

F. Libersat · G. Haspel · J. Casagrand · K. Fouad

Localization of the site of effect of a wasp's venom in the cockroach escape circuitry

Accepted: 19 December 1998

Abstract The parasitic wasp *Ampulex compressa* stings a cockroach *Periplaneta americana* in the neck, toward the head ganglia (the brain and subesophageal ganglion). In the present study, our aim was to identify the head ganglion that is the target of the venom and the mechanisms by which the venom blocks the thoracic portion of the escape neuronal circuitry. Because the escape responses elicited by a wind stimulus in brainless and sham-operated animals were similar, we propose that the venom effect is on the subesophageal ganglion. Apparently, the subesophageal ganglion modulates the thoracic portion of the escape circuit. Recordings of thoracic interneuron responses to the input from the abdominal giant interneurons showed that the thoracic interneurons receive synaptic drive from these interneurons in control and in stung animals. Unlike normal cockroaches, which use both fast and slow motoneurons for producing rapid escape movements, stung animals activate only the slow motoneuron. However, we show that in stung animals, the fast motoneuron still can be recruited with bath application of pilocarpine, a muscarinic agonist. These results indicate that the descending control from the subesophageal ganglion is

presumably exerted on the premotor thoracic interneurons to motoneurons connection of the thoracic escape circuitry.

Key words Insects · Venom · Thoracic interneurons · Wasp · Subesophageal ganglion

Abbreviations *Df* fast coxal depressor motoneuron · *Ds* slow coxal depressor motoneuron · PSP postsynaptic potential · *SEG* subesophageal ganglion · *TIA* thoracic interneuron type A · *vGI* ventral giant interneuron

Introduction

Animals are not automatons which respond in set ways to a specific stimulus. For instance, a stimulus known to initiate a certain behavior in an animal, may or may not elicit that behavior at any given time depending on the intensity of the stimulus and the internal state of the animal. This internal state, which determines, among other behavioral features, the threshold for initiation of behavior, is often referred to as the motivational or arousal state (Marrocco et al. 1994). Our understanding of the neural basis of arousal has undoubtedly benefited from studies on animals with relatively simple nervous systems controlling reproducible stereotypic behaviors (Kravitz 1988; Bicker and Menzel 1989; Teyke et al. 1990; Ziv et al. 1991). The "escape behavior" of the cockroach is one such highly stereotypic behavior. In nature, a common stimulus is the tiny wind gust produced during the strike of a predator such as a toad (Camhi 1984). However, depending on the behavioral context, the same wind stimulus does not always elicit escape behavior (Camhi and Nolen 1981; Libersat 1992; Watson and Ritzmann 1994). Thus, there must be some mechanism operating in the cockroach's nervous system that can alter the excitability of the escape neuronal network such that it is ready to respond at some times and not at others.

The escape behavior of the cockroach is initiated by a wind puff applied to the cerci, two posterior sense organs bearing wind-sensitive hairs. Sensory neurons associated

F. Libersat (✉) · G. Haspel · J. Casagrand¹ · K. Fouad²
 Department of Life Sciences,
 The Zlotowski Center for Neuroscience,
 Ben-Gurion University of the Negev,
 Beer-Sheva 84105, Israel

Present addresses:

¹ Center for Neurosciences and Department of Biology,
 EPO, University of Colorado at Boulder, Boulder,
 CO 80309-0334, USA

² Brain Research Institute, University of Zurich,
 AugustForel-Strasse 1, CH 8029 Zurich, Switzerland

Address for correspondence:

Dr. Frederic Libersat, Division of Neurobiology,
 Arizona Research Laboratories,
 Gould-Simpson Science Building 611,
 University of Arizona, Tucson, Arizona 85721, USA
 e-mail: libersat@manduca.neurobio.airzona.edu
 Fax: +1-520-621-8282

with these hairs monosynaptically excite two groups of giant interneurons in the last abdominal ganglion. The ventral giant interneurons (vGIs) control the initiation of rapid escape movements when the animal is on the ground (Camhi 1984; Comer and Dowd 1993; Ritzmann 1993). The giant interneurons send their axonal projections to the locomotory centers in the three thoracic ganglia where they activate, via pluri-segmental and local interneurons, a pool of motoneurons involved in producing the escape leg movements (Ritzmann and Pollack 1986; Ritzmann 1993; Liebenthal et al. 1994).

The parasitic wasp *Ampulex compressa* hunts cockroaches as a live food supply for her larvae (Williams 1942). In contrast to the venom of most arthropods, the venom of *A. compressa* is injected into the cockroach's prothoracic ganglion and then into the head (Piek et al. 1989; Fouad et al. 1994). The sting does not block the neuromuscular transmission and hence, the cockroach prey is not paralysed (Fouad et al. 1996). Instead, this venom has a dramatic and unique effect on the cockroach locomotory behaviors (Piek et al. 1989; Fouad et al. 1994): the cockroach can still walk, but does not show an escape response to tactile or wind stimuli. The long lasting changes in the cockroach inability to initiate an escape response occur only when the venom is injected into the head but not when injected only into the thorax (Piek et al. 1989; Fouad et al. 1994). It has been shown that in insects, input from the head ganglia – the supraesophageal ganglion (brain) and the subesophageal ganglion (SEG) – appears to be necessary for the expression of the various motor patterns including the escape behavior (Huber 1965; Kien and Altman 1992). Irrespective of where the venom is injected, it could affect a local circuit in the SEG or a distributed circuit located in both head ganglia, the SEG and the brain. For instance, assuming that the neurotoxin is injected in the SEG, as appears to be the case in another parasitoid wasp (Gnatzy and Otto 1996), it could affect neurons in the SEG which project anteriorly to the brain and there control the activity of another group of brain neurons which would control the thoracic escape network. An alternative scenario, which is our hypothesis, is that the neurotoxin is injected inside the SEG, where it affects a local circuit of neurons which controls the thoracic escape network. Thus, the first objective of the experiments presented in this paper was to determine which head ganglion is the most likely target of the venom.

In stung animals, we observed a complete suppression of the escape response. However, the response of the wind-sensitive giant interneurons in the last abdominal ganglion or of the antennal tactile-activated brain interneurons, both of which normally activate escape, is unaffected in stung animals (Fouad et al. 1994, 1996). Therefore, the site of the venom effect on the escape neuronal network is most probably the thoracic portion of the escape circuitry that is involved in organizing the motor patterns of leg movements. Thus, the second objective of this work was to characterize the effects of the venom of *A. compressa* on the thoracic interneurons and

motoneurons of the escape system of the cockroach. More specifically, we investigated whether the escape thoracic interneurons receive normal synaptic drive from the abdominal giant interneurons. Subsequently, we tested whether a specific motoneuron, the fast coxal depressor motoneuron (Df), which is critical for producing rapid leg escape movements, is directly modulated by the venom injection into the head or indirectly via an effect on pre-motor elements of the thoracic escape circuit.

Material and methods

Animals

Wasps, *A. compressa* Fabricius (Hymenoptera: Sphecidae), were raised on a 12L:12D cycle as described by Fouad et al. (1994). All experiments were performed on adult male cockroaches (*Periplaneta americana*) raised at 25–30 °C in plastic barrels and provided with water and cat food pellets ad libitum. Stung cockroaches were obtained by placing an animal in a chamber with a female *A. compressa* until it was stung. All stings were confirmed visually. To ensure that the venom injection was successful, animals were prepared for recording about 2 h after being stung and after testing their responsiveness.

Lesions

For insect anesthesia and surgery, we designed a cooling system consisting of a "Peltier" thermoelectric cooling device (Melcor, N.I, USA) controlled by a digital thermostat (Conrad Electronics; Conrad Electronic Center, Frankfurt, Germany) and mounted on a stainless-steel heat sink. The Peltier device cooled the animal down to 5 °C and kept it motionless for as long as necessary. Animals anesthetized in this way recovered within 10–15 min.

After the cockroach had been anesthetized, a small flap of the cuticle was opened between the compound eyes. The brain was surgically removed and the cuticular flap was sealed back in place as a result of hemolymph coagulation. To disconnect both brain and SEG from the thoracic motor circuitry, a longitudinal incision was made in the ventral cuticle of the neck and the cervical connectives were cut with fine scissors. The wound sealed itself by hemolymph coagulation. All animals were allowed to recover for 1 day before being tested. In some experiments, the lesioned animals were exposed to wasps for stinging.

Recordings

Intact animals

Stung or normal cockroaches were pinned through the lateral parts of the abdominal segments, dorsal side up on a small wax platform in a plastic Petri dish coated with vegetable oil (Fig. 1A). In such a fixed position, cockroaches were able to move their legs in bouts of almost friction-free stationary "walking" and "running" similar to that of free-ranging animals (Camhi and Nolen 1981). The spontaneous walking and escape movements of the cockroach were monitored with electromyogram wire electrodes placed in the coxal depressor muscle M177 of the metathoracic leg (Carbonell 1947). In most preparations we identified two motor units of different amplitude: a very large unit corresponding to the activity of the Df and a smaller unit corresponding to that of the slow coxal depressor motoneuron (Ds). In these tethered-walking-cockroach experiments, we first measured the duration of spontaneous walking immediately after the animal had been tethered for 30 min. To quantify escape behavior we applied calibrated wind stimuli to the cerci via a custom-built wind stimulator.

