REVIEW

What can parasitoid wasps teach us about decision-making in insects?

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Summary

Millions of years of co-evolution have driven parasites to display very complex and exquisite strategies to manipulate the behaviour of their hosts. However, although parasite-induced behavioural manipulation is a widespread phenomenon, the underlying neuronal mechanisms are only now beginning to be deciphered. Here, we review recent advancements in the study of the mechanisms by which parasitoid wasps use chemical warfare to manipulate the behaviour of their insect hosts. We focus on a particular case study in which a parasitoid wasp (the jewel wasp *Ampulex compressa*) performs a delicate brain surgery on its prey (the American cockroach *Periplaneta americana*) to take away its motivation to initiate locomotion. Following a brief background account of parasitoid wasps that manipulate host behaviour, we survey specific aspects of the unique effects of the *A. compressa* venom on the regulation of spontaneous and evoked behaviour in the cockroach host.

Introduction: manipulation of host behaviour in parasitoid wasps

Many parasitoid wasps use insects or spiders as food supply for their developing larvae (Quicke, 1997; O'Neill, 2001). To achieve this, the wasp must lay its eggs in or on the host species and then protect the host from other potential predators or parasites, which may endanger the defenceless larvae and pupae. Most parasitoid wasps use neurotoxic venoms to paralyze the host and lay the egg on its body, and then conceal it inside a nest or burrow for protection (Libersat and Gal, 2007). However, some parasitoid wasp species modulate their host's central nervous system (CNS) to induce various 'tailor-made' behaviours that facilitate the growth and development of the larvae (Libersat et al., 2009). A few superb examples of this phenomenon will be described below, bearing in mind that the underlying neuronal mechanisms of such behavioural manipulations are only now beginning to unravel.

To protect its larvae, the braconid endoparasitoid wasp *Glyptapanteles* coerces its host, the caterpillar *Thyrinteina leucocerae*, to behave as a bodyguard (Grosman at el., 2008). After the wasp lays its eggs inside a caterpillar host, the hatching larvae feed on the living caterpillar and, when ready, emerge from the caterpillar's body and pupate. At this stage the caterpillar stops feeding and remains close to the wasp pupae, defending them against predators with violent head swings. This unique and rather complex behaviour is not observed in un-parasitized caterpillars, suggesting a modulation of the CNS. However, this remains to be proven.

Another exquisite alteration of host behaviour by a parasitoid wasp is that of the ichneumonid wasp *Hymenoepimecis*, which manipulates the behaviour of its spider host *Plesiometa argyra* (Eberhard, 2000). This wasp manipulates the host to literately build a shelter for its future larva: through a yet unidentified mechanism, a spider parasitized by *Hymenoepimecis* stops building its normal orb-shaped web and starts building a new one. The geometry of the new web is strikingly different than that of unparasitized *P. argyra* and is apparently designed to suspend the larva's cocoon in the air, where it will be protected from terrestrial predators. This implies that the wasp's larva must secrete chemicals which manipulate the spider's nervous system to induce the execution of only one subroutine of the normal orb-web construction programme, while repressing all other routines. The nature of the chemicals involved in this extreme alteration of the spider's behaviour is, however, unknown.

The parasitoid jewel wasp: a case study

Probably the best understood example of a wasp-induced behavioural manipulation and its underlying neuronal mechanisms is found in the ampulicine jewel wasp (*Ampulex compressa*), which uses cockroaches as live food supply for its larvae (Fig. 1) (Libersat, 2003). The wasp first grabs its cockroach prey (usually *Periplaneta americana*) at the pronotum or the base of the wing and inflicts a sting into the host's thorax. Chloride channel agonists in the venom inactivate central motor networks temporarily to paralyze the cockroach forelegs for 1–2 min (Moore et al., 2006), which facilitates a second and a more delicate sting into the host's neck. Then, after the neck-sting is complete, the wasp leaves the cockroach for roughly 30 min to search for a suitable burrow; the stung cockroach, however, does not leave the site of the sting, but instead displays an excessive grooming behaviour (Weisel-Eichler et al., 1999). When the wasp returns it clips both antennae of the grooming cockroach and then feeds uninterrupted on the fresh hemolymph oozing from the cut ends. The stung cockroach does not try to fight off the wasp during this process, and fails to struggle even as the wasp grabs one of its antennal stumps and escorts the cockroach to the pre-selected burrow; in fact, the cockroach follows the wasp submissively to the burrow and readily enters its tomb, where it stays immobile as the wasp lays an egg and glues it onto the mid-leg cuticle (Fouad et al., 1994). With the prey staying immobile inside the burrow, the wasp then collects small items (leaves, pebbles, etc.) with which it seals the entrance to the burrow
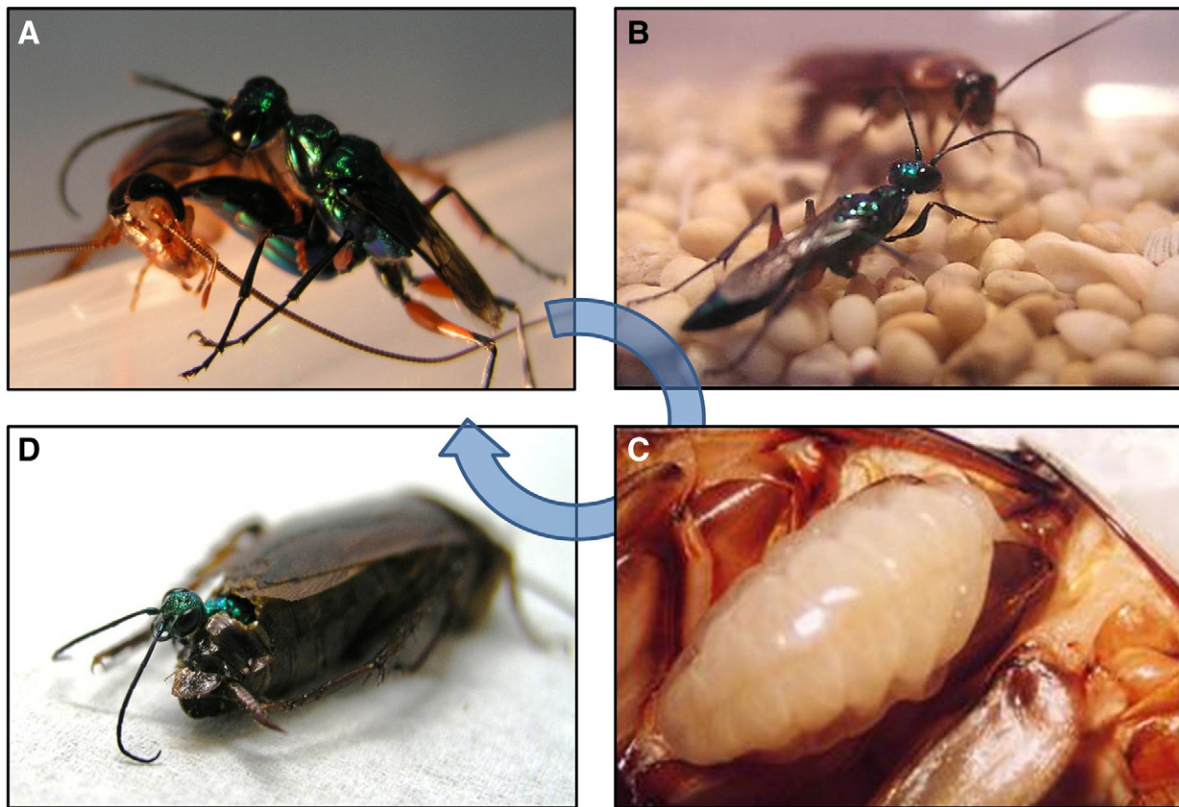
Fig. 1. Life cycle of the parasitoid jewel wasp *Ampulex compressa*. An adult wasp stings a cockroach into the head (A) to manipulate the cockroach behaviour. The wasp then cuts the cockroach's antennae (B) to drink hemolymph, and then leads the stung cockroach into a nest to lay an egg on its cuticle. The hatching larva (C) feeds on the cockroach, pupates inside its abdomen and emerges roughly 30 days later (D).

