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THE ROLE OF NEUROHORMONAL OCTOPAMINE DURING 'FIGHT OR FLIGHT' BEHAVIOUR IN THE FIELD CRICKET *GRYLLUS BIMACULATUS*

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Summary

Octopamine has been called the 'fight or flight' hormone of insects. We tested this hypothesis by measuring octopamine levels in the haemolymph of field crickets after fighting, flying, courting and escape behaviours. Octopamine levels in the cricket *Gryllus bimaculatus* increased during aggressive (agonistic) behaviour from baseline levels of 4.5±2.1 pg μl^{-1} haemolymph to 24.3±15.2 pg μl^{-1} haemolymph, regardless of whether the cricket won or lost the encounter. Octopamine levels also increased after 5 min of flying (to 44.6±22.3 pg μl^{-1}) and during courtship. However, crickets did not exhibit an increase in their haemolymph octopamine levels after performing an escape run. Therefore, neurohormonal octopamine shows some, but not all, of the characteristics that would be expected if it were a component of a non-specific 'arousal' system. Rather, octopamine may be released as a neurohormone to prepare the animal for a period of extended activity or to assist the animal in recovering from a period of increased energy demand. Antennal contact with conspecifics may provide a sensory cue that results in the release of octopamine into the haemolymph.

Key words: aggression, agonistic behaviour, arousal, behavioural state, courtship, copulation, insect, orthopteran, cricket, *Gryllus bimaculatus*.

Introduction

How animals alter their behaviour in order to respond to the demands of a changing external environment remains a central problem for behavioural physiologists. Despite the difficulty in addressing this question, progress has been made in understanding the physiological basis of complex and plastic behaviours, such as feeding and aggression, by using invertebrate systems as models (e.g. Kupfermann and Weiss, 1982; Kravitz, 1990). As has been shown for aggressive (agonistic) behaviour in lobsters (Kravitz, 1988) and during flying in locusts (Orchard *et al.* 1993), shifts in behavioural state are accomplished *via* changes within the central nervous system as well as by changes in the performance of non-neural tissues such as muscles or energy-storing organs. The constellation of changes that occurs to create the new behavioural state for locusts and lobsters relies on both neural and neurohormonal mechanisms.

The courtship and aggressive (agonistic) behaviour of the cricket *Gryllus bimaculatus* have been quantitatively described (Adamo and Hoy, 1994, 1995); however, the physiological bases for these behaviours are poorly understood. In this study, we use high performance liquid chromatography with electrochemical detection (HPLC-ED) to measure changes in the levels of neurohormones such as octopamine during these behaviours. What are some of the hormonal or neurohormonal mechanisms that may contribute to the insect's change in behavioural state? Like flying in locusts or fighting in lobsters, these behaviours in crickets probably also require a cascade of changes resulting in a shift in the performance of both neural and some non-neural tissues.

We focus primarily on the possible role(s) of the biogenic amine octopamine in agonistic and courtship behaviour. Studying the functional roles of octopamine is complicated by its use as both a neuromodulator (i.e. released locally near target sites or within the central nervous system, but not generally into the haemolymph) and as a neurohormone (Orchard, 1982). The two may not have equivalent functions. In this study, we focus on the role of octopamine as a neurohormone. Octopamine is found in the haemolymph of crickets (Woodring *et al.* 1988) and appears to be released from neurohaemal regions on peripheral nerves (Spörhase-Eichmann *et al.* 1992) and from the corpora cardiaca (Woodring *et al.* 1989). As a neurohormone, it can induce increases in circulating levels of both lipids and sugars (Woodring *et al.* 1989). Because octopamine levels were found to increase in the haemolymph of crickets after enforced exercise, Woodring *et al.* (1989) suggested that it may be released during 'stressful' behaviours in order to mobilize energy stores.

Octopamine levels have also been found to increase in the

haemolymph of both locusts (Davenport and Evans, 1984*a*) and cockroaches (Bailey *et al.* 1983) during 'stressful' handling. Because its presence correlates with active or 'stressful' behaviours, octopamine has been dubbed the insect 'fight or flight' hormone (Orchard, 1982). It has also been suggested that octopamine is part of a general arousal system which prepares an insect for vigorous activity (Evans and Siegler, 1982; Davenport and Evans, 1984*a*; Corbet, 1991; Orchard *et al.* 1993). Corbet (1991), for example, suggested that the amount of octopamine released reflects a continuum of levels of arousal from sedentary to active, with small increases occurring during mild stimulation and larger increases occurring as the stimulus, and the need for activity, increases.

These general hypotheses remain largely untested. Although the effects of octopamine on specific target organs such as muscle (Malamud *et al.* 1988) and fat body (Orchard and Lange, 1985) have been studied in some detail, its correlation to arousal has not. This correlation can be studied rigorously in *G. bimaculatus* because quantitative behavioural descriptions exist for this species (Gras and Hörner, 1992; Adamo and Hoy, 1994, 1995). Therefore, concepts such as arousal can be defined for a particular behaviour (e.g. the level of intensity of agonistic behaviour) and measured. *G. bimaculatus* can also be used to test whether octopamine may be involved in a non-specific arousal system by measuring its levels during 'stressful' behaviours such as escape running. This allows us to test whether octopamine levels increase in the haemolymph in a way that is consistent with the hypothesis that it is an effector of the 'fight or flight' response (Orchard, 1982). We also examine which sensory cues can trigger the observed increases in octopamine levels.