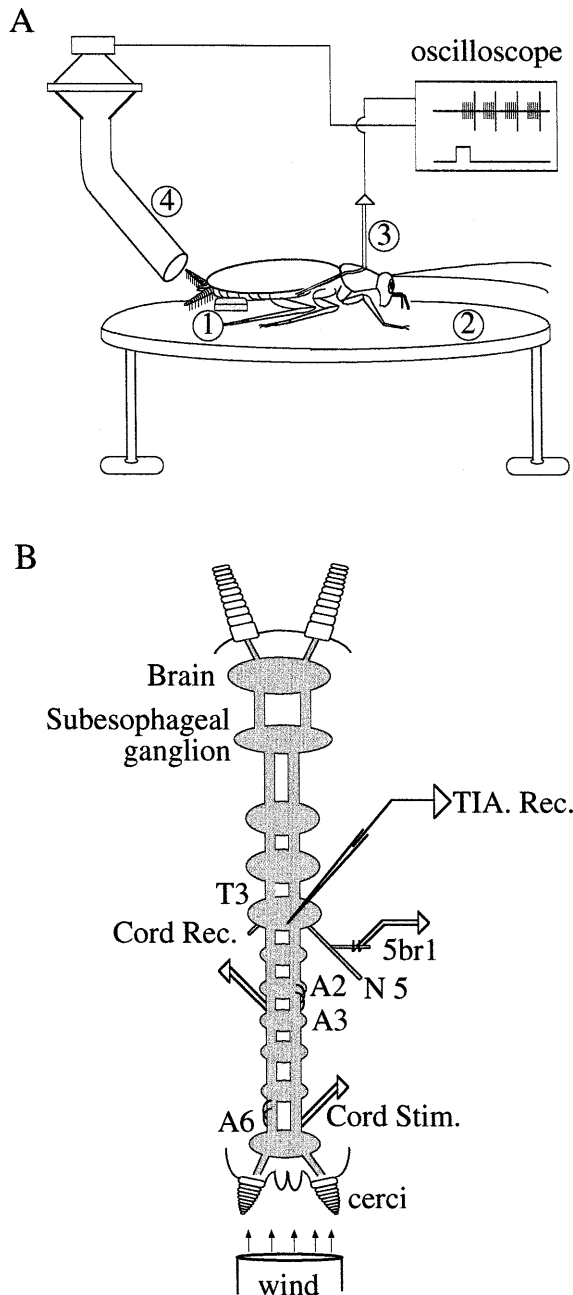


Fig. 1 **A** The experimental set-up for recordings in intact animals. The cockroach was connected to a small waxed platform (1) positioned on a Petri dish (2) coated with vegetable oil. In such a fixed position, cockroaches are able to produce short bouts of stationary walking and running. We monitored the walking and escape movements of the cockroach with electromyogram electrodes (3) placed in the coxal segment of a metathoracic leg. Calibrated wind stimuli were generated by a loudspeaker connected to a tube via a funnel (4) and aimed at the cerci. **B** The experimental set-up for recordings in dissected preparations. The diagram shows the cockroach central nervous system with the positions of all recording and stimulating electrodes. After desheathing, the metathoracic ganglion was supported by a platform during impalement with microelectrodes (*TIA. Rec.*). A Type-A thoracic interneuron (TIA) was impaled near the midline in one of its neuropil branches, and its responses to stimuli were observed in control and stung animals. Bipolar hook electrodes were placed under the A2-3 connectives for extracellular recording (*Cord Rec.*) of spontaneous and evoked activity in the abdominal nerve cord, and under the A5-6 connectives (*Cord Stim.*) for extracellular stimulation of the ventral giant interneurons (vGIs) in some experiments. The activities of the fast and slow motoneurons of the coxal depressor muscle (Df, Ds) were recorded with hook electrodes placed around branch 1 of nerve 5 (*5br1*)

opening a window in the cuticle covering the metathoracic ganglion. The gut was removed to expose the nerve cord (Fig. 1B). Saline was superfused continuously over the nerve cord (Wafford and Sattelle 1986). The dorsal surface of the metathoracic ganglion was desheathed mechanically with a pair of fine forceps, and was supported on a platform during impalement with microelectrodes. A TIA was impaled near the midline in one of its neuropil branches with microelectrodes filled with 2% Neurobiotin (Vector Laboratories, Burlingame, Calif., USA) in 1 mmol l⁻¹ KCl (20–60 MΩ) and the response of the TIA to stimuli were monitored in control and stung animals. Impaled TIAs were recognized by their characteristic activity and responses to wind stimuli (Ritzmann and Pollack 1986, 1988). Wind puffs were generated as described by a loudspeaker connected to a tube via a funnel (Fouad et al. 1994). Generally, wind puffs had a velocity of 1 ms⁻¹. Tactile stimuli were produced manually with a fine-tipped glass rod.

The vGIs were stimulated extracellularly through bipolar silver hook electrodes placed on the abdominal nerve cord between the fifth and sixth ganglia. The stimulus intensity was adjusted so that it was just sufficient to evoke a single action potential in a large-diameter axon (Fig. 1B). Because the vGIs have by far the largest axons in the abdominal nerve cord, when a single action potential is evoked, it is usually from a vGI. The evoked and spontaneous activity was monitored by a second pair of hook electrodes placed anterior to the stimulation site between the second and third ganglia. Both pairs of hook electrodes were insulated with a petroleum jelly/mineral oil mixture.

After the physiological tests, neurobiotin was iontophoresed into the TIAs using a constant depolarizing current of 0.5–5 nA. Preparations were fixed in 2.5% glutaraldehyde and subsequently processed with Vectastain ABC kit (Vector Labs, Burlingame, Calif., USA) to label the cells. Cells were viewed in the wholemount of the ganglion with a compound microscope (Olympus BH2) for identification.

TIAs were identified on the basis of their unique morphology (Ritzmann and Pollack 1986), and were named according to a previously detailed three-digit numbering system (Westin et al. 1988; Ritzmann and Pollack 1990). The TIA population may be further subdivided into three distinct sub-populations, designated the dorsal posterior group (DPG), the ventral median cell and the local interneurons. Some of the TIAs receive inputs from vGIs on both sides, while others only receive inputs from vGIs on one side. The experiments reported here are from 11 TIAs in control animals and 12 TIAs in stung animals (representing 5 different TIAs: Local 131, and DPGs 301, 501, 701, and 703).

The heart rate was measured by an impedance converter (UFI: Model 2991; Morroy Bay, Calif., USA). Briefly, two tungsten electrodes were inserted on each side of the heart between the first and second abdominal segments, and the changes in impedance occurring as a result of changes in the hemolymph flow between the left and right electrode were measured. Stung or normal cockroaches were tethered by fixing a rod to the pronotum with wax and placed on plastic Petri dish coated with vegetable oil. The height of the Petri dish could be moved up and down with a mechanical manipulator to adjust the position of the cockroach's body relative to the platform.

Dissected preparations

Intracellular thoracic interneuron recordings

An animal was prepared for the Type-A thoracic interneuron (TIA) recording by pinning it dorsal side up on a cork platform and

Motoneuron recordings

We monitored the activity of the Df and Ds using hook electrodes as described by Fouad et al. (1996). These electrodes were placed around branch 1 of nerve 5 (5r1) which contains only five motor axons (Fig. 1B), those from the fast and slow excitatory and three inhibitory motoneurons to the coxal muscle (Pearson and Iles 1971). The metathoracic ganglion was exposed and superfused continuously with saline (Wafford and Sattelle 1986). Pilocarpine (Sigma) was dissolved in saline to a concentration of $5 \cdot 10^{-4}$ mmol l⁻¹ and bath applied to the ganglion.

Data analysis

Recordings of electrical signals were recorded on videotape (Data Neurocorder) and digitized with a NB-MIO-16 analog-to-digital board (National Instruments). The data were acquired analysed by a data acquisition/analysis software (Spike Studio; Eli Meir, Cornell University); multiple replicates ($n = 5$) were taken from each experiment, excepting for the number of spontaneous postsynaptic potentials (PSPs) in TIAs. Here, the number of PSPs greater than 1 mV in two 5-s intervals was measured. We therefore measured the number of spontaneous PSPs greater than 1 mV in the TIAs. The PSP amplitude was measured with a cursor function available in the data acquisition/analysis software. The number of spontaneous PSPs and the amplitude of PSPs from control preparations were pooled and compared with those from stung preparations. The Mann-Whitney Rank Sum Test was used for all statistical analyses involving the TIAs. These were performed using Sigmastat for Windows 1.0 (Jandel Scientific, Corte Madre, Calif., USA). The Fisher Exact Test was used for all statistical analyses involving the lesions experiments and the data on heart rate were analysed using A nova with planned comparison of means (GB STAT, Dynamic Microsystems). Data are presented as mean \pm standard deviation (SD). Mean number of spikes and other statistical parameters such as SD were calculated with a commercial statistical program (GB STAT, Dynamic Microsystems).

Results

Localization of the site of effect of the venom in the head ganglia

To localize the site of the venom effect in the head ganglia, we tested the escape behavior of cockroaches with selective lesions of the head ganglia before and after being stung (Fig. 2A). Whereas sham-operated and stung animals showed a significant difference in their ability to respond to wind stimuli (Fisher Exact Test; $P < 0.001$), brainless cockroaches initiated escape responses as often as sham-operated animals (11/16 of brainless animals compared to 14/15 of sham-operated animals; $P = 0.172$). Conversely, only 1/8 of stung, and none of stung brainless (0/10) animals responded to wind puffs ($P = 0.44$; Fig. 2B). In addition, sham-operated and brainless animals exhibited spontaneous bouts of walking, in contrast to the other three groups (stung, brainless stung and neck connectives cut) which showed very little, if any, spontaneous walking. For instance, 8/12 of sham and 7/10 of brainless animals showed spontaneous walking, while none of the stung and only 1/10 of stung brainless animals expressed such behavior (Fig. 2C). None of the neck-connective-cut animals ($n = 8$) responded to the wind stimuli, and in that respect they did not differ from stung animals.

