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Octopamine partially restores walking in hypokinetic cockroaches stung by the parasitoid wasp *Ampulex compressa*

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Summary

When stung by the parasitoid wasp *Ampulex compressa*, cockroaches *Periplaneta americana* enter a hypokinetic state that is characterized by little, if any, spontaneous locomotor activity. In the present study we investigate the effect of an octopamine receptor agonist and an antagonist on the locomotor behavior of stung and control cockroaches. We show that in cockroaches stung by a wasp the octopamine receptor agonist chlordimeform induces a significant increase in spontaneous walking. In good agreement, in control individuals an octopamine receptor antagonist significantly reduces walking activity. Adipokinetic hormone I (AKH-I) promotes spontaneous walking in controls but does not do so in stung individuals, which suggests that the venom effect is most probably not mediated by AKH-I. Dopamine receptor agonists or antagonists had no significant effect on the spontaneous walking of stung or control cockroaches, respectively. The effect of the octopamine receptor agonist was maximal when injected into the brain, suggesting that the wasp venom interferes with octopaminergic modulation of walking initiation in central structures of the cockroach brain.

Key words: *Periplaneta americana*, parasitoid wasp, brain, subesophageal ganglion, walking, octopamine.

Introduction

Unlike most venomous predators, some parasitoid wasps manufacture venoms that manipulate the host behavior to suit the developmental need of their offspring (Libersat and Gal, 2007). The parasitoid wasp *Ampulex compressa* hijacks the nervous system of its host, the cockroach *Periplaneta americana*, and thus controls its motivation to generate specific behaviors (Libersat, 2003). It was shown previously that the wasp injects venom directly into the head ganglia (Haspel et al., 2003). As a result, the cockroach first grooms for 30 min and then enters a hypokinetic state that lasts for 2–5 weeks (Libersat, 2003). Venom-induced hypokinesia is characterized by little, if any, spontaneous walking (Libersat, 2003). One of the neuronal signatures of this hypokinetic state is a change in the activity of neurons secreting octopamine (OA), a monoamine specific to invertebrates. In stung animals the spontaneous firing rate of thoracic octopaminergic neurons is dramatically decreased and the neurons respond more weakly to both sensory stimuli and direct injection of current (Rosenberg et al., 2006). It seems likely that the observed alteration in the activity of octopaminergic neurons is part of the mechanism by which the wasp induces a change in the behavioral state of its prey. In the present study, we explore the possibility that the wasp venom modifies OA levels, which eventually affects spontaneous walking in the cockroach.

OA in invertebrates, like noradrenaline in vertebrates, has been associated with the preparation for and execution of demanding motor behaviors (Evans, 1985; Roeder, 1999; Libersat and Pflüger, 2004; Pflüger and Stevenson, 2005). More specifically, OA is known to generate and enhance motor output patterns in the central nervous system. In *C. elegans* and in earthworms, it causes hyperactivity and stimulates locomotion (Mizutani et al., 2002; Komuniecki et al., 2004). OA applied directly to the thoracic ganglia in a variety of insect species initiates and sensitizes specific neuronal components (Sombati and Hoyle, 1984; Claassen and Kammer, 1986; Stevenson and Kutsch, 1987; Orchard et al., 1993; Weisel-Eichler and Libersat, 1996). OA-null mutant flies display reduced walking, once more suggesting a central role of OA in the initiation and/or modulation of locomotion (Saraswati et al., 2004). Moreover, the walking phenotype can be partially rescued by feeding the mutants with OA (Saraswati et al., 2004). In addition, insect adipokinetic hormone I (AKH-I), a peptide that regulates energy homeostasis, enhances the activity of specific OA neurons that bear AKH receptors and consequently stimulates walking in cockroaches (Wicher et al., 2006). Also in cockroaches, OA changes the gain of specific synapses for escape (Goldstein and Camhi, 1991; Casagrand and Ritzmann, 1992) and modulates the activity of OA neurons (Achenbach et al., 1997). Cockroach octopaminergic cells have been mapped in each ganglion of the nerve cord (Eckert et al., 1992; Sinakevitch et al., 2005).

Not only OA, but also dopamine (DA), has been shown to be involved in the regulation of walking in insects. For example, light induced activation of DA neurons in *Drosophila* elevates walking (Lima and Miesenbock, 2005). Mutations with reduced levels of dopamine, as well as pharmacological interventions

altering dopamine levels, are consistently associated with alterations in walking, although the direction of the effect is variable (Jordan et al., 2006).

In this work, we perform a series of pharmacological experiments involving an agonist and an antagonist of OA receptors to test their effects on the locomotor behavior of stung and control cockroaches. We show that experimental elevation of OA, but not of DA levels, restores walking in stung cockroaches. Moreover, we show that walking can be restored by direct injection of OA into the brain, but not into the subesophageal ganglion (SEG), of stung cockroaches.

Materials and methods

Animals

All experiments were performed on adult male cockroaches *Periplaneta americana* L., 1–2 weeks after adult molt. Cockroaches were raised in crowded conditions in plastic boxes (50 cm×50 cm×70 cm). Experimental animals were collected individually from the colony after the final molt. Individuals were kept at 26°C on a 12 h:12 h L:D cycle and provided with water and food (cat chow) *ad libitum*. Wasps, *Ampulex compressa* Fabricius (Hymenoptera: Sphecidae), were reared in plastic Perspex^{TM} boxes (40 cm×50 cm×50 cm) at 30°C and 60% humidity on a 12 h:12 h L:D cycle and provided with water and honey.

