Spatiotemporal stimulus properties modulate responses to trajectory changes in a locust looming-sensitive pathway

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Dick PC, Gray JR. Spatiotemporal stimulus properties modulate responses to trajectory changes in a locust looming-sensitive pathway. J Neurophysiol 111: 1736–1745, 2014. First published January 29, 2014; doi:10.1152/jn.00499.2013.—The lobula giant movement detector (LGMD) and descending contralateral movement detector (DCMD) constitute one motion-sensitive pathway in the locust visual system that is implicated in collision-avoidance behaviors. While this pathway is thought to respond preferentially to objects approaching on a direct collision course, emerging studies suggest the firing rate is able to monitor more complicated movements that would occur under natural conditions. While previous studies have compared the response of the DCMD to objects on collision courses that travel at different speeds, velocity has not been manipulated for other simple or compound trajectories. Here we test the possibility that the LGMD/DCMD pathway is capable of responding uniquely to complex aspects of object motion, including translation and trajectory changes at different velocities. We found that the response of the DCMD to translational motion initiated in the caudal visual field was a low-amplitude peak in firing rate that occurred before the object crossed 90° azimuth that was invariant to different object velocities. Direct looming at different velocities resulted in peak firing rates that occurred later in time and with greater amplitude for higher velocities. In response to transitions from translational motion to a collision course, the firing rate change depended on both the location within the visual field and the velocity. These results suggest that this pathway is capable of conveying information about multiple properties of a moving object’s trajectory.

THE NATURAL ENVIRONMENT of any animal is a complex combination of sensory stimuli. However, it is adaptive to be vigilant to environmental cues that are salient for a particular animal or species, such as an approaching predator. Rapidly approaching (looming) objects elicit avoidance reactions in many animal species (Gibson 1958). The behavioral and neuronal responses to looming have been studied in many invertebrate and vertebrate systems, such as crabs (Medan et al. 2007; Oliva and Tomsic 2012), frogs (Yamamoto et al. 2003), pigeons (Sun and Frost 1998; Wu et al. 2005), cats (Liu et al. 2011), primates (King and Cowey 1992; Maier et al. 2004), and humans (Vallis and McFadyen 2005; Gray and Regan 2006). Research on looming-sensitive neurons has focused on the mechanisms that underlie responses to objects traveling along simple trajectories, while only recently have the response to increasingly complex environments been explored (Guest and Gray 2006; McMillan and Gray 2012).

Locusta migratoria, the migratory locust, is an ideal system for studying the neural mechanisms that underlie the processing of object visual motion. Gregarious locusts in a swarm fly as close as 30 cm apart (Uvarov 1977) with flight speeds of 3–6 m/s (Baker et al. 1981), which likely requires accurate responses to multiple objects traveling at different velocities, often along changing trajectories. Behaviorally, approaching objects evoke coordinated steering responses from flying locusts (Chan and Gabbiani 2013; McMillan et al. 2013; Robertson and Johnson 1993a; Santer et al. 2006). The descending contralateral movement detector (DCMD) is an identified motion-sensitive neuron in the locust visual system that, along with its presynaptic partner, the lobula giant movement detector (LGMD), constitutes one motion-sensitive neural pathway that responds robustly to looming objects (Hatsopoulos et al. 1995; Judge and Rind 1997; Rind and Simmons 1992; Schlotterer 1997), but is also sensitive to translational motion within its visual field (McMillan and Gray 2012; Pinter 1983; Rind 1987). The LGMD receives integrated retinal inputs that, during looming, evoke a characteristic increase in firing rate that is transmitted to the DCMD in a 1:1 ratio via a mixed chemical and electrical synapse (Rind 1984). The firing rate peaks after a defined subtense angle on the retina is exceeded and occurs near the projected time of collision (TOC) (Gabbiani et al. 2002; Krapp and Gabbiani 2005). Structurally, the DCMD connects to flight interneurons and motoneurons within the thoracic ganglia (Schlotterer 1977; Simmons 1980), implicating this pathway in mediating collision avoidance behaviors (Santer et al. 2006). Modulation of the DCMD firing rate has been correlated with the timing and occurrence of jumps (Fotowat and Gabbiani 2007) as well as with the occurrence of gliding behaviors during flight (Santer et al. 2006; Simmons 1980).

