

Neural Correlates to Flight-Related Density-Dependent Phase Characteristics in Locusts

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ABSTRACT: Locust phase polymorphism is an extreme example of behavioral plasticity; in response to changes in population density, locusts dramatically alter their behavior. These changes in behavior facilitate the appearance of various morphological and physiological phase characteristics. One of the principal behavioral changes is the more intense flight behavior and improved flight performance of gregarious locusts compared to solitary ones. Surprisingly, the neurophysiological basis of the behavioral phase characteristics has received little attention. Here we present density-dependent differences in flight-related sensory and central neural elements in the desert locust. Using techniques already established for gregarious locusts, we compared the response of locusts of both phases to controlled wind stimuli. Gregarious locusts demonstrated a lower threshold for wind-induced flight initiation. Wind-induced spiking activity in the locust tritocerebral commissure giants (TCG, a pair of identified interneurons

that relay input from head hair receptors to thoracic motor centers) was found to be weaker in solitary locusts compared to gregarious ones. The solitary locusts' TCG also demonstrated much stronger spike frequency adaptation in response to wind stimuli. Although the number of forehead wind sensitive hairs was found to be larger in solitary locusts, the stimuli conveyed to their flight motor centers were weaker. The tritocerebral commissure dwarf (TCD) is an inhibitory flight-related interneuron in the locust that responds to light stimuli. An increase in TCD spontaneous activity in dark conditions was significantly stronger in gregarious locusts than in solitary ones. Thus, phase-dependent differences in the activity of flight-related interneurons reflect behavioral phase characteristics. © 2003 Wiley Periodicals, Inc. *J Neurobiol* 57: 152–162, 2003

Keywords: locust; phase polymorphism; flight interneuron; spike adaptation; behavioral plasticity

INTRODUCTION

Locusts are capable of extreme behavioral plasticity; in response to changes in population density, they dramatically alter their behavior. These changes in behavior facilitate the appearance of various morphological and physiological changes, cumulatively

termed density-dependent phase characteristics (Dirsh, 1953; Uvarov, 1966; Pener, 1991; Pener et al., 1997; Applebaum and Heifetz, 1999). At high population density, locusts actively aggregate, forming large hopper bands or adult swarms. In marked contrast, isolated animals are cryptic, demonstrating restricted locomotor activity. They move away from fellow locusts and from crowded groups (Ellis and Pearce, 1962). In the laboratory, crowded-reared and isolated-reared locusts approach the two extreme phases, gregarious and solitary, respectively.

One of the principal behavioral phase changes is the more intense flight behavior of gregarious locusts. Locust swarms exhibit long-range diurnal migratory

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flights, covering up to 1000 km a week (Steedman, 1990). These daily reiterative movements last throughout the active life of the individuals (Uvarov, 1966; Steedman, 1990). Solitary individuals, in contrast, undertake much more limited and short-range nocturnal migrations. Very little is known about solitary locusts' flight behavior. Data are very scarce regarding whether they exhibit sustained high level flights, or even repeated short-range flights (mainly restricted to night time). Phase-dependent differences, both in the tendency to initiate flight and in flight capacities, are also exhibited in the laboratory (Michel, 1970a, 1970b, 1980a, b; Ayali, unpublished results).

Locusts (of both phases) start to fly by jumping into the air. The jump elicits flight by consequence of the loss of tarsal contact with the ground and by setting up an air current to wind sensitive hairs on the locust's forehead (Weis-Fogh, 1949; Camhi, 1969). The tritocerebral giants (TCG) are a prominent pair of interneurons that receive direct excitatory input from the head hairs and make direct synaptic connection with flight motor neurons in the thoracic ganglia (Tyner and Bacon, 1979). These extensively studied interneurons are known to be strongly involved in flight initiation and maintenance (Bacon, 1979; Bacon and Mohl, 1979, 1983; Bicker and Pearson, 1983). A second pair of interneurons, which follow a similar neural path to the TCG, are the GABA immunoreactive tritocerebral dwarfs (TCD). The TCD were reported to be sensitive to the ambient illumination level (i.e., respond to light stimuli) and also respond to the termination of wind stimulus (Tyner et al., 1988).

Flight-related behavioral differences must depend upon phase differences in sensory systems, central neural elements and/or interactions between them. The current study is a first attempt at comparative investigation of flight-related neural elements in the two desert locust phases. The TCG and TCD neurons were used to examine possible differences in neural circuitry between the locust phases. Our findings provide neurophysiological and neuroethological insights that expand the knowledge of locust density-dependent phase polymorphism as well as other cases of environmentally induced behavioral plasticity.

METHODS

Animals

Schistocerca gregaria (Forskål) were reared for many consecutive generations under heavy crowding, 100–160 animals in 60-liter metal cages. Cages were kept in controlled

temperature and humidity conditions (30°C, 35–60%) under a 12D:12L cycle. Direct radiant heat was supplied during daytime by incandescent electric bulbs to reach a final day temperature of 35–37°C. Locusts were fed daily with fresh grass and dry oats. To obtain experimental locusts approaching the solitary phase, hatchlings from eggs laid by crowded-reared locusts were isolated within 12 h posthatching and kept under isolation, one locust per 1.5-liter metal cage. Care was taken to keep locusts of the different phase groups under similar conditions (except density).

Several aspects of locust flight performance change throughout maturation (Uvarov, 1966). Thus, in the behavioral experiments we employed adult females from two age groups, 7- and 30-day-old; immature and fully mature, respectively. One-week-old adult females were employed in all the electrophysiological experiments.

