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VENOM OF A PARASITOID WASP INDUCES PROLONGED GROOMING IN THE COCKROACH

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Summary

The parasitoid wasp *Ampulex compressa* hunts cockroaches *Periplaneta americana*, stinging them first in the thorax and then in the head, the sting penetrating towards the subesophageal ganglion. After being stung the cockroach grooms almost continuously for approximately 30 min, performing all the normal components of grooming behavior. This excessive grooming is only seen after the head sting and cannot be attributed to stress, to contamination of the body surface or to systemic or peripheral effects. This suggests that the venom is activating a neural network for grooming.

We suggest that the venom induces prolonged grooming by stimulating dopamine receptors in the cockroach, for the following reasons. (1) Reserpine, which causes massive release of monoamines, induces excessive grooming. (2) Dopamine injected into the hemocoel also induces excessive grooming and is significantly more effective than octopamine or serotonin. In addition, the dopamine agonist SKF 82958 induces excessive grooming when injected directly into the subesophageal ganglion. (3) Injection of the dopamine antagonist flupenthixol greatly reduces venom-induced grooming. (4) Dopamine, or a dopamine-like substance, is present in the venom.

Key words: grooming, venom, cockroach, wasp, dopamine, suboesophageal ganglion, central nervous system, *Periplaneta americana*, *Ampulex compressa*.

Introduction

Venoms generally act peripherally at the neuromuscular junction or specifically affect conductances underlying the action potential and its propagation, resulting in different types of paralysis. Reports of specific central effects of venoms on behavior have been rare (Adams and Swanson, 1994). One instance where venoms appear to act centrally in the nervous system is the case of insects stung by various parasitoid wasps (Piek and Spanjer, 1986; Steiner, 1986). The venoms of some of these wasps have been reported to block synaptic transmission in the central nervous system and to inhibit specific behavior patterns (Piek and Spanjer, 1986; Piek et al., 1993; Gnatzy and Otto, 1996). Here, in contrast, we describe the induction of a specific type of behavior by the venom of a wasp.

The parasitoid sphecid wasp *Ampulex compressa* preys on cockroaches of the species *Periplaneta americana*, using them as a food supply for its larvae. The wasp stings a cockroach twice, first in the thorax and then in the ventral side of the head, towards and probably penetrating the subesophageal ganglion. Approximately 20–30 min after the sting, the cockroach enters a long-lasting lethargic state, characterized by hypokinesia and the lack of an escape response, although the insect is not paralyzed (Williams, 1942; Piek et al., 1989; Fouad et al., 1994, 1996). During our work with stung cockroaches, we observed that, beginning almost immediately after a sting, a cockroach spends much of the time grooming. We wished to examine whether the cockroach was, indeed, grooming more than normal and, if so, to determine the cause of the extensive grooming behavior.

Grooming in insects serves the function of cleaning the outer body surface and may have other functions as well, such as courtship behavior, social signaling, displacement activity and de-arousal (Spruijt et al., 1992). In the cockroach, a routine grooming response consists of some or all of the following activities: cleaning of the antennae, palpi and legs with the mouthparts; rubbing of the head and the basal segments of the antennae with the forelegs; rubbing of the abdomen and cerci with the hindlegs; and rubbing the underside of the wings with the abdomen (Bobula Smith and Valentine, 1985).

A neural center controlling the entire sequence of grooming movements in insects has not been located. However, in the mantis, head-grooming has been shown to be centrally controlled (Zack, 1978) and, in the locust, this behavior can be elicited by electrical stimulation of the circumesophageal or cervical connectives (Kien, 1983). In the present study, we show that the venom of *Ampulex compressa*, which is injected into the head towards the subesophageal ganglion, activates the entire repertoire of grooming behavior in the cockroach. This

behavior pattern is probably elicited through stimulation of dopamine receptors in the central nervous system of the cockroach. To our knowledge, this is the first report of a venom, injected *via* a sting, eliciting a specific behavior pattern.

Materials and methods

Animals

All experiments were performed on adult male cockroaches *Periplaneta americana* L. raised in crowded conditions in 60 l plastic barrels. The cockroaches were provided with water and cat chow *ad libitum.* The wasps, *Ampulex compressa* Fabricius (Hymenoptera: Sphecidae), were kept in 100 l clear acrylic boxes with 10–30 wasps in each box. The wasps were provided with water and honey *ad libitum.* We also provided female wasps with cockroaches to hunt as a food supply for their offspring and for host-feeding (wasps drank the hemolymph of stung cockroaches), as described by Fouad et al. (1994). All animals were kept at 25–32 °C on a 12 h:12 h L:D cycle.

