Role of an Identified Looming-Sensitive Neuron in Triggering a Flying Locust’s Escape

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Santer, Roger D., F. Claire Rind, Richard Stafford, and Peter J. Simmons. Role of an identified looming-sensitive neuron in triggering a flying locust’s escape. J Neurophysiol 95: 3391–3400, 2006. First published February 1, 2006; doi:10.1152/jn.00024.2006. Flying locusts perform a characteristic gliding dive in response to predator-sized stimuli looming from one side. These visual looming stimuli trigger trains of spikes in the descending contralateral movement detector (DCMD) neuron that increase in frequency as the stimulus gets nearer. Here we provide evidence that high-frequency (>150 Hz) DCMD spikes are involved in triggering the glide: the DCMD is the only excitatory input to a key gliding motor neuron during a loom; DCMD-mediated EPSPs only summate significantly in this motor neuron when they occur at >150 Hz; when a looming stimulus ceases approach prematurely, high-frequency DCMD spikes are removed from its response and the occurrence of gliding is reduced; and an axon important for glide triggering descends in the nerve cord contralateral to the eye detecting a looming stimulus, as the DCMD does. DCMD recordings from tethered flying locusts showed that glides follow high-frequency spikes in a DCMD, but analyses could not identify a feature of the DCMD response alone that was reliably associated with glides in all trials. This was because, for a glide to be triggered, the high-frequency spikes must be timed appropriately within the wingbeat cycle to coincide with wing elevation. We interpret this as flight-gating of the DCMD response resulting from rhythmic modulation of the flight motor neuron’s membrane potential during flight. This means that the locust’s escape behavior can vary in response to the same looming stimulus, meaning that a predator cannot exploit predictability in the locust’s collision avoidance behavior.

INTRODUCTION

When predators attack, prey animals must escape quickly. Often, the startle behaviors they use are triggered by large, identified single neurons or small groups of them (Edwards et al. 1999; Korn and Faber 2005; Levi and Camhi 2000; Nolen and Hoy 1984). The relative simplicity of these networks reflects the need for a quick escape.

Small avian insectivores are common locust predators—carmine bee-eaters, for example, specialize in capturing locusts in flight (Fry and Fry 1992). When such birds attack a flying locust, they appear to it as looming stimuli: their silhouettes expand across its retina (Wheatstone 1852). Looming stimuli particularly excite the lobula giant movement detectors (LGMDs) of locusts, an identified bilateral pair of visual neurons that respond to looming stimuli with trains of spikes that increase in frequency as objects approach (Gabbiani et al. 2002; Hatsopoulos et al. 1995; Rind and Simmons 1992; Schlotterer 1977). The spikes of each LGMD are transmitted faithfully to a second identified neuron, the descending contralateral movement detector (DCMD), which transmits these spikes to the thoracic motor centers (Burrows and Rowell 1973; Killmann and Schürmann 1985; Rind 1984; Simmons 1980).

In response to looming stimuli of predator-like speeds and sizes (Rind and Santer 2004; Santer et al. 2005), flying locusts briefly interrupt flight with a raised-wing gliding behavior (Robertson and Reye 1992; Santer et al. 2005). These glides are interpreted as an escape response because they are similar to the dives used by many insect species to evade bats (e.g., Dawson et al. 2004; Hoy et al. 1989; Roeder 1962). A burst of spikes in the second tergosternal flight motor neuron (MN84, an elevator), raises the locust’s wings into the gliding posture. DCMD spikes cause short-latency excitatory postsynaptic potentials (EPSPs) in this motor neuron that are larger than those mediated by the DCMD in other flight motor neurons (Simmons 1980). Glides are triggered by stimuli that optimally excite the DCMD, during the most vigorous part of its response (Santer et al. 2005).

Identified neurons trigger animals’ startle behaviors in many ways. Occasionally, single spikes trigger complete behaviors, such as Mauthner neuron–triggered C-starts of teleosts (Korn and Faber 2005) and giant interneuron–triggered tail flips of crayfish (Edwards et al. 1999). More usually, spike trains in sets of neurons are integrated to trigger and steer escape, e.g., wind-sensitive giant interneurons triggering cockroach escape running (Levi and Camhi 2000). Often an emergency behavior must occur during another, ongoing behavior, such as flight, and in these cases the trigger signal must be integrated with the ongoing behavior (e.g., corrective steering by flying locusts; Reichert and Rowell 1986).