and flies away. Entombed inside the burrow, the stung cockroach thus serves as living food storage for the developing larva, which hatches a couple of days later and perforates the host's leg cuticle to feed on nutritious hemolymph for the next few days. When ready to pupate, the larva penetrates through the cockroach's cuticle, feeds on its internal organs and pupates inside the abdominal cavity. There, well satiated and safe from predators, the larva metamorphoses and emerges roughly a month later as an adult, ready to restart its life cycle.

This unique behavioural manipulation of the cockroach host by its parasitoid predator raises numerous questions. Why does the stung cockroach, although not paralyzed, fail to fight off the wasp as it cuts the antennae and feeds on its hemolymph? What neuronal modulation does the venom induce that allows the wasp to lead the docile cockroach into its tomb? And how does the venom act to prevent the living cockroach from escaping the burrow while being devoured alive by the developing larva? These questions have been thoroughly investigated during the last 15 years and have yielded some rather surprising and informative answers. Some of the underlying mechanisms responsible for this unique behavioural manipulation, although not yet fully understood, will be discussed below.

The jewel wasp injects venom directly inside the cockroach's CNS

The unique effects of the jewel wasp's venom on the behaviour of its cockroach host, as well as the site of the sting, suggest that the venom affects the cockroach's CNS. However, the CNS of insects is surrounded by a physical protective sheath that forms the insect's 'blood–brain barrier'. The venom, a cocktail of monoamines, peptides and large proteins (Haspel et al., 2003; Weisel-Eichler et al., 1999; Moore et al., 2006), must therefore penetrate this sheath in order to reach the host's CNS. It has been hypothesised that parasitoid wasps uses their sharpened stinger to penetrate through the host's ganglionic sheath (reviewed in Libersat and Gal, 2007). However, until 2003, direct proof of such venom injection into the host CNS was lacking.

