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Brain Control of Mating Behavior in the Male Cricket *Gryllus bimaculatus* DeGeer: Excitatory Control of Copulatory Actions

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ABSTRACT—To understand the functional role of the insect brain in mating behavior, copulatory actions in response to a model stimulus were compared between intact and decerebrated male crickets. Decerebrated males were then tested to examine whether their copulatory actions were modifed by biogenic amines or electrical stimulation. The main difference in copulatory actions between intact and decerebrated males was in the body thrusts consisting of the protraction and retraction of the abdomen for hooking. These movements became slower after the removal of the brain, as measured by the average interval between responses, called the cycle length. The cycle length increased to twice the length in intact males within about 20 min. Intraperitoneal injection of octopamine in decerebrated males shortened the cycle length dose-dependently (10^{-6}–10^{-2}M) and restored it nearly to the level in intact males at 10^{-4}–10^{-2}M. Octopamine-mimicking agents forskolin, IBMX and synephrine, and cyclic AMP analogue DB-cyclic AMP had effects similar to that of octopamine, while serotonin, dopamine, noradrenaline and adrenaline did not. Electrical stimulation of the neck connectives mimicked the effect of octopamine, which was blocked by octopamine antagonist phentolamine. Perfusion of the hemocoel with Ringer's solution to eliminate the octopamine previously injected abolished the effect of extrinsic octopamine, whereas it did not abolish the effect of electrical stimulation. These results suggest that the brain in the male cricket is involved in facilitating the activity of the pattern generator for mating behavior via intraganglionic octopamine.

INTRODUCTION

Various aspects of mating behavior in crickets have been studied in different species since the 1950s (Khalifa, 1950; Huber, 1955; Hörmann- Heck, 1957; Alexander, 1961; Beck 1974; Loher and Rence, 1978; Nagao and Shimozawa, 1987; Sakai and Ootsubo, 1988; Sakai *et al*., 1991; Ureshi and Sakai, 2001; Kumashiro and Sakai, 2001). The sexually active male cricket in the mating stage shows copulatory actions in response to the female's mounting his back. Motor actions in the early stage of copulation consists of cercal vibration, backward walking and hooking. These are the responses to tactile contact of the abdomen and cerci with the female (Sakai and Ootsubo, 1988). Similar responses are also elicited by artificial stimulation with a model of the female (Huber, 1955; Sakai and Ootsubo, 1988; Sakai *et al*., 1990; Sakai *et al*., 1995); these responses are called here the "copulation response". We have recently demonstrated that the copulation response is elicited in decerebrated males as well as decapitated males (Sakai *et al*., 1990; Sakai *et al*., 1995; Matsumoto and Sakai, 2000a). Furthermore, we found that males in the post-copulatory sexually refractory stage and also males under heavy stress in the mating stage, which normally do not show the copulation response, exhibited the copulation response as soon as the brain was removed. These results led us to conclude that the nervous system necessary for the execution of copulation is located completely within the thorax and abdomen, and that the brain has a gating function to switch off the local circuits for the pattern generator responsible for copulatory actions by inhibition (Matsumoto and Sakai, 2000a; Matsumoto and Sakai, 2000b).

It has not been examined, however, whether the copulation response preformed by decerebrated males is exactly the same as that in intact males, though they resemble each other. If there are some differences in movement beween them, the brain may play some role in the execution of copulation actions in addition to gating. To answer this, the back and forth horizontal abdominal movements in hooking called body thrusts were quantitatively analyzed during the copulation response in intact and decerebrated males. As the results indicated that the body thrusts were significantly

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slowed by the removal of the brain, the cause of the slowing was examined by amine injection and electrical stimulation of descending neurons of the head ganglia.

MATERIALS AND METHODS

Animals

Crickets, *Gryllus bimaculatus* DeGeer, of both sexes were used. They were reared in a plastic case in a cycle of 12 hr light-dark at 27±2°C, and given access to insect pellets and water *ad libitum*. Males and females were used a minimum of 5 days after the final molt. To elicit copulatory actions efficiently, males were separated from females for at least 24 h before experiments. The posterior parts of the wings were cut off to facilitate observations of the abdominal end.

Tactile stimulation and copulation response

To elicit copulatory actions, a model made of plasticene mimicking a female abdomen was used as a test stimulus with which the abdominal tergites and proximal cerci were stimulated (Matsumoto and Sakai, 2000a). Sexually active males normally responded to a slight contact of the test stimulus by copulatory actions such as cercal vibration, backward walking and hooking. These responses to the test stimulus are called here the copulation response. In our previous studies, the test stimulus was transiently applied to the male to test whether the male exhibited the copulation response or not (Matsumoto and Sakai, 2000a). In the present experiments, however, the test stimulus was tonically applied as long as the male continued to respond, in order to examine the strength and durability of the response.

Decerebration

Animals were cool anesthetized by dipping the head and thorax into ice cold water for about 40 s. Then the brain, corpus allata and corpus cardiacum were removed after the transection of the connectives anterior to the suboesophageal ganglion. This treatment was finished within 20 s.

Drugs

Biological amines and other pharmaceutical agents related to amines were dissolved in insect Ringer's solution (NaCl 150, KCl 9, $CaCl_2 \cdot H_2O$ 5, $NaHCO_3$ 2, glucose 40 $mmol^{-1}$ in distilled water adjusted to pH 7.2 with NaOH) just before use. Fifty microliters of each drug at different concentrations (10^{-9}–10^{-2} M) was injected into the hemocoel near the terminal abdominal ganglion through the base of the hindleg with a syringe. Insect Ringer's alone was used as a control. The following drugs were used: OA, octopamine (DL-octopamine, Sigma); 5-HT, serotonin (5-hydroxytryptamine, Sigma); DA, dopamine (3-hydroxytrytamine, Sigma); NA, noradrenaline (arterenol, Sigma); AD, adrenaline (epinephrine, Sigma); forskolin, adenylate cyclase activator (Sigma); IBMX, phosphodiesterase inhibitor (3-isobutyl-methylxanthine, Sigma); synephrine (N-methylated derivation of octopamine); DB-cyclic AMP, a cyclic AMP analogue (dibutyryl cyclic monophosphate, Sigma); and PA, phentolamine (α-adrenergic receptor antagonist, Sigma).

Electrical stimulation

Bipolar metal electrodes were placed near the left and right connectives in the neck region in a decerebrated male. Electrical stimulation consisting of 10 train pulses (1 ms duration and 2 ms interval) was given at 1 Hz for 30 s. The stimulus intensity was adjusted to a level at which moderate muscle contractions of the abdomen and legs were elicited.

Movement parameters of body thrusts

Three parameters of the movement during body thrusts were measured manually (Fig. 1). Duration was defined as the time from the first copulation response to the last response in a trial. Response number was defined as the number of elicited copulation responses in a trial. Cycle length was defined as the interval between two responses; it was calculated by dividing the duration (t) by the response number minus one (n-1). In most experiments, the data of 5 successive trials from 10 males were used.

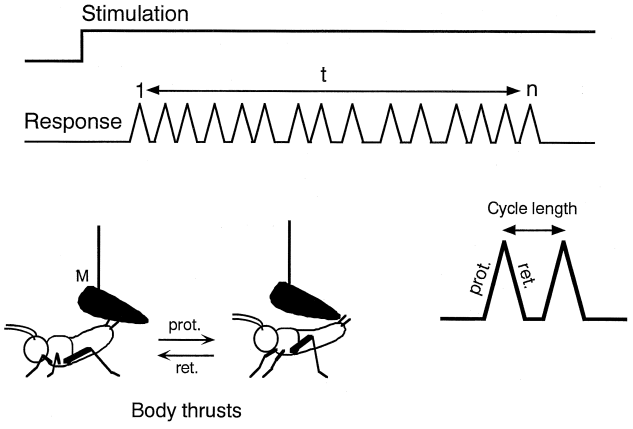

Fig. 1. Movement parameters of the body thrusts. Body thrusts consist of protraction (prot.) and retraction (ret.) of the abdomen with all the legs fixed on the substrate during continuous stimulation of the dorsal regions of the abdomen and cerci with a model (M). Duration was defined as the interval (t) between the first copulation response and the last one. Response number was defined as the number (n) of the repetitive body thrusts in a test trial. Cycle length was defined as the average interval between responses (t/n-1).

