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Wasp uses venom cocktail to manipulate the behavior of its cockroach prey

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Abstract The sting of the parasitoid wasp *Ampulex compressa* is unusual, as it induces a transient paralysis of the front legs followed by grooming behavior and then by a long-term hypokinesia of its cockroach prey. Because the wasp's goal is to provide a living meal for its newborn larva, the behavioral changes in the prey are brought about by manipulating the host behavior in a way beneficial to the wasp and its offspring. To this end, the wasp injects its venom cocktail with two consecutive stings directly into the host's central nervous system. The first sting in the thorax causes a transient front leg paralysis lasting a few minutes. This paralysis is due to the presence of a venom component that induces a postsynaptic block of central cholinergic synaptic transmission. Following the head sting, dopamine identified in the venom appears to induce 30 min of intense grooming. During the long-term hypokinesia that follows the grooming, specific behaviors of the prey are inhibited while others are unaffected. We propose that the venom represses the activity of head ganglia neurons thereby removing the descending excitatory drive to the thoracic neurons.

Keywords *Ampulex compressa* · Grooming · Neurotoxins · Paralysis · *Periplaneta americana*

Abbreviations CNS: central nervous system · DA: dopamine · GI: giant interneuron · PSP: postsynaptic potential · SEG: sub-esophageal ganglion · TI: thoracic interneuron

Introduction

Predators as diverse as snakes, scorpions, spiders, insects and snails manufacture various neurotoxins to incapacitate their prey. These neurotoxins mostly interfere with the ability of the prey's nervous system to generate muscle contractions resulting in an immobilization of the prey (Adams 1996; Olivera 1999). Most venoms contain a cocktail of neurotoxins and each neurotoxin is aimed at different molecular targets in the nervous and muscular systems (Rappuoli and Montecucco 1997). Many neurotoxins are peptides or proteins, which specifically affect conductances underlying the action potential and its propagation or preventing the action potential from invading the synaptic terminal (Na^+ or K^+ voltage-dependent channels) or interfere with the synaptic release of neurotransmitter (Ca^{2+} voltage-dependent channels). The majority of neurotoxins that bind on the ligand gated receptors at the neuromuscular junction act as antagonist of the glutamate receptor in invertebrates or the acetylcholine receptor in vertebrates (Harvey 1993; Adams 1996; Rappuoli and Montecucco 1997). The venom is usually delivered through a spiny device, which may be fangs (snakes, spiders), a sting (scorpions, wasps), pincers (centipedes) or a harpoon (cone shells, jellyfish, sea anemones) (Bettini 1978; Blum 1981). This injection device is connected to a gland or glands, which produce the venom. The venom is in most cases injected into the flesh and rarely into the blood circulation. Most venoms act peripherally at the neuromuscular junction resulting in different types of paralysis (Tipton and Dajas 1994; Rappuoli and Montecucco 1997). However, in a few species of parasitoid wasps, venoms appear to be injected directly into the central nervous system of the prey and act centrally to induce various behaviors (Piek 1990).

Parasitoid wasps belong to the families of Pompilidae, Sphecidae, Mutillidae and Bethylinidae (Rathmayer 1978). These venomous wasps use other insects or spiders as food supply for their offspring. Most wasps

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paralyze their prey and then carry it to a burrow or nest. The wasp subsequently lays one egg on the victim and when the larva hatches, it feeds on the paralyzed host. For example, wasps of the family of Pompilidae are the spider's worst enemies. These wasps paralyze a spider with multiple variable stings, then drag their victim to a prepared burrow and deposit one egg on the spider's abdomen. Upon hatching, the wasp larva feeds on the tarantula's body. After being stung by some wasps, spiders do not resume specific behaviors though they are capable of moving and appear to be "normal". For example, in some cases, oviposition behavior is inhibited while in others, web spinning is inhibited (Steiner 1986).

In those species of Sphecidae where the paralyzing venom is injected through the sting into the hemolymph of the prey, as in the bee wolf (*Philanthus triangulum*), the venom, which contains neurotoxins called philanthoxins (beta, gamma and delta PTX), diffuses to the sites of action. There, these neurotoxins interfere presynaptically at the neuromuscular junctions with the release of excitatory transmitter and block the postsynaptic glutamate receptors at the nerve muscle junction (Rathmayer 1962; Piek and Spanjer 1986; Eldefrawi et al. 1988). The impairment of neuromuscular transmission results in muscular paralysis of the insect prey.

Within the Sphecidae family, a few species do not paralyze but manipulate the behavior of their victims in most interesting ways. Parasitoid wasps that have attracted special interest are those using insects from the Orthoptera group (cockroaches, crickets, mantids and grasshoppers) as preys (Piek 1990; Fouad et al. 1994; Gnatzy 2001). The wasp stings its prey inducing various levels of paralysis and pulls it to a burrow where it lays an egg on the cuticle surface. The larva develops outside of the prey, feeding on the hemolymph through a small hole in the cuticle, and after which it moves inside the prey's body to feed and pupate for completing its development.

The sphecid wasp *Ampulex compressa* applies a unique strategy of behavioral modulation of its cockroach prey (Williams 1942; Piek et al. 1984; Fouad et al. 1994). This parasitoid solitary wasp hunts cockroaches (*Periplaneta americana*) by stinging them first in the thorax and then in the head (Fig. 1). The stung cockroach exhibits three consecutive phases of envenomation. First, the cockroach shows a transient paralysis of its front legs (Fouad et al. 1994; Haspel and Libersat 2003). It then grooms extensively, after which it becomes sluggish and is not responsive to various stimuli (Fouad et al. 1994; Weisel-Eichler et al. 1999, Weisel-Eichler and Libersat 2002). The wasp grabs one of the cockroach's antennae and walks to a suitable oviposition location. The cockroach follows the wasp in a docile manner like a dog on a leash (Williams 1942; Fouad et al. 1994). A few days later, the cockroach serves as an immobilized and fresh food source for the wasp's offspring. These unique effects of the wasp venom on prey behavior suggest that the venom targets the insect's central nervous system. Until recently, the mechanism by which

