Plasticity in the Visual System Is Correlated With a Change in Lifestyle of Solitarious and Gregarious Locusts

Thomas Matheson, Stephen M. Rogers, and Holger G. Krapp
Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom
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Matheson, Thomas, Stephen M. Rogers, and Holger G. Krapp. Plasticity in the visual system is correlated with a change in lifestyle of solitarious and gregarious locusts. J Neurophysiol 91: 1–12, 2004. First published September 17, 2003; 10.1152/jn.00795.2003. We demonstrate pronounced differences in the visual system of a polyphenic locust species that can change reversibly between two forms (phases), which vary in morphology and behavior. At low population densities, individuals of Schistocerca gregaria develop into the solitarious phase, are cryptic, and tend to avoid other locusts. At high densities, individuals develop instead into the swarm-forming gregarious phase. We analyzed in both phases the responses of an identified visual interneuron, the descending contralateral movement detector (DCMD), which responds to approaching objects. We demonstrate that habituation of DCMD is fivefold stronger in solitarious locusts. In both phases, the mean time of peak firing relative to the time to collision nevertheless occurs with a similar characteristic delay after an approaching object reaches a particular angular extent on the retina. Variation in the time of peak firing is greater in solitarious locusts, which have lower firing rates. Threshold angle and delay are therefore conserved despite changes in habituation or behavioral phase state. The different rates of habituation should contribute to different predator escape strategies or flight control for locusts living either in a swarm or as isolated individuals. For example, increased variability in the habituated responses of solitarious locusts should render their escape behaviors less predictable. Relative resistance to habituation in gregarious locusts should permit the continued responsiveness required to avoid colliding with other locusts in a swarm. These results will permit us to analyze neuronal plasticity in a model system with a well-defined and controllable behavioral context.

INTRODUCTION

Visual systems are tuned to signal salient features of the natural environment specific to each animal’s lifestyle. For example, collision-sensitive neurons in birds signal an approaching object (Sun and Frost 1998), and some neurons in the toad tectum preferentially respond to objects that resemble worms (Ewart 1997). In insect species that have different lifestyles, the properties of photoreceptors (Laughlin and Weckström 1993) and interneurons (Egelhaaf et al. 2002; O’Carroll et al. 1996) are spatio-temporally tuned to match the self-motion–induced visual inputs that are generated by the characteristic movements of each animal.

Visual tuning can also change over time (Meinertzhagen 2001). For example, the visual system of dragonflies changes as the aquatic larvae develop into flying adults (Sherk 1978). Polyphenic species that take on a range of forms depending on environmental factors provide the opportunity to analyze how sensory systems are tuned to changing circumstances. We have investigated for the first time whether the tuning of an identified visual interneuron in a polyphenic locust is re-tuned in animals that undergo a remarkable transformation in lifestyle that includes changes in behavior and therefore visual inputs.

Locusts generally live at low densities (<3/100 m²). These “solitarious phase” locusts are highly camouflaged, and if they see other locusts, they generally move away from them. They move slowly and fly infrequently, generally at night. In contrast, when locusts aggregate into swarms (“gregarious phase”), there may be 100,000/100 m². They are brightly colored as juveniles, and if separated from the group, tend to re-join it. They fly frequently and mostly during the daytime. Their aposematic colors advertise their unpalatability to predators (Sword 2000). The behavioral differences between the phases, some of which can occur in 4 h, mean that the genetically similar solitarious and gregarious animals act differently and have different interactions with the environment, conspecifics, and predators (Uvarov 1966, 1977). This provides us with a powerful system with which to analyze the effects of visual and social experience on behavioral and neuronal plasticity.

Visually mediated responses are prominent among behaviors that differ between the phases, so we analyzed the responses of the descending contralateral movement detector neuron (DCMD) (Rowell 1971a; O’Shea et al. 1974) in solitarious and gregarious adult locusts. The pattern of spikes in DCMD reflects 1:1 the activity of the presynaptic lobula giant movement detector neuron (LGMD) (O’Shea and Williams 1974), which is excited most strongly by visual inputs signaling that an object is looming on a direct collision course (Rind and Simmons 1992; Schlatterer 1977; Simmons and Rind 1992). DCMD’s responses can be affected by the visual environment during development (Bloom and Atwood 1980). DCMD excites thoracic interneurons and motor neurons that contribute to flight steering and jumping (Burrows and Rowell 1973; Pearson and Goodman 1979; Pearson et al. 1980; Simmons 1980).

We show that habituation of DCMD is stronger in solitarious than gregarious locusts but that the relationship between mean time of peak firing, angular size, and response delay (Gabbiani et al. 1999) is nevertheless unchanged.

Address for reprint requests and other correspondence: T. Matheson, Dept. of Zoology, Univ. of Cambridge, Downing St., Cambridge CB2 3EJ, UK (E-mail: tm114@hermes.cam.ac.uk).
METHODS

Animal rearing

All experiments were carried out on adult male or female locusts (Schistocerca gregaria Forskål) 2–3 wk after their final molt. They were fed on wheat seedlings supplemented with bran flakes.

Gregarious phase locusts were obtained from our crowded labora-
tory colonies in Cambridge and Oxford. The culture in Cambridge contains 500–1,000 insects per 45 × 50 × 50 cm rearing bin and is maintained under a 18 h:6 h light:dark cycle. The temperature during the light period is 37°C and during the dark period is 25°C. The culture in Oxford contains 450–1,000 insects per 56 × 76 × 60 cm rearing bin and is maintained under a 12 h light:12 h dark illumination cycle at 30 ± 2°C, with additional heat provided by the timed cage lights.

