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AUTO-SPERMATOPHORE EXTRUSION REVEALS THAT THE REPRODUCTIVE TIMER FUNCTIONS IN THE SEPARATED TERMINAL ABDOMINAL GANGLION IN THE MALE CRICKET*

M. SAKAI** and M. KUMASHIRO

Department of Biology, Faculty of Science, Okayama University,
Tsushima-Naka-3-1-1, Okayama, 700-8530, Japan

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Auto-spermatophore extrusion is a kind of spermatophore extrusion without genital coupling in the male cricket. It rarely occurred in intact males paired with a female, while it frequently occurred in all the males with the connectives cut under restraint and dissection. The time interval (SPaSE) between spermatophore preparation and auto-spermatophore extrusion was found to be comparable to that (RS2) of the time-fixed sexually refractory stage measured by the calling song. According to extracellular spike recording, the dorsal pouch motoneuron (mDP), which singly innervates the dorsal pouch muscles and is responsible for spermatophore extrusion, showed a burst discharge in association with auto-spermatophore extrusion with an interval similar to RS2 in males with the connectives transected between the 6th abdominal ganglion and the terminal abdominal ganglion (TAG) after spermatophore preparation. These results strengthened our previous conclusion that the reproductive timer for RS2 is located in the TAG, and demonstrated that it functions normally even in the TAG separated from the rest of the central nervous system.

Keywords: Male cricket – reproduction – sexual refractoriness – timer – terminal abdominal ganglion

INTRODUCTION

Time-dependent behavior in the reproductive cycle is one of the intriguing problems in the male cricket *Gryllus bimaculatus* DeGeer [2, 3, 5, 6]. It has been established that the interval between spermatophore preparation and the re-emission of a calling song is time-fixed (about 1 h), and thus called the post-copulatory sexually refractory stage (RS2). Recently, we have shown that the timer for RS2 is located within the terminal abdominal ganglion (TAG) [5, 6]. However, it is not feasible to induce a calling song or a mating response in the dissected condition with the abdomen opened for extracellular spike recording.

Thus, we focused on a special type of spermatophore extrusion without genital coupling, named auto-spermatophore extrusion in which the male extrudes the

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** Corresponding author; e-mail: masack@cc.okayama-u.ac.jp

mature spermatophore himself without any prior courtship or copulatory actions in spite that the male was paired with a female. This type of spermatophore extrusion seemed to be useful for monitoring the time-keeping of the timer in dissected males because the interval (SPaSE) between spermatophore preparation and auto-spermatophore extrusion was nearly comparable to RS2 measured by the mating response [5].

Here, we describe normal spermatophore extrusion, auto-spermatophore extrusion, and then genital motoneuron activity associated with both types of spermatophore extrusion. The results strengthened our previous conclusion that the reproductive timer is located in the TAG.

MATERIALS AND METHODS

Animals

Adult crickets, *Gryllus bimaculatus* DeGeer.

Spermatophore extrusion and auto-spermatophore extrusion in dissected males

The male was restrained dorsal side up on the substrate with the abdomen opened. The accessory glands, testes and digestive system were removed. Normal spermatophore extrusion was induced by artificial stimulation of cavity hairs in the genital cavity of the epiphallus with a model of the female copulatory papilla [1]. For auto-spermatophore extrusion, dissected males were left for 2 hours without genital stimulation. In order to induce spermatophore preparation, the antennae of the male were contracted with the body of the female restrained in front of the male.

Spike recording

Extracellular spike was recorded from the cut end of the branch (dorsal pouch nerve) of the genital nerve separated from the 10th ventral nerve root (cercal motor nerve) of the TAG with a suction electrode (Fig. 1).