Behavioral testing

We observed the grooming behavior of cockroaches in an opaque plastic box (29 cm×18 cm×13 cm) with a clear plastic cover. The floor of the box was covered with small pebbles. Using a stopwatch, we measured the amount of time spent grooming during the 30 min period immediately following treatment. Animals had never been in the testing box previously, and it was therefore a novel environment in all cases. The temperature in the testing room was maintained at 25–30 °C. Testing was performed 2–8 h after the beginning of the light cycle.

We filmed grooming behavior using a Minolta Master Series V-16 video camera. Control cockroaches were induced to groom by handling and dusting the animals with corn starch. Video films were analyzed on a Daewoo DV-F54DHP system with variable speed control.

Treatments

A number of different treatments were used to study grooming behavior after a sting. (1) Full sting, in which cockroaches were given a full (normal) stinging sequence by the wasp, i.e. they were stung first in the thorax and then in the head. In this case, the wasp was in contact with the cockroach for approximately 2 min. (2) Grabbed, in which cockroaches were grabbed by the wasp but not stung because the wasp grabbed the cockroach on a posterior portion of the wing and could not position itself to sting. In this case, we removed the wasp from the cockroach after approximately 2 min. (3) Thorax sting, in which cockroaches were stung in the thorax only. This was accomplished by interrupting the stinging sequence and removing the wasp from the cockroach after it had delivered the thoracic sting and before it could sting the head. (4) Thorax sting and pierce, in which, following a thorax sting, we pierced the subesophageal ganglion by inserting a 100 μm steel minuten pin through the cuticle of the submentum into the ganglion. The pin diameter was slightly larger than that of the sting of a wasp, which is approximately 80 μm. The pin was placed using landmarks on the cuticle. (5) Untreated, in which cockroaches were kept in small plastic containers for several hours and then introduced into the testing box without being touched.

Cockroaches that were sprayed with irritant received two overall sprays of a solution of 5 % acetic acid, an irritant that elicits prolonged grooming in insects, as described by Hogan-Warburg et al. (1995). We used 5 % acetic acid because this concentration produced prolonged, but not maximal, grooming activity (grooming times after being sprayed were 24.8±2.5 min with a 10 % solution, 16.2±7.0 min with a 5 % solution and 8.7±5.8 min with a 1 % solution of acetic acid (means ± S.D., N=5 for all groups).

Drugs

For drugs administered *via* the hemolymph, 10 μl of solution was injected between the fourth and fifth sternites into the abdominal hemocoel using a Hamilton syringe. Dopamine, octopamine and serotonin (Sigma) were prepared in cockroach saline (Blagburn and Sattelle, 1987) at 10^{-5} mol l^{-1}; cis(z)flupentixol (Research Biochemical International), mianserin (Sigma) and phentolamine (Sigma) were prepared in the same saline at 2×10^{-2} to 6×10^{-2} mol l^{-1}; reserpine (Sigma) was prepared in safflower oil at 5×10^{-3} mol l^{-1}. To inject drugs into the subesophageal ganglion, we immobilized cockroaches by covering them with modelling clay. We made a slit in the ventral cuticle between the neck and the head, and injected 10 nl of solution into the posterior medial area of the subesophageal ganglion using a nano-volumetric injector (Medical Systems Corp., NY, USA). Placement of the pipette was controlled visually using a stereomicroscope (Olympus). Cockroaches that did not begin walking immediately after surgery were considered not to have recovered sufficiently from surgery and were not included in the sample. SKF 82958 (Research Biochemical International) for injection into the subesophageal ganglion was dissolved at 10^{-8} to 10^{-9} mol l^{-1} in a cockroach saline of the following composition (in mmol l^{-1}): NaCl, 214; KCl, 3.1; $CaCl_2$, 9; sucrose, 50; Hepes buffer, 5; pH 7.2, based on Wafford and Sattelle (1986).