We used a novel statistical method to analyze HPLC data. Newer HPLC-ED machines with multiple electrochemical detector electrodes are capable of assessing the amounts of several compounds at once in a single sample. Given the importance of synergistic effects between hormones and/or neurohormones in some systems (e.g. Mani *et al.* 1994), we used a factor analysis to test for correlated changes in the levels of different substances. This may be critical in studying the function of compounds such as octopamine, which appear to be multifunctional. For example, octopamine is released into the haemolymph during flying (see Orchard *et al.* 1993) and during the activation of the immune system (Dunphy and Downer, 1994; S. A. Adamo, C. E. Linn and R. R. Hoy, personal observations). It is possible that the presence or absence of other substances in the haemolymph helps to determine the specific actions of octopamine. This type of analysis can demonstrate where such possible interactions may exist. The potential for synergistic interactions between octopamine and other factors has also been suggested by Orchard *et al.* (1993).

Materials and methods

Animals

Field crickets (*Gryllus bimaculatus* De Geer) were taken from a colony that has been maintained in the laboratory for several years. Crickets were reared as previously described (Adamo and Hoy, 1994). Briefly, crickets were maintained at 28 °C with a 14 h:10 h L:D photoperiod and at approximately 65 % relative humidity. They were fed cat chow (Purina) and water *ad libitum*. Once adult, male and female crickets were marked and placed in individual cages (8 cm high × 10 cm diameter).

Haemolymph collection

Haemolymph was always collected at the same time each day, 2 h after the start of the dark phase. During this time, males show high levels of sexual activity (Simmons, 1988). Haemolymph was collected by inserting the tip of a 10 μl Hamilton syringe through the lateral edge of the membrane between the prothorax and mesothorax. It took about 10 s to remove the haemolymph. About 3 μl of haemolymph was removed from the animal and then 2 μl was immediately added to 40 μl of ice-cold 0.2 mol l^{-1} perchloric acid. Samples were spun at 8500 ***g*** for 5 min at 4 °C. The supernatant was added to 110 μl of HPLC-grade water (Sigma Chemical Co.) and a further 125 μl of mobile phase was added prior to transfer to the autosampler tube. The supernatant was frozen at −80 °C prior to use.

Control (baseline) haemolymph octopamine levels were measured for each of the set of experiments described below, and the octopamine levels measured for each experimental trial were compared with the baseline values that were collected at the same time. Baseline octopamine levels did not differ significantly from each other.

High performance liquid chromatography

Chromatographic separations for octopamine and other compounds were achieved using a Vydac C-18 HS-54-15 HPLC column (15 cm×4.6 mm, 3 μm particles) protected by a Vydac C-18 guard column. The mobile phase (after Downer and Martin, 1987) contained 70 mmol l^{-1} monobasic sodium phosphate, 0.5 μmol l^{-1} EDTA, 0.1 mmol l^{-1} 1-octanesulphonate (sodium salt), with 15 % methanol and 5 % acetonitrile. The pH of the buffer was adjusted to 5.5 (at room temperature) using sodium hydroxide. The mobile phase was throughly stirred and vacuum-filtered through a 0.22 μm filter and degassed. The mobile phase was run isocratically at 0.85 ml min^{-1}. The HPLC pump, autosampler, guard cell and dual-channel coulometric detector were from ESA (ESA Inc., Chelmsford, MA, USA). The first electrode was set at a potential of 0.35 V and the second electrode at 0.73 V. The guard cell was set at 0.8 V. Identification of compounds (e.g. octopamine and dopamine) was based on comparison of retention times with standards (see Fig. 1), which were run at the beginning and end of each daily series. Peak identification was also determined on the basis of changes in retention times or peak height as a function of systematic changes in chromatographic conditions, including pH, percentage organic compounds and applied channel voltage (see Linn *et al.* 1994).

Chemicals were obtained from Sigma Chemical Co. (St

Louis, MO, USA) except for perchloric acid (J. T. Baker Inc., Jackson, TE, USA) and *N*-acetyloctopamine (*N*-acOA) (Research Chemicals Inc.).

Peak heights and/or areas were measured and compared with a calibration curve made from injecting known amounts of each compound, where the peak identity was known. The values for the peaks were then converted to $pg\,\mu l^{-1}$ haemolymph. Chromatograms for octopamine were recorded on a Spectra-Physics dual-channel Data-Jet integrator, which was interfaced with a Spectra-Physics SX-386 computer system for data analysis.

Some peaks in the chromatogram appeared reliably in haemolymph samples from virtually all individuals, but were not from substances we could identify. We measured changes in the amplitude of these peaks as well as changes in the amounts of identified compounds during different behavioural states (described below).

Behavioural manipulations

Agonistic behaviour

Crickets were removed from their individual cages and placed in small glass containers (10 cm×8 cm×6 cm). The two crickets were initially separated by an opaque divider. After a minimum of 5 min, haemolymph was removed from both crickets to measure baseline octopamine levels. We found, as did Woodring *et al.* (1989), that the procedure of blood sampling had no significant effects on the levels of octopamine. Levels remained low (see Results) and did not show any significant change even if haemolymph was removed from the same animal three times in 5 min [N=5; repeated-measures analysis of variance, ANOVA, $F(4,2)$ 1.8, P>0.1]. Other control animals had haemolymph removed from them twice, with the second sample taken 12, 30 or 45 min after the first. These times were similar to those between the baseline measurements and the samples taken from courting and fighting crickets. In all cases, there were no significant changes in the octopamine levels of the second samples (pooled data, paired t-test, N=15, P>0.1).