Anatomy

To confirm the phase of the experimental animals, well-accepted morphometric ratios were measured and compared: the length of elytron/posterior femur (E/F), and the femur/head width (F/C) (Dirsh, 1953; Pener, 1983).

The number of wind-sensitive hairs (70 to 300 μm -long trichoid sensillae on the vertex, occiput, and margins of the frons of the locust) was counted using one of two methods: a stereo-microscope or scanning electron microscopy (SEM). For the latter, whole locust heads were dehydrated and coated with a thin layer of gold before scanning.

Physiology and Experimental Setup

Locusts were briefly anesthetized in CO_2 , their legs removed and wings cut very short. The tritocerebral commissures (TCC) were easily accessible by cutting and removing mouthparts followed by clearing fatty tissue and air sacs as required (Bacon and Tyner, 1978). A fine silver hook electrode (0.003–0.005 inches) insulated to the tip was waxed to the cuticle on the side of the head and placed on one of the TCC nerve branches to monitor action potentials in the TCG and TCD neurons. The hook electrode was electrically insulated from the surrounding hemolymph with petroleum jelly. A reference electrode was inserted into the abdomen through a hole in the cuticle. Flight could be monitored by observing flapping movements of wing stumps. To further demonstrate active flight as well as the recruitment of muscles at flight initiation we recorded the electrical activity (EMGs) of specific flight muscles with fine (0.005–0.007 inches) insulated silver wire. In different experiments we have selected one or two of the following muscles: M 129 and M 119, a metathoracic wing depressor and elevator, respectively, and M99, a mesothoracic wing depressor (Wilson and Weis-Fogh, 1962). The electrodes were glued to the cuticle with a drop of warm wax. Data were recorded using a four-channel differential AC amplifier (Model 1700 A-M Systems), played back in real time, and stored on the com-

puter using an A-D board (Digidata 1320A, Axon instruments) and Axoscope software (Axon instruments).

After electrode implementation and fixing, the locust was mounted in a normal flight position (body axis raised at a 20° angle) in front of a computer controlled shutter, which allowed its exposure to wind stimuli of fixed magnitude and duration. Experiments were carried out at 30°C and (unless otherwise stated) under light condition.

Data Analysis

Spiking activity of individual neurons (TCG and TCD) was identified (spike sorting protocols) and analyzed using "Mini Analysis" software (Synaptosoft Inc., version 5.6.3). Normally distributed data are presented as mean values \pm standard deviation (STD). Other data (percentages, ratios, normalized) are presented as mean values \pm standard errors (STE).

Significance of results was assessed using appropriate statistical tests as indicated including: Two tailed unpaired *t* test, nonparametric Mann-Whitney test, one-way analysis of variance (ANOVA) followed by the Bonferroni test and *F* test for comparing regression lines (Instat, GraphPad software inc, San Diego, CA).

RESULTS

Morphology and Anatomy, Head Hair Count

Morphometric ratios calculated for the adult females used in our experiments were (mean \pm S.D., $n = 20$): E/F = 2.19 ± 0.09 and F/C = 3.27 ± 0.12 for the crowded-reared, and E/F = 2.10 ± 0.07 and F/C = 3.50 ± 0.13 , for the isolated-reared. These findings reveal that our different conditions of density had induced highly significant phase differences ($p < 0.01$, unpaired, two-tailed *t* test). Other previously described density-dependent differences (e.g., typical hopper coloration and an extra larval moult in solitary animals (Uvarov, 1921, 1966) were also demonstrated by our experimental animals.

The number of head sensillae was significantly larger in isolated-reared locusts compared to crowded ones (Fig. 1). This was true for specific receptor fields known to mediate the wind response (the first and second fields; Weis-Fogh, 1956; Sviderskii, 1969), as well as for the total number of sensory hairs on the head.

Behavior

We first sought to confirm the previously reported flight-related behavioral phase differences in our setup. We found that although the wind-induced flight

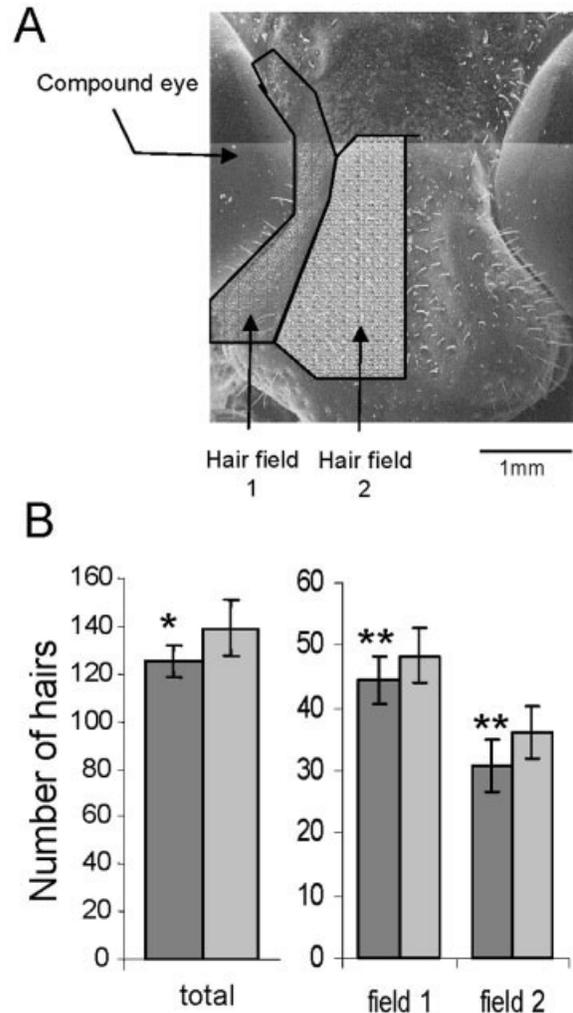


Figure 1 (A) A scanning electron microscope image of the locust frons. Fields 1 and 2 (Sviderskii, 1969) are outlined. (B) The number of sensory hairs on the forehead (70 to 300 μm -long trichoid sensillae counted on one side of the head) was found to be larger in isolated-reared locusts (light bars) than in crowded ones (dark bars). Data show mean values \pm S.D., $n = 10$ (* $p < 0.05$; ** $p < 0.01$, unpaired, two-tailed *t* test).

