Parasitoid Wasps: Neuroethology

F. Libersat, Institut de Neurobiologie de la Méditerranée, Parc Scientifique de Luminy, Marseille, France

Introduction

Predators as diverse as snakes, scorpions, spiders, insects, and snails manufacture venoms to incapacitate their prey. Most venoms contain a cocktail of neurotoxins and each neurotoxin is designed to target-specific receptors in the nervous and muscular systems. Most neurotoxins act peripherally and interfere with the ability of the prey's nervous system to generate muscle contraction or relaxation, resulting in immobilization and often death of the prey to be consumed immediately. However, in a few species of predatory wasps, venoms appear to act centrally to induce various behaviors. These venomous wasps use mostly other insects or spiders as food supply for their offspring. Most parasitoid wasps eat only nectar from flowers and other small insects, but as larvae they eat something totally different. Many of these wasps paralyze their prey and then lay one or more eggs in or on the host, which serves as a food source for the hatching larvae. In a few instances, the parasitoid wasp often manipulates the host's behavior in a manner that is beneficial to and facilitates the growth and development of its offspring. Although the alteration of host behavior by parasitoids is a widespread phenomenon, the underlying neuronal mechanisms are only now beginning to be deciphered. As of today, only a few behavioral alterations can be unambiguously linked to alterations in the central nervous system (CNS).

The direct manipulation of the host nervous system and behavior may take several forms. In some instances, the venom is purely paralytic, affecting either the peripheral or CNS to induce partial or total paralysis, which can be transient (seconds to minutes) or long-term (hours to days). In other instances, the venom might affect behavioral subroutines to produce finer manipulations of the host behavior. In this article, I will discuss selected case studies where the neural mechanisms underlying host manipulation by parasitoid wasps have been identified. I will then focus on one case study where a wasp hijacks the brain of its host to control its motivation to perform specific behaviors.

Most ectoparasitoid wasps incapacitate their prey and then drag it to a burrow or a nest. In this protected nest, the wasp lays its egg on the prey and seals the burrow with the inert prey inside. When the larva later hatches, it feeds on the host, ultimately killing it, and pupates in the nest, sheltered from predators that could harm the cocoon. The hunting and host-manipulation strategies of these wasps are diverse and, at least to some extent, depend on the host natural behavior. Hunters of relatively small or harmless prey usually inflict a single or double sting to the prey item. This typically results in deep paralysis by affecting, for example, the peripheral nervous system (i.e., the neuromuscular junction: synapse between the motoneurone terminals and the muscle). In those species of wasps where the paralyzing venom is injected into the hemolymph of the prey, as in the beewolf (the Egyptian digger wasp *Philanthus triangulum*), the venom has been shown to affect the peripheral nervous system (**Figure 1**). *P. triangulum* feeds its larvae almost exclusively with Honeybees (*Apis mellifera*). The beewolf paralyzes bees by stinging them on the ventral side of the thorax through the membrane between the first and second segments. These wasps are sufficiently strong to airborne cargo the prey item back to the nest (**Figure 1(a)**). After provisioning the nest with a few bees, the wasp lays an egg in it and seals it. The venom of the beewolf contains potent neurotoxins known as philanthotoxins, which evoke neuromuscular paralysis in the bee prey. Such philanthotoxins interfere presynaptically and postsynaptically with glutamatergic synaptic transmission (**Figure 1(b)**). Because glutamate is the neurotransmitter at the insect neuromuscular junction, philanthotoxins in the venom block the neuromuscular transmission to induce flaccid paralysis of the prey. One potent component of the *Philanthus* venom is δ-philanthotoxin, which blocks open ionotropic glutamate receptors in the insect neuromuscular junction (**Figure 1(b)**). Paradoxically, the very same δ-philanthotoxin blocks glutamate uptake (it interferes with the glutamate transporter) at the insect neuromuscular junctions thereby, prolonging the presence of glutamate at the neuromuscular junction (**Figure 1(b)**). This venom-induced hyperexcitation of muscle contraction is presumably responsible for the initial tremor, which immobilizes the prey until flaccid paralysis begins. Hyperexcitation preceding flaccid paralysis is a common venom strategy seen in several types of venomous animals, such as octopus, spiders, coelenterates, and some cone snails where the hyperexcitation is produced by different classes of substances. Apparently, the hyperexcitation immediately immobilizes the prey, so that it cannot get out of reach of the predator, until the slower acting flaccid paralysis begins. The wasp paralyzes several bees and drags them into a concealed burrow. It then lays an egg on one of the bees, seals the burrow, and leaves. The hatching larva is, thus, provided with a large, paralyzed food supply to feed on until pupation.

On the other hand, wasps, which hunt on large prey such as tarantula spiders, face a much more considerable