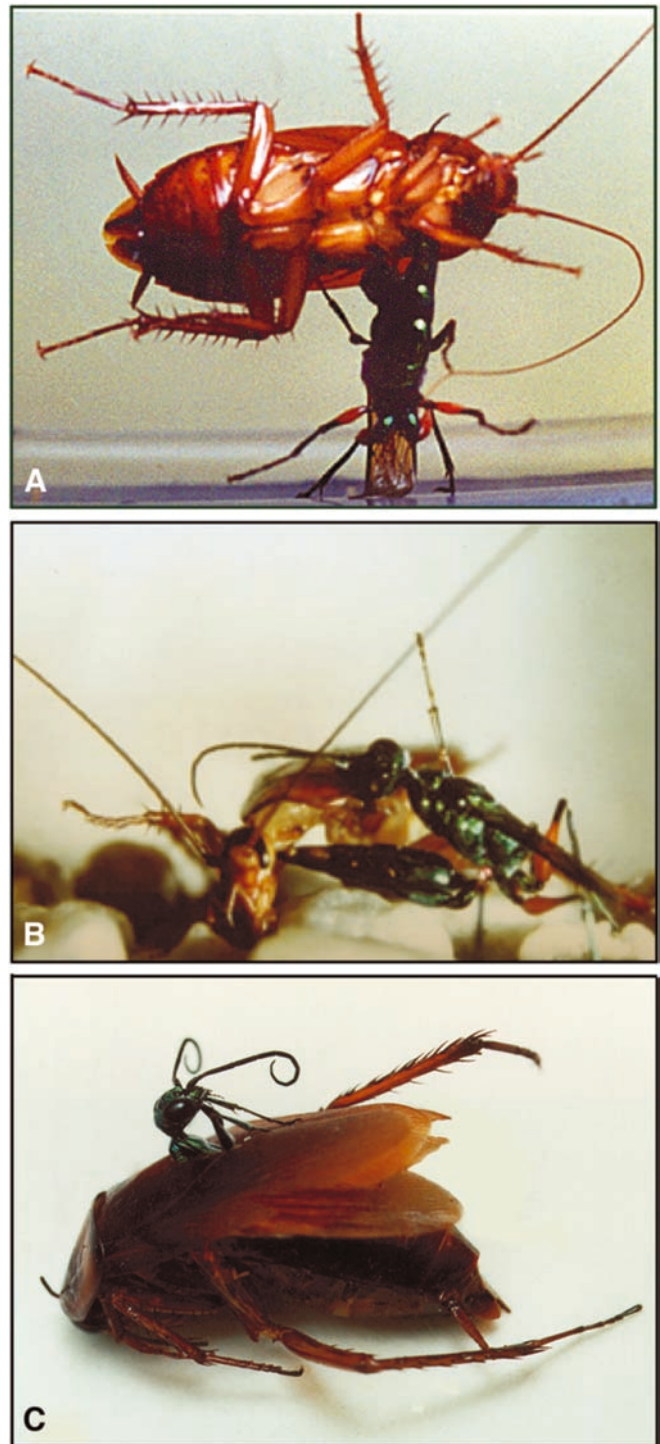


Fig. 1A–C *Ampulex compressa* stinging behavior. The wasp stings a cockroach first into the thorax (A) and then into the head (B). The photograph shown in A was taken from the ventral surface of the cockroach through glass window of the aquarium. C After consuming the entire inside of the cockroach, the larva pupates inside the cuticular shell of the cockroach, and a mature wasp emerges about 6 weeks after the egg is laid (modified from Haspel et al. 2003)

behavior-modifying compounds in the venom reach the central nervous system, given the protective ganglionic sheath around the ganglia, was unknown. Moreover, the molecular and physiological mechanisms by which these compounds induce these central and long-lasting effects remained to be discovered.

A major goal in our laboratory has been to unravel these mechanisms. More specifically, we have explored the possibility that the wasp delivers its venom by stinging directly into the nervous system of its prey. In addition, we have attempted to decipher the neural basis for the three behavioral states that are induced sequentially by the venom injection, namely: (1) the transient paralysis of the front legs, (2) the intense grooming, and (3) the long term-hypokinesia.

The wasp delivers its venom by stinging directly into the nervous system of its prey

The first sting is applied to the first thoracic segment, which houses the pro-thoracic ganglion. Cockroaches stung only once in the prothorax exhibit a flaccid paralysis of the front legs from which they recover within a few minutes (Fouad et al. 1994). Because this first sting prevents the cockroach from using its forelegs to fight off the wasp, it presumably facilitates the subsequent sting into the head of the cockroach. This second sting induces roughly 30 min of grooming behavior, followed by a long-term hypokinesia (Fouad et al. 1994). These two behavioral modifications are induced only when a sting is inflicted into the head.

For more than a century, there has been a controversy over whether or not several species of parasitoid wasps deliver their venom by stinging directly into the nervous system. The French entomologist Jean Henri Fabre observed that wasps sting in a pattern that corresponds to the location and arrangement of nerve centers in the prey and he suggested that the wasp stings directly into target ganglia (Fabre 1879). Others challenged Fabre's idea and claimed that the wasp stings in the vicinity but not into the ganglion (Ferton 1902; Roubaud 1917). These unique effects of the wasp venom on prey behavior suggest that the venom targets the insect's central nervous system (CNS). The wasp's venom consists of a cocktail of proteins and peptides (Haspel and Libersat 1997; Haspel et al. 2003) which are very unlikely to cross the thick and rather selective sheath (the insect's blood brain barrier) around the nervous ganglia (Treherne and Schofield 1979). In our attempt to explore the possibility that the wasp stings into the ganglia to deliver its venom, we have produced so-called "hot" wasps. We injected wasps with C^{14} -radiolabeled amino acids, which were incorporated into the venom. We then used a combination of liquid scintillation and light microscopy autoradiography to trace radiolabeled venom in the prey (Haspel and Libersat 2003; Haspel et al. 2003). In cockroaches stung by radiolabeled wasps, most of the radioactive signal in the

thoracic region was detected in the first thoracic ganglion (Fig. 2A). Only a small amount of radioactivity was detected in other thoracic ganglia and the surrounding, non-neuronal, tissue. Likewise, we found that the levels of radioactivity were significantly higher in the head ganglia, the supra-esophageal and sub-esophageal ganglia (brain and SEG, respectively), than in the surrounding head tissue (Fig. 2B). To determine the precise location of injection, head ganglia of stung cockroaches were embedded in plastic resin, serially sectioned and exposed to radiosensitive emulsion. Radioactivity was observed in the central part of the brain, posterior to the central complex and around the mushroom bodies (Fig. 2C). Furthermore, radioactivity was detected in the sub-esophageal ganglion around the ganglion midline (Fig. 2D; Haspel et al. 2003). This shows that the wasp stings both into the sub-esophageal ganglion, which lies underneath the stinging site in the neck, and separately into the more distant brain (Fig. 2E). To achieve such a precise stereotaxic injection, the wasp's sting must bear sense organs to identify its neuronal targets. Examination of the tip of the sting (ovipositor) in various families of social wasps and bees shows the presence of numerous receptors (Van Marle and Piek 1986). However, such information is not available regarding the solitary wasps and it would be interesting to further categorize the receptors found on the sting of *Ampulex compressa* and examine their possible role in distinguishing nervous tissue from non-nervous tissue. To our knowledge, this is the first direct evidence documenting targeted delivery of venom into the central nervous system of a prey organism. It is possible that other parasitoid wasps follow the same strategy of "drug delivery", injecting venom directly into the central nervous system of their prey.