In this study we investigate the role of the DCMD in triggering escape glides. We find that the DCMD is the sole looming-excited input to MN84 and that high-frequency DCMD spikes >150 Hz cause EPSPs that sum strongly in MN84. Modified looming stimuli that cut short the high-frequency spikes of a DCMD reduce glide occurrence. However, high-frequency DCMD spikes alone cannot trigger a glide—these must occur during the appropriate phase of the wingbeat cycle to be gated into the flight rhythm.

METHODS

Experiments were performed on 40 adult male and female Locusta migratoria L. from a crowded colony maintained at the University of...
Newcastle upon Tyne, or from an outside supplier (Blades Biological, Edenbridge, UK).

Visual stimuli

Locusts were challenged with looming visual stimuli displayed on a Kikusui COS1611 X-Y monitor controlled by a Cambridge Research Systems (Cambridge, UK) VSG2/3 image synthesizer and RG2 raster-generator system. The monitor screen was aligned with the center of one of a locust’s compound eyes, 70 mm from it and parallel with the locust’s long axis. Stimuli were computer-generated 80-mm-diameter black discs that loomed straight toward the eye at a constant speed. The discs loomed over a simulated distance of 2 m and a full loom ended when the stimulus was displayed at the monitor screen, 70 mm from the locust’s eye and subtending 60° at the eye. The delay between the end of image movement and collision with the simulated disc would have been 70, 35, 23.3, and 14 ms at 1, 2, 3, and 5 m/s, respectively.

Intracellular recordings

We made intracellular recordings from the right mesothoracic second tergosternal motor neuron (MN84, nomenclature here and throughout after Snodgrass 1929) and DCMD simultaneously to study excitation reaching the motor neuron during a loom. Intracellular recordings were made from processes of the flight motor neuron in the dorsal neuropil of the mesothoracic ganglion and from the cell body of the DCMD in the protocerebrum. In preparation, a locust was secured upright on a block of modeling clay. The thoracic nervous system was exposed and intracellular recordings were made as described in Simmons (1980). The protocerebrum was exposed as described in Rind (1984). The mesothoracic and metathoracic ganglia were stabilized on a platform manipulated through the thoracic–abdominal connective nerves, and the brain on a platform manipulated from the front. Electrodes (DC resistance 40–60 MΩ, filled with 2 M potassium acetate) were attached to amplifiers with bridge circuits that allowed current to be injected through the recording electrodes. MN84 was identified by correlating single spikes in it, heard over an audio monitor, with twitches of the posterior part of the mesothoracic tergosternal muscle (M84), viewed under the dissecting microscope.

Extracellular recordings from restrained locusts

We made DCMD recordings from minimally dissected locusts to study the effects of manipulations of the looming stimulus on their DCMD responses. A locust was mounted ventral side up and its head was tilted slightly forward to expose the ventral sclerite of the neck. A small hole was made in the right side of the sclerite, and a 50-μm copper wire, insulated but for its tip, was inserted about 1.5 mm from the locust’s eye and subtending 60° at the eye. The delay between the end of image movement and collision with the simulated disc would have been 70, 35, 23.3, and 14 ms at 1, 2, 3, and 5 m/s, respectively.

Behavioral and extracellular recordings from flying locusts

We recorded behavioral responses of tethered flying locusts to looming stimuli. A locust was tethered to a brass rod by its pronotum and placed in front of a laminarized 3 m/s wind source that evokes typical, strong flight behavior at normal wingbeat frequency and posture (Santer et al. 2005). A MotionScope PCI high-speed digital camera (Redlake, San Diego, CA) recorded movements at 125 frames/s or, in one experiment, wing movements were monitored using an infrared beam that was broken by the beating wing (as in Santer et al. 2005). In some experiments, we cut one or the other of a locust’s ventral nerve cords to abolish DCMD input on that side. To cut a ventral nerve cord, a window was cut in the ventral cuticle of the mesothorax and the nerve cord was sectioned between the pro- and mesothoracic ganglia. The thorax was then resealed with wax and the locust’s flight behavior recorded during the approach of simulated objects from the left and right (15 approaches from each side). In other experiments, DCMD spikes and flight muscle activity were recorded during tethered flight. DCMD spikes were recorded as in Santer et al. (2005) using an electrode consisting of two 150-μm silver wire hooks implanted through a small window cut in the ventral cuticle of the mesothorax so that the hooks encircled the right pro-mesothoracic ventral nerve cord. Electromyograms (EMGs) were made from flight muscles using pairs of 50-μm copper wires insulated but for their tips. In all cases a 150-s intertrial interval was used, except in DCMD recordings from flying locusts where a shorter interval was sometimes used. In these experiments no habituation was evident in the DCMD.