Ten minutes after the baseline measures had been taken, the opaque barrier was removed and the duration, intensity and outcome of the agonistic encounter were recorded. Agonistic behaviour is relatively stereotyped and can be divided into stages that correlate with the degree of intensity of the interaction (Adamo and Hoy, 1995). Intensity of the fight was scored as one of two categories. Low-intensity interactions were fight sequences that included antennal fencing and adoption of the threat posture, while high-intensity interactions included grappling, mandible flaring and/or biting. If there was no physical interaction between the two males, the crickets were scored as not having exhibited any agonistic behaviour.

The outcome of an interaction is obvious: winning males chase and stridulate aggressive song while losing males withdraw. At the end of the fight, both winning and losing males had haemolymph samples taken, with the order of sampling (i.e. winners or losers sampled first) alternating between trials. Fights lasted a maximum of 10 s, and haemolymph was collected within 30 s of the end of the fight.

To determine the duration of the changes that occurred during agonistic behaviour, haemolymph was removed from animals immediately after the fight and again at one other time point, 3, 5, 10 or 15 min later. Males were separated at the end of the fight. Fighting males were also separated at various points during the trial and the concentrations of different substances in the haemolymph were measured to determine when during the interaction concentrations of the substances began to change from their baseline levels.

Females also fight, although their agonistic behaviour tends to be shorter in duration and they do not exhibit aggressive singing (Adamo and Hoy, 1995). Females were tested as above and haemolymph was removed if there was an agonistic encounter.

Sexual behaviour

Males and females were placed together as described above for the agonistic behavioural trials. Blood samples were taken from males and females both during courtship and after copulation. After copulation, males often guard females (Simmons, 1986) and they are much more likely to attack other males during this time (Alexander, 1961). To measure the changes that occurred during courtship, blood samples were taken from the male after it began courtship singing and samples were taken from the female once it had mounted the male, but before spermatophore transfer.

Stress

It has been suggested that octopamine may act as an insect 'fight or flight' hormone and be released during stressful situations. To test some aspects of this hypothesis, we induced escape runs in the crickets and then sampled their haemolymph immediately after the escape run.

Crickets were induced to run by pinching either their cerci (N=12) or their hind wings (N=35) with forceps. Crickets that had their hind wings pinched were pinched repeatedly so that the animals continued to run and stop for 1 min.

Flying

Octopamine is released as a neurohormone in flying locusts (Goosey and Candy, 1980). We measured the levels of octopamine in flying male and female crickets. Crickets were tethered at the pronotum and placed upside down so that their legs no longer touched the substratum. Haemolymph samples were taken after the first 5 min of continuous flying.

Sensory cues important for the observed increase in octopamine levels

Males were placed together as described for the agonistic behavioural trials, but were separated after antennal touching but before either animal had expressed any agonistic behaviour. Males were also placed with dead, anaesthetized and plastic-coated (Krylon) crickets and allowed to touch them with their antennae.

Single males were placed in glass containers and one of their antennae was stroked 10 times from the tip to midway from the base either with a metal probe or with the freshly detached antenna of an anaesthetized conspecific. Haemolymph samples were taken immediately after the antenna had been stroked.

Statistics

Where possible, parametric statistics were used (Sokal and Rohlf, 1981); values in the text are means ±1 S.D. unless otherwise stated. For some behavioural states, such as courtship, the octopamine levels were not normally distributed. We used non-parametric statistics in those cases (Meddis, 1984).

We performed a factor analysis (principal components analysis; Hair *et al.* 1987) on the peak levels of octopamine, dopamine and nine unknown compounds during seven behavioural states (baseline, win, lose, escape run, antennal contact, post-copulation and flying, N=272 crickets) to determine whether there was any evidence that levels of these substances were varying in a correlated fashion. We used unidentified peaks in our analysis only if they appeared reliably in most (>80 %) of the individuals sampled. Many unknown peaks appeared in only one or a few animals and these were not analyzed. Calculations were performed using Systat.

Results

Analysis of standards gave a linear response over the range 1–30 pg μl^{-1} injected. Sample chromatograms of standards (50 pg per injection) and selected behavioural categories are shown in Fig. 1. Changes in behavioural state resulted not only in changes in octopamine levels from those observed in baseline samples, but also in changes in the peak heights of compounds of unknown identity. Four such peaks that displayed significant shifts as a function of behaviour (Table 1) are marked (I, II, III, IV) in Fig. 1.

Agonistic behaviour

Octopamine levels increased significantly in the haemolymph in both winning and losing males (baseline levels, 4.5±2.1 pg μl^{-1} haemolymph; winning and losing males, 24.3±15.2 pg μl^{-1} haemolymph, Fig. 2). There were no significant differences in octopamine levels between winning and losing males. Once separated from conspecifics, these levels gradually declined and 5 min later were indistinguishable from baseline levels (Fig. 3).

The increase in octopamine level did not depend on the intensity of the fight. Octopamine levels after brief low-intensity (N=18) and prolonged high-intensity (N=12) interactions were similar (Mann–Whitney U-test 3.4, P>0.1).

Table 1. *Patterns of change in levels (relative to baseline) for octopamine, dopamine and four unidentified substances (I–IV) in response to different behavioural states*

Substance or peak	Win	Lose	Flying	Escape run
Octopamine	Increase	Increase	Increase	None
Dopamine	None	None	Increase	None
I	Increase	None	None	Increase
II	Increase	Increase	Increase	Increase
III	None	None	Increase	Decrease
IV	None	None	Increase	None

Increased and decreased values are those that changed by at least 2 standard deviations above or below baseline.