Wasp venom induces transient leg paralysis through postsynaptic block of central nicotinic synapses

The first sting into the prothoracic ganglion causes a transient paralysis of the cockroach's front legs (Fig. 3A). As a result, the front legs cannot support the cockroach and it assumes a "head down" posture for 2–3 min (Fig. 3A). The artificial injection of milked venom into a thoracic ganglion abolishes spontaneous and evoked responses of the motoneurons associated with leg movements (Fig. 3A; Haspel and Libersat 2003). Because motoneurons receive excitatory cholinergic input (Gundelfinger 1992), we used a well-characterized cholinergic synapse to investigate the possibility that the venom may interfere with central cholinergic neurotransmission. To investigate the physiological mechanisms by which the venom abolishes the motoneuron's responses, we injected venom into the last abdominal ganglion of the cockroach, which houses a well-characterized nicotinic synapse. This synapse connects wind sensory neurons to giant interneurons (GIs) (Callec and Sattelle 1973). In the last abdominal ganglion

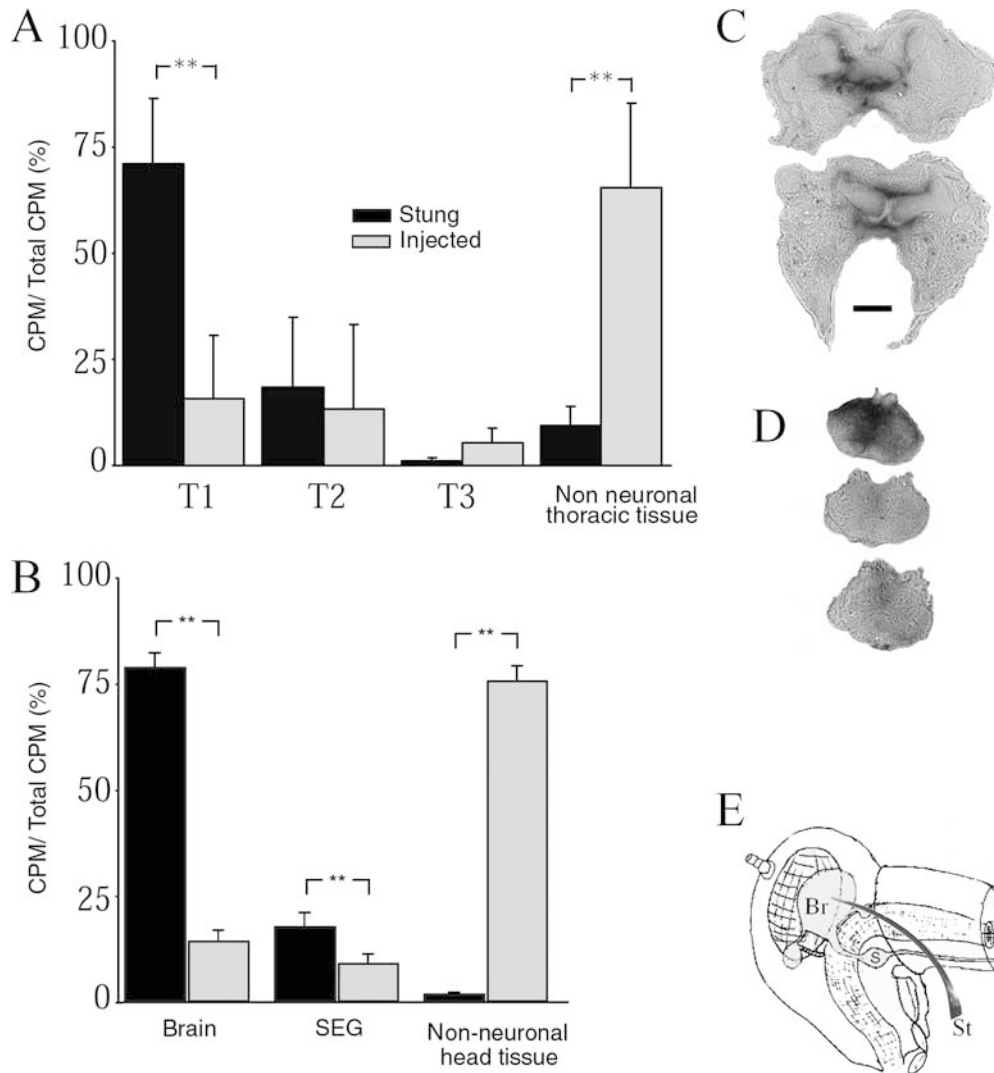


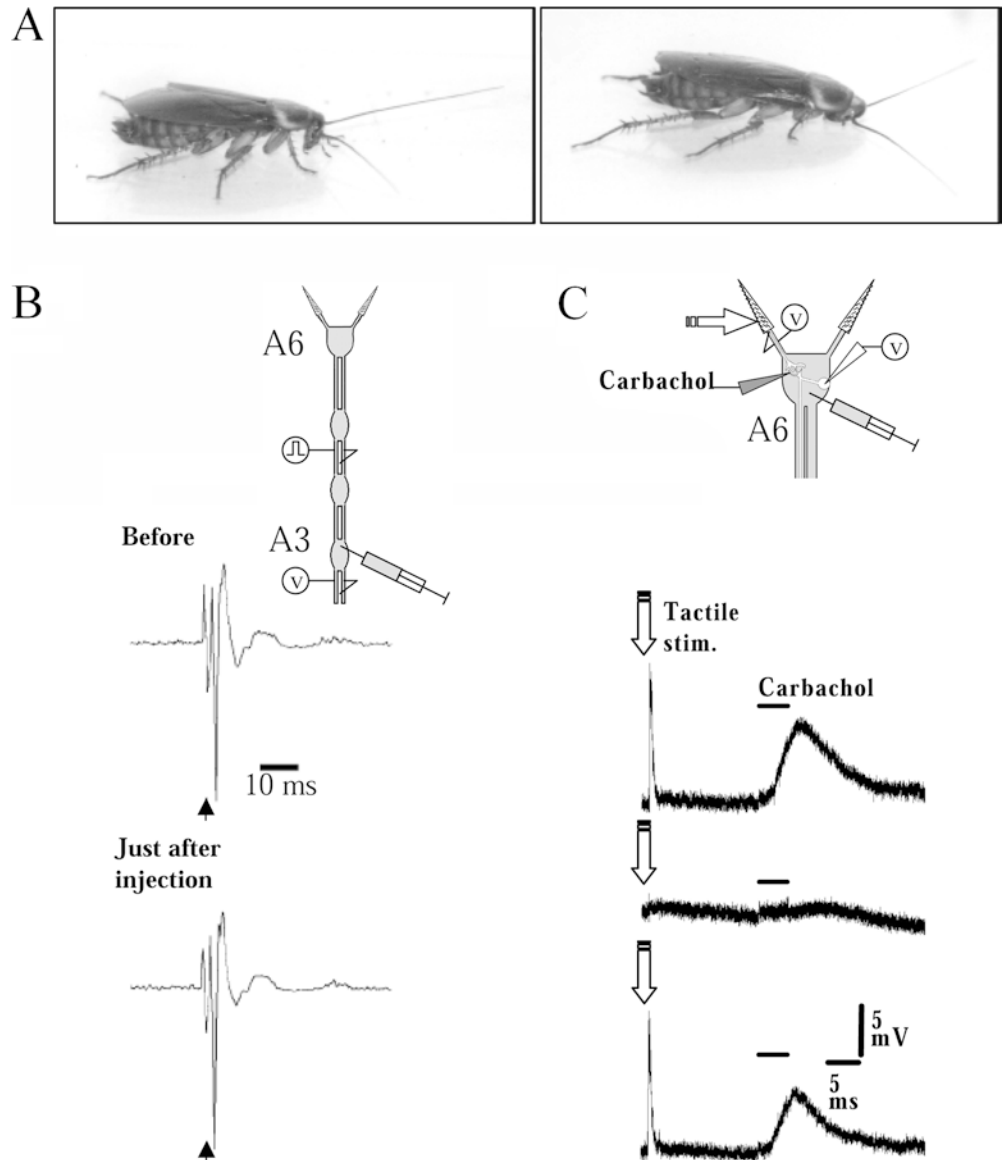
Fig. 2A–E Localization of the site of injection of the venom. **A** In cockroaches stung by radiolabeled wasps (black bars, $n=16$), most of the radioactive signal is detected in the first thoracic ganglion ($T1$). The remaining radioactivity is detected in the second ($T2$) and third ($T3$) thoracic ganglia and in the surrounding, non-neuronal, tissue. In cockroaches injected with radioactive amino acids (grey bars, $n=15$), most of the radioactivity is detected in the surrounding tissue while the rest of the radioactivity is detected in the thoracic ganglia. A significant difference ($**P<0.01$) in radioactivity levels is found between stung and injected cockroaches only in $T1$ and in the surrounding tissue. **B** In stung cockroaches (black bars, $n=16$), the levels of radiolabeled venom are significantly higher in the head ganglia (brain and subesophageal ganglion, SEG) than in non-neuronal head tissue. When radioactive amino acids are manually injected into the head capsule of other cockroaches (grey bars, $n=15$), the levels of radiolabeled venom are significantly higher in non-neuronal head

