deceleration of the fly (Wagner 1982) and pre-landing foot extension

of the pigeon (Lee et al. 1993). These behaviors require accurate control of the timing of interception with the object. Therefore, complicated computation involving acceleration, deceleration and trajectory is required for successful performance. Because the animal is executing its own global motor planning, the neuronal control of these approach behaviors is likely to include sufficient timing for such complicated computations.

On the other hand, retinal threshold size is used frequently to trigger avoidance behavior to approaching objects, particularly, predators or rivals (Robertson and Johnson 1993a, b; Schiff 1965; Yamamoto et al. 2003). Neuronal control of successful avoidance must be based upon rapid responses to cues from an approaching animal. Especially in predator-prey situations, the fastest sensory and motor pathways would be favored, rather than more complex integration pathways which compute abstract variables such as predicted time-to-collision. The angular size of a looming retinal image may well be the fastest way to gain immediate cues to elicit simple and quick avoidance.

For the bullfrog, which is generally sedentary, the latter behavior may be predominant. This could be the reason why the detectors for retinal threshold but not for time-to-collision were found in the bullfrog's optic tectum. Confirmation of this idea could come from searching for τ neurons in the brain of the tree frog, which jumps from

branch to branch with very high accuracy.

A model of tectal and thalamic collision-sensitive neurons in vertebrates

So far, collision-sensitive neurons in vertebrates have been found both in the optic tectum and the thalamus. In the pigeon, putative collision-sensitive neurons were found first in the nucleus rotundus thalami. Of 220 tested neurons 15% showed a preference for movement directed toward or away from the animal (Revzin 1970). Although not found earlier (Wang and Frost 1992), collision-sensitive neurons were recently demonstrated to be present also in the optic tectum of the pigeon (Wu et al. 2005). About 30 % of examined neurons in both the thalamus and the optic tectum were collision-sensitive and were classified as τ, ρ and η neurons (Sun and Frost 1998; Wu et al. 2005). In the toad, although the response properties were not closely examined, the putative collision-sensitive neurons, TH6 and T3 were found in the caudal thalamus and the optic tectum, respectively (Ewert 1971; Grüsser and Grüsser-Cornehls 1976). Like the T3 neurons described above, the TH6 response increased to an object moving towards the eye but not to a translating object.

Here, the relation between the tectal and the thalamic collision-sensitive neurons will be discussed based on their common and different properties. The collision-sensitive neurons in the optic tectum and the thalamus show much stronger responses to an

approaching object than to a translating object (Ewert 1971; Grüsser and Grüsser-Cornehls 1976; Wang et al. 1993; Wang and Frost 1992). Another common feature is the extremely tight tuning to collision trajectories found in the pigeon nucleus rotundus τ neurons (Wang and Frost 1992) and in the collision-sensitive tectal neurons from the present study.

On the other hand, a striking difference has been observed in the sizes of receptive fields of collision-sensitive neurons in the thalamus and the tectum. The collision-sensitive neurons in the pigeon nucleus rotundus have very large receptive fields of approximately 110° of arc (Wang et al. 1993; Wang and Frost 1992), and those of the frog TH6 neurons include the entire visual field (Ewert 1971). In contrast to the thalamic neurons, the tectal collision-sensitive neurons have smaller receptive fields of approximately 30° in the pigeon (Wu et al. 2005) and approximately 10° in the frog. Moreover, in the pigeon, the percentage of each neuron type differs between the two parts of the brain: τ neurons predominate in the thalamus (Sun and Frost 1998; Wu et al. 2005), while η neurons are three-times more frequent than other collision-sensitive neurons in the optic tectum (Wu et al. 2005).

The neuronal projection from the optic tectum to the thalamus is well known. Because the receptive field of the tectal neuron is smaller than that of the thalamic

neuron, it is very likely that the receptive field of thalamic collision-sensitive neurons is composed of a two dimensional array of receptive fields of tectal neurons. Because animals should detect an imminent collision with an approaching object on a collision course from anywhere in the visual field, the collision-sensitive neurons should have large receptive fields and show the same strong response over the entire receptive field.

Frost and Sun (2004) developed a neuronal model to explain how such adaptive properties of collision-sensitive neurons in the pigeon nucleus rotundus are generated. In that model, the receptive field of a rotundal collision-sensitive neuron is composed of a radial arrangement of concentric arrays of receptive fields of tectal neurons. The tectal neurons respond to movements that are oriented radially from the center of the concentric array, and they 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 receptive field radial layout. This arrangement is shown schematically in Fig. 14A.

Based on our experimental results, we propose an alternative neuronal model to explain the physiological response properties of thalamic collision-sensitive neurons. In our study, we demonstrated that some tectal neurons of the frog showed a vigorous response only to a collision stimulus, and not to a straight frontoparallel movement in a

particular direction. In our model, the receptive field of a thalamic collision-sensitive neuron is composed of a radial arrangement of concentric arrays of receptive fields of tectal collision-sensitive neurons. The tectal neurons respond to looming stimuli that expand from the center of their receptive field, and they converge onto the thalamic neurons. Thus, the thalamic collision-sensitive neurons could detect objects approaching on a collision course regardless of object approach angle. This arrangement is shown schematically in Fig. 14B.

