

Habituated visual neurons in locusts remain sensitive to novel looming objects

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Summary

Many animals must contend with visual cues that provide information about the spatiotemporal dynamics of multiple objects in their environment. Much research has been devoted to understanding how an identified pair of interneurons in the locust, the Descending Contralateral Movement Detectors (DCMDs), respond to objects on an impending collision course. However, little is known about how these neurons respond when challenged with multiple, looming objects of different complex shapes. I presented locusts with objects resembling either another locust or a bird approaching on a direct collision course at 3 m s^{-1} while recording from the DCMD axon within the mesothoracic ganglion. Stimulus presentations were designed to test: (i) whether DCMD habituation was related to the frequency of approach, (ii) if habituated DCMDs were able to respond to a novel stimulus and (iii)

if non-looming motion within complex objects (internal object motion) during approach affects habituation. DCMD responses to simulated locusts or birds habituated more when the time interval between consecutive approaches within similar sequences decreased from 34 s to 4 s. Strongly habituated DCMDs were, however, able to respond to the same object approaching along a new trajectory or to a larger object approaching along the same trajectory. Habituation was not affected by internal object motion. These data are consistent with earlier findings that DCMD habituation occurs at localized synapses, which permits maintained sensitivity to multiple objects in the animal's environment.

Key words: locust, *Locusta migratoria*, DCMD, visual cue, neuron, habituation.

Introduction

Generation of adaptive behaviour depends critically on an animal's ability to detect multiple sensory cues from the natural environment. Often, visual information provides the first cue about the saliency of objects that the animal encounters and, in many cases, visual information triggers production of adaptive behavioural responses (Gibson, 1979). Since the visual environment contains contextually variable information, one function of the visual system is to detect potentially threatening cues early enough so that an appropriate behavioural response can be initiated. For example, objects approaching along a direct collision course generate a looming visual stimulus that an animal can use as a cue to trigger an escape reaction. Previous visual stimuli can influence the sensitivity of the underlying detection systems to looming cues and, ultimately, the resulting behaviour. In a complex environment, multiple looming objects of different sizes can move at different velocities and along different approach trajectories, which would produce variable sequences of visual stimulation. Thus, detection systems affected by a sequence of looming motion cues may show different responses when challenged with a new object approaching from a different region of the visual field, enabling the animal to remain sensitive to multiple looming objects.

In a swarm, gregarious locusts fly 0.8–9.0 m apart at flight speeds of about 3 m s^{-1} , and those at the edge tend to turn

inward toward the centre of the swarm (Waloff, 1972). To avoid continual collisions with their nearest neighbours, individuals must be able to react very quickly to generate appropriate collision avoidance manoeuvres. While in flight, locusts also need to detect potential predators, such as the fiscal shrike *Lanius collaris humeralis* and the carmine bee-eater *Merops nubicus*, which can capture locusts on the wing (see Rind and Santer, 2004). These predators have carpal-to-carpal pectoral widths and wingspans of 5 cm and 28 cm, respectively, and often swoop in on flying locusts with the wings held stationary in a gliding posture (Fry et al., 1992). Thus, they present a larger image than a conspecific (wingspan of 7.5–10 cm) during a looming approach. Recent studies examining locust collision avoidance and looming responses of motion-sensitive neurons have used computer-generated stimuli that incorporated aspects of objects thought to be biologically relevant (Mohr and Gray, 2003; Rind and Santer, 2004; Santer et al., 2005).

The visual system of locusts contains identified interneurons that respond strongly to looming stimuli and provide a cue for impending collision (Schlotterer, 1977; Simmons and Rind, 1992; Rind, 1996; Judge and Rind, 1997; Rind and Simmons, 1997, 1998, 1999; Gabbiani et al., 1999, 2001, 2002; Gray et al., 2001). The exact mechanism by which one of these

neurons, the Lobula Giant Movement Detector (LGMD), encodes information about looming stimuli remains contentious. Two current models predict how the output firing of the LGMD represents a looming stimulus. One hypothesis states that the LGMD acts as an angular threshold detector during approach of objects on a direct collision course from within the horizontal plane up to 135° (Gabbiani et al., 2001). According to this model, postsynaptic multiplication of excitatory and inhibitory inputs that converge onto the LGMD produces a peak firing rate that occurs with a fixed delay after the looming object reaches a fixed threshold angular size (Gabbiani et al., 1999, 2002). Accordingly, peak firing occurs before collision (Hatsopoulos et al., 1995; Gabbiani et al., 1999, 2001, 2002; Matheson et al., 2004a). Another model suggests that presynaptic inhibition shapes looming responses of the LGMD (Rind, 1996), which produces a peak firing rate after object motion ceases (see Rind and Simmons, 1999; Rind and Santer, 2004).

In the locust brain, each right and left LGMD synapses onto a Descending Contralateral Movement Detector (DCMD), which projects to the contralateral ventral nerve chord. The DCMD axon branches bilaterally within the thoracic ganglia, and synapses onto flight interneurons and motoneurons (Burrows and Rowell, 1973; Simmons, 1980; Robertson and Pearson, 1983). Each spike in the LGMD elicits a spike in the DCMD (O'Shea and Williams, 1974; Rind, 1984) and thus DCMD activity reflects the spatiotemporal properties of a looming stimulus.

Many earlier studies describing the encoding properties of the DCMD presented looming stimuli at 2–5 min intervals. More frequent stimulation induces habituation of the response (Horn and Rowell, 1968; Rowell, 1971; Bacon et al., 1995), particularly if stimuli are presented 40 s apart (Simmons and Rind, 1992). Recently, however, Matheson et al. (2004a) showed that DCMDs of gregarious locusts maintain up to 85% of a nonhabituated response when stimulated at 60 s intervals. Decreased sensitivity of the DCMD to repeated small-field motion results from habituation of synapses between chiasmatic visual afferents and the dendritic fan of the LGMD (O'Shea and Rowell, 1976; reviewed by Rind, 2002). Moreover, Rowell et al. (1977) suggested that a phasic lateral inhibitory input impinging on the excitatory afferents reduces input to the LGMD during large-field stimulation. The effect of this network would be to reduce saturation of afferent neurons and fatigue of excitatory inputs onto the LGMD during whole-field movements. Gregarious locusts flying in a swarm would encounter many objects, including conspecifics and flying predators, approaching frequently from many directions, which would produce a combination of whole-field and small-field motion. A global habituation mechanism would be maladaptive in these conditions since the system would lose the ability to respond to small-field looming stimuli. In this context, localized habituation of presynaptic inputs to the LGMD would allow each locust within the swarm to remain sensitive to approaches of individual objects within its field of view.

The purpose of the experiments described here was to examine the responses of locust DCMDs to approaches of objects that emulate another locust or a predatory bird at intervals of 34 s and 4 s. Approaches at 4 s intervals were designed to further test whether habituated DCMDs were able to respond to the same object approaching along a new trajectory or to an object with different stimulus parameters approaching along the same trajectory. I show that the DCMDs maintained responses to a simulated locust or bird approaching at 34 s intervals and that habituated DCMDs were able to respond to the same object approaching along a new trajectory and a larger object approaching along the same trajectory. Moreover, internal object motion during an approach did not affect habituation to repeated approaches. Some of the data presented here has been published previously in abstract form (Gray, 2004).

Materials and methods

Experiments were performed on 11 adult male locusts *Locust migratoria* L. selected at least 3 weeks past the imaginal moult from a crowded colony (28–25°C, 12 h:12 h light:dark) maintained at the University of Saskatchewan. Experiments were carried out at room temperature (approximately 25°C).

Visual stimuli

Computer-generated stimuli were created and rear projected onto a dome screen using a Sony VPL-PX11 LCD data projector at 80 frames per second (f.p.s.; Fig. 1A). Stimuli were rendered at 900×768 pixels, which produced an actual pixel size of $0.70 \text{ mm} \times 0.70 \text{ mm}$ when projected onto the center of the dome screen (radius = 35.5 cm). Thus, each projected pixel subtended 0.36° of the locust's field of view, which is below the spatial resolution of the ommatidial array in the acute zone of the eye (1° ; Horridge, 1978). The stimuli were designed to emulate the actual dimensions of either an approaching locust ('locust') or bird ('bird'; Fig. 1C). Each projected object (luminance = $170 \text{ cd m}^{-2} = I_{\text{max}}$) was set against a white background (luminance = $483 \text{ cd m}^{-2} = I_{\text{min}}$), producing a Michelson contrast ratio $(I_{\text{max}} - I_{\text{min}} / I_{\text{max}} + I_{\text{min}})$ of 0.48. Luminance measurements were made using a Quantum Instruments PMLX photometer (B & H Photo, New York, USA) placed at the position of the experimental locust's head (see below). The luminance values were higher than used in previous studies of DCMD responses to looming stimuli (see Gabbiani et al., 1999) and are due to an overall brighter image from a LCD projector compared to a CRT or LCD computer screen. Nevertheless, an object:background contrast ratio of 0.5 is typical for experiments of DCMD responses to looming stimuli. Simulated approaches were created using 3D Studio Max (version 5) animation software (Autodesk Inc., Markham, ON, USA). Each object was scaled to real-world coordinates and designed to approach from 9 m away at 3 m s^{-1} . The object remained stationary for 1 s after the end of approach, thus each approach lasted 4 s. Approaches were rendered in AVI format at 80 f.p.s., slightly above the flicker fusion frequency of the locust eye (Miall, 1978), for a total of 320 frames. Power

