Background complexity affects the response of a looming-sensitive neuron to object motion

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ABSTRACT

An increasing number of studies show how stimulus complexity affects the responses of looming sensitive neurons across multiple animal taxa. Locusts contain a well described, descending motion sensitive pathway that is preferentially looming sensitive. However, the lobula giant movement detector/descending contralateral movement detector (LGMD/DCMD) pathway responds to more than simple objects approaching at constant, predictable trajectories.

In this study, we presented Locusta migratoria with a series of complex 3-dimensional visual stimuli presented while simultaneously recording DCMD activity extracellularly. In addition to a frontal looming stimulus, we used a combination of compound trajectories (non-looming transitioning to looming) presented at different velocities and onto a simple, scattered, or progressive flow field background. Regardless of stimulus background, DCMD responses to looming were characteristic and related to previously described effects of azimuthal approach angle and velocity of object expansion. However, increasing background complexity caused reduced firing rates, delayed peaks, shorter rise phases and longer fall phases. DCMD responded to transitions to looming with a characteristic drop in a firing rate that was relatively invariant across most stimulus combinations and occurred regardless of stimulus background. Spike numbers were higher in the presence of the scattered background and reduced in the flow field background. We show that DCMD response time to a transition depends on unique expansion parameters of the moving stimulus irrespective of background complexity. Our results show how background complexity shapes DCMD responses to looming stimuli, which is explained within behavioural context.
INTRODUCTION

Flying animals are continually challenged with different forms of visual motion, including self-produced flow field motion (self motion in a stationary environment) and motion produced from an object moving within a stationary environment or in a direction opposite to a predicted one. The discrimination between these types of motion is paramount to an animal’s survival, for example predator avoidance. Flying through complex visual environments requires the detection of relevant salient visual cues for successful navigation. Looming objects, for example, provide critical information regarding an oncoming collision or perhaps an approaching predator. While stationary, an approaching visual stimulus is clearly interpreted as noxious, thus detection, and subsequent avoidance behaviour, may be relatively straightforward. However, while generating self-generated optic flow during movement or, in the case of swarming animals, surrounded by conspecifics moving at often unpredictable velocities and directions, detection of noxious stimuli is challenging.

The migratory locust, *Locusta migratoria*, is an established neuroethological model system for studying collision avoidance due to its long research history, easily tractable nervous system, and well-identified looming sensitive neurons (LSNs). During flight, a locust’s visual environment is dynamic. Within a swarm, individual locusts may fly ~3 m/s and in close proximity with each other, while maintaining flight elevations from 1 – 1000 m above ground (Uvarov 1977). Neighbouring locusts approach from different angles and at different velocities while land geography changes below. However challenging the environment, flying locusts are capable of avoiding collisions with conspecifics (Waloff 1972) and aerial attacks of diving birds while swarming (Santer et al. 2012). Indeed, experimental studies on free flying (Dawson et al. 2004) and loosely tethered flying locusts (Chan and Gabbiani 2013; McMillan et al. 2013) show
that locusts use a relatively unpredictable range of avoidance behaviours in response to noxious stimuli. Successful navigation within such a complex environment is, in part, related to a well-developed visual network of movement sensitive neurons, specifically and most widely studied are the lobula giant movement detector (LGMD) and its postsynaptic partner the descending contralateral movement detector (DCMD).

Excitation of the LGMD begins when movement within a locust’s visual field stimulate retinotopically arranged fibers within the ommatidia, which produce excitatory input to one of the three large dendritic fields of the LGMD (Rind 1984). During a looming approach, the number of spikes produced by the LGMD is directly related to an approaching object’s angular velocity and subtense angle and is thus referred to as an angular threshold detector (Gabbiani et al. 1999). As an object approaches the retina, the LGMD firing rate increases to a peak and then decays once object motion stops and before a collision would have occurred (Gabbiani et al. 1999, 2001, 2002; Gray 2005; Guest and Gray 2006; McMillan and Gray 2012; Dick and Gray 2014). Presynaptic lateral inhibition and postsynaptic feed-forward inhibition from the other two dendritic fields control excitation and thus define the peak firing rate of the LGMD (Rowell et al. 1977; Gabbiani et al. 1999, 2001). Each LGMD synapses onto a DCMD within the protocerebrum, generating a one-to-one spike ratio (O'Shea and Williams 1974); for the ease of access, many studies record from the DCMD axon within the contralateral side of the ventral nerve cord.

The LGMD/DCMD pathway is part of a relay system that tracks the approach and signals an impending collision of visual objects to motor centers within the thoracic ganglia (Simmons 1980). Phases of an avoidance jump have been linked to phases of this pathway’s firing rate (Fotowat and Gabbiani 2007) in addition to a possible role in modifying wing beat rhythm.
During flight (Santer et al. 2006), while the DCMD habituates to repetitive stimuli (Horn and Rowell 1968; Palka 1967; Gray 2005), it remains sensitive to a simple looming stimuli following translatory motion within a locust’s field of view and also responds to the transition to and from a looming trajectory (McMillan and Gray 2012; Dick and Gray 2014). Although it remains unknown if the DCMD is responsible for avoidance behaviours in complex environments (such as those found while flying in a swarm), these studies suggest that the DCMD is capable of responding to important aspects of a complex visual environment (see Rind and Simmons 1992). The interest in answering the question of complexity reaches beyond neurobiology and into robotics. MAVs and other robotic control systems are often engineered based on the physiology and circuitry of insect models.

To understand how visual neurons respond in natural environments, it is important to balance quantifiable stimulus parameters (i.e. object motion) with aspects of complex scenes (i.e. optic flow). In addition to a simple looming stimulus, we presented a combination of bilaterally paired non-looming and looming stimuli (i.e. compound trajectories) at varying velocities. All stimuli were presented in a 3-dimensional environment on a specialized dome projection screen using either a simple white background, a scattered background with hundreds of randomly translating dots to represent a “swarm”, or a progressive flow field background representing optic flow produced by forward motion. Consistent with previous work using compound approach trajectories (McMillan and Gray 2012; Dick and Gray 2014), the DCMD responded to a transition to looming with a quantifiable drop in firing rate (valley) that was relatively consistent for all trajectory types, velocities of object approach, and background environment. Response time from transition to DCMD valley was also consistent with previous work (McMillan and Gray 2012; Dick and Gray 2014) and remained relatively invariant across all
stimulus combinations. Moreover, within each stimulus background the DCMD firing rate was capable of tracking and responding to the motion of each stimulus. However, many of the measured response parameters of the DCMD response differed depending on the type of trajectory, velocity, and background. We show that although background complexity affects DCMD responses to looming and transitions to looming stimuli, the collision associated peak response is, in general, remarkably invariant.
MATERIALS AND METHODS

Animals

We used 14 adult male *Locusta migratoria* for experimentation. All animals, at least 3 weeks past the imaginal molt, were obtained from a crowded colony maintained in the Department of Biology at the University of Saskatchewan (25-28°C, 12hr:12hr light:dark). Experiments were carried out at ~25°C during similar times of the animals’ light cycle to avoid potential variations in responsiveness when locusts fly at night (Gaten et al. 2012).