Win and lose denote that the cricket won or lost an agonistic encounter with a conspecific.

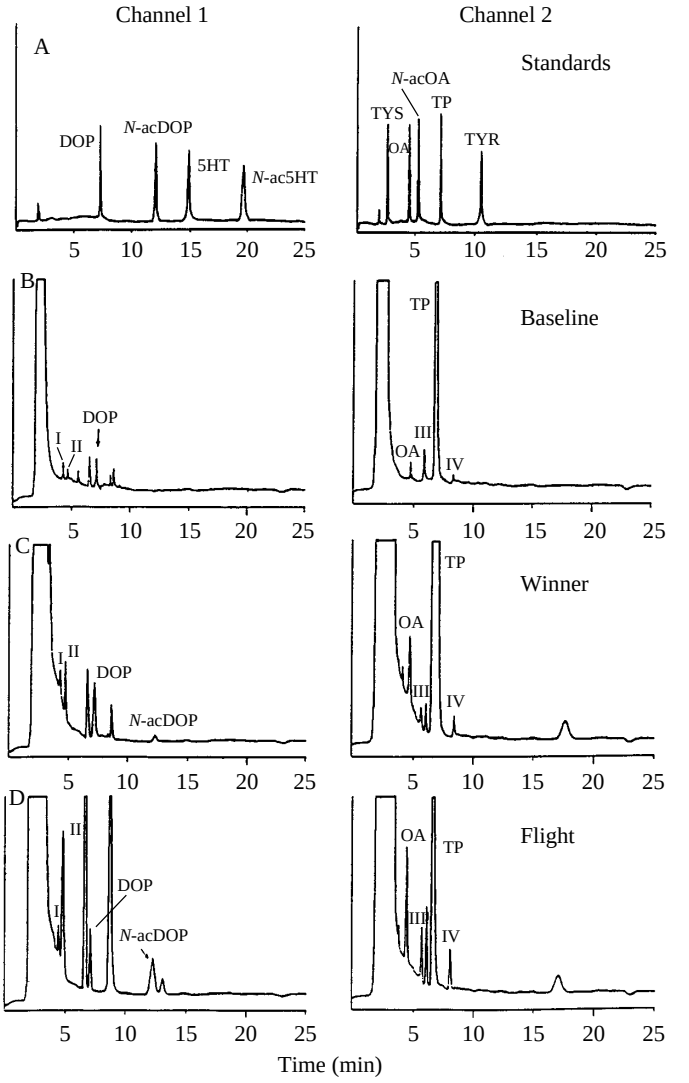

Fig. 1. Selected chromatograms of (A) standards (50 pg per injection), (B) haemolymph from crickets at rest (baseline), (C) haemolymph from crickets that had won a fight and (D) haemolymph from crickets after they had flown for 5 min. The electrode potential of channel 1 was set at 0.35 V and that of channel 2 at 0.73 V. Full-scale sensitivity was 70 nA. Peaks I, II, III and IV represent unknown compounds whose levels change significantly with behavioural state (Table 1). Standards: DOP, dopamine; *N*-acDOP, *N*-acetyldopamine; 5HT, serotonin; *N*-ac5HT, *N*-acetylserotonin; TYS, tyrosine; OA, octopamine; *N*-acOA, *N*-acetyloctopamine; TP, tryptophan; TYR, tyramine.

When fights were interrupted, octopamine levels increased in crickets that had begun to exhibit agonistic behaviour (to 16.3±5.6 pg μl^{-1}, N=21) as well as in those that had not yet shown any behavioural response to the antennal contact of a conspecific (to 9.2±4.2 pg μl^{-1}, N=18, non-parametric test for trends, Z=2.7, P<0.01). Crickets that had exhibited threat posture or antennal fencing (preliminary agonistic behaviours) had higher levels of octopamine than those that had not yet exhibited a visible expression of agonistic behaviour.

Females that chased and bit conspecifics showed an increase in octopamine levels compared with baseline levels (Fig. 4).

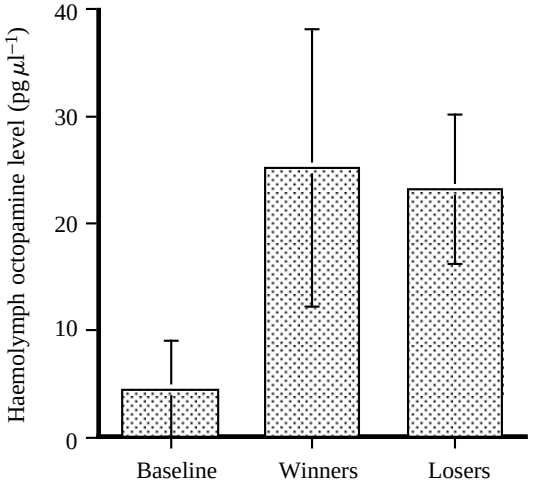

Fig. 2. Octopamine levels in the haemolymph increased after agonistic interactions in both winning and losing males. Columns represent median values and error bars denote first and third quartiles where they differ from the median. (Kruskal–Wallis 5.3, 2 d.f., P<0.01; baseline N=27, winners N=28, losers N=32).