Experimental solitary phase locusts were derived from the Ox-
ford colony and therefore had a similar genotype to gregarious ani-
mals but had been reared in a separate facility under physical, visual, and olfactory isolation from each other for two generations. In other words, both they and their parents were reared in isolation. This permits the development of the full suite of solitary characteristics, since the slowest changes take longer than one generation to be fully expressed. The husbandry procedures for the isolated locusts were the same as those used in Roessingh et al. (1993). The light regimen was 12 h:12 h light:dark. Postnatal mortality was extremely low (<1%).

Locusts raised under these conditions show pronounced solitary phase characteristics (Simpson et al. 1999).

Dissection and recording

Animals were mounted ventral side up in a plastic holder and secured with modeling clay. The head was manipulated into a vertical position using guides on the holder and waxed to the pronotum so that it could not move. The dorsal aspect of the head rested on a narrow extension of the holder that did not restrict the vision of the right eye.

The left eye was covered with modeling clay. The antennae were waxed to the holder so that they were not in the visual field. The soft cuticle of the neck was dissected away to reveal the underlying cervical connectives, and locust saline was added as necessary to keep the tissue submerged. DCMD spikes were recorded using bipolar silver hook electrodes (50 μm diam) placed under the left connective and insulated with petroleum jelly. The spikes have the largest ampli-
tude in the connective and have a characteristic response to visual stimuli. Experiments were carried out at 21–27°C. Signals were captured to computer using a Cambridge Electronic Design (CED) interface and Spike 2 software (CED) at a sampling rate of 25 kHz. Recordings were stable for the duration of the experiments, which could last for several hours. Test stimuli given 15–60 min after the end of the stimulation protocols confirmed that the overall responsiveness of DCMD did not change during these experiments.

Visual stimulation

The animal was mounted 60 mm in front of a Tektronix CRT monitor 100 mm high × 120 mm wide, so that the center of the right eye was aligned with the center of the screen in both azimuth and elevation, and the longitudinal body axis was parallel to the screen surface. The animal and screen were covered by blackout material. The monitor was driven by a Picasso image synthesizer (Innisfree) and the photoreceptor is spatially 1984; Miall 1978), and its spatial resolution was 0.5 mm, giving an angular resolution of 0.48°. This is below the spatial resolution of the locust ommatidial array (1.25°; Horridge 1978) and the photoreceptor acceptance angles of either light- or dark-adapted animals (1.5° and 2.5°, respectively; Wilson 1975).

We simulated the constant velocity approach (looming) of dark squares on a light background (52% contrast) over 5 s prior to collision with the eye as described in detail in Gabbiani et al. (1999). The rate of expansion of the object on the eye is determined by the ratio between the half size of the object (l) and the approach velocity (v, which by convention is negative for approaching objects), i.e., l/v, such that an object of size l approaching at velocity |v| will generate the same pattern of visual stimulation on the eye as an object twice as big approaching twice as fast. For a constant approach velocity, the angular extent increases slowly at first and then more rapidly as the object nears the eye. We simulated objects with values of l/v ranging from 10 to 50 ms. This corresponds to, e.g., squares 40–200 mm across approaching at 2 m/s. The value of l/v affects the timecourse of expansion in two ways. First, larger values of l/v, which are generated by larger or slower objects, have larger initial sizes than for lower values of l/v. Angular extent at the onset of the stimulation ranged from 0.95° (for l/v = 10) to 1.2° (for l/v = 50). Second, larger values of l/v result in more gradual rates of expansion (Fig. 1, right column). The simulated objects appeared in the center of the screen expanded to their maximum size (80° visual angle), where they remained static for 500 ms before vanishing. The final angular extent was the same for all objects.

In one-half of our recordings in all three experimental groups (Cambridge gregarious, Oxford gregarious, and Oxford solitary), there was some alignment of spike times during the early part of each approach (e.g., raster plots in rightmost gray column in Fig. 1). This reflects the spatial resolution of the display screen. The initial slow rate of expansion meant that the first few step increases (of 1 pixel or 0.48° visual angle) occurred at intervals of several hundred milliseconds. As the rate of angular expansion increased, the interval between successive steps rapidly decreased to a few milliseconds. There was no spike alignment during the last hundred milliseconds of the approach in any animal.

We carried out two series of experiments. In the first, each animal was exposed to 30 approaches of five objects with different values of l/v (10, 20, 30, 40, 50 ms), giving 150 approaches in total. Stimuli were repeated at 1 min intervals. The 30 approaches for each object were divided into three blocks of 10, so that the l/v values were presented in the sequence: (10, 20, 30, 40, 50), (50, 40, 30, 20, 10), (30, 40, 10, 20, 50) ms: see Fig. 1), i.e., each value of l/v in this sequence was presented 10 times before moving on to the next value. To avoid confounding habituation with the effect of l/v, we only used the data from the second two blocks. In the second series of experiments, animals were exposed to 30 sequential approaches of an object with l/v = 20 ms at 1 min intervals.