Because brainless animals escape from wind puffs and that venom effect occurs in the absence of the brain, these results suggest that the site of effect of the venom is most likely in the SEG.

We also measured the latency of the escape behavior as being the delay between the start of the wind trigger stimulus and the onset of electrical activity in the coxal muscles, as detected in the muscle recordings. The average escape initiation latency was 42 ± 6 ms ($n = 10$) in sham-operated animals and was not significantly different (t -test $P > 0.02$) from the latency of 53 ± 12 ms ($n = 13$) measured in brainless animals (Fig. 4A). However, we noticed specific differences in the escape responses of sham operated with that of brainless animals. First, sham-operated animals tended to run significantly faster (12 ± 3 steps/s; $n = 9$) than brainless ones (6 ± 3 ; $n = 13$) (Fig. 4B). Although the first motor burst following the wind stimulus always included the fast motoneuron in both the sham-operated and the brainless animals (Fig. 3), the following 10 steps almost always involved the fast motoneuron in each burst in sham-operated (87%, $n = 15$) but not in brainless animals (6%, $n = 16$).

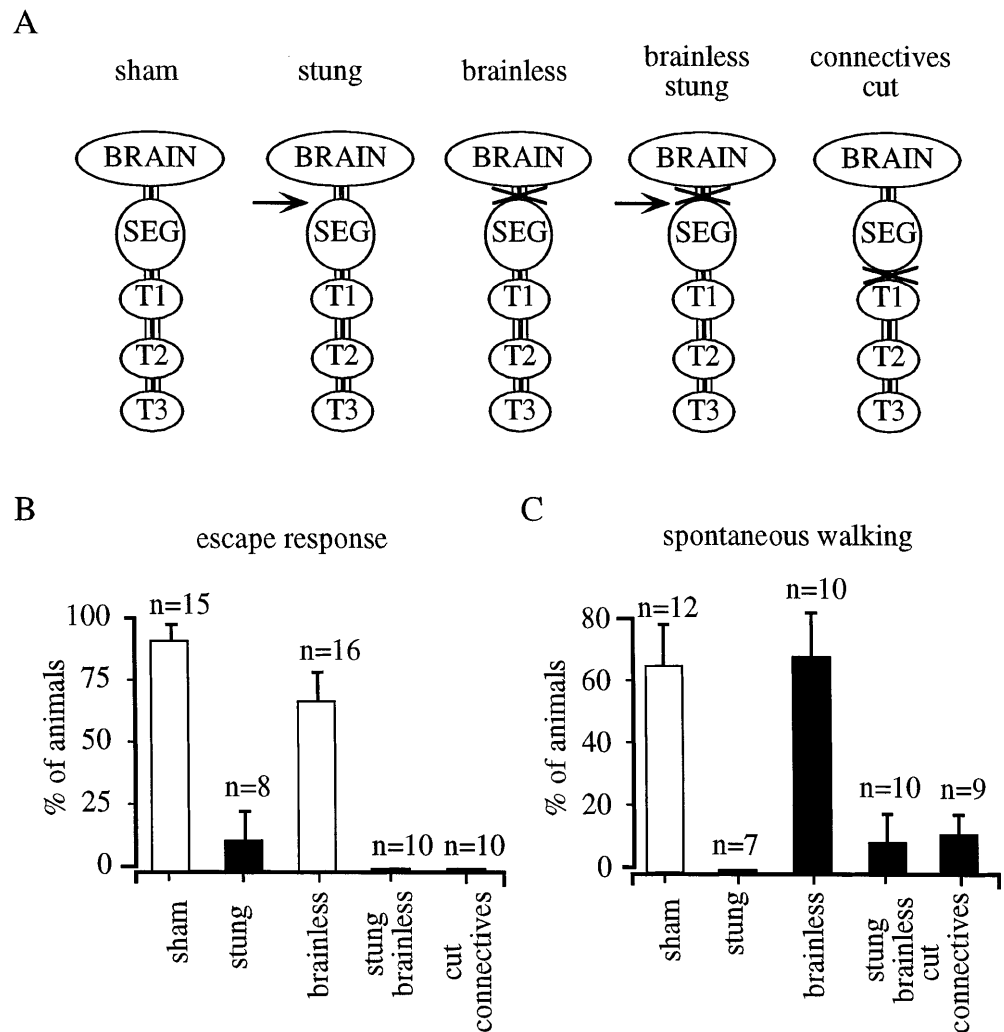
The SEG houses numerous neurosecretory neurons of which some are known to innervate the heart of insects and/or have an effect on heart rate (for review see Burrows 1996). With this fact in mind, we decided to determine if the heartbeat was affected in stung animals. Thus, we measured the heart rate of control ($n = 5$) and stung animals ($n = 6$) before and after an intense period of motor activity (Fig. 5). Our first observation was that the heart rate of resting stung animals was 104 ± 14 beats/min and always significantly higher ($P < 0.01$) than that of control animals (76 ± 9 beats/min). After a brief period of flight, the heart rate of control animals was significantly elevated by $37 \pm 19\%$ ($P < 0.01$). In contrast, the heart rate of stung animals remained unchanged ($P = 0.26$) after a period of flight. Thus, motor activity did not modulate heart rate in the stung animals whereas, as expected, it did in control animals.

Cellular aspects of the venom-induced depression of the thoracic portion of the escape circuitry

In a previous study (Fouad et al. 1996), it was shown that normal cockroaches use both fast and slow motoneurons for producing the rapid escape leg movements in response to a wind puff. In contrast, stung animals activate only the slow motoneuron and do not produce leg movements. In the experiments presented here, we wished to determine which portion of the thoracic circuitry is affected by the venom injected into the head. First, we analyzed the response of identified thoracic interneurons to wind stimuli and to direct electrical stimulation of the vGIs in control and stung animals. We then performed tests to determine if the venom injected in the head affects the thoracic fast motoneuron or

Fig. 2A–C Locomotory behaviors of cockroaches after selective lesions of the head ganglia.

A Different types of lesions performed on the head ganglia. *T1,2* and *T3*: thoracic ganglion 1, 2 and 3; *SEG*: subsophageal ganglion. **B** Histogram of the percentage of animals which showed a wind-evoked escape behavior in differently treated groups of animals. Likewise, the percentage of escape responses in the sham-operated and in the brainless animals was similar. **C** Histogram of the percentage of animal showing spontaneous walking in differently treated groups of animals. Notice that the percentage in the sham-operated and in the brainless was similar



premotor interneurons known to provide input to the fast motoneuron.

Does the venom affect the responses of the TIAs to their inputs?

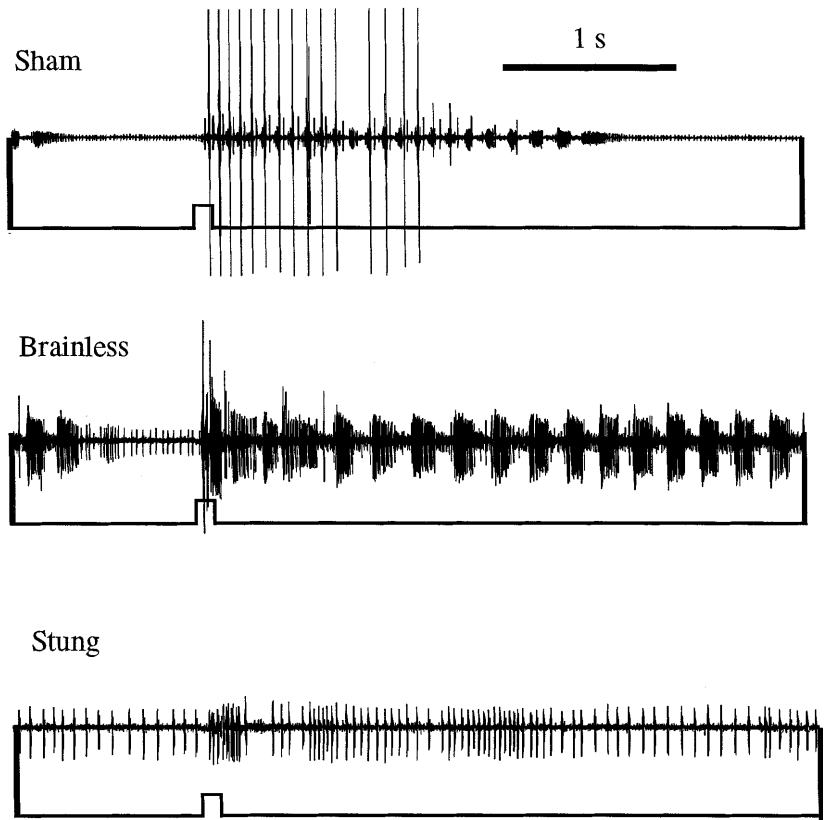
Spontaneous activity

Because the TIAs receive inputs from many sensory modalities (Ritzmann et al. 1991), there is usually considerable subthreshold background activity as shown in Fig. 6a. This background activity is one of the main identifying characteristics used in recognizing these cells during impalements. Considering the dramatic effect of the venom upon the escape response in stung animals, we thought it was possible that the background activity in the TIAs might be significantly depressed. We therefore measured the number of spontaneous PSPs greater than 1 mV in the TIAs, and found that the level of spontaneous background activity was significantly reduced in stung compared to control animals ($P < 0.009$, $n = 11, 12$), with the number of PSPs

occurring in a 5-s period by about half (53.7 ± 43.2 compare to 125.3 ± 46.4 , respectively).