initiation behavior is relatively stereotypic, a wind stimulus may or may not elicit the behavior at any given time, depending on the intensity of the stimulus and the internal state of the animal. The probability of flight initiation in 7-day-old locusts of both phases in response to 1-s wind stimuli directed at the head of the locusts is presented in Figure 2. Each animal ($n = 22$) was tested with eight repetitions for wind stimuli at velocities of 1, 2, and 3 m/s. The data show a clear difference between the phases; for any given wind stimulus the probability of crowded-reared animals initiating flight was significantly higher than that of

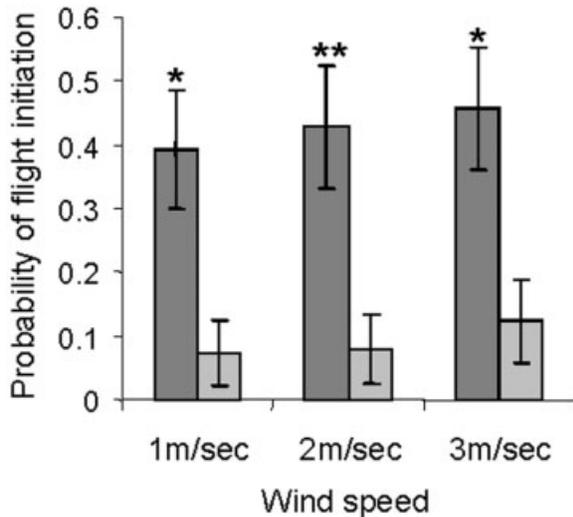


Figure 2 The probability of flight initiation in response to a wind stimulus. Data indicate the mean values \pm S.E. calculated separately for each wind speed ($n = 22$). Crowded-reared locusts—dark bars, isolated—light bars. A significant difference between the phases can be seen for each of the three wind speeds. (* $p < 0.05$; ** $p < 0.01$, Mann-Whitney test).

isolated animals. When testing 30-day-old locusts, flight could never be induced in the isolated animals ($n = 6$).

Muscle and Interneuron Activity Patterns

When no stimulus was applied, irregular spiking activity of both the TCG and the TCD neurons was observed (Fig. 3). The neurons differ in their diameter (ca. 20 μm vs. ca. 5 μm ; (Kutsch and Hemmer, 1994a)) and, hence, in the size of their action potential recorded extracellularly from the anterior branch of the commissure [Fig. 3(A)]. For each locust, a trace of 120 s was recorded, and we calculated the mean spiking rate of both TCG and TCD neurons.

The TCD was previously reported to be sensitive to the ambient illumination level (Tyrer et al., 1988). Therefore, we compared between its spontaneous activity rate under light and dark conditions in both locust phases. Crowded locusts demonstrated a five-fold increase in TCD spontaneous firing rate under dark condition compared to the activity of the TCD in the light. In marked contrast, there was only a slight (nonsignificant) dark induced change in TCD spiking rate in isolated locusts [Fig. 3(B)].

The TCG spontaneous firing rate in crowded females was significantly higher than that calculated for isolated ones [Fig. 3(C); only light conditions tested].

Our results obtained for the activity of crowded locusts' TCG (1.19 Hz) fit previous findings describing a spontaneous activity of 1–2 Hz (Bacon and Tyrer, 1978; Kutsch and Hemmer, 1994a).

Next, we comparatively quantified the response of the locusts to 1-s wind stimuli. As already mentioned, not every locust tested responded to a given wind stimulus with flight initiation. These differences (within and between phases), were of course reflected in flight muscle activity, but also in the TCG response.

