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The role of the cerebral ganglia in the venom-induced behavioral manipulation of cockroaches stung by the parasitoid Jewel Wasp

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Running title: head ganglia and walking initiation

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Abstract

The Jewel Wasp stings cockroaches and injects venom into their cerebral ganglia, namely, the subeosophageal ganglion (SOG) and supraeosophageal ganglion (SupOG). The venom induces a long-term hypokinetic state, during which the stung cockroach shows little or no spontaneous walking.

It was shown that venom injection to the SOG reduces neuronal activity thereby suggesting a similar effect of venom injection in the SupOG. Paradoxically, SupOG-ablated cockroaches show increased spontaneous walking in comparison to control. Yet, most of the venom in the SupOG of cockroaches is primarily concentrated in and around the central complex (CX). Thus, the venom could chiefly decrease activity in the CX to contribute to the hypokinetic state. Our first aim was to resolve this discrepancy by using a combination of behavioral and neuro-pharmacological tools. Our results show that the CX is necessary for the initiation of spontaneous walking and that focal injection of procaine to the CX is sufficient to induce the decrease in spontaneous walking. Furthermore, it was shown that artificial venom injection to the SOG decreases walking. Hence, our second aim was to test the interactions between the SupOG and SOG in the venom-induced behavioral manipulation. We show that, in the absence of the inhibitory control of the SupOG on walking initiation, injection of venom in the SOG alone by the wasp is sufficient to induce the hypokinetic state. To summarize, we show that venom injection to either the SOG or the CX of the SupOG is, by itself, sufficient to decrease walking.

Introduction

The parasitoid Jewel wasp (*Ampulex compressa*) hunts and stings cockroaches (*Periplaneta americana*) to use them as a live fresh food supply for its offspring. In contrast to other parasitoid wasps, the Jewel wasp venom does not paralyze the cockroach. Instead, the venom causes specific changes in the cockroach behavior (Fouad et al., 1994). After the cockroach is stung, it grooms continuously for 30 minutes and then it enters a long-term hypokinetic state, during which it does not voluntarily engage in spontaneous locomotion and escapes neither wind nor tactile stimuli. Such a behavioral manipulation allows the wasp to walk the cockroach to a burrow, where it affixes its egg. The larva that hatches from the egg feeds on the cockroach hemolymph at first and then enters the cockroach abdomen to devour the cockroach's internal organs (Haspel et al., 2005).

Behavioral and electrophysiological studies have demonstrated that the venom effect on locomotion is limited to walking and escape behaviors (Libersat, 2003). Other behaviors, such as flying or righting, remain unaffected and the walking pattern generator, which locally controls the spatiotemporal motor pattern of leg movements during walking, remains functional in stung cockroaches. More specifically, the wasp's sting affects the spontaneous initiation and the maintenance of walking (Libersat, 2003; Gal and Libersat, 2008)

It has been shown that the wasp injects its venom directly into the cockroach cerebral ganglia, namely, the subeosophageal ganglion (SOG) and the supraeosophageal ganglion (SupOG) (Haspel et al., 2003). The SOG and SupOG, which are connected to each other by the circumesophageal connectives (CirC), are considered as 'higher-order' neuronal centers which modulate different aspects of locomotion (Kien and Altman, 1992; Strauss and Heisenberg, 1993; Strausfeld, 1999; Schaefer and Ritzmann, 2001). Since the venom is injected directly into the cerebral ganglia, the behavioral change observed in stung cockroaches must be the result of a pharmacological manipulation of neuronal circuits in these ganglia. However, the contribution of venom injection into each of those ganglia to the observed behavioral manipulation is still unclear.

Based on experiments performed on the SOG, venom injected into the SupOG, almost certainly decreases neuronal activity there (Gal and Libersat, 2010). Paradoxically, SupOG-ablated cockroaches show increased spontaneous walking in comparison to control cockroaches (Kien, 1983; Ridgel and Ritzmann, 2005; Gal and Libersat, 2006) indicating that the SupOG has a general inhibitory role in the control of walking. While the entire SupOG

has a general inhibitory role, it is still possible that the wasp targets sub-regions in the SupOG.

Most of the venom injected by the wasp into the SupOG is predominantly concentrated in its central part of the brain (Haspel et al., 2003), especially in and around the central complex (CX; also know previously as CC (Ito et al., 2014)). Thus, the venom could affect primarily this region to contribute to the hypokinetic state, leaving other regions unaffected. In fact, the CX is a target of choice for the wasp's venom, as many studies suggest that the CX is important for the initiation and maintenance of walking (Huber, 1960; Martin et al., 1999; Strausfeld, 1999; Winther et al., 2006; Bender et al., 2010; Kahsai et al., 2010). In addition, studies carried out on the fruit fly suggest that the CX has a permissive role in the control of walking, whereas the mushroom bodies (MBs), a bilateral region in the SupOG, have a suppressive effect on walking (Martin et al., 1998). We suggest that the venom injected into the SupOG could affect mainly the CX to decrease spontaneous walking. To test this hypothesis and resolve the aforementioned paradox, we first performed a series of experiments in which we injected compounds into discrete regions of the SupOG and evaluated the resulting behavioral deficits.