Behavioral testing

A few hours prior to pharmacological experiments, cockroaches were presented to a wasp for venom injection. Only stung cockroaches that showed the characteristic venom-induced hypokinesia were used for experiments. As described elsewhere (Fouad et al., 1994), hypokinetic cockroaches usually show no spontaneous walking and respond to a touch or wind stimulus with a short startle or a step forward followed by a step backwards. Control cockroaches were screened to ensure normal escape behavior. To test locomotor activity, cockroaches were placed into a round observation arena (diameter 80 cm) and given at least 5 min to adapt to the new environment. Spontaneous walking behavior was monitored by measuring the amount of time spent by the cockroaches walking during 10 min before and after injection.

Statistical analyses of data were performed using Sigmastat© (one-way ANOVA with the Bonferroni *t*-test for multiple comparisons and *t*-test or paired *t*-test; Jandel Scientific, Corte Madre, CA, USA). When data did not pass tests of normality and heterogeneity of variance a parallel non-parametric test was performed.

Drugs

Where not mentioned otherwise, drugs were purchased from Sigma-Aldrich (Rehovot, Israel). Drugs were administered either *via* the hemolymph or directly into a specific head ganglion. Hemolymph injections were performed using a Hamilton microsyringe. 10 μl of solution was injected between the third and fourth sternite into the abdominal hemocoel. Epinastine-HCl (50 mmol l^{-1}; Haorui Pharma-Chem Inc., Edison, NJ, USA), cis(z)flupenthixol dihydrochloride (100 mmol l^{-1}), quinpirole (5 mmol l^{-1}), SKF 82958 (6-chloro-1-phenyl-3-prop-2-enyl-1,2,4,5-tetrahydro-3-benzazepine-7,8-diol; 9 mmol l^{-1}) and chlordimeform-HCl (CDM; 50 mmol l^{-1}) were dissolved directly in cockroach saline [in mmol l^{-1}: NaCl 214, KCl 3.1, $CaCl_2$ 9.0, TES-buffer 10; pH 7.2 (Hancox and Pitman, 1995)].

For injection into the head ganglia, CDM and epinastine were used at a concentration of 2 mmol l^{-1} in cockroach saline, together with an inert tracer (Janus Green B, Sigma). To inject into the SEG, cockroaches were immobilized using modeling clay ventral side up. A slit was made in the ventral cuticle between the neck and the head and 14 nl of solution was injected into the ventral medial area of the SEG using a nano-volumetric injector (Medical Systems, NY, USA). To inject into the brain, cockroaches were immobilized dorsal side up. To prevent hemolymph loss after the cut, the neck was clamped down tightly using a minuten pin bent to form a metal bracket. A window was opened in the cuticle between the ocelli and 54 nl of solution was injected into the medial area of the brain. The injection volume for the SEG and brain was chosen according to the respective ganglion size. After the behavioral test, to verify the success and exact position of the injection site, we exposed the brain and SEG and visualized the localization of injections.

A stock solution of AKH-I (locust; Glp-Leu-Asn-Phe-Thr-Pro-Ans-Trp-Gly-Thr-NH_2, American Peptide Company, Inc., Sunnyvale, CA, USA) was prepared in distilled water. The final concentration was 2 μmol l^{-1} (Wicher et al., 2006).

Results

Effect of systemic injection of an OA receptor agonist on spontaneous walking

This set of experiments investigated the effect of hemolymph injection of the OA receptor agonist chlordimeform (CDM) on spontaneous walking. CDM is a tissue-permeable molecule (Roeder, 1995; Roeder, 1999) with low affinity for other amine receptors, and has been used as an octopamine agonist in many studies (Stevenson et al., 2005). Cockroaches were divided into three treatment groups: (1) controls injected with saline (control/saline), (2) stung cockroaches injected with saline (stung/saline) and (3) stung individuals injected with CDM (stung/CDM). Spontaneous walking differed among the three treatment groups (Fig. 1; $P<0.001$; one-way ANOVA). Pairwise comparisons of saline-injected individuals showed that stung cockroaches walked significantly less than controls [stung/saline, 0±0% (mean% ± s.d.), $N=7$; control/saline, 16.31±9.12%, $N=6$; $P<0.05$; Bonferroni *t*-test]. Injection of CDM significantly increased spontaneous walking in stung cockroaches compared to both saline-injected stung and control cockroaches (stung/CDM, 33.74±15.07%, $N=7$; $P<0.001$ and $P<0.05$, respectively; Bonferroni *t*-test). The effect of CDM on spontaneous walking of stung cockroaches followed a clear time course (Fig. 2). Maximal effect was observed 2 h after injection, while after 4.5 and 24 h spontaneous walking did not differ from pre-injection values (Fig. 2; stung/CDM: 2 h, 33.74±15.07%; 24 h, 4.05±6.52%; $P<0.001$ and $P=1$, respectively; one-way ANOVA, Bonferroni *t*-test).