Venom analysis

We performed gas chromatography/mass spectrometry (GCMS) on a sub-peptidic extract of the crude venom to assess small organic molecules. The venom was milked from the wasps into a solution of 0.4 mol l^{-1} $HClO_4$, centrifuged (9000 ***g***, 5 min), filtered (0.22 μm pore size), lyophilized, neutralized with NaOH, and extracted in $CHCl_3$. We derivatized the sample with pyridine and NO-bis (trimethylsilyl; TMS) acetamide and incubated it for 1 h at 70 °C. For gas chromatographic separation, we used a WCOT-fused silica column with a stationary phase RTX-1 (30 m long, 0.32 mm i.d.). The temperature program was 2 min at 100 °C, ramped to 290 °C at 10 °C min^{-1}. Helium was the carrier gas, at a flow rate of 1 ml min^{-1}. A small amount of the sample

(1 μl) was injected at 250 °C (interface temperature 280 °C). Mass spectrometry analysis was performed on a quadrupole GCMS system (HP-5971A, mass-selective detector), with a 70 eV electron impact ion source. The mass spectra of the venom components were analyzed using a computerized data bank (Wiley/NIST 1998) and were also compared manually with the mass spectra of the following neurotransmitters: dopamine, epinephrine, GABA, glutamate, glycine, histamine, norepinephrine, octopamine, serotonin and tryptophan.

Data analysis

Data were analyzed using *t*-tests or using analysis of variance (ANOVA) followed by planned orthogonal comparison of means (Sokal and Rohlf, 1995), or by Scheffe test, as indicated (GB Stat). Results are presented as mean ± standard deviation (S.D.).

Results

After a cockroach has been stung by a wasp, it usually remains motionless for approximately 5 min. Following this, it grooms almost continuously for 25–35 min. After this grooming period, it enters a state of lethargy in which it shows very little movement. In our experiments, the wasp was removed from the testing box immediately after stinging the cockroach; if the wasp is allowed to remain, it leaves the cockroach after the sting and returns to it approximately 20–40 min later. Cockroaches were observed to continue grooming even while the wasp cut their antennae and drank hemolymph from the cut ends.

Venom and grooming

Cockroaches that received the full stinging sequence by a wasp (stung first in the thorax and then in the head) groomed for 23.0±2.3 min during the 30 min following the sting (Fig. 1). This response was highly consistent among the cockroaches (range 19.6–26.4 min). Untreated cockroaches groomed for 1.1±1.1 min during a 30 min period.

Our hypothesis was that the prolonged grooming was due to venom injection into the head; however, the grooming could have been due to the stress caused by the attack, to contact with the wasp resulting in contamination of the body surface of the cockroach or to injection of venom into the thorax. We examined the first two possibilities by testing cockroaches that were grabbed by the wasp, but not stung; these cockroaches groomed for 5.8±5.2 min. Next, we tested cockroaches that received injections of venom by being stung in the thorax but not in the head, thereby undergoing all the components of a full stinging sequence except for the sting to the head. These cockroaches groomed for 4.0±2.2 min during the 30 min following the thorax sting. We also tested whether the grooming response could be due to mechanical irritation of the subesophageal ganglion by the wasp's sting in addition to all the above factors. Cockroaches that received a thorax sting followed by piercing of the subesophageal ganglion with a minuten pin, to mimic the head sting, groomed for 7.8±5.4 min. In summary, cockroaches receiving a full stinging sequence by a wasp groomed for significantly longer than animals in the four other groups, which did not receive stings to the head (Fig. 1).

Analysis of behavior

To determine whether the grooming movements after a sting are comparable with those of normal grooming, we analyzed video recordings of stung cockroaches grooming and compared them with similar recordings of control cockroaches dusted with corn starch to elicit grooming (N=7 for each group). The same components of grooming behavior were observed in both groups; the following is a complete list of the components observed: (a) rubbing the head, eye and base of the antenna with a foreleg, (b) rubbing the cercus and abdomen with a hindleg, (c) cleaning the antenna with the mouthparts, (d) cleaning the palpi with the mouthparts, (c) cleaning each of the legs with the mouthparts, (f) cleaning the sternum with the mouthparts, and (g) rubbing the underside of the closed wings with the abdomen (for examples, see Fig. 2). The components and the details of the grooming movements in both groups corresponded exactly with the species-specific grooming movements described by Bobula Smith and Valentine (1985) (except that these authors did not observe sternum cleaning in *P. americana*). There was no statistical difference between groups in the time spent cleaning an antenna (stung 18±8 s; control 13±3 s; P=0.2, N=7; *t*-test); however, the frequency of movement of the mouthparts while