RESULTS AND DISCUSSION

Spermatophore extrusion in natural copulation

In natural copulation, the male slips backwards under the female when she mounts. Then, the male attempts to hook the subgenital plate of the female with his epiphal-

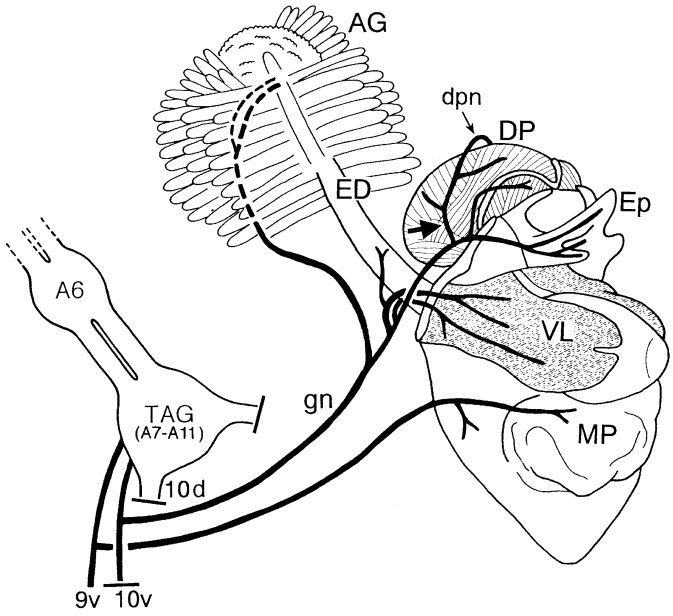

Fig. 1. Innervation of the phallic complex in the male cricket. Genital organs are innervated by nerve roots 9v and 10v from the terminal abdominal ganglion (TAG, consisting of 5 fused abdominal ganglia A7-A11). The dorsal pouch (DP), which contains the attachment plate of the spermatophore (Sp), is innervated by a branch [dorsal pouch nerve (dpn)] of the genital nerve (gn). The thick arrow indicates the recording site for a suction electrode. Backfilling with nickel was carried out through the cut end of the dpn. A6, 6th abdominal ganglion; AG, accessory gland; ED, ejaculatory duct; Ep, epiphallus; MP, median pouch; VL, ventral lobe

lus. After the success in hooking, the male pulls down the subgenital plate of the female, which in turn causes the backward protrusion of the female's copulatory papilla into her genital chamber. The protruded copulatory papilla is naturally inserted into the genital cavity of the epiphallus which stimulates special mechano-sensory hairs (cavity hairs) located near the basal region of the ectoparamer (Fig. 2). Stimulation of the cavity hairs during 4 second-genital coupling causes the contraction of the dorsal pouch which results in the ejection of the attachment plate of the spermatophore, that is, spermatophore extrusion [4].

Response of genital motoneurons to mechanical stimulation of epiphallic sensilla

We have shown that the spermatophore can be extruded artificially with a model of the copulatory papilla [1]. Figure 3 shows the effects of contact stimulation of the epiphallic sensilla on spermatophore extrusion, in which sensilla on different parts of

the epiphallus were stimulated. Stimulation of sensilla (outer hairs) on the lateral and median processes of the epiphallus (Fig. 2) caused a tonic spike response of sensory neurons but failed to activate motoneurons even after 50 seconds of stimulation (Fig. 3A). When contact stimulation was applied to cavity hairs inside the epiphallus, genital motoneurons possibly the dorsal pouch motoneuron (mDP) and two guiding rod motoneurones (mGR) [1] were activated with a latency of 39 seconds, that is, spermatophore extrusion and transfer occurred (Fig. 3B). On the other hand, when both