Test procedures

In order to obtain a uniform level of sexual excitation, males were allowed to copulate with a female once and then separated from females for more than 1 hr. All the males were already in the mating stage before tests since the sexually refractory stage ends within about 1 hr after copulation (Sakai *et al*., 1995). Before the experiments in decerebrated males, body thrusts at different sexual excitation levels were examined in intact males. Based on the results in these experiments, the copulation response test in intact males was carried out 5 times immediately after the male performed courtship for 10 min. Data were obtained from the following 5 groups.

1) Intact males with drug injection: The copulation response test was started 30 s after Ringer's solution injection (control). A male was allowed to copulate with a given female, and then the copulation response test was carried out again using the same male and female 30 s after octopamine injection. A similar test was further performed twice, 30 s and 10 min after phentolamine injection.

2) Decerebrated males: The copulation response test was started soon after decerebration. Males were repeatedly tested twice every 2 or 10 min.

3) Decerebrated males with drug injection: After decerebration, males were left alone for 20 min. The copulation response test was then carried out 30 s after ringer injection (control), and 5 min later, performed again after drug injection.

4) Decerebrated males with electrical stimulation: After decerebration, males were left for 20 min and then their neck connectives were stimulated electrically for 30 s. The copulation response test was performed at 0, 5, 10 and 20 min after stimulation.

5) Decerebrated males with multiple drug injection or drug injection combined with electrical stimulation. Procedures are described in the figure legends.

Hemocoel washing

To eliminate octopamine in the hemocoel, which was previously injected or supposedly released from the ganglia by electrical stimulation, the hemolymph was washed out by perfusion. A large amount of Ringer's solution was injected into the hemocoel with a syringe and ejected at a rate of 0.5 ml/s through a small hole made on the base of the hindleg. The total amount of Ringer's solution perfused was 5 ml which was 50 times the volume of the hemolymph, supposing that the volume of cricket hemolymph is about 0.1 ml.

Elicitation of evasive running

Males which had been left for 20 min after decerebration, were pinched 5 times with an interval of 1s on the tarsus of a hindleg with tweezers 30 s after the Ringer's solution injection (control). They were allowed to rest 10 min and pinched again 30 s after octopamine injection. In response to pinching, the male quickly ran and then stopped. The distance of the running and its duration were measured.

Data analysis

Data were expressed as medians and confidence intervals (C.I.) at 95%. Statistical analysis was performed using by Mann-Whitney *U* test at a significance level of $P=0.05$.

RESULTS

Intact males

Copulatory actions in intact males: The sexually active male slips backward underneath the female when the female steps on the male's back from behind. During the backward walking, the male vibrates his cerci and gropes the female's sternites with his abdominal tip. These are actions to search for the subgenital plate of the female. When his epiproct (the last abdominal tergite) reaches the female subgenital plate, backward walking stops and hooking starts. Hooking consists of a scooping-like backward protoraction and forward retraction movement of the abdomen with all the leg positions fixed on the substrate and the genitalia everted. It is normally repeated 15 times (n=50) at 0.6/s (n=50) until the epiphallus hooks the subgenital plate. This rhythmic body movement for hooking is called here body thrusts. In the copulation response test employed here, hooking attempts continued for more than 10 s after the start of stimulation because the male was unable to hook the plasticine of the model with the epiphallus (see Fig. 1).

Body thrusts in males at different levels of sexual excitation: Copulatory actions are known to differ depending upon the male's sexual excitation (Sakai *et al.*, 1991; Matsumoto and Sakai, 2000a). Thus, to establish conditions producing excitation levels respresentative of those in intact males, the copulation response test was performed at different time after the male had started courtship with a female. Data were obtained 5 times in 30 males (Fig. 2). The duration was 13.0 s (C.I., 11.5–15.0, n=150) in males at 0 min (< 0 min), 17.0 s (C.I., 15.0–19.0) at 10 min and 18.0 s (C.I., 16.0–19.0) at 20 min after the male began to exhibit courtship. The response numbers were 24 (C.I., 22–28), 34 (C.I., 30–37) and 37 (C. I., 34–40) respectively. The cylce lengths were 0.55 s (C.I., 0.53–0.57), 0.51 s (C.I., 0.50–0.53) and 0.50 s (C.I., 0.49–0.51) respectively.

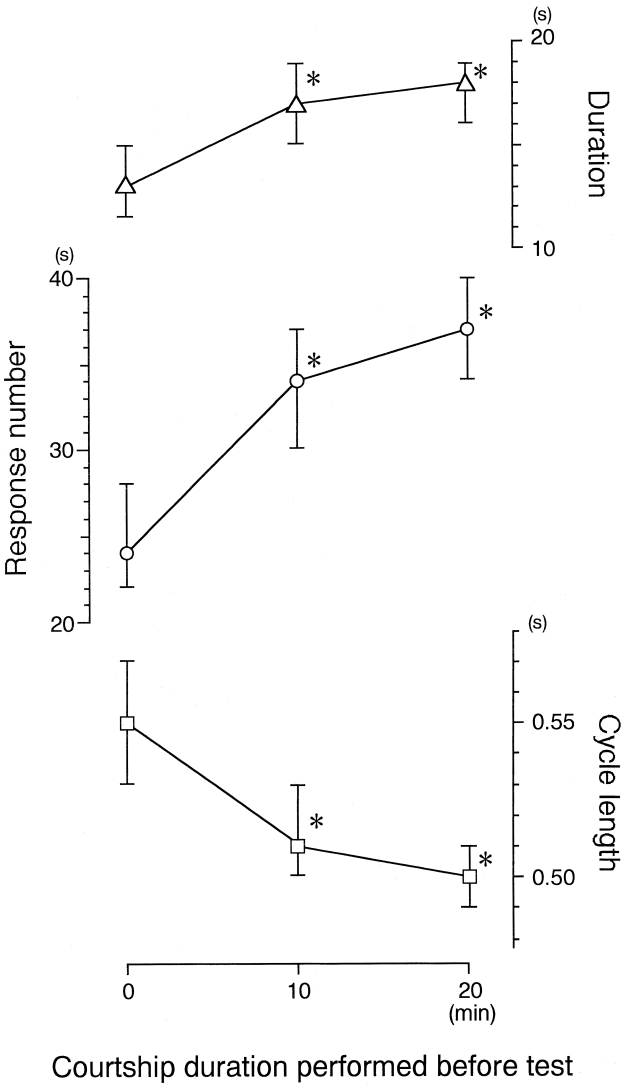

Fig. 2. Changes in the body thrusts in intact males depending upon the duration of courtship performed before the test. Upper panel, Duration; middel panel, Response number; and lower panel, Cycle length. The abscissa indicates the duration of courtship performed before the copulation response test. Each value is a median and each vertical bar is a confidence interval. An asterisk indicates a significant difference compared to the value in males which had performed courtship for only a few seconds (0 min). These conventions also apply in the following figures.

These results indicate that a 10-min courtship increased the duration by 4 s (31%), increased the response number by 10 (42%) and decreased the cycle length by 0.04 s (7%) in comparison with the values at 0 min (<30 s). These values were significantly different from those of the 0-min group but not from those of the 20-min group. Therefore, males which had been allowed to court for 10 min were chosen as representative of intact males and used for all the following experiments.