The SOG, anatomically, is the second cerebral ganglion, located below and connected to the SupOG through the circumesophageal connectives (CirC). A previous study has shown that injection of procaine (a reversible voltage-dependent sodium and potassium channels blocker) into the honey bee brain (Devaud et al., 2007), or into the SOG of cockroaches (Gal and Libersat, 2010) reduces neuronal activity in these ganglia. In the latter, such an injection results in a decrease in spontaneous walking. Likewise, venom injections to the SOG also decrease neuronal activity and, consequently, decrease spontaneous locomotion (Gal and Libersat, 2010). Thus, this study and others show that the SOG exerts a net tonic permissive effect in the control of initiation and maintenance of walking (Kien, 1983; Johnston et al., 1999; Schaefer and Ritzmann, 2001). Hence, the second aim of the present study was to examine the contribution of each cerebral ganglion to the manipulation of walking. To this aim, we performed a series of experiments involving lesions and natural venom injection by wasps into the cerebral ganglia.

Our results show that the CX is predominantly permissive for the initiation of spontaneous walking and that its role in the control of spontaneous walking is antagonistic to that of the MBs. We show that a focal injection of venom or procaine to the CX is sufficient to induce

the decrease in spontaneous walking. However, we also show that when the wasp stings a cockroach with a disabled SupOG but a normally functioning SOG, the cockroach shows decreased spontaneous locomotion. Thus, venom in each of the two cerebral ganglia is sufficient to induce a decrease in spontaneous walking, as seen in stung hypokinetic cockroaches.

Results

A procaine injection to the CX decreases spontaneous walking

First, to determine the role that the CX plays in the control of spontaneous walking, we focally injected procaine to the CX to reversibly decrease neuronal activity specifically in this region (Devaud et al., 2007; Gal and Libersat, 2010; Kathman et al., 2014). Compared to the baseline walking duration, cockroaches that were injected with procaine to the CX walked significantly less, if at all, for 30 minutes after the injections ($p<0.05$; Fig. 1). After this time period, the walking duration returned to baseline, as expected given the reversible nature of procaine. In addition, as compared to Control cockroaches, the walking duration was significantly lower in the 'Procaine-CX' cockroaches, especially 20 and 30 min after injections ($p=0.013$ and $p=0.009$, respectively; Fig. 1). Postmortem verification of the injection site confirmed that the injections were focused in the central body of the CX (Fig. 2). These results show that procaine injections to the CX have an inhibitory effect on walking.

A procaine injection to the MBs increases spontaneous walking

We then tested the effect of a bilateral procaine injection into the MBs, which are also known to be involved in walking. Compared to baseline walking duration, cockroaches that were injected with procaine to the MBs walked significantly more for 30 minutes after the injections ($p<0.005$; Fig. 3). Likewise, compared to Control cockroaches, the walking duration was significantly higher in the 'Procaine-MBs' cockroaches for 30 min after the injection ($p<0.001$; Fig. 3). As expected, the walking duration returned to baseline 30 min following the injections. Postmortem verification of the injection site confirmed the location of the injections to the MBs (Calyces region, Fig. 2). These results show that the procaine injections to MBs have an opposite effect to procaine injections to CX, and that these two regions in the SupOG appear to have antagonistic roles in the control of initiation of walking.

A venom injection to the CX decreases spontaneous walking

Most of the venom injected to the SupOG is concentrated in or around the CX. This means that the wasp could target specifically the CX to decrease spontaneous walking in the cockroach. If true, then venom injection to the CX should have a comparable effect to procaine injections to the CX, and should decrease spontaneous walking.

To test our hypothesis, we focally injected freshly milked venom to CX. Compared to the baseline walking duration, cockroaches injected with venom to the CX walked significantly less for the entire duration of the behavioral test ($p<0.05$; Fig. 4). Likewise, compared to Control cockroaches, the walking duration was lower in the 'Venom-CX' injected cockroaches throughout the entire duration of behavioral testing ($p<0.05$, 20-60 min; Fig. 4). Postmortem verification of the injection site confirmed the location of the injections to the central body in the CX (not shown). These results show that venom injection to the CX alone is sufficient to decrease spontaneous walking.

SupOG-ablated stung cockroaches show decreased spontaneous walking

To examine the contribution of each cerebral ganglion to the drastic effect of the sting on walking, we performed another series of experiments. Although it has been shown that venom injection to SOG is, in itself, sufficient to decrease spontaneous walking, it is not known whether a natural direct injection by the wasp to cockroaches with only SOG will be sufficient to decrease spontaneous walking. If this was the case, the effect of venom in the SOG will need to overcome the increased walking observed previously in SupOG ablated cockroaches (Gal and Libersat, 2006).

To investigate this possibility, we measured spontaneous walking in two groups: 'Crushed CirC - stung' cockroaches and 'Stung - crushed CirC' cockroaches. In the first group, the CirC were crushed before the cockroach was stung whereas in the second group, the CirC were crushed after the cockroach was stung. By crushing the CirC we removed descending inhibitory control of the SupOG on walking (Gal and Libersat 2006). We expected that, if venom in the SOG is sufficient to decrease spontaneous walking, then crushing the CirC before or after the sting would not change the effect of the sting and the cockroach will be hypokinetic in either one of the treatments.