To test the specific location of venom injection, jewel wasps were experimentally injected with a mixture of C14 radiolabelled amino acids that were incorporated into the venom (Haspel et al., 2003). In cockroaches stung by such 'hot' wasps, most of the radioactive signal was localised in three regions: (1) inside the thoracic ganglion; (2) around the midline of the sub-esophageal ganglion (SEG); and (3) in the central part of the supra-esophageal ganglion (SupEG), posterior to the central complex and around the mushroom bodies (Fig. 2A). The location of the radioactive venom indeed corresponds with the first sting into the thorax and with the second sting into the neck. This means that the wasp's stinger must penetrate through the protective ganglionic sheath of the cockroach, such that venom is injected directly onto the neuronal targets (Fig. 2B). Such a precise neuro-anatomical targeting is akin to the most advanced stereotactic delivery of drugs, and preliminary studies (Gal et al., 2005; Gal and Libersat, 2010) suggest that it may be mediated by sensory structures on the wasp's stinger that allow nervous-tissue recognition inside the cockroach head capsule. Although these experiments represent the only unequivocal demonstration to date that a wasp is able to inject venom directly inside the CNS of its prey, the jewel wasp is almost certainly not
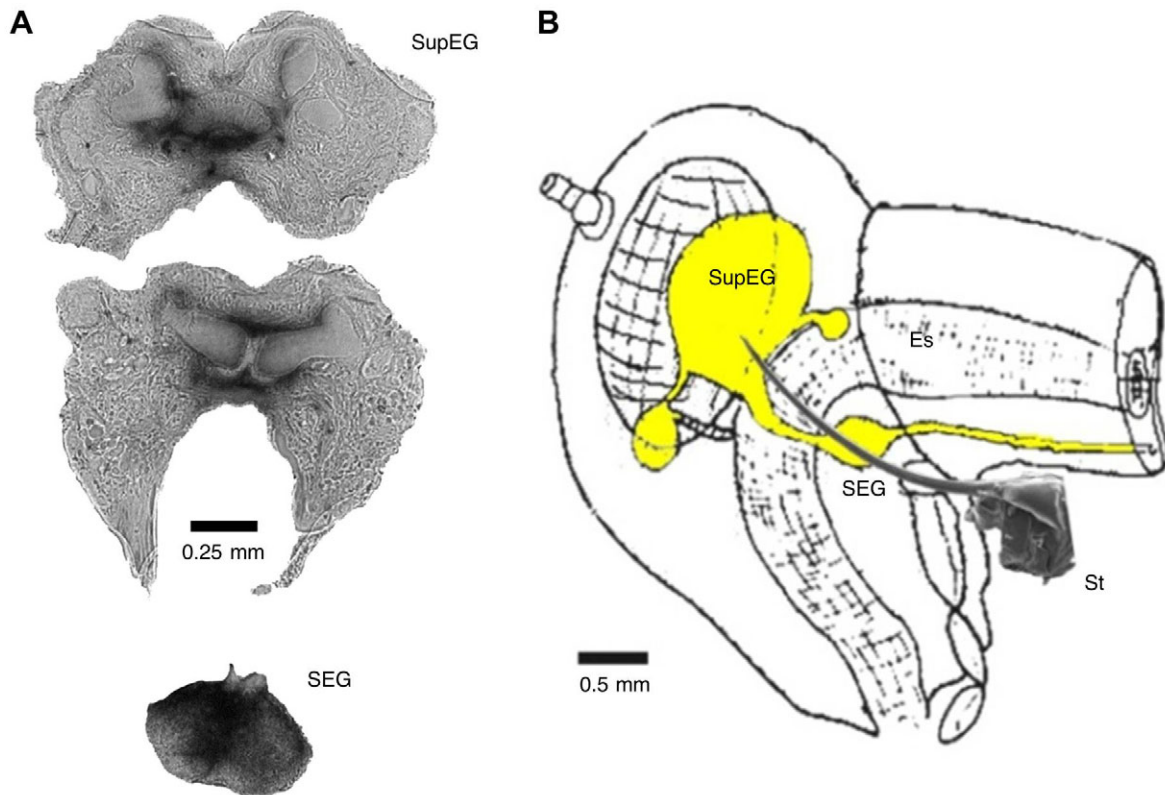
Fig. 2. The wasp stings directly inside the cockroach's cerebral ganglia. (A) Autoradiographs of the supra-esophageal ganglion (SupEG) and sub-esophageal ganglion (SEG) of a cockroach stung by a radio-labelled wasp. Black staining indicates the presence of venom. Adapted from Haspel et al. (Haspel et al., 2003). (B) Suggested mechanism of the stinging process. The wasp's stinger (St; scanning electron micrograph of the stinger, scaled to the schematic drawing of the cockroach's head) penetrates through the neck to reach both cerebral ganglia. Es, esophagus.

the only wasp to use this method of drug delivery. This, however, remains to be proven in other wasp species.

Sensory-motor aspects of the venom-induced hypokinesia

Although the sting into the host's thoracic ganglion induces only a short-term paralysis of the forelegs, the injection of venom into the cerebral ganglia (the SupEG and SEG) produces a long-term hypokinetic state (Fouad et al., 1994). Under laboratory conditions, namely if one experimentally prevents the wasp from laying an egg on the hypokinetic cockroach, the host recovers roughly 5 days following the sting (Fouad et al., 1994; Gal and Libersat, 2008). During this period, however, the stung cockroach does not voluntarily engage in spontaneous locomotion, nor does it escape in response to different stimuli that recruit different sensory and central premotor pathways (Gal and Libersat, 2008). This hypokinetic state is not equivalent to paralysis, as the cockroach demonstrates normal locomotory pattern (the so-called 'alternating tripod gait') as it follows the wasp into its burrow.

Studies of the sensory systems in stung cockroaches revealed that sensory cues reliably propagate along sensory neurons and interneurons, e.g. the descending mechanosensory interneurons from the antennae, or the giant interneurons from the cerci (Fouad et al., 1994; Fouad et al., 1996). Similarly, the muscles and motor neurons are functional in stung cockroaches and are able to produce normal motor outputs (Fouad et al., 1996; Gal and Libersat, 2008). For instance, normal cockroaches typically respond to a tactile or wind stimulus by engaging in a rigorous escape behaviour characterised by the rhythmic recruitment of slow and fast leg coxal depressor muscles (Ds and Df, respectively) (Fig. 3A). Each of these muscles is innervated by a specific set of thoracic motor neurons, hence electromyograms (EMGs) from these muscles provide direct readouts of the associated motor neurons. In stung cockroaches, a stimulus to the cerci evokes only a short burst of Ds potentials (associated with a postural reflex without displacement) and no Df potentials (Fig. 3A), although the stimulus itself evokes neuronal activity that propagates normally to the CNS. However, Ds or Df potentials can be rhythmically evoked when stung cockroaches are immersed in water (Fig. 3C), hence the venom must affect central (rather than peripheral) pre-motor mechanisms to induce hypokinesia. This implies that the venom does not affect all motor outputs similarly, but rather that some aspects of locomotion, e.g. the initiation and/or maintenance of walking, are specifically impaired in stung cockroaches while other motor outputs are spared. These aspects of the venom-induced hypokinesia are discussed in the following section.