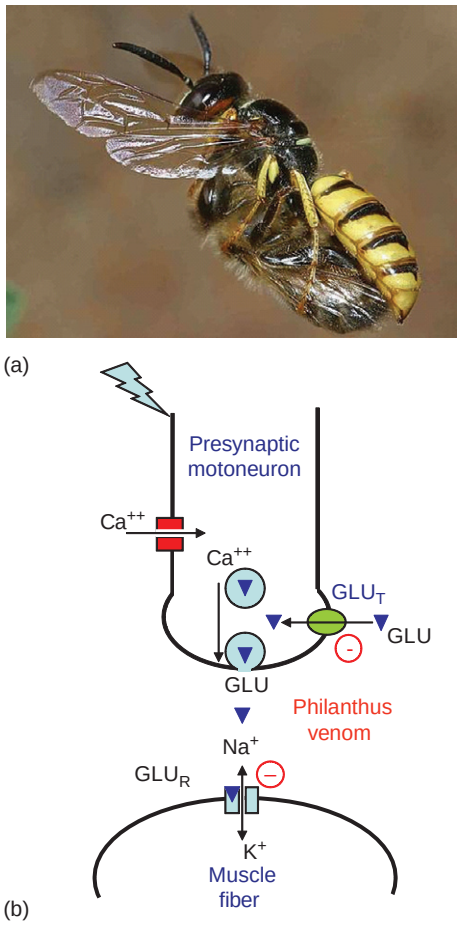

Figure 1 (a) A photograph of an air-borne *Philanthus* wasp carrying its bee prey back to the nest. (b) Schematic representation of the insect neuromuscular junction where *Philanthus* venom affects glutamatergic synaptic transmission. Calcium (Ca++) ions move in when an action potential (blue arrow) reaches the motoneuron terminal and facilitates the vesicular release of glutamate (GLU). One potent component of the *Philanthus* venom (δ-philanthotoxin) blocks open ionotropic glutamate receptors (GLU_R) and glutamate uptake (GLU_T) to induce muscular paralysis.

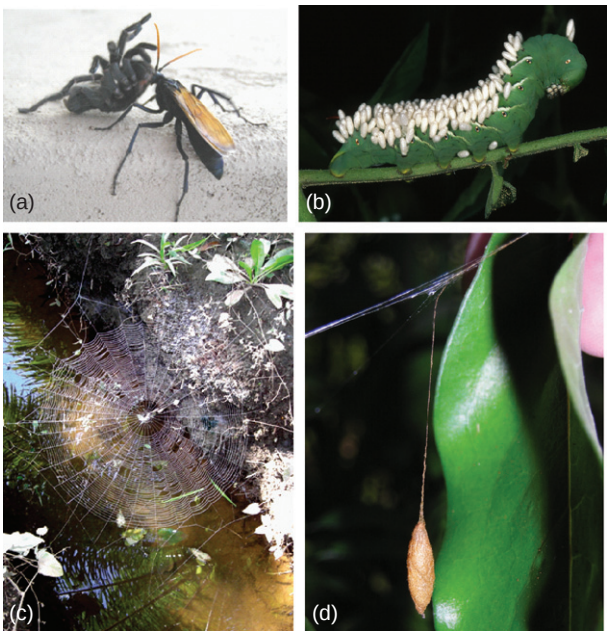

Figure 2 (a) The spider wasp, *Tachypompilus ignitus*, dragging an immobilized *Palystes* spider to her nest. (b) The tomato hornworm, *Manduca*, parasitized by the solitary braconid endoparasitoid wasp *Cotesia* pupae. (c) The normal web of the orb-weaving spider *P. argyra*. (d) The cocoon web of a spider parasitized by the Ichneumonid wasp and wasp cocoon from above.

danger (Figure 2(a)). The tarantula-hawk (*Pepsis*) is the fearsome enemies of spiders. These wasps usually first disarm the spider of its most powerful weapon, the fangs, with multiple stings into the cephalo-thorax but sometimes directly in the mouth. After this stinging sequence, the spider is totally paralyzed, which allows the wasp to drag the spider back to the nest, walking backwards facing its formidable opponent. Once the host is concealed, the wasp lays a single egg on the abdomen of the spider and seals the entrance to the nest. Depending on the species, the spider would completely or nearly completely recover from paralysis within a few hours to 2 months. If the tarantula survives what usually happens next, it can revive and continue living a normal life. But another fate awaits the spider as the larva hatches from the egg after 2 days and feeds on the entombed spider for 5–7 days. The satiated larva then pupates inside the nest, safe from predators.

As we shall see in this article, some parasitoid wasps alter the behavior of their host to the finest degree. The unique effects of such wasp's venom on prey behavior suggest that the venom targets the prey's CNS. A remarkable example of such manipulation is that of the braconid parasitoid wasp (*Glyptapanteles* sp.) that induces a caterpillar (*Thyrinteina leucocerae*) to behave as a bodyguard of its offspring. After parasitoid larvae exit from the host to pupate, the host remains alive but displays stunning modifications in its behavior: it stops feeding and remains close to the parasitoid pupae to defend these against predators with violent head swings. The parasitized caterpillar dies soon after while unparasitized caterpillars do not show any of these behavioral changes. In another example of host manipulation, the wasp *Cotesia congregata* and its host, the tobacco hornworm *Manduca sexta*, we have some information about the underlying mechanisms of manipulation. The female wasp injects a mixture consisting of venom, polydnavirus, and wasp eggs into its caterpillar host (Figure 2(a)). The wasp larvae hatch and develop inside the host's hemocoel, exit through the cuticle, and spin a cocoon which stays attached to the host. One day before exiting the host, host feeding and spontaneous locomotion decline. The host remains in this torpor