Data analysis

Spike times were obtained by applying a threshold to the recorded potential traces to detect the largest spike, which was always that of DCMD, and are expressed here as “time relative to collision,” which is defined as time 0. These data were used to construct raster plots, peristimulus time histograms, and peak spike frequencies, and to calculate the number of spikes elicited by each approach. To examine the timecourse of the response in DCMD, the spike times were transformed to instantaneous spike frequencies by applying a 12.5 ms Gaussian smoothing filter (see Gabbiani et al. 1999), with the integral of the smoothed waveform scaled to equal the number of spikes in the trial. The time of peak firing relative to collision was determined from these smoothed data for each approach. This Gaussian smoothing method means that a single spike gives rise to an apparent firing rate of 32 spikes/s. If spikes occurred at low enough rates that the Gaussian smoothing functions applied to each spike did not overlap, it was not possible to determine a time of peak firing, since all spikes gave rise to the same value of 32 spikes/s. In our analysis of the time of peak firing, we have excluded trials with apparent firing rates of <33
FIG. 1. Patterns of descending contralateral movement detector (DCMD) spikes in the strongest and weakest responding gregarious animals (2 left shaded columns) and the strongest and weakest responding solitarious animals (2 right shaded columns) during the approach of objects with different values of \( l/v \) (right column). Each raster plot illustrates 30 responses of DCMD, with the 1st at the bottom of the stack (Stimulus number). In this series of experiments, stimuli were presented in blocks of 10 so that values of \( l/v \) were interleaved (Block: see METHODS). Time of simulated collision is indicated by the vertical dashed line in right column. Mean pattern of spikes across 30 trials is shown in the corresponding histogram below each raster plot. For the strongest responding gregarious animal, the pattern of activity in DCMD was remarkably similar for each approach of an object with a particular value of \( l/v \). In the weakest responding gregarious animal, DCMD began firing later during the approach for all values of \( l/v \), and peak firing rates were lower. Solitarious animals generally responded with fewer spikes and a lower peak rate than gregarious animals. There was a more marked reduction in responsiveness across trials. In the weakest animal, this meant that for many stimuli, DCMD did not respond at all. Data are taken from 10 solitarious, 10 Cambridge gregarious, and 11 Oxford gregarious animals used in the 1st series of experiments (see METHODS).

spikes (7.2% of the total number of trials). We have noted this in the relevant section of RESULTS.

A model of the changes in spike rate of LGMD and DCMD during the approach of an object suggests that LGMD performs a multiplication on the neuronal representation of two stimulus-related visual parameters: the instantaneous expansion velocity and the instantaneous retinal extent (size) of the approaching object. Excitatory inputs represent the object’s expansion velocity, while the object’s size is represented by an inhibitory input in the form of a negative exponential (Gabbiani et al. 1999; Hatsopoulos et al. 1995). The evidence for this is that the time of peak firing in DCMD always occurs at a fixed delay after the image of an approaching object reaches a constant angular size. This threshold angle and delay are characteristic for each animal, and are independent of object size or velocity. They are also unaffected by body temperature, arousal level, stimulus contrast, or differences in stimulus shape or texture (Gabbiani et al. 1999, 2001). When appropriate, we have used analysis methods that are similar to those described by Gabbiani et al. (1999). In particular, we have determined the threshold visual angles and delay values for both solitarious and gregarious Schistocerca gregaria, and we compare our results for the two phases to the earlier work on ‘gregarious’ S. americana (Gabbiani et al. 1999). This latter (North American) species rarely swarms, and expresses less pronounced behavioral polyphenism than does S. gregaria (Sword 2003).

RESULTS

In the absence of visual stimulation, DCMD fired at a low spontaneous rate that did not differ significantly between the three groups of animals (solitarious: 0.24 ± 0.028 spikes/s, \( n = 10 \); Oxford gregarious: 0.20 ± 0.027 spikes/s, \( n = 11 \); Cambridge gregarious: 0.14 ± 0.028 spikes/s, \( n = 10 \); Kruskall–Wallis \( \chi^2 = 4.76, P = 0.093, 2 \) df).

Response of DCMD to an approaching object

In both solitarious and gregarious locusts, DCMD typically responded to an approaching object with a burst of spikes in which the firing rate increased to a peak and then rapidly declined before the end of the stimulus (rasters and peri-stimulus histograms in Fig. 1). To illustrate the general features of this pattern of firing that are common to both phases and to illustrate the range of variation among animals, we show in Fig. 1 the weakest and strongest responding solitarious and gregarious individuals. For the gregarious animals, both the weakest and strongest were from the Oxford population.

For both phases, peak spike rate was higher for lower values of \( l/v \), corresponding to the more rapid angular expansion of small fast objects, and the response began later during the approach than for larger values of \( l/v \) (Fig. 1). Together, these effects result in broad peri-stimulus time histograms for large values of \( l/v \) and short sharp histograms for small values of \( l/v \) (Fig. 1). In most animals, DCMD often produced a single spike when the stimulus vanished from the screen at time = 0.5 s. This response was not included in our analyses, but is
apparent in three of the four examples in Fig. 1. DCMD of 16 animals (from \(n = 31\)) sometimes responded with one or a few spikes when the simulated object first appeared on the screen at time \(= -5.0\) s, but only for \(l/\lvert v \rvert = 40\) or 50 ms (e.g., Fig. 1, rightmost gray column).