Frost's model and ours correspond to a direction mosaic hypothesis and a vector field hypothesis for optic flow selectivity, respectively (Duffy and Wurtz 1991a,b; Saito et al. 1986; Tanaka et al. 1989; Tohyama and Fukushima 2005).Because tectal collision-sensitive neurons were also found in the pigeon (Wu et al. 2005), we conclude that the receptive field organization of a collision-sensitive neuron in the thalamus is more consistent with the vector field hypothesis.

Spatial integration in the thalamus could reduce the number of efferent fibers to motor pattern generators in the spinal cord and could simplify the efferent pathways to motor output. Recently, the response of telencephalic collision-sensitive neurons of the pigeon was shown to be modulated by optic flow stimulation (Xiao and Frost 2009). Processing of optic flow generated by self-motion of the animal is performed in the

nucleus of the basal optic root and the nucleus lentiformis mesencephali but not in the optic tectum (Fite et al. 1989; Frost et al. 1990; Li et al. 1996; Wylie and Frost 1999). Therefore, the ascending projection from the optic tectum to the thalamus is very important to integrate information about local object motion and optic flow generated by self-motion of the animal, and to facilitate detection of approaching objects in the animal moving in the natural environment, although the indirect pathway delays onset of escape behavior.

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Figure legends

Fig.1. A) Responses of a typical collision-sensitive neuron to an object approaching on a direct collision course (center) and on near-miss trajectories (x-20: forward deviation, x+20: backward deviation, y+20: upward deviation, y-20: downward deviation). Upper traces show voltage changes monitoring size of the approaching objects. Lower traces show neuronal activities to the approaching objects. Black and white squares show the units eliminated for quantitative analysis. The inset shows the shape of spikes of the collision-sensitive neuron.

B) Responses to translating objects with different sizes (trans2: 2x2 cm square, trans10: 10x10 cm square, trans20: 20x20 cm square). Upper traces show voltage changes monitoring position of the translating objects. The first upward and downward deflections represent backward and forward movements of the square, respectively. The second upward and downward deflections represent upward and downward movements of the square, respectively. Lower traces show neuronal activities to the translating objects. C) Responses to three other control stimuli (see Materials and Methods). In the top and bottom panels ("reversed contrast" and "recession" stimuli, respectively), the upper traces show voltage changes monitoring size of an approaching and a receding object, respectively. In the middle panel ("brightness change" stimulus), the upper trace

shows voltage changes monitoring brightness of a stationary square. In all three panels, lower traces show neuronal activities to each control stimulus. D) Responses to an object approaching on a direct collision course and responses to a translating 10 x 10 cm square with a velocity of 30 cm/s, 9 cm/s and 1.5 cm/s. In the upper left panel, upper and lower traces show the size of the approaching object and neuronal activity to the stimulus as shown in A. In the remaining panels, upper and lower traces show the position of the translating object and neuronal activity to the stimuli as shown in B. This neuron responds selectively to a collision stimulus. D was obtained from different preparation than A to C.

Fig.2. Distribution of the foci of expansion of looming stimuli which were presented to 11 collision-sensitive neurons. Seventeen pixels correspond to 1 cm on the display. The eye of the animal was aligned with y-axis but located 8 cm below the origin. The arrows show the directions of horizontal and vertical deviation of the peaks of the tuning curves obtained from four neurons. Two data plots were completely superimposed.

Fig.3. Average number of spikes (mean, SD) per response obtained from 11 collision-sensitive neurons responding to 11 kinds of visual stimuli shown in Fig.1. There is a significant difference between mean number of spikes for the collision stimulus and those for other stimuli ($p<0.01$).

Fig.4. The effects of stimulus repetition on the responses of collision-sensitive neurons to an approaching object and translating objects. A) Average number of spikes (mean, SD) to 3 successive presentations of collision or translating objects obtained from 10 collision-sensitive neurons is shown. B) The mean number of spikes to the first trial is normalized to one. The responses to looming and translating objects were equally susceptible to the effects of stimulus repetition.

Fig.5. A) Responses of the same neuron as in Fig. 1A to C to an object approaching on a direct collision course and near-miss trajectories with deviations from a direct collision path of 1° (x-10: forward deviation, x+10: backward deviation, y+10: upward deviation, y-10: downward deviation). B) Responses of the same collision-sensitive neuron as in A) to an object approaching on a direct collision course and near-miss trajectories with deviations from a direct collision path of 0.5° (x-5: forward deviation, x+5: backward deviation, y+5: upward deviation, y-5: downward deviation). Upper traces show voltage changes monitoring size of the approaching objects. Lower traces show neuronal activities to the approaching objects. Scale bars: 1s

Fig.6. The response profiles of the same collision-sensitive neuron as in Fig. 5 to an object approaching on different trajectories and the corresponding tuning curves fitted by Gaussian function. A) Total spike numbers are plotted against horizontal

displacement from a direct collision path. Positive and negative displacement represents backward and forward deviation, respectively. B) Total spike numbers are plotted against vertical displacement from a direct collision path. Positive and negative displacement represents upward and downward deviation, respectively.