Decerebrated males

Copulation response in decerebrated males: The copulation response in decerebrated males resembled that in intact males. However, close observation indicated that there were some differences. First, decerebrated males usually did not show any response for a few minutes after the operation possibly due to acute shock (see Matsumoto and Sakai, 2000a). Second, they tended to walk backward even with a slight contact of the abdomen with a model, which was rarely seen in intact males. For this reason, the stimulus position had to be adjusted as the male passed by backwards. Third, they had difficulty initiating motor actions even though the model was placed appropriately on the male's back. Slightly changing the position of the stimulus or vibrating the stimulus was effective in eliciting the response. Fourth, hooking in the decerebrated males was not accompanied by genitalia eversion. This is due to the decrease in hydrostatic pressure of the hemolymph (Kumashiro and Sakai, 2001). Finally, the copulation response in decerebrated males was as vigorous as in intact males in the beginning after the operation, but less vigorous later on.

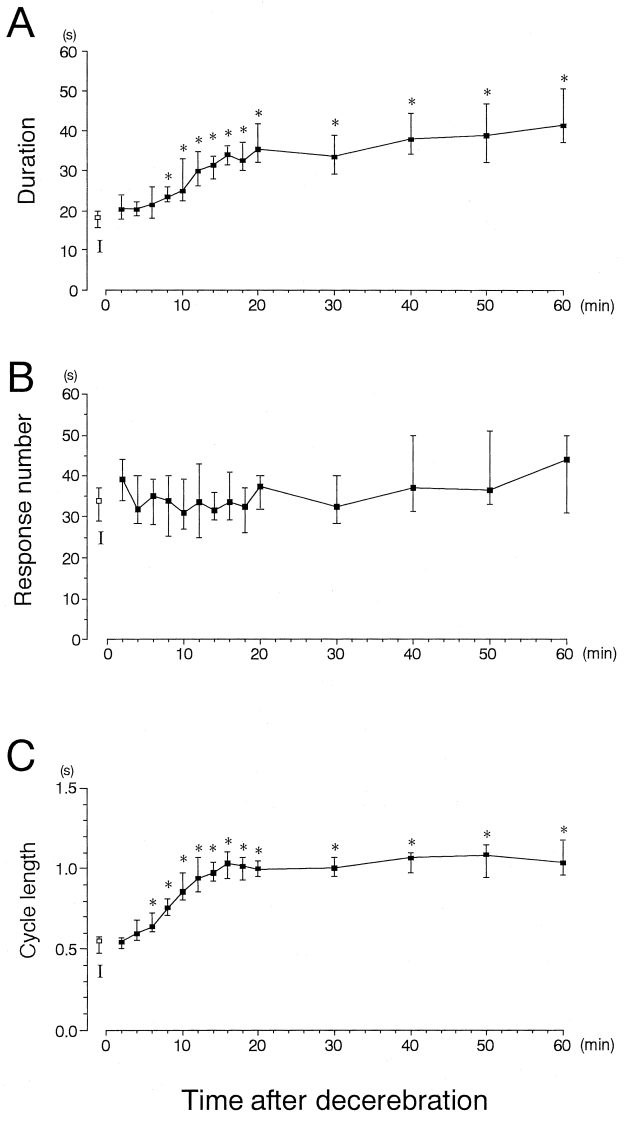

Fig. 3. Changes in the body thrusts in decerebrated males after the operation. A, Duration. B, Response number. C, Cycle length. I indicates intact males and asterisks indicate significant differences compared to the value in intact males.

Body thrusts in decerebrated males: Three movement parameters of the body thrusts in 10 decerebrated males are shown in Fig. 3. Duration gradually increased after decerebration (Fig. 3A) . It was 21.5 s (C.I., 17.5–24.0, n=20) at 2 min after the operation, 23.5 s (C.I., 22.0–26.0) at 8 min and 34.0 s (C.I., 31.5–36.0) at 16 min. The duration at 8 min was significantly longer than that in inact state (18.5 s; C.I., 16.0–20.0). Their response number was not significantly changed after the operation compared to that in intact males (34; C.I., 29–37) (Fig. 3B). The cycle length was 0.55 s (C.I., 0.51–0.57) at 2 min, which was not significantly different from the 0.56 s (C.I., 0.48–0.58) in intact males (Fig. 3C). It gradually increased to 0.64 s (C.I., 0.61–0.72) at 6 min and 0.93 s (C.I., 0.85–1.05) at 12 min both of which were significantly longer than that in the intact state. However, the extent of slowing plateaued within the next 10 min.

These results indicate that the body thrusts were not different in intact and decerebrated males shortly after decerebration, but their cycle length was increased 100% within about 20 min after decerebration. Accordingly, only cycle length was examined in the following experiments.

Intraperitoneal injection of drugs in decerebrated males

Various amine- or octopamine- mimicking agents were administered to 10 decerebrated males each (Fig. 4). Octopamine significantly shortened the cycle length from 1.14 s (C.I., 1.07–1.26, n=50) in the control (20 min after decerebration) to 0.91 s (C.I., 0.79–0.96) after injection at 10^{-6}M, and to 0.49 s (C.I., 0.43–0.50) at 10^{-2}M (Fig. 4A). The dose-response relationship was present. The cycle length reached approximately the level in intact males at 10^{-4}–10^{-2}M. In contrast, four other amines, serotonin (Fig. 4B), dopamine (Fig. 4C), noradrenaline (Fig. 4D) and adrenaline (Fig. 4E), did not change cycle length.

As only octopamine was found to be potent, three agents (forskolin, IBMX and synephrine)whose effect is known to mimic the effect of octopamine via the activation of cyclic AMP and a cyclic AMP analogue DB-cyclic AMP (Evans, 1984; Lange and Orchard, 1986) were used. Forskolin shortened the cycle length significantly from 1.19 s (C.I., 1.08–1.23, n=50) in the control to 0.59 s (C.I., 0.56–0.63) after injection at 10^{-4}M (Fig. 5A); IBMX from 0.91 s (C.I., 0.85–1.00) to 0.44 s (C.I., 0.41–0.46) at 10^{-4}M (Fig. 5B); synephrine from 1.02 s (C.I., 0.93–1.16) to 0.52 s (C.I., 0.50–0.53) at 10^{-2}M (Fig. 5C); and DB-cyclic AMP from 1.32 s (C.I., 1.19–1.38) to 0.59 s (C.I., 0.56–0.62) at 10^{-4}M (Fig. 5D). These results indicated that octopamine mimicking agents had dose-dependent effects similar to those of octopamine, and could induce recovery of the movement in intact males at higher concentrations.