The response strength of DCMD either stayed the same over the 30 approaches (in most gregarious animals) or habituated (particularly in solitarious animals; Fig. 1). The blocked design of the experiment meant that we could not easily analyze the timecourse of habituation, so in a second series of experiments using different animals, we counted the mean number of spikes per approach for each of 30 sequential approaches at 60 s intervals for \(l/\lvert v \rvert = 20\) ms (see Habitation in the response of DCMD differs between the phases).

Habituation in the response of DCMD differs between the phases

The first approach in a sequence elicited equally strong responses in solitarious and gregarious locusts [Fig. 2A; \(t = 0.55, P = 0.59, 21 \text{ df}; 65 \pm 10.1\) spikes for solitarious and \(67 \pm 7.2\) spikes for gregarious locusts, \(n = 11\) solitarious, \(n = 12\) gregarious (9 Oxford, 3 Cambridge)]. By the fifth approach, the responses of solitarious locusts were significantly weaker.

![Graph showing response of DCMD habituates more rapidly and to a lower level in solitarious than in gregarious animals.](image)

**Fig. 2.** Response of DCMD habituates more rapidly and to a lower level in solitarious than in gregarious animals. *A:* number of spikes elicited during the approach of an object with \(l/\lvert v \rvert = 20\) ms. Gray symbols, gregarious animals (mean \(\pm\) SD; \(n = 12\) for 1st 10 approaches then \(n = 7\)); black symbols, solitarious animals (\(n = 11\) for 1st 10 approaches, \(n = 6\) for approaches 11–23, \(n = 5\) for approaches 24–30). Gray and black curves indicate single exponential decay functions fitted through corresponding data. For gregarious animals, \(y = 57.18 + 19.40e^{-0.72t}, r^2 = 0.32\), and for solitarious animals, \(y = 15.74 + 53.92e^{-0.11t}, r^2 = 0.94\). *B:* peak firing rate of DCMD habituates more in solitarious animals (black traces) than in gregarious ones (gray traces). Gaussian smoothed response profiles for all 30 approaches (but only the last 0.5 s of each approach) are concatenated with the intervening 60 s rest periods removed. Numbers and arrowheads indicate selected responses that are expanded in *C* (particularly in solitarious animals; Fig. 1). The blocked design of the experiment meant that we could not easily analyze the timecourse of habituation, so in a second series of experiments using different animals, we counted the mean number of spikes per approach for each of 30 sequential approaches at 60 s intervals for \(l/\lvert v \rvert = 20\) ms (see Habitation in the response of DCMD differs between the phases).
than those of gregarious locusts (Fig. 2A; \( t = 3.34, P = 0.003, 21 \text{ df}; 45 \pm 8.8 \) and \( 58 \pm 9.7 \) spikes, respectively). This difference was even greater after 30 approaches (Fig. 2A; \( t = 4.17, P = 0.002, 10 \text{ df}; 20 \pm 17.6 \) and \( 57 \pm 13.4 \) spikes, respectively). For solitarious animals, this represented a strong habituation to only 30.8% of the first response. In gregarious animals, the habituation was 4.6-fold weaker so that the last response still contained 85% of the number of spikes seen in the first response. For gregarious locusts there was no additional habituation after the fifth approach. Both datasets fitted well with a single exponential decay function of the form \( y = y_0 + ae^{-bx} \), where \( y_0 \) controls the asymptote, \( a \) is a scaling factor, \( b \) describes the rate of decay, and \( x \) denotes the approach number (Fig. 2A). To test the overall significance of the regressions we natural-log-transformed the number of spikes per approach and used a multivariate ANOVA to test for differences in the gradient and intercept. The gradients of the lines differed significantly (\( F_{1,11} = 16.62, P = 0.002 \)), but the intercepts did not (\( F_{1,11} = 1.54, P = 0.24 \)).

Habituation in the response of DCMD was also evident in the peak firing rate (Fig. 2B). During the first of 30 approaches at \( l/|v| = 20 \) ms, the peak instantaneous firing rate did not differ significantly between solitarious animals \((249 \pm 34.6 \) spikes/s\) and gregarious animals \((281 \pm 50.1 \) spikes/s, \( t = 1.85, P = 0.08, 21 \text{ df})\). During the subsequent 29 approaches, the peak rate for solitarious animals declined to \( 82 \pm 47.7 \) spikes/s (33% of the initial rate), whereas for gregarious animals there was little change, so that the rate during the 30th approach was \( 242 \pm 51.1 \) spikes/s, representing habituation to 86% of the initial rate. This is a significantly higher rate than for solitarious animals \( (t = 5.48, P < 0.001, 10 \text{ df}) \).

During the first approach, the ratio of peak firing rate to number of spikes per approach was the same for solitarious and gregarious animals (Fig. 2C; ANOVA on individual regression intercepts, \( F_{1,20} = 2.41, P = 0.136 \)). The ratio increased significantly more for solitarious animals than gregarious animals over the course of 30 approaches (Fig. 2C; \( F_{1,20} = 5.35, P = 0.031 \)). This suggested that the responses for solitarious animals should have become relatively sharper as DCMD habituated over successive approaches. Overlaying the Gaussian smoothed response curves for the 2nd, 15th, and 29th approaches at \( l/|v| = 20 \) ms showed that, for solitarious animals, the peak firing rate (at approximately \( 0.06 \) s) decreased dramatically between the 2nd and 29th approaches, whereas that for gregarious animals did not (Fig. 2D). DCMD began spiking earlier before collision in the nonhabituated state than when habituated (the time at which each response curve falls to a firing rate of 0 is indicated by a dotted line and approach number in Fig. 2D). The firing rates measured at time = \( -0.5 \) s prior to collision nevertheless showed little habituation across trials (and see Firing rate 200 ms prior to collision for further analysis). For gregarious animals, the response curves for the 2nd and 29th approaches overlapped almost completely throughout the approach, reflecting the comparatively weak habituation seen at 60-s interstimulus intervals in these animals (Gregarious in Fig. 2D).