until death. The decline in host feeding and locomotion can be induced by wasp larvae alone which, by an unknown chain of events, target the subesophageal ganglion (SEG) of the host to induce neural inhibition of locomotion. Furthermore, the change in host behavior is accompanied with an elevation of CNS octopamine (OA), a neuromodulator, suggesting that alterations in the functioning of the octopaminergic system may play a role in depressing host feeding or locomotion. But, the most exquisite alteration of behavior ever attributed to a parasitoid wasp is probably the Ichneumonid wasp *Hymenoepimecis*'s manipulation of its spider host. In this exceptional example of host behavioral manipulation, the parasitoid wasp takes advantage of the natural behavior of web waving of its prey to provide the larva with a shelter. Instead of paralyzing and then burrowing into the host, this wasp literately coerces the host to build the shelter for its future larva. The wasp stings its spider host, *Plesiometa argyra* (Araneidae), evoking a total, but transient, paralysis during which the wasp lays its egg on the paralyzed spider and flies away. Soon after the sting, the spider recovers to resume apparently normal activity. It builds normal orb webs to catch prey (**Figure 2(c)**), while the wasp's egg hatches and the larva grows by feeding on the spider's hemolymph. The larva feeds for about 2 weeks and just before it kills the spider, a dramatic behavioral change occurs in the spider. The prey, driven by an unknown mechanism, starts weaving a unique web with a design that seems tailored to fit the needs of the larva for its next stage in development, the metamorphosis. The new web is very different from the normal orb-shaped web of *P. argyra*, and is designed to support the larva's cocoon suspended in the air, rather than lying on the ground (**Figure 2(d)**). In this safe net, the wasp larva consumes the spider, ultimately killing it, and then pupates in the suspended net. Interestingly, if the wasp larva is removed just prior to the execution of the death sentence, the spider continues to build the specialized cocoon web. Hence, the changes in the spider's behavior must be induced chemically rather than by direct physical interference of the wasp larva. The wasp larva must secrete chemicals to manipulate the spider's nervous system to cause the execution of only one subroutine of the full orb web construction program while repressing all other routines. The nature of the chemicals involved in this extreme alteration of the spider's behavior, is unfortunately unknown.

Sphecid wasps often hunt large and potentially harmful orthopteroids (crickets, katydids, grasshoppers, etc.). They usually sting their prey to evoke total transient paralysis, although in some instances, a more specific manipulation takes place. One example of total transient paralysis of the host can be found in the *Larra* – mole cricket system. Mole crickets spend most of their time in a burrow. A larrine wasp (e.g., *L. anathema*) in a hunting mood penetrates the underground refuge of the cricket and attacks it. The frightened cricket may emerge in panic from its burrow pursued by the wasp. The wasp then wrestles with its prey to finally inflict multiple stings, mainly in the thoracic region. The stings induce a total transient paralysis of the legs, lasting just a few minutes. The wasp performs host feeding, sucking some hemolymph before laying a single egg between the first and second pairs of legs of the inert cricket. The wasp then leaves the cricket which fully recovers from paralysis and burrows back into the ground, apparently resuming normal activity. The egg soon hatches and the larva starts feeding on the cricket after piercing the cuticle with its mandibles. The development from egg laying to pupation lasts between 2 weeks and a month, during which the mole cricket appears to behave quite normally, demonstrating complete recovery from paralysis.

An example for central paralysis can be found in the Palearctic Larrine digger wasp *Liris niger* which hunts crickets as food supply for its brood. To transport the cricket to a burrow and lay an egg on its cuticle, the wasp incapacitates the prey with four stings, which are applied near, or perhaps inside, the CNS. First, the wasp disarms the cricket's most powerful weapons, the metathoracic kicking legs, by injecting venom presumably into the metathoracic ganglion. This sting paralyzes the metathoracic legs for several minutes. Successively, the wasp injects venom into the two other thoracic ganglia, transiently paralyzing the legs associated with these ganglia and rendering the stung cricket lying helplessly on its back for several minutes. Last, the wasp stings into the neck, probably directly into, or in the vicinity of, the subesophageal ganglion. This last sting is responsible for the next phase of envenomation, a long-lasting hypokinetic state. The wasp drags the paralyzed cricket to a burrow, glues an egg between its fore and the middle legs, and seals the burrow with soil particles or pebbles. After the burrow has been sealed, the cricket fully recovers from its paralysis and can maintain posture and even walk. However, at this time, a different story unfolds, as the stung cricket never attempts to escape the burrow; rather it stays motionless, although not paralyzed, in its tomb. The wasp larva, after hatching from the egg, feeds on the lethargic cricket and then pupates. If the cricket is experimentally removed from the burrow, no spontaneous and only little evoked activity can be observed in the stung cricket until it dies, probably due to lack of feeding. Thus, *Liris* venom induces not only total transient paralysis but also a partial irreversible paralysis which renders the cricket prey submissive in its future grave. It has been suggested that the latter effect of *Liris* venom is a result of the neck-sting, which is, for comparison, not typical for mole cricket-hunting *Larra* and does not evoke such long-term effects.

The short-term paralysis of the cricket legs has been thoroughly investigated in this *Liris*-cricket system. The venom's effect on the CNS of crickets has been studied in dissected preparations in which venom was manually

applied to thoracic or abdominal ganglia by means of manipulating the wasp to sting directly into the ganglion or by pressure-injecting sampled venom into the ganglion. All experiments with dissected preparations demonstrate two pronounced phases of envenomation. First, the sting typically evokes a short (15–35 s) tonic discharge of the motoneurons innervating the legs. This discharge is most likely responsible for the convulsions of the cricket's limbs, which is the first venom effect observed immediately after the sting. The cellular mechanism by which the motoneurons' discharge rate increases, is not yet fully understood, but it is either due to the presence of an excitatory agonist (e.g., ACh receptors) in the venom or due to the low pH of the venom. The short tonic discharge of the legs' motoneurons then completely disappears, marking the onset of the second effect of the *Liris* venom: total transient paralysis of the legs. This paralysis, lasting from 4 to 30 min, is characterized by a complete absence of spontaneous or evoked activity in the affected neurons. Then, after the total paralysis phase is over, responses of the leg muscles and motoneurons to sensory stimuli recover. However, behaviorally, the prey fails to initiate locomotion, which underlies the beginning of the third, hypokinetic, and irreversible phase of envenomation, at the end of which the cricket dies. The venom's paralytic effects are restricted to the stung ganglion, indicating that the venom affects central (rather than peripheral) targets. For instance, excitation of leg sensory receptors of stung crickets evokes afferent sensory potentials that reach the stung ganglion but fail to engage a motor reflex in that ganglion, demonstrating that the venom's effect is restricted to the stung ganglion. Various physiological experiments have uncovered at least three types of effects in the CNS. First, the venom prevents the generation and propagation of action potentials in the affected neurons, presumably by interfering with voltage-dependant inward sodium currents. Second, the venom decreases central synaptic transmission, the underlying mechanism of which is not yet fully understood. Third, the venom increases leak currents in central neurons and consequently their excitability.