Previous experiments have shown that visual information alone can trigger adaptive behavioral responses in locusts (Chan and Gabbiani 2013; McMillan and Gray 2012; Robertson and Johnson 1993b; Santer et al. 2006), and that the DCMD responds to complex scenes (Rind and Simmons 1992), paired object approaches (Guest and Gray 2006), objects with compound shapes (Guest and Gray 2006), and objects following compound trajectories (McMillan and Gray 2012). These findings strongly suggest that this single pathway may be capable of encoding complex motion, which likely exists in the locust’s natural visual environment. Recently, McMillan and Gray (2012) found that the DCMD responded to transitions between translation and looming with firing rate modulation that depended on direction and distance. To enhance our understanding of the capabilities of this neural pathway, we presented locusts with the image of a black disk...
moving along variable trajectories at different constant velocities. Overall, our findings show that the modulation of the DCMD firing rate reflects aspects of compound visual motion. Stimuli with looming components evoked DCMD responses with characteristic peak timing and firing rate. We found that only the timing of the peak in DCMD responses to noncolliding trajectories was significantly affected by velocity. We also found that the DCMD firing rate modulation at a transition between translation and looming was affected by the velocity of the projected disk, as well as the region of the visual field where the transition occurred. Furthermore, the collision-associated DCMD responses to the looming component of compound trajectories were not affected by the previous translation. Overall, these data describe the response of the DCMD to the additional feature of object velocity while expanding on previously explored responses to translational, looming, and complex visual motion.

MATERIALS AND METHODS

Animals. Adult male Locusta migratoria were obtained from a crowded colony maintained in the Department of Biology at the University of Saskatchewan (25–28°C, 12:12-h light-dark cycle). Locusts selected were at least 3 wk past the imaginal molt. Experiments were carried out at room temperature (~25°C).

Preparation. The experimental setup was similar to that described recently (McMillan and Gray 2012). The locust’s legs were removed, and a rigid tether was attached to the ventral surface of the thorax with melted beeswax while the wings were held in place. A small patch of ventral cervical cuticle was removed to expose the underlying paired connectives of the ventral nerve cord anterior to the prothoracic ganglia. The tissue was bathed in a drop of locust saline (in mmol: 147 NaCl, 10 KCl, 4 CaCl2, 3 NaOH, 10 HEPES, pH 7.2), and the preparation was transferred to the recording stage. Neuronal recordings were obtained from the left ventral nerve connective using two bipolar silver wire hook electrodes insulated with a Vaseline and mineral oil mixture. The preparation was then rotated so that the locust was oriented dorsal side up with the longitudinal axes 10 cm away from and perpendicular to the apex of a rear projection dome screen, so that the right eye was aligned with the azimuthal and elevational axes of the dome apex (see Fig. 1 of Guest and Gray 2006). In this way, azimuthal positions along the equator of the dome at 0°, 90°, and 180° were directed to the frontal, lateral, and caudal visual fields, respectively. The preparation was left for ~15 min in front of a projected white visual field before presentation of stimuli to allow acclimation to the experimental setup. To prevent confounding effects of neural habituation, the interval between each presentation was at least 3 min.

Visual stimuli. The procedure used for visual stimulus generation and data acquisition was similar to that used by Guest and Gray (2006). Visual stimuli were created with Vision Egg visual stimulus generation software (Straw 2008) on a Python programming platform and represented as 1,024 × 1,024 pixel portable network graphics (png) files. Individual pixel sizes on the projection screen were ~0.7 mm, a visual subtense angle of ~0.4°, below the highest spatial resolution of the compound eye (Horridge 1978). A 7-cm-diameter black disk traveling at five different velocities (350, 175, 116.7, 87.5, and 70 cm/s) was scaled in real time at 85 frames/s and projected onto a specialized rear projection dome screen with an InFocus DepthQ LCD data projector, with correction factors embedded in the Vision Egg code to account for the distortion due to projection onto the curved surface of the screen. A 1.2-ms transistor-transistor logic pulse included in each video frame and the vertical refresh synchronization pulse (vsync) from the video card (NVIDIA GeForce4 Ti4200 128 MB) were used to align physiological recordings with events associated with the stimuli (see below). The last transistor-transistor logic pulse was used to determine the final frame of each presentation, which indicated when the object had disappeared from the screen. The corresponding vsync pulse determined the start time of the rendering of the frame. The luminance values of the disk (170 cd/m²) and background (483 cd/m²), as well as the resulting Michelson contrast ratio (0.48), were the same as those used previously (Guest and Gray 2006; McMillan and Gray 2012).