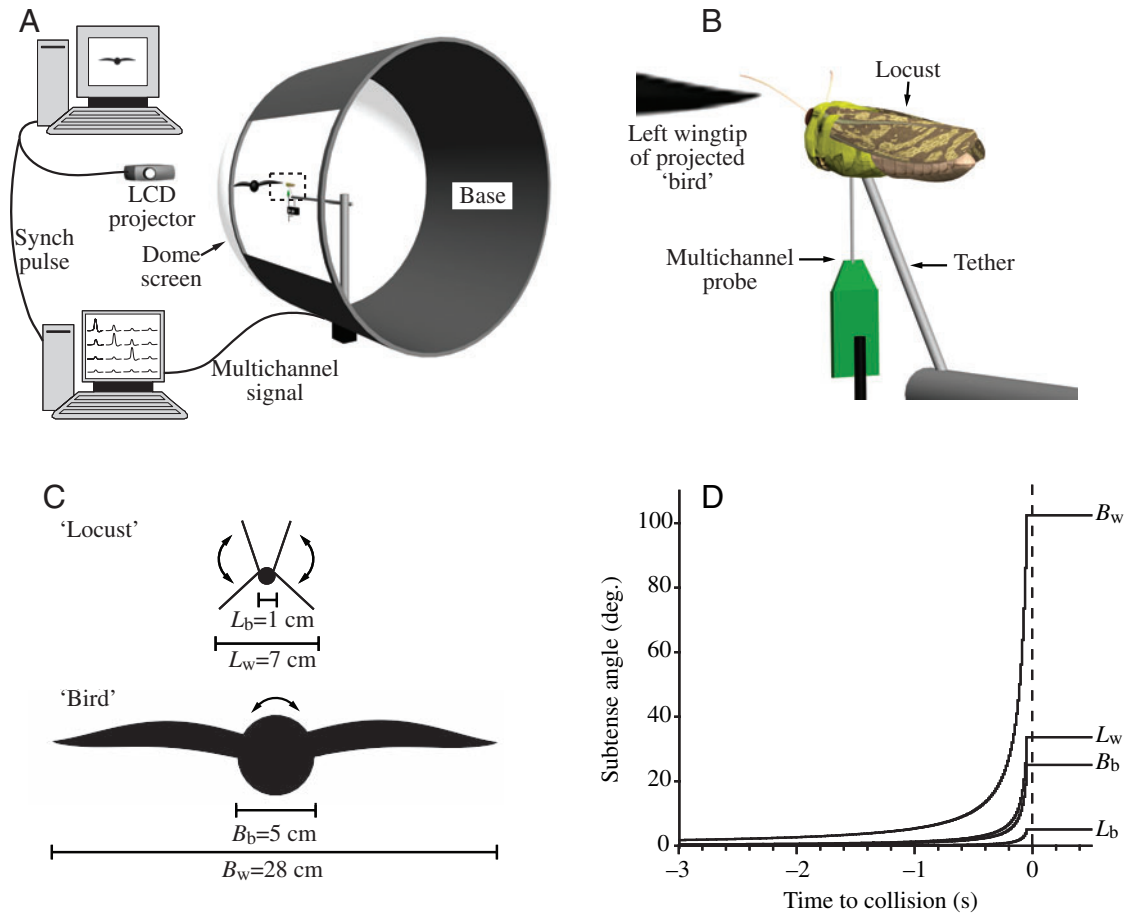


Fig. 1. Experimental setup for presenting looming stimuli. (A) Left rear view of the experimental setup. The rear plate and left side panel of the base were removed from the figure to permit a clear view of the position of the locust in the setup. Computer-generated looming stimuli were projected onto a rear projection dome screen using a LCD projector. The locust was held in place on the inside of the dome with a rigid tether. A synchronization pulse from the stimulus was sent to the TTL input channel of the multichannel neurophysiological recording system. (B) Magnified view of area enclosed by the broken box in A showing the position of the multichannel probes below the tethered locust (see text for details of recording techniques). (C) Scaled images of the 'locust' (top) and 'bird' (bottom) looming stimuli. The 'locust' was designed such that the two sets of wings rotated about the joint with the body in antiphase at 25 beats s^{-1} , which emulated the flapping of real locust wings. The 'bird' was designed with fixed wings to emulate a real bird during a gliding approach. For one sequence of approaches to each experimental animal the 'bird' rotated $\pm 45^\circ$ about its longitudinal axis (arrows) at 1 roll s^{-1} to emulate internal object motion. (D) The angle of the experimental locust's field of view subtended by components of the looming stimuli identified in C. For both types of stimuli the object stopped 37 ms before collision. L_b , 'locust' body; L_w , maximum width of 'locust' wings; B_b , 'bird' body; B_w , 'bird' wing.

spectral density analysis of DCMD spike times showed no discernible peaks at 80 Hz, suggesting that, even at the relatively high luminance values produced by the LCD projector, the visual system did not phase lock to individual frames during approach. Two pairs of wings on the 'locust' were designed to beat in antiphase to each other at 25 beats s^{-1} to emulate flapping flight during approach. This produced a temporal resolution of 3.2 frames wingbeat $^{-1}$, which may have produced artefacts during presentation. However, for approaches of 3 m s^{-1} (i.e. locust flight speed), the rendering rate of the simulated objects (80 f.p.s.) was the maximum that could be played back with high fidelity using the existing hardware. Moreover, DCMD activity (see below) did not phase lock to the motion of the 'locust' wings, suggesting that this relatively crude approximation of flapping flight did not

produce confounding artefacts during an approach. The dimensions of the 'locust' (body diameter=1 cm, maximum wingspan during mid stroke=7 cm) are similar to those of real locusts. The wings of the 'bird' were fixed in place to emulate a gliding approach. The dimensions of the 'bird' (body diameter=5 cm, wingspan=28 cm) are similar to those of small birds known to prey upon locusts (see Fry et al., 1992; Rind and Santer, 2004). The experimental locust was placed such that its head was 11 cm from the apex of the screen and therefore the final visual angle subtended by the general regions of the simulated objects was: 'locust' body=5.2°, 'locust' wings=35.3°, 'bird' body=25.6°, 'bird' wings=103.7°. Each simulated object was rendered to compensate for distortions due to curvature of the rear projection screen and placement of the experimental locust near the apex.

The ratio of the half size of a symmetrical object (l) and the absolute velocity $|v|$ can be used to calculate a single value that relates to the increase in angular subtense during an approach at constant velocity (see Gabbiani et al., 1999). Because the objects described here are composed of complex shapes, I calculated the half size as half the length of the hypotenuse (h) as defined by:

$$\frac{1}{2}h = \frac{\sqrt{x^2+y^2}}{2},$$

where x = object width (i.e. 'wing span') and y = object height (i.e. 'body' height). The ratio $\frac{1}{2}h/|v|$ was 11.8 ms for the 'locust' and 47.4 ms for the 'bird'.

The AVI files were played back with Windows Media Player (version 6.4.09.1128) set to full screen playback using a NVIDIA GeForce4 Ti4200 video card with 128 MB of onboard memory. This configuration maintained the 80 f.p.s. of the original rendering and was confirmed by viewing the video statistics of the player during playback as well as by recording projected images with a high-speed video camera set at 250 f.p.s. The AVI files also contained a 1 ms synchronization pulse that was aligned with the frame in which the object approach stopped (i.e. at time = 3 s). The synchronization pulse was played through the computer's sound card and connected to the TTL channel of the multichannel recording system to allow for synchronization of the stimulus and neurophysiological recordings. The angles subtended by the 'body' diameter and 'wingspan' of each object during an approach (see Fig. 1C) were aligned to the time of collision and are shown in Fig. 1D. These stimuli reliably induce avoidance steering manoeuvres in loosely tethered flying locusts (Mohr and Gray, 2003).

A 'locust' or 'bird' approached along one of three trajectories: either from 0° azimuth in the horizontal plane (straight ahead) or from ±45° azimuth (to the right or left, respectively, of the experimental locust's longitudinal body axis). One sequence of presentations consisted of 30 consecutive approaches with a 34 s interval between the start of each 4 s approach. This sequence was repeated for each object and each trajectory.

Another sequence of approaches was designed to test whether a habituated DCMD was able to respond to the same object approaching along a different trajectory or to a new object approaching along the same trajectory. For each sequence a 'locust' or 'bird' approached 17 times with a 4 s interval between the start of each approach (i.e. no delay between the end of one approach and the start of the next approach). Approaches 1–15 were of the same object approaching along the same trajectory. To test for the effects of a new trajectory the 16th approach was of the same object approaching either from ±45° azimuth, if the first 15 approaches were from 0° azimuth, or of the same object approaching from 0° azimuth, if the first 15 approaches were from ±45° azimuth. The 17th approach was the same as that for the first 15. To test for the effects of a new object size the 16th approach was of a 'bird' if the first 15 approaches were a 'locust' and of a 'locust' if the first 15 approaches were a 'bird'. The effects of a new object size were tested for each trajectory.

A third sequence consisted of a 'bird' approaching from 0°

Table 1. *Presentation order of approach sequences*

Sequence number	Object	Trajectory (° azimuth)	Number of approaches	Approach interval (s)
1	L	0	30	34
2	L	+45	30	34
3	L	-45	30	34
4	B	+45	30	34
5	B	-45	30	34
6	B	0	30	34
7	L, L, L	0 +45 0	17	4
8	L, B, L	+45 +45 +45	17	4
9	L, L, L	0 -45 0	17	4
10	L, B, L	0 0 0	17	4
11	L, L, L	+45 0 +45	17	4
12	L, L, L	-45 0 -45	17	4
13	L, B, L	-45 -45 -45	17	4
14	B, B, B	0 -45 0	17	4
15	B, L, B	+45 +45 +45	17	4
16	B, L, B	-45 -45 -45	17	4
17	B, B, B	0 +45 0	17	4
18	B, L, B	0 0 0	17	4
19	B, B, B	+45 0 +45	17	4
20	B, B, B	-45 0 -45	17	4
21	B	0	15	4

For sequences 7–20 the first letter or number represents the object or trajectory for approaches 1–15, the second letter or number represents the object or trajectory for approach 16 and the third letter or number represents the object or trajectory for approach 17. L, 'locust', B, 'bird'. Sequence 21 is a 'bird' approaching while rotating ±45° about its longitudinal axis.

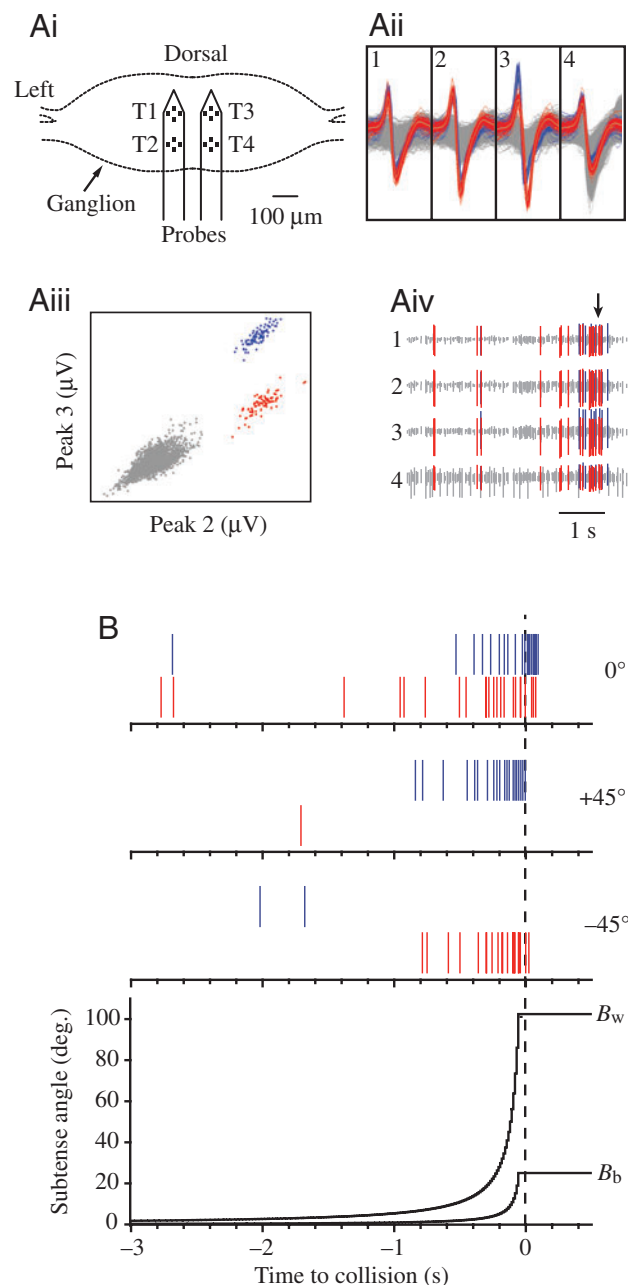
azimuth while it rotated ±45° about its longitudinal axis to emulate roll during approach. Rotations lasted 500 ms and continued until the time at which the approach stopped. This configuration was designed to test whether DCMD habituation is affected by low frequency, non-looming motion of an approaching object (i.e. internal object motion), which might occur during corrective steering manoeuvres of an approaching predator. The time interval between the start of consecutive approaches for this sequence was 4 s.

Including all experimental conditions, each locust ($N=11$) was presented with 433 approaches from 21 sequences for a total of 4763 presentations (see Table 1 for the order of sequence presentations to each experimental animal). The inter-sequence time interval was at least 5 min to reduce potential habituating effects of object size and trajectory between sequences of approaches. At least 5 min after the end of each experiment each locust was presented with a single approach of a 'bird' from 0° azimuth to confirm that DCMD responses were not affected by the duration of the experiment or the total number of presentations (data not shown).

