Dissertation

Neurophysiological Study of the Cercus-to-Giant Interneuron System in the Cricket

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DISSERTATION

NEUROPHYSIOLOGICAL STUDY OF THE CERCUS-TO-GIANT INTERNEURON SYSTEM IN THE CRICKET

BY

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CONTENTS

1 GENERAL INTRODUCTION

GENERAL INTRODUCTION

Some arthropods have served as one of the good experimental animals for the study of fundamental neural mechanism from cellular level. One of the main reasons is that the nervous systems of these animals consist of smaller number of neurons of larger size than those of vertebrates; nevertheless these animals have a lot of fundamental neural mechanisms underlying the behavior.

The cercus-to-giant interneuron system is one of the most extensively studied neural system throughout arthropoda. As to this system, until now there have been done many studies about fundamental neural properties such as synaptic transmission (Harrow and Sattelle 1983; Miller and Jacobs 1984; Boyan and Ball 1989c); target cell recognition and neural plasticity (Edwards and Palka 1971; Palka and Edwards 1974; Murphey 1985; Murphey et al. 1985); the effects of sensory deprivation on GIs (Matsumoto and Murphey 1978; Shankland and Goodman 1982); and output connections to thoracic motor pathways (Ritzmann and Camhi 1978; Ritzmann et al. 1980; Ritzmann 1981; Ritzmann and Pollack 1981; Ritzmann et al. 1982).

The author has been investigating cricket's cercus-to-giant interneuron system. The cerci of cricket are paired, unsegmented, cone-shaped sensory organs arising from a depression on either side of the tip of the abdomen. Each cercus bears a large number of mechanoreceptors including about 500 filiform hairs, which respond wind and low frequency sound (less than 1000Hz). The axons of cercal sensory neurons innervating these receptors conduct mechanical sensory information to second order interneurons within the terminal abdominal ganglion (TAG). Among the second order interneurons, there have been identified GIs, the ascending axons of which are substantially larger than those of

other types of interneurons (Mendenhall and Murphey 1974). And cricket's GI system has been extensively studied as well as other insects' GI systems. However there has been little work for the input pathways from cercal sensory neurons to GIs; for the structural organization of each GI; and for physiological functions. For example, it is not known whether each GI receive sensory information directly or indirectly from cercal sensory neurons; up to where each GI ascends through the ventral nerve cord (VNC); what shape of axonal branches each GI has; and what behavior each GI controls. So the author has been investigating the cercus-to-giant interneuron system to reveal these uncovered problems.

In chapter 2 of this thesis, it is shown that the cricket has eight types of GI in the TAG and that GIs receive sensory information monosynaptically and/or polysynaptically from cercal sensory neurons, characteristic for each GI. In chapter 3, the structural organization of GIs revealed by intracellular dye injection is presented and GIs are classified into two subgroups based on their positions of axons; dorsal types of giant interneurons (DGIs) and ventral types of giant interneurons (VGIs). In chapter 5, it is shown that DGIs have excitatory connections with various motoneurons in thoracic and abdominal ganglia. And DGIs trigger walking movement when the legs of the animal are in contact with the substratum, and flight behavior when the animal is suspended in the air. In contrast, VGIs apparently have no effect on the behavior of cricket.

2 INPUT PATHWAYS TO GIANT INTERNEURONS IN THE TERMINAL ABDOMINAL GANGLION

MATERIAL AND METHODS

Animals

In our laboratory, crickets (*Gryllus bimaculatus* Degeer) are bred from egg to adult, on a 12-12 hr light-dark cycle at a constant temperature of 28°C. Adult male crickets were used throughout the experiments described in this chapter.

Preparation

After removing the head, wings, and legs of an animal, the specimen was pinned to a platform with dorsal side up, an incision was made along the dorsal midline of the abdomen, and the gut, internal reproductive organs, and surrounding fat were removed to expose the TAG. The large abdominal tracheae were disturbed as little as possible. All peripheral nerves of the TAG except for the cercal sensory nerves, were severed. A stainless steel spoon introduced posteriorly between the cercal nerves supported the TAG and served as the indifferent electrode. The specimens remained viable for about 1 hr as long as they were frequently flushed over with saline (NaCl 150 mM, KCl 9 mM, $CaCl_2$ 5 mM, $NaHCO_3$ 2 mM, Dextrose 50 mM, Trizma HCl 40 mM, Trizma Base 0.01 mM; PH 7.2).

Intracellular recording

Glass microelectrodes filled with 5% (W/V) Lucifer Yellow (Stewart 1978) were used for intracellular recording and staining of GIs. The resistance of these microelectrodes ranged from 10 to 50 MΩ. A microelectrode was introduced into obliquely into the TAG through its dorsal sheath. The responses of GIs to electrical stimulation applied to the

cercal sensory nerves were stored on a tape recorder and subsequently photographed with a continuous recording camera.

Electrical stimulation

Electrical stimulation were applied to cercal sensory nerves on each side through a pair of tungsten electrodes placed under cercal sensory nerves. First, to measure the response latency of each GI cercal sensory nerve on each side was repeatedly stimulated over 100 times (50 μsec at 2 Hz). The response latency of each GI was put into histogram with Histogram Analyzer QC-111j (Nihon Kohden) later. Then, to examine the GIs' capacity to follow high frequency activity of cercal sensory neurons, the cercal sensory nerve was stimulated at 50 Hz for several seconds.

Histological identification

After physiological recording, Lucifer Yellow was injected iontophoretically into the GI with a hyperpolarizing current of 2-5 nA for 1-5 min. Then, the TAG containing the GI was isolated, fixed in 4% formaldehyde in phosphate buffer (PH=4.3) for more than 30 min, dehydrated in alcohol and cleared in methylsalicylate. Stained GI was observed and identified in wholemount under a fluorescence microscope (Olympus, BH-RFL) in combination with an excitation filter (BG-12) and drawn using a camera lucida. Some of the preparations were embedded in paraffin and sectioned at 10 μm in order to measure diameter of the axon, and to identify the position of the axon in the VNC.

Backfill staining of filiform hair receptor neurons

One of filiform afferent was stained by placing a drop containing 1M nickel chloride over cut end of a filiform hair, for 24 hr. Then the TAG

was processed according to a Timm's intensification procedure (Bacon and Altman 1977).

RESULTS

Giant interneurons

How many types of GIs dose have the cricket *Gryllus bimaculatus* ? In order to reveal it, the author made transverse sections of the VNC and investigated the size distribution of axons in the VNC (Fig. 1). A transverse section of the VNC at 100 μm anterior to the TAG revealed that there are eight axons substantially larger than the rest (Fig. 1 A). Two axons' diameter are greater than 23 μm, six axons' diameter are between 13-16 μm, and the others are smaller than 8 μm (Fig. 1 B).

Intracellular staining with Lucifer Yellow revealed that these eight large axons are of ascending interneurons whose somata locate in the TAG. The morphology of these ascending interneurons in the TAG are shown in Fig. 2. The morphology of interneuron D in Fig. 2 is homologous to that of 9-1b which was previously identified in the cricket *Gryllus campestris* by Kämper (1984). The morphology of interneurons A, B, C, E, F, G and H is homologous to that of 7-1, MGI, LGI, 9-2, 9-3, 10-2 and 10-3 respectively, which were previously identified in the cricket *Acheta domesticus* by Mendenhall and Murphey (1974). So our ascending interneurons are named according to these previous studies.

It is concluded that these eight ascending interneurons constitute 'giant interneurons' in *Gryllus bimaculatus*. The reasons of this conclusion will be discussed later on.

Arborization of filiform afferents in the TAG

The author has investigated where filiform afferents have their arborization. To stain one of the filiform afferents individually, a filiform hair was cut off which occurs on proximal portion of the cerci and nickel chloride was taken in from the stump of it (in present study, filiform hairs

on distal portion were not investigated). Ninety filiform afferents were stained in total. Filiform afferents' arborizations were found only in posterior half of the TAG, what is called cercal glomerulus (for all ninety preparations). And only five neurons extended arborization beyond midline into contralateral side of origin, but the arborization of the rest eighty-five filiform afferents remained ipsilateral to their side of origin. The area where most (eighty five preparations out of ninety preparations) of filiform afferents' arborization is located is shown by stippling in Fig 2, superimposed on the depiction of GIs. MGI, LGI and 9-2 have their dendrites in this area on the axon side. 9-1b, 9-3, 10-2 and 10-3 have their dendrites in this area on both side. But 7-1 don't have dendrites in this area on either side. It is likely that GIs' dendrites extended in this area form synapses directly with filiform afferents.

Response of GIs to electrical stimulation

To reveal whether GIs have direct connections with cercal sensory neurons, the response of each GI to electrical stimulation to the cercal sensory nerves was investigated. The physiological data on GIs were gathered from over 80 preparations. All types of GI whose physiological data are presented below were recorded a minimum of three times.

1 Response of GIs

7-1 responds with several spikes to one electrical stimulation of low frequency (2 Hz) to cercal sensory nerve on either side. LGI and 9-2 respond with one spike to one electrical stimulation on axon side, but don't respond to soma side (Fig. 3). The other GIs respond with one spike to one electrical stimulation on either side .

2 Response latency to low frequency stimulation

Latency was measured by electrically stimulating the cercal sensory nerve and measuring intracellularly the delay to the commencement of

the evoked spike in each GI. For example, 9-3 responds with short latency (1.8 msec) to stimulation to cercal nerve on soma side and with long latency (3.2 msec) to axon side (Fig. 3). The latencies for each GI are arranged in Table 1 and are put into histogram in Fig 4. In this histogram, it can be seen that there are two types of response in a view of spike latency, namely response with spike of relatively short (1.3-1.8 msec) and constant latency, and response with long (3.0-3.5 msec) and fluctuating latency. It is likely this difference reflects difference in the number of synapses, through which sensory information pass.

3 Synaptic delay

To infer the number of synapses between sensory neurons and GIs, it would be desirable to calculate the synaptic delays and to compare those with synaptic delays revealed in various chemical synapses of other species.

To calculate the synaptic delay, conduction time has to be measured during which spike conduct from the point of stimulation to the sensory neurons' terminals in the TAG. The conduction time was measured as follows.

Two electrodes were placed under the one of the cercal sensory nerves, one at distal side and the other at proximal side, separated from each other by 1 mm. The cercal sensory nerve were electrically stimulated via each of these two electrodes and response latency of a GI to each electrode was measured. The latency of response evoked by proximal electrode is shorter than by distal one by 1.4 msec (average of ten times). This value (1.4 msec) must correspond with time during which spike conduct from the point of distal electrode to proximal one. So conduction velocity of spike is about 1.4 m/sec. In the previous experiments for response latency of GIs, the electrodes were placed under cercal sensory nerves about 0.5 mm apart from the center of the TAG. So the conduction

time is about 0.7 msec. Subtracting this value from the response latency of each GI, the synaptic delay can be obtained, and the results are arranged in Table 1. A synaptic delay less than 1.3 msec would probably represent direct connections between the GI and cercal sensory neurons.

4 Response to high frequency stimulation

Another indicator of whether a direct connection exists between two cells is whether the post synaptic cell can continue to respond 1:1 to presynaptic cells' activity driven by high frequency stimulation. So, each cercal sensory nerve was electrically stimulated at 50 Hz, and was checked whether each GI was able to continue to fire spike 1:1 in response to this stimulation. The recordings of the responses of MGI and 10-3 to this stimulation are shown in Fig. 5, as an example. When the cercal sensory nerve on axon side of MGI was stimulated, MGI continued to fire spike 1:1 to stimulation for several seconds, but couldn't when the cercal sensory nerve on soma side was stimulated. And 10-3 continued to fire, when cercal nerve on either side was stimulated. The results about the other GIs are presented in Table 1.

DISCUSSION

Giant interneurons

Kanou and Shimozawa reported the morphology and threshold curves of *six* types of GI of *Gryllus bimaculatus* (1984). However, in their cross section of the VNC, eight large axons can be seen. The present study also show that there are eight large axons in the VNC. So it is reliable that *Gryllus bimaculatus* have eight substantially large axons in the VNC. And it is revealed that these axons are of wind sensitive ascending interneurons, whose somata are located in the TAG. Seven out of these eight ascending interneurons are homologous in morphology in the TAG to the 'giant interneurons' in *Acheta domesticus* (Mendenhall and Murphey 1974) but the rest interneuron 9-1b has not been classified as to be GI. However, we conclude that the 9-1b interneuron is a member of GIs, because not only its axon diameter in the VNC is as large as other seven types of interneuron, but also its axon run through the VNC up to the brain and terminate in the deutocerebrum, as other seven GIs (see the next chapter). Furthermore, from observation of transverse sections at thoracic ganglia, it is revealed that the axon of 9-1b interneuron run through the ventral intermediate tract (VIT) together with the axons of GIs; 7-1, NGI, LGI, (see the next chapter) So It is concluded that that 9-1b interneuron is a member of ventral giant interneurons (VGIs).

Methods to investigate input pathways

In some insects, researches have been made to elucidate input pathways from cercal sensory neurons to GIs. For example Blagburn showed that monosynaptic connections exist between GIs and cercal sensory neurons in first instar of cockroach (1989), and Boyan and Ball reported the existence of monosynaptic pathways between GIs and cercal

sensory neurons in praying mantids (1986). In these studies, the input pathways were investigated by measuring response latencies of GIs to electrical stimulation to sensory neurons, or by checking GIs for their capacity to follow high frequency stimulation to sensory neurons 1:1. In the present study, these methods are adopted to investigate whether main input pathways from cercal sensory neurons to GIs are monosynaptic or polysynaptic ones.

Main input pathways to GIs

GIs receive input primarily from filiform afferents (Bacon and Murphey 1984; Murphey et al. 1984), so the author has investigated their morphology. As shown in Fig 2, the arborizations of most filiform afferents (85 out of 90 preparations) exist are restricted within cercal glomerulus ipsilateral to their side of origin. It is likely that the filiform afferents form synapses directly on GIs' dendrites extended in this area. If so, 9-1b 9-3 10-2 and 10-3 might receive information from filiform afferents on both side monosynaptically. MGI, LGI and 9-2 might receive information from axon side monosynaptically, but polysynaptically from soma side. 7-1 might receive polysynaptically from both sides. But these dendrites of GIs may not form synapses with ipsilateral filiform afferents directly, or may have synapses from filiform afferents on contralateral side which extend arbolization beyond midline. So, the above inference must be tested by electrophysiological experiments.

Response latencies of each GI to electrical stimulation are arranged in Table 1 and are put into histogram (Fig. 4). It is apparent that there are two types of response in a view of spike latency; the response with relatively short and constant latency and the response with long and fluctuating latency. With regard to the response of short latency, its synaptic delay are about 0.8-1.4 msec, and this value well corresponds to

synaptic delays revealed in other chemical synapses in other species (Yamasaki and Narahashi 1958; Burrows 1975; Silvey and Sandeman 1976). So it is likely that in the case of short latency, GI receives sensory information through monosynaptic pathways from cercal sensory neurons. On the other hand, in the case of long latency, GI receives information through polysynaptic pathways.

Responses of GIs to high frequency (50 Hz) stimulation can be also classified into two types. In some cases, GIs continued to fire 1:1 for several seconds, in other cases GIs come to miss following the stimulation with spike 1:1 and finally come not to fire at all (Fig. 5 and Table 1). In the former cases, it is likely that GIs receive sensory information through monosynaptic pathways, in the later cases, through polysynaptic pathway. And inference from the GI's capacity of following to high frequency stimulation coincides with one from the latency of GIs.

Electrophysiological experiments suggest that 7-1 has monosynaptic connections with cercal sensory neurons, but morphology of filiform afferents suggests that they can't make synapse on 7-1 directly. This contradiction reflects the fact that electrical stimulation excites not only filiform afferents but also other types of cercal sensory neurons which might form synapses on 7-1 directly. Murphey et al. reported that 7-1 is touch-sensitive interneuron and that bristle sensory neurons project their terminals in the anterior half of the TAG, so called bristle neuropile (Murphey and chiba 1990). And in this area, 7-1 has dendrites. So it is very likely that bristle sensory neurons form synapses on 7-1 directly.

Electrophysiological experiments suggest that LGI and 9-2 make no connections with cercal sensory neurons on soma side. But there is possibility that these GIs receive so weak input from sensory neurons on soma side that stimulation to cercal sensory nerve seems to have no effect on these GIs. However, these input pathways have very weak effect on the

GI and may not be important to the organisms, if any. On the contrary, to have no or weak pathways from the cercal sensory neurons on axon side may be very significant to the organism. The same logic can be applied to the putative polysynaptic pathways to 9-3, MGI or 9-1b.