As expected, for 'Crushed CirC - stung', there was a significant increase in walking duration after surgery (70min, $p<0.001$; Fig. 5) compared to baseline. Following the sting, the walking duration dramatically decreased (140 min, $p<0.001$; Fig. 5), similar to when a wasp stung a normal cockroach ('Stung - crushed CirC', 70 min, $p<0.001$ compare to baseline; Fig. 5).

Crushing the CirC after the sting ('Stung - crushed CirC' group) did not affect the walking duration of stung cockroaches as they remained hypokinetic (140 min; Fig. 5).
These results show that venom injection to the SOG alone is sufficient to induce a decrease in spontaneous walking and to overcome the increased walking of SupOG-ablated cockroaches.

Discussion

The parasitoid Jewel Wasp stings cockroaches and injects venom directly into their cerebral ganglia, namely, into the SOG and the SupOG (Haspel et al., 2003). While venom or procaine injection to the SOG alone decreases spontaneous walking, venom or procaine injection to the entire SupOG paradoxically increases spontaneous walking (Gal and Libersat, 2010). Yet, the wasp does not inject venom into the entire SupOG but, rather, mostly into a discrete region of the SupOG known as the CX (Haspel et al., 2003). Hence, the goal of the present study was to resolve this paradox by using behavioral, surgical and neuro-pharmacological methods.

We show here that procaine focally injected to the CX dramatically decreased spontaneous walking in cockroach; in fact, most cockroaches did not show any spontaneous walking (Fig. 1). This clearly indicates that the CX is permissive for the initiation of spontaneous walking. Our result is consistent with work carried out on *Drosophila melanogaster* that shows that flies with genetically manipulated neurons or structural mutations of the CX demonstrate shorter activity duration, lower walking speed, decreased levels of locomotory activity and changes in walking time intervals (Strauss et al., 1992; Strauss and Heisenberg, 1993; Martin et al., 1999; Strauss, 2002; Poeck et al., 2008; Kahsai et al., 2010; Pfeiffer and Homberg, 2014). It is also in good agreement with experiments showing that current injection in the CX can elicit walking in crickets (Huber, 1960) and cockroaches (Bender et al., 2010). While most CX units show an increase in their firing rate preceding the initiation of locomotion some show a decrease indicating that the CX is predominantly permissive on walking (Guo and Ritzmann, 2013). In a recently published study, injection of procaine into the CX of *Blaberus discoidalis* has been performed, to investigate its role on optomotor behavior (Kathman, et al. 2014). They found deficits in the optomotor behavior after procaine injection but no significant change in general activity (i.e. walking). We can only speculate as to what could account for such a difference. First, it is possible that size matters as *Blaberus* CX is much larger then *Periplaneta* CX. A more convincing argument would be a simple

concentration effect. In the present study, we used high concentration solution of procaine (50% versus 20% and 10% in Kathman's study) and a larger volume of diluted procaine (10 nl in the present study versus 2 nl in Kathman's study). Hence, we expect that in the *Blaberus* study, the effect is limited to a smaller portion of the CX whereas in the present study, the effect results from a more complete inhibition of the entire CX.

While the CX is at least necessary for spontaneous walking, the entire SupOG in general has a suppressive role in the control of walking (Gal and Libersat, 2006). The cockroach's head ganglia play a major role in initiating and maintaining the walking pattern generated in the thoracic ganglia (Kien and Altman, 1992; Gal and Libersat, 2006). We have shown previously that the SupOG inhibits walking, whereas the SOG exerts the opposite effect (Gal and Libersat, 2006, 2010). This suggests the existence of other regions within the SupOG that exert inhibitory control on walking initiation. As already suggested by studies on *Drosophila* (Martin et al., 1998), we hypothesized that this observed suppressive effect of SupOG removal is a result of the inhibitory influence of the MBs on walking. Our results showed that the procaine injections to the MBs have the opposite effect on walking to that of injections to the CX. A procaine injection to the MBs reversibly increases spontaneous walking. Our results are in agreement with other studies that suggest that these two major 'higher' brain centers of the SupOG have an antagonistic role in the control of spontaneous walking (Huber, 1960; Strauss and Heisenberg, 1993; Martin et al., 1998; Helfrich-Forster et al., 2002; Svidersky and Plotnikova, 2002).

As it was shown before that the venom decreases neuronal activity similarly to procaine (Gal and Libersat, 2010), we focally injected venom to the CX to mimic the wasp's venom injection in this discrete region. We show that an accurate venom injection to CX alone is sufficient to decrease spontaneous walking. Thus, the Jewel Wasp's venom injection to SupOG must be fairly accurate in order to impact only the CX, without affecting the MBs, to achieve the desired behavioral manipulation.