Experimental setup and multichannel recording

Experimental locusts were mounted ventral side up onto a rigid tether using low melting point beeswax. A small patch of ventral cuticle was removed to expose the underlying

Fig. 2. Identification of DCMD activity within the mesothoracic ganglion. (Ai) Multichannel silicon microprobes were inserted ventrally into the mesothoracic ganglion (outline shows scaled frontal view). Each of the probe's two tines contained two tetrode arrays (T1–T4). The arrangement of the recording sites was as follows: site 1–4 on T1, sites 5–8 on T2, sites 9–12 on T3, sites 13–16 on T4). Thus, sites 1–8 recorded activity from the left side of the ganglion and sites 9–16 recorded activity from the right side of the ganglion. (Aii) Recordings from a sample preparation showing waveforms overlaid in a 1.3 ms time window. The colours of the waveforms correspond to clusters identified in Aiii, which shows a plot of the peak of the waveforms of recording site 3 vs recording site 2 (see details in text for recording parameters). Well-defined clusters were easily identified in all preparations. (Aiv) 5 s time window showing the activity of the discriminated units during the presentation of a simulated looming bird. The blue and red units had the largest spike amplitudes and showed an increase in firing rate during an approach, characteristic of locust DCMDs. The arrow indicates time of collision. (B) Identification of the right and left DCMDs based on physiological responses to looming stimuli. The blue (right DCMD) and red (left DCMD, see Materials and methods for designation) rasters represent the spike times, during different approaches, of the units shown in A. In this example, each raster was time-aligned to the parameters of the approaching 'bird', where 0=time of collision. The top two rasters show responses to a 'bird' approaching from 0° azimuth (0°), which would be seen by both eyes. The middle rasters show responses to a 'bird' approaching from +45° azimuth (+45°) and the bottom rasters show responses to a 'bird' approaching from -45° azimuth (-45°). Because approaches from ±45° azimuth would occupy the field of view of the ipsilateral eye only, these criteria could be used to discriminate unambiguously right and left DCMD activity for all runs for each animal. B_b and B_w as in Fig. 1.



mesothoracic ganglion. The exposed tissue was bathed in a drop of locust saline (147 mmol l⁻¹ NaCl, 10 mmol l⁻¹ KCl, 4 mmol l⁻¹ CaCl₂, 3 mmol l⁻¹ NaOH, 10 mmol l⁻¹ Hepes, pH 7.2) and the preparation was transferred to a flight simulator (Fig. 1B; see Gray et al., 2002 for a complete description). For multichannel recordings I used 2×2 tetrode silicon probes provided by the University of Michigan Center for Neural Communication Technology (<http://www.engin.umich.edu/facility/cnct/>) sponsored by NIH NCRR grant P41-RR09754 (Fig. 2Ai). The probes were connected to a RA16AC 16 channel acute Medusa Bioamp System 3 workstation (Tucker-Davis Technologies Inc., Alachua, FL, USA). Physiological signals were sampled at 25 kHz/channel with Butterworth filter settings of 100 Hz (high pass) and 5 kHz (low pass). An additional TTL channel was used to record synchronization pulses from the visual stimuli (see above) to permit alignment of the physiological recordings with the stimuli. Using a micromanipulator, the probes were inserted ventrally into the mesothoracic ganglion until all sets of tetrode recording sites were within the tissue (Fig. 2Ai). After the initial neuronal injury discharge from probe insertion had ceased I tested the preparation for responses to visual stimuli by waving my hands in front of it. The entire preparation was then rotated 180° to orient the locust dorsal-side up. Following confirmation of responses to

visual stimuli, the rear projection dome screen was placed in front of the preparation such that the head of the locust was 11 cm from the apex of the dome. In this configuration, the dome occupied 250° of the locust's horizontal and vertical field of view. The threshold settings for data capture were adjusted to capture, selectively, spikes with the largest amplitude, which also showed distinct responses to looming stimuli (see below). This configuration produced stable recordings for the duration of each preparation, about 5 h. On occasion, fixed preparations would generate flight-like rhythms and the resulting neural activity in the ganglion would make it impossible to distinguish DCMD activity. Therefore data were collected only if the preparation did not generate these rhythms at least 2 min before and throughout

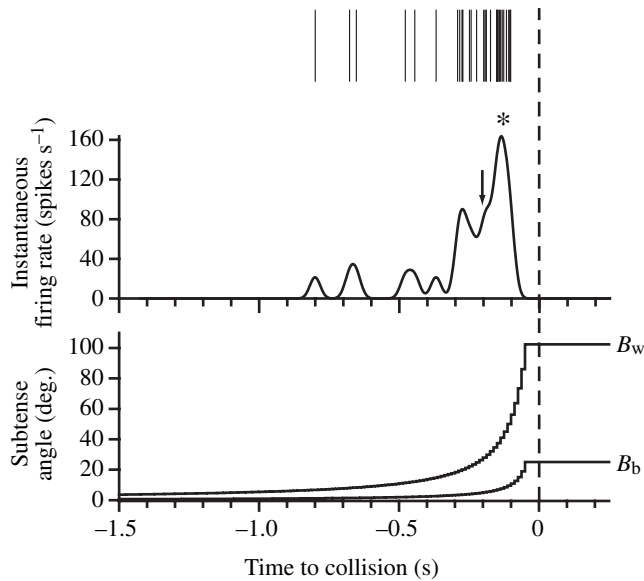


Fig. 3. Quantitative measurements of DCMD firing parameters. This data shows one response of the right DCMD to a 'bird' approaching from $+45^\circ$ azimuth. (Top) The raster plot shows the DCMD spike times. (Middle) A Gaussian smoothed (bin=50 ms) plot of the instantaneous firing rate (see text for details). (Bottom) The subtense angle of the 'bird' body (B_b) and wing (B_w). From the rate histogram plots I measured the time and amplitude of peak firing (asterisk), the instantaneous spike rate 200 ms before collision (arrow), and the total number of spikes during each approach (see Materials and methods). For this example the DCMD produced 29 spikes and reached a peak firing rate of 165 spikes s^{-1} 169 ms before collision. At this time the 'bird wing' subtended approximately 25° of the right eye's field of view. The point at which the angle of the 'bird' wing jumps by more than 3° is 125 ms before collision. Thus in this example the DCMD peak occurred 86 ms (or approximately 7 frames) before a proposed critical subtense angle jump of more than 3° (see Rind and Simmons, 1997).

the stimulation sequence. Data were recorded onto disk and stored for off-line analysis.

Spike sorting and identification of DCMDs

I used the cluster analysis abilities of Offline Sorter (Plexon Inc., Dallas, TX, USA) to discriminate the activity of individual units from the raw multichannel neurophysiological recordings. Typically, 3–8 units could be discriminated. Spike times of these units were imported into Neuroexplorer (NEX Technologies, Littleton, MA, USA) along with the times of the TTL synchronization pulses. Because the approaches stopped 11 cm from the head of the experimental locust, the TTL signal was shifted by 37 ms to indicate time of collision (TOC), which was aligned with the spike time of discriminated units. Two units from each recording were identified as the right or left DCMD, based on two defining criteria: they had the largest amplitude waveforms of all recorded units and they responded to looming stimuli with an increasing spike rate that peaked near TOC. These are standard criteria for identifying DCMD activity from extracellular recordings of the ventral nerve

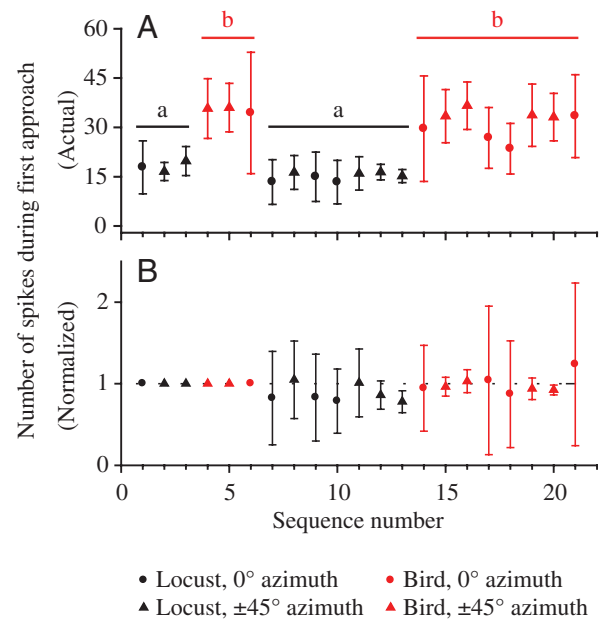


Fig. 4. Dishabituation of DCMD responses between presentations of approach sequences. (A) The number of DCMD spikes for each first approach according to the order of presentation of a sequence (i.e. the order of which the randomized sequences were actually presented, see Table 1). The number of spikes (mean \pm S.D.) was lower for approaches of a 'locust' than for a 'bird'. A Kruskal–Wallis ANOVA on ranks showed that there were no significant differences in the number of spikes within an object type (i.e. 'locust' or 'bird'). In (B) the data were normalized to the first presentation of a particular object and trajectory. For example, the first approach for each sequence of a simulated locust approaching from 0° azimuth was normalized to the first time that approach was presented. If there were no confounding effects of incomplete dishabituation then the normalized values should not be different from 1. A Kruskal–Wallis ANOVA on ranks showed that there were no significant differences in the normalized number of spikes within an object type. These data show that the DCMDs were fully dishabituated prior to each presentation sequence. (See text for statistical parameters; bars with the same letters were not statistically different, $N=11$.)

chord in non-flying animals. Although I recorded from within the mesothoracic ganglion rather than the ventral nerve chord, it is likely that relatively large amplitude spikes of the DCMDs would be maintained when the axons enter the ganglion.

Fig. 2B (top rasters) shows the responses of two units to a 'bird' approaching from 0° azimuth. The same units were also aligned with the time of collision of a 'bird' approaching from $+45^\circ$ (middle rasters) and -45° (bottom rasters) azimuth. Responses to lateral stimuli permitted unequivocal identification of the right and left DCMDs. The distinction between right and left DCMDs was based on the location of the cell bodies within the brain, i.e. ipsilateral to the side of stimulation for objects approaching from $\pm 45^\circ$ azimuth.

Data analysis

To quantify DCMD responses, spike times (Fig. 3, top rasters) were transformed to instantaneous spike rates using