A Case Study in the Neural Mechanisms of Host Manipulation: *Ampulex compressa* and its Prey, the Cockroach *Periplaneta americana*

Ampulex Hunting Strategy and Offspring Development

The best understood manipulation of host nervous system and behavior is the case of the Sphecid cockroach-hunter *A. compressa*. After grabbing its cockroach prey (usually *P. americana*) at the pronotum or the base of the wing, the wasp inflicts a first sting into the thorax. This sting renders the prothoracic legs transiently (1–2 min) paralyzed and presumably facilitates the second sting into the neck, which is much more precise and time-consuming (**Figure 3(a)**). After the neck-sting is complete, the wasp leaves the cockroach for about 30 min and searches for a burrow. During this period, the cockroach is far from being paralyzed but grooms frenetically for about 20 min. When this period is almost over, the wasp returns to the cockroach and pushes him around with its mandibles as if to evaluate the success of the sting. This is when another effect of the venom begins to take place, as the cockroach becomes a submissive 'zombie' capable of performing, but not initiating, locomotion. The wasp cuts the cockroach's antennae with the mandibles and sucks up hemolymph from the cut end. It then grabs one of the cockroach's antennal stumps and leads the host to the preselected burrow for oviposition, walking backwards facing the prey. The stung cockroach follows the wasp in a docile manner, like a dog on a leash, all the way to the burrow. Then, the wasp lays an egg and affixes it on

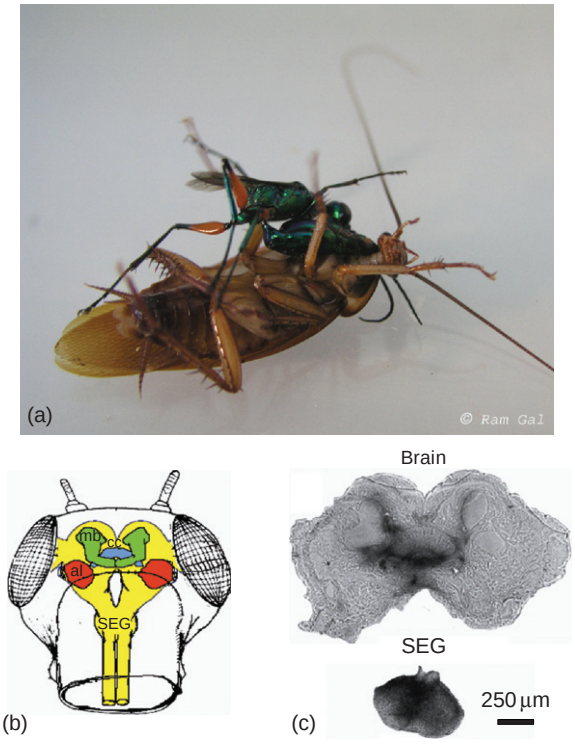

Figure 3 (a) The wasp *Ampulex compressa* stings a cockroach *Periplaneta americana* in the head. (b) A schematic representation of a dorsal view of a cockroach head shows the relative positions of the head ganglia in the head capsule. The brain and SEG are shown in yellow. The major structures of the brain include the central complex (cc, red), the mushroom bodies (mb, green), and the antennal lobes (al, blue). (c) Two sections of representative head ganglia (brain and SEG) preparations 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 and in the center of the SEG.

the cuticle of the coxal segment of the middle cockroach's leg. Having its egg glued on the live food source, the wasp exits the burrow and blocks the entrance with small pebbles collected nearby, sealing the lethargic host inside. The larva hatches within 2–3 days and perforates the cuticle of the cockroach's coxa to feed on hemolymph for the next few days. About 5 days after the egg was laid, the larva moves to the thoracic–coxal junction of the metathoracic leg and bites a large hole along the soft cuticular joint, through which it then penetrates the cockroach. The larva feeds on the internal organs of the cockroach until, 2 days after entering the host, it occupies the entire cockroach's abdominal cavity. Pupation occurs inside the cockroach's abdomen, roughly 8 days after the egg was laid.

The two stings by *A. compressa* induce a total transient paralysis of the front legs followed by grooming behavior and then, by a long-term hypokinesia of the cockroach's prey. In this state, the cockroach remains alive but immobile and unresponsive, and serves to nourish the wasp larva. The long-lasting lethargic state occurs when the venom is injected into the head but not when it is injected only into the thorax. Under laboratory conditions, and if not parasitized by the wasp larva, cockroaches gradually recover from this lethargic state within 1 or 2 weeks, demonstrating a partial long-term paralysis of the cockroach. In nature, cockroaches probably rarely reach recovery as the *A. compressa* larva consumes them before the end of this convalescent time.

Where Is the Venom Injected?

For more than a century, there has been a controversy over whether some parasitoid wasps deliver their venom by stinging directly into the CNS. In 1879, the French entomologist Jean Henri Fabre, who observed that specific wasps sting in a pattern corresponding to the location and arrangement of nerve centers in the prey, suggested that the wasp stings directly into target ganglia. Others challenged Fabre's idea and claimed that the wasp stings in the vicinity of, but not inside, the ganglion. In fact, solitary wasps' venoms usually consist of a cocktail of proteins, peptides, and subpeptidic components, some of which are very unlikely to cross the thick and rather selective sheath (the insects' blood–brain barrier) around the nervous ganglia. Thus, it is most unlikely that neurotoxins in the venom make their way into the CNS by simple diffusion from the hemolymph. It was, therefore, suggested that some wasps use a common strategy of 'drug delivery,' injecting venom directly into a specific ganglion of the CNS of the prey.