Locusts (n = 20) were presented with a randomized set of visual stimuli while neuronal responses were recorded. All visual stimuli were presented at 0° elevation, and the position varied only within the azimuthal plane. The stimulus set consisted of five unique trajectories repeated with five different velocities (Fig. 1). Each presentation sequence began and ended with a direct loom from 90° to test for potential hysteresis effects of the duration of the experiment on DCMD responses. All other trajectories were presented in a different random order for each locust. For each presentation, the disk began movement as soon as it appeared and remained on the screen for 1 s before disappearing within one frame. Direct looms ended 10 cm from the eye of the locust. Trajectory changes occurred over one frame.

Responses to complex motion. This experiment was designed to test DCMD responses to visual motion that translates before changing to a collision course while travelling at different velocities. We define translation as local small-field motion along a straight trajectory parallel to the longitudinal axis of the locust (Fig. 1). This type of translational motion is thus distinct from wide-field optic flow induced during translatory self-motion of the animal and small-field motion within the azimuthal plane at a fixed distance from the animal. Here we used a distance of 80 cm (a subtense angle of 5° at 90° azimuth) for translational motion, an intermediate distance shown to evoke robust DCMD responses (McMillan and Gray 2012). Disks that followed trajectories with no translational component approached from 400 cm at 90° azimuth. Trajectories with no looming component travelled across 168° of the azimuthal plane (174° to 6°).

To test the effects of trajectory changes during object motion, we presented compound trajectories that contained translational and looming components. For all compound trajectories, motion began in the posterior visual field and traveled along a translational trajectory as described above. Translational motion transitioned to looming at 45°, 90°, or 135° azimuth.

To test the effect of velocity, we presented each trajectory type (looming only, translation only, and three compound trajectories) at five different speeds. We used a common measure of a looming stimulus profile which relates the ratio of the object half size (h) and
absolute velocity (\(lv\)) (Gabbiani et al. 1999). The size of the projected disk was constant for all trajectories (7-cm diameter), and therefore velocity was varied to produce different \(lv\) values; velocities of 350, 175, 116.7, 87.5, and 70 cm/s were used, with corresponding \(lv\) values of 10, 20, 30, 40, and 50 ms, respectively. The ratio of size and velocity has been used previously (see Gabbiani et al. 2002) and is based on a biologically relevant object size (Santer et al. 2012).

Spike sorting and quantification of DCMD firing properties. For each stimulus presentation we recorded continuously and stored neuronal activity from the left cervical connective, pulses synchronized with each frame of the stimulus, and vsync pulses from the video card for analysis. Recorded neural activity was amplified with a differential AC amplifier (A-M Systems, model no. 1700, gain = 10,000) and sampled at 25 kHz. An RP2.1 enhanced real-time processor (Tucker-Davis Technologies, Alachua, FL) with Butterworth filter settings of 100 Hz (high pass) and 5 kHz (low pass) was used to store the data to disk. The characteristic large-amplitude DCMD spikes were identified by threshold analysis in Offline Sorter (Plexon, Dallas, TX). Spike times were exported to Neuroexplorer analysis software (NEX Technologies, Littleton, MA), and spike times were transformed into peristimulus time histograms with a 1-ms bin width and smoothed with a 50-ms Gaussian filter (Fig. 2). To characterize DCMD firing properties, we measured the firing rate \((f)\) for each local peak or valley in firing rate as well as the peak width at one-half the maximum firing rate for peaks associated with TOC. We also measured response delays \((\delta)\) from time of transition (TOT), TOC, or time that the object crossed 90° azimuth (T90). Linear regressions of \(lv\) values and response parameters were used to calculate the slope and \(y\)-intercept \((b)\) of the relationships.

In previous work that presented compound trajectories (McMillan and Gray 2012), the change in \(f(\phi')\) and expansion characteristics of the visual stimuli were used to develop a two-dimensional (2D) Gaussian model of the putative relationship. Our data, with the additional manipulation of object velocity, were used to further test the robustness of the model. Briefly, the instantaneous angular acceleration of the disk subtense angle \((\phi')\) and the instantaneous azimuthal acceleration of the leading edge \((\phi'')\) of the projected disks were calculated at TOT from translational to looming motion. The \(f\) over the single frame of transition from translation to looming was averaged from all animals \((n = 20)\) for each of the 15 stimuli that included a change in trajectory. Current and previous (McMillan and Gray, 2012) results were fit with a 2D Gaussian equation of the general form

\[
\hat{z} = a e^{-\frac{1}{2} \left[ \left( \frac{x - x_0}{b} \right)^2 + \left( \frac{y - y_0}{c} \right)^2 \right]}
\]

where \(\hat{z} = f', x = \phi', y = \phi'', x_0\) and \(y_0\) define the center of the peak of the mesh plot, \(a\) is the height of the peak, and \(b\) and \(c\) are related to the width of the curve in the \(x\)- and \(y\)-planes, respectively.