Variation in the time of peak firing increased as the response of DCMD habituated in solitarious animals during 30 successive approaches (black symbols in Fig. 2E and inset). In contrast, the variation in time of peak firing for gregarious animals remained low and nearly constant (gray symbols in Fig. 2E and inset).

Effect of object size and velocity on response profile

To examine the effect of \( l/|v| \) on the pattern of firing in DCMD, we challenged gregarious and solitarious locusts with interleaved trials of objects having different values of \( l/|v| \) at 1 min intervals (Fig. 3; see METHODS and Fig. 1).

The response profiles of DCMD in the two groups of gregarious animals were indistinguishable (Fig. 3, top 2 panels), with smaller values of \( l/|v| \) (smaller, faster objects) giving rise to briefer periods of higher firing rate than did larger values (Figs. 3 and 4A). The time of peak firing was later (closer to the time of collision) for smaller values of \( l/|v| \) (Fig. 3). By contrast, in solitarious animals the flatter broader response profiles differed markedly from those for either gregarious group, with the peak firing rate lower across all values of \( l/|v| \) (Figs. 3 and 4A). For the larger values of \( l/|v| \), there was no sharply defined peak of firing rate (Fig. 3 and Time of peak firing). In gregar-
ious animals, the number of DCMD spikes per approach was smallest for small values of $l/|v|$, rising to a maximum for $l/|v| = 30$ ms and leveling off for larger values of $l/|v|$ (Fig. 4B). In solitarious animals, there was no change in the number of spikes with different values of $l/|v|$.

**Firing rate 200 ms prior to collision**

Flight steering maneuvers begin about 180 ms before collision for objects with $l/|v| = 20$ (recalculated from Robertson and Johnson 1993; for experiments carried out at 26–28°C). The time lag between a visual input and the onset of a behavioral response is approximately 60 ms (Robert and Rowell 1992; for experiments at 19–25°C), and the lag from a visual input to a DCMD spike at our recording site in the neck connective was approximately 40 ms (measured for “off responses” when the stimulus instantaneously disappeared from the monitor, at 21–27°C). For spikes in DCMD to be involved in initiating flight maneuvers, they must therefore occur ≥200 ms prior to collision (180 + 60 – 40 ms).

The firing rate 200 ms prior to collision was the same for gregarious and nonhabituated solitarious locusts (open symbols in Fig. 4C; 104 ± 7.7 vs. 97 ± 9.4 spikes/s, respectively: $l/|v| = 20$ ms, $t = -0.523$, $P = 0.606, 21$ df). In habituated animals, however, the firing rates in Oxford or Cambridge gregarious animals (at $l/|v| = 20$) were significantly higher than that for solitarious animals (Fig. 4C; 73 ± 9 and 69 ± 7 vs. 23 ± 8 spikes/s, respectively; $t = -4.07$, $P = 0.001$, 19 df; $t = -4.29$, $P < 0.001$, 18 df). For gregarious locusts, the firing rate 200 ms prior to collision was significantly lower for $l/|v| = 10$ ms than for $l/|v| = 20–50$ ms (Fig. 4C; Oxford: $t = -3.18$, $P = 0.005$, 18 df; Cambridge: $t = -3.48$, $P = 0.003$, 18 df; see black trace in Fig. 3). In contrast, for habituated solitarious locusts, it was the same for all values of $l/|v|$ and was consistently lower than for gregarious locusts (Fig. 4C).

**Time of peak firing**

The LGMD-DCMD system in gregarious locusts has been suggested to perform a multiplication function on visual inputs representing object velocity and size that drive the response (Hatsopoulos et al. 1995). Of critical importance in this theoretical argument is the timing of the peak firing rate relative to the angular size of the approaching object. We used the method described in detail by Gabbiani et al. (1999) to test whether the time of peak firing relative to a threshold angular size differed between the phases.

The time at which the peak firing rate occurred during the approach of objects with different values of $l/|v|$ was determined from Gaussian smoothed responses for each individual animal (data from the 1st series of experiments). We summed the firing across all 30 trials at each $l/|v|$ and smoothed the pooled response as described in METHODS. For gregarious locusts, increasing $l/|v|$ caused a linear advance in the time of peak firing (Fig. 5, A, B, and insets). The relationship was consistent across animals, and there was little jitter in time of peak firing. For solitarious animals, however, the time of peak firing was substantially more variable (Fig. 5, C and inset), primarily because the total numbers of spikes per approach were lower and the peak firing rates were not sharply defined (Fig. 4). Small fluctuations in the firing rate of DCMD in solitarious animals (e.g., caused by spike doublets) therefore led to erratic peaks (Fig. 5D) and apparently nonlinear relationships between $l/|v|$ and time of peak firing (Fig. 5C). These errors were particularly evident for larger values of $l/|v|$ because the peak firing rates were lowest for these large slow objects (Fig. 3).