The unique effects of *Ampulex*'s venom on prey behavior and the site of venom injection both suggest that the venom targets the prey's CNS. Until recently, the mechanism by which behavior-modifying compounds in the venom reach the CNS, given the protective ganglionic sheath, was unknown. The *Ampulex* stinger, which is about 2.5 mm in length, is certainly long enough to reach the cerebral ganglia that lie 1–2 mm deep in the head capsule. But to obtain a direct proof of the central injection of the venom, we produced so-called 'hot' wasps by injecting them with a mixture of C^{14} radiolabeled amino acids which were incorporated into the venom. In cockroaches stung by 'hot' wasps, most of the radioactive signals were found in the thoracic ganglion and inside the two head ganglia: the supra and the subesophageal ganglia (**Figure 3**). Only a small amount of radioactivity was detected in the surrounding, nonneuronal tissue of the head and thorax. A high concentration of radioactive signal was localized to the central part of the supraesophageal ganglion (posterior to the central complex and around the mushroom bodies) and around the midline of the subesophageal ganglion. The precise anatomical targeting of the wasp stinger through the body wall and ganglionic sheath and into specific areas of the brain, is akin to the most advanced stereotactic delivery of drugs. Sensory structures located on the stinger might be responsible for mediating nervous-tissue recognition inside the head capsule to allow such precise venom injection inside the head ganglia. These experiments represent, to date, the only unequivocal demonstration that a wasp injects venom directly into the CNS of its prey, consistent with Fabre's ideas. *A. compressa* is almost certainly not the only wasp which injects venom in its prey CNS, although the use of such method of drug delivery remains to be proven in other wasp species.

Wasp Venom Induces Transient Paralysis of the Front Legs

The *Ampulex* venom, similar to the *Liris* venom, is a complex cocktail of proteins, peptides, and subpeptidic components. Of this cocktail, only low molecular weight fractions seem to be responsible for the short-lived front legs paralysis. Electrophysiological studies on the *Ampulex* venom have demonstrated that it dramatically affects central cholinergic synaptic transmission. For instance, injection of venom to the cockroach's last abdominal ganglion eliminates synaptically evoked action potentials in the postsynaptic giant interneuron (GI) (**Figure 4(a)**). Likewise, venom injections block the postsynaptic potentials evoked by exogenous cholinergic agents at the same synapse.

To identify the venom components responsible for the total transient paralysis of the front legs, fractions of the venom (based on different molecular weights) were applied to neurons and the responses quantified. The fractions that reduced neuronal activity caused a synaptic block in central synapses. Biochemical screening of the active fractions revealed that the venom contains high levels of the inhibitory neurotransmitter GABA, and

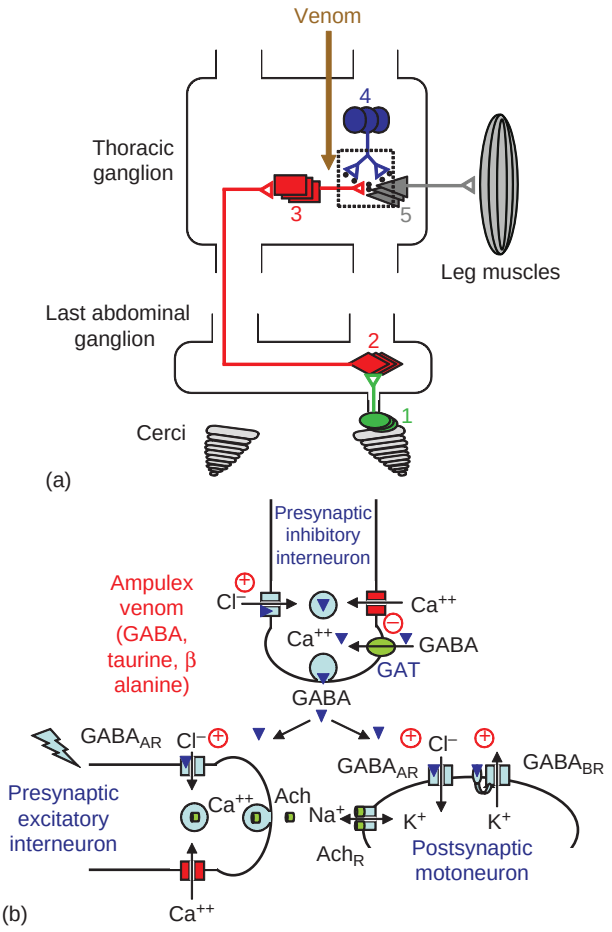

Figure 4 A schematic and simplified drawing of a cockroach's nervous system depicting the circuitry that controls escape behavior and leg movements. For escape, sensory mechanoreceptors of the cerci (1) recruit ascending GIs located in the last abdominal ganglion (2); these GIs converge onto the thoracic interneurons in the thoracic ganglion (3). The thoracic interneurons excite the leg motoneurons (5). Local inhibitory interneurons controls the activity of motoneurons involved in leg movements. The doted rectangle shows the area enlarged in (b). (b) A model of a wasp venom-induced short-term leg paralysis. The natural release of GABA at a synapse causes the opening of the chloride channels in the postsynaptic membrane. When GABA-gated chloride channels ($GABA_{AR}$) are opened by venom components, the resulting GABA current shunts the depolarization generated by the release and binding of acetylcholine (Ach) from the presynaptic excitatory interneuron when an action potential reaches the terminal (blue arrow). GABA can also act on the presynaptic terminals of the cholinergic interneuron by decreasing the synaptic release. Because taurine and β-alanine suppress the reuptake of GABA (GABA transporter: GAT) from the synaptic cleft, they may contribute to a prolongation of chloride channel open times. Together, these effects result in a failure of action potential generation in the postsynaptic motoneuron.