The synaptic activity in the TIAs was correlated with action potentials recorded from the abdominal nerve cord (Fig. 6). Ritzmann and Pollack (1986) demonstrated that the largest-amplitude PSPs recorded in TIAs were correlated with the largest extracellular action potentials observed in the abdominal nerve cord (those of the vGIs), while smaller action potentials were always associated with the smaller amplitude PSPs or with no PSP. For example, Fig. 6 shows that where there are many action potentials, PSP summation occurs. This effect makes the correlation more difficult to visualize. However, when the action potentials occur singly, as in Fig. 7A, the correlation is more obvious. When looking at examples like this, we observed that although the incidence of spontaneous PSPs was reduced in TIAs in stung animals, the amplitude of PSPs associated with large action potentials was similar in TIAs from control and stung animals. These points are summarized in the histogram in Fig. 7C which shows the distribution of PSP amplitudes. The mean amplitudes of PSPs associated with large action

Fig. 3 Electromyogram recordings of a coxal depressor muscle in sham-operated, stung and brainless cockroaches. The slow motoneuron (small amplitude spikes in the electromyogram) is almost always tonically active. In sham-operated animals, the wind stimulus (*square pulse*) elicits an escape response with rapid leg movements which was characterized by rhythmic bursts of fast and slow motoneuron discharge (large- and small-amplitude spikes). In brainless animals, the wind stimulus elicits an escape response which consists of slower leg movements when compared to the sham-operated animals. The escape behavior is characterized by rhythmic bursts in the slow motoneuron and the recruitment of the fast motoneuron is rare and seen only at the onset of the escape response. In stung animals, the wind stimulus elicits a long-lasting burst in the slow motoneuron but no escape behavior; the fast motoneuron is never recruited



potentials was $4.26 \text{ mV} \pm 1.75$ in control and $4.58 \text{ mV} \pm 0.76$ in stung animals. These values were not significantly different from one another ($P = 0.231$, $n = 11, 8$). There was also no difference in the latency of the onset of these PSPs, measured from the time of occurrence of the action potential between A2–3

($1.46 \pm 0.23 \text{ ms}$ versus $1.3 \pm 0.22 \text{ ms}$) ($P = 0.768$; $n = 6, 9$)

vGI-evoked responses

The data described above for large, background action potentials suggested that the TIAs in stung animals were still receiving input from the vGIs, despite the observed reduction in overall spontaneous activity. More direct evidence to support this conclusion was provided by experiments in which the vGIs were

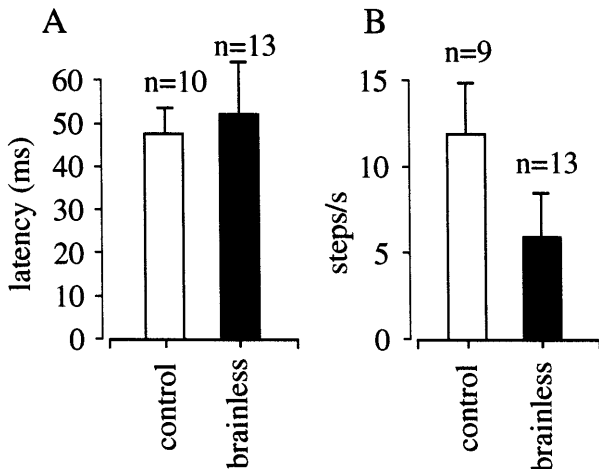


Fig. 4A,B Comparison of the escape responses produced by sham-operated and brainless animals. **A** The latency range of the escape behavior in sham-operated animals (control) is comparable to that of brainless animals. **B** The stepping rate is faster in sham-operated animals (control) compare to that of brainless animals

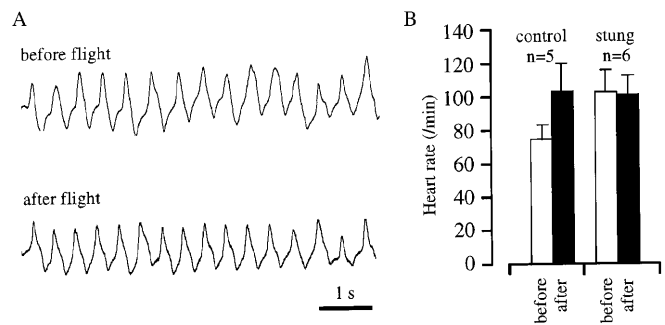
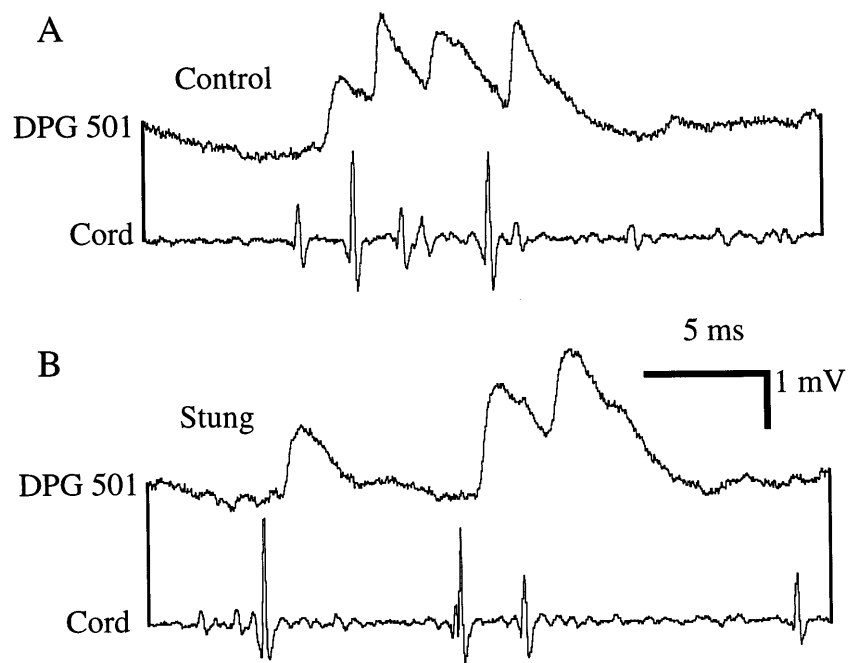


Fig. 5A,B Comparison of the heart rate modulation in control and stung animals. **A** Traces showing the heartbeat measured with electrodes connected to an impedance converter before flight and after flight in a stung cockroach. **B** Histograms of the average heart rate before (*empty bar*) and after (*black bar*) flight in control and stung animals. Notice the increase in heart rate after flight in the control group. In stung animals, heart rate is not different before and after flight

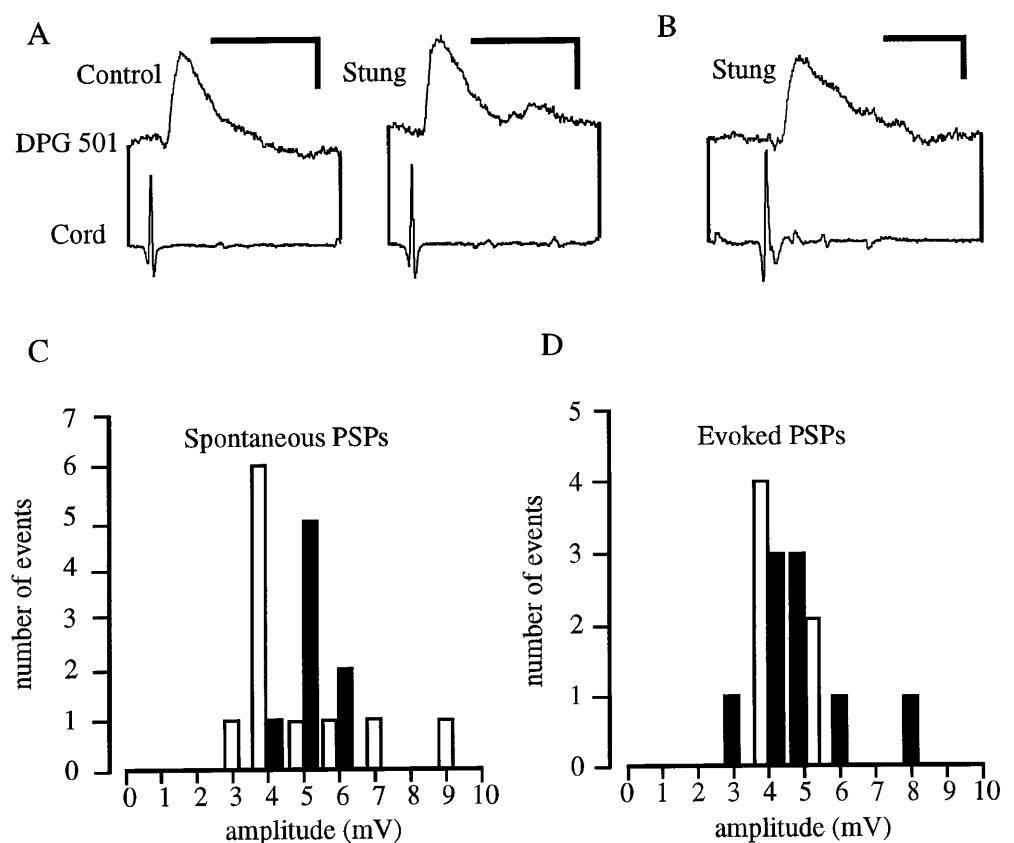
Fig. 6A,B Examples of background activity recorded in TIAs from a control and a stung animal. The *top trace* in each record is the intracellular record from a TIA, DPG 501, in a control (A) and a stung animal (B). The *bottom trace* is the extracellular recording of background activity in the abdominal nerve cord



stimulated extracellularly with hook electrodes. The stimulus was adjusted so that a single action potential was evoked. Because the vGIs have the largest axons in the abdominal nerve cord, when a single action potential is evoked, it is usually from a vGI. The TIAs

responded to extracellular vGI stimulation with a characteristic short-latency, excitatory response (Fig. 7B). There was no significant difference ($P = 0.768$) in the mean amplitude of the evoked PSPs recorded in TIAs from control and stung animals