Effect of systemic injection of an OA receptor antagonist on spontaneous walking

As a 'reverse control' to the previous experiments, we then tested the effect of the OA receptor antagonist epinastine on locomotion in control animals. Hemolymph injection of the OA

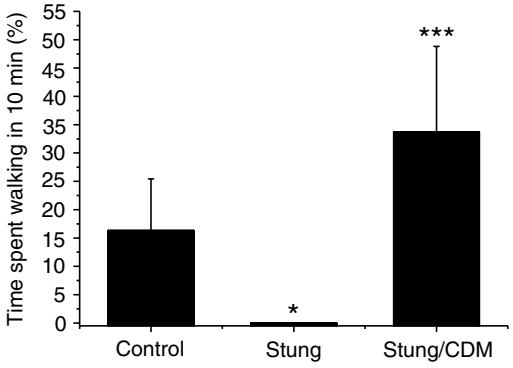

Fig. 1. Hemolymph-injection of an octopamine receptor agonist (CDM) significantly increases spontaneous walking over a 10 min period in stung cockroaches compared to both saline-injected stung and control cockroaches (***P<0.001 and *P<0.05, respectively). Pairwise comparisons of saline-injected individuals shows that stung cockroaches walk significantly less than control cockroaches (*P<0.05).

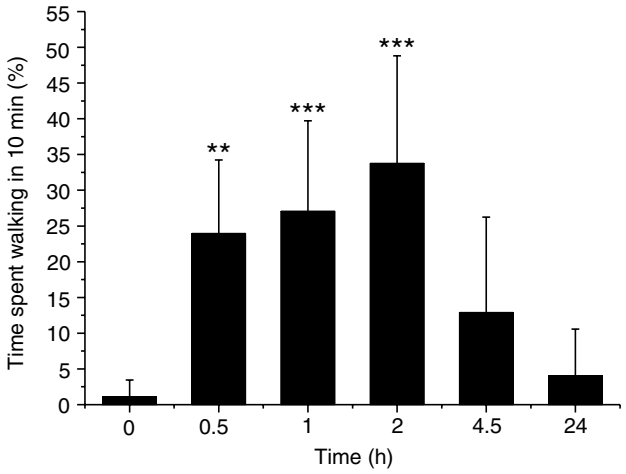

Fig. 2. Time course of the effect of chlordimeform (CDM) in stung cockroaches. The effect of the hemolymph injection of CDM is maximal 2 h after injection (***P<0.001, **P<0.01) and returns to baseline after 4.5 h. Significance levels are calculated with respect to time zero.

receptor antagonist epinastine led to a significant reduction in spontaneous walking (Fig. 3; control/epinastine – before treatment, 18.0±6.9%; after treatment, 9.1±7.1%; P<0.05; paired t-test). In contrast, the DA receptor antagonist flupenthixol injected into the hemolymph of control cockroaches had no significant effect on spontaneous walking (control/flupenthixol – before treatment, 27.38±8.94%; after treatment, 21.62±11.33%; P=0.19; paired t-test).

Effect of systemic injection of AKH-I on spontaneous walking

We demonstrated that an OA receptor agonist injected into the hemolymph of stung cockroaches induces walking to a level comparable to controls. AKH-I modulates the activity of octopaminergic neurons and is known to stimulate locomotor activity in cockroaches (Baumann and Penzlin, 1984; Wicher et

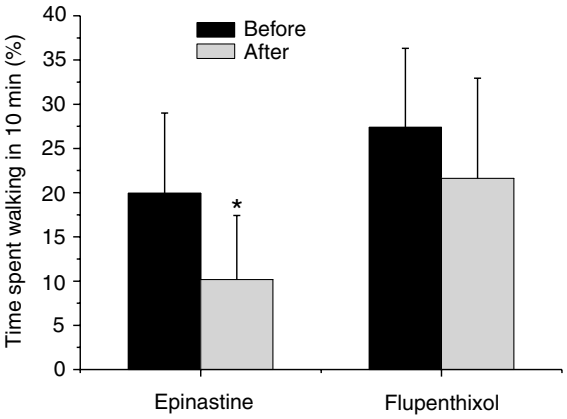

Fig. 3. The octopamine antagonist epinastine significantly reduces the level of spontaneous walking of control individuals (*P<0.05). In contrast, the dopamine antagonist flupenthixol has no significant effect on spontaneous walking (P=0.19).

al., 2006). Here we investigated whether or not the presumed reduced levels of OA in stung animals are related to a reduction in AKH-I levels. AKH-I (2 μmol l^{-1}) injected into the hemolymph had a pronounced effect on spontaneous walking of controls (Fig. 4; control/AKH-I – before treatment, 10.7±9.9%; after treatment, 29.0±12.1%, N=11; P<0.001; paired t-test, Wilcoxon signed rank test). In contrast, no significant effect on spontaneous walking was observed in stung cockroaches (stung/AKH-I – before and after: 0±0%, N=10). Increasing the concentration did not initiate walking in stung individuals (data not shown). Stung cockroaches injected with CDM and control cockroaches injected with AKH-I did not differ in the percentage time spent walking (stung/CDM, 33.74±15.07%; control/AKH-I, 29.0±12.1%; t-test; P=0.22).