a GABA receptor agonist β-alanine. Another component in these fractions was identified as taurine, which is known to impair the reuptake of GABA by the GABA transporter from the synaptic cleft (**Figure 4(b)**). These constituents mimic the transient action of whole venom, synergistically causing a total transient block of synaptic transmission at the cercal-giant synapse through GABA inhibition. Patch-clamp recordings from isolated thoracic motoneurons of the cockroach demonstrate that the *Ampulex* venom induces picrotoxin-sensitive currents, further implicating venom action on cockroach GABA receptors. The natural release or artificial injection of GABA at a synapse causes the opening of chloride channels in the postsynaptic membrane. When GABA-gated chloride channels are opened by venom components, if the sodium channels in the postsynaptic membrane are also opened by a synaptic release of ACh, then for each sodium ion entering the cell, a chloride ion will accompany it. The simultaneous entry of a negative ion and positive ion will produce no change in the membrane potential. The utilization of venom cocktails containing multiple toxins with distinct but joint pharmacological actions has been described previously in venoms of spiders and marine cone snails. To conclude, for the total transient paralysis, the study of *Ampulex* venom has demonstrated a novel strategy for venom-induced synaptic block through chloride channel activation.

Wasp Venom Initiates Prey Grooming Behavior

After the total paralysis effect of the venom is over, the *Ampulex* venom evokes a stereotyped, though excessive, uninterrupted grooming behavior in the stung cockroach. The venom-induced grooming is similar in all respects to normal grooming and involves the coordinated movements of different appendages. The grooming behavior is evoked only if venom is injected into the head, and cannot be accounted for by the stress of the attack, the contact with the wasp, a mechanical irritation or venom injection into a location other than the head. Thus, the *Ampulex* venom appears to engage a central neuronal circuit in the head ganglia responsible for grooming. Experimental manipulation of the monoaminergic system in unstung cockroaches affects grooming behavior. For example, a single injection into the subesophageal ganglion of the alkaloid reserpine, which transiently elevates the concentration of all monoamines in central synapses, induces excessive grooming similar to venom-induced grooming. The specific cause for this is probably an elevation in the levels of the monoamine dopamine (DA), since an injection of DA or DA receptor agonists similarly induced excessive grooming. Moreover, the injection of a DA-receptor antagonist (Flupenthixol) prior to a wasp sting markedly reduced venom-induced grooming. Thus, grooming behavior could result from the existence of a DA (or DA-like) component in the venom, or from a venom component that would activate a DA-releasing mechanism inside the cockroach head ganglia. A gas chromatography–mass spectrometry study has identified

a DA-like substance in the venom. This substance might be responsible for a direct stimulation, via DA receptors, of grooming–releasing circuits within the head ganglia.

One can only speculate regarding the adaptive significance of the grooming phase of envenomation. First, it is possible that grooming is merely a side effect of the venom. For example, the DA-like substance in the venom could be involved in inducing the long-term hypokinetic state, in which case, grooming has no adaptive value to the wasp. Second, and more provocative, is the possibility that excessive grooming cleans off the ectoparasites such as bacteria and fungi on the cockroach's exoskeleton which are potentially harmful to the developing wasp's larva. Interestingly, beewolf females (**Figure 1(a)**) cultivate the *Streptomyces* bacteria in specialized antennal glands and smear them on the ceiling of the brood cell prior to oviposition. The bacteria enhance the survival probability of the larva as they are taken up by the larva later to protect the cocoon from fungal infestation.

Wasp Venom Controls Cockroach Motivation to Walk and Escape

The third phase of cockroach envenomation by *A. compressa* is probably the most interesting in terms of host behavioral manipulation, because a stung cockroach becomes a submissive 'robot,' and fails to initiate spontaneous or evoked locomotion. It is almost certainly of adaptive value to the wasp, since it enables resistance-free host feeding, transportation to the burrow, and oviposition. The venom-induced hypokinesia persists, if the egg is removed experimentally, for at least a week, after which the cockroach resumes a normal activity. In nature, however, the cockroach meets its inevitable fate about a week later.

The long-term hypokinesia is induced only if *A. compressa* stings the cockroach in the head ganglia. Hence, the inability of stung cockroaches to start walking cannot be accounted for by a direct effect of the venom on locomotory centers in the thoracic ganglia of insects. We propose that, unlike most paralyzing venoms, *Ampulex*'s venom affects the 'motivation' of its host to initiate movement, rather than the motor centers. Indeed, the wasp injects its venom directly into the subesophageal ganglion and into the central complex and mushroom bodies in the supraesophageal ganglion of the cockroach, all considered 'higher' neuronal centers modulating the initiation of movement. We investigated whether the venom-induced hypokinesia is a result of an overall decrease in arousal or, alternatively, a specific decrease in the drive to initiate or maintain walking. We found that the venom specifically increased thresholds for the initiation of walking-related behaviors and, once such behaviors were initiated, affected the maintenance of walking. Nevertheless, we show that the thoracic walking pattern generator itself appears to be intact. Thus, the venom, rather than decreasing the overall arousal, manipulates neuronal centers within the cerebral ganglia that are specifically involved in the initiation and maintenance of walking. Furthermore, stung hypokinetic cockroaches show no deficits in spontaneous or provoked grooming, righting behavior, or the ability to fly in a wind tunnel. Hence, the head sting affects specific subsets of motor behaviors, rather than affecting behavior in general. How this comes about is not completely worked out, but we have uncovered some important pieces of the puzzle.