Preparation

After the legs were removed and the wings were clipped, a rigid tether was attached to the ventral surface of the thorax using low melting point bee wax. 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. Locust saline (147·mmol NaCl, 10·mmol KCl, 4·mmol·CaCl$_2$, 3·mmol·NaOH, 10·mmol Heps, pH·7.2) was applied to the exposed tissue and the preparation was moved to the recording stage where two silver wire electrodes were hooked around the left and right ventral nerve connectives; the left and right recording sides are herein referred to as the left and right DCMDs. A mixture of Vaseline and mineral oil was used as an insulator around the recording site once we observed distinct neural responses from each connective to local motion (hand waving). A silver wire was also inserted into the locust’s abdomen and connected to ground. Each locust was oriented dorsal-side up and aligned with the azimuthal and elevation axes of the apex of a rear projection dome screen 10 cm away. In this orientation 0° was directly in front of the locust at the dome apex, ~90° was the center of the left
eye and 90° was the center of the right eye. To allow the animal to acclimate to the experimental setup, the preparation was left for ~10 minutes in front of each background before presenting any visual stimuli. Once experimentation began, we maintained a presentation-to-presentation interval of 3 minutes to prevent confounding effects of neural habituation.

Visual stimuli

The procedure used for visual stimulus generation and data acquisition was similar to that described in (McMillan and Gray 2012). Briefly, visual stimuli were created using Vision Egg (Straw 2008) on a Python programming platform and represented as 1024 x 1024 pixel portable network graphics (png) files. Each pixel on screen subtended approximately ~0.4° of the locust’s eye (well below the 1° resolution of individual ommatidia (Horridge 1978)). Two 7 cm diameter black discs travelling at different velocities and trajectories were presented simultaneously to the left and right side of the locust. Expansion properties associated with different velocities were described as a ratio of the half size of the disc ($l = 3.5$ cm) divided by the absolute velocity ($|v|$). All stimuli were scaled in real-time at 85 frames/sec (fps) and projected in 3 dimensions onto a rear projection dome screen using an InFocus DepthQ LCD data projector. A TTL pulse that was included in each video frame and the vertical refresh synchronization pulse from the video card (NVIDIA GeForce4 Ti4200 128 MB) were used to align neuronal recordings with the stimuli (see below).

All primary visual stimuli (7 cm diameter black disc) were presented at 0° elevation and modified within the azimuthal plane. The initial and final approach trajectory within each background type (see below) was a frontal (i.e. 0° azimuth) looming single disc travelling at 3 m/s ($l/|v| = 12$ ms) (Fig. 1A). Following the initial frontal loom, we presented a randomized series of compound trajectory types consisting of two 7 cm black discs that travelled...
simultaneously along one of three bilaterally-matched trajectories that transitioned from non-
looming to looming (Trajectory 1 (T1), Trajectory 2 (T2), and Trajectory 3 (T3)) (Fig. 1A). The
compound trajectory discs also travelled at one of three different velocities: 0.875 m/s ($l/|v| = 40$
ms), 0.5833 m/s ($l/|v| = 60$ ms), or 0.4375 m/s ($l/|v| = 80$ ms). Each series of compound
approaches was followed by a final frontal loom of a single disc. T1 consisted of a disc that
travelled orthogonal to the long axis of the locust’s body 50 cm anterior to the eyes, transitioned
at $\pm 45^\circ$ azimuthal angle and loomed toward the eye ipsilateral to the visual field of motion; T2
started in front of the locust, offset from direct center by 5 cm, approached to $\pm 90^\circ$ azimuthal
angle and transitioned to a looming approach; and T3 approached from behind the locust and
transitioned to a $\pm 90^\circ$ looming approach. All frontal looms and compound trajectories were
presented onto three stimulus backgrounds (Fig. 1B). The simple background (S) was a white
background with no other object motion other than the 7 cm disc. The scattered background (SC)
was projected over the entire screen and consisted of 600 black discs (0.8 cm diameter; angular
size = $4.6^\circ$) moving randomly in straight trajectories along a single plane orthogonal to the long
axis of the locust at 400 pixels/s (0.028 m/s, or an $l/|v| = 143$ ms). For the flow field background
(FF), we used a modified vertical grating pattern that consisted of vertical bars (width of each bar
= 2 cm; angular size = $11.42^\circ$) moving outwards in the azimuthal plane from the dome apex.
Each bar moved at 0.138 m/s across the dome and extended across the entire length of the dome
screen before disappearing from the field of view at the edge of the dome screen. Each
background, and stimuli presented within each background, maintained a similar contrast ratio,
with the exception of the FF, where the edges of the vertical bars faded from black to white. The
luminance values and Michelson contrast ratio (0.48) of the white background and black discs
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were similar to those previously (Guest and Gray 2006; McMillan and Gray 2012; Dick and
Gray 2014).

Within a randomized background type (S, SC, or FF), each animal was presented with a
randomized set of stimuli based on the trajectory (T1, T2, or T3) and stimulus velocity ($|l|/|v| = 40$
ms, 60 ms, or 80 ms); each different stimulus combination was presented only once per animal.
There was a 10-minute interval between each different background and 3-minute interval
between each stimulus presentation. We did not randomize background changes with stimulus
trajectory and velocity, since we were not concerned with the effect of switching from one
background to another. Moreover, with the exception of a few sporadic DCMD spikes, when the
only visual stimulus present was the SC or FF backgrounds type no DCMD responses were
generated. In total, there were 11 presentations per background per animal (33 presentations
total). For each presentation the 7 cm disc remained on the screen for 1 second before
disappearing within one frame and for all presentations with a trajectory change, the change
occurred over one frame.

Spike sorting and quantification of DCMD firing properties

For each presentation, neuronal activity from the left and right cervical connective, pulses
synchronized with each frame of the stimulus, and vsync pulses from the video card were
recorded continuously and stored for future analysis. All neural activity was amplified with a
differential AC amplifier (A-M Systems, model No. 1700, gain = 10,000) and sampled at 25
kHz. We used 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) to store the data.
Subsequent neuronal activity was analyzed using Off-line Sorter (Plexon, Dallas, TX) and
DCMD activity was isolated using threshold analysis. Spike times were exported to Neuroexplorer (NEX Technologies, Littleton MA) and transformed into peri-stimulus time histograms using a 1-ms bin width and smoothed with a 50 ms Gaussian filter.

We used a similar method described in (McMillan and Gray 2012) to characterize different DCMD firing properties. These included the firing rate \( (f) \) and time \( (t) \) of firing rate relative to time of collision (TOC) or time of transition (TOT) associated with each peak \( (f_p, t_p) \) or valley \( (f_v, t_v) \) firing rate as well as the peak width at 1/2 max firing rate, total spike number during the entire stimulus presentation, the response time \( (\delta) \) and change in firing rate \( (f_{TOT} - f_v) \) from each TOT to the associated valley, and durations of the rise and fall phases for each TOC and TOT-associate peak.