Finally, in the present study, we also addressed the role of the venom in each ganglion in the venom-induced hypokinesia. Since it not possible to coerce the wasp to sting in the SOG alone and prevent it from stinging in the SupOG, we used the following approaches. We surgically crushed the CirC of normal cockroaches or of cockroaches that were already stung by a wasp, thereby removing the inhibitory input from the SupOG. Those 'Crushes CirC' cockroaches tend to be more active due to the removal of inhibition. When these cockroaches

were stung by the wasp, their spontaneous walking significantly decreased comparably to the effect of the sting in both ganglia of normal cockroaches. Crushing the CirC after the sting did not change the spontaneous walking of the cockroaches as they remained hypokinetic. These experiments show that the venom injection into the SOG is sufficient to decrease spontaneous walking, and that the venom's effect in the SOG is sufficient to 'override' the effect of the ablation of the SupOG. Thus, the Jewel Wasp injects venom to both cerebral ganglia, while venom injection to either one would be sufficient to cause a decrease in spontaneous walking in the cockroach. It is only possible to speculate about the adaptive significance of such a double venom injection where one alone would be sufficient to induce hypokinesia. It is possible that the venom injection to both ganglia is a kind of 'insurance policy', in which the wasp maximizes the odds that the cockroach will turn and remains hypokinetic. Indeed, if the wasp were to fail to sting properly in the SupOG, it would still achieve its goal. Similar 'insurance' mechanisms can be found in other venomous organism, such as the cone snail that produces different types of conotoxins, each of which should, in principle, be sufficient for blocking the neuro-muscular junction ensuring paralysis of the prey (Olivera and Cruz, 2001). Another, yet not exclusive, alternative is that the injection in the central portion of the SupOG is involved in other aspects of the manipulation. For instance, an important aspect of the manipulation is the modification of the cockroach's metabolism (Haspel et al., 2005). Venom in the SupOG could slow down the metabolism in the stung cockroach by affecting, directly or indirectly, neuro-peptidergic modulation in the CX (Zornik et al., 1999; Nassel, 2002). Another possibility is that the venom in the SupOG is involved in the stereotypic grooming behavior of stung cockroaches. Indeed, injection of dopamine, which is present in the venom (Weisel-Eichler et al., 1999), of dopamine agonists (Libersat and Gal, 2014), or of venom (data not shown) in the central portion of the SupOG induces intense grooming. One caveat to this possibility is that the benefit of the grooming behavior in this parasitic manipulation remains unclear (Weisel-Eichler et al., 1999).

To summarize, in the present study, we were able to demonstrate the role of different regions in the SupOG in the control of spontaneous walking by using the wasp–cockroach association as a model. We have shown that the venom injection to each of the cerebral ganglia is, by itself, sufficient to decrease spontaneous walking, as observed in stung cockroaches. This presumably insures the wasp with an effective and lasting hypokinetic state of its offspring's host. Our work also provides further insights into the interplay between the SupOG and the

SOG of insects and their role in the processes of motivation and decision to engage in a behavioral act.

Material and Methods

Animals

Ampulex compressa Fabricius (*Hymenoptera: Ampulicidae*) wasps and *Periplaneta americana* cockroaches were reared in crowded colonies under laboratory conditions of 40–60% humidity, 30°C and a 12L:12D cycle. All animals were supplied with water and food (cat chow for cockroaches and honey for wasps) ad libitum.

To obtain stung cockroaches, a single cockroach was introduced to a wasp and the stinging duration was measured to ensure normal stinging behavior (Fouad et al., 1994).

Venom milking

Wasps were immobilized by chilling on ice for 5 min and were confined in a small, conical, plastic tube open at both ends. A modified syringe plunger was fit to one end of the tube and was used to provoke the wasp to sting a small piece of Parafilm held in front of the other end. Venom droplets were collected from the distal side of the Parafilm with a nanovolumetric injector (NVI-570A/V, Medical Systems, Greenvale, NY). From each wasp, approximately 10 nl of crude venom were collected to a glass micropipette containing 10 nl saline.

Pharmacology

Procaine was freshly prepared and dissolved to a concentration of 500 mg/ml in vehicle containing cockroach saline and 0.1% Janus Green dye. Venom was freshly milked and dissolved 1:1 in cockroach saline containing 0.1% Janus Green dye.

Injections and surgical procedures

General

Prior to the procedures, cockroaches were anesthetized with carbon dioxide and immobilized dorsal side up with modeling clay on a wax platform. A staple-shaped pin was softly pressed against the neck to regulate hemolymph flow to the head during the procedure. A U-shaped incision was then performed to open a small flap between the ocelli. Then, we either injected various compounds in specific regions of the SupOG or performed a surgical crush of the CirC. After the procedure, the cuticular incisions were sealed with wax.