Fig. 7 A Example of single postsynaptic potential (PSP) in a DPG 501 correlated with background, large-amplitude action potentials presumably from the vGIs in a control and a stung preparation. Calibrations: vertical, 1 mV; horizontal 5 ms. **B** Example of single PSP in a DPG 501 evoked by extracellular stimulation of a vGI in a stung preparation (calibrations: same as in A). **C** Histograms showing the distribution of amplitudes of PSPs recorded in thoracic interneurons in response to background, large-amplitude action potentials. The *abscissa* represents the amplitude of the PSPs binned by 1-mV increments and the *ordinate* indicates the numbers of PSPs (*empty bars* for control and *filled bars* for stung animals). **D** Same as C but for PSPs evoked by extracellular stimulation of vGIs



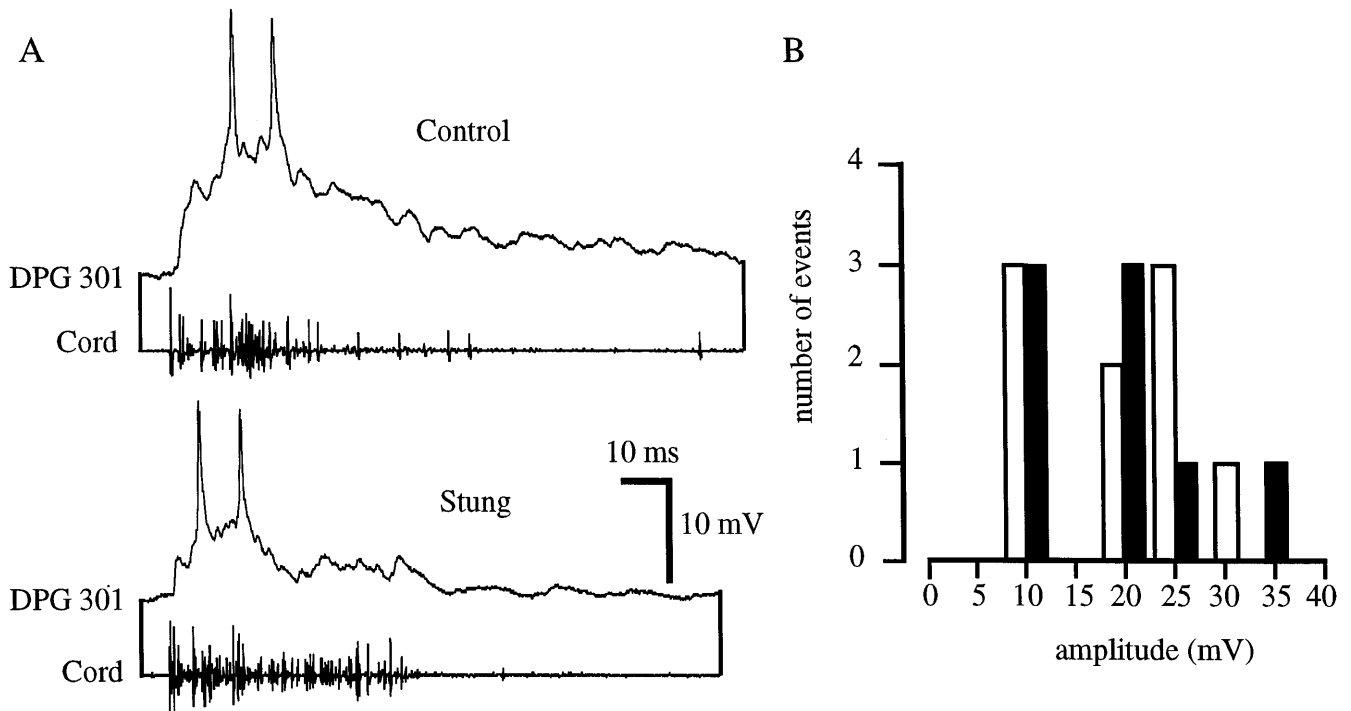


Fig. 8 A Responses to wind stimulus in TIAs from control and stung animals. Wind stimuli produced compound, depolarizing PSPs in the TIAs which were generally supra-threshold. **B** Histogram showing amplitudes of compound PSPs recorded in thoracic interneurons in response to wind puffs. The *abscissa* represents the amplitude of the PSPs in mV and the *ordinate* indicates the number of PSPs (*empty bars* for control and *filled bars* for stung animals). Compound PSP amplitudes were binned by 5-mV increments

(3.94 ± 0.97 mV versus 4.16 ± 1.6 mV; $n = 6, 9$; Fig. 7D). Nor was there a difference in the mean latency from vGI action potential to onset of the PSP in the TIAs (1.37 ± 0.21 ms, 1.42 ± 0.22 ms; $P = 0.953$, $n = 6, 9$) from control and stung animals.

Wind-evoked responses

Stung cockroaches no longer respond to wind or tactile stimuli with escape responses, although previous studies found normal wind-evoked activity in the vGIs (Fouad et al. 1994) and in the descending mechanoreceptive interneurons (Fouad et al. 1996). Since the synaptic responses of the TIAs to electrically evoked activity in individual vGIs was not different in control and stung animals, we next wanted to determine if responses to natural stimuli such as wind puffs was also unaffected in stung animals. These stimuli, which activate many giant interneurons and non-giant interneurons in the last abdominal ganglion, produced compound, depolarizing PSPs in TIAs whose mean amplitude in control animals (16.6 ± 7.43 mV, $n = 9$) was not significantly different ($P = 0.885$) from stung animals (16.1 ± 7.3 mV, $n = 8$). Although there was some individual variability between individual cases, there was again no significant difference in the distribution of the compound PSP

amplitudes of both groups (Fig. 7B). As with the background and evoked PSPs, there was no measurable difference in the onset latency of PSPs to wind stimuli ($P = 0.967$; $n = 10, 9$). The latency for wind activation of the PSPs, as measured from the first action potential in the abdominal nerve cord to the onset of depolarization of the TIAs, was 1.46 ± 0.34 ms in control and 1.47 ± 0.33 ms in stung animals. In addition, the threshold for action potentials was not significantly different in control and stung animals ($P = 0.596$; $n = 9, 9$). There was also no difference in the amplitude of the action potentials evoked by wind ($P = 0.595$; $n = 11, 9$), or in the number of action potentials per response evoked by wind ($P = 0.95$ rear wind, and 0.852 front wind; $n = 6, 8$). Although responses of the TIAs to tactile stimuli were not systematically tested, we observed that the responses to both antennal and cuticular stimuli were similar in control and stung preparations and consistent with previous data in both amplitude and waveform (Ritzmann and Pollack 1994).

Is the fast motoneuron of stung cockroaches recruited by other thoracic motor pattern generating circuits?

It has been shown that pilocarpine, a muscarinic receptor agonist, induces a slow locomotory rhythm in leg motoneurons including the fast coxal motoneuron (Ryckebusch and Laurent 1993; Buschges et al. 1995). With this effect in mind, we did test to determine if pilocarpine induces rhythmic patterns in leg motoneurons including the fast motoneuron of stung cockroaches. We recorded from branch 5r1 of the metathoracic ganglion the activities of the inhibitors, the slow (Ds) and the fast

(Df) motoneurons of a muscle of the coxal depressor group. In both groups of stung ($n = 8$) and control animals ($n = 8$) Df was silent, and Ds as well as the inhibitors were tonically active prior to the application of pilocarpine. At rest, the average firing rate of Ds was similar in both groups. Application of pilocarpine at a concentration of $5 \cdot 10^{-4} \text{ mmol l}^{-1}$ induced patterns of motoneuron discharge after roughly 5–10 min in 50% of the control preparations and 66% of the stung preparations (Fig. 9A). Shortly after applying pilocarpine, the firing rate of the Ds increased in both groups. Roughly 5–10 min after applying pilocarpine to both stung and control preparations, Ds was tonically active between the bursts and the instantaneous firing rate increased until a dense burst of Ds spikes occurred. During these high-frequency bursts of Ds spikes, Df was also activated (Fig. 9B). The burst interval was on average $3.5 \pm 1.5 \text{ s}$ ($n = 8$ stung preparations). Within each of these bursts, Df fired on average 50 ± 7 spikes. In most of the preparations, each burst consisted of the activities of the inhibitors, the slow and the fast motoneurons. To test whether pilocarpine induces changes in the fast

motoneuron excitability in stung preparations, we applied a wind stimulus, thereby recruiting the thoracic escape interneurons. Such a wind stimulus applied between two consecutive pilocarpine-evoked bursts failed to recruit the fast motoneuron (Fig. 9C).