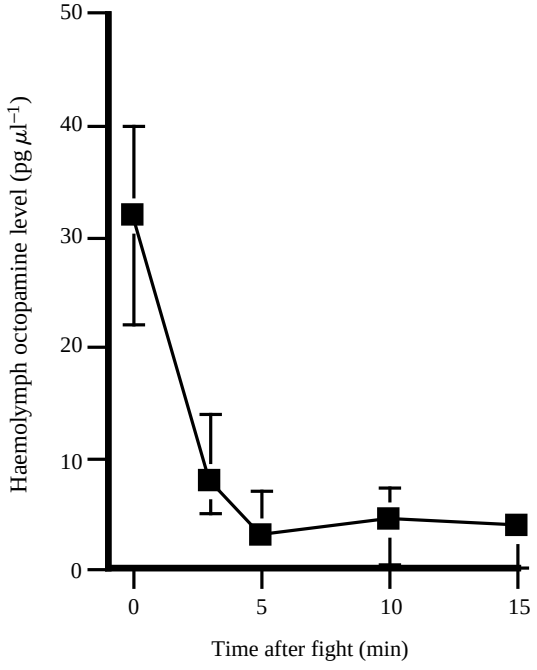

Fig. 3. Octopamine levels in the haemolymph return to baseline levels (median 4.1 pg μl^{-1}, first and third quartiles, 3.9 and 5.8 pg μl^{-1}) 5 min after a fight. Columns represent median values and error bars denote first and third quartiles. (Test for trends, Z=4.3, P<0.01; Meddis, 1984.) Sample sizes: immediately after fight N=18; 3 min N=5; 5 min N=7; 10 min N=7; 15 min N=11.

Sexual behaviour

During courtship, levels of octopamine in males increased over baseline levels [baseline, median 4.8 pg μl^{-1} (first quartile 0 pg μl^{-1}, third quartile 5.2 pg μl^{-1}, N=10); courting males median 7.6 pg μl^{-1} (first quartile 4.3 pg μl^{-1}, third quartile 10.2 pg μl^{-1}, Sign test, P<0.05)]. After copulation, the octopamine levels of isolated males did not differ significantly from baseline values (Fig. 5); however, the males exhibited increased levels of octopamine if they were kept in the same container with either a male or a female. This was true despite the absence of overt courtship or agonistic behaviour directed towards the female, although males tended to fight other males if present.

In females, octopamine levels remained unchanged from baseline levels during courtship until the females mounted males (Fig. 4). Females exhibited significant increases in octopamine levels after copulation (Fig. 4).

Stress and flying

Animals that had their cerci pinched showed no increase in their octopamine levels once they had completed their escape run. Neither male nor female crickets induced to produce a series of escape runs for 1 min showed an increase in their octopamine levels (Fig. 6). However, both male and female

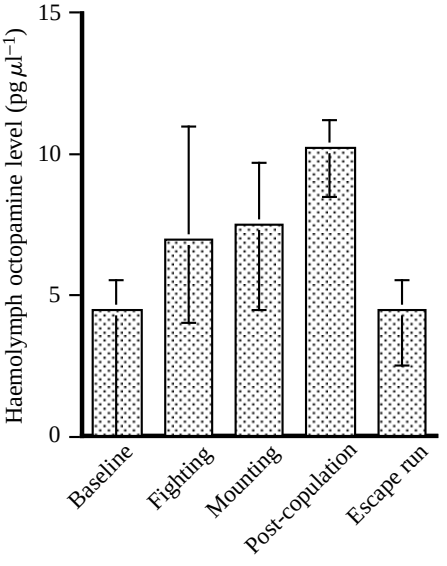

Fig. 4. Octopamine levels in the haemolymph of female crickets. Females also show significant increases in octopamine levels after fighting (N=17), after mounting a male (N=6) and after copulation (N=7), but not after an escape run (N=12). Columns represent median values and error bars denote first and third quartiles. *Post hoc* non-parametric comparisons, alpha error adjusted for multiple comparisons, Z=2.8, 3.2, P<0.05; baseline N=12.

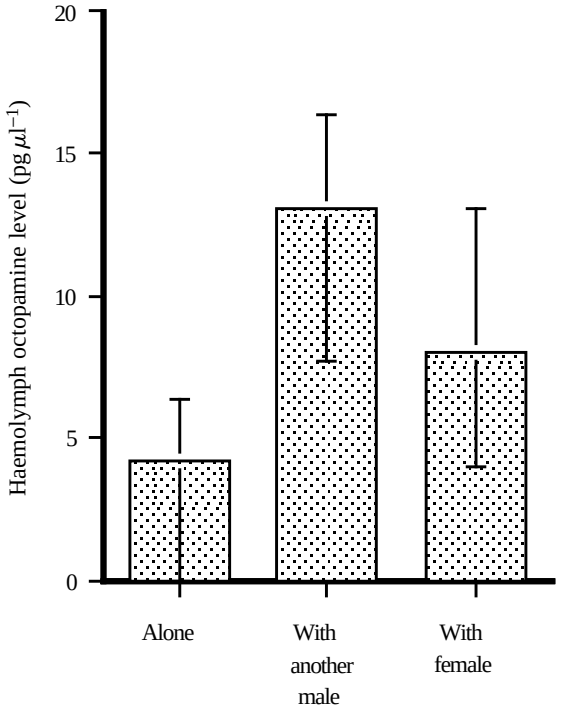

Fig. 5. Octopamine levels in the haemolymph of males after copulation. Columns represent median values and error bars denote first and third quartiles where they differ from the median. Post-copulatory males show no increase in octopamine levels unless conspecifics are present. (Kruskal–Wallis 4.1, $P<0.01$). Sample sizes: male alone $N=12$, male with male $N=8$, male with female $N=8$.