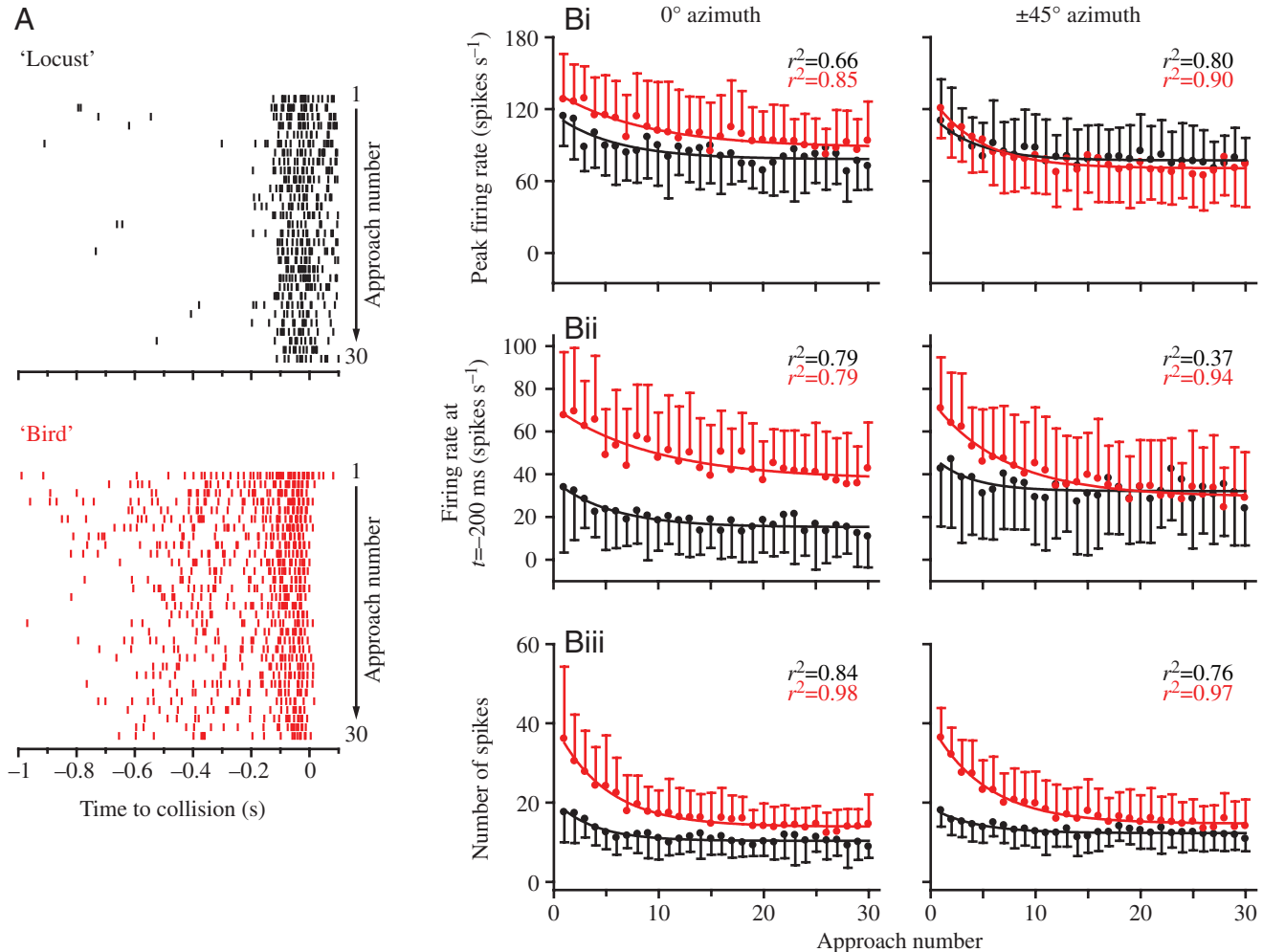


Fig. 5. DCMD responses to 30 consecutive approaches of a 'locust' or 'bird' at 34 s intervals. (A) Sample raster plots from one sequence of 30 approaches showing responses of the right DCMD to a 'locust' (black) and a 'bird' (red) approaching from +45° azimuth. (Bi) The peak spike rate, (Bii) the spike rate at $t=-200$ ms and (Biii) number of spikes decreased following the first approach of either a 'locust' (black circles and line) or 'bird' (red circles and line) and plateaued after 10 or 15 approaches. Data plotted (means \pm s.d.) are from 9 animals. For clarity the s.d. is shown in only one direction. Mean values of each plot were well fit by a single exponential decay of the form $y=y_0+ae^{-bx}$. r^2 values for each plot are indicated. Means were calculated from pooled right and left DCMDs (0° azimuth trajectories) and pooled ipsilateral DCMDs ($\pm 45^\circ$ azimuth).

a 50 ms Gaussian smoothing filter (middle graph; see Gabbiani et al., 1999). A Gaussian filter was used to reduce the artefacts caused by temporal binning of the responses into peristimulus time histograms (Richmond et al., 1990). For each approach I measured the time of the peak firing relative to collision (asterisk in Fig. 3), the amplitude of the peak and the number of spikes. Previous reports suggested that if DCMD spikes are involved in initiating collision avoidance flight manoeuvres then they must occur approximately ≥ 200 ms before collision (Gray et al., 2001; Matheson et al., 2004a). This value takes into account known values of flight reaction times relative to collision, the lag between visual input and the onset of a behavioural response as well as conduction times of DCMD spikes from the brain to the thorax. Moreover, although the DCMDs are likely not solely responsible for initiating or triggering an escape jump, they could have a currently undescribed role early in the

underlying motor program (Burrows, 1996). Energy for a jump is stored *via* co-contraction of tibial flexors and extensors over a period of 200–500 ms (Pearson and O'Shea, 1984). Therefore, I also measured the instantaneous spike rate 200 ms before collision (arrow in Fig. 3) as an approximation of the latest time at which DCMD activity could influence escape behaviour.

Data from responses of the right and left DCMDs to objects approaching from 0° azimuth were pooled, as were data from responses of the right or left DCMD to objects approaching ipsilateral to the DCMD cell body. Plots relating the approach number to the parameters measured were fit with a single exponential decay function of the form $y=y_0+ae^{-bx}$, where y_0 gives the value of the asymptote, a is a scaling factor related to the initial value, b is the rate of decay and x is the approach number (see Figs 5, 7, 9, 11).

Tests for significant differences of DCMD firing parameters

and habituation properties between different objects and different trajectories were conducted using SigmaStat 3.0 (Systat Software Inc., Richmond, CA, USA). Specific tests and resulting statistics are described in appropriate sections of the results. Significance was assessed at $P < 0.05$.

Results

DCMD responses to objects with complex shapes

To test whether DCMD responses presented here were similar to those reported for objects with basic shapes (i.e. squares or circles) I used the regression of the time of peak firing vs the ratio of $\frac{1}{2}h/|v|$ (see Materials and methods) to calculate a delay in the peak firing rate (δ) after the objects reached a threshold subtense angle (θ_{thresh}). The threshold angle was calculated as:

$$\theta_{\text{thresh}} = 2 \times \tan^{-1}(1/\alpha),$$

where α is the slope of the regression line and δ is the y intercept (for derivation, see Gabbiani et al., 1999). To eliminate the effects of strong habituation, when no peak DCMD spike rate could be detected, I calculated θ_{thresh} and δ using the first approach of each sequence. Using values calculated from simulated locusts or birds approaching from 0° or $\pm 45^\circ$ azimuth, the values were: $\theta_{\text{thresh}} = 122.7^\circ$ and 38.6° , respectively, $\delta = 52.0$ ms and 55.0 ms, respectively.

Dishabituation of DCMDs between approach sequences

To determine that the DCMDs were fully dishabituated after a minimum 5 min interval between approach sequences I plotted the number of spikes for the first approach of a sequence against the order in which sequences were presented (see Table 1, Fig. 4A). A Kruskal–Wallis ANOVA on ranks showed that there was a significant difference between treatments ($H_{20} = 152.9$, $P > 0.001$, $N = 11$ approaches/treatment). A Dunn's *post-hoc* multiple comparison showed that there were significantly fewer spikes during approaches of a 'locust' than during approaches of a 'bird' ($P < 0.05$), but that there were no significant differences within 'locust' or 'bird' approaches. To control for the type of object and initial approach trajectory, the number of spikes were normalized to the number for the first approach with a particular combination of parameters. For example, the number of spikes for all sequences in which the initial approach of a 'locust' was from 0° azimuth was normalized to the first sequence in which the first approach was a 'locust' from the same trajectory (Fig. 4B). A Kruskal–Wallis ANOVA on ranks ($H_{20} = 46.3$, $P < 0.001$) showed that there was a significant difference in the median values of the groups (object type combined with trajectory). However, A Dunn's *post-hoc* multiple pairwise comparison showed that there were no significant differences between specific median values from approaches of different objects or different trajectories. These data demonstrate that there were no confounding effects of habituation due to the order of approach sequences during each experiment.

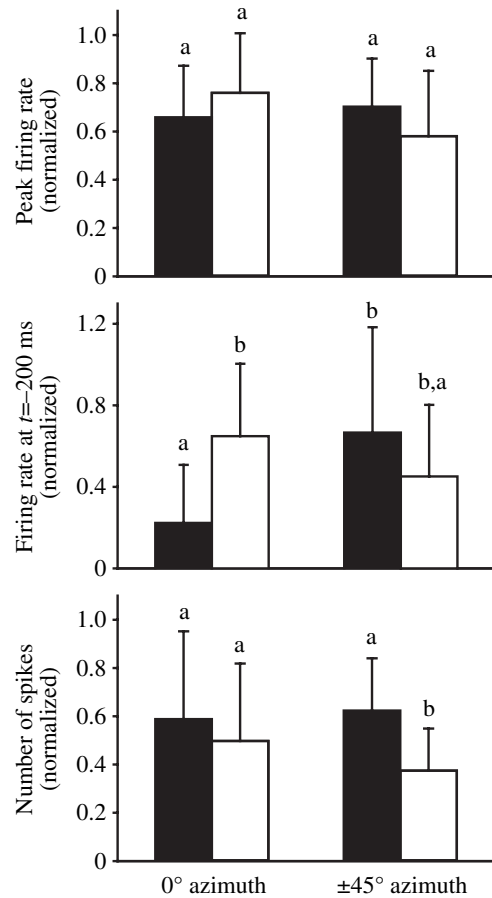


Fig. 6. Assessment of whether DCMD habituation was the same for repeated presentations of a 'locust' (black bars) and 'bird' (open bars). The value of the 30th (habituated) approach was normalized to the first approach. Values are means \pm s.d. A two-way ANOVA revealed that the object size or trajectory did not affect the peak spike rate. A 'locust' approaching from 0° azimuth resulted in a greater decrease of the instantaneous spike rate 200 ms before collision than did a 'bird' from the same trajectory or a 'locust' approaching from $\pm 45^\circ$ azimuth (see text for statistical parameters; bars with the same letters were not statistically different, $N = 9$). Comparisons were made within each graph.

DCMD responses to objects approaching at 34 s intervals

Fig. 5A shows the response of the right DCMD to 30 consecutive approaches of a 'locust' (black rasters) or 'bird' (red rasters) from $+45^\circ$ azimuth at 34 s intervals. For either object the neuron responded to all 30 approaches. The onset of activity during responses to a 'locust' occurred between 200 and 100 ms before collision, and activity continued until after collision (Fig. 5A, black rasters). The responses were qualitatively similar for each consecutive approach. The onset of activity in response to an approaching 'bird' occurred earlier and, for the first approach, continued through collision (Fig. 5A, red rasters). For all subsequent 'bird' approaches, activity ended at or slightly before collision. During the last few approaches the onset of activity occurred later and there were fewer spikes produced. This was consistent for each