Procedures:

1. *Micro-injection* A nanovolumetric injector was used to deliver solutions directly into the CX or MB. Using physical landmarks, a glass needle was inserted to the specific location and the solution was injected. For two groups of cockroaches, we used procaine, a reversible voltage-dependent sodium and potassium channels blocker, which eliminate neuronal activity in the injected area as shown by Devaud (Devaud et al., 2007) and by Gal and Libersat (Gal and Libersat, 2010). 'Procaine-CX' cockroaches were injected with 10 nl of procaine into the CX; 'Procaine-MB' cockroaches were injected with 20 nl of procaine (bilateral injection) into the MB; and 'Venom-CX' cockroaches were injected with 20 nl of venom diluted in saline into the CX. Control cockroaches were injected with saline containing 0.1% Janus Green dye (10-20 nl) to the CX or MBs. The effect of procaine injection on neuronal activity is known to be restricted to the injection site (Kathman et al., 2014).
2. *Surgical crush of the CirC.* Two groups of cockroaches were prepared as follows. For the first group, the CirC were gently crushed at first (carefully to not cut the connectives and to avoid damage to surrounding tissue) and afterward the cockroach was introduced to a wasp for a sting (‘crushed CirC - stung’). For the second group, the CirC were crushed after the cockroach was stung by a wasp (‘Stung - crushed CirC’). The experimental timeline is explained in *‘behavioral assays’*.

Behavioral assays

Micro-injections: Behavioral assays were performed on freely-moving cockroaches in an open-field arena (radius = 30 cm). Spontaneous walking was quantified with a stopwatch in continuous 10 minute bins. Baseline walking duration was measured prior to the nano-injections procedure. After the procedure, the cockroach was given 10 minutes to recover. Then, spontaneous walking was again recorded continuously for one hour.

Surgical crush of the CirC: Freely-moving 'SupOG-ablated' cockroaches in an open-field arena have a tendency to collide with the arena wall and often get turned on their back. This could affect the spontaneous walking measurements. Since 'Crushed CirC' cockroaches were expected to behave similar to 'SupOG-ablated' cockroaches, behavioral assays were performed on tethered cockroaches walking on an oiled glass plate. In such a fixed position, cockroaches are able to move their legs in short bouts of stationary walking or running. The walking and escape movements are similar to those of free ranging animals (Camhi and Levy, 1988). Spontaneous walking was measured in 10 minute bins in three time points. For

both groups, the cockroaches were first tethered and let to acclimate for 20 minutes. Afterwards, baseline walking duration was measured. Following the baseline measurement, the protocol differed for both groups: for 'Stung - crushed CirC' cockroaches, the second time point (70 min) was an hour after the cockroach was stung by a wasp. After the second time point, the CirC were crushed (see *Injections and surgical procedures*) and the third time point (140 min) was an hour afterwards. For 'Crushed CirC - stung' cockroaches, the second time point (70 min) was an hour after the CirC were crushed. After the second time point, the cockroach was stung by a wasp and the third time point (140 min) was an hour afterwards.

Statistical analysis

For the different nano-injections treatments, we used a Two-way Repeated Measures ANOVA, with Treatment as the between-subject factor and Time as the within-subject factor, to compare spontaneous walking between different treatment groups (n=22, n=8, n=8 and n=8 cockroaches in the Control, 'Procaine-CX', 'Procaine-MB' and 'Venom-CX' groups, respectively).

For 'Crushed CirC - stung' and 'Stung - crushed CirC' cockroaches, we used a Two-way ANOVA with a Student-Newman-Keuls posthoc test to identify differences in time and treatment between the groups (n=8 for each group).

Postmortem verification of the injection site

After the behavioral assay, the cockroach head was removed and fixed overnight in formalin. Then, the SupOG was removed from the head, embedded in 6% agarose and sliced (60 µm) with a vibratome. The location of the Janus Green tracer was used to verify the exact injection site.

Abbreviations

SupOG: supraeosophageal ganglion: SOG: subeosophageal ganglion; CX: central complex; MBs: mushroom bodies; CirC: Circumesophageal connectives.

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Competing interests

The authors declare no competing financial interests

Author contribution

Maayan Kaiser contributed to the study conception, design, execution, interpretation of the findings and writing.

Frederic Libersat contributed to the study conception, design, interpretation of the findings and writing.

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References:

Bender, J. A., Pollack, A. J. and Ritzmann, R. E. (2010). Neural activity in the central complex of the insect brain is linked to locomotor changes. *Current biology : CB* **20**, 921-926.

Camhi, J. M. and Levy, A. (1988). Organization of a complex movement: fixed and variable components of the cockroach escape behavior. *Journal of comparative physiology. A, Sensory, neural, and behavioral physiology* **163**, 317-328.

Devaud, J. M., Blunk, A., Podufall, J., Giurfa, M. and Grunewald, B. (2007). Using local anaesthetics to block neuronal activity and map specific learning tasks to the mushroom bodies of an insect brain. *The European journal of neuroscience* **26**, 3193-3206.

Fouad, K., Libersat, F. and Rathmayer, W. (1994). The Venom of the Cockroach-Hunting Wasp Ampulex-Compressa Changes Motor Thresholds - a Novel Tool for Studying the Neural Control of Arousal. *Zool-Anal Complex Sy* **98**, 23-34.

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. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **192**, 1003-1020.

Gal, R. and Libersat, F. (2008). A parasitoid wasp manipulates the drive for walking of its cockroach prey. *Current biology : CB* **18**, 877-882.