Central aspects of the venom-induced hypokinesia
Venom specifically affects walking-related behaviours

Behavioural and electrophysiological studies in stung cockroaches demonstrate that the venom's effect on locomotion is limited to walking behaviour specifically. For instance, stung cockroaches show no apparent deficiency in righting behaviour, which, similar to walking behaviour, involves the synchronized motor outputs of all legs. In addition, EMG recordings from the legs of stung cockroaches during righting attempts reveal rhythmic as well as sporadic Ds bursts, occasionally accompanied by Df potentials that
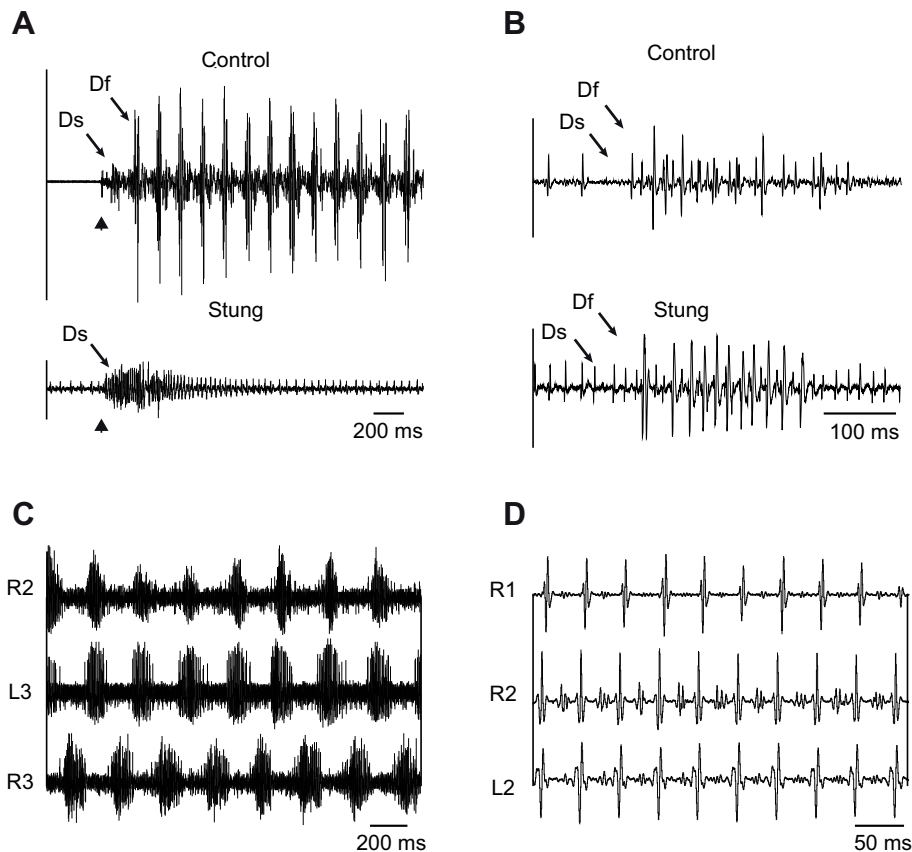
A

B

C

D

Fig. 3. The wasp's venom selectively affects walking. (A) Electromyographic (EMG) recording from the metathoracic coxa of a cockroach before (top) and after (bottom) a wasp's sting. Before the sting, a wind stimulus applied to the cerci (vertical arrows) evokes rhythmic slow (Ds) and fast (Df) coxal depressor activity. After a sting, in contrast, the same stimulus evokes only tonic Ds firing, which is not accompanied by Df potentials. (B) EMG recordings from the metathoracic leg coxa during righting behavior in a control (top) and a stung (bottom) cockroach. Both Ds and Df potentials can be observed. (C) Simultaneous EMG recordings from the coxae of three legs (R2, right mesothoracic; L3/R3, left/right metathoracic) during swimming in a stung cockroach. The alternating tripod gait can be distinguished. (D) Simultaneous EMG recordings from three wings (R1, right forewing; R2/L2, right/left hindwings) demonstrating normal flying motor pattern in a stung cockroach. (B–D) Adapted from Gal and Libersat (Gal and Libersat, 2008).

are rarely observed in stung cockroaches standing on ground (Fig. 3A,B) (Gal and Libersat, 2008). Thus, the sensory-motor and central mechanisms involved in the initiation and maintenance of leg movements *per se* appear to be intact in stung cockroaches. Similarly, stung cockroaches show no difficulties in initiating and maintaining flying behaviour: the stimulus threshold required to induce flying, as well as the wingbeat frequency and inter-wing coordination during flying episodes, are similar in stung and un-stung cockroaches (Fig. 3D). However, these flying episodes are significantly prolonged in stung cockroaches compared with un-stung cockroaches, suggesting that the central pattern generators (CPGs) responsible for flying are hyper-excited in stung cockroaches. One possible explanation for this phenomenon is that the venom may inhibit neuronal activity in the SEG, which sends descending permissive inputs to the walking CPG and inhibitory inputs to the flying CPG (Gal and Libersat, 2006). This issue will be discussed in detail later.

Venom inhibits the initiation of walking

These and other experiments indicate that the hypokinetic state is specific to walking-related behaviours. But does the venom completely prevent the initiation of walking or does it elevate the threshold stimulus required to elicit walking? Or maybe stung cockroaches are able to initiate locomotion but are unable to maintain it? A recent study (Gal and Libersat, 2008) provides answers to some of these questions.

To test the threshold for walking initiation in stung cockroaches, a modified shuttle box was used in which escapable foot shocks were administered to cockroaches' legs without previous training. The voltage required to elicit an escape response, which consists of a 'startle' response followed by a rigorous recruitment of the walking CPG, was noted at different time points after the sting (Fig. 4A). In

stung cockroaches, the threshold voltage required to elicit escape gradually increased following the sting, peaking 2–4 h after the sting and returning to normal approximately 72 h later. At the peak of the venom's effect, stung cockroaches endured voltages more than eightfold higher than un-stung cockroaches before escaping the electric foot shocks, indicating their elevated threshold for walking initiation as well as their basic ability to walk upon reaching this threshold. Similarly, when successive tactile stimuli were applied to the cockroaches' abdomens, and while un-stung cockroaches typically responded with an escape response at the first stimulus applied, stung cockroaches only responded when more than three consecutive stimuli were administered, again demonstrating their elevated walking threshold (Fig. 4B). Furthermore, the walking episodes evoked by such consecutive stimuli were significantly slower and shorter in stung compared with un-stung cockroaches, suggesting that the ability to maintain normal walking speed and duration (once the stimulus reached the threshold) is also tempered in stung cockroaches.