tissue than in the head ganglia. Furthermore, significantly different ($**P<0.01$) levels of radioactivity are measured in stung and injected cockroaches in each of the sampled tissues. The measurements are represented as the percentile fraction of the total CPM (counts per minute) of a specimen. **C** Two sections of brain of a representative preparation of a cockroach, stung by a radiolabeled wasp. Radiolabeled venom is located posterior to the central complex and around the mushroom bodies of the brain. Scale bars: 0.25 mm. **D** Three sections of a representative SEG preparation of a cockroach, stung by a radiolabeled wasp. Radiolabeled venom, indicated as black stain, is located inside the SEG ganglion. **E** A micrograph of the stinger (st) is shown over a schematic lateral view of a cockroach head drawn to scale. The wasp stinger is long enough (2.5 ± 0.2 mm; $n=5$) to reach both the SEG and the brain, which lies 1–2 mm deep in the head capsule (modified from Haspel et al. 2003)

preparation, the venom blocked the wind-evoked responses of the GIs for 2–3 min (Haspel and Libersat 2003). However, the venom did not block the propagation of action potentials in the GIs (Fig. 3B), which indicates that it interferes with cholinergic synaptic transmission. To characterize the effect of the venom on cholinergic synaptic transmission, we recorded intracel-

lularly from a GI soma while alternately inducing an excitatory postsynaptic potential (PSP) by mechanical stimulation of the cercal receptors and by direct iontophoresis of a nicotinic agonist, carbachol. The injection of venom abolished both the sensory and agonist evoked PSPs suggesting that the venom-blocking effect has a postsynaptic component (Fig. 3C), but not ruling out the

Fig. 3A–C The transient paralysis of the front legs. **A** A typical upright posture of the cockroach before the thoracic sting (*left*). A few seconds after the sting, the cockroach's front legs are flaccidly paralyzed and it cannot support its own weight (*right*). **B** The axons of giant interneurons (GIs) are recruited with an electrical stimulus (*arrow*) applied to the nerve cord via an electrode and their propagation through abdominal ganglion A3 is monitored with another electrode on the nerve cord (*diagram*). The average traces of 15 evoked compound action potentials from a typical experiment are represented. Venom injected into abdominal ganglion A3 does not prevent the propagation of action potentials through this ganglion. **C** Two consecutive postsynaptic potentials (PSPs) are evoked 20 ms apart (*top trace*). The first PSP is evoked by mechanical stimulation of cercal sensory receptors (*arrow*, see diagram). The second and slower PSP is evoked by direct application of the nicotinic agonist carbachol. Both PSPs, recorded in the GI soma, are abolished within seconds of the injection of venom into ganglion A6 (*middle trace*) and then both PSPs gradually recover (*bottom trace*) (modified from Haspel and Libersat 2003)



existence of a pre-synaptic component. In the sensory to GI system of the cockroach, both the pre- and postsynaptic neurons depolarize with the application of nicotinic agonists while muscarinic agonists and antagonists have no effect on presynaptic terminals or postsynaptic giant interneurons (Blagburn and Sattelle 1987). Thus, the block of carbachol-evoked potentials by the venom indicates a postsynaptic block of nicotinic receptors rather than a block of muscarinic receptors. Finally, injection of a nicotinic antagonist in the front thoracic ganglion induced paralysis of the front legs, causing the cockroach to assume a “head down” posture for 2–3 min, similar to the effects of the sting in the thorax. We conclude that the transient paralytic effect of the thoracic sting can be mainly accounted for by the presence of a venom component that induces a postsynaptic block of central cholinergic synaptic transmission.