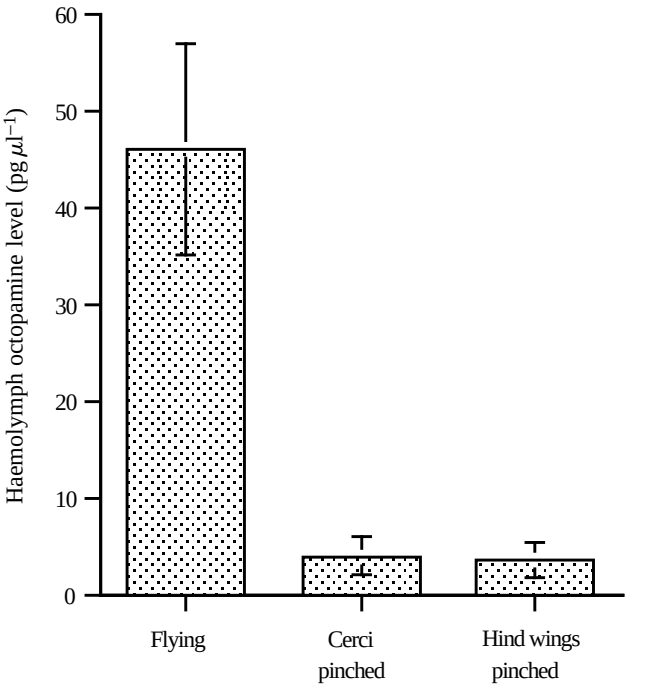

Fig. 6. Octopamine levels increase in the haemolymph when crickets fly but not after escape runs. Columns represent median values and error bars denote first and third quartiles where they differ from the median. (Kruskal–Wallis 6.1, $P<0.01$) Sample sizes: flight $N=8$, cerci pinched $N=12$, hind wings pinched $N=35$.

crickets showed large increases in octopamine levels after 5 min of flying (44.6±22.3 pg μl^{-1}; Fig. 6).

Sensory cues important for the observed increase in octopamine levels

After antennal contact with an anaesthetized male or female, the octopamine levels of a cricket increased (Fig. 7). Contact with a plastic-coated cricket did not result in an increase in octopamine levels over baseline values. Antennal contact with a steel probe never resulted in an increase in octopamine levels (Kruskal–Wallis 7.8, 4 d.f., $P<0.01$, with *post hoc* analysis, alpha error adjusted for multiple comparisons, Fig. 7). Stroking the antenna of a cricket with the steel probe did not cause an increase in octopamine levels ($N=8$), while stroking the same cricket's antenna with the antenna of an anaesthetized conspecific antenna did ($N=10$, Sign test, $P<0.01$, Fig. 7), irrespective of the order of presentation. Two males that were placed in the same glass container but did not come into contact with each other showed no increase in octopamine level (Sign test, $P>0.1$, Fig. 7).

Changes in levels of other compounds

As shown in Fig. 1, changes in behavioural state not only resulted in changes in haemolymph octopamine levels but also in several unidentified peaks. Four of these peaks are shown in the 'winner' and 'flight' chromatograms (I, II, III, IV) and in Table 1.

Table 2. *Factor analysis results of selected chromatographic peaks (octopamine, dopamine and four unidentified substances, I–IV, see Fig. 1) found in cricket haemolymph*

Substance or peak	Rotated loadings			
	1	2	3	4
Octopamine	0.93	0.01	0.02	–0.03
Dopamine	0.15	0	0.11	0.05
I	–0.03	–0.08	–0.01	0.94
II	0.02	0.16	0.95	–0.02
III	0.01	0.92	0.14	–0.08
IV	0.23	–0.03	0.17	0.09
Variance explained by each factor	15.4	15.4	15.1	15.4

A factor analysis found that the data exhibited a high degree of heterogeneity (Table 2). Examining the loading scores after rotating the factors (varimax method) reveals that the levels of none of the substances co-varied to any great extent. Each substance had a unique response pattern to the seven different behavioural states. For example, dopamine levels (baseline values, 10.8±5.3 pg μl^{-1}) increased in the haemolymph during flying (26.5 pg μl^{-1} $P<0.01$) but, unlike octopamine, there was no evidence for increases during fighting, courting or after an

