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WASP MANIPULATES COCKROACH BEHAVIOR BY INJECTING VENOM COCKTAIL INTO PREY CENTRAL NERVOUS SYSTEM*

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The parasitoid wasp *Ampulex compressa* induces behavioral changes in the cockroach prey by injecting venom into its central nervous system. In contrast to most other venomous predators, the wasp's sting does not induce paralysis. Rather, the two consecutive stings in the thoracic and head ganglia induce three stereotypic behavioral effects. The prey behavior is manipulated in a way beneficial to the wasp and its offspring by providing a living meal for its newborn larva. The first sting in the thorax causes a transient front leg paralysis lasting a few minutes. This paralysis prevents the cockroach from fighting with its front legs, thereby facilitating the second sting in the head. A postsynaptic block of central synaptic transmission mediates this leg paralysis. Following the head sting, dopamine identified in the venom induces 30 minutes of intense grooming that appears to prevent the cockroach from straying until the last and third behavioral effect of hypokinesia commences. In this lethargic state that lasts about three weeks, the cockroach does not respond to various stimuli nor does it initiates movement. However, other specific behaviors of the prey are unaffected. We propose that the venom represses the activity of head ganglia neurons thereby removing the descending excitatory drive to specific thoracic neurons.

Keywords: Neurotoxins – *Ampulex compressa* – *Periplaneta americana* – paralysis – grooming

INTRODUCTION

Venomous predators manufacture neurotoxins adapted to affect the nervous system of their prey to subdue it and prevent it from fleeing or fighting back. Predators as diverse as snakes, scorpions, spiders, insects and snails, all manufacture venoms consisting of various neurotoxins to incapacitate their prey. Most neurotoxins in venoms act peripherally at the neuromuscular junction resulting in different types of paralysis [18, 24]. These neurotoxins mostly interfere with the ability of the prey's nervous system to generate muscle contractions resulting in an immobilization of the prey [1, 17]. In many cases, a cocktail of neurotoxins targeting different molecular compo-

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Abbreviations: *PSP,* postsynaptic potential; *GI,* giant interneuron; *TI,* thoracic interneuron; *SEG,* Sub-Esophageal Ganglion; *CNS,* Central Nervous System; *DA,* dopamine; *OA,* octopamine

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nents of the neuromuscular junction is injected, causing a rapid and efficient muscular paralysis [18, 23].

Solitary wasps that belong to the families of Sphecidae, Pompilidae, Mutillidae and Bethylidae [19], are parasitoid using other insects or spiders as a food supply for their offspring. Most parasitoid wasps paralyze and then carry their prey to a burrow or nest. The wasp then lays an egg on the victim and when the larva hatches from the egg, it feeds on the paralyzed host. In contrast to most of the venomous hunters, several Ampulicine wasps, such as *Ampulex bantuae, A. assimilis* and *A. canaliculata*, as well as *A. compressa* do not paralyze their prey. Instead, they manipulate the prey behavior, overcoming its natural instincts to flee from the scene of the attack, and turning it into a submissive "zombie" that behaves in a manner that is most beneficial for the wasp offspring. In all these cases, although the prey remains submissive, it still remains ambulatory and is even capable of short runs if stimulated strongly enough [16].

The wasp *Ampulex compressa* hunts the cockroach, *Periplaneta americana*, to provide its larvae with a live food supply [28]. The wasp subdues cockroaches by stinging them first in the thorax (Fig. 1A) and then in the head (Fig. 1B). The stung cockroach exhibits three consecutive phases of envenomization. First, following the thoracic sting, the cockroach's front legs are paralyzed (Fig. 1C) for about three minutes [8, 12]. After the sting into the head, the cockroach grooms extensively for 30 minutes (Fig. 1D) and then becomes lethargic for about three weeks [8, 9, 15]. In this lethargic state, the cockroach does not respond to various stimuli nor does it initiate movement [8, 9, 15, 27]. Thirty minutes after the sting to its head, the cockroach is lethargic and submissive enough for the wasp to bite off its antennae without putting up a fight. The wasp then drinks some hemolymph from the cut antennae, grabs one of the antennae stumps and, like using a nose ring on a bull, walks the docile prey to a suitable oviposition location (Fig. 1E [8, 28]). A few days later the cockroach serves as an immobilized and fresh food source for the wasp's offspring. The larva feeds on the hemolymph of the living prey and after about a week, burrows inside the cockroach to pupate. Six weeks later, a new wasp hatches from the pupa (Fig. 1F).