We were particularly interested in determining if the underlying relationship between $l/|v|$ and time of peak firing differed between solitarious and gregarious locusts, because this provides key evidence about the underlying computation carried out by the presynaptic LGMD neuron (see Data analysis). We therefore analyzed our data in two ways. First we calculated and tested overall linear regressions from the three datasets shown in Fig. 5, A–C (Fig. 6A). There were no significant differences between the slopes of the three sets of data, suggesting that the underlying relationship between time of peak firing and $l/|v|$ was the same for both solitarious and gregarious locusts.
firing and $l/|v|$ is the same in solitarious and gregarious animals (GLM, $F_{2.148} = 0.722, P = 0.488$). Second, since the large variance in the responses of solitarious animals might have masked any potential difference in slope, we re-examined the smoothed responses of each animal to each object. If there were clear spurious peaks of firing rate before or after a smaller peak that coincided more closely with that predicted by the overall regression we re-assigned the time of peak of firing from the largest to the smaller peak. For example, in one solitarious animal, the time of peak firing recorded during the approach of an object with $l/|v| = 30$ ms clearly distorted the otherwise linear relationship (asterisk in Fig. 5C). Spurious peaks of firing rate (false peaks in Fig. 5D) both before and after a smaller peak (true peak in Fig. 5D) occurred near the time predicted by the linear regression through all of the data from solitarious animals (dotted line in Fig. 5D). We therefore reassigned the time of peak firing from the largest peak (asterisk in Fig. 5D) to the smaller peak. This procedure was repeated for all 31 animals, but subsequent linear regression once again revealed no significant difference between the slopes for any of the three experimental groups (Fig. 6B; GLM, $F_{2.151} = 1.197, P = 0.305$).

The SD of the time of peak firing increased linearly as $l/|v|$ increased for both solitarious and gregarious animals as expected from the broader flatter response curves (Fig. 6C, see also Fig. 6, A and B). The relationships for Cambridge and Oxford gregarious animals had lower slopes than did the relationship for solitarious animals, for both the uncorrected data (data not shown) and the corrected data (ANOVA, $F_{2.9} = 28.2, P < 0.001$). This also means that solitarious animals exhibit a greater variation in time of peak firing at any given value of $l/|v|$ (Fig. 6C). Our results for gregarious animals match closely the comparable data for a gregarious animal reported in Gabbiani et al. (1999) (see Fig. 6C).

Angular threshold and DCMD response delay

The key outcome of the analysis of gregarious locusts presented by Gabbiani et al. (1999) was that the peak firing rate of DCMD always occurred at a fixed delay after an approaching object reached a particular angular size on the retina. This threshold angle $\theta_{\text{thresh}}$ differed somewhat between animals but was constant within each animal over one order of magnitude of $l/|v|$.

The threshold angle is computed from the regression of time of peak firing versus $l/|v|$ as

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

where $\alpha$ is the slope of the regression. The peak firing rate occurs at a delay $\delta$ relative to the time at which this angle occurs. The delay $\delta$ is defined by the intercept of the regression of time of peak firing versus $l/|v|$ (see Gabbiani et al. 1999 for derivation).

The regressions shown in Fig. 6B were first used to compute a mean $\theta_{\text{thresh}}$ and delay $\delta$ for each of the three experimental groups. For Cambridge and Oxford gregarious animals, the values were threshold ($\theta_{\text{thresh}}$), 23.6° and 28.2°, respectively; delay ($\delta$), 27.6 and 19.8 ms, respectively. For solitarious animals $\theta_{\text{thresh}} = 27.7°$ and $\delta = 12.1$ ms. These values are shown in Fig. 7, superimposed on the range of values reported by Gabbiani et al. (2001) for 79 gregarious locusts. Their values were $\theta_{\text{thresh}}$ 15–40°; $\delta$, 3–45 ms. All mean data from both studies are remarkably similar, despite the strong habituation and low firing rates of DCMD in solitarious animals (see Figs. 1–4). To describe the variation in $\theta_{\text{thresh}}$ and delay $\delta$ for the three different experimental groups, we calculated both parameters on an animal-by-animal basis—as was done by Gabbiani et al. (1999).

The time of peak firing was first determined for each indi-
the time of peak firing. For gregarious animals, there were few trials in which the maximum instantaneous firing rate was below 33 spikes/s were discarded (see Data analysis). Data were otherwise uncorrected, so the analysis took into account all the variation in the time of peak firing. The trial-by-trial values of time of peak firing for each of 30 approaches at each value of \(|l|/v|\) are plotted in Fig. 8, and linear regressions fitted for each animal. For gregarious animals, there were few trials in which the time of peak firing occurred earlier than 1 s prior to collision (Fig. 8, Ai and Bi), but for solitarious animals there were many (Fig. 8Ci). This reflects trials with low overall firing rates in which two or more spikes occurring at a short interval
by chance early in the response can yield the highest peak firing rate. All of these points were included in our analysis.

A closer examination of the regressions for the individual animals reveals that only one gregarious animal had a negative intercept (Fig. 8, Aii and Bii), whereas the regressions for the solitarious animals had intercepts scattered on both sides of zero (Fig. 8Cii). The gregarious outlier was the weakest responding Cambridge animal, in which firing rates in several trials were lower than 30 spikes/s. The regression is strongly influenced by just two trials at \( |/| = 30 \) ms (data not shown), which if removed, return the intercept to \(+13\) ms. Using Eq. 1 as before, we computed the threshold angle \( \theta_{\text{thresh}} \) for each animal and plotted this against the intercept of the regression (delay \( \delta \); Fig. 9). The SD of the estimate of \( \theta_{\text{thresh}} \) was obtained by error propagation as described by Gabbiani et al. (1999).