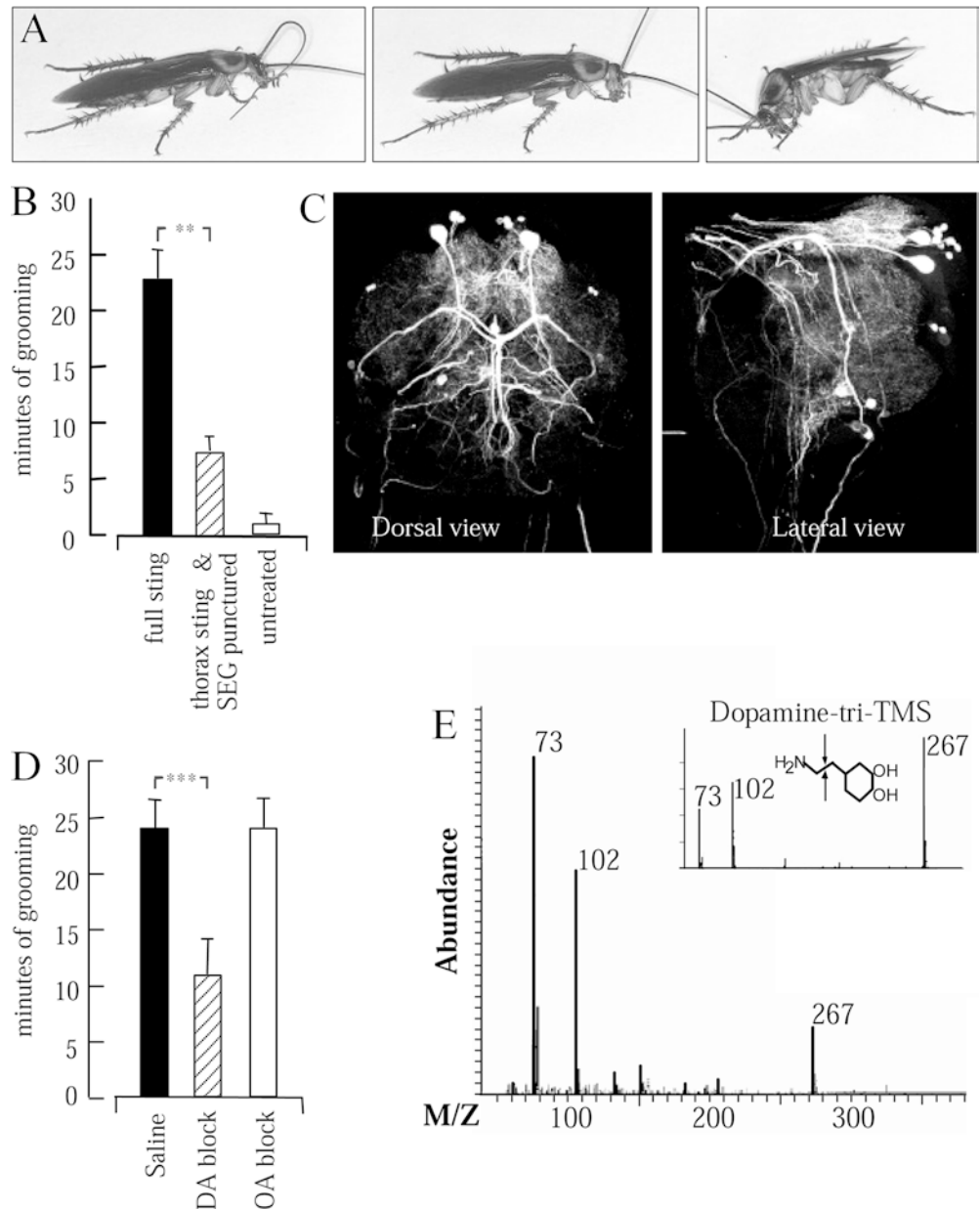
Most solitary Sphecid wasps prey on large orthopteroids equipped with various defense mechanisms (such

as kicks, leaps and bites). In all known cases, the first sting is directed at ganglia involved in locomotion and defense, thereby disarming the prey by inducing 2–60 min of complete paralysis (Steiner 1986). Thus, we suggest that *A. compressa* stings the cockroach directly into the first thoracic ganglion to flaccidly paralyze the front legs, thereby facilitating the more difficult and precise head-sting into the SEG and the brain.

Wasp venom contains dopamine, which induces prolonged grooming behavior in the cockroach

Cockroaches stung by the wasp into the head ganglia groom almost continuously during the 30 min following the recovery from the transient paralysis of the front legs (Fig. 4A). This effect occurs only when venom is injected into the head, and cannot be accounted for by the stress of the attack, by contact with the wasp, by mechanical

Fig. 4A–E Venom-induced grooming in cockroaches. **A** A stung cockroach performing two frequent components of grooming behavior: grooming an antenna (*left photograph*) and grooming a foreleg (*middle and right photographs*) with the mouthparts. **B** Cockroaches that received a full stinging sequence by the wasp groom for 23.0 ± 2.3 min during the 30 min following the sting. This grooming time is significantly longer ($P < 0.001$) than that observed in cockroaches that were stung only in the thorax followed by a puncture of the SEG with a pin (7.8 ± 5.4 min). **C** Dorsal and lateral views of an SEG stained with tyrosine hydroxylase antibody reveals a group of dopaminergic neurons, some of which have axons branching extensively in the SEG while other send their axons to the brain or the thorax. **D** Cockroaches that received flupenthixol, a dopamine (DA) receptor antagonist before a sting groom significantly less ($P < 0.001$) than cockroaches receiving saline before a sting. Mianserin, an octopamine antagonist, does not reduce venom-induced grooming. **E** Mass spectrogram of the large venom peak eluted at 12.68 min during gas chromatography (not shown); this spectrum is comparable to the mass spectrograms (*inset*) of DA. *x*-Axes indicate mass/charge (modified from Weisel-Eichler et al. 1999)



irritation, or by venom injection into a location other than the head (Fig. 4B; Weisel-Eichler et al. 1999). Thus, it appears that venom injection in the head activates a neural network responsible for grooming. The sting-evoked grooming, which is a complex behavior involving coordinated movements of different appendages, exhibits all the components of normal grooming behavior (Weisel-Eichler et al. 1999).

Our studies are consistent with the hypothesis that a monoamine (or its agonist) in the venom is the factor causing excessive grooming. For instance, reserpine injection into the SEG, which causes massive release of the monoamines, dopamine (DA), octopamine and serotonin, induces prolonged grooming (Weisel-Eichler and Libersat 2002). The SEG contains a large number of dopaminergic neurons, which branch extensively in the ganglion (Fig. 4C). Direct injection of a dopamine

agonist into the SEG induces prolonged grooming (Weisel-Eichler et al. 1999). Flupenthixol, which has been found to act as an antagonist at dopamine receptors in the cockroach brain (Notman and Downer 1987; Orr et al. 1992), greatly reduces venom-induced grooming when injected into the cockroach's hemolymph prior to the wasp sting (Fig. 4D; Weisel-Eichler et al. 1999). In contrast, mianserin and phentolamine, which have been found to be effective octopamine receptor antagonists in locust brain and cockroach nerve cord (Roeder 1992; Orr et al. 1992), do not reduce venom-induced grooming at all (Fig. 4D; Weisel-Eichler et al. 1999). In fact, the venom contains a substance, which we identified by Gas chromatography-mass spectrometry as being a dopamine-like substance (Fig. 4F; Weisel-Eichler et al. 1999) and by HPLC-electrochemical detection as dopamine (J. Moore and M.E. Adams, personal communication).

Thus, DA in the venom is likely to be the component that induces prolonged grooming.

