Role of a looming-sensitive neuron in triggering the defense behavior of the praying mantis Tenodera aridifolia

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Sato K, Yamawaki Y. Role of a looming-sensitive neuron in triggering the defense behavior of the praying mantis Tenodera aridifolia. J Neurophysiol 112: 671–682, 2014. First published May 21, 2014; doi:10.1152/jn.00049.2014.—In responses to looming objects, the praying mantis shows a defense behavior, which consists of retracting forelegs under the prothorax. The role of a looming-sensitive neuron in triggering this behavior was investigated by simultaneously recording the activity and behavioral responses of the neuron. The mantis initiated the defense behavior earlier in response to larger and slower looming stimuli. The time remaining to collision at defense initiation was linearly correlated with the ratio of the half-size of an approaching object to its speed (l/v), suggesting that the defense behavior occurred a fixed delay after the stimuli had reached a fixed angular threshold. Furthermore, the results suggested that high-frequency spikes of the looming-sensitive neuron were involved in triggering the defense behavior: the distribution of maximum firing rate for trials with defense was shifted to larger rates compared with trials without defense; the firing rate of the neuron exceeded 150 Hz ~100 ms before the defense initiation regardless of stimulus parameters; when a looming stimulus ceased approach prematurely, high-frequency spikes were removed, and the occurrence of defense was reduced.

sensorymotor transformation; looming; defense; insect

DEFENSE BEHAVIOR IS A GOOD neuroethological model to study the question of how sensory signals are transformed into motor commands (e.g., Allen et al. 2006; Edwards et al. 1999; Korn and Faber 2005). Escape responses to looming objects in the locust are one example that has been intensively studied. The locust possesses a pair of looming-sensitive neurons, the lobula giant movement detector (LGMD; O’Shea and Williams 1974) and descending contralateral movement detector (DCMD; Rowell 1971). There is good evidence that the DCMD is involved with locust escape responses such as gliding and jumping (see Simmons et al. 2010 for a review). During flight, the locust responds to a looming object with gliding behavior (Santer et al. 2005a). Gliding is considered to cause a rapid loss of height, which is interpreted as a last-ditch escape response to the looming stimuli (Santer et al. 2012). The DCMD responds to looming stimuli with a prolonged burst of spikes that tracks stimulus approach, and this burst of high-frequency spikes is a key trigger for gliding (Santer et al. 2005a, 2006). On the ground, the locust evades predatory threats by jumping using its hindlegs. The DCMD is suggested to affect the timing of hindleg flexion in preparation for an escape jump (Santer et al. 2008). It has also been suggested that the preparatory phase (cocontraction) of the jump is triggered after the DCMD firing rate crosses a certain threshold and that the time of DCMD peak firing may contribute to the time of take-off (Fotowat et al. 2011). However, DCMD activity is not essential for triggering an escape jump (Fotowat et al. 2011; Santer et al. 2008).

Alongside the locust, the praying mantis can also be a good model for understanding the neural basis of sensorimotor transformation. When a real object approaches, the mantis shows defense responses consisting of rapid retraction of forelegs under the prothorax (Yamawaki 2011). It has been suggested that a looming-sensitive neuron in the mantis (Yamawaki and Toh 2009b) is involved with this defense behavior. Although it has not been identified yet, extracellular recordings from the cervical connective have revealed that there is at least one looming-sensitive descending neuron in the mantis nervous system. The neuron has similar response properties to the DCMD: it shows the largest response to looming stimuli compared with other visual stimuli such as receding, darkening, and wide-field motion stimuli, and a higher peak firing frequency is elicited by faster or smaller looming stimuli (Yamawaki and Toh 2009b). However, the neuron has a receptive field on the ipsilateral side to its axon at the cervical connective, whereas the DCMD has a receptive field on the contralateral side. Most stimulus parameters (e.g., size, velocity, and trajectory of looming) known to affect the response of a looming-sensitive neuron also affect the defense reaction in the mantis (Yamawaki 2011), suggesting the involvement of the neuron in triggering the defense. However, little is known about the possible mechanism whereby the activity of the looming-sensitive neuron triggers the mantis defense.

Studying the mantis defense has an advantage: the neural pathway involved with it is likely to be relatively simple because the mantis defense is characterized by the retraction of the foreleg, a single flexion movement (Yamawaki 2011; Fig. 1A; Supplemental Video S1, available in the data supplement online at the Journal of Neurophysiology Web site). In contrast, the locust escape jump consists of three distinct phases: cocking, cocontraction, and triggering (e.g., Heitler 1974; Heitler and Burrows 1977; Santer et al. 2005b). In the process of cocking, the hindleg tibiae are tightly flexed against femurs. Then, cocontraction of the flexor and extensor tibiae muscles is performed to store the energy for the jump. Last, triggering releases the energy for the jump by ceasing and inhibiting the flexor muscle activity. Hence, the neural circuitry underlying the “motor pattern” of these three phases consists of several motor neurons and interneurons in the metathoracic ganglion and possibly several sensory neurons for feedback (Burrows 1996). In the case of locust gliding, the contraction of the second tergosternal muscle (M84) raises the forewings and holds them elevated above the hindwings in the stereotyped
gliding posture (Santer et al. 2005a). A burst of spikes in motor neuron 84 (Simmons 1980) can cause this contraction (Santer et al. 2005a). Although this pathway is simpler than that for the forelegs, it can be elicited in the mantis settled on the ground.