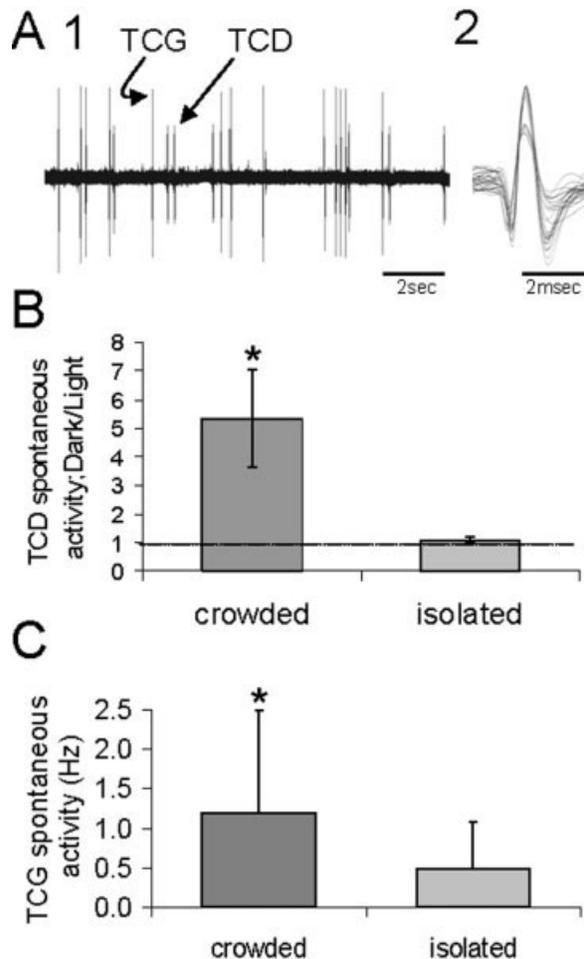


Figure 3 (A) Example of an extracellular recording from the TCC of a 7-day-old crowded locust showing TCG and TCD spontaneous spiking activity (1). An overlaid multi-sweep recording (2) demonstrates the two types of signals; a larger spike from the TCG and a smaller from the TCD interneuron. (B) Phase-related analysis of the TCD's spontaneous activity. Data presented are the average \pm S.E. of the ratio between the spontaneous activity in dark conditions versus light conditions ($p < 0.05$, $n = 7$). (C) A comparison of the average spontaneous activity (\pm S.D.) of the TCG neuron demonstrates a significantly higher firing rate in the crowded-reared animals ($p < 0.05$, Mann-Whitney test, $n = 16$).

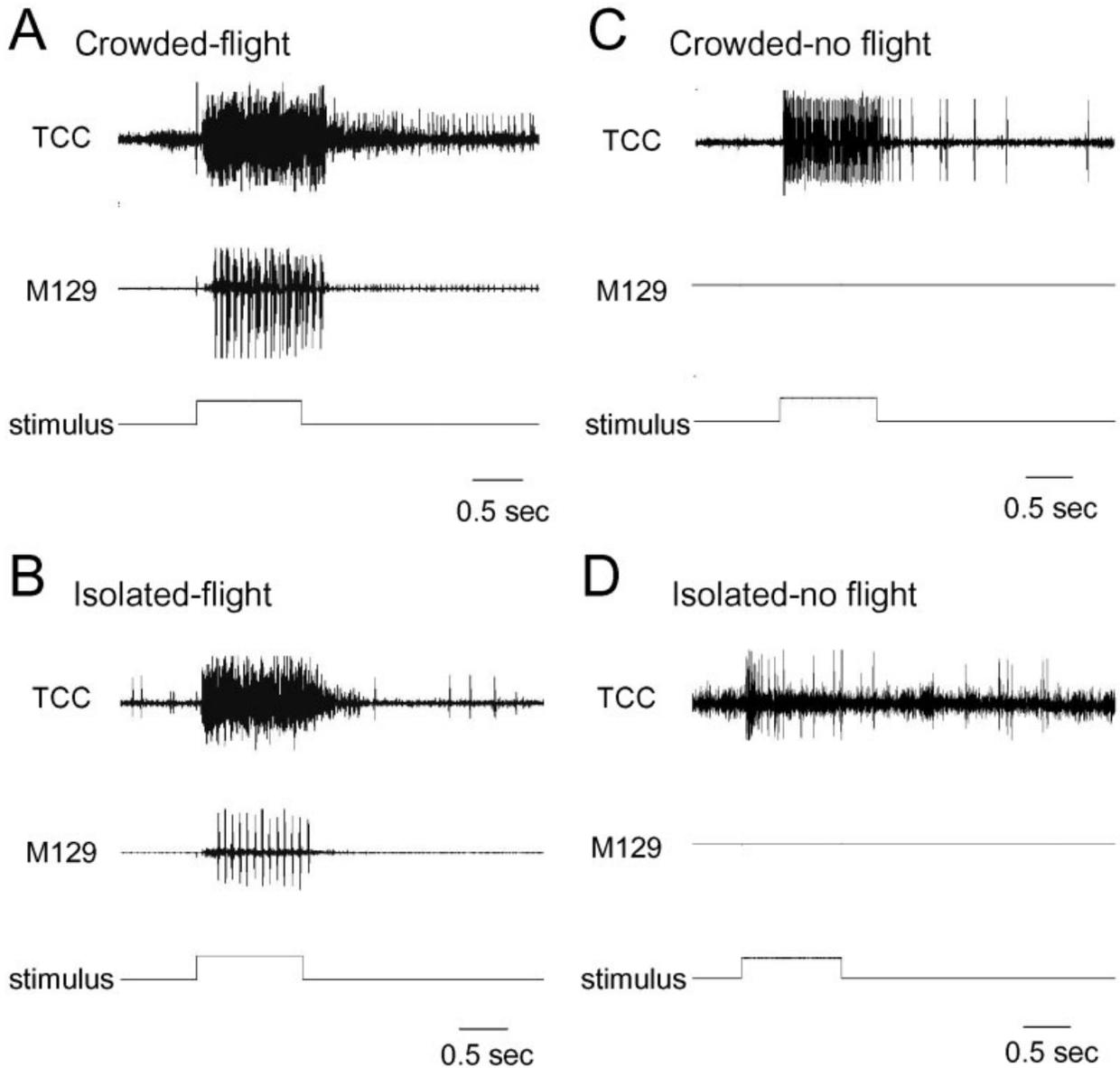


Figure 4 Extracellular recordings from the TCC and simultaneous EMG from a thoracic flight muscle during a 1000-ms wind stimulus of 3 m/s. The TCC neurons exhibited intense spiking activity in response to wind stimuli in cases when flight was demonstrated, independent of the locust phase (A, B). In contrast, in cases when the wind stimulus failed to initiate flight, strong excitation of the TCC was only seen in crowded locusts (C), while in nonflying isolated locusts the TCC response was greatly limited (D).