Gal, R. and Libersat, F. (2010). A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in its cockroach prey. *PloS one* **5**, e10019.
Guo, P. and Ritzmann, R. E. (2013). Neural activity in the central complex of the cockroach brain is linked to turning behaviors. *The Journal of experimental biology* **216**, 992-1002.
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.
Haspel, G., Gefen, E., Ar, A., Glusman, J. G. and Libersat, F. (2005). Parasitoid wasp affects metabolism of cockroach host to favor food preservation for its offspring. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **191**, 529-534.
Helfrich-Forster, C., Wulf, J. and de Belle, J. S. (2002). Mushroom body influence on locomotor activity and circadian rhythms in Drosophila melanogaster. *Journal of neurogenetics* **16**, 73-109.
Huber, F. (1960). Untersuchungen über die Funktion des Zentralnervensystems und insbesondere des Gehirnes bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. Vergl. Physiol.* **44**, 60-132.
Ito, K., Shinomiya, K., Ito, M., Armstrong, J. D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A. et al. (2014). A systematic nomenclature for the insect brain. *Neuron* **81**, 755-765.
Johnston, R. M., Consoulas, C., Pfl ger, H. and Levine, R. B. (1999). Patterned activation of unpaired median neurons during fictive crawling in manduca sexta larvae. *The Journal of experimental biology* **202 (Pt 2)**, 103-113.
Kahsai, L., Martin, J. R. and Winther, A. M. (2010). Neuropeptides in the Drosophila central complex in modulation of locomotor behavior. *The Journal of experimental biology* **213**, 2256-2265.
Kathman, N. D., Kesavan, M. and Ritzmann, R. E. (2014). Encoding wide-field motion and direction in the central complex of the cockroach Blaberus discoidalis. *The Journal of experimental biology* **217**, 4079-4090.
Kien, J. (1983). The Initiation and Maintenance of Walking in the Locust - an Alternative to the Command Concept. *Proc R Soc Ser B-Bio* **219**, 137-174.
Kien, J. and Altman, J. S. (1992). Preparation and execution of movement: parallels between insect and mammalian motor systems. *Comparative biochemistry and physiology. Comparative physiology* **103**, 15-24.
Libersat, F. (2003). Wasp uses venom cocktail to manipulate the behavior of its cockroach prey. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **189**, 497-508.
Libersat, F. and Gal, R. (2014). Wasp voodoo rituals, venom-cocktails, and the zombification of cockroach hosts. *Integrative and comparative biology* **54**, 129-142.
Martin, J. R., Ernst, R. and Heisenberg, M. (1998). Mushroom bodies suppress locomotor activity in Drosophila melanogaster. *Learning & memory* **5**, 179-191.
Martin, J. R., Raabe, T. and Heisenberg, M. (1999). Central complex substructures are required for the maintenance of locomotor activity in Drosophila melanogaster. *Journal of comparative physiology. A, Sensory, neural, and behavioral physiology* **185**, 277-288.
Mizunami, M., Weibrecht, J. M. and Strausfeld, N. J. (1998). Mushroom bodies of the cockroach: their participation in place memory. *The Journal of comparative neurology* **402**, 520-537.
Nassel, D. R. (2002). Neuropeptides in the nervous system of Drosophila and other insects: multiple roles as neuromodulators and neurohormones. *Progress in neurobiology* **68**, 1-84.
Olivera, B. M. and Cruz, L. J. (2001). Conotoxins, in retrospect. *Toxicon : official journal of the International Society on Toxinology* **39**, 7-14.
Pfeiffer, K. and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. *Annual review of entomology* **59**, 165-184.
Poeck, B., Triphan, T., Neuser, K. and Strauss, R. (2008). Locomotor control by the central complex in Drosophila-An analysis of the tay bridge mutant. *Developmental neurobiology* **68**, 1046-1058.

Ridgel, A. L. and Ritzmann, R. E. (2005). Effects of neck and circumoesophageal connective lesions on posture and locomotion in the cockroach. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **191**, 559-573.
Schaefer, P. L. and Ritzmann, R. E. (2001). Descending influences on escape behavior and motor pattern in the cockroach. *J Neurobiol* **49**, 9-28.
Strausfeld, N. J. (1999). A brain region in insects that supervises walking. *Progress in brain research* **123**, 273-284.
Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Current opinion in neurobiology* **12**, 633-638.
Strauss, R. and Heisenberg, M. (1993). A higher control center of locomotor behavior in the Drosophila brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **13**, 1852-1861.
Strauss, R., Hanesch, U., Kinkelin, M., Wolf, R. and Heisenberg, M. (1992). No-bridge of Drosophila melanogaster: portrait of a structural brain mutant of the central complex. *Journal of neurogenetics* **8**, 125-155.
Svidersky, V. L. and Plotnikova, S. I. (2002). Insects and vertebrates: Analogous structures in higher integrative centers of the brain. *J Evol Biochem Phys+* **38**, 627-639.
Weisel-Eichler, A., Haspel, G. and Libersat, F. (1999). Venom of a parasitoid wasp induces prolonged grooming in the cockroach. *The Journal of experimental biology* **202 (Pt 8)**, 957-964.
Winther, A. M., Acebes, A. and Ferrus, A. (2006). Tachykinin-related peptides modulate odor perception and locomotor activity in Drosophila. *Molecular and cellular neurosciences* **31**, 399-406.
Zornik, E., Paisley, K. and Nichols, R. (1999). Neural transmitters and a peptide modulate Drosophila heart rate. *Peptides* **20**, 45-51.