Another advantage of studying the mantis defense is that it can provide a clue to the general architecture of the looming-evoked defense system in insects and its evolution. Although looming-sensitive neurons have been reported in other insects such as fruit flies (Fotowat et al. 2009) and moths (Wicklein and Strausfeld 2000), the locust is a rare case in that both neural and behavioral responses to looming stimuli have been systematically studied. However, most neurophysiological techniques used in the locust are also applicable to the mantis because these two insects have a similar body size and structure. Whereas the locust is prey for several insectivorous predators, the mantis is not only prey, but also a predator. The mantis may have similar but different looming-evoked defense systems that can discriminate an approaching predator from approaching prey. Comparison between the mantis and the locust is expected to help us to understand how the looming-evoked defense system of these insects has been adapted to their lifestyles.

In the present study, we confirmed that the mantis defense against looming stimuli was elicited by its expanding motion as reported in locust escape (e.g., Santer et al. 2005a). Then, the activity of the looming-sensitive neuron was extracellularly recorded from the mantis while executing defense. We attempted to clarify the role of the looming-sensitive neuron in the defense, mainly by analyzing the correlation between neural activity and behavioral responses.

MATERIALS AND METHODS

Animals and preparation. We used adult praying mantises (Tenodera aridifolia). The mantises were reared from eggs collected in the suburbs of Fukuoka, Japan, to adulthood in the laboratory at 25 ± 3°C with a 12:12-h light-dark photoperiod (light phase = 0900–2100). The hatched nymphs of mantises were fed with fruit flies (Drosophila melanogaster) or nymphs of crickets (Acheta domesticus) according to their body size. To avoid cannibalism, nymphs older than the 3rd instar were raised individually.

Mantises were tethered via the dorsal pronotum and settled on a Styrofoam ball (10 cm in diameter) that was kept airborne by a small fan (Fig. 1B). The longitudinal axis of the mantis’ body was inclined at an angle of ~40° relative to the horizontal plane, following Rossel (1980). The mantis showed normal behaviors such as self-cleaning and walking in tethered conditions.

Visual stimuli. Visual stimuli were displayed on the thin-film transistor (TFT) liquid crystal display (Sharp LL-151TR; refresh rate = 60 Hz) and were generated by a computer (Epson MT7900) running a custom-written Visual Basic (Microsoft) program. The computer also generated an electric trigger signal at the offset of visual stimulation using a digital-to-analog converter (Interface PCI-3523A). The TFT display did not flicker during refreshing. The display was placed vertically in front of the mantis at a distance of 40 mm from the eyes, and the subtense of a pixel on the display was 0.425° at the largest. A square region (132 × 132°) of the display was used as a background to present visual stimuli. This square region was covered by a transparent, electrically conductive film (Toray Industries), and the other region was covered by metal boards. The mantis’ head was placed in front of the center of this square region. Experiments were performed under the illumination of fluorescent lamps. The luminance of white and black on the screen were 97 and 5.4 cd/m² (Konica Minolta LS-110), respectively.

We used five kinds of visual stimuli presented on a white square background. First, we used a looming or receding circle. This was a black circle that altered in size to mimic an 8-cm circular disk moving...
toward or away from the mantis at a constant velocity of 2 m/s. The simulated distance at the initial and final positions of the approaching disk were 304 and 4 cm, respectively, and its initial and final subtense were 1.5 and 90°, respectively. These parameters were chosen because this looming circle effectively elicits strong excitation of a looming-sensitive neuron in the mantis (Yamawaki and Toh 2009b). In some experiments, the diameter of the approaching disk ranged from 1 to 16 cm, and its velocity ranged from 0.5 to 4 m/s. The initial and final subtense of the approaching disk also ranged from 0.2 to 3.0° and from 14.3 to 126.9°, respectively, when its diameter was manipulated. The subtense was not changed when its velocity was manipulated. Looming stimuli are characterized by an increasing rate of angular subtense expansion, the time course of which depends on the ratio of the half-size of an approaching object, I, to its speed, lv (Gabbiani et al. 1999). The angular subtense of an approaching object, \( \theta(t) \), is a function of \( l/v : \theta(t) = 2 \tan^{-1}(l/vt) \), where \( t \) denotes the time remaining to collision (Fig. 1C). The \( l/v \) value of looming circles ranged from 2.5 to 80 ms. Second, we used a linearly expanding circle. This was a circle expanding at a constant edge speed of 30°/s. The initial and final subtense and duration of presentation were the same for the looming circle. Last, we used a gradually darkening or lightening circle. This was an 8-cm circle that gradually changed its luminance from white to black or vice versa so that the time course of the luminance change approximated that produced by a looming or receding circle.

**Behavioral analysis.** A high-speed camera (DITECT HAS-220R) positioned on the right side of the mantis recorded its behavior at 200 frames/s. The recording was controlled by the trigger signal from the computer presenting visual stimuli. However, there was a considerable delay (~20 ms) between launching the trigger signal and drawing the graphics on the screen. For this reason, both the mantis behavior and visual stimulus were video-recorded.