Thus experimental animals were categorized by phase and response (Figs. 4 and 5). In cases where an individual animal was induced to fly by one stimulus intensity while it failed to fly following another, it was categorized as “flying” or “nonflying” for the first and second stimuli, respectively. The extremely rare cases, in which a given stimulus to a locust sometimes elicited flight and in others did not, were categorized according to the prevalent reaction. A sequential anal-

ysis did not show any significant change in TCC spike frequency within a series of repeated stimuli (time interval of 30 s between two consecutive stimuli). Thus, the consecutive stimuli were separated enough not to affect each other and the average frequency of each series is sufficient to represent the response within that series.

When flight was induced both phases exhibited rather similar intensive TCC spiking activity, signif-

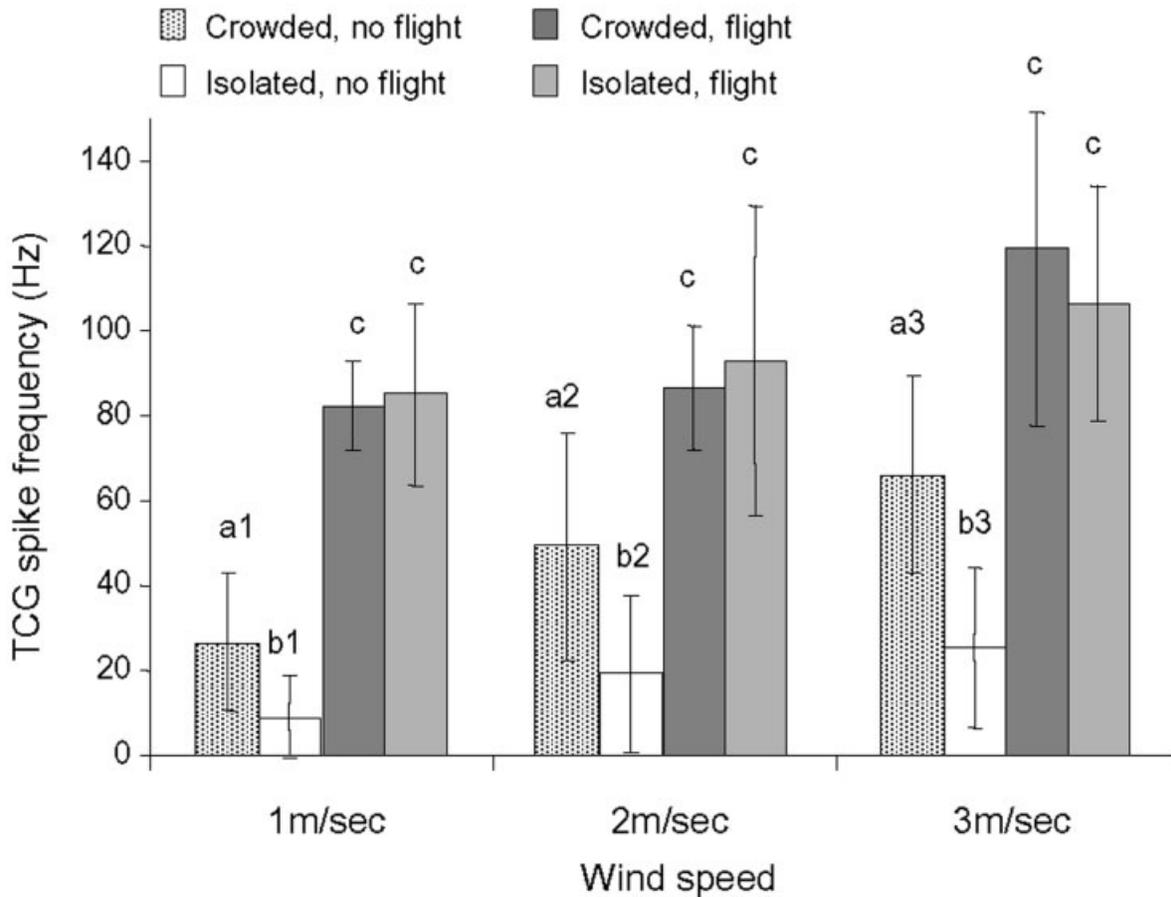


Figure 5 The mean TCG firing rate of the two locust phases during three different wind stimuli. Data calculated separately for cases when flight was elicited and for when it was not. The response of a “nonflying” crowded animal was always significantly more intense than that of an isolated one. Crowded and isolated “flying” locusts had a similar TCG response, independent of the stimulus strength. This response was significantly higher than that exhibited by “nonflying” locusts of both phases. Data show mean values \pm S.D., $n = 13$ for crowded and isolated, no flight; $n = 7$ and 4 for crowded and isolated, flight (respectively). p -Values for significantly different groups are marked by different letter when $p < 0.01$, and by the same letter and different index number when $p < 0.05$ (one-way ANOVA and Bonferroni test).

icantly stronger than that of “nonflying” locusts (Figs. 4 and 5). The response seemed to be independent of stimulus strength and of the locust phase (stronger responses at the higher wind speed and in crowded animals were not significantly different from others). Once flight was initiated EMG recordings of flight muscles also did not reflect significant phase differences in muscle activity ($p > 0.5$ for all wind speeds, $n = 7$ and 4 for crowded and isolated, respectively). In cases where flight was not elicited, both phases showed an increase in TCG response with intensifying wind speeds (Fig. 5). However, for any given wind speed crowded locusts demonstrated significantly stronger TCG responses than isolated ones [Fig. 5; see also Fig. 4(C) and (D)].