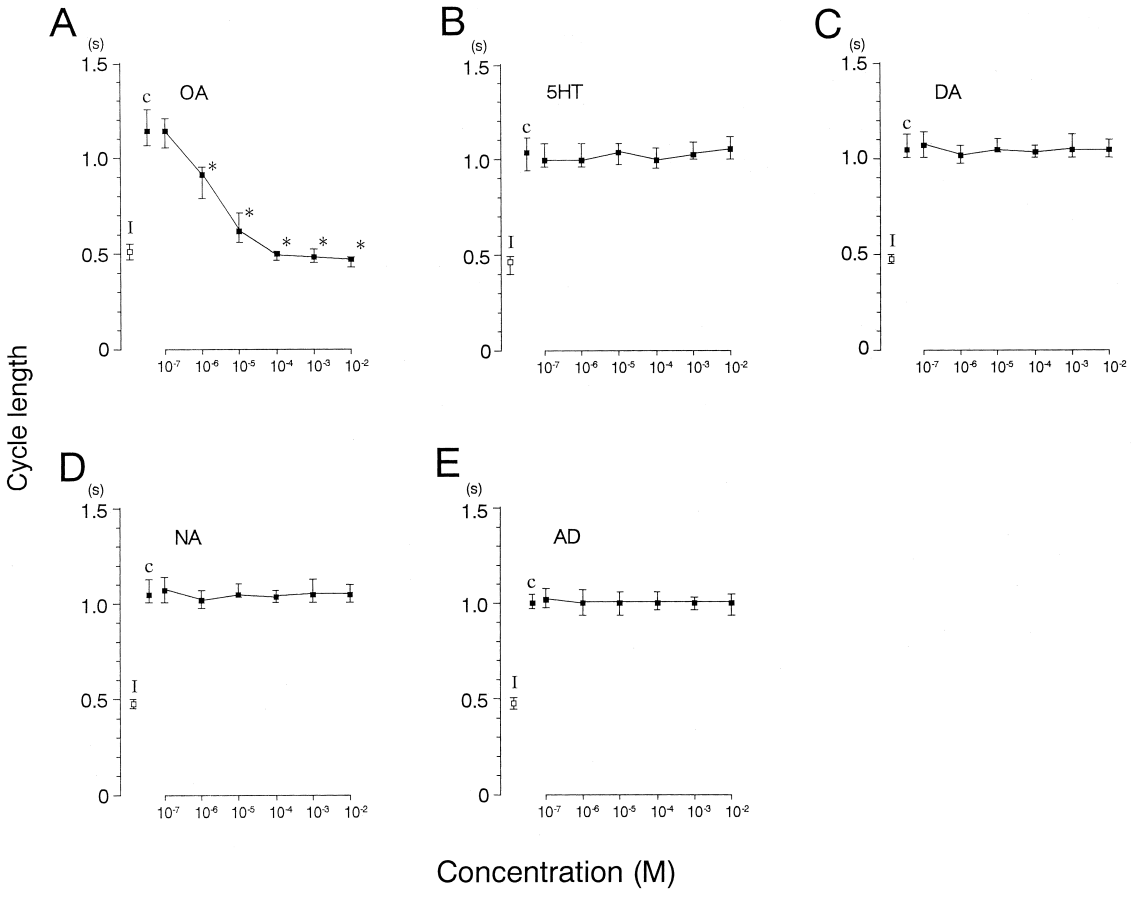

Fig. 4. Effects of biogenic amines on cycle length. A, octopamine (OA). B, serotonin (5-HT). C, dopamine (DA). D, noradrenaline (NA). E, adrenaline (AD). Abscissa, Concentration of amines. I indicates intact males and c indicates control males which were injected with Ringer's solution 20 min after decerebration. Asterisks indicate significant differences compared to the control. These conventions also apply in the following figures.

Electrical stimulation

In order to activate the axons of descending neurons of the head ganglia, electrical stimulation was applied to the neck connectives in 10 decerebrated males. The cycle length was shortened significantly from 1.10 s (C.I., 1.07–1.25, n=50) in the control to 0.54 s (C.I., 0.52–0.59) at 30 s after stimulation (Fig. 6). This was close to the 0.50 s (C.I., 0.48–0.53) in intact males. However, the cycle length returned to 0.96 s (C.I., 0.89–1.00) at 5 min and 1.06 s (C.I.,1.00–1.13) at 10 min after stimulation. These results indicate that electrical stimulation mimicked the effect of octopamine.

Electrical stimulation combined with phentolamine

Electrical stimulation of the connectives had an effect similar to that of octopamine, i.e., an increase of the speed of body thrusts in decerebrated males. The effect of electrical stimulation may have been caused by the release of octopamine as a result of the activation of descending neurons running through the neck connectives. To examine this possibility, electrical stimulation was performed together with injection of an octopamine antagonist, phentolamine. As shown in Fig. 7, electrical stimulation alone shortened the cycle length from 1.35 s (C.I., 1.23–1.46, n=50) in the control to 0.56 s (C.I., 0.52–0.62) which is close to the 0.54 (C.I., 0.50–0.57) in intact males. Treatment with phentolamine nullified the effect of electrical stimulation, as shown by the cycle length of 1.21 s (C.I., 1.05–1.36), which was not significantly different from that of the control. This result is consistent with the possibility that the effect of electrical stimulation is mediated by octopamine.

Effects of washing the hemocoel following octopamine injection or electrical stimulation

Octopamine is known to be released from the central nervous system into the hemolymph when insects are excited (Bailey *et al.*, 1983; Woodring *et al.*, 1988; Adamo *et al.*, 1995). Electrical stimulation may cause the release of octopamine into the hemolymph after the activation of the central nervous system, and then octopamine that permeated into the ganglia through the sheath may act as a neurohormone on neurons responsible for copulation. To examine this possibility, octopamine injection or electrical stimulation was combined with hemocoel washing.

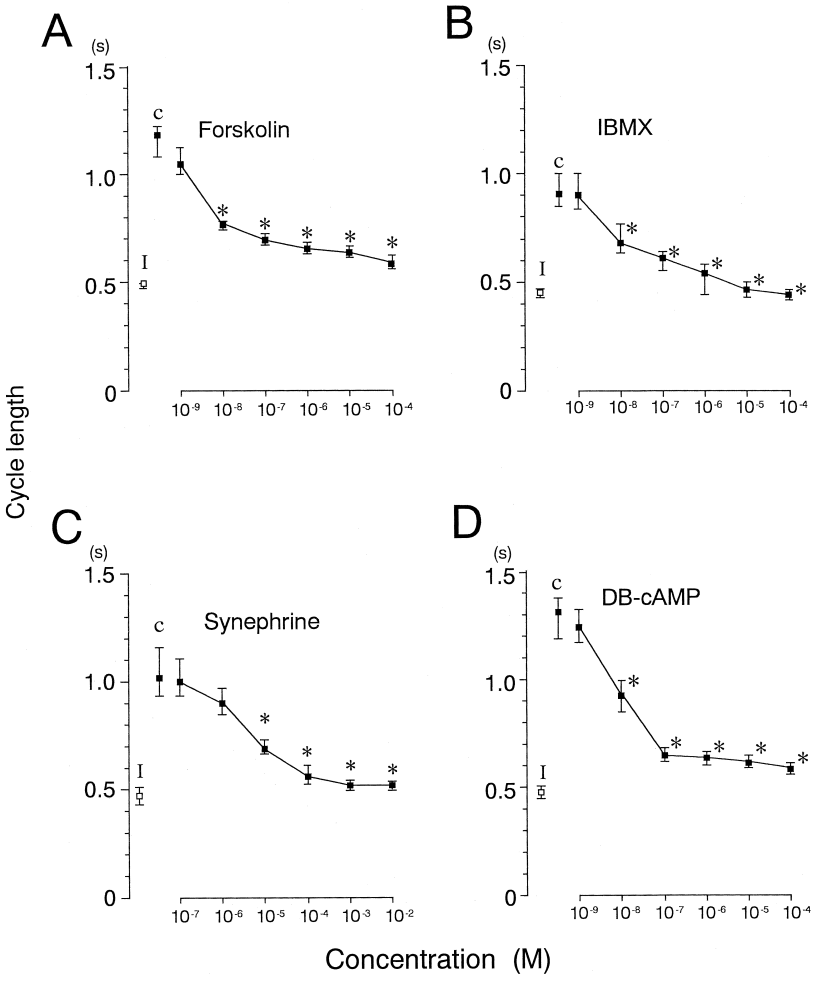

Fig. 5. Effects of agents which mimic the effect of octopamine. A, forskolin. B, IBMX. C, synephrine. D, DB-cAMP.

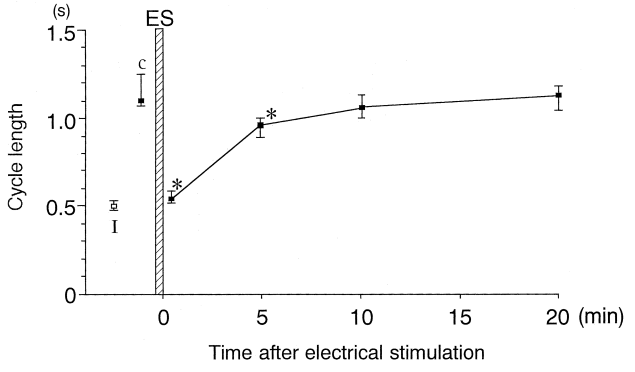

Fig. 6. The effect of electrical stimulation of the neck connectives on cycle length. Electrical stimulation (ES) was applied 20 min after decerebration.