The mantis received each visual stimulus six times. The interstimulus interval was >150 s. The mantis showed three kinds of behavior in response to visual stimuli: defense, fixation, and strike. Defense behavior was defined as retracting the forelegs under the prothorax (Fig. 1A; Yamawaki 2011), which corresponds to subcryptic reaction reported by Watanabe and Yano (2010). Fixation was defined as turning the head toward the stimulus. Strike was defined as extending the forelegs toward the stimulus and retracting them rapidly. The response rate of each behavior was defined as the ratio of the number of presentations where the behavior occurred to the total number of presentations (6). In responses to looming stimuli, the timing of the defense behavior was measured by counting the number of frames from the end of the stimulus presentation to the initiation of the defense behavior. The end of the stimulus presentation was defined as the last frame where the expanding motion was observed. The initiation of the defense behavior was defined as the frame before the first frame where the flexion of coxo-trochanteral joints of forelegs was observed.

**Extracellular recordings.** We used hook electrodes for extracellular recordings of the responses of the neuron sensitive to looming stimuli. Details of the methods have been previously described (Yamawaki and Toh 2009b). After cold anesthesia, a mantis was restrained ventral side up with dental wax to insert the hook electrodes. A small piece (2.0 × 3.0 mm) of cuticle was removed from the ventral prothorax along the midline between the base of the foreleg coxae and the spiracles, exposing the right and left connectives between the pro- and mesothoracic ganglia. The hook electrodes were implanted around the left connective. We replaced the removed piece of cuticle over the aperture and sealed it with beeswax. The hook electrodes consisted of a pair of silver wire hooks (diameter = 0.1 mm) attached together with epoxy resin and insulated with beeswax and silicone rubber except for the recording site. The electrodes were connected to the probe of an alternating current-amplifier (MEG-6108; Nihon Kohden) with 600-μm rubber-insulated copper lead wires (Mogami). The wires were passed from the recording sites in the ventral prothorax to the pronotum, fixed at intervals by small drops of beeswax so as not to disturb the behaviors of the mantis. After these operations, the mantis was released and then tethered to the apparatus described above.

Extracellular responses and the trigger signals indicating the offset of stimulus presentation were stored using the audio channel of a digital video camera (Sony DCR-PC300K). The timing of extracellular responses was compensated for by taking into account the 20-ms delay between sending the trigger signal and drawing the graphics. The video channel of the camera was used to monitor the type of stimulus presented. The simultaneous firing of several different spikes was observed in extracellular recordings. The data were stored on a computer, and spikes were classified into several units by template matching implemented in Spike2 (Cambridge Electronic Design). The unit sensitive to looming stimuli usually produces the largest action potentials in extracellular recordings from the nerve cord (Yamawaki and Toh 2009b) and can be easily identified from its characteristic responses to looming stimuli: a train of spikes that increases in frequency as the image of the looming stimulus expands (Fig. 1D).

We estimated the instantaneous firing rate by convolving the spike trains with a Gaussian window (time resolution = 1 ms; width \( \sigma = 20 \) ms) and normalizing the resulting waveform such that its integral was equal to the total number of spikes over the whole trial (Gabbiani et al. 1999). Then, the peak of the firing rate was found for each trial except trials in which no spikes were observed during stimulus presentation. We also discarded trials as outliers when the peak firing timing was separated from the average by 4 SD.

**Statistics.** SigmaPlot 12 for Windows (Systat Software) was used for all statistical analyses. The occurrence data of behavioral responses were analyzed using repeated-measures binary logistic regression, and the Wald statistic was used to determine whether a tested independent variable was a significant predictor of occurrence. When categorical independent variables (such as subjects and visual stimuli) were used, these were converted into an equivalent set of dummy variables using reference coding. Analyses of correlation were carried out with Spearman rank-order correlation, and its correlation coefficient is denoted by \( r_s \). Least-squares linear regression was used for linear fitting. The differences in the timing of defense initiation and firing rates between groups of trials were analyzed with the Mann-Whitney U test (MWT) or the Kruskal-Wallis test (KWT) by treating behavioral responses as if they were each performed by a separate animal for the following reason. The number of defense occurrences for each stimulus was different among mantises, and some mantises did not respond to a certain stimulus at all. Hence, if we applied repeated-measures statistical tests such as Friedman test, it would average the defense timing for each mantis and generate many missing observations, which can lower the statistical power.

**RESULTS**

**Effects of expanding motion on defense responses in intact mantises.** In the first series of experiments, we confirmed that the defense behavior of the mantis against looming stimuli was elicited by their expanding motion and that the rate of expansion affected the mantis responses. Although defense behaviors in the mantis are elicited by an approaching object (Yamawaki 2011), it was not known whether expanding motion, rather than luminance change, was the main driver of eliciting the defense behaviors. To test the effect of luminance changes, we presented 12 intact mantises with 5 kinds of visual stimuli: looming, receding, linearly expanding, darkening, and lightening circles (Fig. 2A). A looming circle evoked defense behavior most effectively. The effect of looming stimuli on the probability of defense occurrence was significant (logistic regression; \( n = 360; \) Wald \( \chi^2 = 38.608, df = 1, p < 0.001 \)), and its regression coefficient (4.153) was the largest among all factors.
The linearly expanding stimuli also significantly affected the defense occurrence ($\chi^2 = 9.293$, df = 1, $P = 0.002$). These results suggested that luminance change alone was not enough to elicit defense behavior. The response rates of fixation and strike to all stimuli were too small to be statistically analyzed. The mantis occasionally showed strike behavior in response to looming stimuli but not to other stimuli. Hereafter, we focused on defense responses to looming stimuli.