From these considerations, the input pathways to each GI are inferred as follows. 7-1 receives information through polysynaptic pathways from filiform afferents on its either side and through monosynaptic pathways from bristle sensory neurons on either side. MGI receives through monosynaptic pathways from filiform afferents on its axon side and polysynaptic pathways from soma side. LGI and 9-2 receive through monosynaptic pathways from axon side and are not affected by the activity of sensory neurons on soma side. 9-1b receives through polysynaptic pathways from either side. 9-3 receives through polysynaptic pathways from soma side and monosynaptic pathways from axon side. 10-2 and 10-3 through monosynaptic pathways from either side.

Until now, it has been reported that in the cricket there exist polysynaptic input pathways which inhibit GIs (Palka et al. 1977) In this study, it is shown that also excitatory polysynaptic pathways exist which may be significant to the organisms. Recently, various types of local non-spiking interneurons and local spiking interneurons have been identified in the TAG (Baba et al. 1991). The author tried to established whether these local interneurons constitute polysynaptic pathways between GIs and cercal sensory neurons, but all efforts ended in failure. However, the possibilities remains that these interneurons may constitute the polysynaptic pathways. Anyway, each GI has such characteristic input pathways. The reason is not clear. But the diversity of the input pathways may be useful for each GI to code the different features of the environment.

ABSTRACT

1) Eight wind-sensitive interneurons originating in the TAG are found to have relatively large axons ascending through the VNC and therefore are classified as "giant" interneurons (GIs; 7-1, MGI, LGI, 9-1b, 9-2, 9-3, 10-2 and 10-3). The aim in this chapter is to establish the input pathways to GIs from cercal sensory neurons.

2) In the TAG, most of filiform afferents extend arborizations within the cercal glomerulus ipsilateral to their side of origin. GIs except for 7-1 have dendrites in this area.

3) When a cercal sensory nerve on the soma side of a GI was electrically stimulated, 7-1, 9-3, 10-2 and 10-3 responded with spike of relatively short latency (1.3-1.7 msec), MGI and 9-1b responded with relatively long latency (3.0-3.5 msec), LGI and 9-2 didn't respond. When a cercal nerve on the axon side was stimulated, 7-1, MGI, LGI, 9-2, 10-2 and 10-3 responded with short latency (1.3-1.7 msec), 9-1b and 9-3 responded with long latency (3.0-3.5 msec).

4) In the case GIs responded with short latency, the GI continued to fire 1:1 to high frequency electrical stimulation (50 Hz) for several seconds. But in the case of long latency, the GIs immediately came to fail to fire.

5) From these experiments, input pathways to each GI are inferred as follows. 7-1 receives information through polysynaptic pathways from filiform afferents on its either side and through monosynaptic pathways from bristle sensory neurons on either side. MGI receives through monosynaptic pathways from filiform afferents on its axon side and polysynaptic pathways from soma side. LGI and 9-2 receive through monosynaptic pathways from axon side and are not affected by the activity of sensory neurons on soma side. 9-1b receives through polysynaptic pathways from either side. 9-3 receives through polysynaptic pathways

from soma side and monosynaptic pathways from axon side. 10-2 and 10-3 through monosynaptic pathways from either side.

3 STRUCTURAL ORGANIZATION OF GIANT INTERNEURONS IN THE CENTRAL NERVOUS SYSTEM

MATERIAL AND METHODS

Animals

In the experiment described in this chapter, third instar crickets (about 4 mm long) and adult crickets (about 3 cm long) were used.

Preparation

After removing legs of third instar, an incision was made along the dorsal midline of the abdomen and the internal organs were removed to expose the TAG. The animal was pinned to a wax platform dorsal side up, with spines of cactus. A small spoon introduced posteriorly between the cercal sensory nerves supported the TAG served as the indifferent electrode. The specimens remained viable for about several hours as long as they were frequently flushed over with the saline (see previous chapter).

Intracellular staining

Glass microelectrodes filled with 5% (W/V) Lucifer Yellow were used for intracellular recording and staining of GIs. The resistance of these microelectrodes ranged from 10 to 50 MΩ. The microelectrode was introduced obliquely into the TAG through its dorsal sheath. When the microelectrode penetrated a interneuron successfully, the membrane potential was about -30 to -40 mV and wind stimulation was applied to the cerci to check the response of the interneuron. Soon after the check of the response, Lucifer Yellow was injected iontophoretically into the interneuron with a hyperpolarizing current of 2-5 nA for 15-30 min. Then the animal was bathed in the saline and maintained at 4°C temperature for 6-12 hr to allow Lucifer Yellow to spread over the interneuron. Then the VNC containing the labelled neuron was fixed isolated, fixed in 10%

formaldehyde in a phosphate buffer (pH=4.3) for more than 30 min, dehydrated in alcohol and cleared in methylsalicylate. Stained GIs were photographed in wholemount under a fluorescent microscope (Olympus, BH-RFL) in combination with an excitation filter (BG-12). And from the photomicrographs, drawings of GIs were reconstructed. Some of the preparation were embedded in paraffin and sectioned at 10 μm in order to establish the location of the axon of each GI in the VNC. These section preparation were also photographed in the same way.

When adult crickets were used, the methods were same as those described above for third instar crickets.

Backfills staining

With adult crickets, $CoCl_2$ backfillis were performed by cutting the ventral nerve cord between first free abdominal ganglion and second free abdominal ganglion and then filling anteriorly. The filled tissue were silver intensified as whole mount according to a Timm's intensification procedure (Bacon and Altman 1977).

RESULTS

The author has been primarily interested in the anatomy of adult's GIs. So, firstly the author tried to stain each GI of adult cricket intracelluraly. But, even when the author succeeded in penetrating a GI stably and in injecting Luciffer Yellow electropholetically for more than 4 hours, the GI could be visualized from the TAG to the mesothoracic level at best (Fig. 13). So, after revealing morphology of adult's LGI and 9-3 from the TAG to the mesothoracic ganglion, the author gave up staining adult's GIs. Instead the author tried to stain GIs of third instar cricket, of which the VNC is much smaller than that of adult, on the assumption that third instar's GIs have same anatomical feature except for difference in the size. And with third instar, each GI was completely stained as shown in Fig. 6. In this paper, whole morphology of each GI of third instar cricket is presented, the anatomical feature of GI system of the cricket is described, and later the similarities between GIs of adults and GIs of third instar's are shown, which support the above assumption.

The axons of GIs

First of all, the author was interested in the question of *up to where* the axon of each GI ascend. Intracellular filling of each GI shows that axon of each GI ascends from the TAG through the abdominal ganglia, thoracic ganglia, and subesophageal ganglion, pass through lateral side of circumesophageal connective, run into the deutocerebrum of the brain, and there branches off into several collaterals (Fig. 6 and 7). In each ganglion on the way, each GI sends out axonal branches from the main axon. The axons remain to be especially large from the TAG to the metathoracic level, and as the axons ascend anteriorly from there the axons become narrower and narrower.

The location of axons and classification of GIs

It is crucial *where* a neuron exist in the nervous tissue, as well as what shape is the neuron. Therefore, the author tried to make cross sections all thorough the VNC which contained a labeled GI, to reveal the location of axon of each GI. The position of axons for all GIs could be established only in the thoracic ganglia, because the abdominal ganglia are so small that clear sections were rarely obtained and in the brain and subesophageal ganglion Lucifer Yellow was so dim that the axon of the labelled GI could be rarely find out.

In the metathoracic ganglion, individual axon could be discriminated clearly and relative position of each GI's axon was established and is shown in Fig. 8. The relative position is consistent from preparation to preparation. In prothoracic and mesothoracic ganglia, outline of individual axon was not so clear that individual axons couldn't be discriminated and relative position of each GI's axon couldn't be established.

It is evident, however, axons of eight GIs are arranged in two groups also in prothoracic and mesothoracic ganglia, as in the metathoracic ganglion (Fig. 8). The axons of 7-1, MGI, LGI and 9-1b run together through ventral side tract, which is called ventral intermediate tract (VIT) according to the previous study of cockroach's and locust's nervous system (Stubblefield and Comer 1989: Boyan and Ball 1989a). The axons of 9-2, 9-3, 10-2 and 10-3 run through dorsal side tract, which is called dorsal intermediate tract (DIT). Based on this difference, GIs are classified into two subgroups; ventral giant interneurons (VGIs; 7-1, MGI, LGI and 9-1b) and dorsal giant interneurons (DGIs; 9-2, 9-3, 10-2 and 10-3).

In subesophageal ganglion, the locations of MGI, LGI, 9-3 and 10-2 were revealed. The axons of MGI and LGI run through ventral side tract,

those of 9-3 and 10-2 run through dorsal side tract. In abdominal ganglia, the position of only 10-2 was revealed, which run through dorsal side tract.

Consistency of morphology

Next question is whether a given GI identified on the basis of its morphology in the TAG (branching pattern of dendrites and the position of soma), have a consistently recognizable structure in the CNS. Comparisons of the same GI in different animals show that projection pattern of major axonal branches in all thoracic ganglia and of major collaterals in the brain are characteristic for each GI and consistent from preparation to preparation. In Fig. 9, the consistency of some GIs are illustrated in the brain and in the metathoracic ganglion.

In subesophageal and free abdominal ganglia, however, the locations and orientations of the axonal branches vary so much with animals that characteristic branching pattern for each GI can't be find out.

Anatomical differences between VGIs and DGIs

And VGIs and DGIs have very different anatomical feature, namely DGIs send out axonal branches extensively both medially and laterally while VGIs send out only medially except that 7-1 sends out two branches laterally in the metathoracic ganglion (Fig. 11 and 12).

Morphology of individual GI

The compleate morphology of VGIs and DGIs is shown in Fig. 7. And in Fig. 10, 11, and 12, morphology in the brain and thoracic ganglia is presented again, because in these ganglia each GI has characteristic and consistent morphology. For same reason, in this section I point out

anatomical features of each GI in the brain and thoracic ganglia are pointed out.

7-1: In the brain the axon of 7-1 branches off into several collaterals, but these collaterals are less extensive and fewer than those of other GIs. In the pro- and meso-thoracic ganglia, 7-1 sends out short projections only medially as other VGIs. However, in the metathoracic ganglion 7-1 has two lateral branches in addition to many thin medial branches. These lateral branches extend to the periphery of the ganglion, but doesn't branch off so extensively as the lateral branches of 9-2 or 9-3.

MGI: In the brain the axon of MGI branches off into several collaterals, some extend anteriorly and others medially. In the mesothoracic ganglion, there is a rather thick branch at cadual side projecting anteriorly. This branch is very striking viewed in whole mount. In the metathoracic ganglion, MGI send many thin short branches only medially.

LGI: In the brain the axon of LGI branches off into several collaterals, which loop medially. As MGI, in mesothoracic ganglion there is a branch projecting anteriorly in the mesothoracic ganglion, which is rather thick and very striking. In the metathoracic ganglion, unlike MGI, LGI sends out a few branches.

9-1b: In the brain, the axon of 9-1b branches off into three main collaterals. One of them extends laterally and the others anteriorly. In each thoracic ganglion, a few axonal branches extend almost vertically from main axon medially.

9-2: In the brain, the axon of 9-2 branches off into several collaterals. Some extend laterally, some anterioly and the others medially. In the prothoracic ganglion, 9-2 sends out two branches medially and two laterally from the same points. In the mesothoracic ganglion, a thick branch at cadual side extends from the axon to the periphery of the

ganglion, branching into many thin projections. This branch is striking viewed in whole mount. In addition, 9-2 have many lateral branches and two medial branches in this ganglion. In the metathoracic ganglion, 9-2 sends out many projections medially and laterally. One of the lateral projections on rostral side branches off into many thin projections and reaches to the periphery of the ganglion.

9-3: The morphology of 9-3 is so similar to that of 9-2 except for in the TAG that they can't be distinguished only from projection pattern of axonal branches in thoracic ganglia. Therefore to distinguish them by anatomical properties at thoracic level, the position of axons at metathoracic ganglion has to be revealed.

10-2: In the brain the axon of 10-2 branches off into two collaterals. One of them extends anteriorly, and other loop toward medially and extends to the vicinity of the midline. This medial collateral is striking viewed in whole mount. In the thoracic ganglia, 10-2 sends out many branches medially and laterally from the axon, however, all branches are rather short and lateral branches are confined to the vicinity of axon and don't reach to the periphery of the ganglia, in contrast to the case of 9-2 or 9-3.

10-3: In the brain, collaterals of 10-3 extend anterioly and medially. As in the case of 10-2, projection regions of the lateral branches in thoracic ganglia are confined to the vicinity of the axon. In the metathoracic ganglion, however, there is a slightly long lateral branch. This branch is striking, and is characteristic for 10-3.

GIs of adult cricket

Though any GI of adults couldn't be stained wholly intracelluraly, morphology of 9-3 and LGI of adult up to mesothoracic level was revealed (Fig. 13). Compariosns of the anatomy of visualized portions of adult 9-3

and LGI with the counterparts of the corresponding GIs of third instar show that in the meso- and meta-thoracic ganglia projection pattern of axonal branches and the location of axons are very similar between them .

To make sure whether the axons of adult GIs ascend to the brain, interneurons were backfilled anteriorly from the ventral nerve cord between first and second abdominal ganglia (Fig. 14). Among stained interneurons, axons of probable GIs can be found. These axons ascend through thoracic ganglia and subesophageal ganglion, and pass through lateral side of circumesophageal connective, and run into the deutocerebrum of the brain; along the same course as the third instar's GIs.

Furthermore, third instar GIs respond with spikes to wind stimulation on the cerci, as adult GIs. As an example, intracellular recording of the response of third instar's LGI is presented in Fig. 15 with that of adult LGI.

In short, several properties of GI system are common to third instar and adult, except for difference in size. And so, it is very likely that GIs of third instar are miniatures of those of adult and that the morphological features of third instar's GIs applies to adult GIs.

DISCUSSION

Comparison of adult's GIs and third instar's GIs

The author has investigated morphology of third instar's GIs on the assumption that third instar's GIs have same anatomical properties as adult GIs except for a difference in the size. Is this assumption correct?

First of all, GIs of adult seem to ascend to the brain as those of third instar. And in the TAG the anatomical features of each GI are common to adult and third instar's cricket. In the meso- and meta-thoracic ganglia, morphological features of at least LGI and 9-3 are common to adult and third instar. Furthermore, third instar's GIs respond with spikes to wind stimulation to the cerci, as in the case of adult GIs. Bently have reported that in the cricket, the feature of flight pattern began to appear and the main structure of flight motoneurons has been elaborated at least four instars preceding adulthood (1970). This suggests that nervous system of cricket has matured at early stage in the development.

These facts considered, it seems likely that main organization of cercus-to-GI system has been matured until the animal grown into third instar. So it is concluded that the above assumption is correct and that morphological features of third instar's GIs shown in this chapter apply to adult GIs.

Consistency and identification of GIs

In the TAG, it is known that morphology of dendrites and the position of soma are characteristic for each GI and consistent from preparation to preparation. So it was predicted, before this experiments, that in each ganglion, GI might have characteristic and consistent anatomical properties, such as the location of axons and branching pattern of axonal branches.

About location of axons, at only metathoracic ganglion the precise relative position can be established(Fig. 8). And this arrangement is consistent from preparation to preparation.

About the axonal projections or collaterals, the branching pattern is characteristic for each GI and consistent from preparation to preparation in the brain and in all thoracic ganglia. But in the suboesophageal and abdominal ganglia, branching pattern so varied with preparations that I could not find out characteristic for each ganglion. Given the consistency at the thoracic level, the author suspects that these variabilities in the suboesophageal and abdominal ganglia reflect real variabilities rather than artifact of experimental procedure.

Until now, morphology of GIs only in the TAG has been known. And to identify a GI, its morphology at the TAG must be revealed . But from now, identification of a GI can be done based on the anatomical features at thoracic ganglia. It is easy to distinguish 7-1, MGI, LGI, 9-1b, 10-2 and 10-3 based on the projection pattern in thoracic ganglia viewed in whole mount. While 9-2 and 9-3 so resemble each other in projection pattern that it is impossible to tell one from the other. However, the axon of 9-2 run through medial side of axon of 9-3 at least in the metathoracic ganglion (Fig. 8), so they can be distinguished, based on this difference. The author hopes that the anatomical description of GIs presented here facilitates physiological works by providing means of identification of any GIs at thoracic level.

The locations of axons and classification of GIs

It is crucial where the neuron is located in the CNS as well as what shape is the neuron. So, to establish the location of each GI's axon in the CNS, cross sections of the VNC were made which contained a labeled GI.

It is evident that at the thoracic level the axons of the GIs are organized into ventral group (7-1, MGI, LGI and 9-1b) and dorsal group (9-2, 9-3, 10-2, and 10-3).

In cockroach, there has been identified seven GIs (Roeder 1948; Daley et al. 1979). In cockroach's abdominal and thoracic ganglia, two tracts which contain these GIs' axons have been found. And dorsal side tract has been named dorsal intermediate tract (DIT) and ventral side tract has been named ventral intermediate tract (VIT). And GIs running through DIT are classified as DGIs and GIs running through VIT as VGIs. According to these previous studies, two tracts in cricket thoracic ganglia are named DIT and VIT respectively, and GIs are also classified into DGIs and VGIs.