The rise phase of each DCMD response was calculated from the point at which the DCMD firing rate exceeded a 99% confidence interval (sampled from data for the entire stimulus presentation) to the peak of the DCMD firing rate (TOC or TOT-associated). The TOC-associated fall phase duration was calculated from the time of the frame when the stimulus stopped expanding to the time of the last spike following when the firing rate decreased to 15% of the peak DCMD firing rate (see Gabbiani et al. 2005 and Guest and Gray 2006). The TOT-associated falling phase duration was marked from the maximum firing rate prior to the time of the valley to the time of the valley; we used this measure rather than the time of TOT to valley since we defined this parameter as \( \delta \). We normalized the change in firing rate \( (f') \) for our 2D Gaussian fit by dividing the response to a transition (i.e. \( f_v \)) by the time of stimulus change (i.e. \( f_{TOT} \)), such that values between 0 and < 1 represented a relative decrease in firing rate and values > 1 represented an increase in firing rate (see McMillan and Gray 2012).
Statistical analysis

Statistical analysis was performed using SPSS 21.0 (Chicago, IL) and SigmaStat 3.5 and plotted using SigmaPlot 12.5 (Systat Software Inc., Richmond, CA). The statistical treatment of data depended on the number of effects we analyzed and whether the data were parametrically or non-parametrically distributed. In the case of frontal looms, parametric data were tested with one-way repeated measures (RM) ANOVA (reported by $F$ statistic) whereas non-parametric data were tested with Friedman RM ANOVA on ranks (reported by the $\chi^2$ statistic). All pairwise multiple comparisons for head on looming data were performed using a Tukey Test and significant results (i.e. $p < 0.05$) were described using the $q$ statistic and difference of ranks. For the 27 compound trajectories, if the data followed a normal distribution, a three-way RM ANOVA was performed (with factors: Trajectory (with levels: initial approach from the side (T1), front (T2), back (T3)); background (with levels: S, SC, FF); and velocity (with levels: $l/|v|$ = 40 ms, 60 ms, 80 ms). An aligned rank transformation (Wobbrock et al. 2011) was applied to the data if it was not normally distributed. In cases where the data failed Mauchly’s Test of Sphericity (i.e. $p < 0.05$), the test statistic and d.f. were corrected using the Greenhouse-Geisser estimate of sphericity. All compound trajectory statistics were reported with their $F$ statistic (with subscripted d.f. and error d.f.) and associated $p$-value. To refrain from excessive statistical reporting, only the statistics for the main effects and interaction effects of each factor were described. In all cases, $n = 14$ animals (28 neurons) were sampled and significance was assessed at $p < 0.05$. Descriptive statistics are reported as the mean +/- standard deviation (SD).

There were several instances where no TOT-associated DCMD response was detected so we used the SPSS single imputation method for 7 of the dependent variables ($n = 216/756$).
missing values for $f_v$, $t_v$, TOC peak rise phase, TOT peak rising and decay phases, $\delta$, and $f_{TOT} - f_v$). Based on trajectory type, a total of $122/252 = 48\%$, $21/252 = 8\%$, and $73/252 = 29\%$ of recordings in T1, T2, and T3, respectively, did not show TOT-associated responses; FF accounted for most non-responses in T1 and T3.
RESULTS

Regardless of stimulus background or recording side, DCMDs generated a characteristic rapid rise to a peak firing rate in response to a frontal loom (Fig. 2A). Although the DCMD response from different recording sides within the same animal were variable (for example, see Fig. 2A), we found no significant differences between the left and right DCMD responses or between the initial and final frontal looms within each background when comparing $f_p$, number of spikes, peak width at 1/2 max, and $t_p$ ($n = 14$ recordings, 1 presentation per animal, data not shown). Thus, responses were not affected by DCMD location (left or right) or the length of the experiment.

**Frontal looms – effects of increasing background complexity**

All data were pooled for a total of $n = 56$ recordings per background (14 animals, 2 recording sides, 2 looms per background type). We found a significant effect of background on $f_p$ ($\chi^2 = 18.3, p < 0.001$, Fig. 2B) and $t_p$ ($\chi^2 = 40.8, p < 0.001$, Fig. 2C). SC and FF resulted in a significantly lower ($q_{27} = 3.6$ and $q_{45} = 6.0$, respectively) and later ($q_{67} = 8.9$ and $q_{25} = 3.3$, respectively) peak relative to S. The peak also occurred significantly later in the presence of FF than SC ($q_{42} = 5.5$) and the median was slightly after time of collision (TOC). The number of spikes were also affected by different backgrounds ($\chi^2 = 8.6, p = 0.02$, Fig. 2D) with the highest number occurring in SC, which was significantly higher than in the presence of S ($q_{31} = 4.1$). Although we found no significant effect of background on peak width at 1/2 max ($F_3 = 2.7, p = 0.07$, Fig. 2E), the rise and fall phases of the peak firing rate were strongly affected by the type of background (rise phase: $\chi^2 = 76.1, p < 0.001$ and fall phase: $\chi^2 = 59.3, p < 0.001$, Fig. 2F). While SC resulted in a significantly shorter rise phase and longer fall phase relative to S ($q_{59} =$
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7.9 and $q_{48} = 6.4$, respectively), FF resulted in the shortest rise phase (FF vs S: $q_{91} = 12.2$; FF vs SC: $q_{32} = 4.3$) and longest fall phase (FF vs S: $q_{81} = 10.8$; FF vs SC: $q_{33} = 4.4$). Overall, these results show that background strongly affected DCMD firing patterns in response to a frontal looming stimulus. More specifically, increasing background complexity resulted in delayed peak firing, lower peak firing rates, higher spike numbers as well as shorter rise and longer fall phases.

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**Compound trajectories**

Similar to the frontal looming results, simultaneously presented bilateral compound stimuli resulted in no differences between individual left and right DCMD responses for the same trajectory. We subsequently pooled the left and right responses for statistical analysis.

Following a transition to a looming trajectory, the DCMD exhibited a brief drop in the firing rate that created a local valley followed by a TOC-associated firing rate increase (Fig. 3), which occurred regardless of background type and approach velocity and is consistent with previous findings (see McMillan and Gray 2012 and Dick and Gray 2014). We also observed lower peak amplitudes, later peak times, shorter rise and longer fall phases in the more complex backgrounds (Fig. 3). In some trajectories (such as T1 - FF, Fig. 3), TOT-associated responses were masked by the presence of a flow field. Statistical comparisons can be found in Table 1 and valuations of effect weight were based on the resulting $F$ statistic. All TOC-associated parameters are summarized in Fig. 4, TOT-associated parameters in Fig. 5, and the rise and fall phases in Fig. 6.

Table 1 summarizes the statistical analysis of all 13 response variables for all three factors and their interactions (n = 28). Trajectory affected all measured DCMD response variables, changes in stimulus velocity affected all variables except the peak width at 1/2 max, $\delta$,
and TOT fall phase whereas the background type affected all variables except the $f_{TOT}$, $t_v$, and $\delta$. Interactions between stimulus variables also affected DCMD responses. The interaction between trajectory and velocity affected all variables except $f_p$, the interaction between trajectory and background affected all variables except peak width at 1/2 max and $\delta$, and the interaction between velocity and background affected all variables except peak width at 1/2 max, $f_{TOT}$, and the TOC fall phase. The combined effect of all three factors (trajectory, velocity and background) resulted in significant differences in all variables except peak width at 1/2 max, $f_p$, $f_{TOT}$, and $t_v$.

TOC-associated response parameters are affected by trajectory, velocity, and background

Regardless of trajectory or background, higher $l/|v|$ values (lower velocities) evoked a higher number of spikes ($80\text{ ms} > 60\text{ ms} > 40\text{ ms}$, Fig. 4A). For T1, more spikes were evoked depending on the background ($SC > S > FF$). The background type had variable effects on responses to the different trajectory types, where there was little effect of trajectory type in S, while in SC and FF there was an effect of trajectory type ($T1 > T3 > T2$). Overall, velocity had the strongest effect on the number of spikes produced; however the interaction between trajectory and background had the strongest interaction (Table 1).