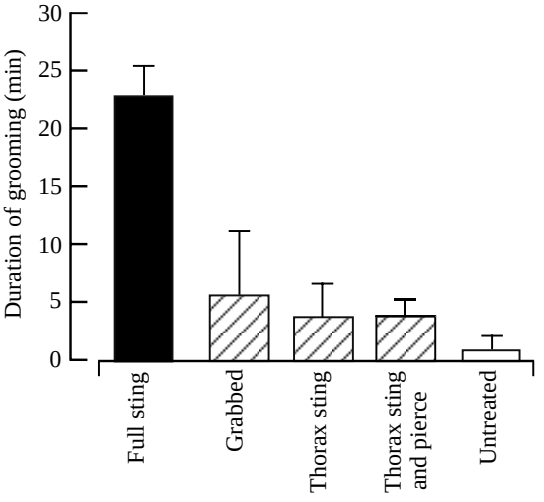

Fig. 1. Stung cockroaches groom extensively. Cockroaches receiving a full stinging sequence by the wasp groom for an extended period after the sting. This grooming time is significantly longer than that observed in cockroaches that were grabbed by the wasp and not stung, stung only in the thorax, stung in the thorax followed by piercing of the subesophageal ganglion with a pin, or were untreated (ANOVA and planned orthogonal comparison of means; P<0.001). Grooming time was measured during the 30 min period following treatment for all animals. Values are means ± S.D. Each bar represents eight animals, except for the grabbed group (N=3).

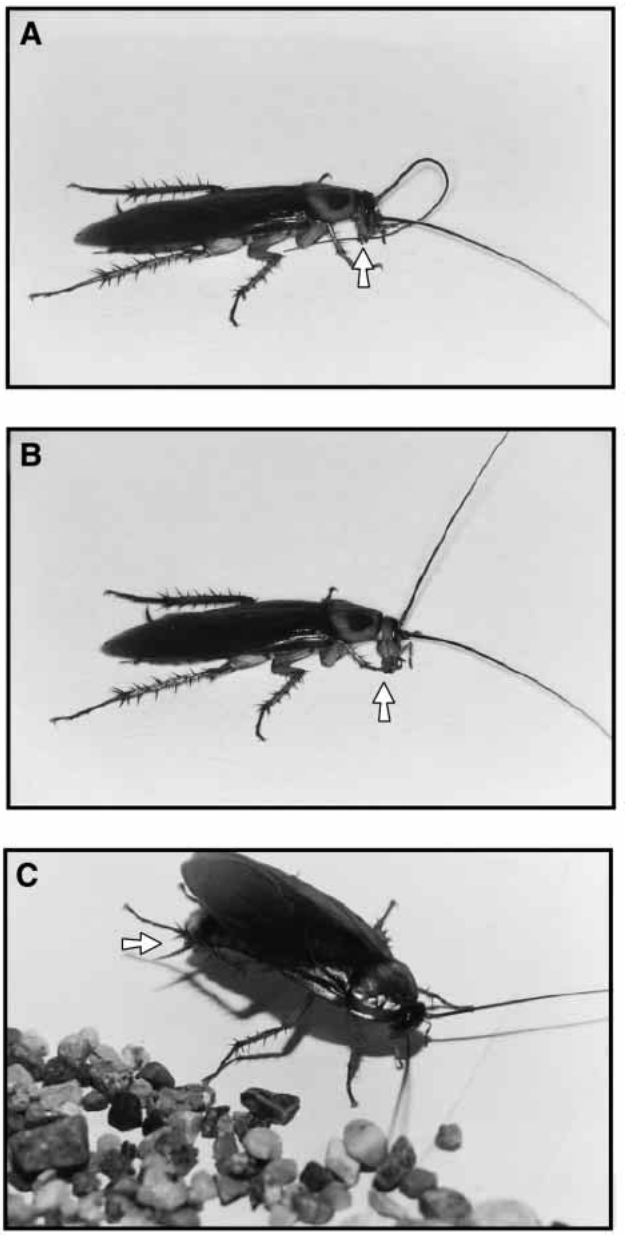

Fig. 2. Stung cockroaches perform the entire repertoire of grooming movements. Photographs show a stung cockroach performing the three most frequent components of grooming behavior (marked with arrows): (A) grooming an antenna with the mouthparts, (B) grooming a foreleg with the mouthparts and (C) grooming a cercus with a hindleg.

cleaning the legs was higher in stung than in control animals (stung 2.5±0.3 s^{-1}; control 2.1±0.2 s^{-1}; $P<0.01$, $N=7$; *t*-test).

There was no consistent order for cleaning various parts of the body in either stung or control animals except that, in both groups, cleaning of the head and the base of the antenna with the foreleg was interrupted intermittently with cleaning of the foreleg. The ranking of the relative frequencies of performance of the components of grooming behavior was the same for both stung and control animals (Table 1).

Table 1. *Relative frequencies of performance of components of grooming behavior*

Part of body groomed	Control* (%)	Stung* (%)
Antennae	35	19
Forelegs	22	17
Cerci	17	17
Midlegs	12	15
Head	7	13
Hindlegs	5	11
Sternum	2	8

*Number of times the indicated body part was groomed during a 30 min period as a percentage of the total number of times all body parts were groomed during that period.