This classification of cricket GIs apparently seems to be based on axonal organization only at thoracic level, however, it is not the case. At subesophageal ganglion and free abdominal ganglia, there could be seen also two tract of probable GIs. Though the position of every GI at these ganglia couldn't be established, a few successful preparations suggest that VGIs run through ventral side tract and DGIs run through dorsal side tract in these ganglia. If it is the case, VGIs and DGIs are separated each other throughout the VNC and the classification of GIs into DGIs and VGIs is very reasonable.

Expectations of function from anatomical feature

The most striking anatomical features of the cricket GI system are that it is consisted from DGIs and VGIs, and that DGIs and VGIs have very different anatomical properties in the thoracic ganglia. Namely, DGIs send out axonal branches extensively both medially and laterally from their main axon, while VGIs project branches only medially except that 7-1 have two lateral branches in the metathoracic ganglion.

The reasons are not clear why there is such a remarkable anatomical differences between DGIs and VGIs in the thoracic ganglia. But it is expected that DGIs and VGIs have quite different physiological functions, for the axonal projections in the thoracic ganglia seems to be mainly output sites. Physiological experiments have shown that there are in fact functional differences between DGIs and VGIs (see next chapter).

VGIs don't have lateral branches except for 7-1, however all GIs have medial branches in each ganglion. So medial branches may be important for GIs' function. These medial branches extends to the vicinity of the midline, therefore as Stubbleefield suggested in the cockroach GI system (1989), some GIs of cricket may have direct connection with contralateral GIs through the medial branches.

Axons of GIs all ascend through suboesophageal ganglion and run into the deutocerebrum of the brain branching off into several collaterals. What physiological functions do GIs have in the suboesophageal ganglion and the brain? 1 GIs may drive various motor systems in the suboesophageal ganglion and the brain. 2 GIs may send sensory information to some multimodal sensory interneurons in the brain. Again in cockroach brain, there have been identified small multimodal interneurons (SM-neurons) which respond to illumination to compound eyes, tactile stimuli and air puff to antennae, vibration to legs and "air puffs to the cerci" (Ohyama and Toh 1986). And in the cricket brain, there may be such interneurons and may receive input from GIs.

Comparison of GIs of cricket with those of cockroach

In the cockroach CNS there have been identified seven types of wind sensitive GIs, which have somata and dendrites in the TAG and ascend axons anteriorly (Roeder 1948; Collin 1985). The anatomy of them has been revealed at TAG level (Daley et al. 1981) and meso- and meta-thoracic level

(Stubblefield 1989). Comparisons of anatomy of cricket's GIs with that of cockroach's GIs shows that projection pattern of axonal branches in thoracic ganglia and morphology of dendrite in TAG are rather different between two species. On the whole as GI system, however, there are common features between them. First, axons of GIs of cockroach ascend in *two groups* through VNC. Four GIs (GI-1, GI-2, GI-3 and GI-4) run through ventral intermediate tract (VIT), while three GIs (GI-5, GI-6 and GI-7) run through dorsal intermediate tract (DIT) (Harris and Smith 1971). Second, in the thoracic ganglia, DGIs (GI-5, -6 and -7) send branches extensively both medially and laterally but VGIs (GI-1, -2, -3 and -4) mainly medially except for GI-1 (Stubblefield and Comer 1989). These similarities in the basic anatomical properties suggest that the GI system had appeared in common putative ancestor of the cricket and the cockroach, and their basic organization has been conserved for a long time.

ABSTRACT

1) The aim in this chapter is to reveal the structural organization of wind sensitive giant interneurons of cricket (GIs; 7-1, MGI, LGI, 9-1b, 9-2, 9-3, 10-2 and 10-3), of which the somata and the dendrites are located within the terminal abdominal ganglion (TAG).

2) Complete visualization of each GI was accomplished in the third instar cricket, of which the VNC is much smaller than that of the adult, by intracellular injection of Lucifer Yellow (Fig. 6).

3) The axon of each GI of third instar ascends from the TAG to the deutocerebrum of the brain, sending out axonal branches in each ganglion on the way (Fig. 7).

4) Transverse sections of the VNC show that GI's are consistently arranged in two distinct groups; 7-1, MGI, LGI and 9-1b ascend through the VIT and are classified as ventral giant interneurons (VGIs), while 9-2, 9-3, 10-2 and 10-3 ascend through the DIT and are classified as dorsal giant interneurons (DGIs) (Fig. 8).

5) GIs have anatomical properties that are unique to their own group. Namely, in the thoracic ganglia all DGIs project axonal branches extensively both medially and laterally, while VGIs project axonal branches almost exclusively medially (Fig. 11 and 12).

6) Projection pattern of branches in the brain and thoracic ganglia are characteristic for each GI, and are consistent from preparation to preparation (Fig. 9). The relative position of axons could be established in the metathoracic ganglion, which is also consistent from preparation to preparation (Fig. 8). So each GI can be identified reliably based on these anatomical features.

7) Backfilling of the VNC of adult cricket reveals that the axons of probable GIs ascend into the brain (Fig. 14). And though it was impossible

to reveal whole structure of a GI of adult cricket with intracellular staining method, morphology of the stained portions of adult GIs are similar to the counterparts of the corresponding GIs of third instar (Fig. 13). Furthermore, GIs of third instar respond with spike to wind stimulation as the adult GIs (Fig. 15). All these facts suggest that GI system of cricket has matured until the animal grown into third instar and that morphological features of third instar GIs presented in this chapter apply to GIs of adult.

8) The basic anatomical properties of GI system of cricket resemble those of cockroach's GI system.

4 PHYSIOLOGICAL FUNCTIONS OF GIANT INTERNEURONS

MATERIAL AND METHODS

Animals

Adult male crickets (Gryllus bimaculatus DeGeer) bred in our laboratory were used throughout the experiment described in this chapter. Two kind of preparation (fixed preparation and tethered moving preparation) were made, according to the aim of the experiment.

Fixed preparation

With fixed preparations, the functional connections between GIs and motoneurons were investigated by stimulating each GI intracellulary while the activity of motor nerves being monitored. The investigated motor nerves were as follows; fifth root of the mesothoracic ganglion (T2R5), third root of the metathoracic ganglion (T3R3), first root of the third abdominal ganglion (A3R1), and seventh root of the TAG (A5R7). For each motor nerve, exclusive preparations were made. The preparations were made as follows.

Preparation for A5R7: After removing all wings and legs of an animal, the abdomen was cut open along the dorsal midline, and the animal was pinned to the wax platform dorsal side up. The gut, internal reproductive organs, and surrounding fats were removed to expose the TAG and A5R7 on both sides.

Preparation for A3R1: An animal was prepared similarly as the preparation for A5R7, then thin muscle and fats around A3 were removed to expose A3R1 on both sides.

Preparation for T3R3: An animal was prepared similarly as for A5R7, then the thorax was cut open and pinned to the wax platform. The muscles and fat were removed which covered the metathoracic ganglion, while taking care that the large flight muscles was not damaged.

Preparation for T2R5: This preparation was made somewhat differently. All wings of an animal were removed and tibia of hind-legs were cut off, but forelegs and midlegs remained intact. The animal was pinned to the wax platform ventral side up and the cuticle over the mesothoracic ganglion and the TAG were cut off to expose these ganglia.

During making these preparations, the saline was poured, for fear that the preparation would dry up. Such a preparation was set in experimental arrangement. A stainless steel spoon introduced posteriorly between the cercal sensory nerve supported the TAG and served as the indifferent electrode. A pair of tungsten bipolar hook electrodes were placed under motor nerves on each side and raised slightly to record their activity. Another bipolar hook electrode was placed under the VNC to monitor the activity of GIs.

Tethered-moving preparation

With tethered moving preparation, the effect of single GIs on behavior was investigated. An animal was anesthetized in a refrigerator at a temperature of -4°C for about 7 min. The dorsal cuticle of abdomen was cut off, and the gut, etc were removed to expose the TAG. A piece of cork was glued onto dorsal surface of the thorax, and a metal holder was glued on a ventral surface of the abdomen. Then the animal was set in the experimental arrangement. The cork on the thorax was picked up by clip which was connected to a manipulator, and the metal holder under abdomen was also fixed to other manipulator. A substratum whose position could be controlled by a manipulator, was covered with aluminum foil to provide slight friction with legs. A stainless spoon was introduced between the cercal sensory nerves, and a bipolar hook electrode was placed under the VNC to monitor the activity of GIs. The animal's

behavior was filmed at 30 frames/sec on videotape. Afterwards, analysis was carried out by profile tracing of single frames.

Intracellular stimulation

GIs were penetrated in the TAG by glass microelectrode filled with 4% (W/V) Lucifer Yellow. The resistance of these microelectrode ranged from 10 to 50 MΩ. Depolarizing current was injected into the penetrated cell via a isolator. The GIs' activities recorded from the VNC (both in fixed and in tethered moving preparations) and the motor activities recorded from each motor nerve (in fixed preparations) were stored on a tape recorder, and then photographed with a continuously recording camera.

Histological identification

After physiological experiments, Lucifer Yellow was injected iontophoretically into the neuron with hyperpolarizing current of 2-5 nA for 1-10 min. Then the TAG was processed described previous chapter, to identify the penetrated interneuron.

RESULTS

The aim of this experiment is to reveal physiological function of GIs of cricket. First, the author investigated whether there are functional connections between each GI and motoneurons not only in the thoracic ganglia, but also in the abdominal ganglion and the TAG. Then the author revealed what behavior is related to these functional connections, if any.

Motor nerves and their response to wind

To establish the functional connections between GIs and motoneurons, each GI was stimulated intracellularly while monitoring the activity from a pair of motor nerves on both sides. The investigated motor nerves are as follows.

1. Fifth nerve roots of the mesothoracic ganglion (T2R5), which innervate midleg muscles.

2 Third nerve roots of the metathoracic ganglion (T3R3), which innervate flight muscles.

3 First nerve roots of third free abdominal ganglion (A3R1), which innervate abdominal transverse muscles.

4 Seventh nerve root of the TAG (A5R7), which innervate cercal muscles.

Soon after the animal was prepared for the physiological experiment, breath was blown on the cerci to check the response of the motoneurons. There were two purposes. One was to see whether the preparation was damaged or not during the operation. Another purpose was to compare the motor responses evoked by wind stimulation with those evoked by intracellular stimulation of single GIs. If cercus-to-GI system constitute significant part of information pathway from cerci to the motor centers,

the motor responses evoked by intracellular stimulation of single GIs parallel those evoked by wind stimulation.

The typical response of each motor nerve to wind stimulation are shown in Fig. 16. The response recorded from T2R5 were not consistent and varied in the duration, strength, or pattern; from preparation to preparation and from trial to trial even in the same preparation. While the response from T3R3, A3R1, and A5R7 were consistent.

In T2R5, several units respond vigorously. Some units seem to be of excitatory leg motoneurons, for the spike discharges in T2R5 were almost always accompanied by abrupt movement of the middle legs.

In T3R3, many units responded vigorously and spike discharges outlasted wind stimulation. Some units is likely to be of excitatory flight motor neurons, for spike discharges occurred simultaneously with the activity of flight muscles.

In A3R1, on the other hand, perhaps only one unit responded sporadically in any preparation. Activity of this unit could be recorded from more peripheral site, at the vicinity of a abdominal transverse muscles. So this motoneuron is likely to innervate these muscles; though it is not certain whether this is an excitatory or inhibitory neuron.

In A5R7, also perhaps only one unit fired in any preparation. This unit is surely of cercal closer motoneurons. Because this unit fired whenever the cerci moved inwardly and remained close whether the cercal movement was evoked by wind stimulation, or occurred spontaneously synchronizing with respiratory abdominal movement.

Motor response to single GIs stimulation

Intracellular stimulation of a member of DGIs evoked spike discharges in some motor nerves. However, the responsiveness of motor systems to GI stimulation were not consistent and varied from

preparation to preparation and from trial to trial even in the same preparation. For example, in some trial, spike activities was evoked in A5R7 by a GI stimulation of less than 100 msec duration, but in other trial no response appeared, no matter how long the GI was stimulated in the same preparation. This suggests that GI-motor connections are modulated by higher control center. As it was impossible to eliminate or control these putative modulatory effects, the threshold and latency of GI-motor connections were not measured.

10-3: Intracellular stimulation of 10-3 elicited spike discharges in both sides of T2R5 and T3R3, as shown in Fig. 17. In T2R5, though response pattern and duration varied from preparation to preparation and even from trial to trial in the same preparation, several units were seen to fire vigorously in any preparation. In T3R3, vigorous motor response were evoked. The action potentials seemed to be of motoneurons which responded to the wind stimulation. And spike discharges outlasted the excitation of 10-3. Intracellular stimulation of 10-3, however, never evoked response neither in A3R1 nor A5R7, as far as investigated.

10-2: Intracellular stimulation of a 10-2 elicited spike discharges in both sides of T2R5, T3R3, A3R1, and A5R7 as shown in Fig. 18. In T2R5, though the pattern of spike activity varied from trial to trial, several units responded vigorously. In T3R3, the responding units seemed to be those which responded to wind. And again, the response outlasted the activity of 10-2. 10-2 also evoked spikes in A3R1, perhaps of only one unit. The spikes were sporadic and surely are of a motoneuron which responded to the wind stimulation. On both sides of the ganglion, spike discharges began at the almost same time, lasted during excitation of a 10-2, and ceased soon after the termination of excitation of 10-2. Also in A5R7, spike discharges of perhaps only one unit were evoked on both sides of the ganglion. The motor activity began at almost same time on both sides and ceased soon

after the excitation of 10-2 terminated. Evoked spikes were surely of motoneurons which responded to wind. And as soon as the motoneurons began to response, the cerci were observed to move inwardly and remained closed as long as the motoneurons fired.

9-3: Intracellular stimulation of 9-3 evoked spike discharges in both sides of T2R5, T3R3, A3R1, and A5R7 (Fig. 19). Response feature and responding units in each motor nerves were almost same as in the case of 10-2, but for A5R7. In the A5R7, motoneurons on axon sides fired earlier and more vigorously than on the soma side. This asymmetry of motor response is characteristic for 9-3.

9-2: Intracellular stimulation of 9-2 evoked spike discharges in both sides of T2R5, T3R3, A3R1, and A5R7. The response feature and responding units were almost same as in the case of 10-2

VGIs: Fig. 20 shows the record from each motor nerve during the intracellular stimulation of MGI (a member of VGIs). As this example, MGI never evoked spike discharges in these motor nerves. And the other VGIs (7-1, LGI, 9-1b) never evoked spike discharges. VGIs seemed to have no excitatory connections with thoracic and abdominal motor systems as far as investigated in this experiment.

Motor responses to GI stimulation of varying duration

As described above, even in the same preparation, stimulation of a GI evoked spike in motor nerves in some trials, and did not at all in other trials. However, there were some preparations for T3R3, A3R1, and A5R7, in which the responses of motoneurons were relatively stable and reproducible. In the experiments with these preparations, the duration of stimulation was varied (Fig. 21, 22, and 23). As shown in Fig. 21, the motoneurons in T3R3 fired vigorously and almost equally in response to 9-2, regardless of the stimulus duration (50, 100, 200 msec). And similar

results were obtained for 10-3, 10-2 and 9-3. In contrast to the thoracic motoneurons, abdominal motoneurons fired as long as a DGI was exciting. As shown in Fig. 22, the longer the duration of the stimulation of 9-3 was, the longer the response of motoneurons in A3R1, and the response ceased as soon as the termination of the excitation of 9-3. For 10-2 and 9-2, similar result was obtained. The response of cercal motoneurons in A5R7 to a DGI were also dependent upon the duration of stimulation of the DGI (10-2, 9-3 and 9-2; Fig.23).

Site of GI-cercal motor interactions

It was expected that GIs of cricket have output connections with motoneurons in the thoracic ganglia. However, it was not expected at all that GIs have functional connection with cercal motoneurons in the TAG, for the TAG seemed to be exclusively input site for GIs. So, once the connection was established, the author wanted to determine whether the connection was made within the TAG.

An alternative connection may be mediated by descending interneurons, which receive sensory information within anterior ganglion (brain, suboesophageal, or thoracic ganglia), and then elicit cercal motoneurons via their descending axons. To distinguish between these two possibilities, after a DGI was impaled and stimulated, the connectives were cut between the TAG and the fourth abdominal ganglion. Then after checking that the micro-electrode was still in the GI, the GI was re-stimulated. The result for 9-3 is shown in Fig. 24. As this example, for 9-2 and 10-2, the motor response was reproduced. This indicates that connections between DGIs and cercal motoneurons, whether monosynaptic or polysynaptic, is made within the TAG.