With the exception of trajectory (where $T3 > T1 > T1$) and background (where $S > SC$ and FF), we found few differences in peak width at 1/2 max (Fig. 4B). Table 1 shows that the type of trajectory had the greatest effect on peak width at 1/2 max followed by the type of background. Although changes in velocity did not affect the peak width at 1/2 max, we did find an interaction effect with trajectory, which is likely related to the strong effect of trajectory.
Generally, regardless of trajectory or background, lower $l/|v|$ values (higher velocities) evoked a higher $f_p$ ($40 \text{ ms} > 60 \text{ ms} > 80 \text{ ms}$, Fig. 4C). The type of background also affected $f_p$ within each trajectory and velocity ($S > SC > FF$). Although there wasn’t as great of an effect, trajectory type also influenced $f_p$ (T2 and T3 > T1). Regardless of trajectory type FF resulted in the lowest $f_p$.

The main effects of velocity and background appear to have caused the greatest change in $f_p$ (Table 1).

For any given trajectory or background, higher $l/|v|$ values (lower velocities) evoked an earlier $t_p$ ($80 \text{ ms} > 60 \text{ ms} > 40 \text{ ms}$, Fig. 4D) and for any given trajectory or velocity, FF generated the latest $t_p$. We also found that trajectory had some effect on $t_p$, occurring earlier for T1, relative to T2 and T3. The velocity of approach had the greatest effect on the time of $t_p$, although all main effects and their interactions influenced $t_p$ (Table 1).

In summary, we found that more spikes were produced in response to T1 and T3 relative to T2, at lower velocities, and for SC. Increasing stimulus velocity evoked a higher $f_p$ and earlier $t_p$. Relative to S, the SC and FF backgrounds caused lower $f_p$ and later $t_p$. In general, T2 and T3 evoked a higher $f_p$ and earlier $t_p$ relative to T1. With the exception of T1 generating a wider peak width at 1/2 max relative to T2 and T3, we found little other differences in peak width.

TOT-associated response parameters are affected by trajectory, velocity, and background

Different stimulus trajectories evoked clearly different $f_{TOT}$ (T2 > T3 > T1, Fig. 5A) and higher $l/|v|$ values (lower velocities) evoked lower $f_{TOT}$ ($80 \text{ ms} < 60 \text{ ms} < 40 \text{ ms}$, Fig. 5A).

Overall, background had a strong effect on $f_{TOT}$ (FF < SC < S), although several recordings showed no TOT-associated response, specifically when the stimulus followed T1 within FF, and the data were quite variable (see error bars in Fig. 5A). Although there was an effect of velocity
and significant interactions, the type of trajectory and background had the greatest effects on $f_{TOT}$ (Table 1).

Trajectory (T1 < T2 and T3), velocity (80 ms < 60 ms < 40 ms), background (FF < SC < S), and their interactions affected $f_v$ (Fig. 5B). Trajectory and velocity were the strongest main effects on $f_v$ and their interaction yielded the strongest interaction effect (Table 1).

Different trajectories significantly affected $f_{TOT-v}$ (T2 and T3 > T1, Fig. 5C). Although there was a significant effect of velocity, no clear trends were observed. We found very similar $f_{TOT-v}$ for different backgrounds (S = 43 ± 27, SC = 42 ± 21, and FF = 44 ± 32 spikes/s), although T1 in FF showed very little change, likely contributing to this result as T2 and T3 in FF had the highest $f_{TOT-v}$. All interactions affected $f_{TOT-v}$ with the interaction between trajectory and background resulting in the greatest effect. Overall, it was the type of trajectory that resulted in the greatest differences in $f_{TOT-v}$ (Table 1).

Regardless of velocity or background, T1 evoked an earlier $t_v$ than T2 and T3 (Fig. 5D). Higher $|l/v|$ values (lower velocities) also evoked earlier $t_v$ (80 ms > 60 ms > 40 ms). Velocity and trajectory had the greatest effect on $t_v$ followed by the interaction between trajectory and velocity (Table 1).

We found that $\delta$ was only affected by trajectory (T1 > T2 > T3, Fig. 5E). However, we also found significant interactions between velocity and background, trajectory and velocity and a three way interaction (Table 1). For stimuli that followed T1, the highest velocity ($l/|v| = 40$ ms) generated the longest $\delta$ relative to T2 and T3, where as for T2 and T3, the longest $\delta$ was at an $l/|v| = 60$ ms. Moreover, in T1 only, lower velocities caused shorter $\delta$. Thus, although we did find significant interaction effects, trajectory type appears to be the greatest determining factor in affecting $\delta$ (Table 1).
In summary, we found that T2 and T3 generated a higher $f_{TOT}$, $f_v$, and $f_{TOT-v}$ relative to T1. The $t_v$ also occurred later in T2 and T3 relative than T1 and the $\delta$ was longest in T1, followed by T2 and then T3. Lower velocities evoked lower $f_{TOT}$ and $f_v$ amplitudes and earlier $t_v$ in all trajectories. Background only affected $f_{TOT}$ and $f_v$, with S and FF resulting in the highest and lowest amplitudes, respectively.

$TOT$- and $TOC$-associated DCMD peak rise and fall phases depend on the trajectory and velocity of object approach and type of background

Trajectory type significantly affected TOT peak rise phase (T1 > T3 > T2, except for FF, Fig. 6A) and fall phase (T1 > T2 > T3 in S and SC, Fig. 6B). Generally, faster object velocities resulted in shorter TOT peak rise phases (particularly for T1 and T3, S and SC), but did not affect the fall phases. FF resulted in shorter TOT peak rise and fall phases regardless of trajectory or velocity. Although the type of background and velocity of object motion had the greatest effect on the rise phase of the TOT peak, all interactions showed an effect (Table 1). For the TOT fall phase, the type of background had the greatest effect followed by the type of trajectory and interaction between the trajectory and background (Table 1).

Regardless of background or velocity, T1 evoked a much longer TOC peak rise phase than T2 and T3 and slightly longer TOC peak fall phase relative to T2 and T3 in FF (Fig. 6C, D). Within each trajectory and background, higher $l/|v|$ values caused longer TOC peak rise phases (80 ms > 60 ms > 40 ms). Regardless of trajectory or velocity, background affected both the TOC peak rise phase (FF > SC > S) and TOC peak fall phase (FF > SC > S). Whereas velocity and trajectory most strongly affected the TOC rise phase, the strongest effect on TOC fall phase was background type (Table 1).
In Summary, changes in trajectory, velocity, and background strongly affected TOT- and TOC-associated peak rise and fall times. T1 typically had the longest TOT and TOC peak rise and fall phases of all three trajectories. While T2 and T3 shared similar TOC peak rise and fall phases, T2 resulted in shorter TOT peak rise phases and longer TOT peak fall phases. Slower stimulus velocities caused longer TOT and TOC peak rise phases and shorter TOC fall phases. In general, S caused the longest TOT peak rise phase and shortest TOC fall phase, while FF caused the shortest TOT peak rise phase and longest TOC fall phase; SC evoked intermediate values.