To obtain a more detailed analysis of the TCG response profile, the stimulation period was divided into 10 bins of 100 ms each, and the firing rate was calculated separately for each bin. The wind-induced spiking activity of the TCG within a stimulus that did not elicit flight, showed continuous spike frequency adaptation. This was true for both phases at any given stimulus strength. The strength of adaptation did not depend on activity level (no correlation was found between the adaptation level and mean firing frequency in the first 100 ms of the stimuli). Therefore, it was possible to apply a measure of spike frequency adaptation by calculating normalized activity level: the ratio of the number of spikes within each 100-ms bin versus the number of spikes at the onset of the response [Fig. 6(A)].

As the data in Figure 6 suggest, there was a clear phase-dependent difference in the adaptation properties of the TCG between the two locust phases. Spike frequency adaptation was much stronger in isolated locusts compared to crowded ones. As the stimulus intensity increased, the differences in the strength of adaptation between the phases became more decisive and significant.

The major decline in spike frequency (more than 75% of the change) occurred within the first 200 ms of the stimulus period [Fig. 6(A)]. To obtain a more detailed insight into the firing pattern in this time window it was examined at a higher resolution, using 20-ms bins. As shown in Figure 6(B) for wind stimuli of 3 m/s, the TCG response of the two phases showed significantly different adaptation rates. The time constant of adaptation (derived from the exponential fit) of the crowded locusts TCG was twice as long as that of the isolated locusts TCG [Fig. 6(B)]. Similar differences were obtained for the other wind speeds tested.

In contrast to the strong spike adaptation the TCG exhibited when flight failed to be induced, the TCG response to “flight-inducing” stimuli remained at a high-frequency level throughout the stimulus (less than 20% decline from frequency at the onset of the response), both in crowded and isolated locusts [Fig. 4(A) and (B)].

Next, we examined the rate at which the wind stimulus is conducted to flight muscles in both phases by measuring the onset of wind-induced flight muscle activity (in cases where flight was initiated). Due to small variations in the exact distance between the shutter and the locust head, and some minor inconsistency in the opening response of the shutter, we concentrated on the latency between the first TCG action potential and the onset of flight muscle activity [Fig. 7(A)]. Previous results had indicated that the latency between stimulation of head wind-sensitive hairs and the first TCG spike (4 ms in crowded-reared locusts; Kutsch and Hemmer, 1994a) is negligible relative to the total period from hair stimulation to flight initiation. The onset of flight muscle activity was calculated for each animal at the three different wind speeds and was found to be significantly shorter in crowded locusts compared to isolated ones [Fig. 7(B) shows the results for 3 m/s].

Flight usually terminated with the termination of the wind stimulus [Fig. 4(A) and (B)]. At higher wind speeds crowded-reared locusts showed a nonsignificant tendency to sustain muscle activity several milliseconds beyond the end of the stimulus (data not shown). Thus, we characterized the TCG activity pattern at stimulus termination. Baseline activity was

defined and calculated as twice the average TCG spontaneous frequency recorded during a 20-s time window just prior to the stimulus. The time from termination of a stimulus until the TCG resumed this baseline activity was measured [marked by an arrow in Fig. 8(A)]. Data were calculated separately for stimuli that initiated flight and for those that did not. In addition to a pronounced difference between “flyers” and “nonflyers,” crowded-reared locusts always took longer to return to prestimulus activity compared to isolated ones [Fig. 8(B)].

DISCUSSION

Behavioral plasticity is an important adaptive process that occurs during the lifetime of an individual as a response to environmental variations. Neural correlates of behavioral plasticity are a major topic of neurobiological research, as they are highly instructive regarding neural mechanisms of behavior (e.g., Balaban, 2002; Hobert, 2003; Rohrbough et al., 2003). Locust density-dependent phase polymorphism is, among other things, a remarkable and extreme case of behavioral plasticity. It is unique, because the behavioral changes are, on the one hand, a response to an environmental change, and on the other, a stimulant-catalyst of various other environmentally induced physiological changes. Furthermore, the changes are continuous and reversible at any stage.

One component of locust behavioral phase difference, which is a key feature of locust biology and is central to their occasional yet catastrophic impact on humans, is the more intense flight behavior of gregarious locusts in comparison to that of solitary ones (reviewed in Pener, 1991). The current study was aimed at finding density-dependent differences in neural properties and mechanisms that correspond to the phase-dependent behavior.

The role of wind stimuli in generating locust flight has been thoroughly studied and reported (Weis-Fogh, 1949; Bacon and Mohl, 1979; Bicker and Pearson, 1983; Kutsch and Hemmer, 1994b). We focused on a very well-characterized neural track connecting wind receptors to flight motor centers, which includes a pair of identified descending brain interneurons the TCGs (Bacon and Tyrer, 1978). Our findings reveal various differences in TCG spiking activity between the locust phases; i.e., more intense spontaneous as well as wind-induced firing in crowded-reared locusts compared to isolated ones. By counting the number of wind-sensitive hairs in excitatory fields on the locust head, we confirmed that the phase differences in TCG

activity levels do not result merely from a higher number of wind-sensitive receptors in crowded-reared animals. On the contrary, based on the number of sensory receptors, one could expect a stronger response in the isolated animals. Because the number of facial setae increases throughout successive larval in-

stars up to the adult stage (Sviderskii, 1969) it was not surprising that isolated locusts (which have an additional larval molt) were found to have a higher number of head hairs. Thus, the firing properties of the TCG interneuron reflect well the higher excitability and higher capacity of gregarious locusts to initiate both repeated and sustained flights.