To test whether AKH-I elevates spontaneous walking in controls *via* the octopaminergic system, we injected control individuals with a mixture of AKH-I and the OA receptor antagonist epinastine. Epinastine blocked the effect of AKH-I on spontaneous walking (Fig. 5; control/AKH-I, 29.0%±12.1, N=11; control/epinastine/AKH-I, 8.9%±11.1, N=7; P<0.01; one-way ANOVA, Bonferroni t-test). Epinastine (no AKH-I)-injected controls walked significantly less than AKH-I injected controls (control/epinastine, 9.1±7.0%, N=10; P<0.001; Bonferroni t-test). The two experimental groups, epinastine with AKH-I and epinastine alone, were not significantly different from each other (P=1; Bonferroni t-test). When comparing spontaneous walking before and after treatment, epinastine significantly reduced the percentage time that control cockroaches spent walking (control/epinastine – before treatment, 18.0%±6.9; after treatment, 9.1%±7.0, N=10; P<0.05; paired t-test). In contrast, epinastine injected together with AKH-I did not affect the percentage time controls spent walking (control/epinastine/AKH-I – before, 15.0±11.4%; after, 8.9±11.1%, N=7; P=0.36; paired t-test).

Effect of systemic injection of DA receptor agonists on spontaneous walking

There is considerable evidence that DA is also a potent modulator of walking in insects. Thus, we tested the effect of

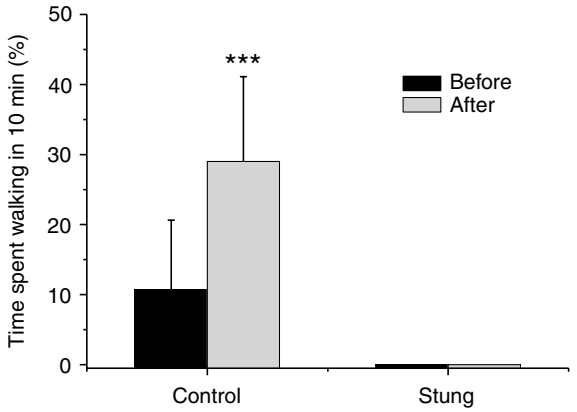

Fig. 4. Adipokinetic hormone-I (AKH-I) injected into the hemolymph enhances the duration of spontaneous walking in control cockroaches (***P<0.001). However, AKH-I has no effect on the walking behavior of stung cockroaches.

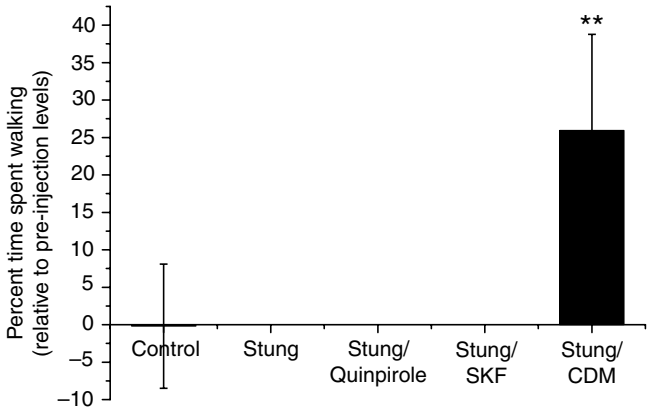

Fig. 6. Dopamine agonists do not increase spontaneous walking in stung animals. Stung cockroaches injected with chlordimeform (CDM) walk significantly more (**P<0.01) than stung cockroaches that were injected with either saline, quinpirole or with SKF.

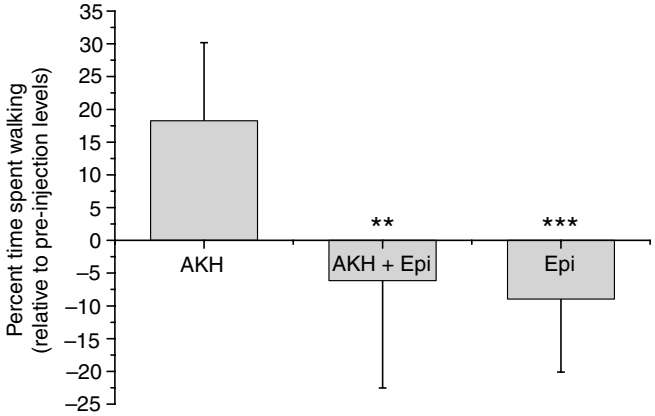

Fig. 5. AKH-I stimulates locomotion *via* the octopaminergic system. Control cockroaches injected with AKH-I walk significantly more than control cockroaches injected with epinastine (Epi) alone (***P<0.001) or with AKH-I and epinastine simultaneously (**P<0.01). Cockroaches injected with both drugs simultaneously walk as much as cockroaches injected with epinastine alone (P=1).

DA receptor agonists on spontaneous walking of stung cockroaches. Unlike CDM, neither the D2 receptor agonist quinpirole nor the D1 receptor agonist SKF 82958 affected spontaneous walking of stung cockroaches (Fig. 6; P=1.0; Kruskal–Wallis one-way ANOVA). Cockroaches injected with quinpirole (N=6) or SKF (N=7) remained motionless for the entire 2 h of observation (stung/DA agonist, 0±0%). These results clearly indicate that the venom acts on walking *via* the octopaminergic and not *via* the dopaminergic system in the cockroach.