The unique effects of the wasp venom on prey behavior suggest that the venom targets the insect's central nervous system. We have explored this possibility and searched for the neural basis for the three behavioral states that are induced sequentially by the venom injection. Here, we will focus on the first two behavioral states, namely: the transient paralysis of the front legs and the intense grooming.

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

The unique effects of the wasp venom on prey behavior suggest that the venom targets the insect's central nervous system. However, the wasp's venom consists of a cocktail of proteins and peptides [11, 12], which are unlikely to cross the thick and rather selective sheath (the insect's blood brain barrier) around the nervous ganglia

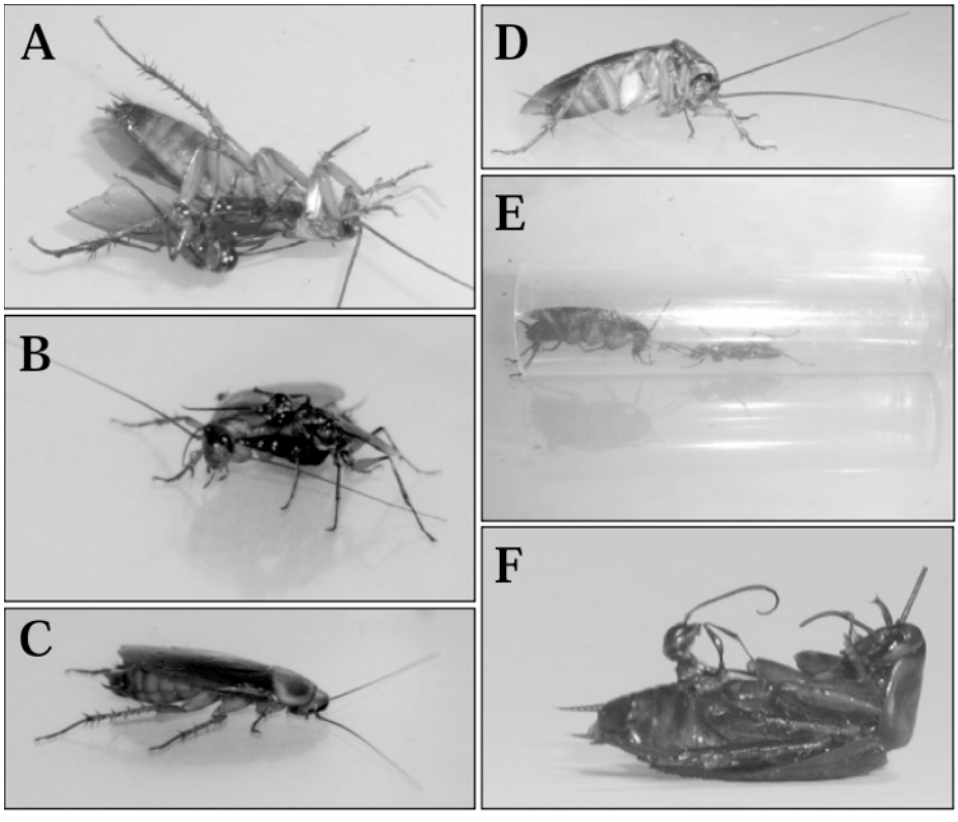

Fig. 1. Ampulex compressa stinging behavior: **A** The first thoracic sting is inflicted beneath one of the front legs. **B** The head sting is inflicted in the neck. **C** The cockroach's front legs are paralyzed for 2–3 minutes resulting in a postural change. **D** The cockroach grooms extensively for 30 minutes. **E** The wasp leads the submissive cockroach into a burrow by gripping an antenna stump and walking backwards. **F** After six weeks, a new wasp emerges from its cocoon spun inside a cockroach