Venom inhibits the maintenance of walking

To directly test the ability of stung cockroaches to maintain walking upon receiving a supra-threshold stimulus, stung and un-stung cockroaches were subjected to a modified forced swimming test (Fig. 5). This paradigm, in which an animal is placed in the centre of a water-filled cylinder and its active swimming duration is measured, has been traditionally and widely employed to evaluate depression-like phenotypes in non-human mammals. Because *Periplaneta* are terrestrial insects, water immersion provides a reliable and continuous stressful stimulus that typically produces a rigorous walking-like motor pattern (Gal and Libersat, 2008).

Similar to un-stung cockroaches, stung cockroaches readily initiate normal swimming patterns and express rhythmic Df and Ds
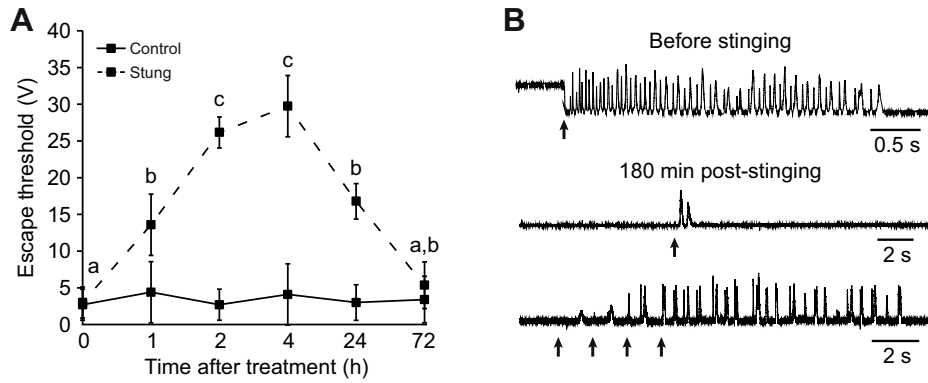
Fig. 4. Stung cockroaches show elevated behavioural thresholds for walking. (A) Cockroaches were subjected to escapable foot shocks in a modified shuttle box before (time 0) and at different time points after the sting (or after handling, in controls). The threshold voltage required to elicit escape responses gradually and reversibly increases in stung but not in control individuals. Data points labelled with the same letter are not significantly different. (B) Stung cockroaches were positioned in a walking posture on a slippery surface to allow tethered walking, recorded with a photoresistor placed beneath the mesothoracic leg. Motion traces of the mesothoracic leg show the response to trains of brief (arrowheads) tactile stimuli in the same cockroach before and 180 min after a sting. A single stimulus evokes walking before (top) but not after (middle) the sting. However, four consecutive stimuli applied to the stung cockroach (bottom) evoke a walking episode that outlasts the stimuli train. Walking evoked by this supra-threshold stimulus is significantly slower in the stung cockroach (note the different time scales in the top and bottom panels). Adapted from Gal and Libersat (Gal and Libersat, 2008).

potential in the modified forced swimming test (Fig. 3C). However, in marked contrast with un-stung cockroaches, the active swimming durations are significantly decreased in stung compared with un-stung cockroaches, such that stung cockroaches stop swimming a short while after immersion (Fig. 5) and, rather, float passively on the water while occasionally moving their antennae about in an exploratory manner. At least two observations indicate that the reduction in swimming durations upon water immersion is not due to direct motor deficits or muscle fatigue: first, wind or tactile stimuli applied to the abdomen or antennae during periods of passive floating typically re-evoke short periods of swimming; and second, after removal from the water cylinder, stung cockroaches placed on their backs are able to right themselves, i.e. turn over using their legs vigorously and stand upright. Therefore, it appears that the central inhibition of pre-motor centres affects both aspects of walking, namely the initiation and maintenance of walking. The central mechanisms that may be responsible for these deficits, as well as possible interpretations, will be discussed in the following sections.

Venom inhibits the drive for walking

Insects are not automatons that react in the same way every time the same stimulus is applied. Although variations in behavioural

outputs in response to similar stimuli have been traditionally attributed to 'noisy circuits in a complex system', recent evidence indicates that the internal state (also referred to as the 'rest state') of insects may profoundly affect, and even determine, the behavioural output in response to a given stimulus (van Swinderen, 2005; Maye et al., 2007). Namely, similar to vertebrates, it seems that the specific state of an insect (e.g. its metabolic state, arousal, attention, etc.) at a specific time point may dramatically influence its decision to initiate a certain behaviour in response to a specific internal or external stimulus, as well as the maintenance of this behaviour. This can be therefore regarded as the drive (or, analogically, the 'motivation') of the insect to engage in a specific behaviour in response to a specific stimulus.

Because the jewel wasp's venom specifically affects the initiation and maintenance of walking without affecting other behaviours, and because walking can be initiated and, to some extent, maintained in stung cockroaches when the stimulus reaches a certain (elevated) threshold, it seems that stung cockroaches have a specific deficit in reaching the decision to walk rather than in the ability to walk. Such a manipulation may be beneficial in the investigation of several key questions in the study of the neurobiology of behaviour, such as which circuits determine
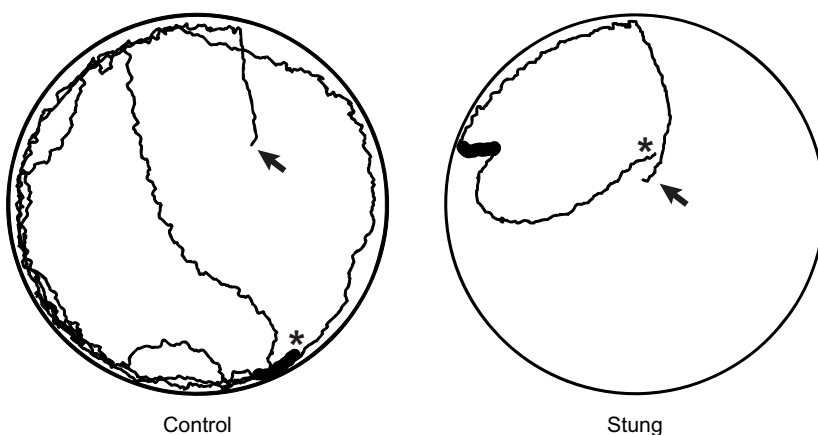