Fig.7. A cross section of the frog optic tectum in which a lesion of recording site was made by current injection. The lesion is observed in the tectal layer 7, the main efferent layer of the tectum (arrow). The pial surface is at the top, and the ventricular surface is at the bottom. Scale bar: 200 μ m.

Fig.8. A typical response profile of a collision- sensitive neuron in response to a black square of 35 x 35 cm approaching at a velocity of 2m/s through a direct collision path of 6m. The number of spikes within a bin width of 50 ms was obtained from 5 blocks in which 3 successive stimuli were presented. Collision would be at 0 ms. The activity started increasing about 1 s before predictive collision. The activity peaked about 400ms before predictive collision, when the retinal image of the stimulus ranged from 25° to 28° , and then declined.

Fig.9. Response of the collision-sensitive neuron shown in Fig.8 (thin line) and model prediction which is superimposed on the data (thick line). In this particular case, the response can be fitted with the equation: $f(t)=61.6\cdot\theta'(t)\cdot e^{-4.1\theta(t)}$. Collision would be at 0

ms.

Fig.10. Response profiles of the same collision- sensitive neuron as in Fig.8 in response to a black square of 35 x 35 cm approaching at a velocity of 1m/s, 3m/s and 4m/s through a direct collision path of 6m are shown in A, B and C, respectively. The number of spikes within a bin width of 50 ms was calculated from 3 successive sweeps with an interval of 1 min. Collision would be at 0 ms.

Fig.11. A) Response of the collision-sensitive neuron shown in Fig.10A (thin line) and model prediction which is superimposed on the data (thick line). The response can be fitted with the equation: $f(t)=12.2\cdot\theta'(t)\cdot e^{-4.7\theta(t)}$. B) Response of the collision-sensitive neuron shown in Fig.10B (thin line) and model prediction which is superimposed on the data (thick line). The response can be fitted with the equation: $f(t)=27.9\cdot\theta'(t)\cdot e^{-4.4\theta(t)}$. C) Response of the collision-sensitive neuron shown in Fig.10C (thin line) and model prediction which is superimposed on the data (thick line). The response can be fitted with the equation: $f(t)=34.5\cdot\theta'(t)\cdot e^{-5.0\theta(t)}$. Collision would be at 0 ms.

Fig.12. Plot of the peak time relative to the predicted collision as a function of visual parameter l/v (l: object's half size, v: approaching velocity) obtained from the same collision-sensitive neuron shown in Fig8. A strong linear relationship is found between the two parameters (R^2=0.98).

Fig.13. Plot of the mean peak time relative to the predicted collision as a function of visual parameter l/v (l: object's half size, v: approaching velocity) obtained from 11 collision-sensitive neurons. Error bars indicate SD. A strong linear relationship is found between the two parameters (R^2=0.99).

Fig.14. Two neuronal models to explain how tectal inputs integrate and build up the wide receptive field of thalamic collision-sensitive neurons. An outer large circle represents the receptive field of a thalamic collision-sensitive neuron. A series of concentric circles represents receptive fields of tectal neurons. A. The model proposed by Frost and Sun (2004). The tectal neurons give selective responses to movements that are oriented radially from the center of the concentric array. B. Our model based on the results obtained from the present experiments. The tectal neurons respond selectively to looming stimuli that are expanded from the center of their receptive fields irrespective of the locations.

Table 1. Angular thresholds calculated from model fit of responses of 8 collision-sensitive neurons to an object approaching at four different velocities.

neuron	approaching velocity				mean±SD
	1m/s	2m/s	3m/s	4m/s	
CS13	22.7°	25.2°	26.2°	19.9°	23.5° ±2.8°
CS18	23.8°	27.5°	25.6°	22.8°	24.9° ±2.1°
CS19	26.6°	26.7°	19.3°	25.1°	24.4° ±3.5°
CS22	23.8°	34.0°	29.5°	37.6°	31.2° ±6.0°
CST2	19.8°	31.1°	24.3°	29.1°	26.1° ±5.1°
CST5	13.3°	18.1°	12.4°	11.9°	13.9° ±2.8°
CST7	16.6°	27.7°	30.2°	24.1°	24.7° ±5.9°
CST8	19.9°	27.0°	23.9°	27.5°	24.6° ±3.5°
mean±SD	27.5° ± 4.4°	21.5° ± 4.5°	24.3° ± 5.5°	25.1° ± 7.0°	24.6° ±2.5°