Monoamines and grooming

The next question we addressed was the identity of the venom component eliciting grooming. A variety of rhythmic motor patterns in insects and other invertebrates have been shown to be induced by biogenic monoamines (dopamine, octopamine and serotonin) (for reviews, see Bicker and Menzel, 1989; Selverston, 1993). We therefore began by examining the possibility that one of the monoamines (or an agonist of a monoamine) is present in the venom and is inducing grooming in the stung cockroaches. One way to achieve a transient, elevated concentration of monoamines experimentally in the subesophageal ganglion, without the problems associated with surgery, is by using reserpine, a plant alkaloid known to cause massive release (and subsequent depletion) of monoamines (Oates, 1996). It has been found that reserpine crosses the blood–brain barrier and affects monoamine levels in the subesophageal ganglion of the cockroach (Sloley and Owen, 1982). We were interested to determine whether reserpine, like the venom, elicits grooming in insects. We found that injection of 30 µg of reserpine into the hemocoel induced prolonged grooming in cockroaches (Fig. 3); during the 30 min following injection, the time spent grooming was significantly longer after reserpine injection than after injection of vehicle (after reserpine 18.2±3.3 min; after vehicle 6.7±3.7 min). The components of the grooming behavior were the same as in stung or normal cockroaches.

To determine which of the monoamines (dopamine, octopamine or serotonin) is most likely to be the factor underlying reserpine-induced grooming, we injected each of the three monoamines, individually, into the hemocoel and measured the duration of grooming after injection. During the 30 min after injection, dopamine induced prolonged grooming which lasted significantly longer than grooming induced by octopamine, serotonin or saline (dopamine 16.6±4.1 min; octopamine 9.7±6.9 min; serotonin 9.5±4.4 min; saline 4.0±2.7 min) (Fig. 4A). The components of the grooming behavior were identical to those of stung or normal cockroaches. To determine whether dopamine is inducing grooming *via* an effect in the subesophageal ganglion, we injected the dopamine D_1 agonist SKF 82958 [(±)-6-chloro-

APB] directly into the posterior medial area of the subesophageal ganglion using pressure injection (no preceding thorax sting or injection was given). SKF 82958 injected into the subesophageal ganglion induced grooming which lasted significantly longer than that induced by saline injection (SKF 82958 14.6±3.0 min; saline 2.4±2.4 min) (Fig. 4B). Precise placement of SKF 82958 within the posterior medial area of the subesophageal ganglion was not required since all animals that recovered from surgery groomed excessively after SKF 82958 injection (range 11.7–18.5 min; N=7) while none of the animals injected with saline groomed for more than 6 min (N=7).

If, in fact, a factor in the venom stimulates dopamine receptors to induce prolonged grooming, then a dopamine antagonist, administered prior to a sting, should block the grooming effect of the venom. We injected the dopamine D_1/D_2 receptor antagonist flupentixol 1 h prior to subjecting cockroaches to a sting. These cockroaches groomed significantly less than cockroaches injected with saline prior to a sting (flupentixol 11.8±3.3 min; saline 24.1±2.6 min) (Fig. 5). This reduction in grooming time is not due to a general impairment of the ability of the flupentixol-treated cockroaches to groom, because these cockroaches groomed as much as untreated cockroaches after being sprayed with an irritant (flupentixol-treated 18.8±6.9 min; untreated 16.3±5.6 min; N=7, P=0.6; t-test) (results not shown). The octopamine antagonist mianserin did not block grooming (22.7±2.6 min) (Fig. 5) nor did the octopamine antagonist phentolamine (23.2±2.0 min; N=5) (results not shown).

Analysis of venom

We analyzed the sub-protein fraction of the venom by gas chromatography/mass spectrometry (GCMS). Gas chromatographic analysis indicates the presence of many peaks, including six abundant components (Fig. 6A). The peaks at 15.32, 16.83, 16.90 and 17.16 min were identified as being fatty acids, while the peak at 14.09 min was identified as benzenedicarboxylic acid. The retention time of the remaining abundant component was 12.68 min, which was comparable with the retention times of the dopamine (12.94 min) and octopamine (12.45 min) references.