Fig. 5. Swimming motion tracks during a 1-min trial in a modified forced swimming test. A control cockroach (left) continuously swims to escape the water-filled arena, demonstrating a period of immobility (thick line) only towards the end of the trial (asterisk). In contrast, a stung cockroach initiates swimming similar to that of the control but 'despairs' faster, spending most of the trial passively floating on the water surface. Adapted from Gal and Libersat (Gal and Libersat, 2008).

the internal state and regulate the decision to engage in a specific motor behaviour, and what are the neuronal substrates responsible for determining the 'motivation' for walking specifically? Although these questions are at the core of ongoing research, some answers have already begun to emerge thanks to the study of this unique parasitoid–host system.

Involvement of the SEG in the venom-induced hypokinesia

In insects, the SupEG and SEG comprise the cerebral ganglia and have been implicated, among other functions, in integrating multimodal sensory inputs and modulating locomotion (e.g. Kien and Altman, 1992; Strauss and Heisenberg, 1993; Martin et al., 1998; Martin et al., 1999; Ridgel and Ritzmann, 2005; Gal and Libersat, 2006; Wessnitzer and Webb, 2006; Bender et al., 2010). Interneurons descending from the cerebral ganglia to the thoracic regions of the CNS express specific neuronal firing patterns to select certain thoracic pattern generators while inhibiting others. The thoracic pattern generators, in turn, orchestrate the specific spatio-temporal patterns required to execute the selected behavioural output. The SupEG of cockroaches generally exerts a tonic inhibitory effect on walking and a tonic permissive effect on flying, while the SEG generally acts antagonistically and exerts a tonic permissive effect on walking and a tonic inhibitory effect on flying (Gal and Libersat, 2006). Because stung cockroaches show decreased excitability of the walking CPG and increased excitability of the flying CPG (see above), it seems that the venom may inhibit neuronal circuits within the SEG to decrease the drive for walking. Hence, if true, focal inhibition of neuronal activity in the SEG of un-stung cockroaches should exert effects on the drive for walking comparable to those observed in stung cockroaches. Injections of procaine (a reversible voltage-dependent sodium-channel blocker) into the SEG reversibly decreased spontaneous and evoked walking in un-stung cockroaches, a behaviour comparable to that of cockroaches stung by the jewel wasp (Gal and Libersat, 2010). A similar result was observed when crude venom milked from jewel wasps was injected focally into the SEG neuropile of un-stung cockroaches; like procaine, the venom inhibited the drive for walking in un-stung cockroaches in a manner similar to that seen in naturally stung cockroaches (Gal and Libersat, 2010). This strongly (though indirectly) suggests that the SEG is involved in the decrease of spontaneous and evoked walking in stung cockroaches.

To directly test whether the sting modulates neuronal activity within the SEG, spontaneous and evoked spiking activity within the SEG of stung cockroaches was recorded using extracellular bipolar electrodes (Gal and Libersat, 2010). Compared with un-stung cockroaches, spontaneous and stimulus-evoked neuronal spiking activity in the core of the SEG was dramatically decreased in stung cockroaches (Fig. 6). The correlation between the decreased neuronal activity in the SEG and the behavioural decrease in the drive for walking suggests that the SEG may be an important part of the decision-making system; therefore, it may play a central role in determining the 'rest state' and the drive for walking in cockroaches, and probably in other insects as well. In locusts, for example, decision-making with respect to the selection and maintenance of walking has been examined using intracellular recordings of neurons in the SEG and SupEG. The spontaneous initiation of walking is accompanied by changes in the firing pattern of several SEG and SupEG descending interneurons. However, while SEG and SupEG interneurons both fire during walking, and are thus both involved in walking, predominantly SEG interneurons fire during the preparatory phase of walking. This observation

suggests a prime role for SEG neuronal circuits in determining the motivational level to engage into walking in locusts as well (Kien and Altman, 1992).

Although it seems that the jewel wasp has evolved to target the SEG of its cockroach host to maximize the chances of survival of its offspring, numerous questions still remain open. What is the role of the SupEG, in which a large amount of venom is also injected, in suppressing the motivation of stung cockroaches to engage in walking? Which neuronal circuits within the SEG may be specifically involved in determining the insect's 'rest state'? Which factors determine the motivation of insects to engage in walking? And how are external and internal determinants integrated in the insect CNS to affect the animal's decisions? The majority of these and other questions remain unanswered and are currently under intensive investigation. However, several answers can be proposed based on previous research in cockroaches and other insects, as will be discussed below.

Possible role of the SupEG in the venom-induced hypokinesia

The exact role of the SupEG in the venom-induced manipulation of the cockroach motor behaviour is currently under rigorous investigation. Several possibilities come to mind, e.g. a role in evoking the excessive grooming behaviour seen in stung cockroaches (Libersat, 2003), or a role in the venom-induced changes in cockroach metabolism (Haspel et al., 2005). It is also possible that the SupEG, in concert with the SEG, plays a role in inducing certain aspects of venom-induced hypokinesia. For instance, the venom could affect the SupEG directly, by affecting specific circuitries in this ganglion, or indirectly, by affecting ascending SEG interneurons, which, in turn, modulate SupEG circuitries that control motor behaviour. A direct effect of the venom on the SupEG apparently contradicts a previous study that showed that insects in which the SupEG has been surgically removed tended to walk uninhibitedly (Gal and Libersat, 2006), suggesting a generally inhibitory effect of this ganglion on locomotion. However, within the SupEG, some neuronal structures [e.g. the central body complex (CBC)] seem to upregulate while others (e.g. the mushroom bodies) apparently downregulate thoracic motor centers (Martin et al., 1998; Martin et al., 1999; Wessnitzer and Webb, 2006). The CBC, specifically, is known to affect some finer aspects of locomotion, including the frequency, duration and coordination of walking, turning behaviour and obstacle climbing (Bender et al., 2010). The venom could thus, in principle, specifically manipulate the CBC, in addition to manipulating SEG activity, to further inhibit the initiation of locomotory behaviour in the cockroach prey. In support of this hypothesis is the observation that the wasp injects its venom directly in the medial area of the SupEG, namely in the region of the CBC (Fig. 2A).