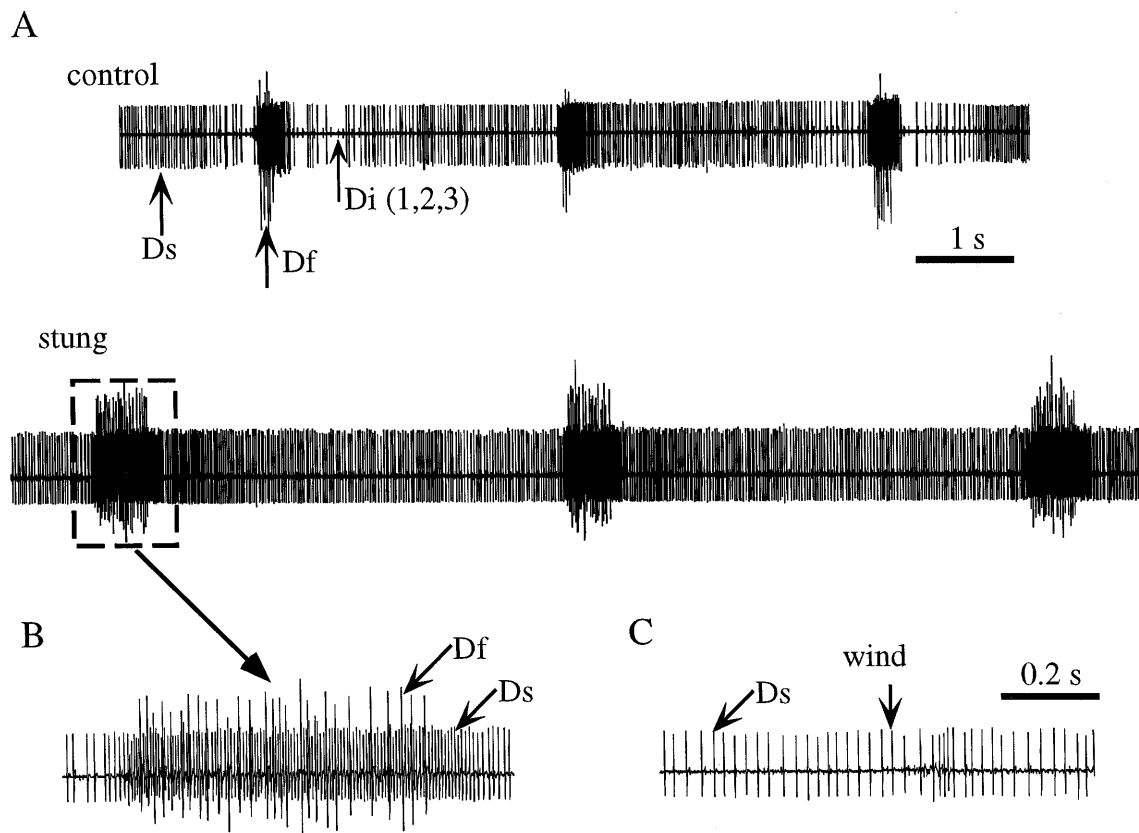
Discussion

The wasp *A. compressa* exerts control on specific locomotory behaviors of the cockroach by injecting venom into the head of its prey. In the present paper, we present evidence indicating that the venom targets the SEG and impairs the thoracic portion of the escape circuitry.

Control of the escape circuitry by head ganglia

Although locomotory motor patterns are produced in the thoracic ganglia of insects, the brain and the SEG appear to exert descending control on the expression of the various motor patterns (Kien and Altman 1992). Descending interneurons located in the brain and the SEG project to the thoracic ganglia. Although some of these descending interneurons in both the brain and the SEG are involved in the preparatory phase preceding walking and during walking, the SEG interneurons seem to contribute more than the brain interneurons to the preparatory phase (Kien and Altman 1992). During walking, the brain and SEG are necessary for the

Fig. 9A,B Pilocarpine initiates rhythmic patterns in both fast and slow coxal motoneurons of stung cockroaches. **A** In control and stung animals, pilocarpine ($5 \cdot 10^{-4} \text{ mmol l}^{-1}$), a muscarinic agonist induces bursts of action potentials in the inhibitors, the slow and the fast motoneurons. **B** One of the bursts occurring during the pilocarpine-evoked rhythm in the stung preparation expanded in time showing both Df and Ds spikes. **C** A wind stimulus fails to recruit Df



regulation of, among other things, the speed of walking. For the escape behavior, Roeder (1948) reported that the cockroach escape behavior is much reduced in headless cockroaches. Correlated with this, the response of fast coxal motoneuron to cercal stimulation is reduced in headless or neck-connectives-cut animals (Pearson and Iles 1970). Thus, there appears to be some descending influence from the head ganglia to control the excitability of the thoracic escape motor circuitry. We found that brainless animals generated escape responses to wind puffs, albeit about half as fast as control animals (Figs. 3, 4). In addition, after the onset of escape, the leg escape movements of the brainless animals did not involve the activity of the fast motoneuron (Fig. 3). A cockroach responds to the approach of a predator with rapid turning and then fast running (Camhi 1985). Considering that the escape behavior is composed of these two sequential components, our results indicate that the SEG is critical in the execution of rapid turning or the first component of the response. We reached this conclusion because in brainless animals, the first motor burst always included the fast motoneuron but in animals with their neck connectives cut and in stung animals, the first motor burst never included the fast motoneuron (Fig. 3). In this regard, brainless animals resemble normal animals in the initial phase of the escape. However, once the escape motor pattern has been activated, the brain appears to play an important role in generating fast escape movements. This role of the brain is indicated by the fact that subsequent motor bursts very often included the fast motoneuron in control but rarely did so in the brainless animals. In addition, stung and brainless stung animals both failed to escape wind stimuli. Thus, the fact that the venom effect took place in the absence of the brain indicates that the site of effect of the venom is likely the SEG. In addition, the fact that stung, brainless stung and neck-connective-cut animals responded little to wind stimuli suggest that the nature of the descending influence from the SEG on the thoracic circuitry is permissive. We suspect that direct injection of the venom in the SEG is almost certainly required for inducing the long-lasting effect as shown by Gnatzy and Otto (1996) for the parasitoid wasp *Liris niger* which uses crickets as prey.

Modulation of the escape circuitry in the thorax

We have examined specific aspects of the mechanisms by which the thoracic portion of the escape circuitry might be impaired in stung animals. The escape thoracic interneurons (TIAs) receive inputs from the ascending vGIs (Ritzman and Pollack 1986, 1988; Ritzman 1993) and descending mechanoreceptive interneurons (Comer and Dowd 1993; Ye and Comer 1996). These TIAs are probably involved in performing the final processing stage of localizing the wind stimulus so as to prepare an appropriate directional motor response. Previous studies have shown that the venom of *A. compressa* does not

affect the responses of neither the vGIs to wind stimuli nor the descending mechanoreceptive interneurons to tactile stimulation of the antennae (Fouad et al. 1994, 1996). Given the dramatic suppression of the escape response in stung animals, experiments concerned with TIA excitability were aimed at determining whether the responses of TIAs were significantly affected, i.e., affected to an extent consistent with the drastic change in the escape behavior. These experiments were not designed to look for subtle differences because these would not explain the dramatic changes observed in the escape behavior of the stung animals.

One such subtle difference was the reduction in the level of spontaneous background activity by about half in TIAs in stung cockroaches. This observation is very interesting, particularly given that the amplitude and latency of spontaneous, and wind- and tactile-evoked responses was unchanged. Schaefer and Ritzmann (1998) recently reported a similar decrease in the frequency (about 50%) of large (> 3 mV), spontaneous PSPs in TIAs in decapitated cockroaches. Although this data is preliminary, it lends support to the idea that descending input from the sub- and/or supraoesophageal ganglia is affected by the venom, and would impact the threshold for escape in stung animals. Nevertheless, whether this reduction acts to significantly increase the threshold for escape in ways consistent with a drastic change in the initiation of escape behavior is yet not known. It seems unlikely that this reduction is the major cause of the absence of escape behavior in stung animals.

In this study, we sampled the wind sensitivity of several TIAs including TIA 301 and TIA 701, which are known to make extensive connections with many motoneurons (Schaefer et al. 1997). These TIAs are likely targets of descending influences from the brain and SEG. However, we show that these cells respond to vGI input no differently in stung and control animals (Figs. 6, 7, 8). The synaptic transmission between the giant interneurons and the thoracic interneurons is mediated by acetylcholine and is subject to modulation (Casagrand and Ritzmann 1992a, b). Putative octopaminergic, dopaminergic and serotonergic neurons are found in all three thoracic ganglia (Tyrer et al. 1984; Eckert et al. 1992; Stevenson et al. 1992; Watson 1992). Octopamine and dopamine enhance, and serotonin decreases, synaptic efficacy between the giant interneurons and the thoracic interneurons (Casagrand and Ritzmann 1992b). Consistent with the results, Goldstein and Camhi (1991) have shown that, when bath applied to the metathoracic ganglion, octopamine and dopamine enhance and serotonin decreases the leg motor output evoked by recruiting the giant interneurons. Thus, one possibility is that the effect on descending control of the thoracic portion of the escape circuitry in stung animals is mediated by these three monoamines. A fact is that the response of the TIAs onto which converge the vGIs and brain descending neurons is not impaired by the venom injection into the head. Therefore, it is possible that the endings of a given set of monoaminergic

neurons are specifically on the TIAs to motoneurons connections and not the connections of the vGIs to TIAs; thus, monoamines could exert their control selectively at the connections of TIAs to motoneurons. This possibility remains to be examined using paired recording of TIAs and motoneurons.