We suggest that the venom induces prolonged grooming by stimulating DA receptors in the cockroach's SEG. The DA D1 agonist SKF82958, which has been shown to be an agonist of DA receptors of the locust and the fruit fly (Ali and Orchard 1994; Reale et al. 1997) induces prolonged grooming in the cockroach when injected directly into the SEG (Weisel-Eichler et al. 1999) as well as in the fly *Drosophila melanogaster* (Yellman et al. 1997). The grooming response of the cockroach to SKF 82958 is similar to the excessive grooming seen in mammals in response to injection of DA D1 agonists (Molloy and Waddington 1984). Both effects occur in the central nervous system in the head, and both involve induction and coordination of a complex grooming behavior, involving many different parts of the body. There appears to be no other report in the literature of a venom injected via a sting that elicits a specific behavior pattern such as the venom-induced grooming that we observed. However, experimental injection of certain snake, scorpion and cone snail venoms into the brains of rats has been found to elicit scratching and other stereotypical behaviors (Silveira et al. 1988; Mello and Cavalheiro 1989; Dorce and Sandoval 1994; Olivera et al. 1999). Furthermore, in invertebrates, certain neuromodulators, particularly the monoamines, have been found to activate specific neural circuits and release well-defined behaviors, such as feeding in the leech (Lent et al. 1989), stridulation in the grasshopper (Ocker et al. 1995), and flight in the moth and locust (Sombati and Hoyle 1984; Claasen and Kammer 1986; Stevenson and Kutsch 1987).

It is challenging, if not impossible, to establish if the grooming effect of the *A. compressa* venom is in any way evolutionarily advantageous to the wasp or if it is simply a consequence of the location of the sting. The primary function of the sting is, surely, to produce the long-lasting lethargic effect in the cockroach, and the location of the sting is likely to be one that maximizes the hypokinetic effect. However, it is interesting to note that the extensive grooming behavior after the sting lasts for about 30 min, while the venom-induced hypokinesia is fully developed only after approximately 30 min (Fouad et al. 1994). During this initial period the cockroach tends to remain in the place where it was stung, perhaps because the escape response threshold is elevated, and locomotion is depressed, during grooming (Camhi and Nolen 1981; Hogan-Warburg et al. 1995). Cone snails use of a combination of venoms components to induce hyperactivity followed by flaccid paralysis. Apparently, this uncoordinated and frantic motor excitation immediately immobilizes the prey in place, so that it cannot get out of reach of the predator, until the slower acting paralysis begins. The identification of such a strategy in other venomous animals may shed some light on the adaptive significance of the venom induced grooming before hypokinesia sets in the cockroach prey.

Wasp venom induces long term-hypokinesia by modulating descending input to the thorax

Besides grooming behavior, the second sting in the head ganglia causes hypokinesia, which we define as a long lasting change in the threshold for initiation of various locomotory behaviors (Fouad et al. 1994, 1996). This hypokinesia starts after the 30 min of continuous grooming and lasts for 2–5 weeks (Fouad et al. 1994, Weisel-Eichler and Libersat 2002). Stung cockroaches show very little spontaneous or provoked activity such as escaping from wind stimuli. Their locomotion is also different (Weisel-Eichler and Libersat 2002). By contrast, our results did not show any differences between stung and control cockroaches in spontaneous or provoked grooming, righting behavior, or ability to fly in a wind tunnel (Weisel-Eichler and Libersat 2002). Hence, the head sting affects specific motor behaviors while leaving others unaffected.

The most striking effect of the second sting concerns the escape behavior. Wind stimuli directed at the cerci, which normally produce strong escape responses, are no longer effective in stung cockroaches (Fouad et al. 1994, 1996; Libersat et al. 1999). Wind-sensitive hairs on the cerci that detect the minute air movements produced by a predator's strike (Camhi 1984), excite GIs in the last abdominal ganglion (A6) (Fig. 5A), which mediate running escape behavior (Camhi 1984; Ritzmann 1993; Liebenthal et al. 1994). The GIs activate various thoracic interneurons (TIs) in the thoracic locomotory centers (Ritzmann and Pollack 1986; Ritzmann 1993). A sub-group of TIs, the TI_{AS}, excite various local interneurons or motoneurons, which are associated with escape running (Ritzmann and Pollack 1986; Ritzmann 1993). The cockroach escape behavior can be initiated by wind stimuli but also by tactile stimuli applied at various loci on the cuticle of the animal's body or the appendages other than the cerci such as the antennae (Comer and Dowd 1993; Comer et al. 1994). Although tactile and wind sensory information is carried by distinct populations of interneurons, all seem to converge on the same thoracic premotor circuitry (Ritzmann et al. 1991; Ritzmann and Pollack 1994) which controls similar escape leg movements (Camhi and Levy 1989; Nye and Ritzmann 1992; Schaefer et al. 1994). Thus, the TI_{AS} represent a common premotor pathway on which different sensory modalities converge (Fig. 5A). Our results show that the sting affects neither the response of the ascending GIs nor that of the descending interneurons (Fouad et al. 1994, 1996). All tactile and wind sensory pathways must carry signals to the premotor circuitry in the thoracic ganglia. Thus, we propose that the ultimate effect of the venom injected into the head ganglia is a modulation of the thoracic portion of the escape circuitry.

The venom could affect the initiation of the escape behavior by changing either (1) the synaptic drive from