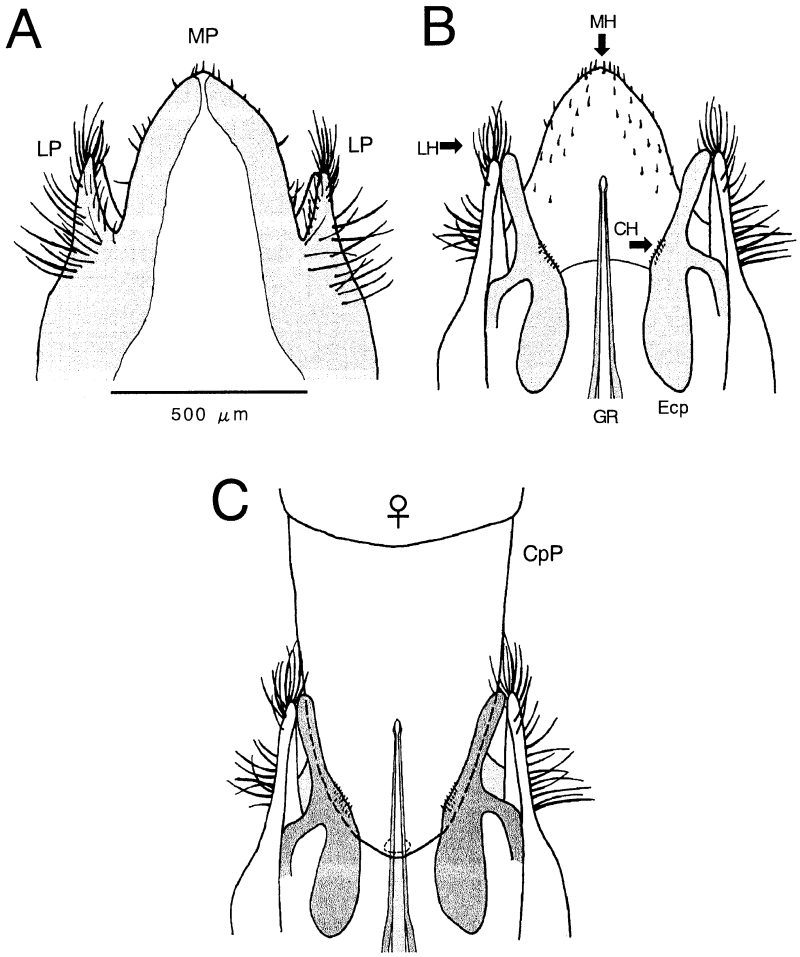

Fig. 2. Epiphallus and copulatory papilla. A: dorsal view of the epiphallus. B: ventral view of the epiphallus. C: ventral view of the epiphallus and copulatory papilla (genital coupling). The copulatory papilla (CpP) is inserted into the genital cavity, which stimulates cavity hairs (CH) near the basal region of the ectoparamere (EcP) in the genial cavity. Stimulation of the cavity hairs causes spermatophore extrusion via dorsal pouch contraction. GR, guiding rod; LH, lateral hairs; LP, lateral process; MH, median hairs; MP, median process

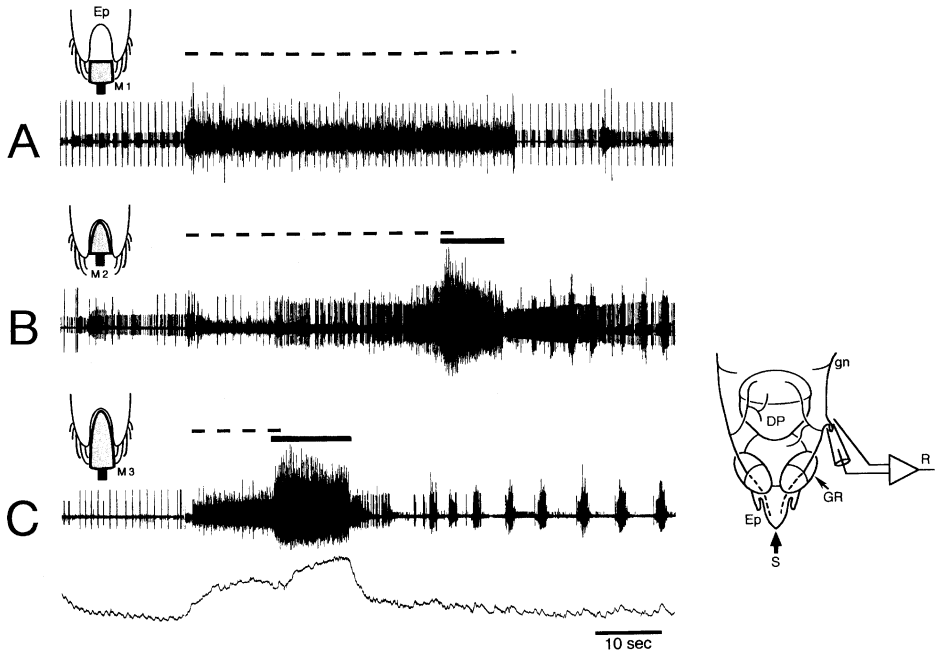

Fig. 3. Responses to stimulation of epiphallic sensilla with a model of the female copulatory papilla. A: Response to stimulation (broken bar) of the outer hairs (M1) on the epiphallus (Ep) with a model. Sensory component continued to discharge for 50 seconds but dorsal pouch contraction was not triggered. B: Responses to stimulation of cavity hairs (M2). Spermatophore extrusion and transfer (solid bar) was triggered after 39 seconds as shown by additional responses of genital motoneurons. This preparation was the same as in A. C: Responses to both cavity hairs and outer hairs (M3). Spermatophore extrusion was triggered 11 seconds after the onset of stimulation. This preparation was different from in A and B. The bottom line is movement of the genitalia. Right inset shows set-ups for recording. See Fig. 1 legend for abbreviations