The behavioral outputs evoked by single GIs stimulation

The author have demonstrated that trains of action potentials in single DGIs evoke spike discharges in various motoneurons. How do these functional connections relate to the animal's behavior under natural conditions? And what are the functions of VGIs? To reveal these obscurities, tethered-moving preparations were made which could move legs as if they had walked, when the legs of the animal were in contact with substratum, and express flight behavior when the animal was suspended in the air. With this preparation, each GI was stimulated intracellulary and behavior of the animal was filmed on the video-tapes.

Diagram of the experimental set up is shown in Fig. 25. The substratum could be moved up and down. When footing was up and the legs were in contact with it, slight blow on the cerci of the preparation drove the animal to move all legs abrupt. And then, the animal move all legs reciprocally, which would produce forward movement of the animal if not tethered, though the direction of movement could not be deduced. On the other hand, when the substratum was down and the same animal was suspended in the air, blow on the cerci triggered flight behavior (Fig. 26). As shown in Fig. 26, within 30 msec from the commencement of wind stimulation, the animal begin to raise all legs. After about 100 msec the legs is at highest position above body level, then as lowering the legs the animal opened fore wings and hind wings and began to flutter (about 1 msec after onset of the stimulation), finally it folded up all legs and came into stable flight posture.

Before begging GI's stimulation experiment, breath was blown on the cerci to check the response as in the case of the fixed preparation. When the response was 'normal' (namely the animal flew and walked), the experiment was went on with. If the behavior was abnormal, this preparation was abandoned, and new preparation was made.

The responsiveness of crickets to single GIs' activity was not consistent and varied from preparation to preparation, as that of motoneurons did so in the fixed preparation. So also with tethered-moving preparation, the threshold and latency of the response were not investigated.

When the legs were in contact with the substratum, stimulation of any single DGIs (9-2, 9-3, 10-2, 10-3) elicited first abrupt movement of legs, then regular movement of all legs on both sides as if the animal had walked. This movement of legs would propel the animal forward if not tethered. At this time, the head and the antennae were moving up and down. The pattern of behavior was almost same, whether which DGI was stimulated. Fig. 27 illustrates the behavioral output evoked by intracellular stimulation of 9-3, when the legs was in contact with the substratum. The minimum duration of stimulation needed to elicit walking movement was 30 msec for 9-2 and 9-3, 50 msec for 10-2 and 10-3. In the case the legs were not in contact with the substratum, namely the preparation was suspended in the air, stimulation of any single DGIs initiated flight behavior, the sequence of which was identical to the behavior initiated by wind stimulation, regardless of the types of stimulated DGIs. Fig. 28 illustrates behavioral output evoked by intracellular stimulation of 9-3. The minimum duration needed to elicit flight behavior was 20 msec for 9-2 and 9-3, 30 msec for 10-2 and 10-3.

On the other hand, VGIs had no efect on behavior, whether the legs were in contact with the substratum or not.Furthermore, stimulation of single VGIs didn't change behavior at all, even when the animal was walking or flying. So physiological function of VGIs still remains to be uncovered.

DISCUSSION

The purpose of this experiment is to clarify the physiological functions of the cercus-to-giant interneurons system. The most striking features of the results are that DGIs (9-2, 9-3,10-2,10-3) have excitatory connection with wide variety of motoneurons and elicit flight behavior or walking movement. In contrast, VGIs have no apparent effect on the animal, as far as investigated.

The functions of DGIs

With fixed preparation, the functional connections only with motoneurons running in T2R5, T3R3, A3R1, and A5R7 have been investigated. So the connections with other motor systems remains to be unknown. But in the experiment with tethered-moving preparation, the movement of all legs, wings, antennae and head was observed during flight or walking evoked by stimulation of single DGIs. Therefore, it is expected that 9-2, 9-3, 10-2, and 10-3 have output pathway in the brain, the suboesophageal ganglion and the thoracic ganglia. Further 9-2, 9-3, and 10-2 perhaps have output connections in every abdominal ganglion. Abdomen is constructed from homologous segments, and A1, A2, A3 and A4 resemble each other in structure. So these DGIs likely to have output connections not only in A3 but also in A1, A2, and A4.

And surprisingly, 9-2, 9-3, and 10-2 have output pathway in the TAG. Why do the DGIs have the output connection in the TAG? A putative reason is that the cerci, abdomen, thorax, and head need to act simultaneously and/or in harmony with each other. Therefore it seems to be appropriate that related motor systems in each ganglion are controlled by the same neural elements.

Activity of single DGIs could elicit complete flight behavior or walking movement identical with that evoked by wind stimulation. And the minimum duration needed to elicit these movement is relatively short (20~50 msec) And there are eight DGIs on both sides in total. These facts suggest that DGIs play a chief role in transmitting sensory information from cerci to motor centers throughout the body under natural condition.

Under natural condition, how do these DGIs relate to the animal's behavior ? DGIs are likely to elicit and to maintain flight behavior, when the animal is in the air and sense air current, for example, after jumping. And when the animal is on the ground, DGIs are likely to trigger escape behavior from the source of air current, which perhaps means predator for the cricket.

Baba has reported that during flying or walking, DGIs receive excitatory input from motor center (1991). So once the behavior is initiated, positive feedback loop of DGIs and the motor centers may begin to function, to maintain the behavior. Therefore DGIs' function seem to be not only to initiate the behavior but also to maintain the behavior. Gillete et al. reported the command neurons which receive excitatory synaptic feedback from motor center they excite (1978). Such command neurons may exist widely among animals.

Though the axonal branches are restricted to the hemisphere of axonal side in each ganglion (see previous chapter), motor response and behavioral response to single DGIs appeared on both sides simultaneously and similarly. This fact suggests that the connections between DGIs and motoneurons are mediated by unknown interneurons, or DGIs trigger CPG(s) which control motoneurons on both sides. At thoracic ganglia, DGIs perhaps trigger CPG(s) for flight or walking, because the flight behavior or walking movement outlasted firing of single DGIs (Fig. 27 and 28). On the other hand, the motor system in the abdominal ganglia fired

as long as 9-2, 9-3, or 10-2 fired. So, DGIs don't seem to trigger neural circuits such as CPG(s) in the abdominal ganglia.

By now, what is called command neurons have been classified into subclasses based on various criteria, one of which is the duration of evoked motor responses. According to this criterion, if the motor response outlasts the activity of a command neuron themselves independent of their duration, this command neuron is called 'trigger neuron'. And if duration of motor response is restricted to the duration of activity of command neuron, this neuron is called 'gating neuron'. However, 10-2, 9-3, and 9-2 have both functions; function as trigger neuron for thoracic motor systems, and function as gating neuron for abdominal motor system. Therefore it may be improper to classify a command neuron, which have connections with various motor system, as gating or trigger neuron. Rather it may be proper to say that this connection is controlled by gating mode and that connection is controlled by triggering mode.

The function of VGIs

VGIs seem to have quite different function from that of DGIs, which the author couldn't established in this experiment. The putative functions of VGIs are as follows, 1, VGIs might have so weak excitatory connections with flight or walking motor system that exert subthreshold effects, or might modulate DGIs' effect. 2, VGIs might inhibit ongoing behavior which couldn't be reproduced in the preparation and investigated in this experiment, such as feeding or courtship behavior. 3, VGIs might excite modulatory neurons in the CNS, such as dorsal unpaired medium (DUM) neurons. In cockroach, it has been shown that VGIs excite thoracic DUM neurons. So VGIs of cricket may have such function.

Modulation of DGIs-motor conncections

In some preparations, single DGIs never evoked motor response or behavioral change, no matter how long the stimulation was. These inconsistencies perhaps result from slight mistakes during dissection of the preparations. So, if the dissection had been done well in these preparations, DGIs would have evoked motor responses or behavioral changes. In other preparations, the situations were more complicated. In these preparations, at some trials, brief stimulation of the DGI elicited motor response or behavior, but at other trials no response was evoked no matter how long the stimulation of the DGI was.

In these cases, some defect in the preparation was not evidently the cause of the inconsistency. it is likely that this inconsistency reflect the mocification of DGIs-motor connections. In general, stereotyped behavior of animals is not always released by same stimulation, but only under some suitable conditions, in other word the threshold of the behavior to the stimulation fluctuates.

For examples, the threshold of escape behavior of crayfish, which is triggered by touch to the abdominal cuticle, fluctuates. Namely, the stimulation of same intensity trigger escape in some case, and don't in other case. This reflex behavior is mediated by medial giant interneuron (MGI), or lateral giant interneuron (LGI); the command neurons , which receive sensory input from mechanical sensory neuron and trigger tail flip escape behavior. A single spike in LGI always results in tail flip, but the responsiveness of LGI themselves varies with time, and as a result, the animal escapes in some occasions, and doesn't in other occasions, to the stimulation of same intensity. The responsiveness of LGI are found to be controlled by the brain, because the decerebration stabilizes the responsiveness. In short, the modification of escape behavior of crayfish is controlled by brain at the level of the connection between sensory neurons to LGI.

As this example, the threshold of flight behavior or walking movement of the cricket also seems to be modified, at least at the level of DGIs-motoneurons connections, though the significance of putative modification can't be estimated.

Properties peculiar to each GI

The author has been interested in whether each GI has peculiar function. In this experiment, DGIs seemed to have similar function one another, except that 10-3 don't have connections in abdominal ganglia. There may be some essential functional differences between GIs (for examples, direction of walking triggered by each DGI may differ), though in this experiment any differences couldn't be detected. To reveal the obscurity, more elaborated preparations need to be made. But clearly VGIs differ from DGIs in physiological functions, though functions of VGIs couldn't be established.

DGIs and VGIs

In previous chapter, the author showed that DGIs and VGIs have morphological features characteristic for each groups in thoracic ganglia. And in this experiment, it is revealed that DGIs and VGIs have different physiological functions. In short, GI system is constituted from two separate subgroups; DGIs and VGIs, which differ both morphologically and physiologically. Why is GI system constituted from such subgroups? Are there any advantages? The reason is a quite mystery. However, it may be suggestive to note that cockroach has cercus-to-GI system similar to that of cricket. The similarities are as follows. 1. GIs of cockroach can be classified into three DGIs (GI-5, -6 and -7) and four VGIs (GI-1, -2, -3 and-4), based on the axonal positions (Harris and Smith 1971). 2. DGIs extend axonal branches to both side of thoracic ganglia, while VGIs mainly

medially (Stubblefield and Comer 1989). and 3. DGIs elicit flight motorneurons when the legs are not in contact with substratum and elicit legs' motoneurons when the legs are in contact with substratum, while VGIs never elicit flight motorneurons (Ritzmann et al. 1980). Therefore basic feature of GI system are common to the two species and perhaps has been conserved through history of evolution. This suggests that there are some advantages of such construction of GI system for these insects.

ABSTRUCT

1. In the experiments with fixed preparations, it was shown that 10-2, 9-3, and 9-2, evoked spike discharges in the flight motoneurons, leg motoneurons, abdominal motoneurons and cercal motoneurons and 10-3 evoked spike discharges in the flight and leg motoneurons, when each GI was depolarized with depolarizing current pulse (Fig. 17, 18, and 19).

2. Activity of flight motoneurons outlasted the artificial activity of a DGI (Fig. 21). On the other hand, the activity of abdominal motoneurons and cercal motoneurons lasted as long as a DGI was fired, and terminated as soon as cessation of a DGI stimulation (Fig. 22 and 23).

3. In contrast, ventral types of GIs (7-1, MGI, LGI, 9-1b) never evoked spike discharges in the motoneurons as far as investigated.

4. In the experiment with tethered-moving preparations, The activity of single DGIs (10-3, 10-2, 9-3, 9-2) triggered complete flight behavior when the legs of the animal were in contact with the substratum, and triggered walking movement when the animal was suspended in the air (Fig. 27 and 28).

5. In contrast to DGIs, VGIs never evoked any movements in tethered-moving preparation, no matter how long single VGIs was stimulated.

6. Therefore DGIs seems to constitute pathway of sensory information from cercus to motor systems throughout the body, to trigger flight or escape behavior. While VGIs perhaps have unknown function different from that of DGIs.

7. GI system of cricket resembles that of cockroach in the view point of physiological function.

5 GENERAL DISCUSSION

Giant interneurons

What is called giant interneurons are identified in various animals, such as crayfish (Wiersma 1947), fish (Eaton et al. 1982), fruit fly (Tanouye and Wyman 1980), cockroach (Roeder 1948), praying mantids (Boyan and Ball 1986), locust (Boyan and Ball 1989a) and bush cricket (Shen 1983). The axons of these interneurons are substantially larger than other types of interneurons, and so they are named 'giant' interneurons (GIs). And they are all somehow related the escape behavior. And in this study, it is shown that cricket GIs likely to trigger escape behavior (flight and walking). It is likely that natural selection has pressured nervous system to have large axons of neurons in order to quicken the speed of transmission in relatively small animals as above.

The feature of cricket GI system is that GIs are constructed from DGIs and VGIs, which differ from each other anatomically and physiologically. These feature seems to be characteristic to insects GI system which receive sensory information of air current from the cerci, though concrete results are available only for cockroach system.

The role of DGIs of the crickets

DGIs trigger walking movement when the leg is contact with the substratum, and trigger flight behavior when the legs is not in contact with substratum. Namely, DGIs are bifunctional neurons. Why are two different behavior triggered by the same neurons, not by separate neurons ? Some neurons may have multifunctions to save the total number of the neurons in the nervous system. And it is interesting to note that cockroach' DGIs are bifunctional neurons that can trigger the activity of flight motoneurons when all legs are not in contact with the substratum,

and trigger the legs' motoneurons when a leg is in contact with the substratum (Ritzmann et al. 1980).

Then, why are there as many as four types of DGIs, which apparently have similar functions ? One type of trigger neuron seems to be sufficient for the control of the behavior. But in this system redundancy of sensory information may be important for reliance and so many DGIs exist. Or, there may be essential and important differences in their functions. There is found a functional difference among DGIs. Namely, 10-3 don't have connections with motoneurons in abdominal ganglia, with which the other DGIs have connections. But this difference seems not to be so essential. However, there may be further functional differences which have not been known. Kanou and Shimozawa showed that 10-2 and 10-3 encode the velocities while 9-2 and 9-3 encode both the velocities and acceleration, and that each have preferred directions of wind stimulation (1974). And in this study, it is revealed that each GI have characteristic input pathways. In short, each DGI have characteristic physiological properties. Therefore these properties may be reflected in their output connections. For example, a DGI which respond well to wind from right direction, may control motor center to turn the animal away from the right side. DGIs may have such ability, to trigger escape behavior from the source of the air current, which means predator for the cricket.

Axons of DGIs extend throughout the VNC, sending out axonal branches in each ganglion on the way. And perhaps DGIs have output connections in every ganglion except that 10-3 doesn't have in abdominal ganglia. Why do DGIs have connections with so many motoneurons throughout the body ? Flight or escape behavior consist not only of thorax but also of various parts of the body, such as antennae, head, abdomen and cerci, although the propulsive power is produced certainly by thoracic organs. And they need to act simultaneously and/or in harmony with

each other. therefore it seems to be appropriate that related motor systems throughout the body are controlled by the same neural elements such as DGIs.

By now, what is called command neurons have been classified into subclasses based on various criteria, one of which is the duration of evoked motor responses. According to this criterion, if the motor response outlasts the activity of a command neuron themselves independent of their duration, this command neuron is called 'trigger neuron'. And if duration of motor response is restricted to the duration of activity of command, this neuron is called 'gating neuron'. However, 10-2, 9-3, and 9-2 have both functions; function as trigger neuron for flight or walking motor systems, and function as gating neuron for abdominal motor system. therefore it may be improper to classify a command neuron, which have connections with various motor system, as gating or trigger neuron. Rather we should say that this connection is controlled by gating mode and that connection is controlled by triggering mode.

The role of VGIs of the cricket

Physiological functions of DGIs could be established to some extent. In contrast, VGIs' functions remain to be entirely obscure. The most probable function of VGIs is modulatory one, for example, VGIs may excite thoracic DUM neurons as cockroach VGIs do (Pollack and Ritzmann 1988). Anyway it is certain that VGIs and DGIs have different physiological functions.

Feature of GI system

The most striking feature of GI system of cricket is that it consist of DGIs and VGIs, which differ from each other physiologically and anatomically. It is interesting that the axons of physiologically and

anatomically different neurons are separated into two groups. But why is GI system constructed from such separated subgroups ? Are there any advantages of such constructions ? It may be suggestive that cockroach has cercus-to-GI system similar to that of cricket. The similarities are as follows. 1. GIs of cockroach can be classified into four types of VGIs(GI-1, -2, -3, and -4) and three types of DGIs(GI-5, -6, and -7) based on the position of the axons (Harris and Smith 1971). , 2. DGIs extend axonal branches to both side of thoracic ganglia, while VGIs mainly medially (Stubblefield and Comer 1989), and 3. DGIs elicit flight motoneurons when all legs are not in contact with substratum and elicit legs' motoneurons when a leg is in contact with substratum. In contrast, VGIs never elicit flight motoneurons (Ritzmann et al. 1980). Therefore basic feature of GI system are common to these two species and perhaps has been conserved throughout history of evolution. So, these feature of GI system seems to have some advantages, which has not been revealed yet.