[25]. We have explored the possibility that the wasp injects its venom directly into the nervous ganglia by injecting wasps with C^{14} radiolabeled amino acids, which were incorporated into the venom. Then, we let the "hot" wasps sting cockroaches and used a combination of liquid scintillation and light microscopy autoradiography to trace radiolabeled venom inside the prey [12, 13]. The levels of radioactivity in stung cockroaches were significantly higher in the first thoracic ganglion, than in other thoracic ganglia and the surrounding, non-neuronal, tissue (Fig. 2A). Likewise, most of the radioactive signal in the head was detected in the head ganglia, the supra-esophageal and sub-esophageal ganglia (brain and SEG, respectively) (Fig. 2B). Only a small amount of radioactivity was detected in the surrounding head tissue. To determine the precise location of injection, head ganglia of stung cockroaches were embedded in plastic resin, serially sectioned and exposed to radiosensitive emulsion.

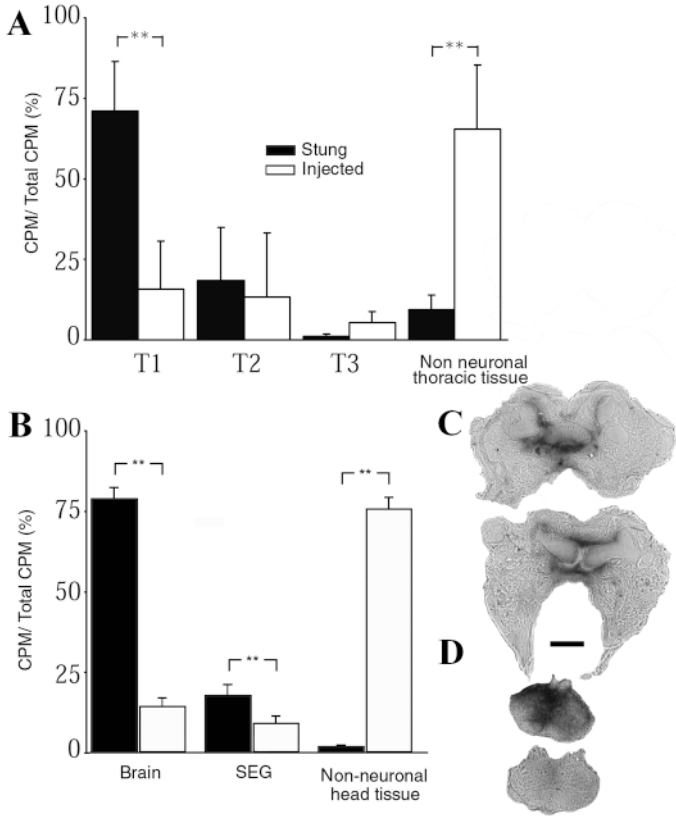

Fig. 2. Localization of the site of injection of the venom. **A** In cockroaches stung by radiolabeled wasps (solid bars, n = 16), most of the radioactive signal is detected in the first thoracic ganglion (*T1*). The remaining radioactivity is detected in the second (*T2*) and third (*T3*) thoracic ganglia and in the surrounding, non-neuronal, tissue. As a control for diffusion, radioactive amino acids were manually injected into the thoracic cavity of other cockroaches (open bars, n = 15). In those cockroaches, 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. The measurements are represented as the percentile fraction of the total CPM *(counts per minute)* of a specimen. **B** In stung cockroaches (solid bars, n = 16), the levels of radiolabeled venom are significantly higher in the head *ganglia (brain and SEG)* than in non-neuronal head tissue. In cockroaches injected with radioactive amino acids (open bars, n = 15), the levels of radiolabeled venom are significantly higher in non-neuronal head tissue than in the head ganglia. Significantly different ($P < 0.01$:**) levels of radioactivity are measured in stung and injected cockroaches in each of the sampled tissues. **C** Two sections of brain of a representative preparation of a cockroach, stung by a radiolabeled wasp. Radiolabeled venom, indicated as black stain, 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 sub-esophageal ganglion *(SEG)* preparation of a cockroach, stung by a radiolabeled wasp. Radiolabeled venom is located inside the SEG ganglion (modified from [12, 13])