Statistical analysis. DCMD firing parameters were tested for putative effects of velocity and direction with R (The R Foundation for Statistical Computing) and SigmaStat 3.5 and plotted with SigmaPlot 10.0 (Systat Software, Richmond, CA). Data were initially tested for normality and equal variance to determine the use of parametric or nonparametric tests. The correlation of the data were quantified using Pearson product-moment correlation coefficients \((r_p)\) for parametric data or Spearman’s rank correlation coefficients \((r_s)\) for nonparametric data to determine the strength and direction of linear dependence. Differences between velocities and trajectory and their effects on firing parameters were tested with a two-factor repeated-measures analysis of covariance (ANCOVA) on ranks and plotted as line graphs with means ± SD. For all tests, significance was assessed at \(P < 0.05\).

RESULTS

Translation. Translating disks evoked slow increases in the DCMD spike rate, with a low-amplitude peak near T90, followed by a decrease to the resting \(f\) (Fig. 3). While there was no effect of velocity on the peak \(f(\bar{r_p} = -0.076; P = 0.45)\), we
found a weak correlation between object velocity and the timing of the peak ($r_p = -0.21; P = 0.034$; Fig. 4A).

**Looming.** Looming disks evoked consistent and characteristic DCMD responses with a spike rate that increased during object approach and peaked near TOC (Fig. 3). Comparing data from the first and final approaches at 90°, we found no significant differences in the peak firing amplitude (Student’s paired $t$-test; $t_{18} = -0.040; P = 0.97$), peak time ($t_{18} = 0.0015; P = 0.89$), or peak width at half-maximum amplitude ($t_{18} = 0.0016; P = 0.87$). Therefore, there was no hysteresis effect on the duration of each experiment. The velocity of the projected disk was significantly correlated with the peak $f$ ($r_p = -0.50; P < 0.001$), peak time ($r_p = -0.89; P < 0.001$), and peak width at half-maximum ($r_p = 0.38; P < 0.001$; Fig. 4B).

**Compound trajectories.** Similar to previous findings (McMillan and Gray 2012), we found that disks following trajectories that transitioned from translational motion to looming evoked a DCMD response with a TOT-associated decrease in $f$ and a subsequent increase in $f$ leading to a TOC-associated peak (Fig. 5). The translational component of the trajectory evoked a slow increase in $f$ as the disk approached 90° azimuth. For trajectories where the disk passed through 90°, the $f$ decreased again as the object continued translation (Fig. 5A).

**Approach angle and velocity.** To determine the relationship between velocity and the measured neuronal parameters, Pearson or Spearman’s coefficients were calculated for all trajectories. For all approach angles (Fig. 6), there was a significant correlation between object velocity and the peak $f$ ($r_p = 0.40, 90° = -0.54, 45° = -0.58; P < 0.001$), peak time ($r_p = 0.45 = -0.90; r_p = 45° = -0.93; P < 0.001$), and peak width at half height ($r_p = 0.3, 45° = -0.55; r_p = 90° = 0.42; P; 135° = 0.003, 45° < 0.001, 90° < 0.001$). The $f$ at TOT was correlated with velocity for 135° approaches ($r_p = -0.50; P < 0.001$) and weakly correlated for 45° approaches ($r_p = -0.28; P = 0.005$), while the valley $f$ was significantly correlated for all angles ($r_p = 0.61, 90° = -0.54, r_p = -0.62; P < 0.001$). The difference between TOT and valley $f$ values was only weakly correlated with velocity for a 90° approach ($r_p = 0.25; P = 0.008$). The timing of the valley was correlated for 135° ($r_p = -0.41; P < 0.001$) and 90° ($r_p = -0.46; P < 0.001$), but not for 45° approaches. These results show the significant effects of both velocity and approach angle on numerous measures of the DCMD response to visual motion.