Effect of direct injection of OA into the head ganglia

In the cockroach central nervous system octopaminergic neurons provide dense innervation to the central complex of the brain and to specific regions of the SEG (Sinakevitch et al., 2005). These regions of octopaminergic innervation coincide well with the areas where the wasp injects its venom (Haspel et al., 2003). With this in mind, we injected the OA receptor agonist CDM directly into the head ganglia of stung and control cockroaches with behavioral observations following after 5 min. Stung cockroaches injected with CDM into the medial protocerebrum of the brain walked significantly longer than stung cockroaches injected with saline in the same area (Fig. 7A; stung/CDM/brain, 36.73±25.68%, N=10; stung/saline/brain, 0±0%, N=10; P<0.001; Kruskal–Wallis one-way ANOVA). Control cockroaches injected with CDM into the brain did not walk significantly more than those injected with saline (Fig. 7A; control/saline/brain, 22.92±17.71%, N=14; control/CDM/brain, 53.31±35.84%, N=14; P>0.05; Dunn's *post hoc* test). This was almost certainly due to the variability in walking duration of CDM injected controls, since walking behavior was significantly elevated when comparing walking before and after the injection (control/CDM/brain – before treatment, 11.83±11.85%, N=14; after treatment: 53.31±35.84%; P<0.001; paired t-test). Walking was not significantly different when comparing before and after saline injection in the brain. Stung cockroaches injected with CDM into the SEG did not differ from stung cockroaches injected with saline (Fig. 7B; stung/CDM/SEG, 0±0%, N=14; stung/saline/SEG, 0±0%, N=10; P>0.05; Dunn's *post hoc* test). Furthermore, unlike control cockroaches injected with CDM into the brain, injection of CDM into the SEG did not increase walking in control cockroaches (Fig. 7B; control/saline/SEG, 10.41±8.12%, N=12; control/CDM/SEG – before treatment, 13.99±5.29%, N=13; after treatment, 10.22±7.67%; P=0.18; paired t-test). In good agreement with the CDM results in stung individuals, the OA receptor antagonist epinastine injected into the brain of control individuals reduced spontaneous walking [control/epinastine/brain – before treatment, 16.20±9.29%, N=10; after treatment (Fig. 7A), 0.47±1.49%; P<0.001; paired t-test].

Discussion

In the present study, we show that an OA receptor agonist can effectively restore spontaneous walking in stung cockroaches. The time course of the effect of chlordimeform

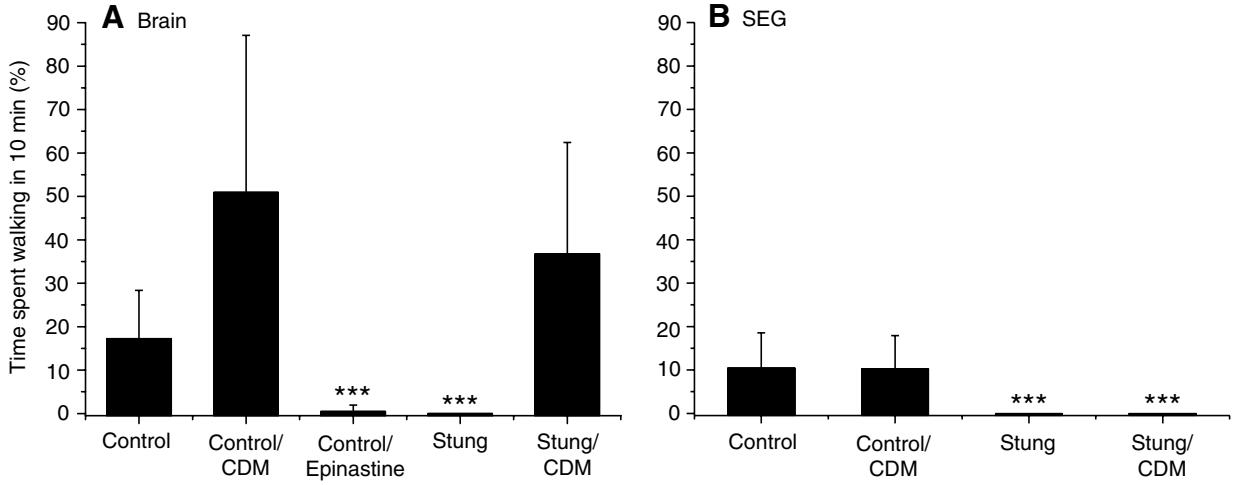

Fig. 7. An octopamine agonist chlordimeform (CDM) injected into the brain but not into the SEG stimulates walking in stung cockroaches. (A) Brain injection. Stung/CDM individuals walk significantly longer (***P<0.001) than stung/saline or control/epinastine cockroaches. There is no significant difference in walking behavior between stung/saline and control/epinastine cockroaches. Furthermore, no significant difference is found between control animals injected with saline or CDM or stung cockroaches injected with CDM. (B) SEG injection. CDM injected into stung cockroaches has no effect on walking behavior. Stung cockroaches injected with CDM or with saline walk significantly less than control cockroaches injected with saline or CDM (***P<0.001). Injecting CDM into control cockroaches has no significant effect on walking behavior when compared to control cockroaches injected with saline.