First, decerebrated males were tested 10 s after perfusion with Ringer's solution, and 10 min later they were tested again 30 s after octopamine (10^{-2}M) injection, and then 10 min later tested again 30 s after washing the hemolymph following the octopamine injection. The results indicated that washing out the hemolymph did not change the cycle length, as shown by the cycle lengths of 1.00 s (C.I., 0.96–1.07) in the control and 0.98 s (C.I., 0.92–1.06) after perfusion (Fig. 8A). Octopamine injection shortened the cycle length to 0.50 s (C.I., 0.47–0.52) and subsequent washing restored it to 0.95 s (C.I., 0.90–1.00), indicating that perfusion successfully washed out octopamine from the hemolymph (Fig. 8A).

On the other hand, electrical stimulation shortened the cycle length from 1.13 (C.I., 1.04–1.30) in the control to 0.52 s (C.I., 0.50–0.58), which was not significantly different from the 0.47 s (C.I., 0.46–0.50) in intact males (Fig. 8B). In contrast to the effect of octopamine injection, the cycle length was not changed by perfusion; it was 0.53 s (C.I., 0.50–0.56) which was not significantly different from the pre-perfusion level. That is, the effect of electrical stimulation remained even after the elimination of octopamine.

Intraperitoneal injection of octopamine or phentolamine in intact males

So far, the experiments were conducted on decerebrated males. Next, octopamine (10^{-2}M) was injected into 10 intact males. As shown in Fig. 9, controls which were

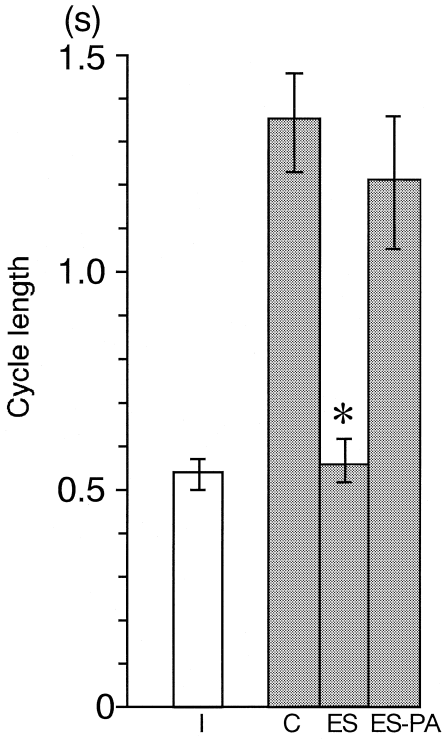

Fig. 7. Cancellation of the effect of electrical stimulation on cycle length by phentolamine. Males were tested first (I) in the intact state, tested again (C) 20 min after decerebration, then tested (ES) immediately after electrical stimulation. Thirty minutes later, they were tested again (ES-PA) after phentolamine injection following electrical stimulation. The asterisk indicates a significant difference between controls and experimentals. This convention also apply in the following figures.

injected with Ringer's solution did not differ from males with no injection: the cycle lengths were 0.44 s (C.I., 0.42–0.46, n=50) and 0.44 s (C.I., 0.43–0.47), respectively. Octopamine shortened the cycle length slightly to 0.40 s (C.I., 0.38–0.42). On the other hand, phentolamine significantly increased the cycle length from 0.44 s (C.I., 0.42–0.46) to 0.73 s (C.I., 0.69–0.77), an increase of nearly 50%. In the second test, which was carried out 10 min later without phentolamine (10^{-4}M), the cycle length was 0.43 s (C.I., 0.38–0.44), showing no effect of the phentolamine previously applied. Electrical stimulation was not performed because it might have resulted in complicated effects owing to inhibition via the brain due to heavy stress (Matsumoto and Sakai, 2000a). These results indicated that octopamine slightly shortened the cycle length in intact males, while the octopamine antagonist phentolamine had a major effect similar to that of decerebration.

Evasive running in decerebrated males injected with octopamine

To examine whether octopamine may facilitate motor actions other than the body thrusts, running performed by reciprocal leg movements was analyzed. To elicit evasive running, a nociceptive stimulus was given to the tarsus of a hindleg in 20 males 20 min after decerebration. The distance of the initial run and its speed were compared 5 times between the controls and the experimental males treated with octopamine (Fig. 10). The control males ran 5.5 cm (C.I., 5.0–6.5, n=100) and the experimental males also ran 5.5 cm (C.I., 4.5–7.5) (Fig. 10A). The speed of running was

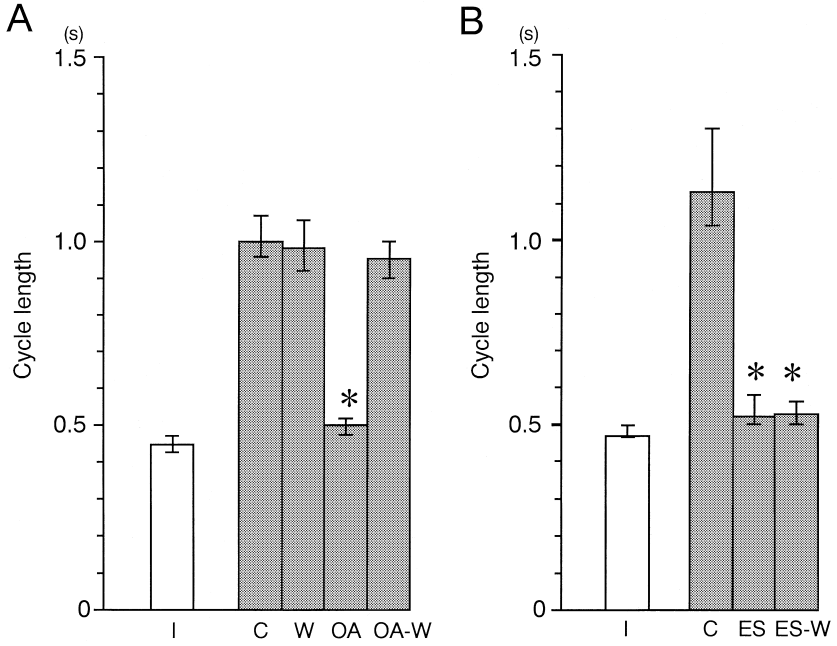

Fig. 8. Effects of hemocoel washing on cycle length in males treated with octopamine or electrical stimulation. A, The males were tested (C) 20 min after decerebration, and a few minutes later were tested again (W) 10 s after hemocoel washing. They were subsequently tested (OA) 30 s after OA (10^{-2}M) injection, and 30 min later, tested again (OA-W) 10 s after washing following OA injection. B, Males were tested (C) 20 min after decerebration, then tested (ES) immediately after electrical stimulation, and 30 min later tested again (ES-W) immediately after washing following electrical stimulation.

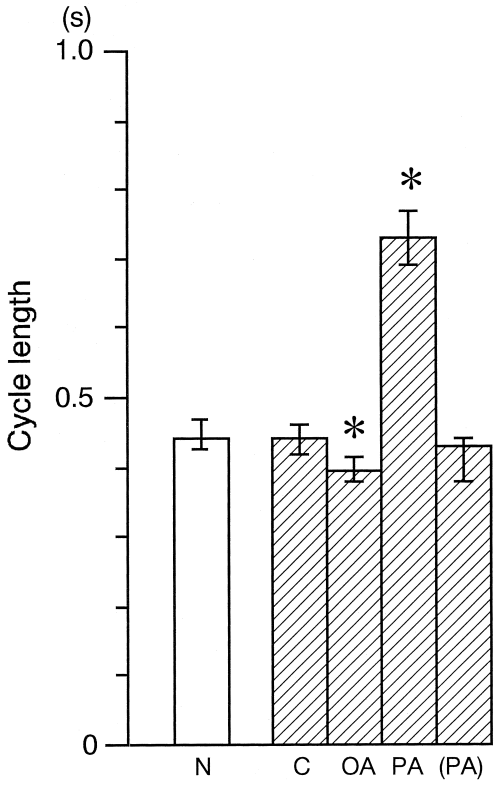

Fig. 9. Effects of octopamine and phentolamine on cycle length in intact males. Males were first tested without injection (N). They were allowed to copulate with females, and about 1 hr later were tested (C) 30 s after Ringer's solution injection. Then they were allowed to copulate with females, and 1 hr later were tested (OA) 30 s after octopamine injection (10^{-2}M). A similar procedure was performed (PA) with phentopamine (10^{-4}M). Finally, 10 min later, males were tested ((PA)) with no injection.