Next, we confirmed that the rate of image expansion in looming stimuli affected the defense responses in the mantis. To manipulate the expansion rate, we altered both the size and velocity of looming and examined their effects on defense. In locusts, for example, the velocity of looming affects the rate of gliding responses: faster looming stimuli elicit gliding responses more frequently (Santer et al. 2005a). When the looming velocity was kept at 2 m/s and the diameter of the looming circle ranged from 10 to 160 mm, there was no significant effect of looming size on the defense occurrence (11 mantises, $n = 330$; Wald $\chi^2 = 1.508$, df = 1, $P = 0.219$; Fig. 2B). When the looming diameter was kept at 80 mm and the looming velocity ranged from 0.5 to 4 m/s, the looming velocity significantly affected the defense occurrence (12 mantises, $n = 288$; Wald $\chi^2 = 16.060$, df = 1, $P < 0.001$; Fig. 2C): faster looming elicited the defense more frequently. Defense rates to looming stimuli tended to be lower than those in the previous experiment. Because these experiments were performed with different mantises, it is possible that individual differences between mantises affected defense rates. Nevertheless, the results clearly indicate the effects of looming velocity on defense occurrence.

Significant effects of expansion rate were observed on the timing of defense initiation, too. Larger looming circles elicited defense responses earlier relative to the end of stimulus presentation (KWT; $n = 92$ behavioral responses by a total of 11 mantises; $H = 45.05$, $P \leq 0.001$; Fig. 2D). There was a weak tendency toward slower looming eliciting the defense earlier ($n = 67$ behavioral responses by a total of 12 mantises; $H = 5.17$, $P = 0.0219$; Fig. 2E). The effects of size and velocity of looming on defense timing were examined more intensively in the next experiment. The earliest and latest defense timings
were \(-375\) and \(45\) ms relative to the end of looming, respectively. Then, in the following experiments, we defined defense behavior as rapid foreleg flexion that was initiated within the time range from \(-500\) to \(500\) ms relative to the end of looming. The analysis of the firing pattern of the looming-sensitive unit was performed within this time range, too.

**Effects of \(|lv|\) on timing of behavioral and neural responses.**

In the second series of experiments, we attempted to clarify the role of the looming-sensitive neuron in triggering defense behavior by recording neural and behavioral responses simultaneously from the same mantises. First, we reexamined the effects of the size and velocity of looming on defense timing by using six mantises implanted with electrodes for each experiment. Reexamination of these effects revealed again that mantises initiated the defense behavior earlier in response to larger \((KWT; n = 54\) behavioral responses; \(H = 26.4, P \leq 0.001;\) Fig. 3A) and slower \((KWT; n = 46\) behavioral responses; \(H = 20.4, P \leq 0.001;\) Fig. 3B) looming stimuli. Although the distribution pattern of defense timing for each stimulus might appear to be different from that in the previous experiment, general tendency was the same. This result might be caused by electrode implantation or individual differences in mantises.

The effects of size and velocity of looming on defense timing suggested that the defense behavior occurred a fixed delay after the stimuli had reached a fixed angular subtense. According to Gabbiani et al. (1999), this occurs if the time remaining to the collision at the initiation of the defense behavior is linearly correlated with the ratio of the half-size of an approaching object to its speed \((lv|v|)\). To test this, we pooled the data from both experiments manipulating velocity and size simultaneously from the same mantises. First, we reexamined the effects of the size and velocity of looming on defense timing by recording neural and behavioral responses in the present study for the following reasons. First, most defense responses occurred after these bursts ceased. Second, each burst lasted for only a short duration (approximately \(10–20\) ms), and its instantaneous firing rate rarely reached a high frequency when convolved with a Gaussian window. Last, even if these bursts might have affected the occurrence rate or timing of defense responses, the results still can provide a clue to how the activity of the neuron triggers the defense response.

Among several possibilities that can explain the coincidence between neural and behavioral responses, we first tested the possibility that the peak of the firing rate of the looming-sensitive unit generally coincides with the timing of the initiation of the defense behavior in all cases, whether the looming velocity was manipulated from \(0.5\) to \(4.0\) m/s (Fig. 4) or the diameter of looming circle was manipulated from \(1\) to \(16\) cm (Fig. 5). In response to slower looming stimuli, high-frequency firing of the looming-sensitive neuron occurred earlier, and defense behavior occurred earlier, too (Fig. 4).