Data capture and analysis

Electrophysiological data were recorded using Spike2 v.5 (Cambridge Electronic Design, Cambridge, UK). In tethered flying locusts DCMD spikes were obscured by flight muscle activity, which was removed using a high-pass filter with a low cutoff point determined from power spectra of typical DCMD recordings (Santer et al. 2005). Recordings in which DCMD spikes were not clear and the largest in the filtered data were discarded.

Statistical analyses were carried out using either SPSS v.11 (SPSS, Chicago, IL) or Minitab v.13.1 (Minitab, State College, PA). To analyze gliding occurrence in response to prematurely ending looming stimuli, a one-way ANOVA of glide occurrence with time removed from end of loaf (a fixed factor) was carried out using SPSS. A post hoc Dunnett t-test was used to compare glide occurrence to prematurely ending stimuli with that to the full looming stimulus. To analyze differences in the DCMD responses of locusts during gliding and nongliding trials, spike trains were compared in Minitab using a three-factor repeated-measures ANOVA of “individual locust” (the repeated measure), “behavior” (glide or nonglide), and “time bin nested within gliding and nongliding behaviors.” We then compared differences in mean DCMD spike frequencies between gliding and nongliding trials for each locust at each 10-ms time bin, using post hoc Student–Newman–Keuls (SNK) tests. To analyze the timing of high-frequency DCMD spikes within the wingbeat cycles of flying locusts, we plotted instantaneous DCMD spike frequencies for each recorded trial using wingbeat phase, rather than time, as the x-axis. We used forewing depression before a glide, or at an equivalent time where no glide occurred, to synchronize all trials. We then arranged these plots into groups according to the behavior of the locust in that trial, and for each group we plotted the instantaneous DCMD spike frequency by wingbeat phase data on the same axes. This gave us plots of instantaneous DCMD spike frequency against wingbeat phase for each behavior group. We analyzed these by using a MATLAB (The MathWorks, Natick, MA) script to overlay a 30 x 30 grid (with 0.067 x 0.33.6 on August 28, 2017 http://jn.physiology.org/ Downloaded from...
motor neurons (Simmons 1980), we focused our investigation on the role of this pathway in the forewing movements integral to the gliding behavior.

**DCMD input to MN84 during a loom**

Our first step was to assess the role of DCMD excitation of MN84 in the gliding behavior. Although MN84 is known to be excited by the DCMD (Simmons 1980), the way that this motor neuron responds to visual stimuli has not been described. We wanted to discover whether MN84 was excited by the approach of a looming stimulus and whether the DCMD neuron was the sole source of this excitation. To do this, we made simultaneous intracellular recordings from MN84 and both intra- and extracellular recordings from the DCMD in restrained locusts. By injecting steady depolarizing currents into the DCMD, we evoked spikes that mediated EPSPs one for one in MN84 (Fig. 1A). The maximum frequency of spikes evoked by current injection into the DCMD cell body was 85 Hz, and at this frequency there was little or no summation of the EPSPs, which had a duration of about 10 ms, in MN84 (Fig. 1A, inset). In response to looming stimuli, the EPSPs mediated by a DCMD summed in a frequency-dependent manner for instantaneous frequencies ≥100 Hz (Fig. 1B). Sometimes, in response to looming stimuli, the EPSPs summed sufficiently to trigger a spike (Fig. 1C).

To assess whether DCMD spikes were the only excitatory input to MN84 during a looming stimulus, we injected hyperpolarizing current into a DCMD cell body, which caused DCMD excitation to occur in bursts rather than following its normal pattern in response to a looming stimulus (we could not completely prevent spikes in the DCMD in response to the looming stimulus by injecting hyperpolarizing currents into its cell body). A 2 m/s looming stimulus induced a steady increase in DCMD spike frequency when no current was injected (Fig. 1D, top trace); however, the same stimulus induced three distinct bursts of DCMD spikes when −20 nA was injected into the DCMD (Fig. 1D, second trace and main part of the figure). With hyperpolarizing current injected, the pattern of EPSP summation in MN84 closely followed the altered pattern of spikes in the DCMD (Fig. 1D), indicating very strongly that the DCMD is the only source of visually generated excitation to MN84 in response to a looming stimulus.