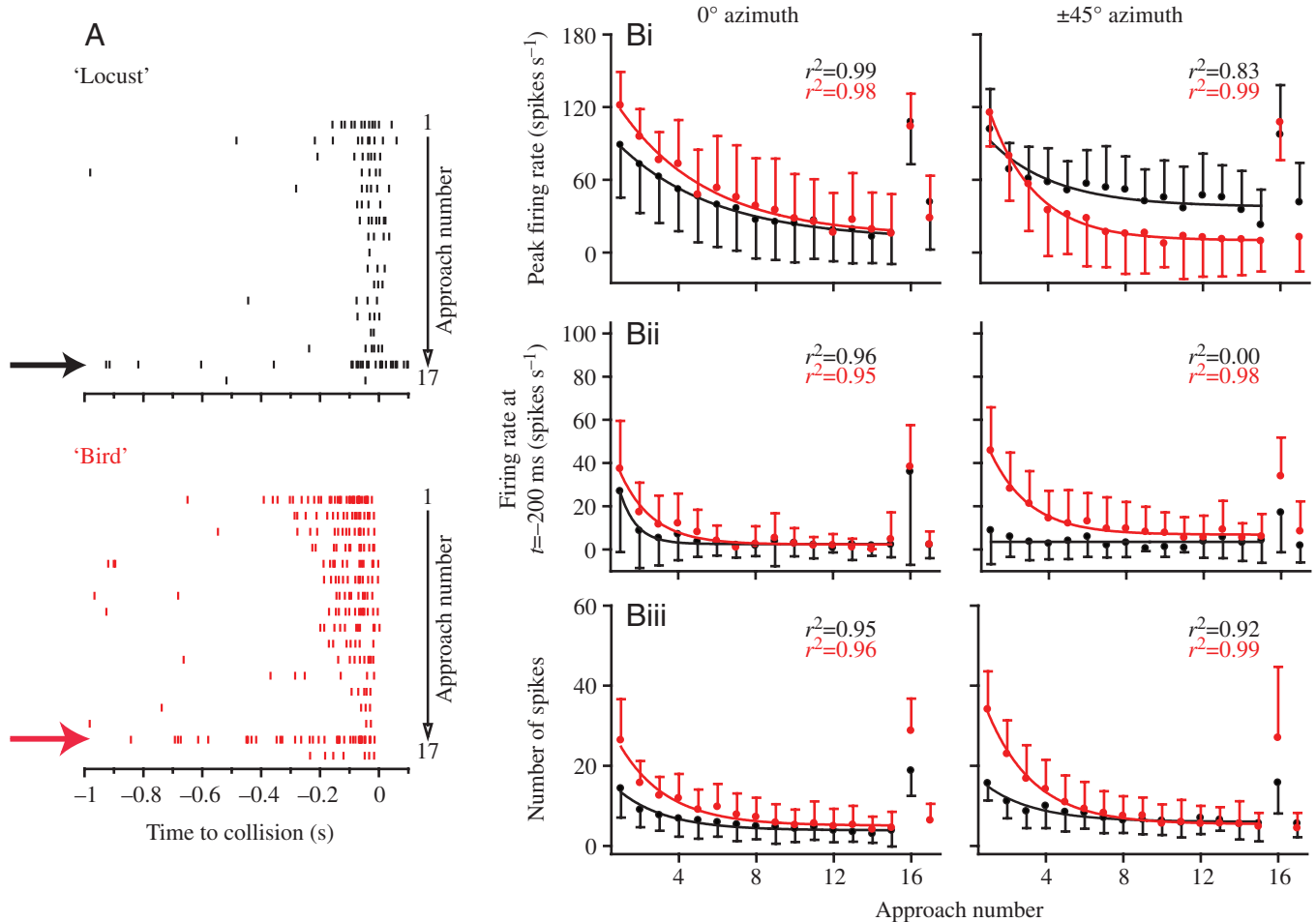


Fig. 7. DCMD responses to 17 consecutive approaches of a 'locust' (black) or 'bird' (red) at 4 s intervals. Approach 16 is the same object approaching along a different trajectory. (A) Sample raster plots of the right DCMD to 17 approaches of a 'locust' and 'bird' showing a stronger response to approach 16 (arrows). In this example approaches 1–15 and 17 are from 0° azimuth and approach 16 is from +45° azimuth. (Bi–iii) Data ($N=11$) were plotted as in Fig. 5 except that the curve was fit for approaches 1–15. For clarity the s.d. is shown in only one direction.

animal tested. Fig. 5B shows changes in the peak firing rate, the instantaneous spike rate 200 ms before collision and the number of spikes for consecutive approaches for all animals tested ($N=9$). Conditions were separated based on whether the object approached from 0° or ±45° azimuth (pooled right and left approaches). For both object types each of the measured parameters decreased and plots of the mean values during consecutive approaches were well fit by a single exponential decay (Fig. 5B). There was little qualitative difference in a decrease of the peak spike rate whether the preparation was presented with a 'locust' or 'bird' (Fig. 5B, top plots). For each approach from 0° azimuth, a 'bird' induced a higher spike rate 200 ms before collision than did a 'locust'. However, the relative decrease of the spike rate 200 ms before collision was similar during repeated approaches of a 'locust' or 'bird' from 0° azimuth. The first approaches of a 'locust' or 'bird' from ±45° azimuth were similar to those in response to approaches from 0° azimuth. However, the spike rate decreased further during repeated approaches of a 'bird'. The number of spikes also decreased during consecutive approaches of either object

type (Fig. 5B, bottom plots). For either approach trajectory the 'bird' invoked more spikes during the first few approaches and these decreased to levels similar to those induced by a 'locust' after 10–15 approaches.

To quantify the extent of DCMD habituation within a sequence I normalized the values of the last approach to those of the first approach (Fig. 6) and used a two-way ANOVA to test the effects of object type and trajectory on the peak spike rate, the instantaneous spike rate 200 ms before collision and the number of spikes. Neither object type nor trajectory affected a decrease of the peak spike rate. While the object type or trajectory alone did not affect a decrease of the instantaneous spike rate 200 ms before collision there was a significant combined effect of these two parameters ($F_{1,59}=10.472$, $P=0.002$). Holm-Sidak *post-hoc* multiple comparisons revealed that a 'locust' approaching from 0° azimuth produced a greater decrease of the instantaneous spike rate 200 ms before collision than did a 'bird' from the same trajectory or a 'locust' approaching from ±45° azimuth. There was also a significant effect of the object type on a decrease of

the number of spikes produced during an approach along either trajectory ($F_{1,63}=6.28$, $P=0.015$). The same *post-hoc* multiple comparisons revealed that a 'bird' approaching from $\pm 45^\circ$ azimuth produced a greater decrease of the number of spikes than did a 'bird' from 0° azimuth or a 'locust' from either trajectory. These data show that the DCMDs were able to maintain 60–80% of the peak spike rate and 40–60% of the number of spikes in response to repeated approaches of a 'locust' or 'bird' presented at 34 s intervals. However, the firing rate 200 ms before collision, when a behavioural response would be initiated, decreased to 60% or lower.

Responses to the same object approaching along a new trajectory

DCMD responses decreased dramatically during 15 approaches of a 'locust' or 'bird' at 4 s intervals (Figs 7 and 9). In Fig. 7A, approaches 1–15 and 17 are from 0° azimuth and approach 16 is from $+45^\circ$ azimuth. The response to approach 15 of a 'locust' or 'bird' began later and was much weaker than the response to approach 1. Qualitatively, the response decrement was greater during the first 15 approaches than it was to approaches at 34 s intervals (Fig. 5). The response to approach 16 (new trajectory, arrows in Fig. 7A) was much stronger than to approach 15 and qualitatively similar to the response to approach 1 whereas the response to approach 17 (original trajectory) was similar to the response to approach 15.

Data from 11 animals showed that the peak spike rate, the instantaneous spike rate 200 ms before collision and the number of spikes during approaches 1–15 decreased with repeated approaches and were well-fit with an exponential decay (Fig. 7B) as described above. There was little qualitative difference in a decrease of the peak spike rate during repeated approaches of either object type from 0° azimuth whereas the DCMD habituated further during repeated approaches of a 'bird' from $\pm 45^\circ$ azimuth (Fig. 7Bi). The decrease of the instantaneous spike rate 200 ms before collision was similar for a 'locust' or 'bird' approaching from 0° azimuth whereas during approaches from $\pm 45^\circ$ azimuth the spike rate was consistently low for each approach of a 'locust' (Fig. 7Bii). This habituation profile is due to the fact that the DCMD firing rate does not increase until after a 'locust' is within 200 ms of collision. The number of spikes also decreased during consecutive approaches of either object type (Fig. 7Biii). As with the 34 s interval data, an approaching 'bird' evoked more spikes during the first approach, but these decreased to a level that was the same for either object type. For either object type or trajectory, the 16th approach evoked a higher peak spike rate, a higher spike rate 200 ms before collision and more spikes than approach 15, whereas the response to approach 17 was the same as to response 15.

Data for approaches 15–17 were normalized to those for approach 1 (Fig. 8), as described above. To test the effects of the object type and approach number on the parameters measured I used a two-way ANOVA to compare data within each initial trajectory (0° or $\pm 45^\circ$ azimuth). For either

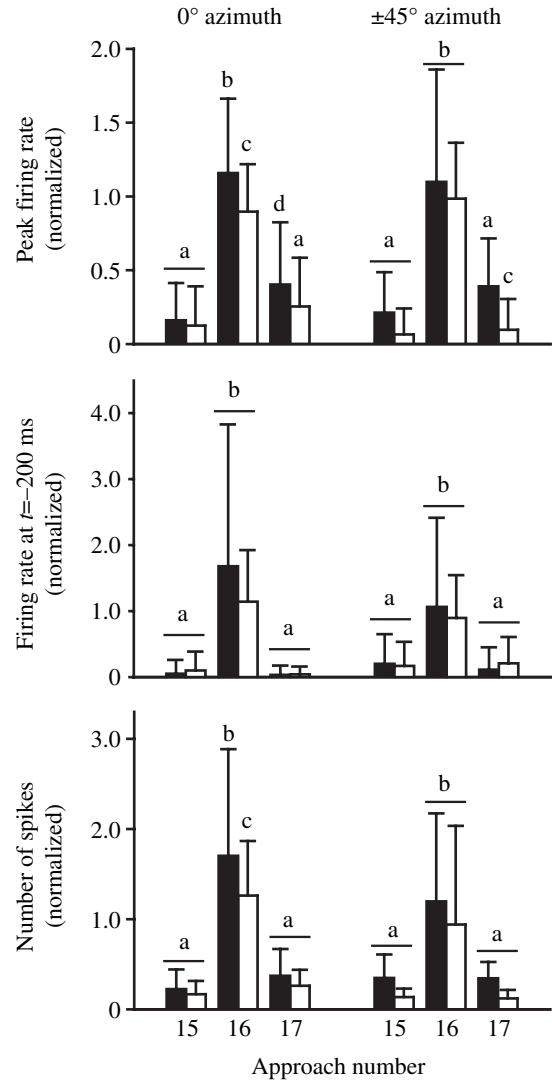


Fig. 8. Habituated DCMDs (approach 15) were able to respond to objects approaching along a new trajectory (approach 16) with a significantly higher peak spike rate, spike rate 200 ms before collision and number of spikes. The new trajectory did not dishabituate the response to the original trajectory (approach 17). (See text for statistical parameters, significance assessed as in Fig. 6). 'locust', black bars; 'bird', open bars. Data are plotted as the mean \pm s.d. ($N=11$).

trajectory there was a significant effect of the stimulus type or approach number on a decrease of the peak spike rate and number of spikes, whereas a decrease of the spike rate 200 ms before collision was affected by the approach number but not by the object type (see Table 2 for two-way ANOVA parameters). There was no combined effect of object type and approach number on habituation of the responses. I used a Holm–Sidak multiple comparison to test for significant differences between object types within a specific approach number or for differences between approach numbers within a given object type. Results showed that for all parameters measured, regardless of the object type, responses to the 15th and 17th approaches were lower than responses to the first

Table 2. Two-way ANOVA parameters for tests of effects of object type and approach number on habituated DCMD responses

Test condition	Peak spike rate			Spike rate at $t=-200$ ms			Number of spikes		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
New trajectory									
0° azimuth									
Object type	1,204	8.80	0.003	1,103	0.10	0.337	1,208	9.58	0.002
Approach no.	2,204	94.77	<0.001	2,103	28.69	<0.001	2,208	125.13	<0.001
Combined	2,204	1.56	0.212	2,103	1.25	0.292	2,208	2.69	0.070
±45° azimuth									
Object type	1,126	6.95	0.009	1,99	0.01	0.926	1,125	4.58	0.034
Approach no.	2,126	65.89	<0.001	2,99	21.71	<0.001	2,125	26.42	<0.001
Combined	2,126	0.62	0.540	2,99	0.817	0.445	2,125	0.02	0.984
New object									
0° azimuth									
Object type	1,123	25.64	<0.001	1,90	41.01	<0.001	1,122	76.32	<0.001
Approach no.	2,123	46.51	<0.001	2,90	43.33	<0.001	2,122	68.50	<0.001
Combined	2,123	35.10	<0.001	2,90	52.19	<0.001	2,122	53.59	<0.001
±45° azimuth									
Object type	1,126	105.96	<0.001	1,102	20.09	<0.001	1,125	218.39	<0.001
Approach no.	2,126	33.20	<0.001	2,102	10.75	<0.001	2,125	104.64	<0.001
Combined	2,126	25.87	<0.001	2,102	11.68	<0.001	2,125	104.90	<0.001

Shaded cells indicate no significant effect at $P=0.05$.

approach (i.e. normalized values <1). Responses to the 16th approach were as high as or higher than responses to the first approach (i.e. normalized values ≥ 1) and were significantly greater than the normalized responses to approaches 15 and 17. The only significant differences between different objects were in responses of the peak spike rate (approaches 16 and 17 from 0° azimuth and approach 17 from $\pm 45^\circ$ azimuth) and the number of spikes (approach 16 from 0° azimuth). These differences should be interpreted with caution, however, as the P -values for each comparison ranged from 0.45 to 0.5 (with significance set at $P=0.05$), suggesting that they may not be biologically significant. Nevertheless, these data demonstrate that DCMD habituation was not maintained for targets approaching along a new trajectory which, in turn, did not dishabituate the response to the initial object parameters. Moreover, there were few differences between habituated responses to a 'locust' or 'bird'.