The mass spectrum of the venom component whose retention time was 12.68 min showed major peaks at 73, 102 and 267 m/z, where m is mass and z is charge (Fig. 6B). The mass spectra of both dopamine and octopamine references (derivatized) showed the same three peaks. The peak at 73 m/z corresponds to trimethylsilyl (TMS), the derivatizing molecule, the peak at 102 m/z corresponds to methylamine-TMS, and the peak at 267 m/z corresponds to dihydroxy-toluene-di-TMS. Thus, GCMS analysis of the venom component peaking at 12.68 min is consistent with it being either dopamine or octopamine. It is possible, however, that this venom component is a substance related to dopamine and octopamine, namely a dihydroxy-phenethylamine or an α-(aminomethyl)hydroxybenzyl alcohol, which are also consistent with the GCMS analysis of the venom. Other than dopamine and octopamine, none of the nine members of the dihydroxy-phenethylamine and α-(aminomethyl)hydroxybenzyl alcohol families has been reported to be biologically synthesized, as far as we could determine. None of the venom components (including the less abundant ones) matched serotonin or other common neurotransmitters (see Materials and methods).

Discussion

Cockroaches stung by the wasp *Ampulex compressa* groom almost continuously during the 30 min immediately following

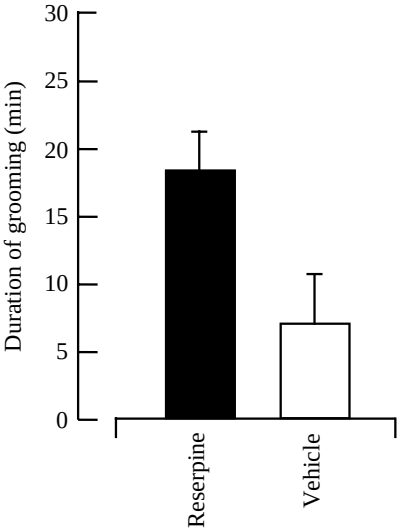

Fig. 3. Reserpine induces grooming. Reserpine elicits significantly more grooming than vehicle (safflower oil) when injected into the hemolymph. Values are means ± S.D. (P<0.0001; N=7; t-test).

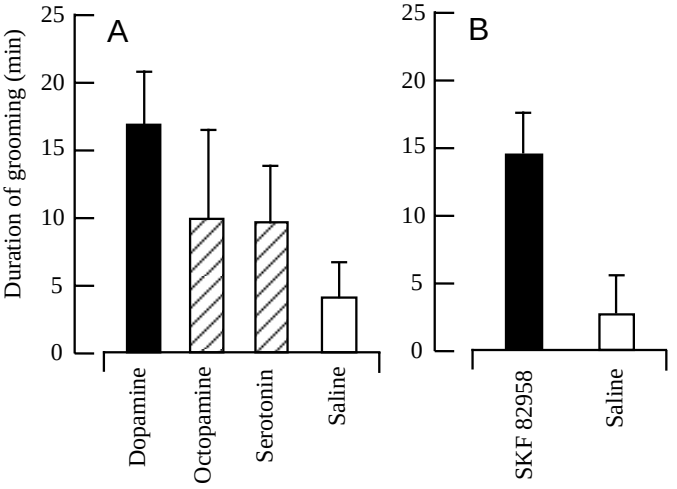

Fig. 4. Dopamine induces excessive grooming. (A) Injection of dopamine into the hemocoel induces significantly more grooming than injection of octopamine, serotonin or saline (P<0.01; N=8; ANOVA and Scheffe method). None of the other groups differ significantly from each other. (B) The dopamine agonist SKF 82958 induces significantly more grooming than saline when injected into the subesophageal ganglion (P<0.0001, N=7, t-test). Values are means ± S.D.

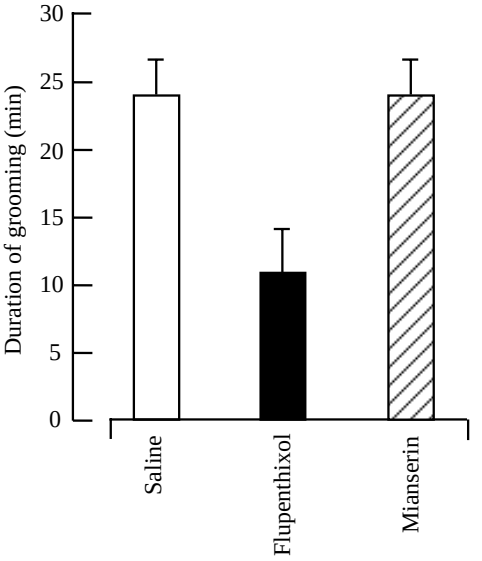

Fig. 5. Flupentixol reduces venom-induced grooming. Cockroaches that received flupentixol before a sting groom significantly less than cockroaches receiving saline before a sting (P<0.0001; N=7; t-test). Mianserin does not reduce venom-induced grooming (N=5). Values are means ± S.D.