**Manipulation of the DCMD response and effects on behavior**

Next, we wanted to investigate whether a burst of high-frequency DCMD spikes, which would cause strong summation EPSPs in MN84, was necessary for the production of a gliding behavior in a flying locust. If this were the case, abolishing or altering this element of the DCMD response should alter the occurrence of gliding behavior. Because the dissection necessary to expose the dorsally located DCMD axon and then kill it by intracellular injection would have prevented flight, we used two less direct methods to interfere with the normal DCMD response.

In our first manipulation, we altered the response of the DCMD by showing locusts looming stimuli that were modified to stop moving earlier than usual. In response to a complete 5
m/s loom, the DCMD of an aroused, restrained locust follows the expansion of the object and reaches high spike frequencies (>150 Hz) at the approximate time that the stimulus ceases moving (Fig. 2A). By incrementally reducing this stimulus, so that instead of ceasing movement at the monitor screen 70 mm from the locust’s eye it stopped moving 10 or 20 ms earlier, we could remove the final high-frequency spikes from the DCMD response without altering the early part of its response before the end of stimulus movement (Fig. 2B and C). Because our previous results showed the DCMD to be the only looming-excited input to MN84, this manipulation specifically affected this pathway. When we challenged undissected, tethered flying locusts with full and incrementally reduced 5 m/s looming stimuli, we found that gliding behavior was elicited in nearly 70% of trials in response to the full loom and to looms reduced by 5 and 10 ms (Fig. 2D). However, when the final 15 ms or more were omitted, the frequency of occurrence of gliding behavior was significantly reduced (Fig. 2D). Omitting the final 15 ms of a looming stimulus can remove three or more DCMD spikes at instantaneous frequencies >200 Hz (Fig. 2C).

In our second manipulation, we prevented spikes from one of the DCMDs from reaching the thoracic ganglia by sectioning one of the locust’s ventral nerve cords. Under these conditions, locusts flew with reduced wingbeat frequencies (about 14 Hz), and lower than usual wingbeat amplitudes and flight durations. However, with looming stimuli delivered ipsilaterally to their cut nerve cord, locusts still produced gliding behaviors in nearly 55% of trials (Fig. 2E), which is slightly less frequently than that in undissected locusts. When looming stimuli were delivered contralaterally to the cut nerve cord, the frequency of occurrence of gliding behavior was significantly reduced to around 15% of trials (t-test; df = 6, t = 11.104, P < 0.001; Fig. 2E). These data implicate a contralaterally descending looming-sensitive neuron, such as the DCMD, in the production of the gliding response. They also indicate that the behavior only occurs occasionally in the absence of this input.

**Correlating high-frequency spikes and behavior**

So far, our data suggest an important role for high-frequency (>150 Hz) DCMD spikes in the production of gliding behavior. If these spikes act as a trigger for gliding, their occurrence should differ between spike trains recorded during trials with glides and spike trains recorded during trials where no glide

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**FIG. 2.** Manipulation of the DCMD response alters the occurrence of looming-elicited gliding behavior. A: a full 5 m/s looming stimulus simulating an approach of an 80-mm-diameter disc over 2 m and ending at the stimulus monitor screen, 70 mm from the locust’s eye (subtense shown in bottom left), elicited a normal DCMD response in restrained locusts. B and C: when successive 10-ms intervals were removed from the loom, the DCMD’s final high-frequency spike burst was cut short. Example stimulus subtenses for incrementally reduced looming stimuli are plotted in the bottom left and the time that the stimulus stopped moving is indicated by a triangle superimposed on the x-axis of plots A–C. D: when presented to tethered flying locusts, full 5 m/s looms and looms cut short by 5 or 10 ms resulted in similar occurrences of gliding behavior (as recorded by an infrared wingbeat sensor; see METHODS). When looms were cut short by > 15 ms, the occurrence of gliding behavior was significantly reduced (**, behavioral occurrences that were significantly different (P < 0.001) from those elicited by the full looming stimulus as determined by one-way ANOVA (showing a significant effect of premature loom ending on glide occurrence; F_{12,117} = 21.35, P < 0.001) and post hoc Dunnett t-test). Labels A, B, and C indicate stimuli used in the production of A–C. E: occurrence of gliding behavior was reduced when stimuli were delivered to the eye contralateral to a cut nerve cord (with only ipsilaterally descending inputs to the thorax intact), but unchanged when stimuli were delivered ipsilaterally to it (with only contralaterally descending inputs intact). A–D: 6 stimulus presentations to each of 10 locusts. E: 15 ipsilateral and 15 contralateral looms displayed to each of 4 locusts (2 sectioned each side).
occurred. In response to the repeated presentation of identical 5 m/s looming stimuli, glides do not always occur (Fig. 2D; in response to a full loom, glides occur in about 70% of presentations). If the DCMD triggers glides, then differences in DCMD responses between trials should reflect whether a glide occurs in a particular trial. Using implanted hook electrodes and simultaneous high-speed video recordings, we were able to obtain clear DCMD and strong flight data from four locusts (from a total of 10 experiments) responding to a total of 104 presentations of a 5 m/s looming stimulus. The low sample number was partly attributable to the low success rates of the experiment and the need to analyze in fine detail the behavior and neuronal data from a low number of samples. As previously noted, gliding behaviors occurred less often when locusts had electrodes implanted (Santer et al. 2005). Example DCMD and flight recordings from a tethered flying locust are shown in Fig. 3A.