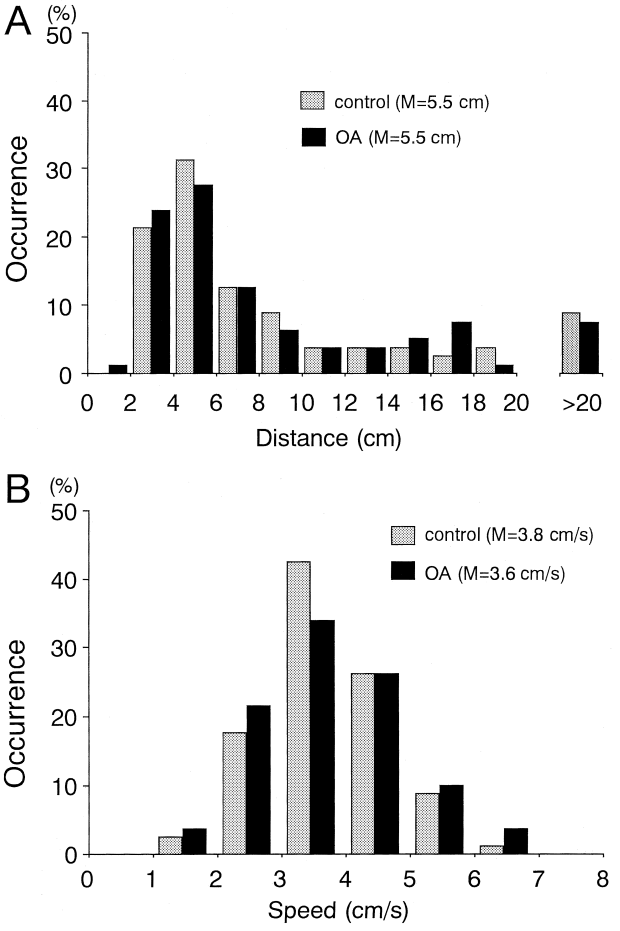

Fig. 10. The effect of octopamine on evasive running. Males were left for 20 min after decerebration. They were tested 30 s after ringer injection (control), and 10 min later, tested again (OA) 30 s after OA (10^{-2}M) injection. A The distance run in response to pinching a hindleg. B The speed of running. Gray bar, males injected with ringer (controls). Black bar, males injected with OA (experimentals). M indicates median. No significance was present in running distance or speed between controls and experimentals.

3.8 cm/s (C.I., 3.5–4.0, n=100) and 3.6 cm/s (C.I., 3.4–4.1) respectively (Fig. 10B). No difference was found in the running distance or speed between the control and experimental males. Thus, octopamine had no effect on evasive running.

DISCUSSION

The brain plays a key role in behavior in insects as well as vertebrates. Empirical findings show that decerebrated crickets do not fly, swim, run away from the wind or stridulate, although they jump and run in response to strong nociceptive stimuli such as leg pinching. This can be explained by the fact that command signals to start adaptive behaviors are derived from the brain (Burrows, 1996). However, there is one exception, reproductive behavior. Some orthopteran insects such as praying mantises (Roeder *et al.*, 1960), cockroaches (Roeder *et al.*, 1960; Grossman and Parnas, 1973), crickets (Carrow *et al.*, 1982) and locusts (Thompson, 1986; Facciponte and Lange, 1996) show longlasting spontaneous rhythmic movements of the abdomen and genitalia when they are decapitated or when their connectives are transected. These are the components of motor actions for copulation or oviposition. Furthermore, decapitated male mantises and crickets show more complex and well-coordinated motor actions for copulation when they are placed with a female or stimulated with a female model (Fabre, 1910; Aoki, 1927; Roeder, 1935; Roeder 1937; Huber, 1955; Roeder *et al.*, 1960). This may be because the copulatory mechanism is essentially local: motor actions are performed with the legs, abdomen, cerci and genitalia as a response to mechanical stimulation of a specific region on the abdomen and cerci, and copulation is accomplished by a chain reaction (Sakai and Ootsubo, 1988). Thus, the brain may be unnecessary for the execution of copulation.

In contrast, it was found that the cricket brain plays a key role in gating the neural circuits for copulation by inhibition. These circuits are swiched off when the male extrudes the spermatophore at the final stage of copulation or is under heavy stress in the mating stage (Matsumoto and Sakai, 2000a). However, is the functional role of the brain in copulation only gating ? If that is the case, the movements

in the copulation response in decerebrated males should be virtually the same as those in intact males. On the contrary, if the brain participates in controlling the performance of copulation, some deficits should arise in decerebrated males. Our results have shown that the latter is the case.

Copulatory actions in decerebrated males

Although the copulation response in decerebrated male crickets resembles that in intact males, the body thrusts for hooking, consisting of the protraction and retraction of the abdomen, were found to be slower in decerebrated males than in intact males, as shown by the increase of the cycle length. This slowing of the repetitive actions gave one the impression that the copulation response became less vigorous. No other differences were observed in the posture or movement pattern during the copulation response. The increase in cycle length began within a few minutes after decerebration and reached a maximum (100% increase) in about 20 min. This increase of cycle length does not seem to be due to the progressive deterioration of the physical condition leading to acute death because decerebrated males usually continued to exhibit the copulation response for more than 1 hr after the operation (Matsumoto and Sakai, 2000a). In fact, in most of the males, the movement was restored to that in intact males by some treatments, as described below.

These results suggest that the activity of the pattern generator for the body thrusts is maintained by some excitatory factors which are constantly supplied by the spontaneous activity of the brain. In other words, the slowing of the body thrusts occurs when the excitatory factors are depleted by the interruption of communication between the brain and pattern generator. Although electrical stimulation activates axons of both the brain and suboesophageal ganglion neurons, the former plays a major role in facilitation because electrical stimulation restored the speed of the body thrusts which had been maintained before the removal of the brain.

In addition, decerebrated males tended to show excessive backward walking and less sensitivity to contact stimulation. These findings suggest that the brain may also be involved in inhibition of backward walking and facilitation of the receptivity of mechanoreceptors.

Octopamine as an excitatory factor for body thrusts

It has been established that octopamine acts as a neurotransmitter, neuromodulator and hormone (Evans, 1980; Orchard and Loughton 1981; Orchard 1982; Orchard *et al*., 1993; Roeder, 1999). Injection of octopamine induces specific behaviors in invertebrates, for example, defensive flexion posture in lobsters (Livingstone *et al*., 1980) and flight in locusts (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987). Octopamine also modifies various types of motor actions (Long and Murdock, 1983; Harris-Warrick,1985; Mulloney *et al*., 1987; Goldstein and Camhi, 1991; Weisel-Eichler and Libersat, 1996; Parker, 1996), and has thus been likened to adrenaline in mammals (Hoyle, 1975). So far, however, no reports have been available on the effect of octopamine on insect copulation. Our results indicated that octopamine restored the decreased speed of the body thrusts after decerebration to the level of the intact state. In addition, some agents mimicking octopamine, namely, forskolin, IBMX, synephrine and DB-cyclic AMP, were also as effective as octopamine, while other amines such as serotonin, dopamine, noradrenaline and adrenaline were not. These findings suggest that the basic activity of the pattern generator for copulatory actions in the male cricket is specifically maintained by octopamine.