It is possible that the looming-sensitive neuron is strongly excited when the subtense of an object reaches the threshold, and this high activity triggers the defense response in the mantis. Indeed, the timing of high-frequency firing of the looming-sensitive unit generally coincided with the timing of the initiation of the defense behavior in all cases, whether the looming velocity was manipulated from \(0.5\) to \(4.0\) m/s (Fig. 4) or the diameter of looming circle was manipulated from \(1\) to \(16\) cm (Fig. 5). In response to slower looming stimuli, high-frequency firing of the looming-sensitive neuron occurred earlier, and defense behavior occurred earlier, too (Fig. 4). Similarly, in response to larger looming stimuli, both the firing of the unit and the defense occurred earlier (Fig. 5). Although an intermittent burst of spikes occurred during the presentation of each stimulus, and these bursts were an artifact of the low screen refresh rate (60 Hz; Yamawaki and Toh 2009b), they were considered to have substantially little effect on the results in the present study for the following reasons. First, most defense responses occurred after these bursts ceased. Second, each burst lasted for only a short duration (approximately \(10–20\) ms), and its instantaneous firing rate rarely reached a high frequency when convolved with a Gaussian window. Last, even if these bursts might have affected the occurrence rate or timing of defense responses, the results still can provide a clue to how the activity of the neuron triggers the defense response.

**Fig. 3.** Effects of size and velocity of looming on the timing of defense initiation in mantises implanted with electrodes. A and B: the timing of defense initiation relative to the end of presentations of looming varied in diameter from \(1\) to \(16\) cm (A) or in velocity from \(0.5\) to \(4.0\) m/s (B). For each experiment, 6 mantises were used. Stimuli were presented to each mantis 6 times. C: the time remaining to expected collision at defense initiation are plotted as a function of stimulus size to speed ratio \((lv|v|)\). The mean and SE of all defense responses to each stimulus is indicated, combining the data of A and B. \(n = 100\) Behavioral responses.
T2), and defense responses are controlled by motor neurons in the prothoracic ganglion (T1). Hence, peak timing must be compensated by supposing that recording had been made from the connective between the subesophageal and prothoracic ganglia (SG-T1). It has been reported that there is a one-to-one correspondence of the spike firing of the looming-sensitive neuron between SG-T1 and T1-T2 recordings with a fixed delay of ~12 ms (10–14 ms; Yamawaki and Toh 2009b). Then, the compensated delay can be estimated at 42 ms, and a linear regression suggested that the firing rate of the unit (recorded from SG-T1 connective) reached the peak 42 ms before the angular subtense of looming objects had reached 47°. There was significant correlation in timing between the firing peak and the defense (n = 99 responses; r_S = 0.508, P ≤ 0.001; Fig. 6B), although the coefficient of determination of linear regression was low (r^2 = 0.158). The mean and SD of peak timing relative to defense initiation were −10.0 ± 73.8 ms, and its compensated relative timing was −22 ms (Fig. 6C). These results might imply that the firing peak of the looming-sensitive unit is one of the factors determining the timing of defense initiation, but the larger angular threshold of firing peak (47°) than of defense initiation (38°) did not positively support this possibility.

The difference in angular threshold suggested that firing peak generally occurred after the defense initiation during the expansion of object image. However, firing peak must precede it to trigger the defense.

Comparing the maximum firing rate between trials where defense occurred and other trials without defense (Fig. 6D) suggested the other possibility that reaching a certain firing rate threshold was important for triggering defense. The distribution for trials with defense was significantly shifted to larger firing rates compared with trials without defense (MWT; n = 320 behavioral responses; U = 9180.5, P = 0.022), and the peak of its distribution was ~150 Hz in trials with defense. To test the above possibility, the correlation coefficient between firing rate of the looming-sensitive unit and |v| was calculated for each moment in time relative to defense initiation using the data from all trials where defense occurred (Fig. 7A; n = 100 behavioral responses, time resolution = 5 ms). When the correlation coefficient is 0, the firing rate must be almost the same in all trials irrespective of |lv|. Then, it is possible that the firing rate at that time is an important feature for triggering the defense. Indeed, the correlation coefficient was nearly 0 at a timing of −95 and 5 ms relative to defense initiation (Fig. 7A). The compensated timings for these were −107 and −7
Because the latter timing was too late to trigger defense and the mean firing rate passed its peak at that timing, we focused on the former timing. The mean and SD of the firing rate were 147 ± 77 Hz at the former timing, and there were no significant differences in firing rate among different \( |v| \) values (KWT; \( n = 100 \) behavioral responses; \( H = 11.09, P = 0.135 \)). Then, we measured the timing when the instantaneous firing rate exceeded 150 Hz for each trial in which defense occurred (\( n = 88 \) responses; Fig. 7B). Although the firing rate did not exceed 150 Hz in some trials with defense (\( n = 12 \)), this timing (\( >150 \) Hz) preceded the defense initiation in most trials. The mean and SD of its timing relative to defense initiation were \(-119 ± 105\) ms, and the compensated mean timing was \(-131\) ms. Next, we measured this timing (\( >150 \) Hz) for all trials. This timing (\( >150 \) Hz) was significantly correlated with \( |v| \) (\( n = 248 \) neural responses; \( r_S = 0.747, P ≤ 0.001 \); Fig. 7C). The mean and SE of the slope and intercept of the linear regression were \( 3.58 ± 0.25 \) and \( 93.78 ± 8.02 \), respectively, indicating an angular threshold of \( 31° \) and a compensated delay of \(-106\) ms. However, the relationship between this firing timing and \( |v| \) did not appear to be linear.