(CDM) on cockroach locomotion agrees well with the effective time reported for CDM on cricket aggressive behavior (Stevenson et al., 2005). In control cockroaches, the OA receptor antagonist epinastine significantly reduced the amount of spontaneous walking. It was shown previously that in headless cockroaches and decapitated flies injection of OA increases coordinated walking (Yellman et al., 1997; Ridgel and Ritzmann, 2005). In crickets, hemolymph injection of CDM induces a state of hyperactivity (Stevenson et al., 2005). In locusts, OA injected into the metathoracic ganglion produces walking movements (Sombati and Hoyle, 1984). Moreover, flies with a deficit in neural OA show reduced walking activity (Saraswati et al., 2004; Roeder, 2005; Fox et al., 2006). Therefore, together with the present results there is strong evidence that OA initiates walking behavior by activating command-like circuits in the central brain. Furthermore, it was shown that OA evokes walking movements in insects when applied onto the thoracic nerve cord. It therefore appears likely that OA acts on several levels within the distributed neuronal network for walking and thus acts as a true neuromodulator as proposed by Libersat and Pflüger (Libersat and Pflüger, 2004). The increase in walking duration observed in OA-injected stung cockroaches suggests that OA might be either depleted or not secreted after a sting. In support of this hypothesis, we showed previously that the activity level of octopaminergic neurons in the thoracic ganglia is strongly depressed in stung cockroaches (Rosenberg et al., 2006). The question as to whether or not this also occurs in octopaminergic neurons in the head ganglia remains to be investigated by direct intracellular recording.

It was demonstrated that *Drosophila* mutants with an impaired central complex show locomotor deficits (Strauss and Heisenberg, 1993). In the present study, we show that in stung cockroaches focal injection of a potent OA receptor agonist around the central complex area restores walking. Conversely, in controls, focal injection of a selective OA receptor antagonist into the same area reduces walking. However, it appears that the relevant neurons that modulate walking reside in the SEG and send axons to innervate the motor centers, such as the central complex. In locusts and cockroaches a specific group of octopaminergic neurons in the SEG sends (i) ascending axons towards the brain, innervating all its major neuropiles (Bräunig, 1991; Sinakevitch et al., 2005), or (ii) descending axons that form extensive ramifications in the neuropiles of the thoracic and abdominal ganglia (Bräunig and Burrows, 2004; Sinakevitch et al., 2005). Within the group of octopaminergic ascending SEG neurons, at least three provide dense innervation in the protocerebral bridge and ellipsoid body of the central complex (Sinakevitch et al., 2005), a region implicated in control of walking (Strausfeld, 1999; Strauss, 2002) and the location of venom injection (Haspel et al., 2003). The wasp injects venom into both the SEG and into the brain (Haspel et al., 2003). Thus, the SEG sting might be affecting the activity of SEG OA ascending neurons to cause reduced OA levels in the walking centers of the brain. Alternatively, reduced OA levels in these brain areas might be the consequence of the sting directly into the central brain. This issue should be resolved by direct measurements of OA levels in central areas of the brains of stung and control cockroaches. Our study provides additional evidence for the role of OA in the control of walking mediated *via* the central complex.

We demonstrated previously that removing the brain in cockroaches significantly enhances walking behavior, suggesting that the brain has an overall inhibitory effect on walking (Libersat et al., 1999; Gal and Libersat, 2006). However, the present data show that injection of OA towards or into the central complex initiates and maintains walking. Thus, we can think of two possible alternative explanations to account for this. The first is that the brain may have both,

inhibitory and excitatory components that are responsible for initiation and maintenance of walking – with the inhibitory component, however, being more dominant. The second is that OA may drive neurons that in turn release the inhibition that the brain normally exerts onto the thoracic locomotory circuits for walking maintenance.

DA receptor agonists had no measurable effects on spontaneous walking of stung cockroaches. In flies DA induces grooming and walking behavior (Yellman et al., 1997). The rate-limiting enzyme required for catecholamine biosynthesis is tyrosine hydroxylase. Pharmacological inhibition of this enzyme with α-methyl-*P*-tyrosine results in a dose-related inhibition of walking activity in adult flies (Pendleton et al., 2002). Similar results were found with reserpine, an inhibitor of catecholamine uptake into vesicles, in both flies (Pendleton et al., 2002) and cockroaches (Weisel-Eichler and Libersat, 2002). Conversely, light induced activation of DA neurons in *Drosophila* elevates locomotor activity (Lima and Miesenbock, 2005). However, the effect of DA appears to be receptor specific. SKF 82958, a D1 DA receptor agonist, had no effect on walking, whereas quinpirole, a D2 DA receptor agonist, enhanced locomotion (Yellman et al., 1997). In the present study quinpirole did not restore walking in stung cockroaches, which could have one of several possible explanations. It might be that DA receptor agonists are not permeable to the neuroepithelium. This seems unlikely though, since we found that even direct injection of DA receptor agonists into the brain or SEG of stung cockroaches does not elevate walking, although it enhances grooming (F.L., unpublished observations) (Weisel-Eichler et al., 1999). It thus seems that these DA receptor agonists are ineffective in activating walking in stung cockroaches, possibly because DA receptors are impaired in stung individuals (Weisel-Eichler and Libersat, 2002). Weisel-Eichler and Libersat showed that injecting a DA receptor agonist into a stung cockroach failed to elicit a grooming response. It is also possible, however, that cockroach walking is simply not affected or regulated by DA receptor agonists. Importantly, we show that DA receptor agonists do not restore walking in stung cockroaches, indicating that the venom does not affect walking *via* interfering with the dopaminergic system.