6 CONCLUSION

CONCLUSION

The purpose of this study is to clarify properties of the cercus-to-GIs system of the cricket, such as the input pathways to this system, structural organization, and the physiological function.

1) Eight wind-sensitive interneurons originating in the TAG were found to have relatively large axons ascending through the ventral nerve cord (VNC) and therefore are classified as giant interneurons (GIs; 7-1, MGI, LGI, 9-1b, 9-2, 9-3, 10-2 and 10-3).

2) 7-1 receives informations through polysynaptic pathways from filiform afferents on its either side and through monosynaptic pathways from bristle sensory neurons on either side. MGI receives through monosynaptic pathways from filiform afferents on its axon side and polysynaptic pathways from soma side. LGI or 9-2 receives through monosynaptic pathways from axon side and receives no information from soma side. 9-1b receive through polysynaptic pathways from either side. 9-3 receives through polysynaptic pathways on soma side and monosynaptic pathways from axon side. 10-2 and 10-3 through monosynaptic pathways from either side.

3) The axon of each GI ascends from the TAG to the deutocerebrum of the brain, sending out axonal branches in each ganglion on the way GIs ascend separated in two groups. Namely, 7-1, MGI, LGI and 9-1b ascend through the VIT in group and are classified as ventral giant interneurons (VGIs), while 9-2, 9-3, 10-2 and 10-3 ascend through the DIT in group and are classified as dorsal giant interneurons (DGIs). In the thoracic ganglia, GIs have anatomical properties that are unique to their own group. Namely, every DGIs project axonal branches extensively both medially and laterally, while VGIs project axonal branches exclusively

medially, except that 7-1 have lateral branches in the metathoracic ganglion.

4) 10-2, 9-3, and 9-2, evoke spike discharges in the flight motoneurons and leg motoneurons in thoracic ganglia, and motoneurons in abdominal ganglia, and cercal motoneurons in the TAG. And 10-3 evoked spike dischrges in the flight and leg motoneurons in the thoracic ganglia. Perhaps through these output connections, DGIs triggered complete flight behavior when the legs were in contact with the substratum, and triggered walking movement when the animal was suspended in the air. In contrast, ventral types of GIs (7-1, MGI, LGI, 9-1b) never evoked spike discharges in the motoneurons and never evoked any movements as far as investigated. VGIs seem to have unknown functions different from those of DGIs.

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8 FIGURES AND FIGURE LEGENDS

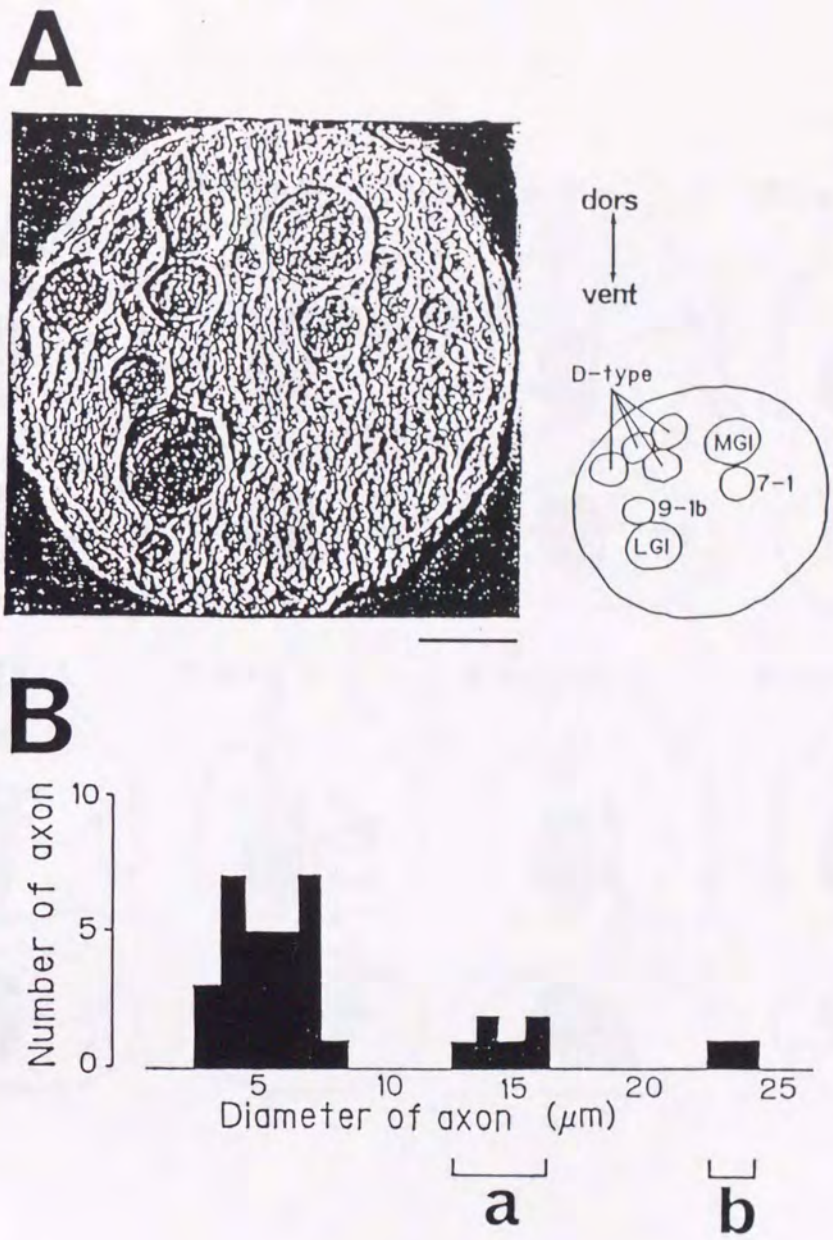

Fig. 1. A. Transverse section of a ventral nerve cord (VNC) at 100 μm anterior from the terminal abdominal ganglion (TAG). The section is viewed posteriorly with dorsal side at the top, medial side at the right. Eight large axons can be seen. The inset shows identity of the eight large axons. Scale bar:20 μm. B. Histogram of diameter of axons seen in the above section. Diameter of 7-1, 9-1b, 9-2, 9-3, 10-2, 10-3 are 13~16 μm(a), and those of MGI and LGI are 23~25 μm(b).

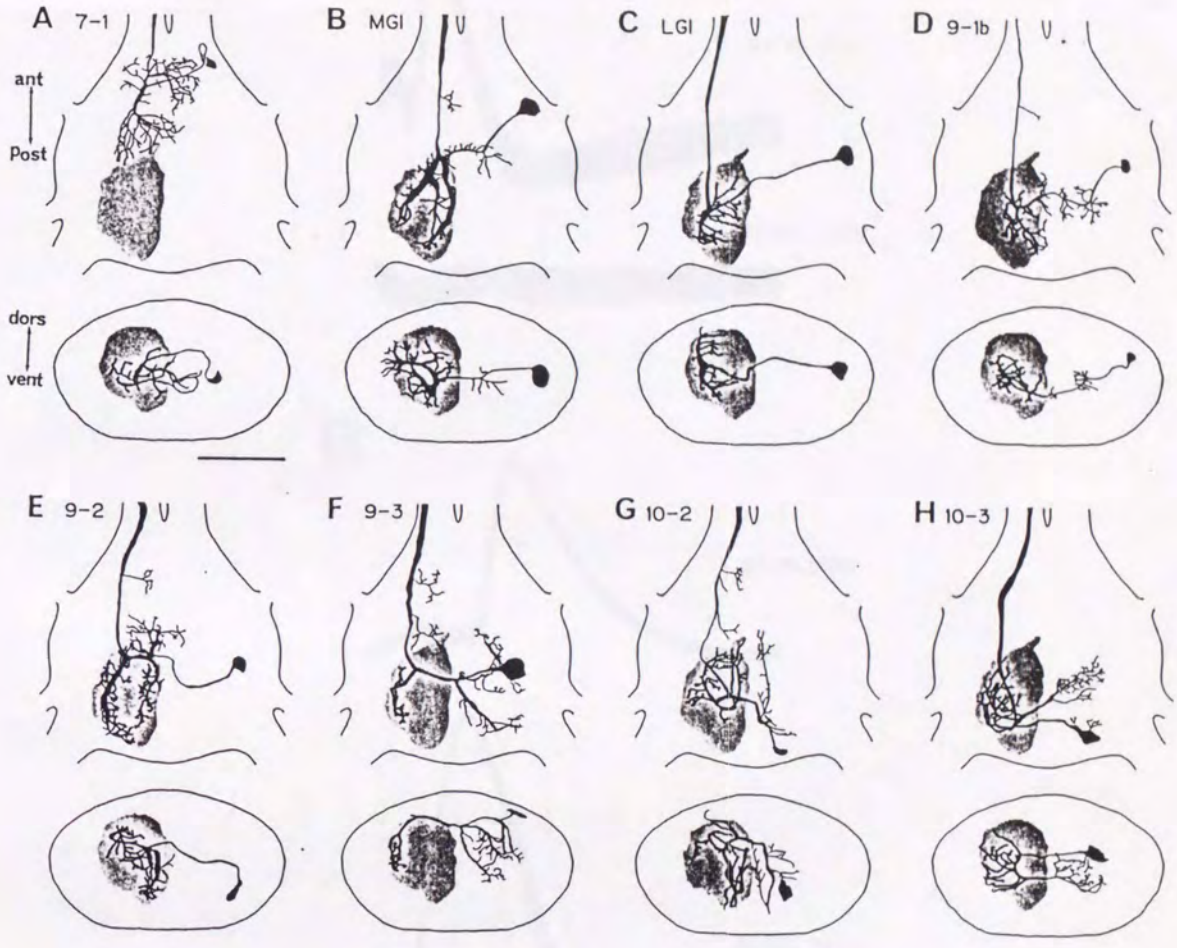

Fig. 2. Morphology of eight giant interneurons whose axons in the VNC are substantially large. Each neuron was stained intracellularly with Lucifer Yellow and drawn from wholemount. The ganglion is viewed dorsally (upper), and posteriorly (lower). The area where arbolizations of most of filiform afferents exist is shown by stippling. Scale bar:200 μm.

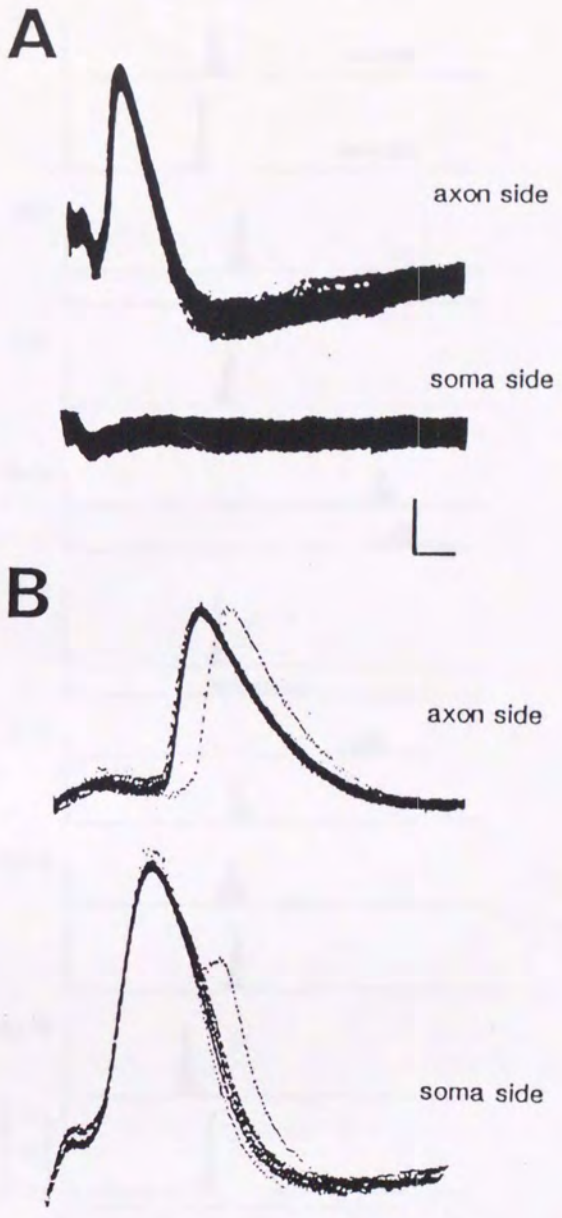

Fig. 3. Intracellular recording from 9-2 (A), and 9-3 (B). Responses of these giant interneurons were evoked by 2 Hz electrical stimulation to the cercal sensory nerve on each side. Initial deflections in each record are artifacts, and each trace consists of multiple sweeps (10 times) of the oscilloscope. Note that the 9-2 did not fire when the cercal sensory nerve on the soma side was stimulated. Scale bar; vertical 10 mV, horizontal 1 msec.

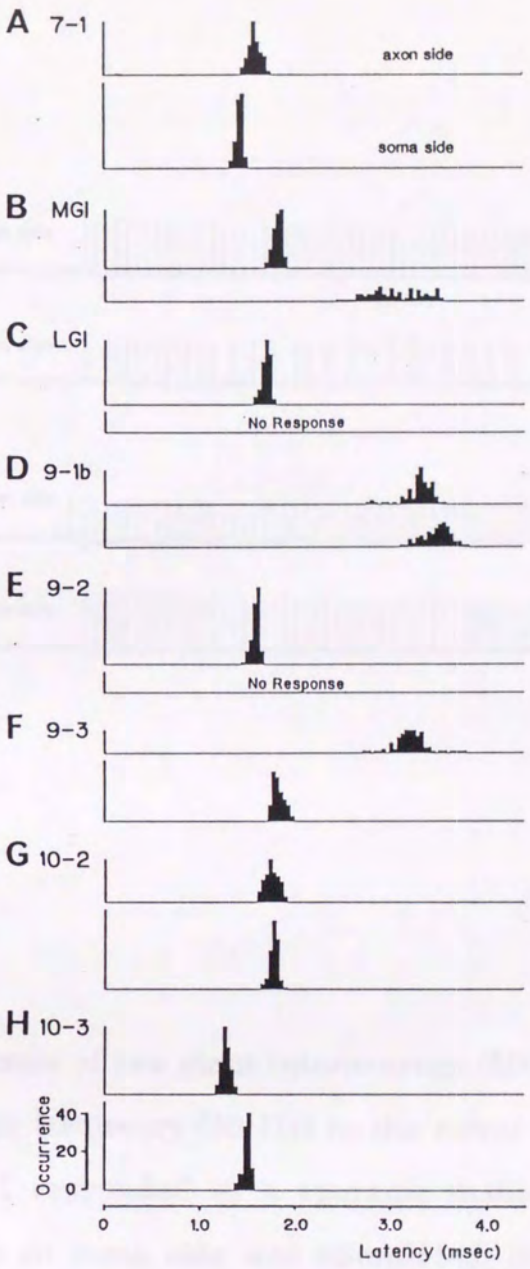

Fig. 4. Histogram showing response latency of the spike response in each giant interneuron evoked by 2 Hz electrical stimulation to the cercal sensory nerve on each side.

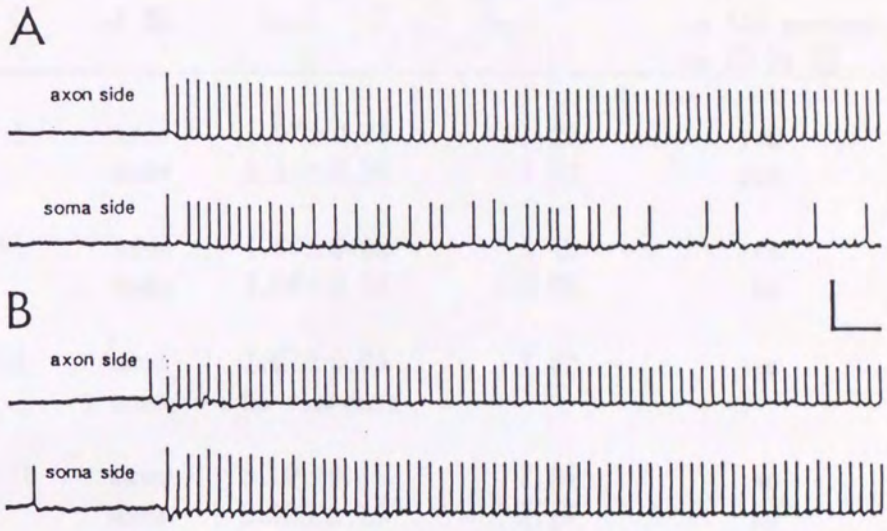

Fig. 5. Responses of two giant interneurons (MGI in A, 10-3 in B) to stimuli of high frequency (50 Hz) to the cercal sensory nerve. Note that the MGI responded in a sporadic fashion when the cercal sensory nerve on soma side was stimulated. Scale bar; vertical 40 mV, horizontal 100 msec.