We divided the DCMD responses of the flying locusts into two groups based on whether a gliding behavior was observed. We defined a glide as a pause in flight >1.25 × the mean wingbeat duration of the preceding 10 wingbeat cycles (as in Santer et al. 2005). If gliding behavior was triggered solely by the DCMD neuron, we hypothesized that DCMD responses should differ between trials where glides were performed and trials where glides were not performed (Fig. 3B). To test for differences in the DCMD spike frequencies of gliding and nongliding trials, we focused on a 100-ms period between 60 ms before and 40 ms after the end of stimulus movement (from 74 ms before collision until 26 ms after collision) because this was the period in which high-frequency DCMD spikes that could cause summing EPSPs in MN84 occurred. This is also the period during which glides are triggered (Santer et al. 2005). The mean frequency of DCMD spikes, averaged over all locusts and all time bins, was slightly higher during gliding than during nongliding trials over the analysis period (242 vs. 226 Hz, respectively). To test for any significant differences in spike frequency between gliding and nongliding trials, and to account for potential interactions between the different behaviors shown during trials (gliding and nongliding), the different locusts and the different time bins, a three-factor repeated-measures ANOVA was performed. “Time bin” was nested within “behavior” (this was done to allow variation between the time bins to be taken into account because we expect the DCMD responses to change over time as a result of the looming stimulus, and still test for differences between DCMD responses in gliding and nongliding trials in spite of this variation). “Locust” was designated a repeated measure because both gliding and nongliding behaviors occurred in trials

**FIG. 3.** DCMD responses in flying locusts differ when they respond to a looming stimulus with a glide and when they produce no apparent response. A: an extracellular recording of DCMD activity in a tethered flying locust responding to a 5 m/s looming stimulus (subtense shown in C). Forewing movements are shown in the top trace (forewing tip position, at maximum elevation and depression only), measured from a recording at 125 frames/s. Locust performs a glide during the most vigorous period of the DCMD response. Bottom trace: DCMD activity. B: mean DCMD responses to 5 m/s looming stimuli (10-ms bins) during trials where locusts did and did not glide. C: looming stimulus subtense at the locusts’ eyes. D: number of consecutive >200-Hz DCMD spikes from spike trains recorded during glides and nonglides. Boxes show the 25th and 75th percentiles and bisecting lines indicate the median value. Whiskers indicate the 5th and 95th percentiles and outliers are plotted. A: a single recording from a tethered flying locust. B and D: 104 stimulus presentations to 4 flying locusts divided into 50 glides and 54 no responses using the gliding criterion of Santer et al. (2005) (see RESULTS). In all panels, stimulus is a simulated 80-mm-diameter disc looming over 2 m at 5 m/s.
indicate that triggering of the gliding behavior is a stochastic process or that the criterion DCMD response for triggering the complete behavior is more complex than a combination of spike frequency and number.

Flight-gating of the DCMD response

A likely reason that a high DCMD spike rate does not always trigger a glide is that, during flight, the membrane potential of flight motor neurons is modulated in a cyclical fashion by inputs from the flight central pattern generator (Hedwig and Becher 1998; Robertson and Pearson 1982, 1985). As a result, the effects of EPSPs from a DCMD will depend on the wingbeat phase in which they arrive (Reichert and Rowell 1985, 1986; Reichert et al. 1985). Consequently, a glide could be gated appropriately into flight, preventing elevator activity during wing depression and thus potential musculoskeletal damage.