Expansion properties at the time of transition predict changes in the DCMD firing rate

Transitions to looming causes a decrease in firing rate (i.e. \( f' < 1 \)) that is correlated to unique expansion properties of the stimulus (McMillan and Gray 2012; Dick and Gray 2014). During a transition to looming, there is an increase in subtense angle velocity \((\theta')\) and decrease in leading edge velocity \((\psi')\) (Fig. 7A). This decrease in \(\psi'\) (which is related to where the transition occurs in 3-dimensional space and speed of approach (McMillan and Gray 2012; Dick and Gray 2014)) is responsible for the observed TOT response. However, DCMD responses to approaching objects are better matched to changes in angular acceleration of the image rather than velocity (Rind and Simmons 1992; McMillan and Gray 2012). To confirm that a decrease in \(f'\) is correlated to a positive change in the acceleration of the subtense angle \((\theta'')\) and a negative change in the acceleration of the leading edge \((\psi'')\), data were pooled from (McMillan and Gray, 2012) and (Dick and Gray, 2014) and those presented here. Data failed to converge when we attempted to fit our data set to predetermined constraints from the previous model (i.e. (McMillan and Gray 2012; Dick and Gray 2014)), and thus the data did not satisfy tolerances of the 2D Gaussian model. Given that this current data set only included transitions to looming and
the original model included values from transition to and away from looming, there could be a 
weighted effect of data points falling below a \( f' = 1 \). Subsequent fitting of an unconstrained 2D 
Gaussian model resulted in a good fit to the pooled data (\( r^2 = 0.75 \), Eq. 1, Fig. 7B):

\[
f'(x, y) = 3.0e^{-0.5\left(\frac{(x+6.6)^2}{32.3} + \frac{(y+157.7)^2}{136.1}\right)}
\]

(1)

While the mean normalized \( f' \) evoked by S (0.31 ± 0.18), SC (0.27 ± 0.16), and FF (0.27 ± 0.17) 
backgrounds were relatively invariant for each background, we wanted to determine if there was 
an effect of background using the new constraints established in Eq. 1. When isolating the data 
based on background but using previous data we found that all backgrounds had similar \( r^2 \) values 
(\( S = 0.77 \), SC = 0.75, FF = 0.73), suggesting that background type does not have an affect on \( f' \) 
at TOT for the same values of \( \theta'' \) and \( \psi'' \). Although \( f' \) changed according to unique stimulus 
parameters (velocity, angle of transition, and also stimulus background), the response time (\( \delta \)) 
was relatively invariant and weakly fit a 2D Gaussian model using the same stimulus parameters 
(\( \theta'' \) and \( \psi'' \)) with an \( r^2 = 0.24 \) (Eq. 2, Fig. 7C):

\[
f'(x, y) = 0.1e^{-0.5\left(\frac{(x+4.0)^2}{43.2} + \frac{(y-378.1)^2}{728.7}\right)}
\]

(2)

With the addition of background complexity, unique stimulus trajectories and velocities 
to previous data, we confirm that at TOT an increase in \( \theta'' \) and decrease in \( \psi'' \) correlates to a 
decrease in \( f' \). The relative changes in \( \theta'' \) and \( \psi'' \) are related to the point of transition in 3-
dimensional space (McMillan and Gray 2012) and velocity of approach (Dick and Gray 2014) 
but, as our results suggest, not background complexity. Consistent with previous work, we also 
confirm that \( \delta \) remains relatively invariant regardless of stimulus complexity and is only weakly 
correlated to changes in \( \theta'' \) and \( \psi'' \) (however, see above regarding the effect of trajectory on \( \delta \)).
We show that the type of visual background influences DCMD responses to different object motion. We characterize typical TOT-associated drops in firing rate and subsequent rises to peak firing prior to TOC that are affected by the object’s velocity, trajectory, and type of presentation background. Irrespective of trajectory or background, increasing stimulus velocity generally evoked higher spike numbers, larger peak firing rates, later peak firing and longer TOT- and TOC-associated peak rise phases. Although each trajectory was unique, T2 and T3 shared identical looming components, which accounts for similarities in some response parameters. Overall, the type of trajectory had a large, and varying, impact on both TOT and TOC-associated responses. More complex backgrounds delayed peak firing, evoked lower peak firing rates and generated shorter TOT-associated peak rise phases and longer TOC-associated peak fall phases. The strong correlation between relative increases in $\theta''$, decreases in $\psi''$, and decreases in $f'$ at TOT (Fig. 7) was invariant to background types. Moreover, $\delta$ remained relatively invariant to all stimulus parameters. Although we attribute no biophysical mechanism by which this process operates, our data support the hypothesis that the LGMD/DCMD pathway is capable of conveying information regarding unique expansion properties of a moving object (McMillan and Gray 2012; Dick and Gray 2014) regardless of background complexity.

Although our stimuli and background environments were relatively slow when considering the natural flight speed of *Locusta migratoria* or predatory bird attacks (Santer et al. 2012), they were designed to increase the level of visual complexity compared to our previous studies (McMillan and Gray 2012; Dick and Gray 2014) and to coincide with background velocities used in a developing computational (Silva et al. 2012) and real world robot (Silva et al. 2012) model of object motion detection. Additionally, although the LGMG/DCMD pathway
responds preferentially to monocularly presented small objects (Rind and Simmons 1992; Rowell and O’Shea 1980), we simultaneously presented all compound stimuli bilaterally, a situation which locusts could conceivably encounter in a swarm or during flight (Rowell and O’Shea 1980). However, contralateral DCMD stimulation may affect ipsilateral responses; an affect which may originate from the optic lobe or within the thoracic ganglia (Rowell and O’Shea 1980). Thus, each DCMD may have produced a different response if presented with unilateral stimuli. Given the relatively small area of stereoscopic overlap in locusts (Rowell 1971), in the case of the compound trajectories, the discs would have moved out of the contralateral eye’s field of view before affecting ipsilateral DCMD responses, with any overlap early on (such as in T2, Fig. 2) only marginally affecting the responses. However, in the case of our frontal looming presentation, object expansion would spread equally across each eye. Gray et al. (2001), who recorded the activity from each DCMD simultaneously, also reported relatively low peak firing rates to head-on looms but attributed the weaker response to relatively low omnitidial density in the anterior portion of the eye. Based on comparisons with previous work (i.e. McMillan and Gray 2012 and Dick and Gray 2014), we found no qualitative evidence to suggest that simultaneous bilateral stimulation greatly affects unilateral and contralateral responses. For example, the looming component of our T2 (a lateral loom) generated comparable TOC-associated peak firing rates as did the 40 ms lateral loom used in Dick and Gray (2014). Moreover, our intention was not to compare the effect of one side of the pathway on the other but to test more complex stimuli based on previously used types of stimuli within the working confines of a robot model. Our progressive FF pattern reliably suppressed DCMD responses (see also Gabbiani et al. 2004) and regardless of recording side, our compound and frontal looming stimuli generated DCMD responses that are similar to those previously described. We did not
compare the putative effect of switching backgrounds that, in the case of the FF, would occur as
the animal begins moving forward. However, Rind and Santer (2004) found no response to the
start of motion of their drifting grating stimuli. Moreover, we were primarily concerned with
comparing differences between the same type of object motion within a different background
and not direct comparisons of the backgrounds themselves (although we anecdotally noted more
spiking in the SC background). We also did not provide any feedback from the locust, which
may have altered the perceived approach of the object (or motion of the background, such as a
turn).