In contrast to its effects in decerebrated males, octopamine had only a minor effect on the body thrusts in intact males: octopamine shortened the cycle length by only 9% in intact males while it shortened it by nearly 100% in decerebrated males. In the experiments to examine the effect of sexual excitation on the body thrusts in intact males, the cycle length was shortened only 7% by a 10-min courtship. These results may be explained by the fact that octopamine is constantly released into the pattern generator to maintain the basic level of activity in intact males. However, there may be an upper limit of the speed of the back-and-forth movements such as body thrusts and thus they cannot be accelerated so much in intact males. On the other hand, octopamine antagonist phentolamine acted to similar extents in intact and decerebrated males. This strengthens our hypothesis that the effect of decerebration is caused by the depletion of octopamine.

Electrical stimulation of the neck connectives in decerebrated males caused a similar effect on the body thrusts as octopamine. This facilitation may be mediated by octopamine because additional application of phentolamine cancelled the effect of electrical stimulation.

Although the body thrusts were facilitated by octopamine, it is not certain to what extent the octopamine effect is specific. To answer this, various types of motor actions have to be tested with octopamine. At least, however, evasive runnning was not facilitated by octopamine.

Does octopamine act on neurons as neurohomones?

The central nervous system in insects has octopaminergic neurons such as dorsal unpaired median (DUM) neurons and some neurons with ventrally located small somata (Dymond and Evans, 1979; Pflüger and Watson 1988; Konings *et al*., 1988; Ferber and Pflüger 1990; Gras *et al*., 1990; Eckert *et al*., 1992; Stevenson *et al*., 1992; Spörhase-Eichmann *et al*., 1992; Bräunig *et al*., 1994; Stevenson and Spörhase-Eichmann, 1995; Duch and Pflüger H-J, 1999). These octopaminergic neurons release octopamine centrally as well as peripherally (Watson, 1984).

On the other hand, it is known that octopamine in the hemolymph increases rapidly after insects fly (Goosey and Candy, 1980; Bailey *et al*., 1983; Woodring *et al*., 1988; Adamo *et al*., 1995) or fight (Adamo *et al*., 1995). A similar increase of octopamine occurred when female male crickets were forced to run (Woodring *et al*., 1988) or male crickets were bought into contact with a conspecific male (Adamo *et*

al., 1995). Extraganglionic octopamine may partly contribute to facilitating copulatory actions in the male cricket by modifying the muscle contractility (Evans and O'Shea, 1978; O'Shea and Evans, 1979; Evans and Siegler, 1982) or/and sensory receptivity (Pasztor and Bush, 1989; Ramirez and Orchard, 1990; Ramirez *et al*., 1993; Matheson, 1997; Pophof, 2000), although the sources of such octopamine have not been determined yet. However, the extraganglionic octopamine concentration in the hemolymph was very low (10^{-8}–10^{-7}M) even at the peak of its increase during animal's excitation (Bailey *et al*., 1983; Woodring *et al*., 1988; Adamo *et al*., 1995), which was much lower than the level of octopamine used for intraperitoneal injection in the present study. The minimum concentration of octopamine for the facilitation of the body thrusts was 10^{-6}M and the maximum effect was obtained at 10^{-4}–10^{-2}M. Thus, it does not seem possible that octopamine which had been released into the hemolymph from the central nervous system entered the ganglia and acted as a hormone on the pattern generator for copulatory actons.

It should be noted that the effect of octopamine was lost when injected octopamine was washed out with Ringer's solution (octopamine at a high concentration (10^{-2}M) would certainly permeate the ganglia through the sheath, act on neurons in the ganglia and then be quickly eliminated). In contrast, the facilitation induced by electrical stimulation, which may have been mediated by octopamine, was not lost after perfusion. In this case, intraganglionically released octopamine should have been eliminated together with extraganglionic octopamine. Nevertheless, the facilitatory effect was not affected at all. This may be explained by the possibility that electrical stimulation potentiated octopaminergic neurons even for some time after the stimulation and subsequent perfusion were terminated, so that octopamine was continuously released and accumulated within the ganglia. However, the possibility that octopamine intraganglionically released by electrical stimulation may somehow be prevented from being eliminated by perfusion cannot be excluded.

We conclude that octopamine, intraganglionically released by the activity of descending brain neurons, produces the basic activity of the pattern generator for copulation actions. This is the first demonstration that the brain is involved in excitatory control of mating behavior in insects.

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REFERENCES

Adamo SA, Linn CE, Hoy RR (1995) The role of neurohormonal octopamine during 'fight or flight' behavior in the field cricket *Gryllus bimaculatus*. J Exp Biol 198: 1691–1700

Alexander RD (1961) Aggressiveness, territoriality, and sexual behaviour in the field crickets (Orthoptera: Gryllidae). Behaviour 17: 130–223

Aoki B, Tateishi S (1927) On the copulatory behavior of Japanese mantis (*Tenodera aridifolia* Stoll.), and the seat of its nerve centre (Prerimiary note). Zool Mag (in Japanese) 39: 114–129

Bailey BA, Martin RJ, Downer RGH (1983) Haemolymph octopamine levels during and following flight in the American cockroach, *Periplaneta americana* L. Canad J Zool 62: 19–22

Beck R (1974) The neural and endocrine control of mating behavior in the male cricket *Acheta domesticus.* L. University of Nottingham, Doctoral thesis.

Bräunig P, Stevenson PA, Evans P (1994) A locust octopamine-immunoreactivtive dorsal unpaired median neurone forming terminal networks on sympathetic nerves. J Exp Biol 192: 225–238

Burrows M (1996) The neurobiology of an insect brain. Oxford Univeristy Press, Oxford

Carrow GM, Cabeza RdeJ, Flores G (1982) Isolation of the abdomen releases oviposition behaviour in females of the cricket, *Acheta domesticus*. J Insect Physiol 28: 401–404

Duch C, Pflüger H-J (1999) DUM neurons in locust flight: a model system for amine-mediated peripheral adjustments to the requirements of a central motor program. J Comp Physiol A 184: 489–499

Dymond GR, Evans PD (1979) Biogenic amines in the nervous system of the cockroach, *Periplaneta americana*: association of octopamine with mushroom bodies and dorsal unpaired median (DUM) neurones. Insect Biochem 9: 535–545

Eckert M, Rapus J, Nürnberger A, Penzlin H (1992) A new specific antibody reveals octopamine-like immunoreactivity in cockroach ventral nerve cord. J Comp Neurol 322: 1–15

Evans (1980) Biogenic amines in the insect nervous system. Adv Insect Physiol 15: 317–474

Evans PD (1984) A modulatory octopaminergic neurone increases cyclic nucleotide levels in locust skeletal muscle. J Physiol 348: 307–324

Evans PD, O'Shea (1978) The identification of an octopaminergic neurone and the modualtion of a myogenic rhythm in the locust. J Exp Biol 73: 235–260

Evans PD, Siegler MV (1982) Mediated relaxation of octopamine maintained and catch tension in locust skeletal muscle. J Physiol 93–112

Fabre JH (1910) Souvenirs Entomologiques. Vol. 5, DeLagrave Paris

Ferber M, Pflüger H-J (1990) Bilaterally projecting neurones in pregenital abdominal ganglia of the locust: anatomy and peripheral targets. J Comp Neurol 302: 447–460

Facciponte G, Lange AB (1996) Control of the motor pattern generator in the VII abdominal ganglion of *Locusta*: Descending neural inhibition and coordination with the oviposition hole digging central pattern generator. J Insect Physiol 42: 791–798

Goldstein RS, Camhi JM (1991) Different effects of the biogenic amines dopamine, serotonin and octopamine on the thoracic and abdominal protions of the escape circuit in the cockroach. J Comp Physiol A 168: 103–112