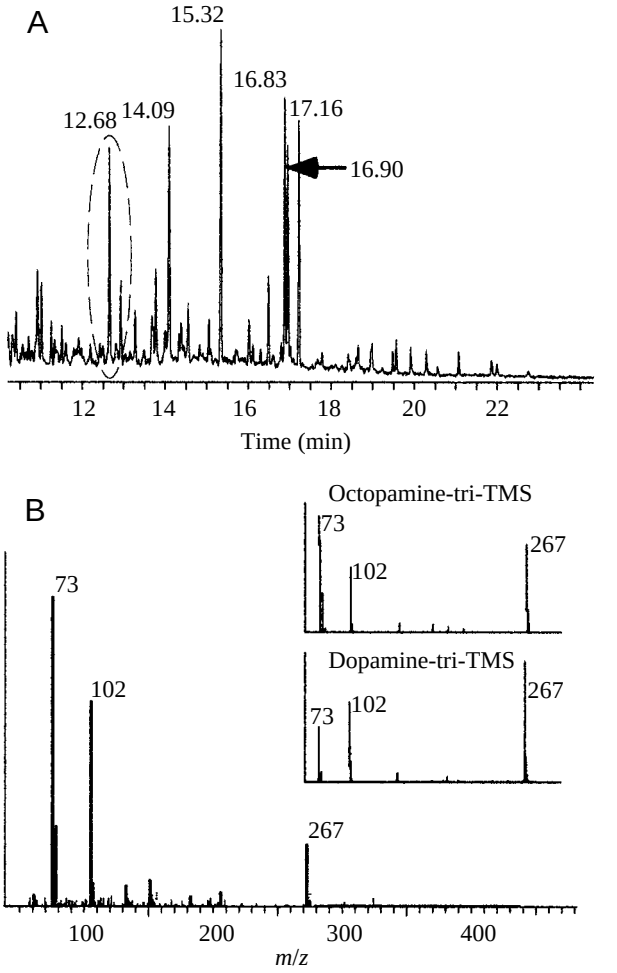

Fig. 6. Gas chromatography/mass spectrography (arbitrary units). (A) Gas chromatographic analysis of the sub-protein fraction of the venom (derivatized) shows many peaks, including six abundant substances. The x-axis indicates retention time. (B) Mass spectrographic analysis of the large venom gas chromatographic peak at 12.68 min is shown; this spectrum is comparable with the mass spectrograms (inset) of both dopamine and octopamine (the derivatized forms, dopamine-tri-TMS and octopamine-tri-TMS). The x-axes indicate mass/charge (m/z).

the sting. This effect occurs only when venom is injected into the head and cannot be accounted for by the stress of the attack, by contact with the wasp, by mechanical irritation or by injection of venom into a location other than the head. This grooming behavior includes all the normal components of grooming. Injection of dopamine induces similar prolonged grooming, while injection of the dopamine antagonist flupentixol, prior to a sting, greatly reduces venom-induced grooming. We suggest that the venom is stimulating dopamine receptors in the central nervous system of the cockroach, thereby inducing prolonged grooming.

Animals receiving a thorax sting tend to groom more than untreated animals (Fig. 1). However, they do not groom more than animals that were grabbed by the wasp, but not stung at all. Therefore, we attribute this small increase in grooming time to stress and/or to contact with the wasp, rather than to a systemic or peripheral effect of the venom following a thorax sting. Animals that are subjected to all the components of a full stinging sequence except for venom injection into the head (thorax sting followed by piercing of the subesophageal ganglion with a pin) also do not groom significantly more than animals that were only grabbed. Thus, it appears that injection of venom into the head is activating a grooming network. The grooming seen after a sting is a complex pattern of behavior (Fig. 2). It exhibits all the usual components of, and generally resembles, normal grooming but differs in the duration of the activity.

We have found no other report in the literature of a venom, injected *via* a sting, that elicits a specific behavior pattern. However, experimental injection of certain snake and scorpion venoms into the brain of rats has been found to elicit scratching and other stereotypical behavior patterns (Silveira et al., 1988; Mello and Cavalheiro, 1989; Dorce and Sandoval, 1994). In invertebrates, certain neuromodulators, particularly the monoamines, have been found to activate specific neural circuits and to release well-defined types of behavior, such as biting in the leech (Lent et al., 1989), gastric mill activity in the lobster (Heinzel, 1988), adoption of aggressive and submissive postures in the lobster (Kravitz, 1988), stridulation in the grasshopper (Ocker et al. 1995) and flight in the moth and locust (Sombati and Hoyle, 1984; Claasen and Kammer, 1986; Stevenson and Kutsch, 1987). Of particular interest here is the recent study of Yellman et al. (1997), working with decapitated flies, who found that grooming is elicited by dopamine D_1 and D_2 agonists, dopamine, octopamine or serotonin applied to the nerve cord.