Whereas previous studies have tested the locust LGMD/DCMD pathway to compound
approaches (McMillan and Gray 2012) varying in velocity (Dick and Gray 2014), looming
approaches combined with optic flow (Rind and Simmons 1992; Gabbiani et al. 2002), other
moving periodic patterns (O'Shea and Rowell 1975; Pinter 1977; Rowell et al. 1977), or
examined steering torques using pseudo-swarm and progressive flow field stimuli (Preiss and
Spork 1993), this study is unique in a few ways. First, we used bilaterally presented stimuli and
recorded from both ventral nerve cords (isolated right and left DCMD axonal responses), which
allowed us to test the effect of simultaneous DCMD stimulation spiking between opposite sides; a
situation that a locust may experience while flying in a swarm. Second, during forward motion, a
more realistic flow field would have motion parallax with translating motion in a single,
predictable direction, and stationary objects expanding at different rates and directions (Xiao and
Frost 2013). Our compound approach trajectories travelled at different velocities and were
presented in multiple background environments, including a progressive flow field. Finally, we
had the advantage of presenting in virtual 3-dimensional space, encompassing a large area of the
visual field (270°). Preiss and Spork (1993) presented simulated gratings and spotted patterns to
the longitudinal body axis of *Schistocerca gregaria*, yet their stimuli did not cover the entire visual field. Rind and Simmons (1992) used moving gratings presented laterally to a single eye and Gabbiani et al. (2002) used a background flow that consisted of constantly expanding concentric rectangles presented 2-dimensionally and perpendicular to a single eye. Our stimuli further represented motion in depth that simulated translational gaze shift and rotational gaze shift (Eckmeier 2013).

Although typical LSN responses show little to no activity within optic flow, they remain sensitive to objects that approach the eye (Rind and Simmons 1992; Sun and Frost 1998; Gabbiani et al. 2002, 2004; Xiao and Frost 2009). However, the responses are generally suppressed (Rowell et al. 1977; Rind and Simmons 1992; Gabbiani et al. 2002, 2004). Figure 4A illustrates that relatively fewer spikes are generated in response to FF, which has been shown to be related to the velocity of a flow field (Rind and Simmons 1992). While some studies have indicated that flow fields have little effect on time of peak firing (Rind and Simmons 1992), other findings and ours reported here, showed a slight delay in peak firing time (Gabbiani et al. 2002; Xiao and Frost 2009). In the FF background, longer lasting feed-forward inhibition would delay the build up of excitation, thus delaying peak firing. We also found a significantly shorter TOT-associated peak rise phase and longer TOC-associated fall phase in the presence of a flow field (Fig. 6A and D). Without the presence of a background, feed-forward inhibition terminates the DCMD response to object approach (Rind 1996) by controlling excitation (Gabbiani et al. 2004). Although Rind and Simmons (1992) did not measure DCMD peak firing rise and fall phases, their Fig. 8A is consistent with our results, where the rise phase is shorter in the presence of a flow field. However, their fall phase is not longer. In addition, Gabbiani et al. (2002) also found a shorter rise phase, although the fall phase seemed unaffected (see their Fig. 2a). This
result, however, is likely related to differences in our visual background stimuli, since our FF covered a larger field of view. Several studies have shown that higher velocity looming stimuli result in fewer spikes, and narrower, larger amplitude peak firing rates that occur later (Rind 1996; Rind and Simmons 1997; Gabbiani et al. 1999, 2001; Gray et al. 2001; Matheson et al. 2004; Gray 2005; Guest and Gray 2006; Dick and Gray 2014), which is consistent with our findings in all backgrounds.

Activation and termination of the DCMD response is strongly affected by the kinematics of the stimuli. For example, higher $l/|v|$ values (slower velocities) may cause a longer and weaker activation of excitation that is overcome earlier by feed-forward inhibition (Gabbiani et al. 2004), since the edges of the approaching object are increasing more slowly. Indeed, we found that higher $l/|v|$ stimuli resulted in longer rise phases (TOT and TOC) and shorter TOC peak fall phases (Fig. 6). Differences in the trajectory leading up to a transition also affected some TOT and TOC response parameters. For example, T2 was the shortest trajectory and thus had the fewest spikes. T2 also contained the greatest change in angle of transition, which occurred closer to the animal and from the anterior, thus generating the highest level of excitation and the largest drop in firing rate (i.e. $f_v$); this result may be related to the relative optical density across (Krapp et al. 1998) the retina in addition to the relative differences in object expansion at TOT.

Consistent with Dick and Gray (2014) who reported little difference between their $f_{TOT}$ and $f_v$ for any angle or $l/|v|$ they tested (see their Fig. 6), we found similar results with the exception of the FF background, which generated the largest $f_{TOT-v}$ (except for T1).

Optic flow direction is known to affect LSN responses in pigeons; when flow and direction of object motion are opposite, no looming response is detected (Xiao and Frost 2009). In our study, the non-looming, translational component of the compound trajectories originated
from different locations around the locust and the presence of FF decreased DCMD responses to
the translational component in all trajectories. However, the reduction was much more
pronounced in the trajectories where object motion travelled opposite to the flow field (overall,
T2 generated the highest $f_{TOT}$). Moreover, the looming component of all compound trajectories
travelled in a different direction of FF and all peak firing rates were substantially reduced. In fact
the most robust translational response was from T2 (Fig. 3), where translational motion was in
the same direction as FF.

The different rates of object expansion (i.e. relative $\theta^*$ and $\psi^*$ values) at TOT for each
trajectory affected the relative timing and amplitude of DCMD responses. Although $\delta$ was
relatively variable among all animals and stimuli, they are consistent and within the range of $\delta$ in
previous studies using different compound trajectories, object sizes, and object velocities
(McMillan and Gray 2012; Dick and Gray 2014).

The LGMD/DCMD pathway responds to more than simple looming objects/shapes:
directionality (Peron et al. 2009; McMillan and Gray 2012), near-miss trajectories (Judge and
Rind 1997; Gray et al. 2001; McMillan and Gray 2012; Dick and Gray 2014), compound shapes
(Guest and Gray 2006), proximity and direction of non-looming, translatory trajectories, and
changes to and from a direct collision course (McMillan and Gray 2012), and different stimulus
velocities during a transition to looming (Dick and Gray 2014). Locusts, particularly in flight and
in a swarm, are not likely to only encounter simple objects that move in a predictable direction
and whose edges expand uniformly across the retina. Moreover, locusts generate collision
avoidance behaviours in response to objects that approach along non-looming trajectories
(Robertson and Johnson 1993) and thus an object does not need to approach on a direct collision
course to be important in collision detection. Although our backgrounds do not replicate all
aspects of complex natural motion within a swarm, DCMD responses within our SC and FF backgrounds still provide valuable insights into how it may encode aspects of an emulated swarm or during forward flight. We found that the DCMD was still remarkably responsive to object motion (both to transitions and to a loom) within SC and FF, even though the LGMD receives wide field inhibition (O'Shea and Rowell 1975) and habituates to stimuli repeatedly presented in the same area of the retina (Palka 1967; Horn and Rowell 1968; Gray 2005).