Type of GI	Location of ES	Latency (ms)	Synaptic delay (ms)	Response in 1:1 pattern to 50 Hz ES
7-1	axon	1.57±0.07	1.22	yes
	soma	1.41±0.04	1.06	yes
MGI	axon	1.80±0.05	1.45	yes
	soma	3.04±0.28	2.69	no
LGI	axon	1.67±0.05	1.32	yes
	soma	No response		
9-1b	axon	3.29±0.15	2.94	no
	soma	3.49±0.15	3.14	no
9-2	axon	1.57±0.04	1.22	yes
	soma	No response		
9-3	axon	3.21±0.17	2.86	no
	soma	1.81±0.06	1.46	yes
10-2	axon	1.73±0.07	1.38	yes
	soma	1.76±0.05	1.41	yes
10-3	axon	1.25±0.05	0.55	yes
	soma	1.50±0.04	0.80	yes

Table 1. Table showing response latency and synaptic delay to 2 Hz electrical stimulation of the cercal sensory nerve, and capacity to follow 50 Hz stimulation of the same nerve 1:1 in eight GIs. Each latency value are the mean ± SD of one hundred measurements. Synaptic delay was calculated from the mean value of latency by subtracting the conduction time of the spike (see text).

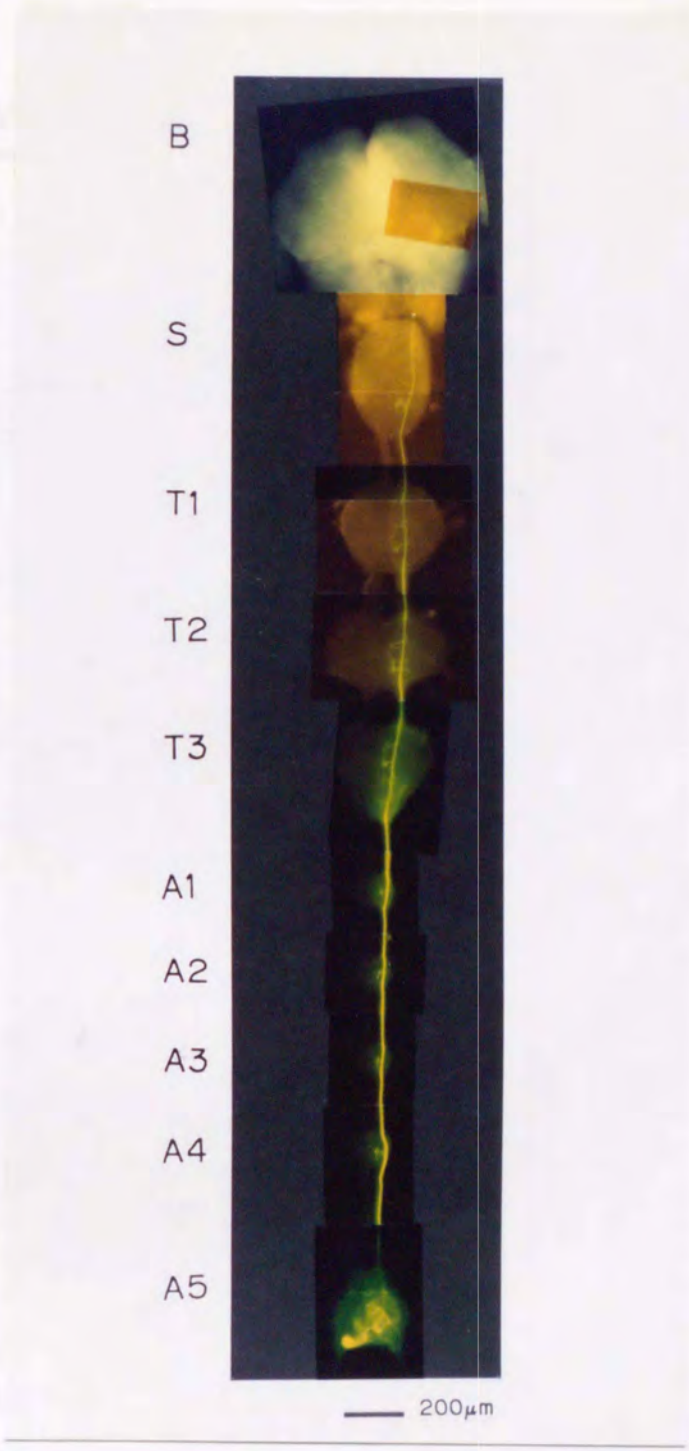

Fig. 6. Photomicrograph of a third instar's GI (10-2) of which the whole structure was stained with Lucifer Yellow running from the terminal abdominal ganglion (A5) to the deutocerebrum of brain. B, brain; S, suboesophageal ganglion; T1~T3, pro-, meso-, and metathoracic ganglia; A1~A5, abdominal ganglia.

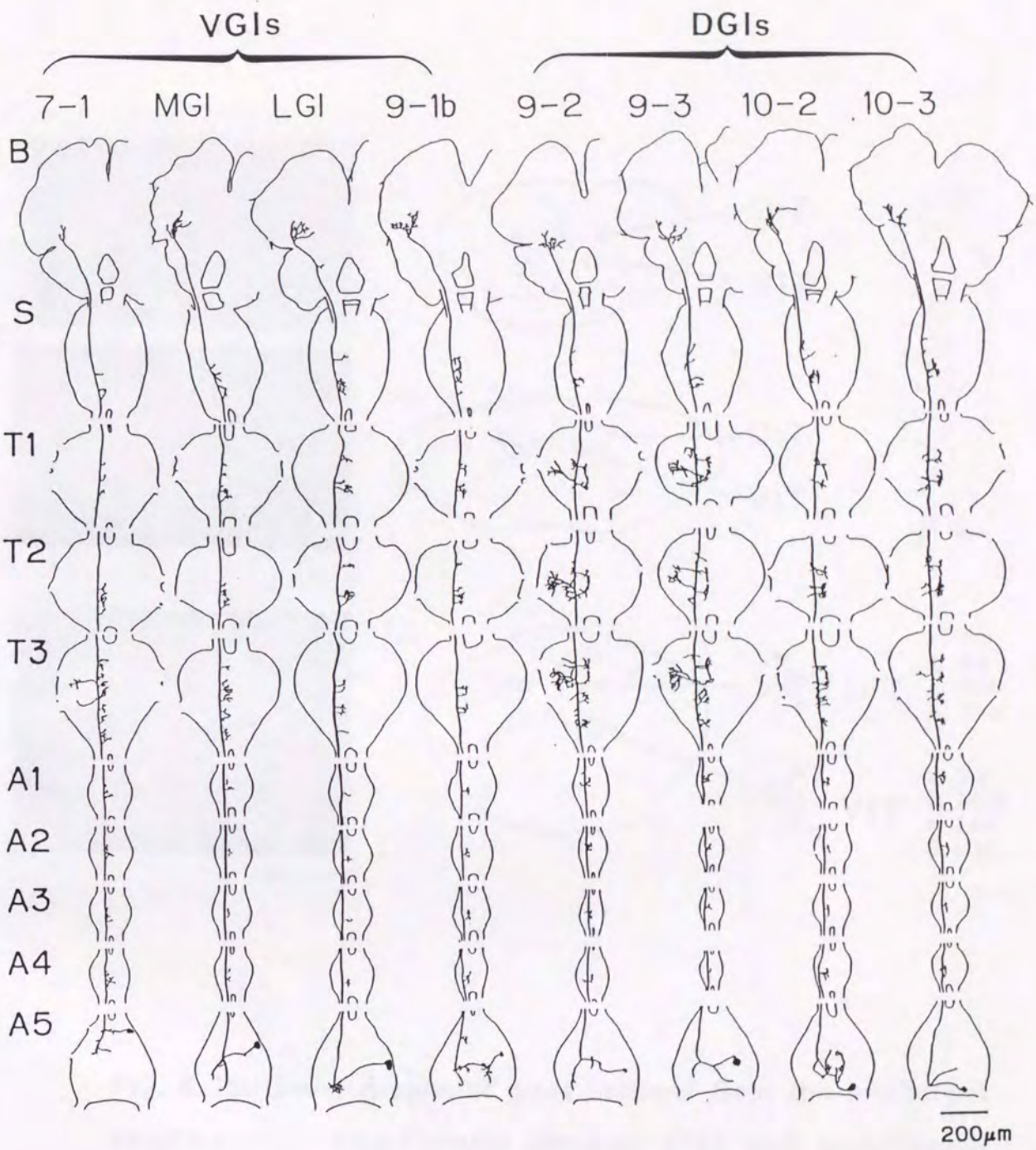

Fig. 7. Whole structure of eight GIs. The axons of all GIs ascend from the terminal abdominal ganglion to the deutocerebrum of the brain and spread out the axonal branches in each ganglion on their ways. For abbreviation, see Fig. 6.

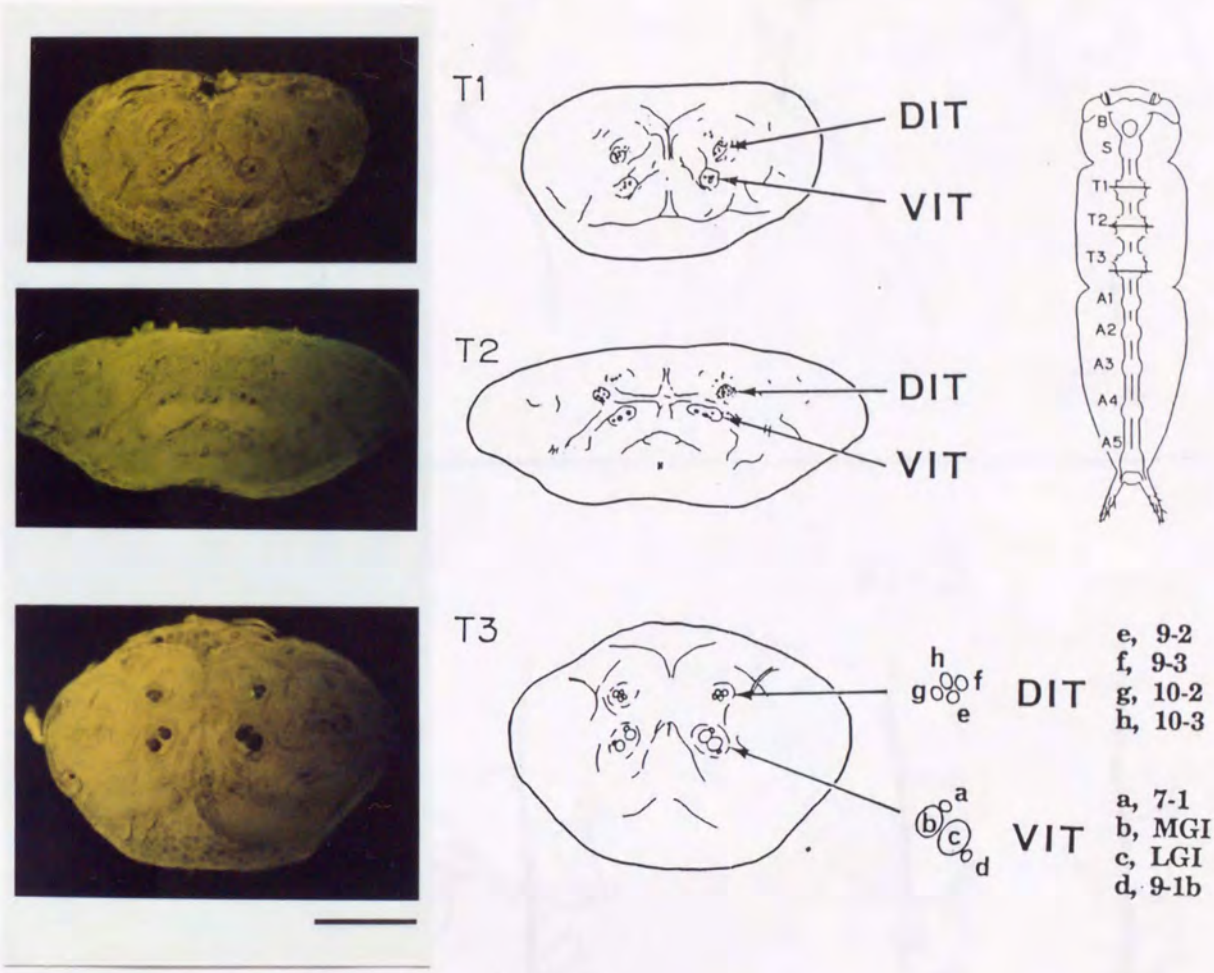

Fig. 8. Photomicrographs of cross sections from the prothoracic ganglion (T1), mesothoracic ganglion (T2), and metathoracic ganglion (T3) of an animal in which 10-2 on right side was labeled. Diagram of the sections show the location of the DIT (dorsal intermediate tract) and VIT (ventral intermediate tract). In the metathoracic ganglion, each axon can be distinguished clearly and the relative position of each GI was s established. Inset shows the levels of the sections. Scale bar;100 μm.

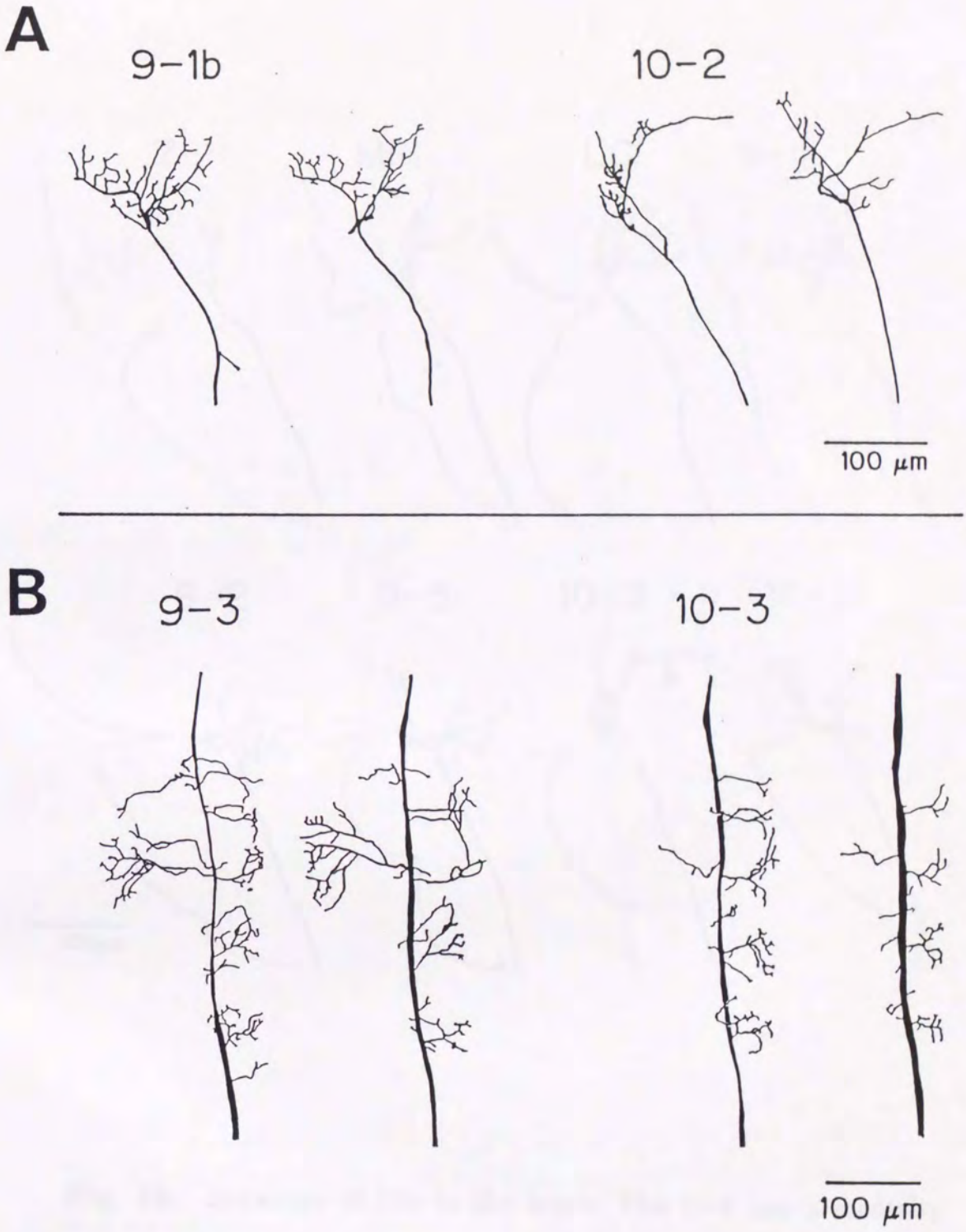

Fig. 9. Consistency of GI branching patterns. (A) Branches of the GIs, 9-1b and 10-2 in the brain. (B) Branches of the GIs, 9-3 and 10-3 in the metathoracic ganglion. The comparison between two examples in each GI shows the consistency of branching pattern.