Does the venom affect bioaminergic neurons to induce hypokinesia?

As mentioned above, the specific neuronal circuitries within the cockroach CNS that are targeted by the venom to induce hypokinesia are currently under investigation. However, the prime candidates are neuromodulatory interneurons and, in particular, monoaminergic interneurons, which descend from the SEG to thoracic motor centers and/or ascend from the SEG to the SupEG. With respect to motivation, the role of monoaminergic systems (which are also major targets for human mood stabilisers) may have been conserved throughout evolution. In insects as in mammals, CNS dopamine (DA) and octopamine (OA, which is the
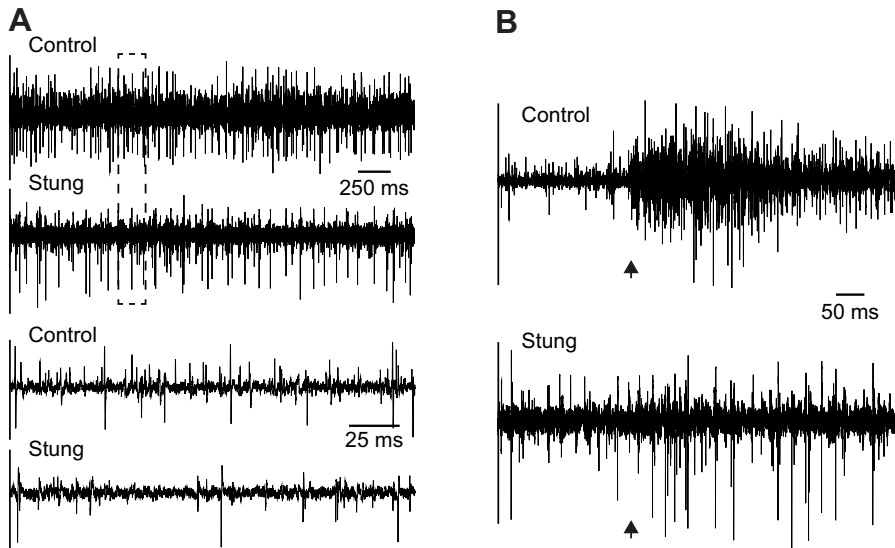
Fig. 6. Spontaneous (A) and evoked (B) neuronal activity in the sub-esophageal ganglion (SEG) is inhibited in stung cockroaches. Neuronal activity was recorded with an extracellular bipolar stereode from the middle of the SEG. The dashed area in the top traces in A is expanded in the bottom traces. Arrowheads in B represent the onset of a wind stimulus applied to the cerci. Adapted from Gal and Libersat (Gal and Libersat, 2008).

invertebrate analogue of noradrenaline) have been shown to profoundly affect motivation, arousal and locomotion (Libersat and Pflüger, 2004). Moreover, in insects as in mammals, it is the interplay between different neuromodulators that seems to affect behavioural choices.

Recent evidence suggests that the monoamine pair DA/OA, in particular, plays an important role in the induction of hypokinesia in the stung cockroach host. In the SEG, one specific population of monoaminergic neurons that may be affected by the venom is the octopaminergic unpaired median neurons. The axons of some of these neurons innervate segmental ganglia while others innervate major neuropiles in the SupEG (Bräunig and Burrows, 2004). Similar to noradrenaline in mammals, OA in insects has been previously implicated in regulating locomotion (Pflüger and Duch, 2000; Saraswati et al., 2004) and, recently, activity in SEG-OA neurons in *Manduca* larvae has been correlated with fictive locomotion (Cholewa and Pflüger, 2009), further highlighting these neurons as major candidates for the venom-induced hypokinesia. Accordingly, the OA receptor agonist chlordimeform has been shown to induce a significant increase in spontaneous walking when injected into the SupEG of stung cockroaches (Rosenberg et al., 2007). This suggests that the jewel wasp's venom interferes with octopaminergic modulation of walking initiation in central structures of the cockroach SupEG, which receive inputs from the SEG. Supporting this hypothesis are the findings of previous studies showing that the activity of thoracic dorsal unpaired median (DUM) neurons, a population of thoracic OA-releasing neurons, is decreased in stung cockroaches (e.g. Rosenberg et al., 2006). Because OA is an important excitatory neuromodulator in insects, it appears that the cerebral circuits targeted by the wasp's venom affect at least, directly or indirectly, the excitability of thoracic DUM neurons to decrease the drive for walking. A similar role for OA in depressing host locomotion following a wasp's sting has been also proposed for the wasp *Cotesia*, a parasitoid of *Manduca* (Adamo and Shoemaker, 2000). In addition, in *A. compressa*, there are some experimental data suggesting the possible involvement of the DA modulatory system: the injection of DA induces grooming in an un-stung cockroach, but not in a stung one. Notably, the effect of the sting on the DA-induced grooming wears off according to the same time course as the escape recovery (Weisel-Eichler, 2000).