To determine whether there was individual or combined effects of velocity and trajectory on the measured neuronal parameters, we used two-factor repeated-measures ANCOVA on ranks integrating both $l/h/v$ value and the approach angle. For transitioning stimuli, there was a significant effect of velocity ($F_1 = 103.45; P < 0.001$) on the peak $f$, while the peak time and width were both affected by the velocity ($F_1 = 1.024.47, 66.86; P < 0.001$) and a combined effect of velocity and approach angle ($F_1 = 8.56, 4.47; P < 0.001, 0.035$). The $f$ at TOT was affected by the velocity ($F_1 = 34.42; P < 0.001$) and the angle ($F_1 = 10.07; P = 0.002$), and a combination of the two ($F_1 = 3.91; P = 0.049$), while the $f$ at the valley was significantly affected by the velocity ($F_1 = 204.69; P < 0.001$) and the angle ($F_1 = 10.79; P = 0.001$) with no interaction. The difference between the $f$ values at TOT and the valley was only significantly affected by the angle of approach ($F_1 = 19.42; P < 0.001$). The timing of the valley was affected by the velocity ($F_1 = 2.187.89; P < 0.001$), the approach angle ($F_1 = 7.15; P = 0.008$), and the interaction ($F_1 = 12.69; P < 0.001$). No significant effect was found for the interaction on valley $f$ ($F_1 = 0.29; P = 0.593$), peak $f$ ($F_1 = 2.76; P = 0.098$), or the difference between $f$ at TOT and the valley ($F_1 = 1.76; P =
0.186). The trajectory had no significant effect on the peak \( f (F_1 = 3.86; P = 0.050) \), peak timing \( (F_1 = 0.94; P = 0.334) \), or the width of the peak at half height \( (F_1 = 2.06; P = 0.153) \), and the velocity had no effect on the difference between \( f \) at TOT and the valley \( (F_1 = 0.044; P = 0.834) \). The results of the ANCOVA show that, for all measured parameters, there was a significant effect of either velocity or approach angle.

**Expansion properties at trajectory changes.** Previous experiments have described the expansion properties of objects following trajectories that transition between translation and looming that differed with respect to trajectory prior to transition, the proximity of the transition, and TOT within stimulus presentation. We combined our transitional data here with those of McMillan and Gray (2012) and fit them with the 2D Gaussian equation as they described:

\[
f' = 2.9e^{-0.5 \left( \frac{\theta + 10.1}{28.9} \right)^2 \left( \frac{\psi + 169.8}{211.6} \right)^2}
\]

The amplitude change \( (f') \) was predicted by \( \theta' \) and \( \psi' \) \((r^2 = 0.56)\), whereas the \( \delta \) was poorly fit \((r^2 = 0.18, \text{Fig. 7})\). These data further suggest that modulation of the DCMD \( f \) was related to the unique trajectory changes of a moving object (Fig. 7B), whereas the delay between TOT and the response was invariant (Fig. 7B).

**DISCUSSION**

This is the first study to quantify and compare the firing of a locust visual interneuron to objects that transition from translation to looming while travelling at different velocities. DCMD responses to objects looming with different velocities were consistent with previous findings, showing that slower objects evoked lower, wider, and earlier peaks in the \( f \). We found that, in response to translational motion, the DCMD peak \( f \) was invariant to object velocity and that peak time was only weakly correlated, with earlier peaks occurring at lower velocities (higher \( l/hv \)). These data suggest that the DCMD is not sensitive to different velocities of translational objects. For objects that transitioned to looming, we found that the DCMD \( f \) at TOT, the timing and width of the peak response, and the timing of the valley were all dependent on both trajectory and velocity. This suggests that the response of the DCMD, resulting from properties of a presynaptic network of retinal inputs to the LGMD, reflects aspects of object motion that may be perceived as a threat. Finally, our data support the previously proposed relationship between \( f' \) and unique expansion properties \((\theta' \) and \( \psi' \)), with invariance in \( \delta \) (McMillan and Gray 2012).