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). This shows that the wasp stings not only into the sub-esophageal ganglion, which lies underneath the stinging site in the neck, but also separately into the more distant brain. 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.

The transient leg paralysis is mediated by a postsynaptic block of central nicotinic synapses

The first sting into the prothoracic ganglion causes a transient paralysis of the cockroach's front legs. As a consequence, the cockroach's posture is affected and it assumes a "head down" posture for about three minutes (Fig. 1C). If the stinging sequence is interrupted before the head sting is applied, the cockroach will fully recover after a transient front leg paralysis. The direct injection of milked venom into a thoracic ganglion abolishes spontaneous and evoked responses of the motoneurons associated with posture and fast leg movements [12]. Because motoneurons receive excitatory cholinergic input [10], we used a well-characterized cholinergic synapse to investigate the possibility that the venom may interfere with central cholinergic neurotransmission. The last abdominal ganglion of the cockroach's nervous system, in which we injected milked venom, contains a well-characterized nicotinic synapse that connects wind sensory neurons to giant interneurons (GIs) [6]. Direct injection of venom blocked the wind-evoked responses of the GIs for about three minutes [12], but left the propagation of action potentials in the GIs unaffected (Fig. 3A). This indicates that the venom might target synapses. To characterize the effect of the venom on cholinergic synaptic transmission, we intracellularlly recorded from a GI soma while alternately inducing an excitatory postsynaptic potential (PSP) by stimulation of the cercal receptors and by direct iontophoresis of a nicotinic agonist, carbachol. The injection of venom abolished both PSPs suggesting that the venom blocking effect has a postsynaptic component (Fig. 3B) without ruling out the possibility of a presynaptic effect. Because muscarinic agonists and antagonists have no effect on the presynaptic synaptic terminals or the postsynaptic giant interneurons [5], the block of Carbachol evoked potentials by the venom indicates a postsynaptic block of nicotinic receptors rather than a block of muscarinic receptors. Finally, the motor impairment induced by the thoracic sting is mimicked by injection of a nicotinic antagonist (b-tubocurarine) in the front thoracic ganglion. 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.