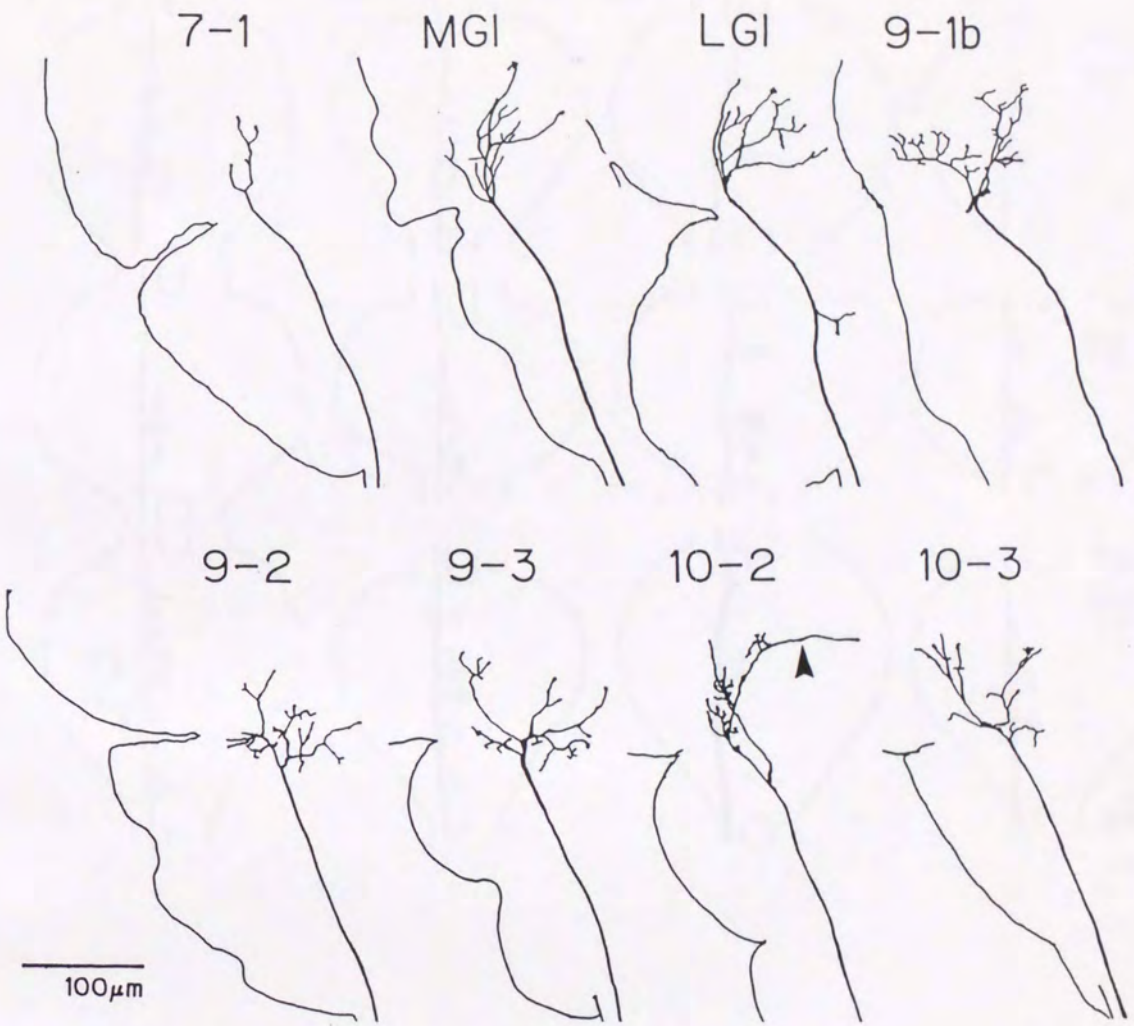

Fig. 10. Drawings of GIs in the brain. The 10-2 has a medially extending branch (arrowhead), which shows a distinct profile.

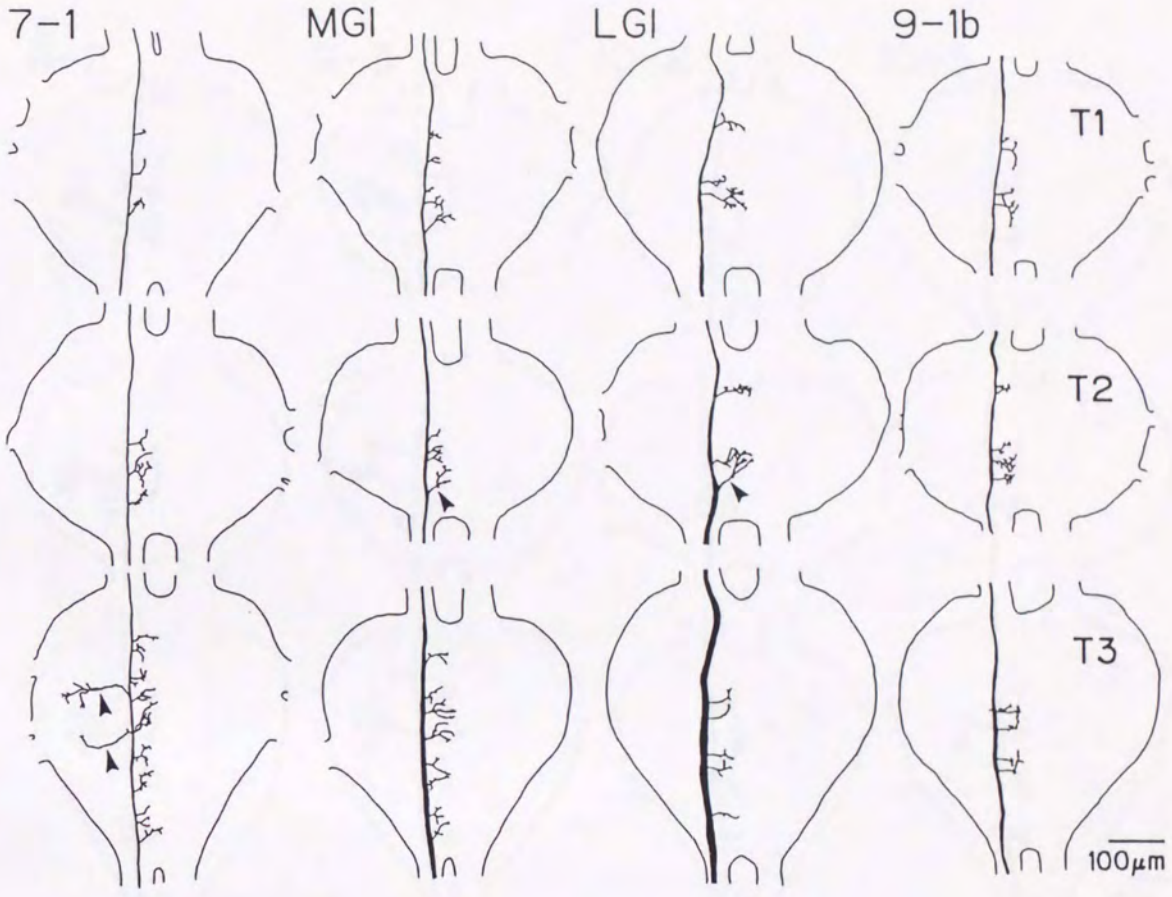

Fig. 11. Drawings of VGIs in the prothoracic (T1), mesothoracic (T2) and metathoracic (T3) ganglia. All axonal branches are restricted medially, except for two branches (arrowheads) of 7-1 in the metathoracic ganglion. The branches of MGI and LGI indicated by arrowheads, as compared with other branches, are thick.

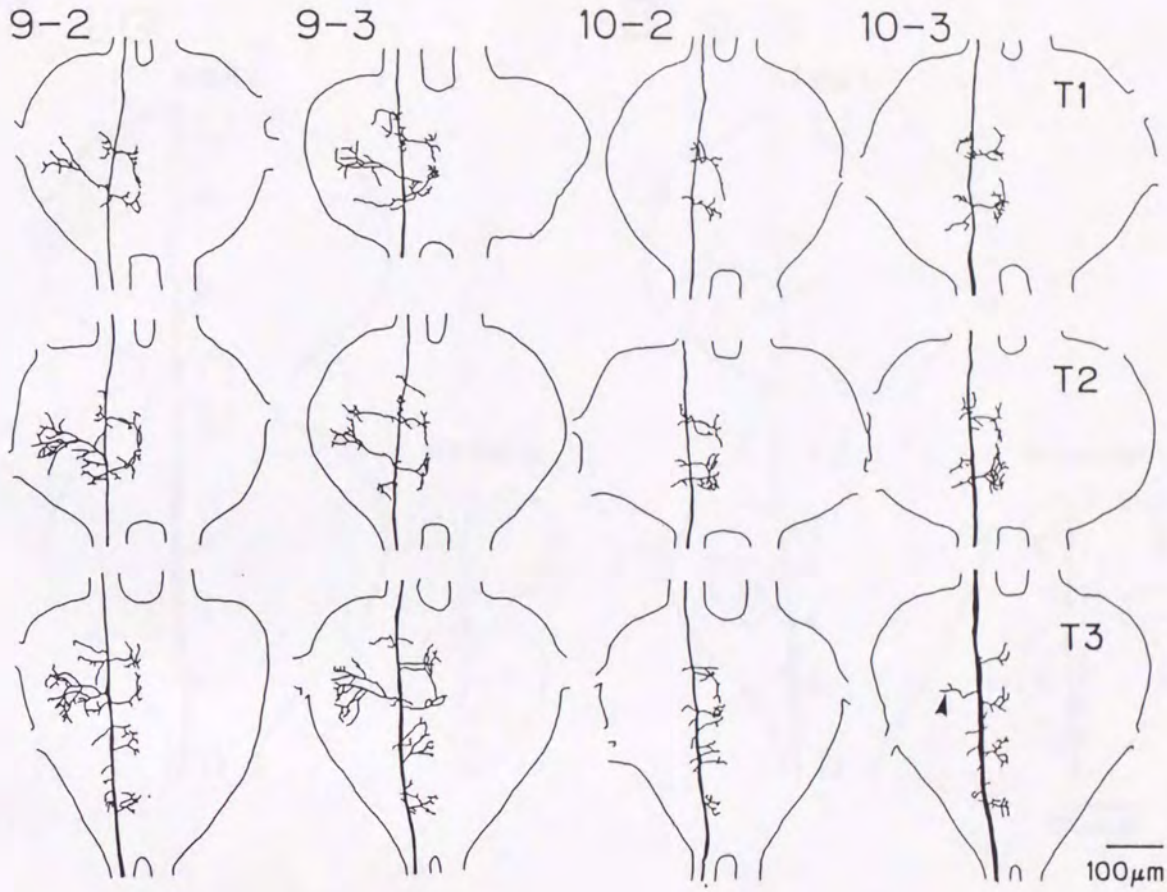

Fig. 12. Drawings of DGIs in the prothoracic (T1), mesothoracic (T2) and metathoracic (T3) ganglia. In contrast to the VGIs, each DGI extends its axonal branches medially and laterally. The GIs, 9-2 and 9-3 have well developed branches and look alike so much that they can't be distinguished based on morphological characteristic in the thoracic ganglia (see text). There is also a close resemblance between the GIs, 10-2 and 10-3, except for a laterally extending branch (arrowhead) in the metathoracic ganglion.

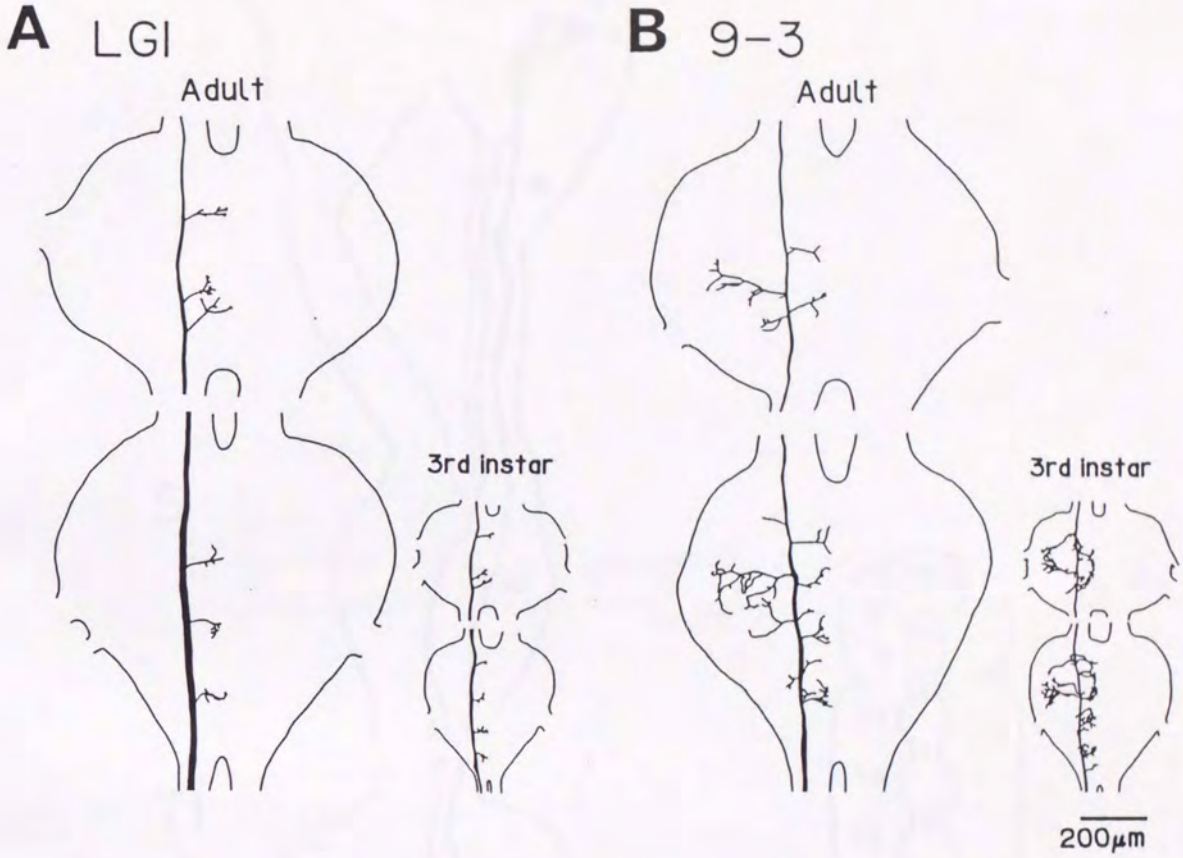

Fig. 13. Projection pattern of adult and third instar GIs in the meso- and metathoracic ganglia. (A) Projection pattern of 9-3. (B) Projection pattern of LGI. Note the similarity between the adult and third instar GIs. The staining of the adult 9-3 was so pale that the counterpart of branch on the rostral side was invisible. All drawings are shown in the same scale.