FIGURE LEGENDS:

Figure 1. **A procaine injection to the CX decreases spontaneous walking.** Spontaneous walking duration measured in seconds, in 10 minute bins, for baseline walking and for a continuous hour after nano-injection treatments. Data points represent mean ± s.e.m. Data points with same capital letter are not significantly different, whereas data points with different letters are significantly different ($p<0.05$). Asterisks represent significant difference between treated and control groups (*=$p<0.05$). n=22 and n=8 cockroaches in Control and 'Procaine-CX' groups, respectively.

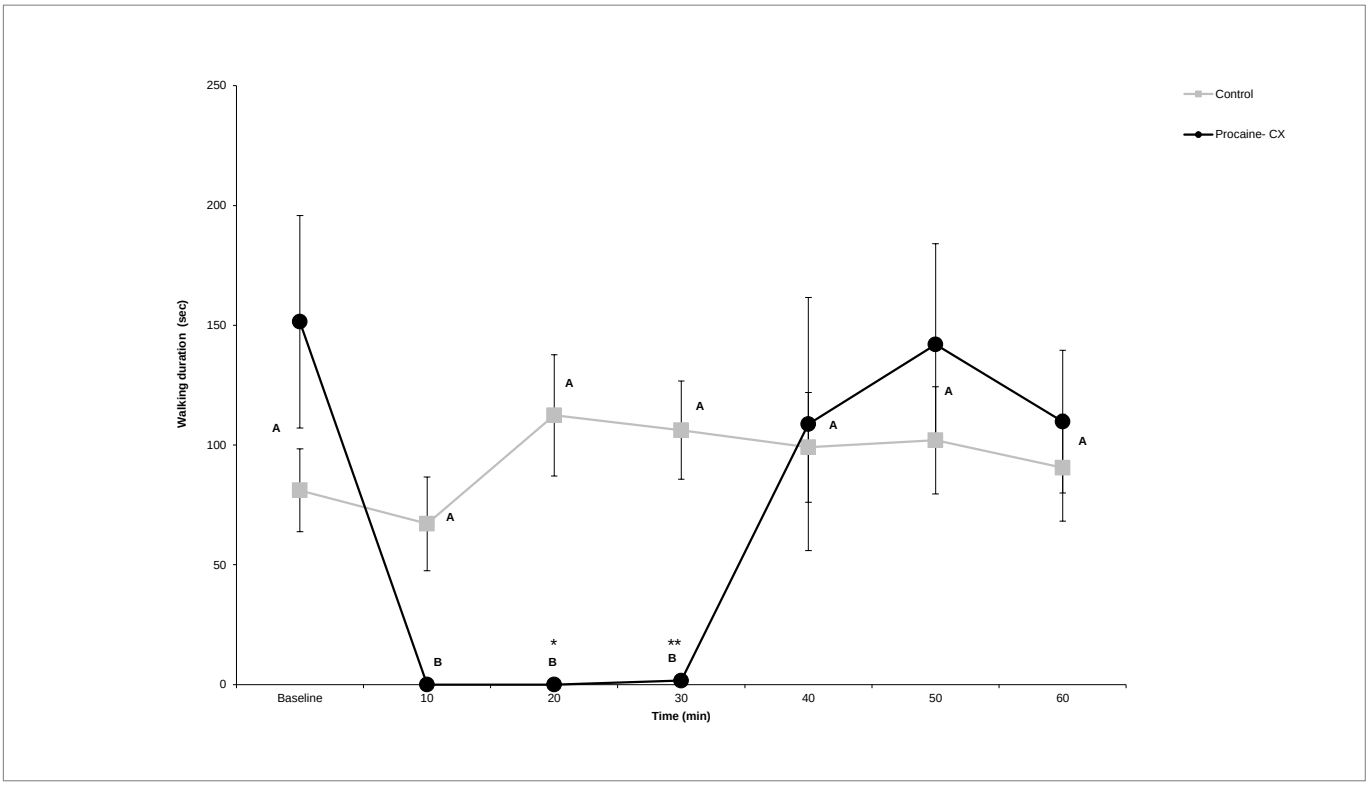
Figure 2. **Representative images for postmortem verification of injection site.** In the center, a schematic drawing showing the general anatomy of the SupOG (Mizunami et al., 1998). Darker spots in the drawing indicate the injections site as shown in the representative images (bottom and top). Bottom photograph shows the injection site in the central body of the Central Complex and top photograph shows the injections sites in the Calyces of the Mushroom Bodies. CX: central complex, MB: mushroom body.