For both Cambridge and Oxford gregarious animals, mean delay times \( \delta \) ranged from 12 to 88 ms, with the one outlier at –29 ms (Fig. 9, A and B; arrow in Fig. 9A indicates the outlier). Threshold angles \( \theta_{\text{thresh}} \) ranged from 13 to 34° (Fig. 9, A and C). For solitarious animals, mean delay times \( \delta \) ranged from –109 to 110 ms (Fig. 9, A and B), with more than one-half of the values being negative (Fig. 9B). Mean delay times were highly variable as evidenced by the large SDs for each animal (Fig. 9A). Threshold angles \( \theta_{\text{thresh}} \) ranged from 7 to 37°, with one outlier at 122° (Fig. 9, A and C).

**DISCUSSION**

We have shown that signaling of object approach by an identified neuron, the DCMD, habituates more strongly in solitarious than gregarious locusts (Figs. 1–3). When habituated, the peak firing rate and overall number of spikes per stimulus are lower and the time of peak firing is more variable (Figs. 4–6). The mean time of peak firing is nevertheless the same in both phases.

**Plasticity in the visual system**

In gregarious locusts, the response of DCMD habituates (Horn and Rowell 1968), particularly if stimuli are repeated at intervals of \(<40\) s (Simmons and Rind 1992). We used an interstimulus interval of \(60\) ms to ensure that little habituation in gregarious locusts from two laboratory populations. This stimulus interval caused pronounced habituation in solitarious locusts that were derived from, and therefore closely related to, one of these gregarious populations. The differences that we describe cannot be due to different genotypes in the populations, but are presumably due to differential gene expression that accompanies phase transition. Habituation in the LGMD-DCMD pathway occurs at theafferent synapses onto LGMD (O’Shea and Rowell 1976; reviewed by Rind 2002). We assume that the differences in DCMD habituation in solitarious and gregarious locusts result from changes in the properties of these synapses. The monotonic timecourse of habituation in both phases suggests that this response decrement results from a single process. Moreover, since we can fit the data from both phases with equations of the same form, we predict that the phase-related difference is generated by a modification of this single underlying process.

**Effect of visual environment**

The visual environments experienced during development by solitarious and gregarious locusts differ strongly in both natural and laboratory populations. Gregarious animals are subjected to almost continuous visual stimulation from the movements of many surrounding locusts, and they themselves move frequently, causing repeated self-motion-induced retinal image shifts. In contrast, solitarious locusts rarely see a conspecific, and they move less frequently (Uvarov 1966). Rearing locusts in the dark for their entire nymphal development is reported to reduce the excitability of DCMD and increase its habituation (Bloom and Atwood 1980), but this observation cannot explain our results, since DCMD in solitarious locusts is initially as responsive as that in gregarious animals. In contrast, the overall response properties of fly visual interneurons that process optic flow patterns do not depend on early rearing conditions (Karmeier et al. 2001).

Bloom and Atwood (1980) attempted to separate the effects...
of rearing conditions and visual environment (continuous dark vs. natural light cycle) by testing locusts that they called “intermediate phase conditions.” Unfortunately, the conditions used to rear these animals caused “extremely high nymphal mortality and reduced adult life span,” and the behavioral phase state was not tested. For this reason, we consider the data of Bloom and Atwood (1980) to be inconclusive, but for completeness, we note that DCMD in these animals produced fewer spikes than in gregarious animals and had the same rate of habituation. Our data for healthy solitarious locusts of proven phase state show the opposite. The visual environment to which locusts are exposed during development can clearly affect some aspects of the responsiveness of DCMD (Bloom and Atwood 1980), but it is difficult to separate the effects of visual environment from the effects of phase change per se. This is because some visual stimuli themselves influence phase state (Häggele and Simpson 2000; Roessingh et al. 1998). For example, solitarious juvenile locusts (fifth instar) exposed to the sight of a crowd of other locusts become partly gregarized within 4 h (Häggele and Simpson 2000). We have not attempted here to analyze the timecourse of changes in the visual system that accompany phase change, but it is possible that they may begin to occur within a few hours. There was no evidence from our analyses of DCMD that solitarious animals began to gregarize as a result of the visual stimulation that we used.

Escape behavior

We show that for nonhabituated solitarious locusts the strength of DCMD response (spike number or peak spike rate) is the same as for gregarious locusts. For a solitarious animal in its sparsely populated natural environment, this means that DCMD will signal a novel approaching object as vigorously as will DCMD in a gregarious locust. This may be of high survival value since, for solitarious locusts, an approaching object is likely to be a predator, whereas for gregarious locusts, approaching objects will generally be conspecifics. What is the value of DCMD habituating strongly in solitarious animals? We suggest that the progressive increase in variability concomitant with the habituation should make a solitarious locust’s behavior progressively more unpredictable, thus making it more difficult for a predator to catch the animal (see Arnott et al. 1999; Jabłoński and Strausfeld 2000, 2001). This argument must be tempered by the observation that, at least in gregarious locusts, there is no evidence that firing of DCMD is by itself sufficient to trigger an escape jump (Burrows 1996). We have, however, already shown that an output synapse made by DCMD onto the fast extensor tibiae motor neuron in the metathorax is stronger in solitarious than in gregarious locusts (Rogers et al. 2001), so this may provide a mechanism for compensating for the lower responsiveness of the DCMD pathway (Matheson et al. 2003). This difference was demonstrated for single action potentials elicited in DCMD at low rates. We do not yet know if the difference persists (or is even enhanced) when DCMD fires at high-frequency during a visual approach.