In contrast, but again in agreement with their inferior flight behavior, the dominant property of the TCG of isolated-reared locusts was that of strong spike frequency adaptation. Spike adaptation is an important adaptive property of neurons, which is attributed to Ca-activated K currents (Hille, 1992; Guckenheimer et al., 1997). More work is needed to locate the site of adaptation, which could be at the level of the TCG itself, at the level of the sensory receptors, or at the level of the synaptic connections between the two (or in any combination of the above). Camhi (1969) had demonstrated that the sensory cells innervating each locust wind-receptor hair respond to a persistent wind stimulus with a slowly adapting train of impulses. The sensory cells are excited when the sensillae are inclined, and the frequency of their slowly and incompletely adapting responses is proportional to loss of incline by the hair (Varanka and Svidersky, 1974). The rate of adaptation reported by Camhi (a decrease of 37.5% from the initial rate within 250 ms, followed by a plateau) was quite similar to the adaptation exhibited by the TCG responses of crowded locusts in our study. However, it was not sufficient to explain the rapid adaptation results we obtained in the TCG of isolated locusts; a decrease in firing rate of more than 77% within the first 250 ms.

Spike frequency adaptation has been reported to be a major target for neuromodulation, both in invertebrates and vertebrates neurons (e.g., Pedarzani and Storm, 1993; Cox et al., 1994; Katz and Frost, 1997). Most interestingly, Lorenzon and Foehring (1993) have reported changes in the intensity of adaptation in rat neocortical neurons during the animals' postnatal

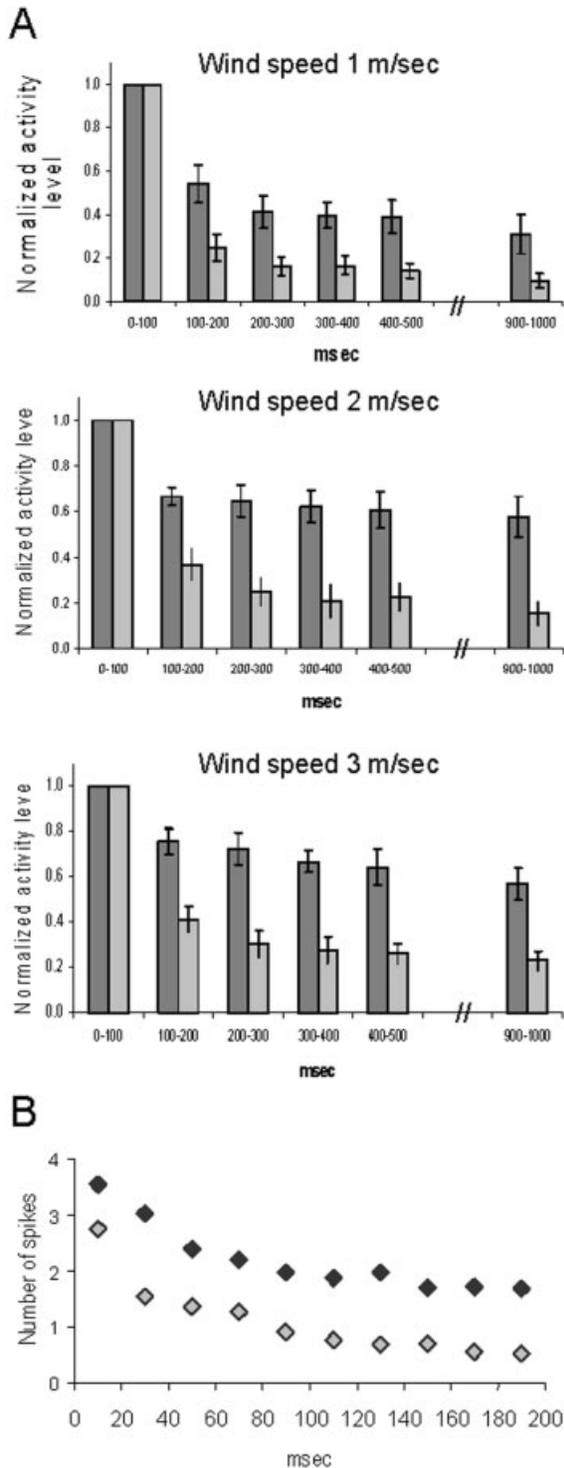


Figure 6 (A) Spike frequency adaptation of the TCG interneuron during a 1000-ms wind stimulus in cases when flight was not induced (three different wind speeds). Data show the mean spiking rate (\pm S.E., $n = 11$) in 100-ms bins normalized by the activity in the first bin. Crowded-reared locusts—dark bars, isolated—light bars. (B) The TCG spiking activity during the first 200 ms of a 3 m/s wind stimulus. The average number of spikes was calculated in 20 ms bins. A single exponential fit was calculated for the isolated (light gray) and crowded locust TCG data (dark). The time constant derived from the exponential fit was 119.0 s and 243.9 ms, for the isolated and crowded locust respectively.

ontogeny. This was attributed to the effects of the neuromodulator norepinephrine.