However, the DCMD is not suppressed during walking (Rowell 1971), which would produce a progressive flow field and is only strongly suppressed during a saccade in the optomotor response (Zaretsky 1982). The optic flow generated from rotation (such as a saccade) and translation (such as objects moving in the context of a single reference point, i.e. the moving animal) is quite different. The LGMD is inhibited if the entire field of view shifts at once, such is the case in a rotational gaze shift which would cause all objects to appear to move at the same angular velocity, whereas during straight flight, objects in the flight path would expand at different rates (Koenderink 1986). Since the LGMD/DCMD response is related to a balance between excitation and lateral inhibition (Rowell et al. 1977), during forward flight with translational optic flow, the DCMD may remain sensitive since the objects in the flight path have varying expansion rates, thus the spread of excitation is faster than the corresponding inhibition. Indeed, the DCMD response to a moving object is suppressed with an increase in temporal frequency of periodic vertical bars (Rowell et al. 1977; Rind and Santer 2004). The LGMD remains responsive to new movements since recovery from habituation is not only rapid, but it is localized to the area on the retina that was repetitively stimulated (O'Shea and Rowell 1975). Thus, a habituated DCMD is still responsive to the approach of the same object on a different approach trajectory (i.e. different part of the visual field) or a larger object on the same trajectory.
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(Gray 2005). This would explain how the DCMD was still able to respond to trajectory changes within the complex backgrounds and generate a relatively large peak firing rate. Interestingly, although we found a lower $f_p$ in the SC relative to the S background, there were higher overall spike numbers. Perhaps the lower $f_p$ within SC is simply related to a reduced number of stimulated ommatidia, since part of the visual field would expectedly be shadowed by the moving dots. Meanwhile, the moving dots in other parts of the visual field would be stimulating corresponding ommatidia in that particular region, leading to a higher spike number over a wider time window.

Considering edge expansion alone may explain why we observed lower $f_p$ in both complex backgrounds. Thus, with similar contrasts, when a looming object exceeds the size of moving objects around it (in this case, the subtense angle of the dots in SC (~5°) or bars in FF (~12°)) we should observe a DCMD response. Indeed, relative to S and SC where the TOT-associated rise phase of the DCMD response begins before the 7 cm disc subtends either 5° or 12° (Fig. 3), the FF background resulted in a much later TOT-associated rise phase that began closer to 12°. The lateral inhibition network presumably prevents fatigue of individual small-field elements (Rowell et al. 1977), thus during the SC background, more spikes were produced and the DCMD was still able to respond robustly to the moving visual stimuli while in the FF background, fewer spikes were produced due to relatively larger cover area of the bars.

Summation of responses to multiple looming stimuli are sublinear (Guest and Gray 2006) and controlled by postsynaptic mechanisms, such as the absolute refractory period within the LGMD spike initiating zone (see Guest and Gray 2006), that compensate for the afferent inputs onto the LGMD (Krapp and Gabbiani 2005). Regardless of the background we used, the type of trajectory, and thus the motion of the disc and not the background, had the largest main effect on
DCMD response parameters, significantly affecting all 13 measured variables (Table 1 and Figs 4, 5, and 6). Thus, one of our major findings is that while DCMD responses are sensitive to background complexity, they are more sensitive to object motion complexity, i.e. trajectory changes and velocity.

We found that DCMD responses within FF had a delay in peak firing. As suggested regarding pigeons (Xiao and Frost 2009), locusts would benefit from a timing delay since in flight they need to react faster to avoid a predator. Moreover, while in flight (emulated, in part, by our FF) and potentially surrounded by conspecifics (emulated, in part, by our SC), locusts would have difficulty isolating approaching visual stimuli and may respond later to ensure that the predator is on a collision course and that the animal does not continually perform emergency avoidance responses to conspecifics. However, the DCMD may also assist in subtle course deviations while in swarming flight, which may be related to the relatively lower firing rates in the presence of FF compared to S. Yet the importance of peak firing remains unclear, since it often occurs after the initiation of behavioural responses during flight (Burrows 1996; Gray et al. 2001; Matheson et al. 2004; McMillan et al. 2013) and before looming-evoked jumps (Fotowat et al. 2011). The DCMD response leading up to the peak is likely quite important and has been related to phases in an avoidance jump (Rind and Santer 2004; Fotowat and Gabbiani 2007; Santer et al. 2007). However, if this was the case for avoidance responses during flight, our observed short rise phase within the FF relative to S also suggests that locusts would still need to respond more rapidly to an approaching predator while flying.

We show how responses of a single neuron are modulated by approach velocity, trajectory, and background type. Since relative object motion can represent different visual stimuli, the rapid detection and performance of appropriate responses are paramount. Many
animal groups use different aspects of the same sensory stimuli to extract behaviourally relevant information, such as velocity, contrast, texture, and more (Kern et al. 2000). For example, flies use flow field motion to provide cues regarding self motion, discriminate objects from background and estimate the relative distance of objects (Tammero and Dickinson 2002). Nevertheless, it is likely that several LSNs in the locust’s visual system (e.g. LDCMD (Gray et al. 2010)) are also involved in detecting salient stimuli and responsible for producing appropriate avoidance responses. In fact, in studies where the DCMD neuron has been ablated, animals are still capable of performing avoidance responses (Santer et al. 2007; Fotowat et al. 2011).

Since our moving disc and dots in SC and vertical gratings in FF were similar in contrast, it is possible that there was a shadowing effect of the moving disc each time it overlapped with the background. In addition, although the FF and the 7 cm looming disc were distinct stimuli, looming objects should contribute to increased translational flow, if the object travelled in the same direction and irrespective of its velocity. Therefore, it would be interesting to determine how DCMD responds to a disc traveling slower or at the same velocity as the flow field. The relative velocity and trajectory of objects in an animal’s visual environment may represent very different things. If the looming object was designed to travel slower than the flow field it would appear to the locust that it was catching up to a slower moving object and we would expect a weakened DCMD response compared to an object that was traveling faster than the flow, which may indicate the trajectory of a predator. Conversely, we would expect that no response would be detected if the disc was traveling the same velocity, as it would appear as a stationary part of the flowing environment. In addition, we only presented a flow in one direction, so it would be interesting to see what happens if presented in opposite direction or if the flow stopped during the approach of an object. Visual gratings that simulate progressive motion causes landing
responses in flies when the change in expansion velocity of the pattern occupies a large enough area of the retina (Eckert 1982), which has also been suggested to occur in locusts (Baader 1991). Categorizing the LGMD/DCMD pathway as being simply looming sensitive may underestimate what this and potentially other similarly responding neurons encode. Ultimately, coupling behavioural and electrophysiological recordings to these types of stimuli will elucidate the importance of different aspects of complex stimulus environments.
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A.C.S. designed and performed the experiments and did preliminary data analysis. G.A.M. assisted in experimental design, analyzed data, interpreted results, prepared figures, drafted and revised the manuscript. C.P.S. assisted with conception and design of research and finalized the manuscript. J.R.G. overviewed experimental design, contributed to data analysis and interpretation of results, and edited, revised and finalized the manuscript.
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Table 1

Statistical comparison of measured variables for all TOC and TOT-associated DCMD responses. Trajectory type, velocity of object motion, and background type were the three major factors compared. Shaded cells indicate a non-significant effect and each cell contains the corresponding F statistic, with degrees of freedom and associated p value, generated by a three-way RM ANOVA (see METHODS).