The wasp venom clearly affects the escape motor program of cockroaches. Of the two excitatory motoneurons innervating the coxal muscle, only the fast motoneuron is not recruited by wind stimuli in stung animals (Fouad et al. 1996; Fig. 3). Yet, in stung animals this motoneuron still receives a sub-threshold synaptic drive from the escape thoracic interneurons (Fouad et al. 1996). One possibility is that the descending control from the SEG circuitry is exerted directly on the fast motoneuron by changing its excitability to the input of the TIAs; an alternative possibility is that this descending control is exerted on the synaptic drive provided by the TIAs to the fast motoneuron. To discriminate between these alternatives, we checked whether the fast motoneuron could be recruited by some other interneuronal circuitry. We found that the fast motoneuron is not the likely target of the effect of the venom in the thorax. We have come to this conclusion because the muscarinic agonist pilocarpine recruits the fast motoneuron in slow bursting rhythm but that under the same pharmacological conditions, a wind stimulus does not activate this motoneuron (Fig. 9). The frequency of the rhythmic motor pattern induced by pilocarpine in the cockroach metathoracic ganglion was roughly 0.3 Hz and similar to pilocarpine-induced motor pattern in locusts and stick insects (Rykebusch and Laurent 1993; Buschges et al. 1995). In these insects, like in cockroaches, pilocarpine activates Df and Ds in rhythmic bursts. However, in our pilocarpine experiments, we can not rule out the possibility that bath application of pilocarpine changed the excitability of the fast motoneuron. Given that muscarinic receptors are both pre-synaptic and post-synaptic (Trimmer and Weeks 1989, 1993), pilocarpine may act post-synaptically on the motoneuron by increasing its excitability (Trimmer and Weeks 1993). Nevertheless, this change in excitability, if any, was not sufficient for the fast motoneuron to produce action potentials in response to the input of the TIAs (Fig. 9C). Thus, we can conclude that pilocarpine induced a central generated rhythm capable of activating the fast motoneuron but that the thoracic escape interneurons failed to do so. Whether the thoracic premotor local interneurons or the output of the TIAs to the fast motoneuron are indeed the target of descending influences modulated by the venom are questions currently being investigated.

Arousal and the neuromodulation of the cockroach escape behavior

We can only speculate as to how SEG neurons may control the thoracic escape network (Fig. 10). It is

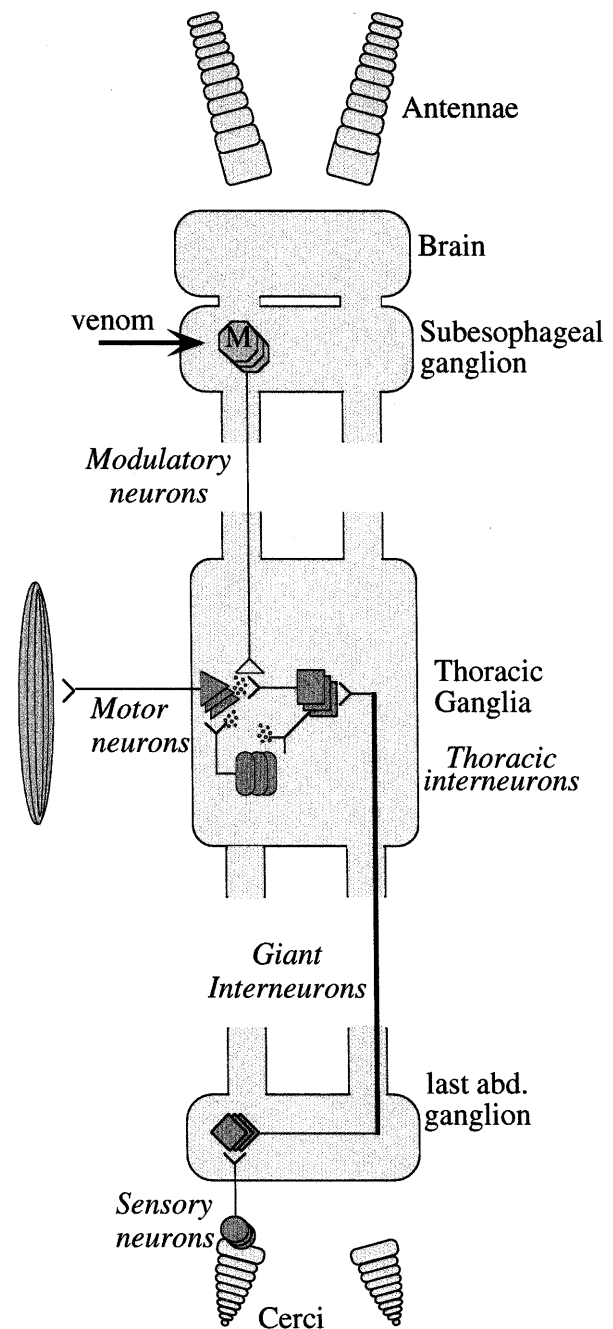


Fig. 10 Model of venom-induced depression of the escape circuit by the wasp neurotoxin. The venom could inactivate neuromodulatory neurons (*M*) in the SEG. These neuromodulatory neurons could regulate the excitability of the thoracic portion of the escape circuitry by modulating the synapses between thoracic interneurons and specific motoneurons such as the fast motoneurons involved in producing rapid leg movements

possible that the venom affects cells located in the SEG and that these control the thoracic premotor circuitry. Interneurons located in the SEG have been shown to be associated with various rhythmic motor pattern including flight (Ramirez 1988), ventilation and stridulation (Otto and Hennig 1993), but besides

these interneurons, the SEG also houses numerous types of neuromodulatory neurons (for review see Burrows 1996). Using immunocytochemical staining, octopaminergic cells in the nervous system of the cockroach have been mapped, and a large cluster of these cells is found in the SEG (Eckert et al. 1992). Octopamine could have a general arousal effect on insects (Orchard 1982; Orchard et al. 1993) and has been found to sensitize escape behavior (Goldstein and Camhi 1991; Casagrand and Ritzmann 1992b) and flight initiation threshold (Weisel-Eichler and Libersat 1996) in cockroaches. An antiserum against a vasopressin-like peptide has revealed a pair of vasopressin-like neurons with projections throughout every thoracic and abdominal locust ganglia (Thompson et al. 1991). These brain cells show a circadian rhythm entrained to the light-dark cycle and has been suggested that they may be involved in the control of the arousal state in locusts (Thompson and Bacon 1991).

Also consistent with our hypothesis that the SEG is the main target of the venom is our data on its effect on heart rate. We found that the heart rate of resting stung cockroaches is higher than the heart rate of resting control animals (Fig. 5). In addition, after about of flight activity, control cockroaches show an increase in heart rate whereas the heart rate of stung animals remains unchanged (Fig. 5). The SEG of insects contains various neuropeptidergic neurons some of which have been directly or indirectly implicated in the control of heartbeat: vasopressin (Davis and Hildebrand 1992), corazonin (Veenstra and Davis 1993), FMRFamide (Robb and Evans 1990, 1994), bovine pancreatic peptide (Brauning 1991). By testing various antisera directed at different peptides, one might be able to find changes in neuropeptide immunoreactivity of the brain-SEG complex that are associated with the stinging. Once the identity of the targeted neuromodulatory neurons is established, one should be able to determine how these neurons control the excitability of the escape circuitry in the thorax.

Parasitoid wasps are interesting because very little is known about venomous animals that inject their toxins in the prey's nervous system. By studying the effect of the neurotoxin on the relatively simple cockroach escape system, it may be possible to determine some principles by which the nervous system controls changes in responsiveness.

Acknowledgements We are grateful to N.T. Davis, A. Weisel-Eichler and A. Mizrahi for valuable comments on the manuscript and to Mr. Schulten of the Lobbeke Museum and Aquazoo of Dusseldorf, Germany for his kind gift of wasps. This work was supported by grant (18-97) from the National Institute for Psychobiology in Israel and a grant No 96-00472 from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel. K.F. was supported by a DFG grant (Ra 113/7-1) to Werner Rathmayer and J.L.C. was supported by a Human Frontiers Science Organization short-term fellowship (SF 0511/96-B). These experiments comply with "Principles of animal care", NIH publication No. 86-23, revised 1985, and also with the current laws of the State of Israel.