Responses to a new object approaching along the same trajectory

Fig. 9A shows that habituation of the responses during approaches 1–15 (the same object traveling along the same trajectory) was consistent with that shown in Fig. 7A. The 16th approach, however, produced different responses based on the initial object type. For responses that had habituated to a 'locust' (black rasters), the 16th approach (a 'bird', red arrow) produced a more intense response, whereas for responses that had habituated to a 'bird' (red rasters), the 16th approach (a 'locust', black arrow) did not evoke a more intense response. For both conditions the response to approach 17 was

indistinguishable from the habituated response (approach 15). In these examples all approaches were from $+45^\circ$ azimuth. Data from 11 animals showed that a decrease of the peak spike rate, the instantaneous spike rate 200 ms before collision and the number of spikes during approaches 1–15 was qualitatively similar to that described in Fig. 7B. However, when the new object (approach 16) was a 'bird', the responses were consistently larger than the habituated responses compared to when the new object was a 'locust'.

Data were normalized and plotted as in Fig. 8 (Fig. 10). A two-way ANOVA within each trajectory showed that there was a significant effect of the stimulus type or approach number as well as a combined effect of both factors on habituation of DCMD responses (Table 2). Multiple comparisons (Holm–Sidak) within the object type or approach number showed similar differences for responses as described in Fig. 8 (Fig. 10), except for one important point. For approach 16 the peak spike rate, the spike rate 200 ms before collision and number of spikes was significantly higher when the new object was a 'bird'. When the new object was a 'locust', neither parameter differed from the habituated state. These data show that habituated DCMDs are able to produce an increased response to a larger object approaching along the same trajectory and that a new object, of either type, did not dishabituate the response.

Effects of target size, trajectory and presentation frequency on habituation

To test for the effects of the approach interval, object type and object trajectory on the rate of habituation I plotted the

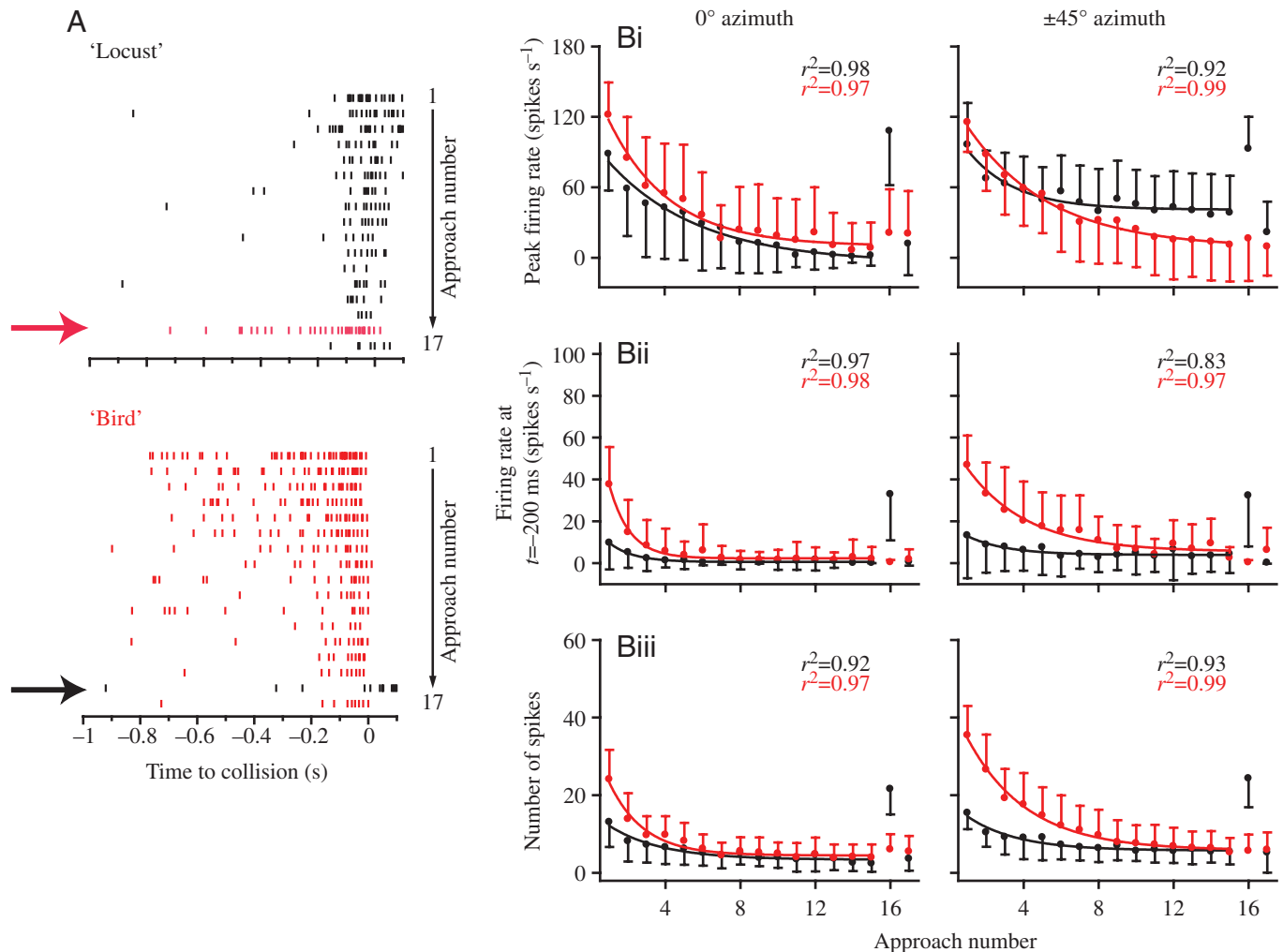


Fig. 9. DCMD responses to 17 consecutive approaches of a 'locust' (black rasters) or 'bird' (red rasters) at 4 s intervals. Approach 16 is a new object ['bird' following 'locust' (red arrow) or 'locust' following 'bird' (black arrow)] approaching along the same trajectory. All data plotted as in Fig. 7. (A) The habituated right DCMD responded more strongly to a 'bird' (red arrow) than to a 'locust' (black arrow) approaching from +45° azimuth. (Bi) The peak spike rate, (Bii) the spike rate 200 ms before collision and (Biii) the number of spikes increased only when the DCMD was presented with a new larger object (note approach 16, $N=11$). For clarity the s.d. is shown in only one direction.

natural log of the peak spike rate and number of spikes from each approach against the approach number and measured the slope of the linear regression ($N=11$; data not shown). A three-way ANOVA for the peak spike rate and number of spikes showed that there was a significant effect of all three factors combined on the rate of decrease of the peak spike rate ($F_{1,136}=4.13$, $P=0.044$). Individually, there was no effect of object type or trajectory on the slope, whereas objects presented at 4 s intervals resulted in a significantly more negative slope (-0.17) than objects approaching at 34 s intervals (-0.01 , $F_{1,136}=21.89$, $P<0.001$). This suggests that the peak spike rate decreased at an equal rate to a 'bird' and 'locust' approaching from 0° or ±45° azimuth at 34 s intervals, but decreased 17 times faster when the object approached in rapid succession. Similarly, there was a significant combined effect of the factors on the rate of decrease of the number of spikes ($F_{1,136}=21.48$, $P<0.001$).

Specifically, the object size and approach frequency had a significant effect on the slope of the line for the number of spikes ($F_{1,136}=26.71$, $P<0.001$) whereas the object trajectory did not. However, for a given trajectory a 'bird' produced a significantly more negative slope compared to a 'locust'. These data demonstrate that the peak spike rate and the number of spikes remained more stable when presented with 'locusts' at longer time intervals than when presented with a 'locust' at 4 s intervals or a 'bird' presented at either interval.

For many initial and subsequent approaches of a 'locust', DCMD activity did not increase until after 200 ms before collision. Therefore, these approaches could not be used to calculate the slopes of the transformed data. The resulting inequality of the number of observations eliminated the possibility of testing the effects of all three factors simultaneously. Therefore I used a two-way ANOVA within

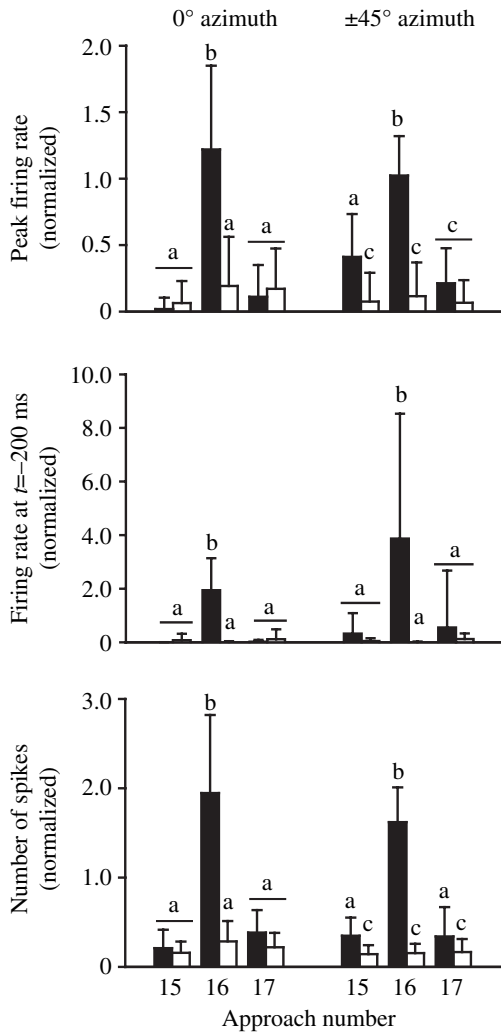


Fig. 10. Habituated DCMDs respond to a new, larger object. Data plotted as in Fig. 8. Note that the open bar for approach 16 is not significantly different from the habituated condition. Significance assessed and indicated as in Fig. 8.

each trajectory to test for effects of the object type and approach interval on the rate of decrease of the instantaneous spike rate 200 ms before collision. The results suggest that, of the approach sequences presented here, DCMD habituation was more sensitive to different objects approaching from 0° azimuth and to the approach frequency along trajectories from ±45° azimuth. For approaches from 0° azimuth there was a significant combined effect of the object type and approach frequency ($F_{1,89}=8.50$, $P=0.004$) and, individually, there was an effect of the object type ($F_{1,89}=8.76$, $P=0.004$), but no effect of the approach frequency. For approaches from ±45° azimuth there was a significant effect of the approach frequency ($F_{1,104}=12.402$, $P<0.001$), but no effect of object type or a combination of the two factors. Taken together, results from calculating habituation rates suggest that the DCMD is better able to respond upon repeated presentations of a 'locust'.