**Looming.** The DCMD response to a looming disk was a stereotyped increase in \( f \) with a peak just before TOT (Fig. 2), which is consistent with previous reports (Gabbiani et al. 1999; Gray 2005; Gray et al. 2001; McMillan and Gray 2012; Rind and Simmons 1992). While the velocity-dependent peak \( f \) and time resemble findings from Gabbiani et al. (2002), we report higher peak \( f \) values that decayed more slowly with an increase in \( l/hv \) values. Rind and Santer (2004) attribute the low firing frequency to postsynaptic feed-forward inhibition (Rind and Bramwell 1996) caused by the exaggerated size of the visual

Fig. 4. Parameters of the DCMD response to translational (A) and looming (B) trajectories. Peak \( f \) and time for five different \( l/hv \) values are shown for both translational and looming stimuli, with the addition of peak width for responses to looming. Data represent means ± SD \((n = 20)\). Peak times are relative to T90 (translation) or TOC (looming). Equations of the line and Pearson’s correlation coefficients \((r_p)\) or Spearmann’s coefficients \((r_s)\) are given for each. A: translational stimuli evoked earlier peaks with higher \( l/hv \) values, but had no significant difference in peak amplitude. B: peaks in the \( f \) occurred earlier and were lower and wider for looming stimuli with higher \( l/hv \) values. δ. Response delays. *Significant correlations, assessed at \( P < 0.05 \).
of translational velocity of small-field objects on DCMD re-
ver, this is the first experiment to directly examine the effect
responses to moving edges (Rind and Simmons 1992). How-
), which is consistent with
response to slower objects (Fig. 3
A
), indicating
in a manner dependent on the size, distance, and velocity
of the object. In combination with data from McMillan and
Gray (2012), we found that $f$ was significantly correlated with
$\theta^*$ and $\psi^*$ ($r^2 = 0.56$), while the timing of the response was
invariant ($r^2 = 0.18$). Although these data support the previ-
ously reported correlation for a wider range of stimuli, the
underlying biophysical mechanism remain to be determined.

Fig. 5. Response profiles for the average DCMD response ($n = 20$) to visual
stimuli transitioning between translation and looming at 45° (A) or 135° (B)
azimuth with five different $l/v|l$ values (indicated in A). For both approach
angles, transition-induced valleys were most distinct for lower $l/v|l$ values (10
and 20 ms). A: for $l/v|l$ values of 30 to 50, the response is similar to responses
to pure translation, with the $f$ decreasing almost to a resting value after the disk
crossed 90° azimuth. B: the translational component has little effect on the $f$ for
higher $l/v|l$ values.

Translational motion. While the LGMD/DCMD pathway responds vigorously to looming objects (Gabbiani et al. 1999; Krapp and Gabbiani 2005; Schlotterer 1977), it also responds to small-field, translating objects (McMillan and Gray 2012; Palka 1967; Peron and Gabbiani 2009b; Pinter et al. 1982). Consistent with previous reports (McMillan and Gray 2012), the projected translating disks evoked an increase in the DCMD $f$ with a peak near T90. We found weak negative correlations between the $l/v|l$ of the translating disk and the timing of the peak $f$, such that the peak occurred earlier in response to slower objects (Fig. 3A), which is consistent with responses to moving edges (Rind and Simmons 1992). However, this is the first experiment to directly examine the effect of translational velocity of small-field objects on DCMD res-
ponses over such a large region of the locust’s visual field
(168°). Previous studies either manipulated velocity with non-
translational motion (Krapp and Gabbiani 2005) or with wide-
field stimuli (Simmons and Rind 1992) or did not change the velocity of the translational stimuli (Peron and Gabbiani 2009a). True translation in three-dimensional space would contain a looming component (see McMillan and Gray 2012); thus these results may be indicative of translational motion being a nonoptimal stimulus (Simmons and Rind 1992), with $f$ increasing only as a byproduct of the increase in retinal size as the translational object approached the minimum distance. As such, it is not surprising that the DCMD is relatively insensitive to a suboptimal stimulus moving at different velocities.

Trajectory changes and object velocity. Transition from translation to looming affects the expansion properties of a disk in a manner dependent on the distance and type of trajectory change (McMillan and Gray 2012). Our data show that the change in expansion properties is also affected by the velocity of the disk. At a transition from translation to looming, the $\theta^*$ increased while the acceleration of the leading edge ($\psi^*$) decreased, coinciding with a decrease in the DCMD $f$ (Fig. 7A). Accordingly, the changes in these stimulus parameters over a transition are uniquely related to the size, distance, and velocity
of the object. In combination with data from McMillan and
Gray (2012), we found that $f$ was significantly correlated with
$\theta^*$ and $\psi^*$ ($r^2 = 0.56$), while the timing of the response was
invariant ($r^2 = 0.18$). Although these data support the previ-
ously reported correlation for a wider range of stimuli, the
underlying biophysical mechanism remain to be determined.