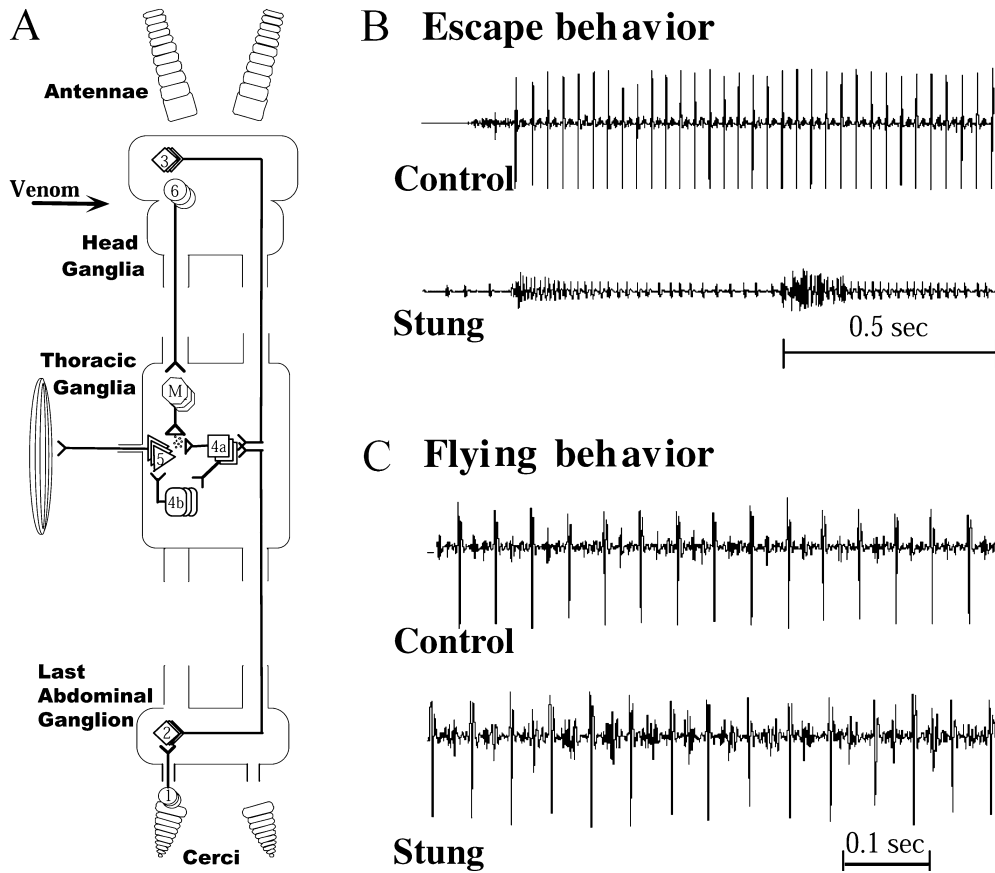


Fig. 5A–C Model of wasp venom-induced, long-term hypokinesia. **A** Schematic drawing of the cockroach's nervous system and escape neuronal circuit. Sensory mechanoreceptors of the cerci (1) recruit ascending GIs (2); these giant interneurons together with brain descending interneurons (3) converge onto the thoracic interneurons (4a). The thoracic interneurons excite via local interneurons (4b) or directly the leg motoneurons (5) involved in fast leg movements. Neurons (6) in the head ganglia provide descending permissive input to neuromodulatory neurons (M) located in the thoracic ganglia. These modulate the synapses between the thoracic interneurons and specific motoneurons. The venom represses the activity of head ganglia neurons thereby removing the descending excitatory drive to the neuromodulatory neurons. **B** Comparisons of the effect of the wasp's head sting onto a fast (*large unit*) and slow (*small unit*) motoneuron recorded with EMG electrodes implanted in a bifunctional muscle that spans the coxa and inserts into the thorax in stung and control animals. A wind stimulus is delivered to the cerci when the tethered cockroach is standing still on a slippery platform. In controls, the stimulus elicits an escape response with rapid leg movements. Both the fast and the slow motoneurons are recruited. In stung animals, the same wind stimulus elicits a burst in the slow but no response of the fast motoneuron. **C** The same cockroach is lifted up from the slippery platform in front of a wind stream and starts flying upon the release of leg contact with the substrate. During flight, the fast motoneuron is recruited in both control and stung animals (modified from Libersat et al. 1999)

the abdominal ascending and/or brain descending interneurons to thoracic interneurons, or (2) the synaptic drive from specific thoracic interneurons to specific leg motoneurons (Fig. 5A). To investigate these alternatives, we recorded the response of thoracic interneurons to the input from GIs. Our results show that the thoracic

interneurons receive a comparable synaptic drive from the giant interneurons in control and stung animals (Libersat et al. 1999). However, unlike normal cockroaches, which use both fast and slow motoneurons for producing rapid escape movements, stung animals activate only slow motoneurons and do not produce rapid movements (Fig. 5B; Fouad et al. 1996; Libersat et al. 1999). However, we show that in stung animals a fast motoneuron (Df) can still be recruited with a bath application of pilocarpine, a muscarinic agonist (Libersat et al. 1999). In insects, several muscles are bifunctional and are activated during walking and flight (Ramirez and Pearson 1988). We took advantage of this feature to test whether another fast motoneuron innervating such a bifunctional muscle could be recruited by thoracic flight interneurons. We show that, in stung animals, this fast motoneuron, which is not recruited for escape, can still be recruited in a rhythmic pattern during flight (Fig. 5C). This suggests that the descending control of the head ganglia on the thoracic escape circuitry is exerted on the premotor thoracic interneurons to motoneurons connections.

It is not yet clear what effects the wasp venom injected in the head ganglia may have on the thoracic portion of the neuronal circuitry controlling the initiation of escape behavior. It is certainly parsimonious to focus the venom indirect effect on the premotor thoracic circuitry rather than on sensory elements such as the GIs. First, the wasp approaches the cockroach

and even bites off its antennae without eliciting escape behavior. In so doing, the wasp applies tactile stimuli that would, in a normal cockroach, immediately trigger an escape and run. To prevent the cockroach from escaping to sensory stimuli, the venom would have to block all sensory inputs arising from cerci, antennae and the entire cuticular surface of the cockroach. Alternatively, since tactile or wind evoked escape behaviors share the use of a pre-motor interneuron pool (TI_{AS}) in the thoracic ganglia (Ritzmann et al. 1991; Ritzmann and Pollack 1994), inhibiting this portion of the escape circuitry appears to be a more efficient way to prevent the cockroach from escaping. Further experimental evidence for this hypothesis is the specificity of the inhibition whereby the venom depresses mostly the escape behavior. For instance, whereas wind stimuli always fail to elicit escape behavior in stung animals, these wind stimuli do initiate flight in stung animals (Fouad et al. 1994). Given these facts, escape-specific depression appears to require that inhibition be exerted on the pre-motor or motor elements dedicated to the escape circuit (Fig. 5A).

This specificity may be achieved by targeting a certain neuromodulatory system that controls a specific subset of behaviors. Such specificity of neuromodulatory systems has often been observed in invertebrates, where specific neuromodulators, particularly the monoamines, have been found to modulate the release of well-defined behaviors such as cockroach flight (Weisel-Eichler and Libersat 1996) or aggression in lobsters and crickets (Kravitz 1988, 2000; Hofmann and Stevenson 2000). Thus, it is possible that the venom could regulate the activity of neurons located in the head ganglion, which in turn, control the activity of neuromodulatory neurons in the thoracic ganglia. These neuromodulatory neurons would ultimately control the synaptic gain between the TIs and motoneurons (Fig. 5). Alternatively, neuromodulatory neurons in the head ganglia could directly affect this synapse in the thorax. Consistent with this hypothesis, experiments using reserpine to deplete the synaptic content of mono-aminergic neurons show impairment in the ability of cockroaches and crickets to generate escape behavior (Weisel-Eichler and Libersat 2002; Stevenson et al. 2000). Moreover, crickets depleted of octopamine and DA with AMT (alpha-methyl-*p*-tyrosine) show a similar impairment in their escape behavior (Stevenson et al. 2000). Goldstein and Camhi (1991) report that application of DA to the thoracic ganglia increases the excitability of the thoracic portion of the escape circuitry. Likewise, octopamine and DA can control the synaptic gain between the GIs and the TI_{AS} (Casagrand and Ritzmann 1992). Moreover, in headless fruit flies, the thorax is capable of generating coordinated leg movements if chemically stimulated with biogenic amines (Yellman et al. 1997). Finally, we have recently found that the spontaneous activity of thoracic octopaminergic neurons, which is modulated by descending input, appears to be depressed in stung cockroaches (Rosenberg and Libersat 2002). Together,

these pieces of experimental evidences suggest a role of DA or octopamine as chemical modulators of escape behavior in cockroaches.