FIGURE LEGENDS

Figure 1

Computer-generated stimuli. A) Following an initial and preceding a final frontal looming stimulus using a single 7 cm black disc, each locust was presented, in random order, with 3 compound trajectories (T1, T2, and T3) consisting of a bilateral pair of non- looming transitioning to looming 7 cm black discs travelling at 3 different velocities at two azimuthal angles (T1, 45°; T2 and T3, 90°). B) Each trajectory was presented onto one of three stimulus backgrounds (simple, scattered, or flow field). Arrows in panel A and B represent the relative direction of object motion. Further description of stimuli in METHODS.

Figure 2

Looming disc (l/|v| = 12 ms) were presented head on to each locust (n = 14) within three background types (n = 56 recordings per background). A) Individual raw neural recordings from the left (L) and right (R) DCMDs, pooled mean DCMD responses (black lines) and SD (grey shading) to a looming black disc approaching from the in three visual backgrounds (simple [top],
scattered [middle], and flow field [bottom]), and change in subtense angle aligned to the time of collision (TOC; dashed, red vertical line). Labeled points on the mean DCMD response panels \((t_{99}, t_p, \text{ and } t_{15})\) and \(t_{LF}\) on the subtense angle panel represent specific times used for the calculation of the rise and fall phases (see figure legend and METHODS). B-F represent, respectively, the statistical comparisons of the TOC-associated firing rate \((f_p)\), time of peak firing \((t_p)\), number of spikes, peak width at 1/2 max, and phase duration for each background type. Different letters above bars represent significant differences between parameters. Significance assessed at \(p < 0.05\).

Figure 3

Example DCMD responses (pooled left and right) to three compound trajectory types (columns: left, T1; middle, T2; and right, T3) at an \(l/|v| = 40\) ms presented in three visual backgrounds (rows: top, simple (S); middle, scattered (SC); and bottom, flow field (FF)). The grey shading in each panel represents the looming phase of the stimulus. The blue line in each panel represents the change in subtense angle, relative to the eye of the locust, over each frame and three seconds prior to the end of stimulus. The vertical red dotted line in each panel represents TOC. Each DCMD response was averaged from \(n = 28\) recordings. With but a few exceptions (see RESULTS), transitions to looming caused a valley in the firing rate followed by a TOT-associated rise and peak firing around the time of collision. Irrespective of background, the most robust response was found for T2, when the non-looming component of the moving disc travelled in the opposite direction, the DCMD response to a transition was quite subtle (i.e. T1). In general, DCMD peak firing rates were diminished and delayed in the presence of either SC or FF. In addition, the rise and fall phases were greatly affected by background in all trajectories.
Figure 4

TOC-associated peak response variables. A) Higher number of spikes were present in the SC and lower numbers in FF relative to S. Higher $l/|v|$ values also generated more spikes, as did T1 and T3. B) The peak width at half 1/2 max was generally unaffected by trajectory type, velocity, or background. C) Higher peak firing rates ($f_p$) were observed at lower $l/|v|$ values, in S, and in T2 and T3. D) Delays in the time of peak ($t_p$) were observed in FF, at higher $l/|v|$ values, and for T2 and T3. Each column represents the mean (+SD) for each response (n = 28 recordings) to one of 27 stimulus combinations (see METHODS). The bars are colour coded based on the velocity of the 7 cm disc (blue ($l/|v|$ = 40 ms), green ($l/|v|$ = 60 ms), and orange ($l/|v|$ = 80 ms)) and grouped based on trajectory type (1, 2 and 3) on the z-axis and background (S = simple, SC = scattered, and FF = flow field) on the x-axis. See TABLE 1 and RESULTS for statistical comparisons.

Figure 5

TOT-associated peak and valley response variables. The firing rate at TOT ($f_{TOT}$) (A) and at the valley ($f_v$) (B) were higher for lower $l/|v|$ values, in S, and for T2 and T3. C) The change in firing rate from TOT to valley ($f_{TOT-v}$) was unaffected by background type, although the smallest change was observed for T1 FF. Although individually significant differences were found, no trend was observed for the effect of trajectory type or velocity. D) The time of valley ($t_v$) was clearly related to when the disc transitioned to looming in each trajectory; higher $l/|v|$ values and T1 had earlier times. E) Although variation existed between the different trajectories (particularly for T1), the response time ($\delta$) was relatively invariant of background and velocity. Similar labeling and colouring scheme as in Fig. 4.
Rise and fall phases of the TOT and TOC-associated peak firing rates. A) Longer TOT peak rise phases were observed in T1 and for higher $l/|v|$ values, while the FF caused significantly shorter TOT rise phases. B) The FF also caused shorter TOT fall phases, as did T3. C) The TOC peak rise phase was longest in T1 and for higher $l/|v|$ values. D) The FF and SC resulted in significantly longer TOC peak fall phases, while the trajectory and velocity had little effect.

Similar labeling and colouring scheme as in Fig. 4.

Correlation of DCMD firing modulation with select expansion parameters of a disc during a transition to looming. A) An example illustrating the relationship between a decrease in subtense angular velocity ($\theta'$, blue line), increase in rotational velocity of the leading edge of the disc ($\psi'$, red line) and resulting drop in DCMD firing rate ($f'$, black line) at the time of transition; sample data represents a 7-cm disc travelling along Trajectory 2 at an $l/|v| = 40$ ms within a simple background (see Fig. 1). The grey shaded area indicates when the disc is on a looming trajectory; the first transition from white to grey represents TOT and the second transition represents when object motion stops. B) 3-dimensional scatter plot representing simple (blue), scattered (green), and flow field (red) data plotted with previous data (black; McMillan and Gray (2012) and Dick and Gray (2014)) and fit to a 2D Gaussian equation (mesh plot). Data represent the mean firing rate change ($f'$) in response to a transition plotted against the subtense angular acceleration ($\theta''$) and the rotational acceleration of the leading edge ($\psi''$); data fit with an $r^2 = 0.75$. C) Correlation of mean response time ($\delta$) to $\theta''$ and $\psi''$; data weakly fit to a Gaussian equation with an $r^2 = 0.24$ (mesh plot).
<table>
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<th>Velocity</th>
<th>Background</th>
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<th>Trajectory x background</th>
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<td>$F_{(2,54)} = 34.9$</td>
<td>$F_{(4,108)} = 8.4$</td>
<td>$F_{(2,8,77.7)} = 21.6$</td>
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</table>

**Silva, McMillan, Santos, and Gray - Table 1**
Silva, McMillan, Santos and Gray - Figure 1
A  
Simple

10 mV

L
R

150
100
50

f (spikes/s)

Scattered

150
100
50

f (spikes/s)

Flow field

150
100
50

f (spikes/s)

\( t_{09} \) = time when response exceeds a 99% CI (start of rise phase)
\( t_{f} \) = time of last frame of expansion (start of fall phase)
\( t_{p} \) = time of peak firing rate (end of rise phase)
\( t_{15} \) = time of nearest spike to 15% of peak firing rate (end of fall phase)

B

\( f_{r} \) (spikes/s)

C

\( f_t \) (s)

D

Number of spikes

E

Peak width at 1/2 max (s)

F

Phase duration (s)

\( \theta \) (°)

TOC (s)

Simple (S)

Scattered (SC)

Flow field (FF)

Silva, McMillan, Santos, and Gray - Figure 2
Silva, McMillan, Santos, and Gray - Figure 4
Silva, McMillan, Santos, and Gray - Figure 6