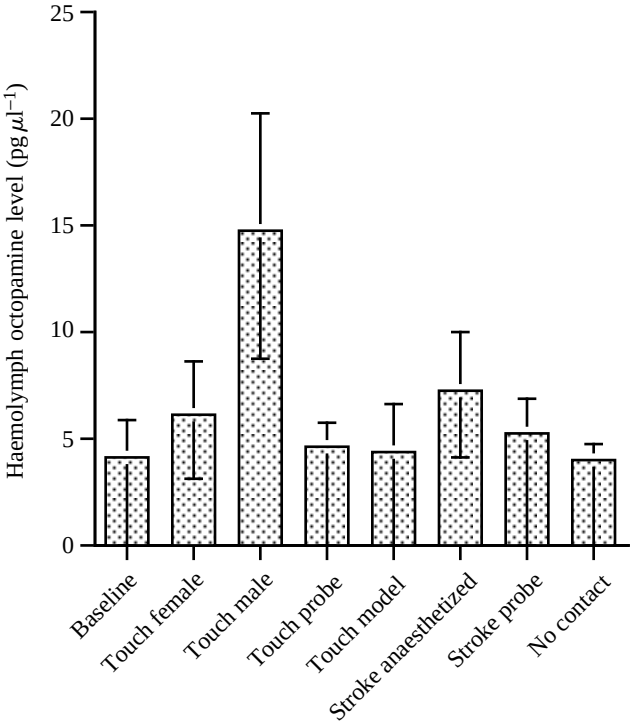

Fig. 7. After antennal contact (touch) with an anaesthetized conspecific (N=23; N=15 males, N=8 females), octopamine levels increased over baseline values (median 4.6 pg μl^{-1}, first and third quartiles 4.2 and 5.4 pg μl^{-1}), but not after contact with a steel probe (N=15) or with a model (i.e. a plastic-coated cricket) (Kruskall–Wallis, P<0.01, N=12). Octopamine levels also increased after a cricket's antenna was stroked by the freshly detached antenna of a conspecific (N=10), but not after being stroked by a steel probe (P<0.01, N=8). Levels remained at baseline values when two males were in the same cage but did not touch each other (Sign test, P>0.1, N=16). Columns represent median values and error bars denote first and third quartiles where they differ from the mean.

escape run when these levels were compared with baseline values (Kruskal–Wallis with *post hoc* analysis, alpha error adjusted for multiple comparisons).

Discussion

Octopamine and stress

Octopamine levels increased after antennal contact with conspecifics and increased to a greater extent once agonistic behaviour had been initiated and to even higher levels during flying. This supports the suggestion of Corbet (1991) that the amount of octopamine released may reflect a continuum from slight to more intense arousal; in this case, arousal would reflect the level of activity. However, levels of octopamine did not increase as the intensity of fighting behaviour increased. Therefore, the degree to which octopamine levels correlate with the level of arousal depends on the definition of arousal. This word needs to be precisely defined because of its many uses (see Adamo, 1990).

In the literature, the term 'central arousal' has been used to describe the relative level of responsiveness of an animal to stimuli. This may or may not correlate with its general level of locomotor activity, which is often called the animal's behavioural state (Adamo and Chase, 1991). Changes in the intensity of a particular behaviour, such as feeding or fighting, should not be confused with the concept of central arousal. The two phenomena may not necessarily share the same underlying physiological mechanisms and therefore the parameter being measured should be stated explicitly.

Examining the pattern of octopaminergic changes over a variety of behaviours suggests possible functional roles for this neurohormone. Because octopamine levels did not change significantly in the haemolymph of *G. bimaculatus* during escape runs or startle responses, its function is not exactly analagous to the 'fight or flight' systems (i.e. those physiological mechanisms mediating a non-specific response to a novel or intense stimulus) in animals such as mammals (Levine and Ursin, 1991). And, unlike other 'fight or flight' systems, octopamine levels in the haemolymph do not correlate with changes in arousal in the form of an increased proclivity for attacking conspecifics. Post-copulatory crickets, which are highly 'aggressive', show no increase in haemolymph octopamine levels unless they are in contact with conspecifics.

Octopamine levels increase in the haemolymph during extended bouts of activity. For example, courtship, which requires courtship singing, can last for many minutes (Adamo and Hoy, 1994). Although agonistic behaviour itself is very brief, it has long-lasting effects (10–15 min) on subsequent behaviour, including an increased tendency to attack conspecifics (Adamo and Hoy, 1995) and an increased level of locomotion in both winning and losing males for several minutes after the fight (S. A. Adamo, personal observations). In contrast, escape runs tend to be very brief (less than 15 s, Gras and Hörner, 1992; S. A. Adamo, personal observations).

As a neurohormone, octopamine may be released principally when energy stores need to be mobilized. The best documented effect of neurohormonal octopamine in crickets is that it induces an increase in both lipid and sugar levels in the haemolymph (Woodring *et al.* 1989). This increase does not occur immediately after an octopamine injection, but several minutes later, and does not reach a peak until 30 min after the injection (Woodring *et al.* 1989), suggesting that, in crickets, octopamine may not mobilize energy stores fast enough to be of any use during an escape run which ends within seconds. Octopamine release may also aid in stimulating the fat body to replenish circulating levels of sugar and lipid. This would help to explain why it is released during the active behaviours of aggression and courtship where the increase in activity lasts several minutes, but not usually for 30 min. Davenport and Evans (1984*a*) suggest a similar function for neurohormonal octopamine in locusts. They postulated that in locusts it regulates lipid release during 'stressful' situations. It is also released into the haemolymph during food deprivation in locusts (Davenport and Evans, 1984*b*), which is consistent with the hypothesis that neurohormonal octopamine is important for mobilizing energy stores.

In locusts, however, octopamine has also been implicated in

mediating an acute stress response. Davenport and Evans (1984*a*) found that stressful handling for 1 min could increase octopamine levels in the haemolymph of locusts. This seems to differ from the results reported here in crickets, which show no increase in octopamine levels even after an escape run. However, although 'stressful' handling (e.g. tumbling in a 1 l drum at 40 revs min^{-1}) does increase haemolymph levels of octopamine in locusts (Davenport and Evans, 1984*a*) and crickets (Woodring *et al.* 1988), it is unclear what the natural correlate for this abnormal stress would be. It is possible that abnormal stresses cause pathological responses and that this response would never occur in the animal in the field. Our more realistic experiments, which allowed the animals to make an escape run and stop on their own, suggest that octopamine is not released in large quantities into the haemolymph during acute stress (Fig. 6). It is also possible that hormonal octopamine does not function in exactly the same way in all insects. Bailey *et al.* (1983) found increases in octopamine levels in the haemolymph of cockroaches if they were held for 1 min. If cockroaches deplete their haemolymph levels of sugars and lipids faster than crickets, then cockroaches may release octopamine under conditions that would not elicit release in a cricket. For example, cockroaches (Davenport and Evans, 1984*a*) but not locusts (Davenport and Evans, 1984*b*) exhibit an increase in their octopamine levels at the same time of day that their locomotor activity tends to increase. It is likely that the function of octopamine will vary depending on the animal's physiological adaptations to its ecological niche.