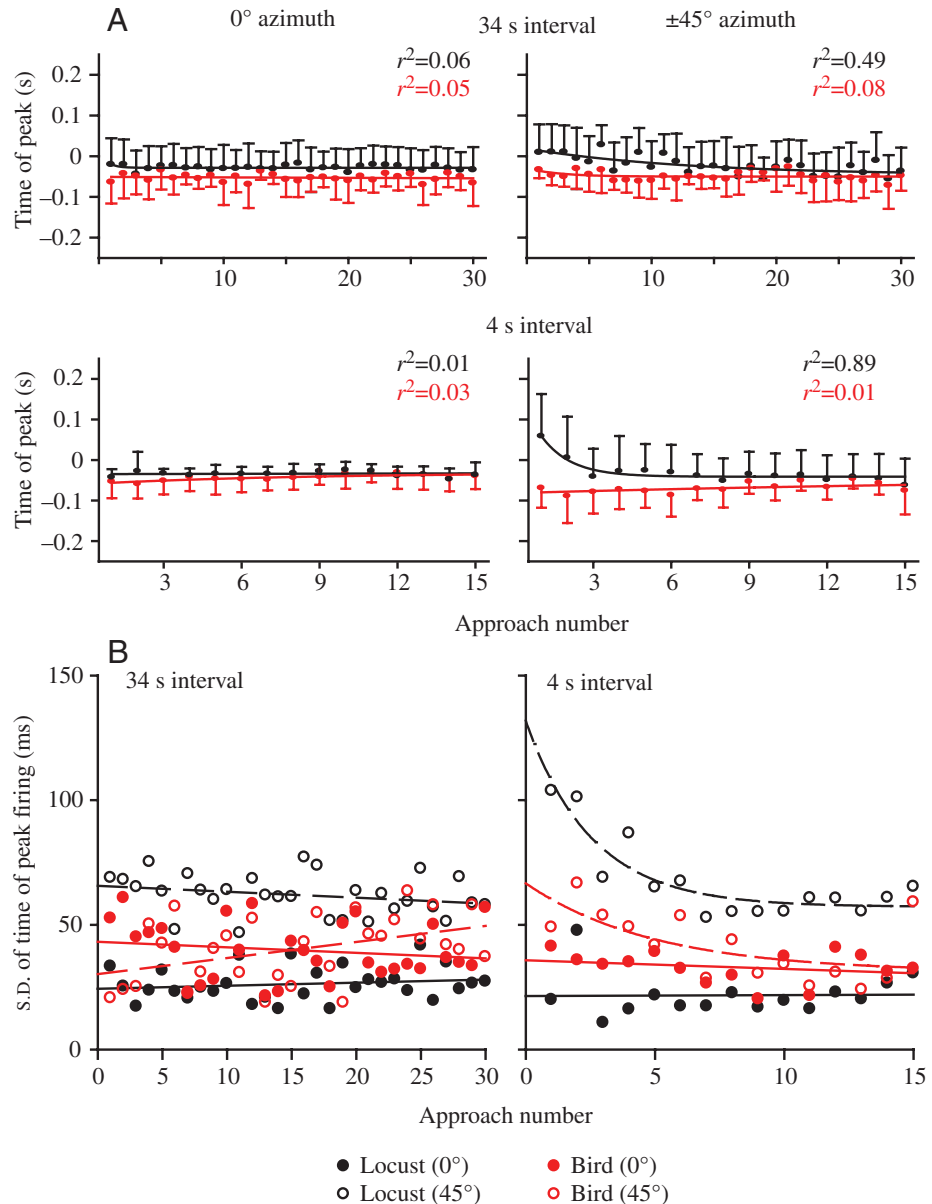
Habituation of peak firing times

Matheson et al. (2004a) showed that variability of the time of the peak DCMD spike rate was low for gregarious locusts presented with objects approaching at 60 s intervals. Therefore, I used a Pearson Product Moment Correlation to measure the relationship between the approach number and the time of the peak DCMD spike rate under the experimental conditions used here. Results revealed that during repeated approaches at 34 s intervals, the mean peak firing time relative to collision was invariant for a 'locust' and 'bird' from 0° azimuth, and a 'bird' from ±45° azimuth. The peak time was, however, significantly negatively correlated to the approach number for a 'locust' approaching from ±45° azimuth ($r=-0.669$). For approaches at 4 s intervals from 0° azimuth, the time of the peak was invariant for a 'locust' and positively correlated to a 'bird' ($r=0.733$). For approaches at 4 s intervals from ±45° azimuth, the time of the peak was negatively correlated to a 'locust' ($r=-0.771$) and positively correlated to a 'bird' ($r=0.595$). Fitting a single exponential decay function (Fig. 11A, see Materials and methods) showed that the peak time occurred earlier during approaches of a 'locust' from ±45° azimuth at 4 s and 34 s approach intervals and, significant relationships described above notwithstanding, was relatively insensitive to other combinations of object type, approach trajectory and approach interval. To examine the variability of peak firing time with respect to the object size, trajectory and the state of habituation I plotted the standard deviation (S.D.) of the peak time against the approach number (Fig. 11B). A Pearson Product Moment Correlation revealed that variability of the time of peak firing was not affected by repeated presentations at 34 s intervals of a 'locust' approaching along either trajectory or a 'bird' approaching from 0° azimuth, whereas the S.D. was positively correlated with repeated approaches of a 'bird' from ±45° azimuth, albeit weakly ($r=0.429$, $P=0.019$). For 4 s interval data there was no effect of repeated approaches of either object from 0° azimuth, whereas the variation decreased for either object approaching from ±45° azimuth ('locust': $r=-0.698$, $P=0.004$, 'bird': $r=-0.551$, $P=0.03$). These data suggest that the time of peak DCMD firing is sensitive to repeated approaches of small objects from ±45° azimuth and relatively insensitive to repeated approaches of a large object from ±45° azimuth, or to either object type from straight ahead.

Effects of internal object motion on habituation

To test for the effects of internal object movement on habituation I normalized data from the 15th approach, as described above, of a 'bird' that rolled about its longitudinal axis (see Materials and methods) and compared the mean to results from the pooled 4 s interval data of a 'bird' approaching from 0° azimuth (Fig. 12). Internal motion during approach did not influence decreases of the peak spike rate, the instantaneous spike rate 200 ms before collision or the number of spikes (Kruskall-Wallis ANOVA on ranks). The time of the peak for either stimulus type was also invariant (data not shown).

Fig. 11. (A) Regardless of the approach interval, the mean time of peak DCMD firing occurred earlier after repeated approaches of a 'locust' from $\pm 45^\circ$ azimuth and was relatively insensitive to repeated approaches of either object from 0° azimuth or a 'bird' from $\pm 45^\circ$ azimuth ($N=11$). For trajectories and intervals in which the time of the peak was invariant, it occurred 28 ± 27 ms (mean \pm s.d.) before collision of a 'locust' and 50 ± 40 ms before collision of a 'bird'. For clarity the s.d. is shown in only one direction. r^2 values as in Fig. 5. (B) The s.d. of time of peak firing was not affected by repeated presentations at 34 s intervals of a 'locust' approaching along either trajectory or a 'bird' approaching from 0° azimuth (left) whereas the s.d. increased during repeated approaches of a 'bird' from $\pm 45^\circ$ azimuth ($r=0.429$, $P=0.019$). (Right) For approaches at 4 s intervals the s.d. was not affected by repeated presentations of a 'locust' or 'bird' from 0° azimuth whereas it decreased upon repeated approaches of a 'locust' ($r=-0.698$, $P=0.004$) or 'bird' ($r=-0.551$, $P=0.033$) from $\pm 45^\circ$ azimuth. For approaches from $\pm 45^\circ$ (4 s intervals) the rate of decrease of the s.d. (b from the single exponential decay function) was greater during repeated approaches of a 'locust' ($b=0.390$) compared to a 'bird' ($b=0.200$).



Discussion

The data presented here show that the locust DCMDs are able to respond to repeated approaches of looming stimuli that emulate birds and other locusts. Moreover, habituated DCMDs are able to respond to approaches of a looming object along a new trajectory and to a larger object approaching along the same trajectory. Except for small objects approaching from $\pm 45^\circ$ azimuth, the time of peak firing was invariant during repeated approaches. Habituation was also unaffected by low frequency internal movements of a looming object.

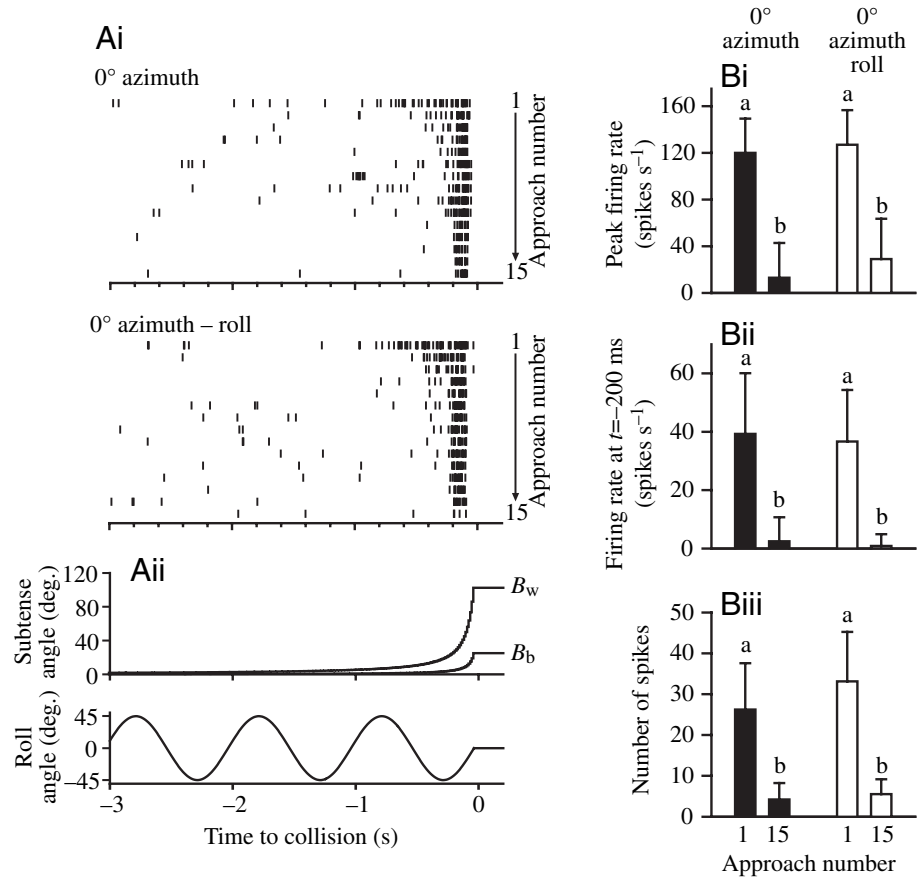
DCMD responses to complex looming objects

For objects traveling along the same trajectory, lower ($\frac{1}{2}h/|v|$) values (i.e. a simulated locust) generated shorter duration responses that began and ended later during an approach (Figs 5A, 7A, 9A). These observations are consistent with previous findings (Judge and Rind, 1997; Gabbiani et al., 1999,

2001; Gray et al., 2001; Matheson et al., 2004a; Rind and Santer, 2004) and are likely due to weak activation of the feedforward inhibition to the LGMD as the expanding edges stimulate presynaptic inputs relatively late in the approach. This may also explain why, for a constant object size, approaches from 0° azimuth produced a later response compared to approaches from $\pm 45^\circ$ (see Fig. 2B). However, the frontal region of the eye is less sensitive to motion (Rowell, 1971; Krapp and Gabbiani, 2004; Matheson et al., 2004b) and the relationship between $h/|v|$ and LGMD firing parameters is non-linear during frontal approaches (Gabbiani et al., 2001). Therefore further experiments are needed to determine how presynaptic inputs influence LGMD firing across different regions of the field of view.