One possible candidate for a neuromodulator that could be partially responsible for the differences observed in the interneurons' physiological properties between the locust phases (adaptation as well as other) is the biogenic amine octopamine. Octopamine is often correlated with arousal state in insects (Davenport and Evans, 1984; Parker, 1996). It also plays an important role in locust flight (Stevenson and Kutsch, 1988; Parker, 1996; Pfluger and Duch, 2000). Released at the onset of flight, from DUM neurons in the locust segmental ganglia into the hemolymph, octopamine has a variety of effects on the central and peripheral nervous systems (Ramirez et al., 1989; Parker, 1996; Pfluger and Duch, 2000). Moreover, it has been suggested that octopamine, when injected to the hemolymph, increases the TCG response to frontal wind puffs (Ramirez et al., 1989). Further inves-

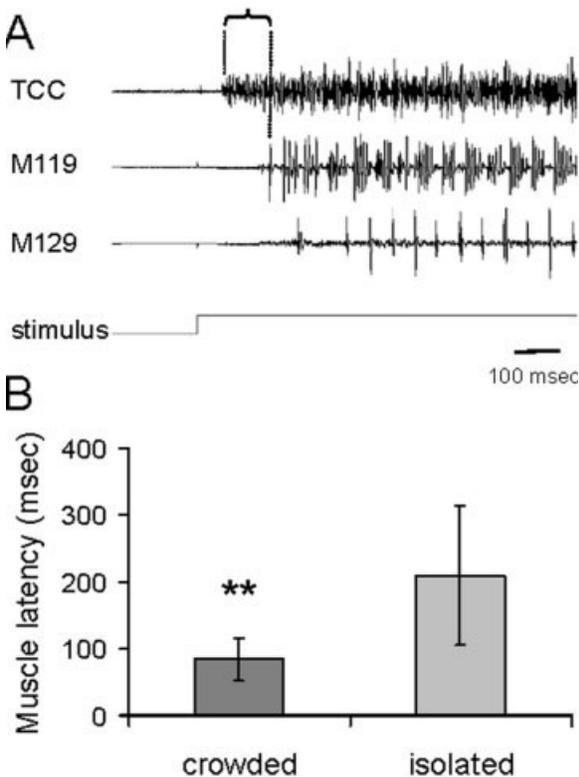


Figure 7 (A) Extracellular recordings from the TCC and simultaneous EMG from a pair of metathoracic flight muscles of a crowded reared locust during wind stimuli. (B) The latency of wind-induced flight muscle activity calculated as the time between the first TCG action potential and the electrical activation of elevator flight muscles [marked in (A)]. Data show mean values \pm S.D., $n = 10$ and 4 for crowded and isolated, respectively, $p < 0.01$, unpaired, two-tailed t test.

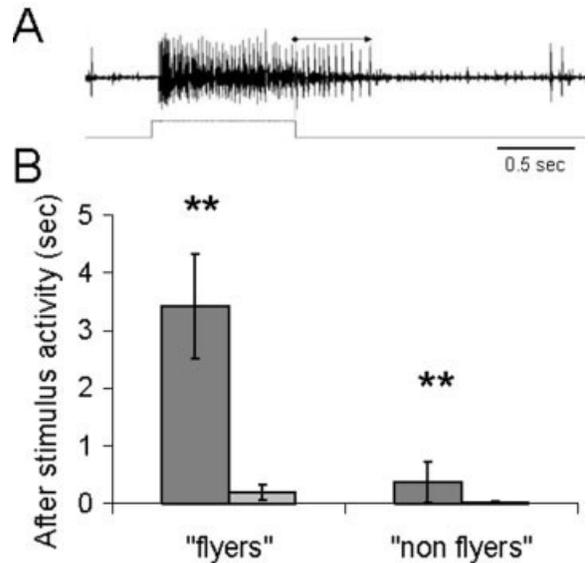


Figure 8 (A) Extracellular recordings from the TCC of a crowded locust during wind stimulus. An arrow indicates the activity of the TCG after stimulus termination. (B) Time from stimulus termination until the TCG resumed its baseline activity (defined and calculated as twice the average TCG spontaneous frequency recorded during a 20-s time window just prior to the stimulus). Data (mean \pm S.D.) shown separately for stimuli that elicited flight and for stimuli that did not. Independent of flight the TCG of crowded locusts (dark) remained in an excited (spiking) state much longer than that of isolated locusts (light) $p < 0.01$, Unpaired, two-tailed t test. $n = 13$ for crowded and isolated, no flight; $n = 7$ and 4 for crowded and isolated, flight (respectively).

tigation is needed to determine whether the higher excitation level of crowded locust TCG results from higher levels of octopamine in the hemolymph.

Recent preliminary studies on crowded locust free flight capacities under various illumination conditions revealed positive correlation between flight initiation, flight duration, and light intensity, with a clear light threshold (Fuchs, Ayali, and Kutsch, unpublished). These findings are consistent with the diurnal gregarious flight behavior previously described in field observations. Based on our current findings, the TCD interneuron appears to be a good candidate to serve as part of the neural basis of this behavior. No behavioral role has yet been assigned to this GABA immunoreactive (=inhibitory) interneuron. Tyrer et al. (1988) reported that its activity is suppressed by wind on the head and periods of tethered flight. In addition these authors suggest that the TCD is sensitive to illumination level, generally showing greater spontaneous activity in the dark. We found that while the TCD firing rate in isolated locusts did not change in the dark, a

remarkable and highly significant increase in TCD activity was observed in crowded locusts when the light was switched off. Could the TCD interneuron be (partially) responsible for limiting the swarms' migratory flights to increased light conditions, while not restricting isolated locusts' night flights?

One should not make the mistake of associating a specific interneuron with a discrete behavioral function while forgetting that many neural components work in parallel to produce and control behavioral patterns. The basic flight rhythm is generated within the thoracic ganglia and modified not only by facial hair receptors but also by the antennae and of course by sense organs associated with the wings (Bacon and Mohl, 1979, 1983). However, the TCG (and perhaps also the TCD) have an important role in flight initiation and control (Bacon, 1979; Bacon and Mohl, 1979, 1983; Bicker and Pearson, 1983). One can positively conclude that the observed phase-dependent differences in the interneurons' activity well reflect flight-related behavioral phase characteristics. More research is needed to confirm causative relations between the specific neural mechanisms studied and the behavioral phase changes.

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