References

- Bicker G, Menzel R (1989) Chemical codes for the control of behavior in arthropods. *Nature (Lond)* 337: 33–39
- Bräuning P (1991) A suboesophageal ganglion cell innervates heart and retrocerebral complex in the locust. *J Exp Biol* 156: 567–582
- Burrows M (1996) Neurotransmitters, neuromodulators and, neurohormones. In: Burrows N (ed) *The neurobiology of an insect brain*. Oxford University Press, Oxford, pp 168–228
- Buschges A, Schmitz J, Bassler U (1995) Rhythmic patterns in the thoracic nerve cord of the stick insect induced by pilocarpine. *J Exp Biol* 198: 435–456
- Camhi JM (1984) A case study in neuroethology: the escape system of the cockroach. In: Camhi JM (ed) *Neuroethology*. Sinauer, Sunderland, Massachusetts, pp 79–105
- Camhi JM (1985) Feedback control of an escape behaviour. In: Barnes WJP (ed) *Feedback and motor control in invertebrates and vertebrates*. Croom Helm, London, pp 93–112
- Camhi JM, Nolen TG (1981) Properties of the escape system of cockroaches during walking. *J Comp Physiol A* 142: 339–346
- Carbonell CS (1947) The thoracic muscles of the cockroach *Periplaneta americana*. *Smithson Misc Collect* 107: 1–23
- Casagrand JL, Ritzmann RE (1992a) Evidence that synaptic transmission between giant interneurons and identified thoracic interneurons in the cockroach is cholinergic. *J Neurobiol* 23: 627–643
- Casagrand JL, Ritzmann RE (1992b) Biogenic amines modulate synaptic transmission between identified giant interneurons and thoracic interneurons in the escape system of the cockroach. *J Neurobiol* 23: 644–655
- Comer CM, Dowd JP (1993) Multisensory processing for movement: antennal and cercal mediation of escape turning in the cockroach. In: Beer RD, Ritzmann RE, McKenna T (eds) *Biological neural networks in invertebrate neuroethology and robotics*. Academic Press, Boston, pp 89–112
- Davis NT, Hildebrand JG (1992) Vasopressin-immunoreactive neurons and neurohemal systems in cockroaches and mantids. *J Comp Neurol* 320: 381–393
- Eckert M, Rapus J, Nürnberger A, Penzlin H (1992) A new specific antibody reveals octopamine-like immunoreactivity in cockroach ventral cord. *J Comp Neurol* 322: 1–15
- Fouad K, Libersat F, Rathmayer W (1994) The venom of the cockroach-hunting wasp *Ampulex compressa* changes motor thresholds: a novel tool for studying the neural control of arousal? *Zoology* 98: 23–24
- Fouad K, Libersat F, Rathmayer W (1996) Neuromodulation of the escape behavior in the cockroach *Periplaneta americana* by the venom of the parasitic wasp *Ampulex compressa*. *J Comp Physiol A* 178: 91–100
- Gnatzy W, Otto D (1996) Digger wasp vs. cricket: application of the paralytic venom by the predator and changes in behavioural reactions of the prey after being stung. *Naturwissenschaften* 83: 467–470
- Goldstein RS, Camhi JM (1991) Different effects of the biogenic amines dopamine, serotonin and octopamine on the thoracic and abdominal portions of the escape circuit in the cockroach. *J Comp Physiol A* 168: 103–112
- Huber F (1965) Brain controlled behavior in orthopterans. In: Treherne JE, Beament JWL (eds) *The physiology of the insect central nervous system*. Academic Press, London, pp 233–246
- Kien J, Altman JS (1992) Decision-making in the insect nervous system: a model for selection and maintenance of motor programmes. In: Kien J, McCrohan CR, Winlow W (eds) *Neurobiology of motor programme selection*. Pergamon Press, Oxford. Pergamon studies in neuroscience, No 4, pp 147–169
- Kravitz EA (1988) Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. *Science* 241: 1775–1781
- Libersat F (1992) Modulation of flight by the giant interneurons of the cockroach. *J Comp Physiol A* 170: 379–392
- Liebethal E, Uhlman O, Camhi JM (1994) Critical parameters of the spike trains in a cell assembly: coding of turn direction by

- the giant interneurons of the cockroach. *J Comp Physiol A* 174: 281–296
- Marrocco RT, Witte EA, Davidson MC (1994) Arousal systems. *Curr Opin Neurobiol* 4: 116–170
- Orchard I (1982) Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. *Can J Zool* 60: 659–669
- Orchard I, Ramirez JM, Lange AB (1993) A multifunctional role for octopamine in locust flight. *Annu Rev Entomol* 38: 227–249
- Otto D, Henig RM (1993) Interneurons descending from the cricket subesophageal ganglion control stridulation and ventilation. *Naturwissenschaften* 80: 36–38
- Pearson KG, Iles JF (1970) Discharges patterns of coxal levator and depressor motoneurons of the cockroach, *Periplaneta americana*. *J Exp Biol* 54: 215–232
- Pearson KG, Iles JF (1971) Innervation of coxal depressor muscles in the cockroach, *Periplaneta americana*. *J Exp Biol* 54: 215–232
- Piek T, Hue B, Lind A, Mantel P, Marle J van, Vissier JH (1989) The venom of *Ampulex compressa* – effects on behavior and synaptic transmission of cockroaches. *Comp Biochem Physiol* 92 C: 175–183
- Ramirez JM (1988) Interneurons in the subesophageal ganglion of the locust associated with flight initiation. *J Comp Physiol A* 162: 669–686
- Ritzmann RE (1993) The neural organization of cockroach escape and its role in context-dependent orientation. In: Beer RD, Ritzmann RE, McKenna T (eds) *Biological neural networks in invertebrate neuroethology and robotics*. Academic Press, Boston, pp 113–137
- Ritzmann RE, Pollack AJ (1986) Identification of thoracic interneurons that mediate giant interneurons-to-motor pathways in the cockroach. *J Comp Physiol A* 159: 639–654
- Ritzmann RE, Pollack AJ (1988) Wind-activated thoracic interneurons of the cockroach. II. Patterns of connection from ventral giant interneurons. *J Neurobiol* 19: 589–611
- Ritzmann RE, Pollack AJ (1990) Parallel motor pathways from thoracic interneurons of the ventral giant interneuron system of the cockroach, *Periplaneta americana*. *J Neurobiol* 21: 1219–1235
- Ritzmann RE, Pollack AJ (1994) Responses of thoracic interneurons to tactile stimulation in the cockroach, *Periplaneta americana*. *J Neurobiol* 25: 1113–1128
- Ritzmann RE, Pollack AJ, Hudson SE, Hyvonen A (1991) Convergence of multi-modal sensory signals at thoracic interneurons of the escape system of the cockroach, *Periplaneta americana*. *Brain Res* 563: 175–183
- Robb S, Evans PD (1990) FMRamide-like peptides in the locust: distribution, partial characterization and bioactivity. *J Exp Biol* 149: 335–360
- Robb S, Evans PD (1994) The modulatory effect of on heart and skeletal muscle in the locust *Shistocerca gregaria*. *J Exp Biol* 197: 437–442
- Roeder KD (1948) Organization of the ascending gnat fibre system in the cockroach, *Periplaneta americana*. *J Exp Zool* 108: 243–261
- Ryckebusch S, Laurent G (1993) Rhythmic patterns evoked in locust leg motor neurons by the muscarinic agonist pilocarpine. *J Neurophysiol* 69: 1583–1595
- Schaefer PL, Pollack AJ, Ritzmann RE (1997) Analysis of descending influences on motor control of cockroach escape. *Soc Neurosci Abstr* 23: 613.11
- Schaefer PL, Ritzmann RE (1998) Supraesophageal influence on the thoracic circuitry of cockroach escape. Proceedings of the 5th International Congress of Neuroethology, August 23–28, 1998, University of California, San Diego, La Jolla, California, USA
- Stevenson PA, Pfluger HJ, Eckert M, Rapus J (1992) Octopamine immunoreactive cell populations in the locust thoracic-abdominal nervous system. *J Comp Neurol* 315: 382–397
- Teyke T, Weiss KR, Kupfermann I (1990) An identified neuron (CPR) evokes neuronal responses reflecting food arousal in *Aplysia*. *Science* 247: 85–87
- Thompson KSG, Bacon JP (1991) The vasopressin-like immunoreactive (VPLI) neurons of the locust, *Locusta migratoria*. II. Physiology. *J Comp Physiol A* 168: 619–630
- Thompson KSG, Tyrer NM, May ST, Bacon JP (1991) The vasopressin-like immunoreactive (VPLI) neurons of the locust, *Locusta migratoria*. I. Anatomy. *J Comp Physiol A* 168: 605–617
- Trimmer BA, Weeks JC (1989) Effect of nicotinic and muscarinic agents on a identified motoneurone and its direct afferent inputs in larval *Manduca sexta*. *J Exp Biol* 144: 303–337
- Trimmer BA, Weeks JC (1993) Muscarinic acetylcholine receptors modulate the excitability of an identified insect motoneuron. *J Neurophysiol* 69: 1821–1836
- Tyrer NM, Turner JD, Altman JS (1984) Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. *J Comp Neurol* 227: 313–330
- Veenstra JA, Davis NT (1993) Localization of corazonin in the nervous system of the cockroach *Periplaneta americana*. *Cell Tissue Res* 274: 57–64
- Wafford KA, Sattelle DB (1986) Effects of amino acid neurotransmitter candidates on an identified insect motoneurone. *Neurosci Lett* 63: 135–140
- Watson AHD (1992) The distribution of dopamine-like immunoreactivity in the thoracic and abdominal ganglia of the locust (*Shistocerca gregaria*). *Cell Tissue Res* 270: 113–124
- Watson JT, Ritzmann RE (1994) The escape response versus the quiescent response of the American cockroach: behavioural choice mediated by physiological state. *Anim Behav* 48: 76–478
- Weisel-Eichler A, Libersat F (1996) Neuromodulation of flight initiation by octopamine in the cockroach. *J Comp Physiol A* 179: 103–112
- Westin J, Ritzmann RE, Goddard DG (1988) Wind-activated thoracic interneurons of the cockroach. I. Responses to controlled wind stimulation. *J Neurobiol* 19: 573–588
- Williams FX (1942) *Ampulex compressa* (F), a cockroach-hunting wasp introduced from New Caledonia to Hawaii. *Proc Hawaii Entomol Soc* 11: 221–233
- Ye S, Comer CM (1996) Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, *Periplaneta americana*. *J Neurosci* 16: 5844–5853
- Ziv I, Lustig C, Markovitch S, Susswein AJ (1991) Sequencing of behaviors in *Aplysia fasciata*: integration of feeding, reproduction and locomotion. *Behav Neural Biol* 56: 148–169