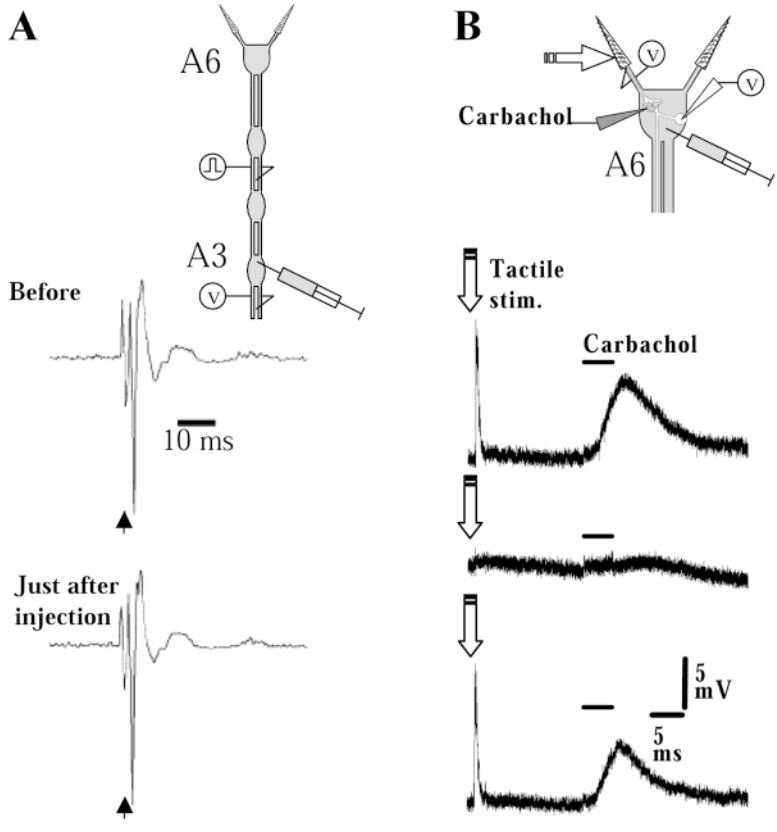

Fig. 3. The transient paralysis of the front legs. **A** The axons of GIs are recruited with an electrical stimulus *(arrow)* applied to the nerve cord via one electrode and their propagation through abdominal ganglion A3 is monitored with another electrode on the nerve cord *(diagram)*. The average 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. **B** 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 [12, 13])

Most solitary Sphecid wasps prey on large insects equipped with various defense strategies (such as kicks, leaps and bites). The first sting disarms the prey by inducing 2–60 minutes of complete paralysis of the appendages involved in flight or fight [21]. Thus, we suggest that *Ampulex 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.

Dopamine in the venom induces prolonged grooming behavior

Cockroaches stung by the wasp into the head groom almost continuously during the 30 minutes following recovery from the transient paralysis of the front legs (Fig 1D). Grooming is evoked only when venom is injected into the head, and cannot be attributed to the stress of the attack, contact with the wasp, mechanical irritation, or venom injection into a location other than the head (Fig. 4A; [26]). Thus, it appears that the sting in the head ganglia evokes a complex behavior involving coordinated movements of different appendages to generate all the components of natural grooming behavior [26].

Our studies are consistent with the hypothesis that a monoamine agonist in the venom is the factor causing excessive grooming. For instance, reserpine injection into the SEG, which causes massive release of all monoamines: dopamine, octopamine and serotonin, induces prolonged grooming [27]. Moreover, direct injection of a dopamine agonist into the SEG induces prolonged grooming [26]. Injection of an antagonist of dopamine receptors prior to the wasp sting greatly reduces venom-induced grooming (Fig. 4B). In contrast, an octopamine receptor antagonist does not reduce venom-induced grooming (Fig. 4B). Finally, dopamine was identified in the wasp venom by Gas Chromatography-Mass Spectrometry (Fig. 4C) and