While our data show that the initial translational motion did not affect the TOC-associated peak in DCMD $f$, it does show location-dependent modification of TOC-associated features of the DCMD response (Fig. 6). In fact, of all the parameters examined, only the peak in $f$ was not significantly affected by the angle of approach. The $f$ at TOC and the valley were both affected by velocity and location of the transition, while the difference between them was only affected by the final angle of approach (Fig. 6). The timing of the valley was also affected by velocity and angle. In addition, there was an interaction be-
tween velocity and angle for both the $f$ at TOC and the timing of the valley. These results together show that transition-
induced modulation of the LGMD/DCMD pathway is velocity
and location dependent.

DCMD responses to compound trajectories and velocity. A current model suggests that LGMD activity is controlled by excitation spreading through retinotopic inputs from the eye (Peron et al. 2009), which is modulated by feed-forward inhibition onto a branch of the LGMD (Gabbiani et al. 2002; Rind and Bramwell 1996). This suggests that the LGMD performs a multiplicative computation resulting in an increase in $f$ during object approach related to $l/v|l$ (Gabbiani et al. 2002). This model also explains how the DCMD is able to respond to novel stimuli after habituation (Gray 2005) and to multiple object approaches (Guest and Gray 2006). Our data are also consistent with this model. Translational motion results in an increased $f$ as the object expands with an increase in the velocity of the leading edge (Fig. 3). The $f$ decreases when the leading-edge expansion decreases across the ommatidia, either at the point of transition to looming or as the object crosses 90° azimuth. This decrease in expansion would allow feed-forward inhibition to counteract excitation in synchrony with a reduction in presynaptic excitation, with additional
adaptation at the level of the LGMD membrane (Peron and Gabbiani 2009b), ultimately resulting in the observed reduction in $f$. In the case of a translational trajectory, $\theta$ would continue to decrease, allowing inhibition to dominate, causing a decrease in $f$ to the resting level. This could also explain the difference in peak time for translation at different velocities. A slower moving object with identical size will have a lower leading edge velocity, allowing feed-forward inhibition greater influence on the $f$. Concurrently, the slower moving object allows more time for adaptation, resulting in the overall inhibition overcoming the excitation due to expansion more quickly, which is presented as an earlier peak time (Fig. 3).

While the response of the LGMD/DCMD pathway to direct looming in different regions of the locusts’ visual field was not directly tested here, previous results have shown no difference in response to looming over 135° azimuth (Gabbiani et al. 2001). The data presented here support these findings for all looming motion. However, components of the LGMD/DCMD response allows more time for adaptation, resulting in the overall inhibition overcoming the excitation due to expansion more quickly, which is presented as an earlier peak time (Fig. 3).

**Fig. 6.** Comparison of DCMD firing parameters between responses to compound trajectories with three different angles of approach (based on angle at point of transition) (blue: 135°, red: 90°, green: 45°) for five $l/|v|$ values. Data represent means ± SD ($n = 20$). Linear regression and $r_p$ or $r_s$ are given for each. A: peak $f$. B: peak $\delta$. C: peak width. D: TOT $f$. E: valley $f$. F: TOT valley $f$. G: valley $\delta$. All correlations were significant except for the valley timing for 45° approaches, $f$ at TOT for 90° approaches, and TOT-valley $f$ for 135° and 45°. *Significant correlations, assessed at $P < 0.05$. 
that are associated with transitions show local sensitivity. In particular, correlations between $l/|v|$ and the timing of the valley were reversed for transitions at 45° compared with transitions at and 135° and 90° (Fig. 6). For transitions in the frontal region of the eye, the decrease in $f$ after TOT reached its minimum sooner than for transitions in the caudal region. The eye of the locust has an acute zone of maximal ommatidial density in the frontal equatorial region (Horridge 1978; Krapp and Gabbiani 2005), but has a lower sensitivity to motion that is attributed to a greater electrotonic distance from the spike initiation zone of the LGMD, resulting in attenuation of electrical signals (Krapp and Gabbiani 2005; Peron et al. 2007). The more rapid drop in $f$ could be related to the decrease in $\psi'$ occurring over a larger subset of ommatidia than at either 135° or 90°. A simultaneous drop in excitation of many ommatidia, particularly those with the greatest electrotonic distance and thus the most dependent on synchronized membrane depolarization, could result in a more rapid decrease in $f$. When coupled with adaptation resulting from a slower-moving stimulus, this would result in $f$ reaching the minimum more quickly, explaining the observed negative correlation (Fig. 6).