Contrary to what might be expected from the results of this paper, Gras *et al.* (1990) found that octopaminergic dorsal unpaired median (DUM) cells are strongly activated during escape running in *G. bimaculatus*. These DUM cells send processes into the peripheral nerves and may be able to release octopamine from neurohaemal regions (Spörhase-Eichmann *et al.* 1992). Hoyle and Dagan (1978) reported a similar result for locusts. Octopaminergic DUM cells, some of which may be neurosecretory (Bräunig *et al.* 1994), are excited by stimuli that elicit escape jumps. However, because haemolymph octopamine levels do not increase during escape runs in *G. bimaculatus*, it is possible that the increase in DUM cell firing measured by Gras *et al.* (1990) may result in an increase in local levels of octopamine in the neuropile and/or muscle, but not in large quantities being released into the haemolymph. This illustrates the complexity of the octopaminergic system, in which octopamine can act at different levels (i.e. as a neurotransmitter, neuromodulator or neurohormone; Orchard, 1982), and each level may subserve a different function and therefore be used in different behaviours. Octopamine, when released in such a manner as to increase the levels found in the haemolymph, has the potential to affect many more target sites than it does when its release is primarily local. Therefore, these two types of release may represent functionally separate phenomena. For example, Orchard *et al.* (1993) suggest that the octopamine-mediated increases in stretch receptor sensitivity and muscle function during locust flight are probably a function of locally released octopamine and are not caused by octopamine circulating in the haemolymph. Neurohormonal octopamine, they suggest, is more important for stimulating the fat body. In the cockroach, octopamine appears to be released as a neuromodulator during an escape response but not as a neurohormone (Goldstein and Camhi, 1991), even though escape runs in cockroaches are of longer duration than those of crickets (Gras *et al.* 1994). It may be that the neuromodulatory release of octopamine that primes muscle and sensory responsiveness (Evans and Siegler, 1982) will correlate more closely to the concept of a 'fight or flight' system (i.e. a system that is activated non-specifically during intense or novel stimuli) than does neurohormonal octopamine.

Nagao *et al.* (1991) found that the effect of octopamine injected into the haemocoel of post-copulatory crickets was to decrease the time before they began to re-emit calling song. Because we found that post-copulatory crickets had elevated octopamine levels in the presence of conspecifics, it might be expected that post-copulatory males would decrease the time between mating bouts in the presence of other crickets. This does not appear to be the case (Nagao *et al.* 1991). However, to achieve the shortened interbout period, Nagao *et al.* (1991) injected 10^{-4} mol l^{-1} octopamine. Given that the volume of haemolymph in the cricket is about 100 μl, the effective concentration in the animal was at least in the micromolar range. This is two orders of magnitude above levels actually existing in the animal. The effect observed by Nagao *et al.* (1991) may have involved the stimulation of receptors in the central nervous system and may not have been related to the function of octopamine as a neurohormone.

Sensory cues

Antennal contact with a conspecific appears to be an important sensory stimulus for the increase in octopamine levels. Increases occurred when the animal contacted an anaesthetized cricket, suggesting that movement or other behavioural cues are not required. Plastic-coated crickets did not elicit an increase in octopamine levels, suggesting that a visual stimulus is not sufficient. Interestingly, visual stimuli are also not sufficient to induce courtship behaviour (Adamo and Hoy, 1994). Touching a cricket's antenna with a freshly detached antenna can elicit an increase in octopamine level, but a steel probe is ineffective. This suggests that chemoreception of conspecifics may be an important sensory stimulus for the observed increases in haemolymph octopamine levels. *G. bimaculatus* is probably capable of recognizing conspecifics after contacting them. Rence and Loher (1977) have found evidence for contact pheromones in a related cricket, *Teleogryllus commodus*. This sensory stimulus is probably a reliable enough predictor of future activity to have been selected to initiate increases in octopamine levels before there is a need for an increased energy supply.

Possible synergistic interactions

Octopamine levels increase in the haemolymph under some very different conditions, such as when crickets fly (Fig. 6) and when they are infested by parasitoids (S. A. Adamo, C. E. Linn

and R. R. Hoy, personal observations). Do both increases activate the same subset of systems? Or do co-factors modulate the response depending on how the increase in octopamine level is initiated? It is known that taurine reduces the release of octopamine into the haemolymph during handling stress (Hayakawa *et al*. 1987). We found that the changes in the levels of some compounds correlated with only one or two behavioural states (Fig. 1; Table 1). Some of these substances may work synergistically with octopamine and/or other compounds to alter target specificity or to co-modulate their effects. The chemical identities of these compounds await future experiments.

The factor analysis showed that most of the compounds that we could measure had a unique pattern of response to the seven different behavioural states examined. The same chromatograms had over 20 identifiable peaks; we have narrowed down the number of potential candidates for future study to four. This ability to reduce the data set will become more critical as biochemical methods become more comprehensive. By using an ultraviolet detector as well, for example, we could detect many more of the compounds that exist in the haemolymph. Factor analysis is one potential tool for analyzing the large amounts of data now available from modern HPLC machines.

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