the sensilla (cavity hairs and outer hairs) were stimulated simultaneously, dorsal pouch contraction was elicited in only 11 seconds (Fig. 3C). These results indicated that cavity hairs play an essential role in triggering spermatophore extrusion, while outer hairs play a supplementary role.

The occurrence rate of auto-spermatophore extrusion and SPaSE under dissection

In intact males paired with a female, almost all the males showed spermatophore extrusion during normal copulation. However, about 2% of them showed auto-spermatophore extrusion without any prior courtship or copulatory attempts after spermatophore preparation. The spermatophore was ejected as it were waste. Males with

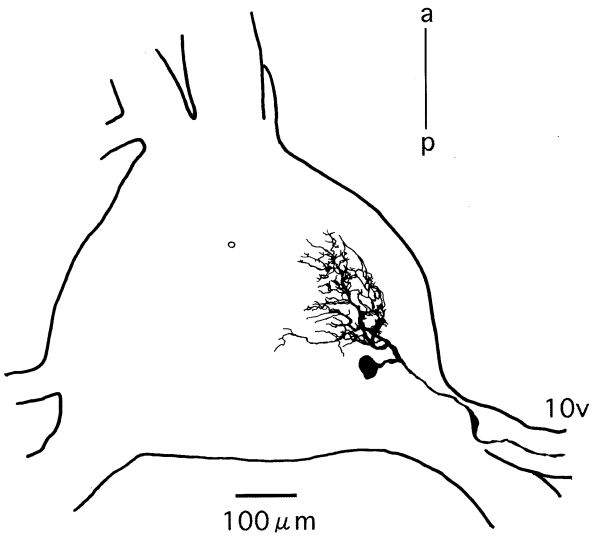

Fig. 4. Morphology of the dorsal pouch motoneuron. a, anterior; p, posterior. 10v, the 10th ventral nerve root (cercal motor nerve) of the TAG

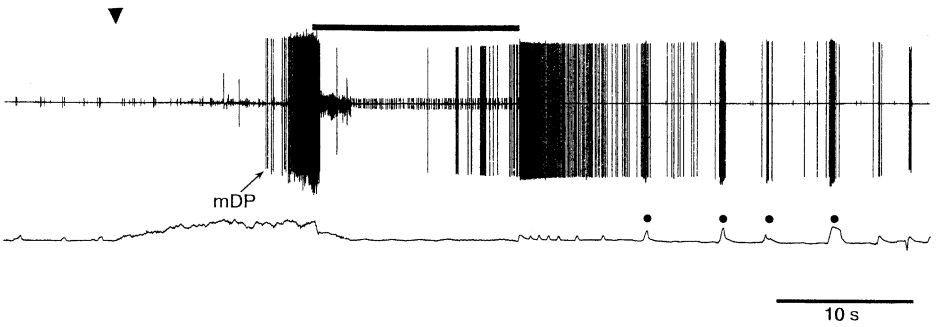

Fig. 5. Spike bursting of mDP in normal spermatophore extrusion. The discharge pattern of mDP (top trace) around spermatophore extrusion which was elicited by genital stimulation (inverted triangle). mDP did not respond to the first 13 seconds, but then exhibited a strong burst, and gradually changed into rhythmic burst after the spermatophore transfer phase (horizontal thick line). Top trace, mDP spike; bottom trace, movement of the phallic complex. Each upward deflection (dot) indicates the movement of the phallic complex

the abdomen dissected showed a higher rate of auto-spermatophore extrusion up to 81%. For the interval (SPaSE) between spermatophore preparation and auto-spermatophore, the median value was 70 min (n = 34) which was comparable to 64 min (n = 42) for RS2 measured by the calling song as control. Similar results were obtained in males with the TAG separated from the rest of the central nervous system by cutting the connectives.