Previous studies using either expanding circles or squares to stimulate the LGMD/DCMD pathway have allowed investigators to describe precise relationships between

Fig. 12. Internal object motion does not affect the initial response or habituation of DCMDs to repeated looming stimuli. (Ai) Sample raster plots of sequences of 15 approaches (4 s intervals) of a 'bird' from 0° azimuth (top rasters) or of a 'bird' from 0° azimuth with an additional roll component (bottom rasters). The subtense angle of the 'bird' wing (B_w) and 'bird' body (B_b) during an approach (Aii, top) are the same for both stimulus types. The roll angle about the 'bird's longitudinal axis during an approach is shown in (Aii, bottom). Note that the spike trains for each approach number were similar for each type of stimulus and that spike trains did not phase-lock to the roll angle. (Bi) The peak spike rate, (Bii) the spike rate 200 ms before collision and (Biii) the number of spikes of the pooled right and left DCMDs during approaches 1 and 15 were compared between the different stimulus types. There were no significant differences (Kruskal–Wallis ANOVA on ranks; see text) in any of the measured parameters compared during approach 1 or during approach 15. Data plotted are the mean \pm s.d. ($N=11$; significant differences as in Fig. 6).



stimulus parameters and neuronal responses (see Rind and Simmons, 1997; Gabbiani et al., 2002). While the stimulus parameters of an expanding circle resemble the frontal profile of a bird approaching with wings folded to the side, locusts in their natural environment would encounter many complex objects in which the edges do not expand uniformly across the retina. Although the angular threshold computation of the LGMD is invariant for expanding circles and squares up to an azimuth of 135° within the horizontal plane (Gabbiani et al., 2001), it was not known if DCMD responses are conserved for spatially complex objects or objects that produce internal motion during an approach. To compare DCMD responses to complex objects, presented here, with responses to simple objects, reported previously, I calculated θ_{thresh} and δ based on a linear measurement ($\frac{1}{2} h/|v|$) of the stimuli used (see Materials and methods). The values for θ_{thresh} and δ (see Results) are higher than reported by Gabbiani et al. (1999, 2001, 2002; their values: $\theta_{\text{thresh}}=15\text{--}40^\circ$ and $\delta=15\text{--}35$ ms). In a separate study, Matheson et al. (2004a) reported that for two populations of gregarious locusts presented with squares approaching from 90° azimuth, $\theta_{\text{thresh}}=13\text{--}34^\circ$ and $\delta=12\text{--}88$ ms. The threshold angle is also larger here (see Results), whereas the delay is within the range of the latter study. Two main factors could contribute to the different values presented here and those reported previously. First, θ_{thresh} is influenced by the approach trajectory within the horizontal plane. Gabbiani et al. (2001) showed that the two-dimensional linear model relating $h/|v|$ and

the time of the peak spike rate does not explain approaches from 0° and 45° azimuth. Their data show that for a square approaching from 0° or 45° azimuth $\theta_{\text{thresh}}=180^\circ$ or 90° , respectively (compared to 122.7° and 38.6° , respectively, reported here). Secondly, textured objects, containing multiple (>4) expanding edges, do not fit the linear model (Gabbiani et al., 2001). Therefore, it is reasonable to expect that the relationship between stimulation parameters of a looming object and DCMD responses would vary depending on object trajectory and complexity. Although not within the scope of this study, it would be interesting to explore how the LGMD responds to multiple complex objects approaching from various trajectories at varying time intervals within the entire field of view.

Rind and Simmons (1997) have suggested that DCMD responses to looming stimuli decline dramatically when a digitized object expands more than 3° in a given step (however, see Gabbiani et al., 1999). For the objects described here, this jump occurs at distinct times before collision: 'locust' body = 37.5 ms, 'locust' wings = 62.5 ms, 'bird' body = 50 ms, 'bird' wings = 125 ms. The lag time from visual stimulation to the occurrence of a DCMD spike in the cervical connective is approximately 40 ms (see Matheson et al., 2004a). Assuming a DCMD conduction velocity of 3.3 m s^{-1} at 25°C (see Money et al., 2005), it would take approximately 2 ms for a spike to travel from the midpoint of the cervical connective to the mesothoracic ganglion (6–7 mm). Therefore, with electrodes

in the mesothoracic ganglion (see Materials and methods), a rapid decrease of the DCMD spike rate would be detected approximately 42 ms after an angular jump of 3° . Using the longest axis of the simulated objects as an indication of when the angular subtense first jumps by 3° (i.e. using the dimension of the 'locust' or 'bird' wings), then an effect on DCMD firing, i.e. an artefactually induced peak, should occur, invariably, at -20.5 ms ('locust') and -85 ms ('bird'). To compare these times to DCMD activity I pooled the time of peak firing during the first approach (to avoid putative habituation) of each object from each trajectory. The peak times for these stimulus parameters are: 'locust' from 0° azimuth, -58.5 ± 36 ms (range, -184 – 14.4 ms), 'locust' from $\pm 45^\circ$ azimuth, 21.3 ± 111 ms (range, -233.0 – 261.9 ms), 'bird' from 0° azimuth, -77.9 ± 60 ms (range, -298.5 – 23.7 ms), 'bird' from $\pm 45^\circ$ azimuth, -80.5 ± 47 ms (range, -224 – 14.4 ms). While these ranges bracket times at which the 'locust' or 'bird' wings jump by 3° there is a high level of variability, suggesting that the peak times were not produced by this single stimulus parameter (see also Gabbiani et al., 1999).

Discrepancies in the time of peak DCMD firing relative to collision have received much attention in recent literature and are thought to be due to differences in target size between different preparations. The data presented here suggest that object complexity is also involved in defining the response. In non-habituated locusts the mean time of peak firing occurred before the end of the approach of the simulated bird, which was designed to emulate the size and dimensions of small birds known to catch flying locusts on the wing (see Materials and methods). Rind and Santer (2004) used pectoral diameters of avian locust predators to suggest the DCMDs are tuned to detect objects with diameters in the range of 50–90 mm, which would result in peak firing occurring after the end of a loom. These birds, however, may also approach flying locusts while gliding with outstretched wings (Fry and Fry, 1992), which, for a given approach velocity, would produce a larger $l/|v|$ ratio and an earlier time of peak DCMD firing, as reported here (see also Gabbiani et al., 1999). Further investigation is required to identify specific effects of looming object complexity on DCMD firing responses.

Matheson et al. (2004a) showed that in gregarious locusts the time of peak DCMD firing was invariant during repeated approaches of a dark square ($l/|v|=20$ ms) from the equivalent of 90° azimuth. In agreement with their findings, I show that the mean and s.d. of time of peak firing is stable during repeated approaches of a 'bird' ($\frac{1}{2}h/|v|=47.4$ ms), regardless of the approach trajectory or interval (Fig. 11). However, the peak time occurred earlier after repeated approaches of a 'locust' ($\frac{1}{2}h/|v|=11.8$ ms) approaching from $\pm 45^\circ$ azimuth. It is difficult to attribute this apparent discrepancy to a single factor since Matheson et al. (2004a) did not describe, explicitly, stability of the time of the peak during habituation for each value of $l/|v|$ that they used. It would be interesting to compare the stability of the time of peak firing during habituation to their smallest sized objects ($l/|v|=10$ ms, assuming their sample approach speed of 2 m s^{-1}) with the

earlier peak times described above. Although earlier peak times after repeated approaches of a small object has been observed independently (F. C. Rind, personal communication), it is not clear if it would be of any biological significance. This effect occurs in conjunction with a decrease in the peak firing rate and the number of spikes (Figs 5, 7, 9), which makes it difficult to interpret the effects of habituation on this single parameter. Moreover, the time of peak firing typically occurs after the initiation of escape responses (see Materials and methods).

Mechanisms of habituation

Increased responses of habituated DCMDs to objects approaching along a new trajectory (Fig. 8) or larger objects approaching along the same trajectory (Fig. 10) are consistent with a presynaptic mechanism of habituation between chiasmatic visual afferents and the dendritic fan of the LGMD (O'Shea and Rowell, 1976; Rowell et al., 1977). Objects approaching from a new trajectory would stimulate a different, nonhabituated, array of ommatidia and thus provide input to the LGMD through a different series of visual afferents. Similarly, the edges of a larger object approaching along the same trajectory would expand beyond the subtense angle of the original, smaller, object and thus stimulate nonhabituated local input elements to the LGMD. However, the edges of a new, smaller object would expand within the visual field of a habituated array of input elements, resulting in a continued 'habituated' DCMD response (see Fig. 10). In agreement with previous findings (Rowell, 1971; Bacon et al., 1995), a new trajectory or object did not dishabituate DCMD responses. This is also consistent with a model of local synaptic habituation because a new, larger object or the same object approaching from a new trajectory would not influence a separate set of habituated synaptic inputs to the LGMD. In this context, responses to internal object motion, such as the beating wings of the simulated locust or the roll movements of the simulated bird (Fig. 12), are not surprising since the same regions of the retina would be stimulated often enough to induce habituation to these specific motions.

Implications for avoidance responses

The ability of habituated DCMDs to respond to the same object on a new trajectory could be advantageous for gregarious locusts. While there is no information on specific patterns of visual stimulation experienced by individual locusts in a swarm, there would be a complex combination of translating, receding and looming stimuli produced by self motion (i.e. whole-field optic flow, especially during flight) and object motion (e.g. conspecifics and predators). On the ground or in the air, an individual within a swarm would be surrounded by many conspecifics that would stimulate various regions of the field of view. In the air, those that fly along non-colliding trajectories would contribute to a background motion that is known to influence LGMD responses to looming stimuli (Gabbiani et al., 2002). The lateral inhibition network among input elements to the LGMD prevents saturation and fatigue of

individual small-field elements (Rowell et al., 1977), and optic flow sharpens the peak of LGMD firing during looming by activating feed-forward inhibition pathways (Gabbiani et al., 2002). The limitations of the hardware used here for stimulus presentation precluded the use of a visual flow field during approach. Therefore, putative effects on DCMD habituation to the stimuli used here are not known. However, the results are consistent with the mechanisms of habituation described above.

Although the time of peak DCMD firing occurs after the initiation of escape responses, earlier activity could be important for modulating avoidance behaviours (Burrows, 1996; Gray et al., 2001; Matheson et al., 2004a). Nonhabituated DCMD responses 200 ms before collision are lower during the approach of a 'locust' than during the approach of a 'bird' (Fig. 5, 7, 9), which is consistent with previous findings (Matheson et al., 2004a) and may be important in distinguishing a potential predator from a conspecific. It should be noted, however, that in the presence of a constant velocity visual flow field, the LGMD did not fire 200 ms before collision of a looming object (Gabbiani et al., 2002).

The data presented here suggest that complex object shapes and differing collision trajectories influence DCMD response parameters that define responses to approaching objects (i.e. θ_{thresh} and δ). This effect is likely due to non-linear integration of local inputs to LGMD as images of looming objects expand differentially across the eye. The results also demonstrate that the LGMD/DCMD pathway encodes looming approaches irrespective of internal object motion, suggesting that fine movements may not be involved in activating the collision detection circuitry. Responses of habituated DCMDs to novel looming stimuli imply that the LGMD/DCMD pathway should remain sensitive to multiple objects approaching along different trajectories, which would occur in a swarm. Testing this assumption requires a better understanding of a locust's visual input structure during swarming. Flying locusts generate self-motion across the eye resulting from forward motion and steering manoeuvres, making it necessary to incorporate complex visual flow patterns into an experimental paradigm. Current studies are underway to describe habituation of behavioural and kinematic responses of loosely tethered locusts presented with the same stimuli used here, which will allow us to emulate closed-loop visual stimuli while recording DCMD activity during looming approaches. Stimulating the locust visual system with complex objects and flow fields, in conjunction with multichannel recording techniques, will make it possible to better understand how a motion sensitive neuron, or a putative population of descending visual neurons, function in a complex visual environment.

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