Glides usually follow a normal and complete wingbeat cycle and, before a glide, a locust’s wingbeat frequency declines during the approach of the looming stimulus (Santer et al. 2005). We analyzed the forewing movements of gliding locusts and synchronized them using the time of forewing elevation into the glide as a reference (Fig. 4). In all trials where a glide occurred, it followed a complete, although very occasionally reduced amplitude, wingbeat (Fig. 4A); wingbeat adjustments were evident during the approach of a looming stimulus (Fig. 4B). These data suggest that DCMD input must be gated into an appropriate phase of the wingbeat cycle for gliding to be triggered because glides can only occur at one point in the wingbeat cycle. This may also explain why the timing of differences in the DCMD responses from gliding and nongliding trials differed between locusts.

To investigate whether the DCMD response is flight-gated, we examined further the flight data of the four locusts used in the previous analysis. If high-frequency DCMD spikes were gated into the flight rhythm, we expected to find their distribution—within the wingbeat cycle and relative to normal MN84 activity—to be different between trials where glides were performed and trials where glides were not performed.

We plotted DCMD responses relative to wingbeat phase for all trials in which glides occurred and for all trials in which no glides were performed and trials where glides were not performed.

![Figure 4](https://example.com/fig4.png)

**FIG. 4.** Gliding behaviors occur at a set point in the flight rhythm. A: forewing elevation (marked only during maximum wing elevation and depression) during the approach of a looming stimulus for trials in which a locust performed glides. Trials are aligned so that wing depression before the glide is at $t = 0$. Glides always followed a complete and apparently normal wingbeat cycle. B: wingbeat frequency declines during the approach of a looming stimulus. Plot shows mean instantaneous wingbeat frequency for the 6 wingbeats preceding a glide for the trials plotted in A.
glides occurred. One wingbeat phase unit described the period from one wingbeat depression to the next, and phase 0 was the phase at which the forewing was last fully depressed before a glide, or the equivalent relative to stimulus movement for trials in which there was no glide (in these trials the forewing was often elevated more than usual at the time a glide would normally occur). For each trial we plotted the instantaneous DCMD spike frequencies for each spike in a response against wingbeat phase (Fig. 5A, left). For each behavior category (glides and no glides), we then combined data from all trials into a plot of instantaneous DCMD spike frequencies against wingbeat phase. This plot included a total of 1,343 individual DCMD spikes recorded during nongliding trials and 1,518 recorded during gliding trials over the two wingbeat analysis period. As described in METHODS, we overlaid each plot with a 30 × 30-sector grid (indicated schematically in Fig. 5A). Within each grid box, we counted the number of spike occurrences. These numbers were divided by the number of trials constituting the group and transferred into shading density on contour plots of DCMD instantaneous spike frequency against wingbeat phase (Fig. 5, C and D). The most densely shaded area on each of these plots (indicating the most commonly occurring spike frequencies and timings) occurred at a spike frequency >200 Hz, but at a wingbeat phase that differed between behaviors. In trials that ended with glides (Fig. 5C), the area with densest shading occurred during and after phase 0, when the wing was elevating into a glide and an extended burst of spikes in MN84 was occurring (Fig. 5, B and C). In trials where a glide was not performed, high-frequency DCMD spikes still occurred but were distributed during the downstroke, before normal MN84 spikes and rarely extending past phase 0 (Fig. 5, B and D). Therefore the timing of the DCMD’s high-frequency spikes, relative to the wingbeat cycle and normal MN84 activity was a good predictor of gliding behavior occurrence. The necessity for these spikes to occur at (and past) the time that muscle 84 is normally active indicates that they must be gated into the flight rhythm by modulations in MN84 membrane potential resulting from flight central pattern generator activity.