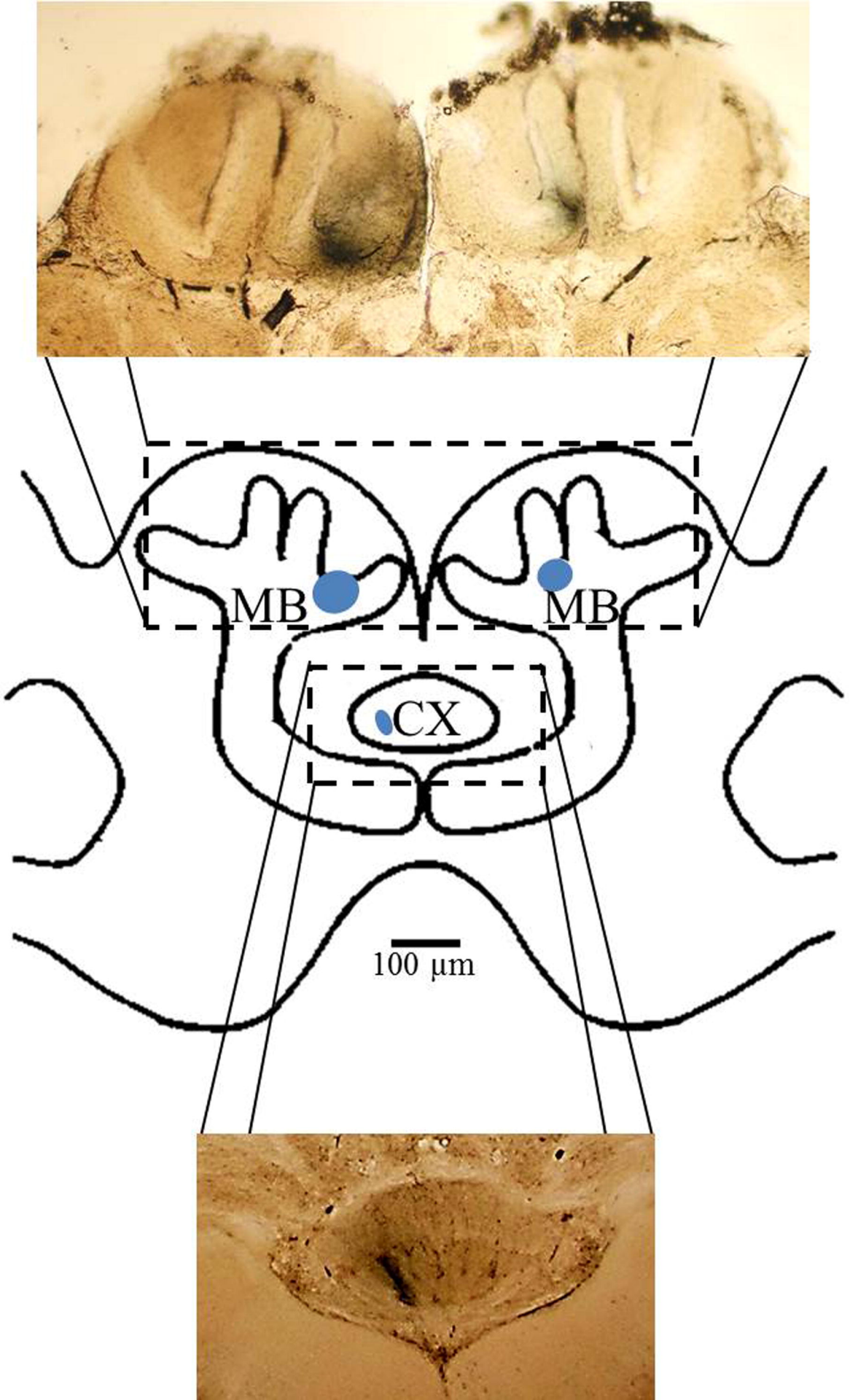
Figure 3. **A procaine injection to the MBs increases spontaneous walking**. Spontaneous walking duration measured in seconds, in 10 minute bins, for baseline walking and for a

continuous hour after nano-injection treatments. Data points represent mean ± s.e.m. Data points with same capital letter are not significantly different, whereas data points with different letters are significantly different ($p<0.05$). Asterisks represent significant difference between treated and control groups (***=$p<0.001$). n=22 and n=8 cockroaches in Control and 'Procaine-MBs' groups, respectively

Figure 4. **A venom injection to the CX decreases spontaneous walking**. Spontaneous walking duration measured in seconds, in 10 minute bins, for baseline walking and for a continuous hour after nano-injection treatments. Data points represent mean ± s.e.m. Data points with same capital letter are not significantly different, whereas data points with different letters are significantly different ($p<0.05$). Asterisks represent significant difference between treated and control groups (*=$p<0.05$, **=$p<0.01$). n=22 and n=8 cockroaches in Control and 'Venom-CX' groups, respectively

Figure 5. **SupOG-ablated stung cockroaches show decreased spontaneous walking.** Spontaneous walking duration measured in seconds, in 10 minute bins in 3 time points: baseline (before treatment), 70 min (an hour after the first treatment) and 140 min (an hour the after second treatment). For 'Crushed CirC stung' cockroaches, the first treatment was crushing the CirC and the second one was stinging by a wasp. For 'Stung Crushed CirC' cockroaches, the first treatment was stinging by a wasp and the second one was crushing the CirC. Data points represent mean + s.e.m. Data points with same capital letter are not significantly different, whereas data points with different letters are significantly different ($p<0.05$). Asterisks represents significant difference between treatments (***=$p<0.001$). n=8 cockroaches in each treatment.




MB
MB
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