Goosey MW, Candy DJ (1980) The D-octopamine content of the haemolymph of the locust, *Schistocerca americana gregaria* and its elevation during flight. Insect Biochem 10: 393–397

Gras H, Hörner M, Runge L, Schürmann F-W (1990) Prothoracic DUM neurons of the cricket *Gryllus bimaculatus*–responses to natural stimuli and activity in walking behavior. J Comp Physiol A 166: 901–914

Grossman Y, Parnas I (1973) Control mechanisms involved in the regulation of the phallic neuromuscular system of the cockroach *Periplaneta americana*. J Comp Physiol 82: 1–21

Harris-Warrick RM (1985) Amine modulation of extension command

element-evoked motor activity in the lobster abdomen. J Comp Physiol 156: 875–884

Hörmann-Heck S (1957) Untersuchungen über den Erbgang einiger Verhaltensweisen bei Grillenbastarden (*Gryllus campestris* L., *Gryllus bimaculatus* DeGeer). Z Tierpsychol 14: 137–183

Hoyle G (1975) Evidence that insect dorsal unpaired median (DUM) neurons are octopaminergic. J Exp Zool 193: 425–431

Huber F (1955) Sitz und Bedeutung nevöser Zentren für Instinkthandlungen bein Männchen von *Gryllus campestris*. L. Z Tierpsychol 12: 12–48

Khalifa A (1950) Sexual behaviour in *Gryllus domesticus* L. Behaviour 2: 264–274

Konings PMN, Vullings HGB, Geffard M, Buijs RM, Diederen JHB, Jansen WF (1988) Immunocytochemical demonstration of octopamine-immunoreactive cells in the nervous system of *Locusta migratoria* and *Schistocerca gregaria*. Cell Tissue Res 251: 371–379

Kumashiro M, Sakai M (2001) Reproductive behaviour in the male cricket *Gryllus bimaculatus* DeGeer. I Structure and function of the genitalia. J Exp Biol 204: 1123–1137

Lange AB, Orchard L (1986) Identified octopaminergic neurons modulate contractions of locust visceral muscle via adenosine 3'5'-monophosphate (cyclic AMP). Brain Res 363: 340–349

Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce oppsite postures in lobsters. Science 208: 76–79

Loher W, Rence B (1978) The mating behaviour of *Teleogryllus commodus* (Walker) and its central and peripheral control. Z Tierpsychol 46: 225–259

Long TF, Murdock LL (1983) Stimulation of blowfly feeding behavior by octopaminergic drugs. Proc Natl Acad Sci USA 80: 4159–4163

Matheson T (1997) Octopamine modulates the reponses and presynaptic inhibition of proprioceptive sensory neurons in the locust *Schistocerca Gregaria*. J Exp Biol 200: 1317–1325

Matsumoto Y, Sakai M (2000a) Brain control of mating behaviour in the male cricket *Gryllus bimaculatus* DeGeer: the center for inhibition of copulation actions. J Insect Physiol 46: 527–538

Matsumoto Y, Sakai M (2000b) Brain control of mating behaiour in the male cricket *Gryllus bimaculatus* DeGeer: brain neurons responsible for inhibition of copulation actions. J Insect Physiol 46: 539–552

Mulloney B, Acevedo LD, Bradbury AG (1987) Modulation of the crayfish swimmeret rhythm by octopamine and neuropeptide proctolin. J Neurophysiol 58: 584–597

Nagao T, Shimozawa T (1987) A fixed time-interval between two behavioural elements in the mating behaviour of male crickets, *Gryllus bimaculatus*. Anim Behav 35: 122–130

Orchard I (1982) Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. Can J Zool 60: 659–669

Orchard I, Loughton BG (1981) Is octopamine a transmitter mediating hormone released in insects? J Neurobiol 12: 143–153

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

O'Shea M, Evans PD (1979) Potentiation of neuromuscular transmission by an octopaminergic neruone in the locust. J Exp Biol 79: 169–190

Parker D (1996) Octopaminergic modulation of locust motor neurons. J Comp Physiol A 178: 243–252

Pasztor VM, Bush BM (1989) Primary afferent responses of a crustacean mechanoreceptor are modulated by proctolin, octopamine, and serotonin. J Neurobiol 20(4): 234–254

Pflüger H-J, Watson AHD (1988) Structure and distribution of dorsal unpaired median (DUM) neurones in the abdominal nerve cord of male and female locusts. J Comp Neurol 268: 329–345

Pophof B (2000) Octopamine modulates the sensitivity of silkmoth pheromone receptor neurons. J Comp Physiol A 186: 307–313

Ramirez J-M, Büschges A, Kittmann R (1993) Octopaminergic modulation of the femoral chordotonal organ in the stick insect. J Comp Physiol A 173: 209–219

Ramirez J-M, Orchard I (1990) Octopaminergic modulation of the forewing stretch receptor in the locust *Locusta migratoria*. J Exp Biol 149: 255–279

Roeder DK (1935) An experimental analysis of the sexual behavior of the praying mantis (M. L.). Biol Bull 69(2): 203–220

Roeder DK (1937) The control of tonus and locomotor activity in the praying mantis (*Mantis religiosa* L.). J Exp Zool 76: 353–374

Roeder DK, Tozian L, Weiant EA (1960) Endogenous nerve activity and behavior in the mantis and cockroach. J Insect Physiol 4: 45–62

Roeder T (1999) Octopamine in invertebrates. Prog Neurobiol 59: 533–561

Sakai M, Katayama T, Taoda Y (1990) Postembryonic development of mating behavior in the male cricket *Gryllus bimaculatus* DeGeer. J Comp Physiol A 166: 775–784

Sakai M, Matsumoto Y, Takemori N, Taoda Y (1995) Post-copulatory sexual refractoriness is maintained under the control of the terminal abdominal ganglion in the male cricket *Gryllus bimaculatus* DeGeer. J Insect Physiol 41: 1055–1070

Sakai M, Ootsubo T (1988) Mechanism of execution of sequential motor acts during copulation behavior in the male cricket *Gryllus bimaculatus* DeGeer. J Comp Physiol A 162: 589–600

Sakai M, Taoda Y, Mori K, Fujino M, Ohta C (1991) Copulation sequence and mating termination in the male cricket *Gryllus bimaculatus* DeGeer. J Insect Physiol 37: 599–615

Sombati S, Hoyle G (1984) Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. J Neurobiol 15(6): 481–506

Spörhase-Eichmann U, Vullings HGB, Buijs RM, Hörner M, Schürmann F-W (1992) Octopamine-immunoreactive neurons in the central nervous system of the cricket, *Gryllus bimaculatus*. Cell Tissue Res 268: 287–304

Stevenson PA, Kutsch W (1987) A reconsideration of the central pattern generator concept for locust flight. J Comp Physiol A 161: 115–126

Stevenson PA, Pflüger H-J, Eckert M, Rapus J (1992) Octopamine immunoreactive cell populations in the locust thoracic-abdominal nervous system. J Comp Neurol 315: 382–397

Stevenson PA, Spörhase-Eichmann U (1995) Localization of octopaminergic neurones in insects. Comp Biochem Physiol A110(3): 203–215

Thompson KJ (1986) Oviposition digging in the grasshopper. J Exp Biol 122: 413–425

Ureshi M, Sakai M (2001) Location of the reproductive timer in the male cricket *Gryllus bimaculatus* DeGeer as revealed by local cooling of the central nervous system. J Comp Physiol A 186: 1159–1170

Watson AHD (1984) The dorsal unpaired median neurons of the locust metathoracic ganglion: neuronal structure and diversity, and synaptic distribution. J Neurocytol 13: 303–327

Woodring JP, Meier OW, Rose R (1988) Effect of development, photoperiod, and stress on octopamine levels in the house cricket, *Acheta domesticus*. J Insect Physiol 34: 759–765

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

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