McMillan and Gray (2012) suggested that the drop in $f$ after a trajectory change may reset the LGMD/DCMD pathway to a $f$ below that associated with the threshold angle implicated in looming detection (Gabbiani et al. 1999). They also found that the size of the drop was dependent on the distance that the transition occurred relative to the locust. Our data supports that the $f$ may reset the system, but found the timing of the change, rather than the magnitude, to be related to both $l/|v|$ and location within the visual field. While there was variation in the $f$ both at TOT and the subsequent valley (Fig. 5), there was limited change in the difference between TOT and valley $f$ for any of the angles or $l/|v|$ values tested (Fig. 6). This suggests that, through a combination of $f$ at TOT, $f$ at the valley, and the timing of the valley, LGMD/DCMD activity may reflect object distance, velocity, size, location, and direction during transition between looming and translation. In light of recent studies showing the behavioral implications of multiplexed information in the locust during visually-triggered escape jumps (Fotowat et al. 2011), these data may present a similar mechanism during locust flight. The instantaneous $f$ change ($f'$) may be related to the presence of a trajectory change, while the $f$ over the subsequent tens of milliseconds is related to additional information associated with the looming phase of object motion. In addition, while escape behaviors are triggered by DCMD activity exceeding a threshold (Fotowat and Gabbiani 2007; Santer et al. 2006), another motion-sensitive neuron (late DCMD) with coincident firing has been identified in the locust visual system (Gray et al. 2010). Multiple visual neurons have been implicated in relation to jumping behaviors in locusts (Fotowat et al. 2011; Santer et al. 2008), which presents the possibility that the activity of other visual neurons may act in concert with DCMD to form a population code for complex object motion, including trajectory transitions.

Fig. 7. Correlation of DCMD firing modulation with expansion parameters during trajectory transitions. A: sample data from presentation of a 7-cm disk travelling along a compound trajectory consisting of translation from the posterior with a transition to looming at 90° with an $l/|v|$ of 30 ms. The subtense angular velocity ($\theta'$, blue line) and rotational velocity of the leading edge of the disk ($\psi'$, red line) changed abruptly at the trajectory transition and preceded modulation of the DCMD $f$ (black line). The gray shaded area indicates a looming trajectory with the margin at TOT. TOC, time of collision. B: current (red) data from the mean $f$ change ($f'$) at TOT were plotted against the subtense angular acceleration ($\theta''$) and the rotational acceleration of the leading edge ($\psi''$) with previous data (black; McMillan and Gray 2012). The resulting scatterplot was fit with a Gaussian equation (gray mesh). C: data for the mean response time did not satisfy the tolerance of a Gaussian equation. Axes were scaled according to the range of values for each variable.
Our suggestion that the locust LGMD/DCMD pathway is capable of responding to unique aspects of complex object motion is supported by findings in other systems. Lobula plate tangential cells in the hoverfly *Eristalis* are capable of coding directionality of both elementary and figure motion (Lee and Nordström 2012). Dragonflies use small target motion detector neurons to track prey targets, with individual neurons that are selective to targets even within clutter (Nordstrom et al. 2006), and other neurons that are active on a longer time scale may enhance overall sensitivity of the system (Dunbier et al. 2012). In primates, neurons in the medial superior temporal area of the visual cortex respond to expansion, rotation, and deformation to control important behavioral functions (Mineaulet et al. 2012). It is important to note that, while many individual neurons in these systems show the capability to encode complex motion, they always work in conjunction with other neurons within their system. To this end, future experiments with the locust system will investigate other motion-sensitive neurons, both individually and as a population. In addition, wind tunnel experiments using similar stimuli to that used here are necessary to correctly correlate neuronal and behavioral responses.

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AUTHOR CONTRIBUTIONS

Author contributions: P.C.D. and J.R.G. conception and design of research; P.C.D. performed experiments; P.C.D. analyzed data; P.C.D. and J.R.G. interpreted results of experiments; P.C.D. prepared figures; P.C.D. drafted manuscript; P.C.D. and J.R.G. edited and revised manuscript; J.R.G. approved final version of manuscript.

REFERENCES