The hypokinetic state is characterized by very little spontaneous or provoked activity; an important hallmark of this hypokinetic state is the inability of stung cockroaches to produce normal escape responses. Wind stimuli directed at the cerci, which normally produce strong escape responses, are no longer effective in stung cockroaches. Normally, wind-sensitive hairs on the cerci detect the minute air movements produced by a predator's strike and excite GIs in the terminal abdominal ganglion (TAG) to mediate escape running behavior (**Figure 5**). The GIs activate various thoracic interneurons in the thoracic locomotory centers, which, in turn, excite various local interneurons or motoneurons associated with escape running. In addition, escape running can be triggered by tactile stimuli applied the antennae that recruit GIs descending from the head ganglia to the thorax. Tactile and wind information is carried by two distinct populations of interneurons, each located at the far and opposite ends of the nervous ganglionic chain, to converge on the same thoracic premotor circuitry which controls similar escape leg movements. Studies on stung cockroaches show that the sting affects neither the response of the sensory neurons and associated ascending GIs nor that of the descending interneurons. Moreover, thoracic interneurons receive comparable synaptic drive from the GIs in control and stung animals. Thus, the ultimate effect of the venom injected into the head ganglia must take place at the connection between the thoracic interneurons and specific motorneurons.

Unlike normal cockroaches, which use both fast and slow motoneurons for producing rapid escape movements, stung cockroaches activate only slow motoneurons, which are also important to maintain posture, and do not produce rapid movements. This lack of response of fast motoneurons appears to be due to a reduction in the synaptic drive they receive from premotor interneurons. Such reduction could be due to a modulation of a particular neuromodulatory system that controls a specific subset of behaviors. In this case, the venom would chemically manipulate specific pathways in the head ganglia which themselves regulate neuromodulatory systems involved in the initiation and/or execution of movement (**Figure 5**). Monoaminergic systems are again probable candidates, as alterations in these systems are known to affect specific subsets of behaviors. For instance, depletion of the synaptic content of monoaminergic neurons, and especially of dopaminergic or

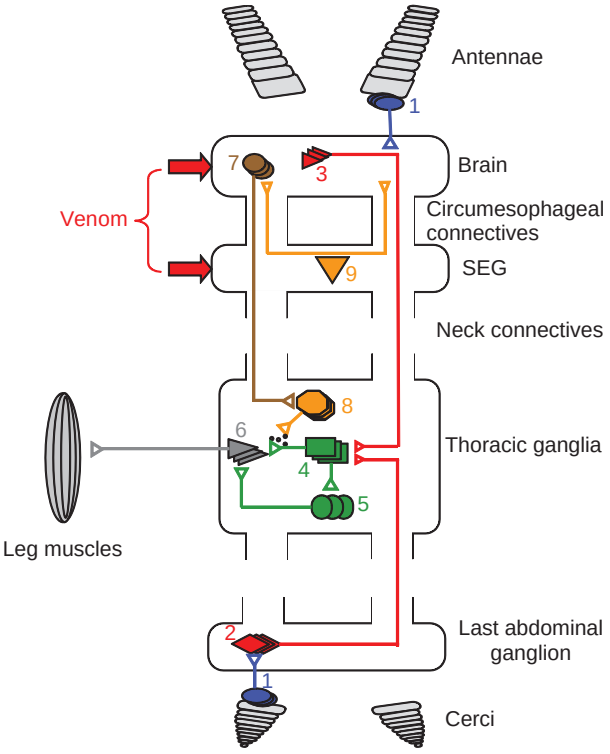

Figure 5 A current model of the neurophysiological events leading to venom-induced hypokinesia in cockroaches stung by *Ampulex compressa*. A schematic and simplified drawing of a cockroach nervous system depicting circuitries that affect walking-related behaviors. The walking pattern generator that orchestrates leg movements is located in the thorax. It consists of motor neurons innervating leg muscles (6), sensory neurons associated with sensory structures on the legs (not shown) and thoracic interneurons (TIAs; 4), which synapse onto the motor neurons directly and indirectly via local interneurons (5). The TIAs receive inputs from several interneurons. For example, sensory neurons in the antennae or cerci (1) recruit ascending (2) or descending (3) GIs, which converge directly onto the TIAs to ultimately evoke escape responses. In addition, neurons of the pattern generator receive input from thoracic neuromodulatory cells (8). One example of these is the DUM neurons, which secrete OA and modulate the efficacy of premotor-to-motor (4-to-6) synapses. The neuromodulatory cells, in turn, receive tonic input through interneurons descending from the brain (7) and SEG (not shown). This tonic input affects the probability of the occurrence of specific motor behaviors by modulating the different thoracic pattern generators. The wasp *A. compressa* injects its venom cocktail directly into both cerebral ganglia to modulate some specific yet unidentified cerebral circuitries. Our current hypothesis states that in the SEG, the venom suppresses the activity of brain-projecting DUM neurons (9), which control the activity of brain-descending interneurons (7) that modulate, either directly (not shown) or indirectly via the neuromodulatory cells (8), the walking pattern generator. Hence, the venom injected into the cerebral ganglia decreases the overall excitatory input to the thoracic walking pattern generator. As a result, walking-related behaviors are specifically inhibited, and the stimuli to the antennae or cerci fail to evoke normal escape responses.

octopaminergic neurons, induces impairment in the ability of cockroaches and crickets to generate escape behavior. The activity of octopaminergic neurons, known to modulate the excitability of specific thoracic premotor neurons in the cockroach, is compromised in stung cockroaches. The alteration in the activity of OA neurons could be part of the mechanism by which the wasp induces a change in the excitability of thoracic premotor circuitries.