DISCUSSION

We have investigated the role of the DCMD neuron in looming-elicited emergency gliding behavior of flying locusts, concentrating on the DCMD–MN84 pathway and forewing movements. We found that DCMD spikes are the only source of visually generated excitation to MN84 during a loom, and that EPSPs resulting from these spikes sum strongly—because of their duration—only when the DCMD spikes at >150 Hz. When these high-frequency spikes are removed from the DCMD response, the frequency of occurrence of gliding is reduced. Although the mean DCMD spike frequencies across a 100-ms period at the end of a loom are higher when locusts glide than when they do not, the times at which significant differences occur between gliding and nongliding spike trains, relative to the loom, are variable between locusts. The point in the wingbeat cycle where these high-frequency DCMD spikes occur is crucial to their effectiveness, probably arising from the large rhythmic input to MN84 during normal flight (Robertson and Pearson 1982, 1985). Thus we propose that high-frequency

FIG. 5. Effect of DCMD spikes on M84 activity depends on the wingbeat phase at which they occur. A: schematic illustration of data preparation. DCMD spike trains of gliding and nongliding locusts were compared by plotting the instantaneous frequencies of each DCMD spike during a trial against wingbeat phase (1 unit = 1 complete wingbeat cycle). Left: trials were then plotted together on the same axes for each behavior group (gliding and nongliding). Right: grid was overlayed over the plots and spike occurrence within each wingbeat phase/frequency bin was counted. In analysis, we used a 30 × 30 grid rather than the 3 × 3 illustrated here. Spike occurrence was standardized between the 2 groups by dividing spike counts per bin by number of trials. B: during a normal wingbeat cycle, elevator muscles 83 and 84 contract just before wing elevation. During a glide, muscle 84 continues to contract for a sustained period, holding the forewing elevated. C: during glides, high-frequency DCMD spikes >200 Hz occurred at the same time as the muscle 84 burst that defines a glide and are thus able to sum with normal rhythmic excitation of MN84 by the flight central pattern generator. D: when locusts did not glide, high-frequency DCMD spikes occurred before the elevator muscle burst and not during wing elevation (out of phase with normal MN84 activity). Data are as plotted in Fig. 3.
DCMD spikes, gated into the flight rhythm, trigger MN84 to raise a locust’s forewings into the gliding posture.

**High-frequency DCMD spikes and glide triggering**

Looming-elicited glides are characterized by a burst of potentials in elevator M84 that cause it to contract and raise the locust’s forewings into its gliding posture (Santer et al. 2005). The DCMD is the only source of excitation to MN84 during a loom and high-frequency DCMD spikes are sufficient to cause MN84 to spike in a restrained locust. In tethered flying locusts, shortening a 5 m/s loom by 15 ms caused a reduction in glide occurrence from about 70 to 30% of trials. This procedure selectively removes the final high-frequency spikes from the DCMD response. Also, the DCMD spike trains of the same flying locusts contained higher spike frequencies at variable times during the terminal period of a loom in trials where they performed a glide than in trials where they did not, providing further evidence for the need for a burst of high-frequency DCMD spikes during flight for a glide to occur.

The requirement for several high-frequency DCMD spikes contrasts with some well-characterized escape behaviors that are triggered by single spikes in identified neurons (Edwards et al. 1999; Korn and Faber 2005). It is similar to the cat-cry evasion behavior of field crickets that is triggered by sustained high-frequency spikes in an identified auditory interneuron (Nolen and Hoy 1984). Because high-frequency DCMD spikes occur late in its response to a looming stimulus, the requirement for them may reflect the need to trigger a glide when a predator is at close quarters, permitting the glide to be used as a last-chance escape behavior. Similar last-chance evasive behaviors are used by many nocturnally flying insects in response to bat cries with high pulse repetition rates, indicating that the attacking bat is close (Triblehorn and Yager 2005).

Sectioning all descending inputs to the thorax on the side contralateral to a looming stimulus greatly reduced the occurrence of gliding behavior, whereas sectioning ipsilateral inputs did not. This evidence strongly implicates a contralaterally descending looming detector and is consistent with our proposal that the DCMD triggers a glide. However, that glides could still occasionally be performed when contralaterally descending neurons were sectioned indicates that either these glides were chance occurrences, because flight in these animals was weaker and more erratic than normal, or that other pathways, perhaps involving the DIMD—an ipsilaterally descending movement detector (Burrows and Rowell 1973)—may trigger glides under some circumstances. This arrangement would be similar to that in telesots, where the Mauthner neuron is the first to trigger a C-start, but is reinforced by additional, slower pathways that can trigger the behavior when the Mauthner neuron is ablated (Di Domenico et al. 1988; Eaton et al. 1982; Korn and Faber 2005).