OA receptor agonists stimulate and maintain walking behavior in stung cockroaches. A similar effect was observed in control individuals injected with AKH-I and, moreover, the effect of AKH-I on walking was blocked by an OA receptor antagonist. This suggests that AKH-I activates OA neurons to increase walking. AKH also stimulates walking in *Drosophila* and is thought to be the starvation signal bringing about food foraging (Lee and Park, 2004). We expected that AKH-I would stimulate walking in stung cockroaches by elevating OA levels. The lack of effect suggests that AKH-I is not sufficient to activate OA neurons in stung cockroaches to a level that can trigger walking. The fact that OA itself does activate walking in stung individuals indicates that the walking central pattern generator is not impaired; however, it might have an elevated threshold for activation. There are additional modulators that could neutralize the effect of AKH-I on OA neurons in stung cockroaches, and these include γ-aminobutyric acid (GABA), as well taurine and β alanine. GABA has been found to be present in the venom in large quantities (Moore et al., 2006) and is known to have an inhibitory effect on OA neurons (Washio, 1994). AKH is known to affect the activity of octopaminergic neurons *via* calcium channels, whereas GABA inhibits the activity of octopaminergic neurons *via* activating chloride channels (Washio, 1994; Wicher, 2001). This GABAergic inhibitory pathway could be the cause of reduced activity in octopaminergic neurons found in stung cockroaches and, consequently, the inability of AKH to activate walking in stung individuals.

To conclude, we have shown in the present study that changes in levels of OA are likely to account for the modulation of spontaneous walking of cockroaches stung by the wasp *A. compressa*. To our knowledge, this is the first direct evidence that OA agonist injected into the insect brain activates walking. The present results imply that OA has a more prevalent role than DA in the regulation of spontaneous walking in the cockroach. We further show that AKH-I evokes walking *via* the octopaminergic system. However, AKH-I is most probably not the main modulatory substance affecting the activity of octopaminergic neurons in stung cockroaches. We propose that venom injection into the head ganglia selectively depresses the initiation and maintenance of walking by modifying the release of OA as a neuromodulator in restricted regions of the cockroach brain. This seems likely considering that specific octopaminergic neurons provide extensive innervation of neuropiles that are targeted by the wasp venom injection, and are involved in the control of initiation of walking.

List of abbreviations

AKH-I	adipokinetic hormone I
CDM	chlordimeform-HCl
DA	dopamine
GABA	γ-aminobutyric acid
OA	octopamine
SEG	subesophageal ganglion
SKF	dopamine D1 receptor agonist

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References

Achenbach, H., Walther, C. and Wicher, D. (1997). Octopamine modulates ionic currents and spiking in dorsal unpaired median (DUM) neurons. *Neuroreport* **8**, 3737-3741.

Baumann, E. and Penzlin, H. (1984). Sequence-analysis of neurohormone-D, a neuropeptide of an insect, *Periplaneta americana*. *Biomed. Biochim. Acta* **43**, K13-K16.

Bräunig, P. (1991). Suboesophageal DUM neurons innervate the principal neuropiles of the locust brain. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **332**, 221-240.

Bräunig, P. and Burrows, M. (2004). Projection patterns of posterior dorsal unpaired median neurons of the locust subesophageal ganglion. *J. Comp. Neurol.* **478**, 164-175.

Casagrand, J. L. and Ritzmann, R. E. (1992). Biogenic amines modulate synaptic transmission between identified giant interneurons and thoracic interneurons in the escape system of the cockroach. *J. Neurobiol.* **23**, 644-655.

Claassen, D. E. and Kammer, A. E. (1986). Effects of octopamine, dopamine, and serotonin on production of flight motor output by thoracic ganglia of *Manduca sexta*. *J. Neurobiol.* **17**, 1-14.

Eckert, M., Rapus, J. and Nurnberger, A. (1992). A new specific antibody reveals octopamine-like immunoreactivity in cockroach ventral nerve cord. *J. Comp. Neurol.* **322**, 1-15.

Evans, P. D. (1985). Octopamine. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Vol. 11 (ed. G. A. Kerkut and L. I. Gilbert), pp. 499-530. Oxford: Pergamon Press.

Fouad, K., Libersat, F. and Rathmayer, W. (1994). The venom of the cockroach-hunting wasp *Periplaneta americana* changes motor thresholds: a novel tool for studying the neural control of arousal? *Zoology* **98**, 23-34.

Fox, L. E., Soll, D. R. and Wu, C. F. (2006). Coordination and modulation of locomotion pattern generators in *Drosophila* larvae: effects of altered biogenic amine levels by the tyramine beta hydroxylase mutation. *J. Neurosci.* **26**, 1486-1498.

Gal, R. and Libersat, F. (2006). New vistas on the initiation and maintenance of insect motor behaviors revealed by specific lesions of the head ganglia. *J. Comp. Physiol. A* **192**, 1003-1020.

Goldstein, R. S. and Camhi, J. M. (1991). Different effects of the biogenic amines dopamine, serotonin and octopamine on the thoracic and abdominal portions of the escape circuit in the cockroach. *J. Comp. Physiol. A* **168**, 103-112.

Hancox, J. C. and Pitman, R. M. (1995). Spontaneous bursting induced by convulsant agents in an identified insect neurone. *Gen. Pharmacol.* **26**, 195-204.

Haspel, G., Rosenberg, L. A. and Libersat, F. (2003). Direct injection of venom by a predatory wasp into cockroach brain. *J. Neurobiol.* **56**, 287-292.

Jordan. K. W., Morgan, T. J. and Mackay, T. F. (2006). Quantitative trait loci for locomotor behavior in *Drosophila melanogaster*. *Genetics* **174**, 271-284.

Komuniecki, R. W., Hobson, R. J., Rex, E. B., Hapiak. V. M. and Komuniecki, P. R. (2004). Biogenic amine receptors in parasitic nematodes: what can be learned from *Caenorhabditis elegans*? *Mol. Biochem. Parasitol.* **137**, 1-11.