We did the same analysis with different firing rates of \( 160, 180, \) and \( 200 \) Hz. There was a significant linear regression, but the coefficient of determination was \( 0.2 \) for each case (data not shown). These results suggested that defense could be triggered 110–130 ms after the firing rate of the looming-sensitive unit exceeds 150 Hz.

Fig. 5. Effects of the looming diameter on responses of the looming-sensitive unit (PSTHs) and the defense initiation (filled circles). The looming diameter ranged from 1 to 16 cm, whereas the velocity was kept at 2.0 m/s. Data were from 6 presentations to each of 6 mantises implanted with electrodes.
Manipulating responses of the looming-sensitive neuron and its effects on behavior. To test further the hypothesis of firing rate threshold, we modified looming stimuli to stop moving 0.14, 0.06, or 0.02 s earlier than usual ($n = 320$ mantises; Fig. 8). By incrementally reducing the looming stimuli, we expected that the final high-frequency spikes of the looming-sensitive unit would be removed (Santer et al. 2006). Then, if the hypothesis were true, the frequency of occurrence of the defense behavior would be decreased. As expected, the defense behaviors were not observed when stimuli ceased moving 0.14 s earlier. Comparing the maximum firing rate between trials with and without defense indicated again that the distribution of firing rates was significantly different.

Fig. 6. Effects of peak firing of the looming-sensitive unit on defense response. $A$: the time remaining to collision at peak firing rate (mean and SE) is plotted as a function of the stimulus size to speed ratio, $l/v$. $n = 99$ Responses. $B$: the time remaining to collision at defense initiation is plotted as a function of time at peak firing rate. $n = 99$ Responses. $C$: the time of peak firing rate relative to defense initiation. Negative values indicate that firing rate reached a peak before defense initiation. $n = 99$ Responses. $D$: maximum firing rate at peak in trials where defense occurred (gray, $n = 99$ responses) and did not (black, $n = 221$ responses). The peak firing rate was calculated for each trial for data used in Figs. 4 and 5.

Fig. 7. Effects of firing rate of the looming-sensitive unit on defense response. $A$: the correlation coefficient between firing rate and $l/v$ was calculated for each moment in time relative to defense initiation (bottom). $n = 100$ Behavioral responses. Time resolution was 5 ms. The mean firing rate for each moment is also shown (top). Vertical broken lines indicate the time when correlation coefficient was nearly 0. When the correlation coefficient is 0, the firing rate must be almost the same in all trials irrespective of $l/v$. Thus it is possible that the firing rate at that time is an important feature in triggering the defense. $B$: the timing relative to defense initiation when firing rate exceeded 150 Hz. $n = 88$ Responses. $C$: the time remaining to collision when firing rate exceeded 150 Hz (mean and SE) is plotted as a function of the stimulus size to speed ratio, $l/v$. $n = 248$ Neural responses. $D$: the time remaining to collision at defense initiation is plotted as a function of that time when firing rate exceeded 150 Hz. $n = 88$ Responses. The same data as in Figs. 4 and 5 were used for analysis.
bution for trials with defense was significantly shifted to larger firing rates compared with trials without defense (MWT; \( n = 144 \) behavioral responses by a total of 6 mantises; \( U = 849, P < 0.001; \) Fig. 9A). In addition, the firing rate exceeded 150 Hz in most (84%) trials with defense. In contrast to previous results, there was no significant correlation between firing peak timing and defense initiation (\( n = 25 \) responses; \( r_s = 0.176, P = 0.395; \) Fig. 9B). The timing when the firing rate exceeded 150 Hz tended to correlate with defense initiation but not significantly (\( n = 21 \) responses; \( r_s = 0.420, P = 0.058; \) Fig. 9C).

Fig. 8. Effects of stopping looming stimuli on responses of the looming-sensitive unit (PSTHs) and the defense initiation (filled circles). The image expansion in looming stimuli (8 cm in diameter, 2.0 m/s in velocity) was stopped 0, 0.02, 0.06, and 0.14 s before its end (vertical lines). The final angular subtense were 90, 52, 28, and 14°, respectively (bottom traces). Horizontal broken line superimposed on bottom traces shows the hypothesized angular threshold subtense (38°). Data were from 6 presentations to each of 6 mantises implanted with electrodes.

Fig. 9. Firing rate threshold was a better predictor of defense timing than peak firing. A: maximum firing rate at peak in trials where defense occurred (gray, \( n = 25 \) responses) and did not (black, \( n = 119 \) responses). B and C: the time remaining to collision at defense initiation is plotted as a function of that time at peak firing rate (\( B; n = 25 \) responses) or when firing rate exceeded 150 Hz (\( C; n = 21 \) responses). The same data as in Fig. 8 were used for analysis.
DISCUSSION

We have investigated the role of the looming-sensitive neuron in defense responses of the mantis to approaching objects. The results suggest the possibility that the activity of the looming-sensitive neuron can be a trigger for defense behavior and considerable potential of the mantis as a model for investigating the neural basis of sensorimotor transformation. By applying the neurophysiological techniques established in the locust, we succeeded in long-lasting recording of both neural and behavioral responses simultaneously in the tethered mantis. Although in the present study we recorded responses of only the descending neurons that extend their axons from the brain to the thoracic ganglia, we expect that it will be possible to record from brain neurons and motor neurons innervating foreleg muscles in tethered but behaving mantis with techniques established in insects.