**Flight-gating of high-frequency DCMD spikes**

The DCMD provides the only visually generated input to MN84 during a loom and high-frequency DCMD spikes can cause this motor neuron to spike, although high-frequency DCMD spikes can often be seen in trials where a locust does not glide. This is probably because the gliding behavior occurs only at the end of a normal, complete wingbeat cycle, so DCMD input must be gated into the appropriate phase of the flight cycle (Santer et al. 2005). During glides, the high-frequency DCMD spikes that cause summing EPSPs in MN84 occur coincidentally with the onset of the MN84 burst that elevates the forewings into the gliding posture and continue throughout it. They are thus summed with rhythmic modulations of the motor neuron’s membrane potential during normal flight (Hedwig and Becher 1998; Robertson and Pearson 1982, 1985) to trigger powerful and sustained elevator muscle contraction. In contrast, where the locust does not glide, high-frequency DCMD spikes usually arrive before MN84 activity during the time when it is not strongly excited. In these cases DCMD input is insufficient to cause prolonged excitation of MN84, but an increased amplitude forewing elevation is often seen in these trials that might result from subtle modulation of MN84 activity by preceding DCMD spikes. DCMD spikes trigger glides on most occasions (approximately 70%) but where they cannot, potential musculoskeletal damage may be preclusive and forewing modulations an alternative tactic. In a similar way, it has been proposed that spike trains from the locust’s deviation detector neurons are gated into the flight rhythm to evoke course correction during flight (Reichert and Rowell 1985, 1986; Reichert et al. 1985). DCMD activity could also directly affect the flight central pattern generator during a glide.

**Behavioral significance**

Locusts respond to an approaching object with steering behaviors and, if these fail, a last-chance emergency glide (Gray et al. 2001; Robertson and Johnson 1993; Robertson and Reye 1992; Santer et al. 2005). Low DCMD firing rates 200 ms before collision might trigger collision avoidance steering (Gray 2005; Matheson et al. 2004), whereas higher rates later in the response trigger a glide. Although tethered flying locusts most often perform glides in response to a loom, occasionally they do not. In these trials the forewing was often elevated more than usual. On a trial-by-trial basis, the behavior of the locust cannot be predicted from its DCMD response alone (or thus from the type of stimulus the locust encounters) because of the variation in the effectiveness of these spikes at different phases of the flight rhythm. In this way, the locust exhibits a form of protean behavior (Edut and Eilam 2004; Humphries and Driver 1967, 1971) that might prevent predictability in its escape responses being exploited by a predator (e.g., Jablonski and Strausfeld 2001). This range of behaviors, from steering to diving, also indicates that locusts’ evasive behaviors vary with the level of threat posed by a looming stimulus. The evasive responses used by some nocturnal insects to evade bats also vary with the perceived danger posed by the stimulus (Miller and Olesen 1979; Roeder 1967; Yager et al. 1990).

Looming-elicited gliding by flying locusts appears to be an emergency response suited to the evasion of fast aerial predators such as the carmine bee-eater *Merops nubicus*, the lanner falcon *Falco biarmicus*, and the black kite *Milvus migrans*, which are all reported to capture locusts in flight (Fry and Fry 1992; Nickerson 1958; Smith and Popov 1953). When these birds attack locusts, they appear as looming stimuli and thus the DCMD neuron is ideally suited to their detection (e.g., Rind and Simmons 1992). Furthermore, the DCMD is most sensitive to objects moving in its caudal rather than frontal field.
of view (Krapp and Gabbiani 2005), suiting it to the detection of pursuing predators.

The relatively small diameter looming stimuli used in our study are close to the pectoral widths of typical avian locust predators and result in a DCMD response that increases in frequency until after stimulus movement has ceased (e.g., Money et al. 2005; Rind and Santer 2004; Santer et al. 2005). However, the DCMD’s response to larger and/or slower stimuli is curtailed earlier in the looming as a result of feedforward inhibition to the LGMD (Gabbiani et al. 2005; Rind and Santer 2004). The important role for high-frequency spikes at the end of a looming stimulus in triggering an emergency glide raises the interesting question of whether the variation in DCMD response with object speed and size suits it to the triggering of different emergency behaviors according to the perceived nature of a looming threat. Because large but not small head-on looming objects trigger flight steering behaviors (Gray et al. 2001), it has been proposed that different avoidance reactions may be triggered by predators and conspecifics (Gray 2005; Matheson et al. 2004). However, rather than being very large stimuli, during flight the most specialized of these predators are small, fast birds with small pectoral diameters (about 30–45 mm) and thin profile wings that may be best evaded by gliding rather than steering (Santer et al. 2005).

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