Lee, G. H. and Park, J. H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics* **167**, 311-323.

Libersat, F. (2003). Wasp uses venom cocktail to manipulate the behavior of its cockroach prey. *J. Comp. Physiol. A* **189**, 497-508.

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

Libersat, F. and Pflüger, H. J. (2004). Monoamines and the orchestration of behavior. *BioScience* **54**, 17-25.

Libersat, F., Haspel, G., Casagrand, J. and Fouad, K. (1999). Localization of the site of effect of a wasp's venom in the cockroach escape circutry. *J. Comp. Physiol. A* **184**, 333-345.

Lima, S. Q. and Miesenbock, G. (2005). Remote control of behavior through genetically targeted photostimulation of neurons. *Cell* **121**, 141-152.

Mizutani, K., Ogawa, H., Saito, J. and Oka, K. (2002). Fictive locomotion induced by octopamine in the earthworm. *J. Exp. Biol.* **205**, 265-271.

Moore, E. L., Haspel. G., Libersat, F. and Adams, M. E. (2006). Parasitoid wasp sting: a cocktail of GABA, taurine and β-alanine opens chloride channels for transient synaptic block and paralysis of a cockroach host. *J. Neurobiol.* **66**, 811-820.

Orchard, I., Ramirez, J. M. and Lange, A. B. (1993). A multifunctional role for octopamine in locust flight. *Annu. Rev. Entomol.* **38**, 227-249.

Pendleton, R. G., Rasheed, A., Sardina, T., Tully, T and Hillman, R. (2002). Effects of tyrosine hydroxylase mutants on locomotor activity in *Drosophila*: a study in functional genomics. *Behav. Genet.* **32**, 89-94.

Pflüger, H.-J. and Stevenson, P. A. (2005). Evolutionary aspects of octopaminergic systems with emphasis on arthropods. *Arthropod Struct. Dev.* **34**, 379-396.

Ridgel, A. L. and Ritzmann, R. E. (2005). Effects of neck and circumoesophageal connective lesions on posture and locomotion in the cockroach. *J. Comp. Physiol. A* **191**, 559-573.

Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* **59**, 1-31.

Roeder, T. (2005). Tyramine and octopamine: ruling behavior and metabolism. *Annu. Rev. Entomol.* **50**, 447-477.

Rosenberg, L. A., Pflüger, H. J., Wegener, G. and Libersat, F. (2006). Venom of wasp injected into the prey's brain modulates thoracic identified bioaminergic neurons. *J. Neurobiol.* **66**, 155-168.

Saraswati, S., Fox, L. E., Soll, D. R. and Wu, C. F. (2004). Tyramine and octopamine have opposite effects on the locomotion of *Drosophila larvae*. *J. Neurobiol.* **58**, 425-441.

Sinakevitch, I., Niwa, M. and Strausfeld, N. J. (2005). Octopamine-like immunoreactivity in the honey bee and cockroach: comparable organization in the brain and subesophageal ganglion. *J. Comp. Neurol.* **488**, 233-254.

Sombati, S. and Hoyle, G. (1984). Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. *J. Neurobiol.* **15**, 481-506.

Stevenson, P. A. and Kutsch, W. (1987). A reconsideration of the central pattern generator concept for locust flight. *J. Comp. Physiol. A* **161**, 115-129.

Stevenson, P. A., Dyakonova, V., Rillich, J. and Schildberger, K. (2005). Octopamine and experience-dependent modulation of aggression in crickets. *J. Neurosci.* **25**, 1431-1441.

Strausfeld, N. J. (1999). A brain region in insects that supervises walking. *Prog. Brain Res.* **123**, 273-284.

Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Curr. Opin. Neurobiol.* **12**, 633-638.

Strauss, R. and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. *J. Neurosci.* **13**, 1852-1861.

Washio, H. (1994). Effects of putative neurotransmitters on dorsal unpaired median neurons of cockroach (*Periplaneta americana*) thoracic ganglia. *J. Insect Physiol.* **40**, 841-847.

Weisel-Eichler, A. and Libersat, F. (1996). Neuromodulation of flight initiation by octopamine in the cockroach *Periplaneta americana*. *J. Comp. Physiol. A* **179**, 103-112.

Weisel-Eichler, A. and Libersat, F. (2002). Are monoaminergic systems involved in the lethargy induced by a parasitoid wasp in the cockroach prey? *J. Comp. Physiol. A* **188**, 315-324.

Weisel-Eichler, A., Haspel, G. and Libersat, F. (1999). Venom of a parasitoid wasp induces prolonged grooming in the cockroach. *J. Exp. Biol.* **202**, 957-964.

Wicher, D. (2001). Peptidergic modulation of insect voltage-gated Ca^{2+} currents: role of resting Ca^{2+} current and protein kinases A and C. *J. Neurophysiol.* **86**, 2353-2362.

Wicher, D., Agricola, H. J., Sohler, S., Gundel, M., Heinemann, S. H., Wollweber, L., Stengl, M. and Derst, C. (2006). Differential receptor activation by cockroach adipokinetic hormones produces differential effects on ion currents, neuronal activity, and locomotion. *J. Neurophysiol.* **95**, 2314-2325.

Yellman, C., Tao, H., He, B. and Hirsh, J. (1997). Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated *Drosophila*. *Proc. Natl. Acad. Sci. USA* **94**, 4131-4136.