Flight steering

Gregarious locusts flying in swarms space themselves at distances of 0.8–9.0 m (median 3.6 m) and fly parallel to one another, although those at the edge of a swarm tend to turn and fly back toward the middle, increasing their chances of colliding with other animals (Waloff 1972). Solitarious locusts fly alone so they do not face this problem. The stimuli that regulate the spacing behavior of gregarious locusts are not known, but one possibility is that DCMD, by virtue of its connections to flight motor neurons (Simmons 1980), contributes to turning maneuvers when adjacent locusts loom closer. A prerequisite for such a mechanism would be maintained sensitivity of DCMD to repeated stimuli, which is what we see in gregarious locusts—at least for stimuli presented at intervals of 60 s. DCMD of gregarious locusts habituates when stimuli are presented at shorter intervals (e.g., 40 s, Simmons and Rind 1992), but in the absence of information about the patterns of visual stimulation experienced by locusts flying in a swarm, it is difficult to assess the importance of these differences in the timescale of habituation.

Flight steering maneuvers are relatively slow because it takes time for wing kinematics to change and for aerodynamic forces to turn the animal. The time of peak firing in DCMD occurs too late to initiate these maneuvers, although spikes at this time may modify the ongoing movement. The time at which the firing rate reaches any arbitrary threshold value (e.g., 50 spikes/s) is, like the time of peak firing, related linearly to the value of $|l/v|$ and therefore precedes with a fixed delay the time at which the approaching object reaches a specific angular extent on the retina (Gabbiani et al. 2002). Such a threshold-crossing mechanism could permit earlier DCMD spikes to trigger escape when the firing rate exceeded a certain level. We therefore analyzed the firing rate of DCMD 200 ms prior to collision (Figs. 2–4), at which time the spikes could initiate turning. There are three key points. First, the firing rate in nonhabituated solitarious locusts is the same as that in gregarious locusts so, if DCMD is involved in predator avoidance during flight, then we predict that the two phases should be equally good at escaping. Second, DCMD’s responsiveness measured 200 ms prior to collision habituates more strongly in solitarious than gregarious locusts, providing the latter with the ability to maintain sensitivity in a swarm. Third, in gregarious locusts the smallest fastest object elicited lower firing rates than did the larger slower objects (although these rates still exceeded those of solitarious locusts). This may permit gregarious locusts to respond differently to conspecifics (small and fast) and larger predators like birds and could help to explain the observation that large but not small objects evoke steering maneuvers (Gray et al. 2001). In contrast to our finding, Gray et al. (2001) found no difference in DCMD firing rate 214 ms prior to collision in resting gregarious locusts presented with targets having $l/v = 16.7$ or 33.3 ms, but in their experiments, the firing rates were very low; only one to three spikes typically occurred earlier than 200 ms prior to collision. The responses we recorded began 1–3 s prior to collision, and by 200 ms prior to collision, the firing rate could exceed 100 spikes/s (Figs. 1 and 2D).

Variability in threshold angles and response latency

We show that because DCMD in solitarious locusts habituates more strongly than that in gregarious locusts, the response of solitarious animals to repeated looming stimuli has a lower overall firing rate and a broader peak. We show that despite the
differences in firing between phases, DCMD of solitarious locusts can still encode a threshold angle that is comparable to that found in gregarious animals. This is consistent with the hypothesis that DCMD’s primary visual input neuron, the LGMD, carries out a multiplication on visual inputs (Gabbiani et al. 1999, 2002; Hatsopoulos et al. 1995). There is greater variability of the signaling in solitarious locusts, and in the most habituated state in which a stimulus elicits only a few spikes at low rates, there is no single peak of activity. For solitarious locusts, this must mean that behaviors that are dependent on the peak of DCMD firing rate also become more variable. For a response curve like that of DCMD, with a gradually increasing firing rate that shuts off suddenly, increasing the noise by decreasing the number of spikes inevitably leads to estimates of the time of peak firing that precede the true value rather than lag behind.

The relationship between time of peak firing and $l/(l^2)$ (and thus the computation of a threshold angle) is not affected by target shape, contrast, texture, or approach angle, within reasonable limits (Gabbiani et al. 1999, 2001). Moreover, the relationship is the same in gregarious phase Schistocerca gregaria, S. americana, and Locust migratoria (Gabbiani et al. 2001). We show that the responses of DCMD in nonhabituated solitarious and gregarious locusts are indistinguishable but become more variable in solitarious animals in the face of repeated stimuli. Despite this, the underlying computation carried out in the visual system is conserved in the two phases.

Our data show that environmentally and socially induced phenotypic differences in locusts are accompanied by differences in neuronal function that must affect the animals’ behavior. The behavioral changes occur over timescales ranging from a few hours to generations. We can drive behavioral phase change in restrained locusts within 4 h (Rogers et al. 2003), so a key question is now to determine the timescale of the changes in the visual system. Our ability to drive phase change and analyze neuronal responses at many levels in this system will provide a powerful model for analyzing neuronal plasticity in vivo.

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