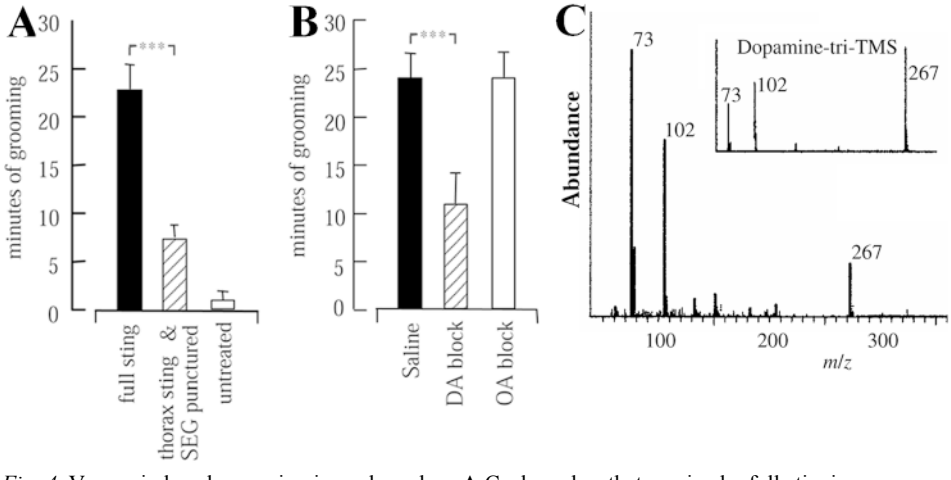

Fig. 4. Venom-induced grooming in cockroaches. **A** Cockroaches that received a full stinging sequence by the wasp groom for 23.0 ± 2.3 min during the 30 min following the sting. This grooming time is significantly longer ($P < 0.001$:***) than that observed in cockroaches that were stung only in the thorax followed by a puncture of the SEG with a pin (7.8 ± 5.4 min). **B** Cockroaches that received flupenthixol, a dopamine (DA) receptor antagonist before a sting groom significantly less ($P < 0.0001$:***) than cockroaches receiving saline before a sting. Mianserin, an octopamine (OA) antagonist, does not reduce venom-induced grooming. **C** Mass spectrogram of one of the large venom peak separated by Gas chromatography (not shown); this spectrum is comparable to the mass spectrograms *(inset)* of dopamine (modified from [26])

by High Performance Liquid Chromatography-Electrochemical Detection (M.E. Adams, personal communication) in the venom. Thus, dopamine is the likely venom component that induces prolonged grooming.

The venom induces prolonged grooming presumably by stimulating dopamine receptors in the cockroach's SEG. The dopamine D1 agonist SKF82958 that we used is an agonist of dopamine receptors of the locust and the fruit fly [3, 20] induces prolonged grooming in the cockroach when injected directly into the SEG [26] as well as in the fly *Drosophila melanogaster* [29]. 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.

At this point, it is only possible to speculate the adaptive value of the induced grooming. For instance, it is interesting to note that the extensive grooming behavior after the sting lasts for about 30 minutes, while the venom-induced hypokinesia is fully developed only after approximately 30 minutes [8]. During grooming, the escape response threshold is elevated, and locomotion is depressed [7, 14]. Consequently, during this initial 30 minutes period the cockroach tends to remain in the place where it was stung while the wasp explores the burial site for its larva's food supply.

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 commences within 30 minutes and lasts for about three weeks [8, 27]. Stung cockroaches show very little spontaneous or provoked activity such as escape [15]. 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 [27]. Hence, the head sting affects specific motor behaviors while leaving others unaffected.

This specificity may be achieved by targeting a specific neuromodulatory system that regulates the expression of a single behavior or a specific subset of behaviors. Such specificity of neuromodulatory systems has been well established in invertebrates, in particular for the monoamines, which have been found to modulate the release of well-defined behaviors [4]. Consistent with this hypothesis, reserpine, which depletes the synaptic content of monoaminergic neurons, induces impairment in the ability of cockroaches and crickets to generate escape behavior [22, 27]. Moreover, a similar impairment in their escape behavior is observed in crickets depleted of octopamine and dopamine with AMT (alpha-methyl-*p*-tyrosine) [22]. This suggests a role of dopamine or octopamine as chemical modulators of escape behavior in cockroaches. We propose that the venom injected into the head ganglia removes a descending excitatory input to these neuromodulatory systems thereby decreasing the excitability of specific motor behaviors.

CONCLUSION

The specificity and effectiveness of neurotoxins are the outcome of evolutionary selection on one animal's strategy to incapacitate another [2]. 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. First, by direct injection of synaptic blocker into a specific area of the prey's nervous system, the wasp achieves a precise control over the motor command of specific appendages. Second and regarding the head sting, monoamines must act on distributed networks of neurons in the central nervous system and in the periphery to yield the "appropriate" behavior. Monoaminergic neurons of invertebrate nervous systems are well suited for this task, with extensive branching terminals and diffuse release of monoamines in large areas of the central nervous system. By manipulating one of these monoaminergic system, the wasp insures to modulate the expression of a single specific behavior, grooming. Still much work needs to be done and one could envisage that the other *Ampulex*'s neurotoxins to be identified might have interesting novel effects on the excitability of neurons or on synaptic transmission. 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 and their corresponding molecular targets by their introduction *in vivo* and *in vitro* into the cockroach's central nervous system. 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 and the neurobiology of initiation of motor behaviors.

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