Discussion

What has the wasp taught us about the organization of locomotory systems?

The long-lasting changes in the threshold for initiation of various locomotory behaviors occur only when the venom is injected in the head ganglia but not in the thoracic ganglia (Fouad et al. 1994). The head ganglia have been implicated in controlling the expression of locomotory patterns which are generated in the thoracic ganglia in insects (Kien and Altman 1992). The fruit fly *D. melanogaster* is becoming a promising model for genetic dissection of the control of locomotion by higher centers in the head. First of all, headless fruit flies do not generate spontaneous locomotory movements (Yellman et al. 1997). The genetics of the fruit fly provides for mutations that alter the mushroom body or central body structures. Analysis of walking in fly strains with mutations affecting the mushroom bodies or central complex structures have clearly demonstrated the role of these structures with higher locomotor control (Martin et al. 1998; Strauss 2002; Zars 2002). In addition, focal chemical stimulation of the central complex in the brain affects the threshold of initiation of a specific motor act, suggesting a role for this brain area in arousal (Heinrich et al. 2001). Therefore, the labeled venom detected in the SEG and around the central complex and mushroom bodies in the brain provides further support for the role of these neuronal structures in the control of insect locomotion and arousal. Recently, Schaefer and Ritzmann (2001) demonstrated the importance of descending neural influences on the escape behavior by examining the behavior following removal of descending neural inputs. As in stung cockroaches, the reduction of leg movement is accompanied by a reduction of a fast motoneuron activity suggesting a reduction of excitability within the thoracic portion of the escape circuitry. In addition, again as in stung cockroaches (Weisel-Eichler and Libersat 2002), the legs retain the ability to move vigorously during righting. Our results indicate that the venom affects motor systems selectively, rather than affecting all motor system and behaviors (Weisel-Eichler and Libersat 2002). Thus, the effect of the wasp's venom on cockroach locomotion may provide us with a tool to study the control of insect locomotion by higher centers.

There are close analogies between the roles of the vertebrate brainstem and the insect SEG. During walking, both are essential for initiation and detailed ongoing control of stepping and both carry out these functions through many separate and parallel pathways. They are also both involved in local motor activities, such as driving neck muscles, as well as in overall

regulation of motor excitability (respiration, posture, locomotion) and integration with autonomic activity (heart rate, salivation and gut activity) (for review, see Kien and Altman 1992). For example, a descending catecholaminergic system from the reticular formation affects the excitability of spinal locomotion generators (reviewed in Bjorklund and Lindvall 1986). In our relatively simple system, the neuronal circuitry and the cellular mechanisms by which neuromodulatory neurons control the initiation and maintenance of locomotory activity are much more accessible to experimentation. Thus, the discovery of clear behavioral roles for neuromodulators in invertebrate locomotion might shed light on patterns in the evolution of these behavioral neurochemicals as well as their function in vertebrate locomotion.

The wasp-cockroach association as a model system to study parasite-induced alterations of host behavior

Many parasites alter the behavior of their hosts in many ways including phototaxis, locomotion, behavioral fevers, foraging behavior, reproduction and a variety of social interactions, to name a few (see Moore 2002 for review). Although the alteration of host behavior by parasites is a widespread phenomenon, underlying mechanisms are only beginning to be deciphered (Beckage 2002; Moore 2002). Whereas alterations in host reproduction and development (Beckage 2002) are known to be caused by manipulation of the host's endocrine or neuroendocrine system, very few altered behaviors can be unambiguously linked to alterations in the central nervous system. Here again, the most fascinating examples of behavioral manipulation are arthropods parasitized by specific species of wasps. For example, orb-weaving spiders (*Plesiometa argyra*) parasitized by the wasp *Hymenopimecis* sp. spins an unusually shaped web to support the wasp offspring's cocoon. This is almost certainly the most sophisticated alteration of behavior ever attributed to an insect parasitoid (Eberhard 2000). Of particular interest for this review is the change in locomotory behavior observed in *Manduca sexta* parasitized by the braconid wasp *Cotesia congregata*, which resembles the hypokinesia of stung cockroaches. These changes in locomotory behavior are attributable to a modulation of the inhibitory control of a locomotion-initiating network in the SEG (Beckage 2002). Nonetheless, wasps are not the only parasites known to alter locomotion in their hosts. For instance, the cockroach escape behavior is altered by an infective acanthocephalan parasite *Moniliformis moniliformis* (Libersat and Moore 2000). The worm-parasite has a two-host life cycle. It matures to an infective stage called a cystacanth in the cockroach hemocoel, and infects a rodent when the cockroach is consumed. The escape response is modified in ways that probably increase predation risk for infected animals (Libersat and Moore 2000). An understanding of the neuronal basis of

parasite induced alterations of host behavior would greatly assist us in placing such alterations in an evolutionary context that could then be used as a predictive framework for unstudied host-parasite associations. Cockroaches have proven to be among the most fruitful subjects for investigating the neural mechanisms of animal behavior, and the wasp-cockroach model may prove equally rewarding to decipher the neural basis of host-parasite associations.

Future prospects

The specificity and effectiveness of neurotoxins are the outcome of evolutionary selection on one animal's strategy to incapacitate another (Adams and Olivera 1994). Here we highlight the selection of an amazing behavioral strategy by a venomous predator for the delivery of these neurotoxins into the central nervous system of its prey to cause specific and effective behavioral modifications. The unique effect of the wasp venom on the cockroach locomotory behavior contrasts markedly with the paralyzing actions of other known solitary wasp venoms, which interfere at the neuromuscular junction. We know that, unlike these other species of wasps, *A. compressa*'s venom has no effect on the cockroach neuromuscular junction (Fouad et al. 1996). Thus, because of its specific behavioral effects, one could envisage that *ampulex*' neurotoxins might have interesting novel effects on the excitability of neurons or on synaptic transmission. The venom components and their corresponding molecular targets that are involved in these behavioral modifications remain to be identified. With the available battery of biochemical and molecular biology techniques, it should be possible to characterize the biochemical composition of *A. compressa* venom and identify bioactive components by their introduction in vivo and in vitro into the cockroach's central nervous system.

To conclude, by studying the effect of the wasp's venom on its host, it should be possible to increase our understanding of several important biological issues such as, the neuronal basis of parasite induced alterations of host behavior, the neurobiology of initiation of motor behaviors and the neural mechanism for change in responsiveness, which is a prime question in the study of motivation.

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