However, compared with the locust, there are many open questions on the neural mechanism underlying the mantis defense. Hereafter, we will first discuss the visual parameters that affect the mantis defense and then the role of the looming-sensitive neuron in triggering the defense. Finally, we will mention the perspective of further research on mantis defense.

Critical visual cues for eliciting the mantis defense. We demonstrated that not only real approaching objects, but also computer-generated looming stimuli could elicit the defense behavior in the mantis. In addition, we confirmed that the types of stimuli that elicit the mantis defense were the same types that excite the looming-sensitive neuron in the mantis (Yamawaki and Toh 2009b) and the locust (DCMD; Rind and Simmons 1992; Simmons and Rind 1992). For example, Simmons and Rind (1992) have reported that the critical image cues for the selective response of the DCMD neuron are an increase in the amount of the edge (expanding image) and a continual increase in edge velocity. This characteristic coincided with the present result that the mantis defense was elicited by the expanding motion of a looming image, not by a luminance change (Fig. 2A). Defense response rates to looming stimuli tended to be larger than that to linearly expanding stimuli, suggesting the importance of the continual increase in edge velocity. It has also been reported that locust gliding is triggered by stimuli that optimally excite the DCMD (Santer et al. 2005a, 2006).

Faster looming stimuli tended to cause the mantis defense more frequently in the present study (Fig. 2C). In flying locusts, the frequency of glide occurrence increased as looming velocity increased, reaching a plateau at a stimulus velocity of 3 m/s and above (Santer et al. 2005a). Hence, the mantis defense responses showed a tendency similar to those in locust gliding.

Effects of size and velocity of looming on the timing of defense initiation. The results of experiments manipulating the size and velocity of looming (Fig. 3) suggested that the mantis defense occurred a fixed delay after the subtense of a looming image had reached a certain angular threshold. However, the accuracy of both delay (−6.5 ± 7.3 ms) and angular threshold (38°) is still questionable. First, it is unlikely that the delay is negative or nearly 0. Between the detection of stimuli and the behavioral reaction, there must be an inevitable delay imposed by neural processing. For example, flying locusts initiate collision avoidance behaviors 65 ms after the stimulus has reached an angular size of 10° (Robertson and Johnson 1993). In case of take-off reaction in locust jumps, delay is 57 ms on average, and angular threshold is ~60° (Fotowat and Gabbiani 2007). Second, some results did not support the angular threshold being 38°. For example, the maximum subtense of an image that 2-cm looming generated was 28° (Fig. 5), which did not reach the estimated angular threshold of 38°, but this stimulus elicited the defense to some extent. Similarly, the looming stimulus that stopped approaching 0.06 s before its end finally generated a 28° circle (Fig. 8, 3rd plot from top) but elicited the defense. Hence, the threshold value might be ~30°.

Accurate estimation of the delay and angular threshold might be possible by recording muscle activity directly. The initiation timing of leg movements not only is controlled by active muscular contractions, but also can be affected by gravity, inertia, and, especially, passive forces intrinsic to the leg (e.g., Ache and Matheson 2013). Page et al. (2008), for example, have reported the extension of the femorotibial joint of the locust hindleg in the absence of any extensor motor activity. They have suggested that passive (springlike) joint force moves the tibia toward the neutral position. It is possible that some foreleg movements recognized as defense responses in the present study might be accidentally caused by similar passive joint forces rather than by motor activity presumably elicited by the looming-sensitive neuron. Thus excluding such passive movements is important for precise analysis of looming-evoked response. Hence, we are planning to record the electromyogram of foreleg muscles in response to looming stimuli.

Nevertheless, we cannot preclude the possibility that delay is variable depending on stimulus in the mantis defense. If this is the case, the formulation established by Gabbiani et al. (1999) is not applicable for estimating the delay and angular threshold.

Two strategies for collision avoidance. For collision avoidance, two different strategies are typically used (e.g., Nakagawa and Hongjian 2010; Rind and Simmons 1999). The first is to use estimated time remaining to collision as a sensory cue for avoidance. The second is to use an angular threshold value for the size of the retinal image subtended by an approaching object. Nakagawa and Hongjian (2010) have suggested that time to collision is used for approach behaviors such as the control of prelanding reactions (e.g., Lee et al., 1993; Wagner 1982), whereas angular threshold is used frequently to trigger avoidance behaviors to approaching objects such as predators or rivals (e.g., Robertson and Johnson 1993; Santer et al. 2008; Yamamoto et al. 2003). In other words, behaviors requiring the accurate control of the timing of interception with an object use time to collision for the complicated control of motor pattern, whereas behaviors requiring the fastest responses to approaching objects use angular threshold for simple and quick motor action.