We hypothesized that a monoamine (or its agonist) in the venom is the factor causing excessive grooming, and our results are consistent with this hypothesis. Reserpine injection, which

causes massive release of the monoamines dopamine, octopamine and serotonin, induces prolonged grooming (Fig. 3). Injecting dopamine into the hemocoel induces significantly more grooming than injecting octopamine, serotonin or saline (Fig. 4A), suggesting that dopamine is the monoamine underlying reserpine-induced grooming. In addition, we found that the dopamine D_1 agonist SKF 82958, which has been shown to be an agonist at locust and *Drosophila melanogaster* dopamine receptors (Ali and Orchard, 1994; Reale et al., 1997), induces prolonged grooming when injected directly into the subesophageal ganglion (Fig. 4B). This response of the cockroach to SKF 82958 resembles the excessive grooming seen in mammals in response to injection of dopamine D_1 agonists (Molloy and Waddington, 1984). Both effects occur in the central nervous system in the head, and both involve the induction and coordination of a complex grooming behavior pattern involving many different parts of the body.

We injected SKF 82958 into the posterior medial area of the subesophageal ganglion, but did not place the injection precisely. Despite this, all animals that recovered from surgery groomed excessively after SKF 82958 injection. Thus, it appears that precise placement of the agonist, and perhaps of the venom as well, is not necessary to elicit grooming. This is consistent with results showing that specific behavior patterns can be elicited in the moth and the grasshopper when neuromodulators are injected into sites that are not highly specific in the mesothoracic ganglion and the protocerebrum, respectively (Claasen and Kammer, 1986; Ocker et al., 1995). This finding contrasts, however, with the results of others showing that very precise placement, in the metathoracic ganglion, of ionophoretically applied neuromodulator is required to elicit flight in the locust (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987). Possible sources of the difference between our study and these latter studies are that they used a natural neuromodulator, while we used a synthetic agonist. Also, they injected into the metathoracic ganglion, where the central pattern generator for flight is found, while we injected into the subesophageal ganglion, which may be the location of a control center for grooming. In addition, the latter studies do not state the amount or volume of neuromodulator injected (only the concentration), and we do not therefore know whether this might be another potential source of differences between these studies.

Our results with dopamine and its agonist suggest that, if dopamine were present in the venom, it could produce the observed grooming after a sting. In fact, the venom contains a substance that we identified as being dopamine, octopamine or a related compound (Fig. 6). Flupentixol, which has been found to act as an antagonist at dopamine receptors in the cockroach brain (Notman and Downer, 1987; Orr et al., 1987), greatly reduced venom-induced grooming (Fig. 5). In contrast, mianserin and phentolamine, which have been found to be effective octopamine receptor antagonists in locust brain and cockroach nerve cord (Roeder, 1992; Orr et al., 1992), did not reduce venom-induced grooming. These results indicate that dopamine in the venom is very likely to be the component that induces prolonged grooming. It is, however, possible (1) that the dopamine component in the venom is actually a dopamine-like substance which is more potent than dopamine itself, and/or (2) that the venom contains an additional component (perhaps a peptide) that induces prolonged grooming or that potentiates the effect of dopamine. This would explain our findings that dopamine and SKF 82958 induce less grooming than the venom, and also that flupentixol does not completely block venom-induced grooming. We are currently working on a more detailed analysis of the venom components.

Dopamine has been found to be a component of the venom of several species of social Hymenoptera (for reviews, see Nakajima, 1986; Banks and Shipolini, 1986). The effect of the dopamine in these other venoms has not yet been determined. It is difficult, if not impossible, to determine whether the grooming effect of the *Ampulex compressa* venom is evolutionarily advantageous to the wasp or whether it is simply a consequence of the location of the sting. The primary function of the sting is surely to produce the long-lasting lethargy in the cockroach, and the location of the sting is likely to be that which maximizes this effect. However, it is interesting to note that the extensive grooming behavior after the sting lasts for approximately 30 min, which corresponds with the time taken for the venom-induced lethargy to become fully developed (Piek et al., 1989; Fouad et al., 1994). During this initial period, the cockroach tends to remain in the place where it was stung, perhaps because the escape response threshold is elevated, and locomotion depressed, during grooming (Camhi and Nolen, 1981; Hogan-Warburg et al., 1995).

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