These results indicate that auto-spermatophore extrusion rarely occurs natually but it frequently occurs when males are under stress especially with the connectives transected, and that SPaSE was not significantly different from RS2.

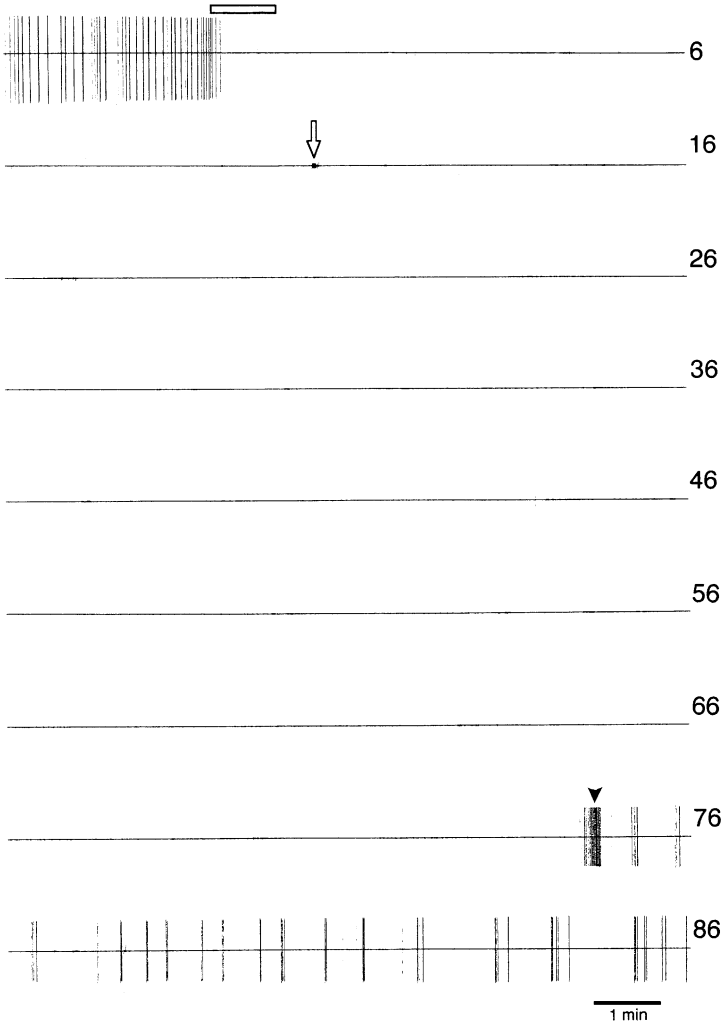

Fig. 6. The activity of mDP associated with auto-spermatophore extrusion in males with the TAG separated from the central nervous system. Spike discharge of mDP is seen in the first and the last two lines. White bar on the first line is spermatophore preparation and white arrow is the time of the connective cut. Numbers to the right indicate time in minutes after spermatophore preparation. Arrowhead indicates the onset of auto-spermatophore extrusion

Dorsal pouch motoneuron activity associated with auto-spermatophore extrusion in males with the TAG separated

Dorsal pouch motoneuron (mDP) is located far laterally in the TAG (Fig. 4). The soma was located ventrally and the dendrites arbolized dorsally. mDP has no spontaneous discharge in the mating stage and showed a strong burst discharge at a frequency of more than 100 Hz a few seconds after the onset of stimulation of the epiphallic sensilla (Fig. 5). The discharge then decreased during the spermatophore transfer phase and increased thereafter rhythmically at a frequency of 1 per 6 seconds. This burst continued for more than 1 hour if no female stimulation was given. However, if the male antennae were contacted with the female body, the rhythmic burst was gradually accelerated and finally stopped just before the onset of spermatophore preparation (white horizontal bar in the first line in Fig. 6). Then, the connectives were cut bilaterally just anterior to the TAG (white arrow). No change occurred and silence continued for approximately one hour. At 74 min after spermatophore preparation, mDP abruptly started discharging which was associated with auto-spermatophore extrusion (see arrow head in Fig. 6). In total of the 16 males, the interval between spermatophore preparation and spike bursting associated with auto-spermatophore extrusion was comparable to RS2 measured by the calling song.

These results strengthened our previous conclusion that the reproductive timer is located in the TAG and functions normally even in the TAG separated from the rest of the central nervous system.

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