The wasp injects its venom directly into the SEG and in and around the central complex in the brain. We have shown that, in stung cockroaches, the focal injection of a potent OA receptor agonist around the central complex area partially restores walking. Conversely, in controls, the focal injection of a selective OA receptor antagonist into the same area reduces walking. However, it appears that the relevant neurons that modulate walking reside in the SEG and send axons to innervate the motor centers, such as the central complex. Within this group of ascending interneurons, at least three OA ascending SEG neurons provide dense innervation in the central portion of the brain. Thus, the SEG sting might be affecting the activity of SEG octopaminergic ascending neurons to reduce OA levels in the walking centers of the brain (**Figure 5**).

To conclude, we propose that venom injection into the head ganglia selectively depresses the initiation and maintenance of walking by modifying the release of OA as a neuromodulator in restricted regions of the cockroach's brain. Then, it is likely that *A. compressa* alters some yet unidentified descending pathways in the cockroach head ganglia which affect, at the least, OA secretion from thoracic dorsal unpaired median (DUM) neurons (**Figure 5**). The latter are known to dramatically affect locomotion, which can explain the long-lasting hypokinetic state induced in stung cockroaches.

Conclusions

In this article, I introduce the reader to the astonishing world of parasitoid wasps and their insect hosts. There are several reasons to be interested in these wasps. First, they are increasingly used as a biological control of crop pests to preserve the environment. But, the most relevant reason is that these wasps are considerably better than we are at manipulating the neurochemistry of their prey with specific neurotoxins. Thus, for those interested in the neural mechanisms of animal behavior, parasitoid wasps have evolved, through years of co-evolution with their prey, a better 'understanding' of the neuromodulatory systems of their insect hosts than insect neuroethologists. Moreover, neurotoxins are invaluable as tools to reveal the physiological mechanisms underlying nervous system functions. Because neurotoxins are the outcome of one

animal's evolutionary strategy to incapacitate another, they are usually highly effective and specific. Chemical engineers can generate hundreds of neurotoxins in their labs, but these products are random and often useless, whereas any natural neurotoxin has already passed the ultimate screening test, over millions of years of co-evolution. As such, wasp neurotoxins may provide us with new highly specific pharmacological tools to investigate cell and network function. Although, the alteration of host behavior by parasitoids is a widespread phenomenon, the underlying mechanisms are beginning to be revealed only now. I have focused here on the neuronal mechanisms by which parasitoid wasps manipulate the behavior of other insects using chemical warfare. In a case study, I have surveyed the unique venom effect of an unusual predator, the parasitoid wasp *A. compressa*. *Ampulex* does not kill its prey but instead performs a delicate brain surgery to take away the 'free will' of its prey to initiate locomotion. Much work remains to be done until we know the exact neuro-chemical cascade taking place in the host's CNS to alter its behavior. Given the breath of such investigations, a multidisciplinary approach, combining molecular techniques with cellular electrophysiology and behavior analysis, is essential. It is my great hope that such host–parasite interactions will stimulate the curiosity of young and talented minds to investigate the neuronal basis of parasite-induced alterations of host behavior, with the goal of increasing our understanding of the neurobiology of the initiation of behaviors and the neural mechanisms underlying changes in responsiveness, which are prime questions in the study of arousal and motivation.

See also: Evolution of Parasite-Induced Behavioral Alterations; Experimental Approaches to Hormones and Behavior: Invertebrates; Neuroethology: Methods; Parasite-Modified Vector Behavior; Parasitoids; Predator Evasion.

Further Reading

Adamo SA (2002) Modulating the modulators: Parasites, neuromodulators and host behavioral change. *Brain Behaviour and Evolution* 60(6): 370–377.

Eberhard WG (2000) Spider manipulation by a wasp larva. *Nature* 406 (6793): 255–256.

Fabre JH (1879) *Souvenirs Entomologiques* (1945 ed.), vol. 1, pp. 108–112. Paris: Delagrave.

Gal R and Libersat F (2008) A parasitoid wasp manipulates the drive for walking of its cockroach prey. *Current Biology* 18(12): 877–882.

Gnatzy W (2001) Digger wasp vs. cricket: (Neuro-) biology of a predator–prey-interaction. *Zoology* 103: 125–139.

Grosman AH, Janssen A, de Brito EF, et al. (2008) Parasitoid increases survival of its pupae by inducing hosts to fight predators. *PLoS ONE* 3(6): e2276.

Libersat F (2003) Wasp uses venom cocktail to manipulate the behavior of its cockroach prey. *Journal of Comparative Physiology A* 189: 497–508.

Libersat F, Delago A, and Gal R (2009) Manipulation of host behavior by parasitic insects and insect parasites. *Annual Review of Entomology* 54: 189–207.

Libersat F and Gal R (2007) Neuro-manipulation of hosts by parasitoid wasps. In: Yoder J and Rivers D (eds.) *Recent Advances in the Biochemistry, Toxicity and Mode of Action of Parasitic Wasp Venoms*, pp. 96–114. Kerala, India: Research Signpost.

Libersat F and Pflueger HJ (2004) Monoamines and the orchestration of behavior. *Bioscience* 54(1): 17–25.

O'Neill KM (2001) *Solitary Wasps: Behavior and Natural History,* p. 58. Ithaca and London: Comstock Pub., Cornell University.

Piek T (1990) Neurotoxins from venoms of the Hymenoptera – twenty-five years of research in Amsterdam. *Comparative Biochemistry and Physiology Part C* 96: 223–233.

Quicke DLJ (1997) *Parasitic Wasps*. London: Chapman and Hall.

Zimmer C (2000) *Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous Creatures*. NY, USA: Free Press/Simon & Schuster.