In case of the mantis defense, this behavior is considered as defense against rapidly approaching predators, which requires the fastest response. Hence, it is not surprising if the mantis uses the angular threshold to trigger defense responses against
an approaching object. It should be noted that this does not preclude the possibility that some behaviors in the mantis use time to collision. For example, the mantis sometimes catches flying insects such as flies. In the case where the fly is approaching the mantis, this task might require the estimation of the timing when the fly enters the scope of predatory strike, i.e., time to collision.

A slightly different strategy from detecting angular threshold has been reported in predator avoidance of some crustaceans. For example, the crab *Neohelice granulata* initiates an escape run when the stimulus angular increment reaches 7° (Oliva and Tomsic 2012), and the crayfish defensive reflex occurs when the angular size of the approaching object increases by 7.5–10° (Glantz 1974). In these cases, the critical stimulus parameter to initiate defense is an increase in the apparent size of the stimulus rather than its absolute value. Thus the initial angular subtense of an approaching object affects the timing of defense initiation. The experiments manipulating initial angular subtense are required to test whether this is the case for the mantis defense.

**Role of the looming-sensitive neuron in triggering the mantis defense.** Here, we propose that high-frequency spikes of the looming-sensitive neuron are involved in triggering the defense behavior, especially the initiation of foreleg flexion, for the following reasons. First, the timing of high-frequency firing of the looming-sensitive neuron generally coincided with the timing of defense initiation when either the size or speed of looming was manipulated (Figs. 4 and 5). Second, the distribution of maximum firing rate for trials with defense was shifted to larger rates compared with trials without defense (Figs. 6D and 9A). Last, when a looming stimulus ceased approach prematurely, high-frequency spikes were removed, and the occurrence of defense was reduced (Fig. 8).

However, high-frequency spikes of the looming-sensitive neuron were not sufficient for triggering the defense. Indeed, we observed high-frequency spikes in some trials where defense did not occur (Figs. 6D and 9A). This suggests that the looming-sensitive neuron observed in the present study is only one of the factors that contribute to causing the defense and that several other descending neurons might be involved in it. In the locust, high activity of the looming-sensitive neuron (DCMD) is not necessary for triggering either the cocking (Santer et al. 2008) or escape jump (Fotowat et al. 2011), and the involvement of other neurons such as the descending ipsilateral movement detector (DIMD; e.g., Burrows and Rowell 1973) is suggested.

The looming-sensitive neuron in the mantis might contribute to the precise control of the timing of defense initiation rather than causing it, as suggested in the role of the DCMD in the cocking phase of locust jumps (Santer et al. 2008). The results of the present study suggested that the timing when the firing rate of the looming-sensitive neuron exceeded 150 Hz was a better predictor of defense initiation than its peak timing. Defense timing was correlated with this timing (>150 Hz) better than peak timing in either when we manipulated l/hv l (Figs. 6B and 7D) or we reduced looming stimuli (Fig. 9, B and C). In addition, firing rate generally exceeded 150 Hz ~100 ms before the defense initiation, irrespective of l/hv l (Fig. 7, A and B), suggesting that defense is triggered ~100 ms after the firing rate exceeds 150 Hz. The control of initiation timing by firing rate threshold has also been suggested in the cocontraction phase of the locust jump (Fotowat et al. 2011). Still open to question is how the timing when the firing rate exceeds 150 Hz contributes to angular threshold strategy for triggering defense. The relationship between this timing (>150 Hz) and l/hv l did not appear to be linear (Fig. 7C). In addition, although the angular threshold of this firing timing (31°) was smaller than that of defense initiation (38°), and this does not contradict our hypothesis, its delay (~106 ms) was much lower than 0. Hence, it is less likely that firing rate is directly used for detecting a certain angular subtense. Several other neurons might be involved in detecting an angular subtense.

**Future perspectives.** Our goal is to understand the neural mechanism underlying the mantis defense at the cellular level. Its neural circuit can consist of, at least, visual interneurons in the brain, descending neurons extending their axon from the brain to the thoracic ganglia, and local interneurons and motor neurons in the prothoracic ganglion. Although there are a few studies investigating visual interneurons (Yamawaki and Toh 2003) and descending neurons (Yamawaki and Toh 2009a,b) in the mantis, little is known about these neurons, especially the motor neurons controlling the foreleg movements. In the locust, contrary to the mantis, not only visual interneurons and descending neurons (such as LGMD and DCMD), but also many motor neurons in the metathoracic ganglion have been identified (e.g., Burrows and Hoyle 1973; Hoyle and Burrows 1973a,b). In addition, many synaptic connections between a descending neuron and motor neurons (e.g., Burrows and Rowell 1973) and between different motor neurons (e.g., Hoyle and Burrows 1973a) have been investigated. Therefore, we plan to identify these neurons and synaptic connections between them in the mantis, in particular, the output connections of looming-sensitive neurons. Furthermore, we are attempting simultaneous recording of both responses of the looming-sensitive neuron and the electromyogram of foreleg muscles for investigating the role of the DCMD in evoking muscle activity more intensively.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Y.Y. conception, design, and supervision of research; K.S. performed experiments; K.S. and Y.Y. analyzed data; Y.Y. interpreted results of experiments; K.S. and Y.Y. prepared figures; K.S. drafted manuscript; Y.Y. edited and revised manuscript; Y.Y. approved final version of manuscript.

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