Concluding remarks

In this Review, we highlighted experimental evidence indicating that the sting of the parasitoid jewel wasp *A. compressa* specifically modulates the motivation of its cockroach prey to walk. To achieve this unique manipulation, the wasp injects its venom cocktail directly into discrete regions of the cockroach cerebral ganglia, namely the SEG and the medial area of the SupEG. As a result, and although sensory-motor systems are not directly affected, stung cockroaches show a deficit in producing the decision to walk. Anecdotally, some behavioural characteristics of stung cockroaches are analogous to those observed in mammalian models for decreased motivation; for instance, the unresponsiveness to escapable foot shocks observed in stung cockroaches is also a hallmark of the mammalian model of depression often referred to as 'learned helplessness'. Surprisingly, learned-helplessness-like phenomena have been shown to manifest in insects, even at the level of individual ganglia (Eisenstein and Carlson, 1997; Harris and Eisenstein, 1999). Stung cockroaches also show decreased active swimming durations in the forced swimming test, a hallmark of mammalian depressed-like states, which presumably signals a decrease in motivation. Stung cockroaches also demonstrate decreased excitability of the walking CPG, resulting in slower and shorter walking bouts, which somewhat resembles the 'psycho-motor retardation' symptoms observed in mammalian models for depression. Such analogies are clearly more figurative than substantial; however, it is not impossible that insects possess a precursor of what we refer to as motivation in mammals. Unlike motivational deficits in mammals, however, the venom-induced hypokinesia in stung cockroaches can be traced to specific neuronal structures within the cockroach CNS. More specifically, it seems that the jewel wasp modulates certain neuronal circuitries within (at least) the cockroach SEG, which generally exerts a tonic permissive effect on walking-related thoracic CPGs. The SEG may therefore be involved in determining the 'motivation' of the insect to engage in spontaneous and evoked walking.

In this Review, we tried to introduce the reader to the astonishing world of parasitoid wasps and their insect hosts. These wasps are much better at manipulating the neurochemistry of their prey with specific neurotoxins than are the neuroscientists who study them. As such, wasp neurotoxins may provide us with new and highly specific pharmacological tools with which to investigate cell and

network function. Much work remains to be done until we know the exact neuro-chemical cascade taking place in the host's CNS to alter its behaviour in such exquisite ways. In addition, given the time scale of the manipulation, we suspect that the venom has genomic effects on the cockroach's CNS. Owing to the breath of such an investigation, a multidisciplinary approach combining molecular techniques with cellular electrophysiology and behavioural analysis is essential.

Future prospects: cellular and molecular mechanisms of the venom-induced behavioural manipulation
The CBC and the drive for walking

The CBC in the protocerebrum of the SupEG has been implicated in modulating locomotion in various insects (Strauss and Heisenberg, 1993; Martin et al., 1999; Strausfeld, 1999; Strauss, 2002). It receives bilateral sensory relays and resides immediately upstream of premotor areas, suggesting that it may be involved in the context-dependent control of behaviour. In addition, many descending neurons that supply leg motor circuits also receive connections from the CBC. Walking analysis of *Drosophila* strains with mutations affecting the CBC associates this central structure with functions related to higher locomotor control. In several behavioural paradigms, flies from mutant strains with altered structure of the CBC walk more slowly than wild-type flies, react less quickly to changing stimuli during flight and show altered orientation behaviour toward landmarks (Strauss, 2002). Harley and Ritzmann (Harley and Ritzmann, 2010) reported recently that cockroaches with lesions to the CBC exhibit abnormalities in turning and climbing, and Bender et al. (Bender et al., 2010) used chronic multi-neuron extracellular recording, a technique that provides suitable temporal resolution, to show that neural activity in the CBC is strongly correlated with and sometimes predictive of stepping frequency. Last but not least, electrical stimulation of the CBC evokes or modifies walking. One of the current research goals in our laboratory is therefore to implement this powerful technique, which has been developed and refined over the last 10 years to sample assemblies of neurons in the insect SupEG, and specifically to test neuronal activity in the CBC of a cockroach prior to, during and following a wasp's sting. Employing the high temporal resolution of chronic extra-cellular multi-neuron recordings, we hope to identify the microcircuits in the cockroach's SupEG that are involved in providing descending control over thoracic locomotory circuits, and which the venom manipulates to decrease locomotion.

Molecular targets underlying behavioural manipulation: a proteomics investigation

Another approach used to study behavioural manipulation is the study of protein expression. Post-genomic era approaches have led to great advances in our understanding of the proximate mechanisms mediating host behavioural changes. In particular, recent parasito-proteomics studies of infected host brains have yielded important data (Ponton et al., 2006). These new techniques are important especially in light of the complex mechanisms that are typically involved in host behavioural changes. Such an approach involves difference gel electrophoresis multi-fluorescent dye approaches, where multiple protein samples are labelled and proteins are then separated on a two-dimensional gel according to their isoelectric focusing point and their size. Proteins shared by samples co-migrate, whereas differentially expressed or post-transcriptionally modified proteins occupy unique spots on the gel. Such proteins can then be extracted and their mass and amino acid

sequence can be determined *via* mass spectrophotometry and aligned against available databases. Pioneer proteomics studies on manipulated hosts have been carried out on seven arthropod host-parasite associations, among which two Orthoptera–hairworm associations (Ponton et al., 2006; Biron et al., 2006; Lefevre et al., 2007). These studies have contributed to the discovery of candidate genes and of biochemical pathways altered in host CNS during the parasite manipulation for three aberrant host behaviours: suicidal behaviour, increase in probing rate and alteration of evasive behaviour. The simultaneous study of host and parasite proteomes during their interaction is a promising avenue to decipher and understand the manipulative tactics used by parasites and to reveal new products, such as neuropeptides and neurotransmitters, related to the alteration of host behaviour. Given the time scale of the cockroach manipulation by *A. compressa*, we suspect the venom to have genomic effects on the cockroach's CNS. Using the proteomics technique, we hope to identify the molecular targets of the venom components in the cockroach cerebral ganglia. We hope that these lines of investigations will provide useful information regarding the neuronal underpinnings of behavioural spontaneity, which is a prime issue in behavioural neurobiology.

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