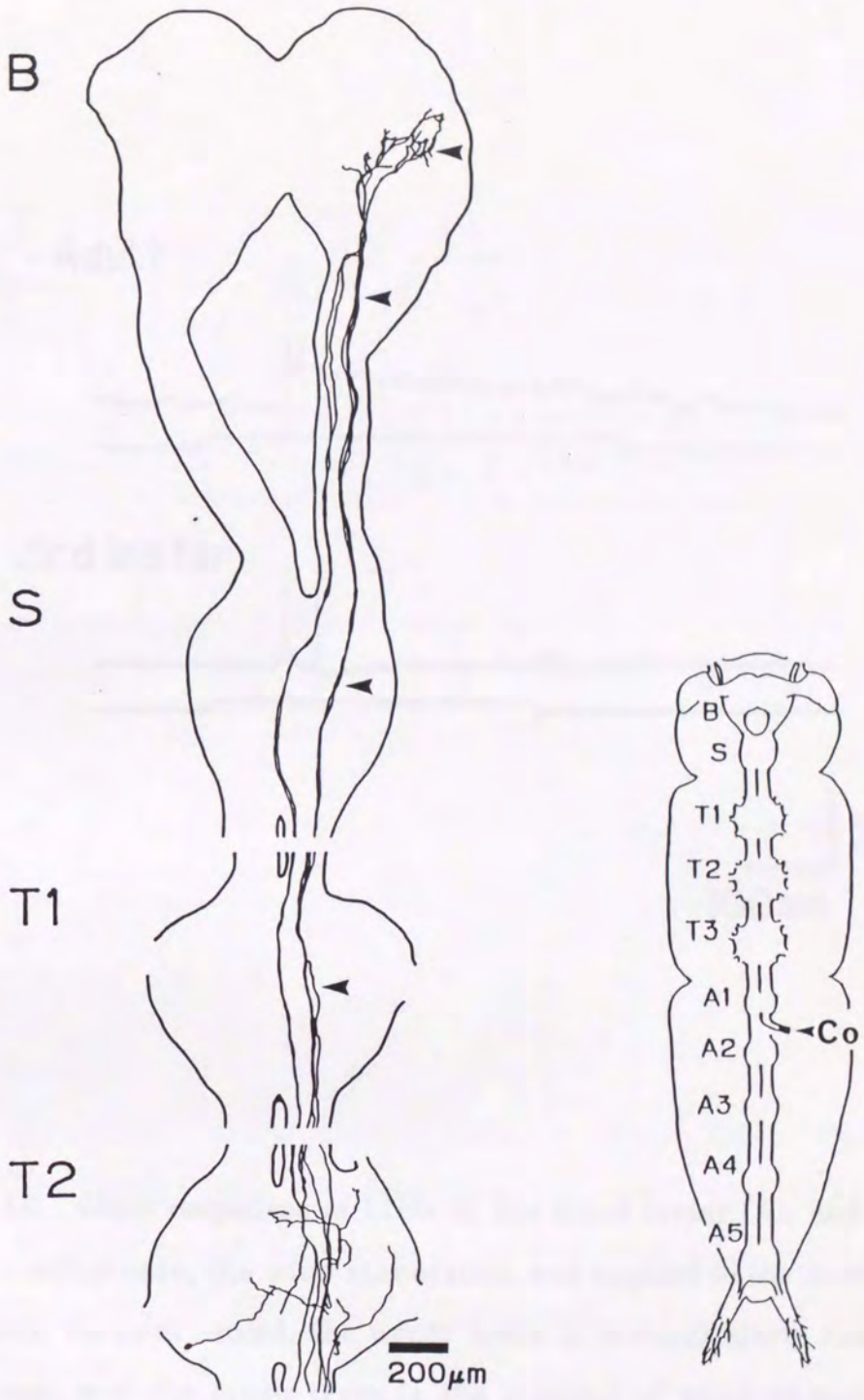

Fig. 14. Camera lucida drawing of several interneurons in the adult cricket, based on anterograde cobalt fills from the cut end of one of the connective between the first and second abdominal ganglion. Among these stained interneurons, there can be seen a group of axons running through the ganglion and nerve cord (arrowhead).

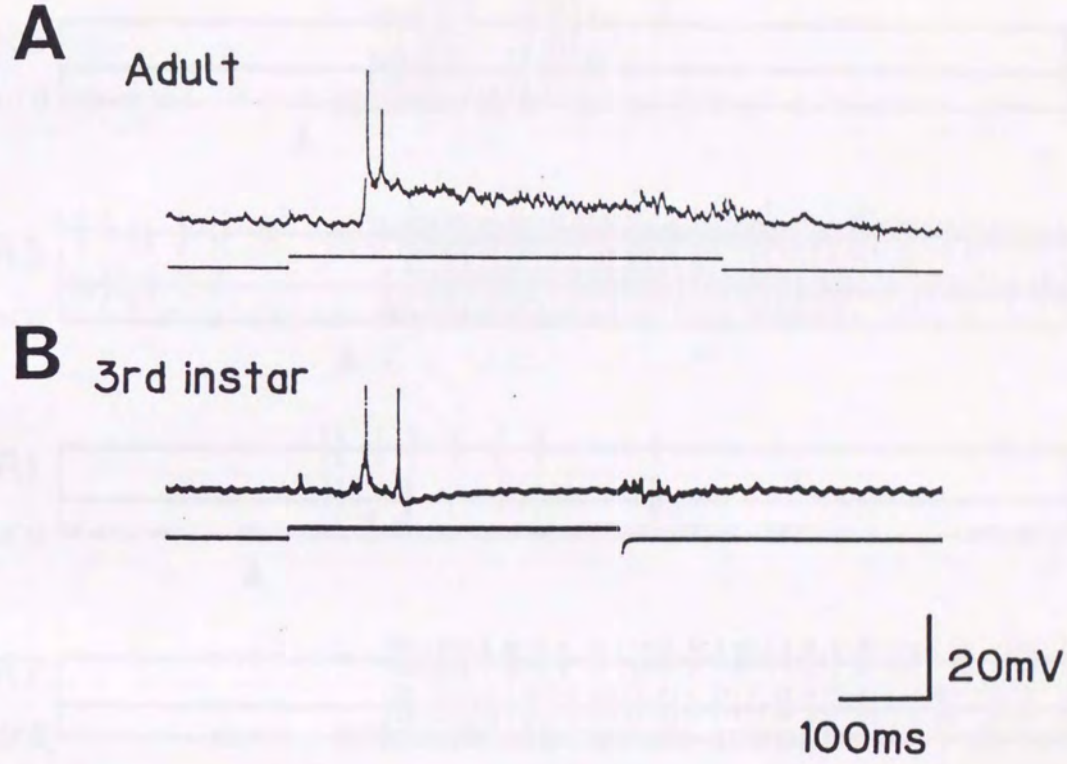

Fig. 15. Wind responses of LGIs in the third instar (A), and adult (B). In either case, the wind stimulation was applied to the cerci from the rear. In each record, the upper trace is intracellularly recorded response, and the lower trace is the monitor of wind stimulation. Note the similarity between the responses, irrespective of age.

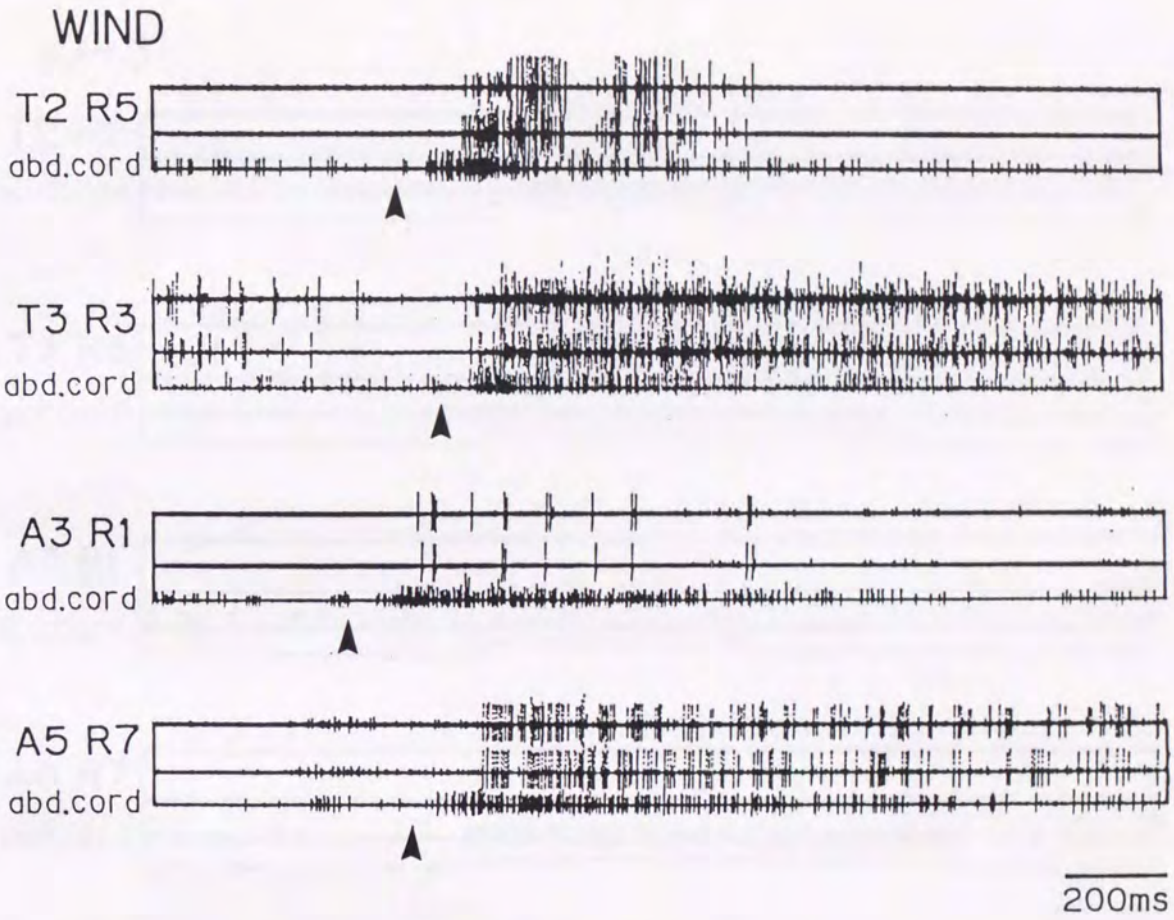

Fig. 16. Response of motoneurons to wind stimulation. Wind puff was applied to the cerci from the rear. Stimulus onset is indicated by arrows. In each record, top trace is extracellular recording from each motor nerve on the right side, middle trace from each motor nerve on the left side, and bottom trace from the abdominal nerve cord. T2R5, fifth nerve root of mesothoracic ganglion; T3R3, third nerve root of metathoracic ganglion; A3R1, first nerve root of third free abdominal ganglion; A5R7, seventh nerve root of TAG. These records were each other obtained from different preparations. It should be noted that the responses of GIs to wind stimulation occurred prior to the responses of motoneurons.

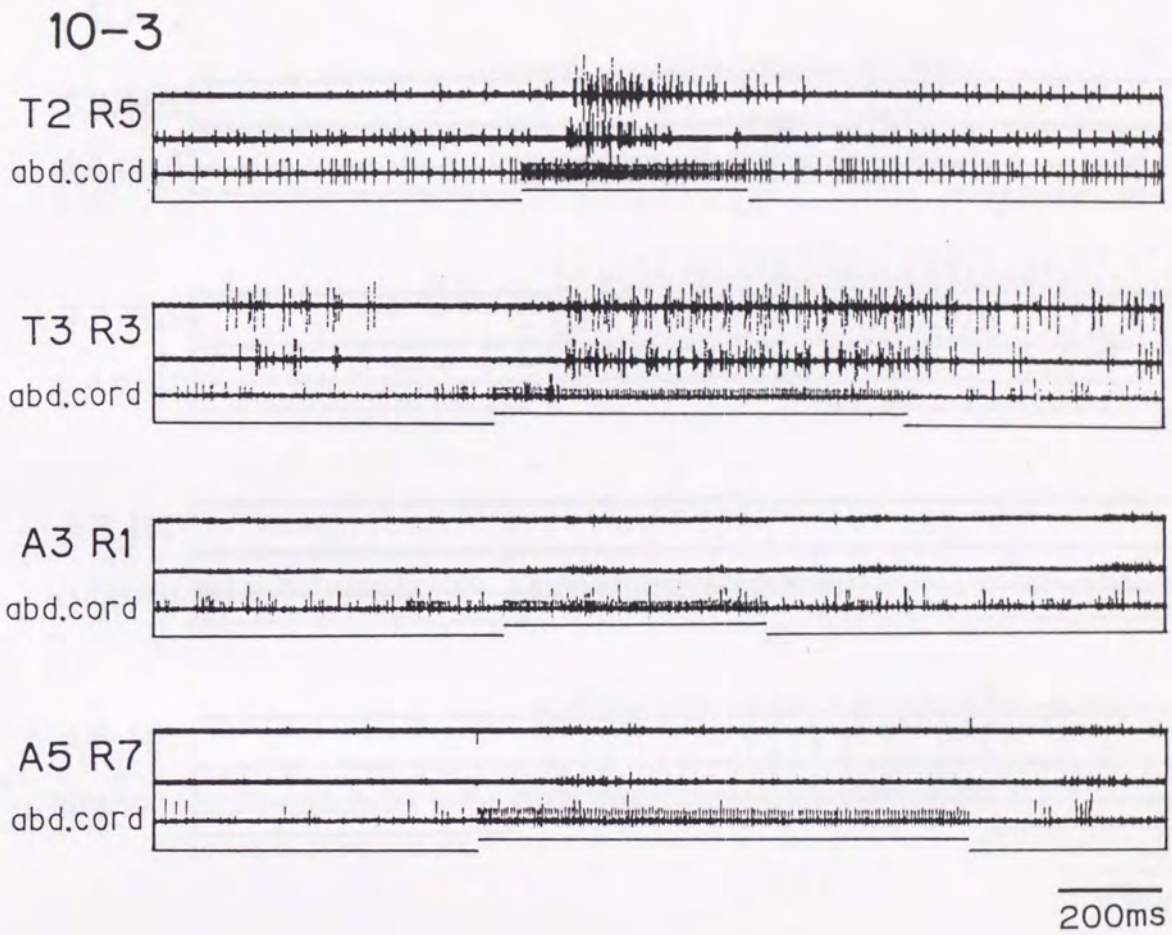

Fig. 17. Response of motoneurons to the intracellular stimulation of 10-3. In each record, the top, second and third traces are extracellular recordings from the nerve roots on the axon side and soma side and from the abdominal nerve cord, and the bottom trace is the monitor of current injection. For abbreviation see Fig. 16.,

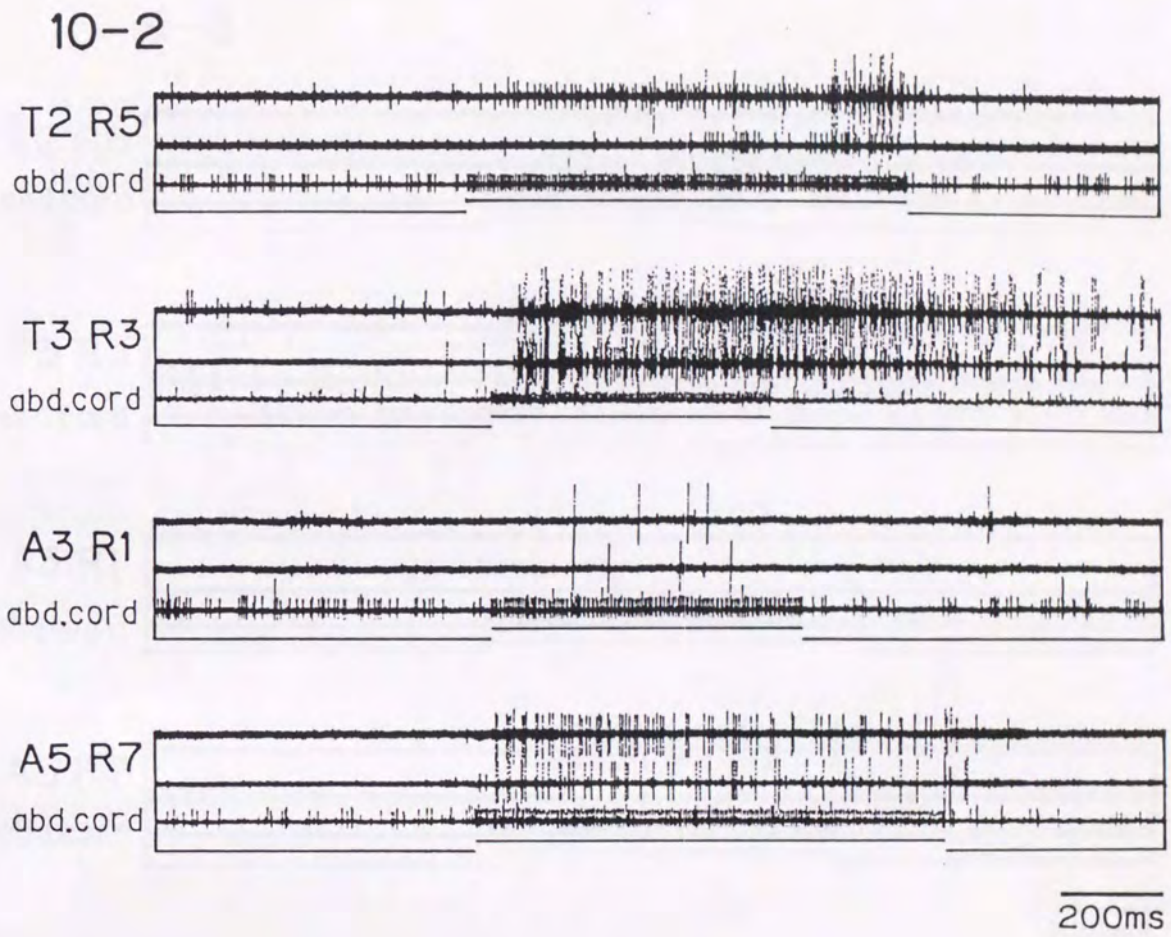

Fig. 18. Responses of motoneurons to the intracellular stimulation of a GI, 10-2. The stimulation to the 10-2 elicits spike discharges in all motor nerves examined in this recordings.

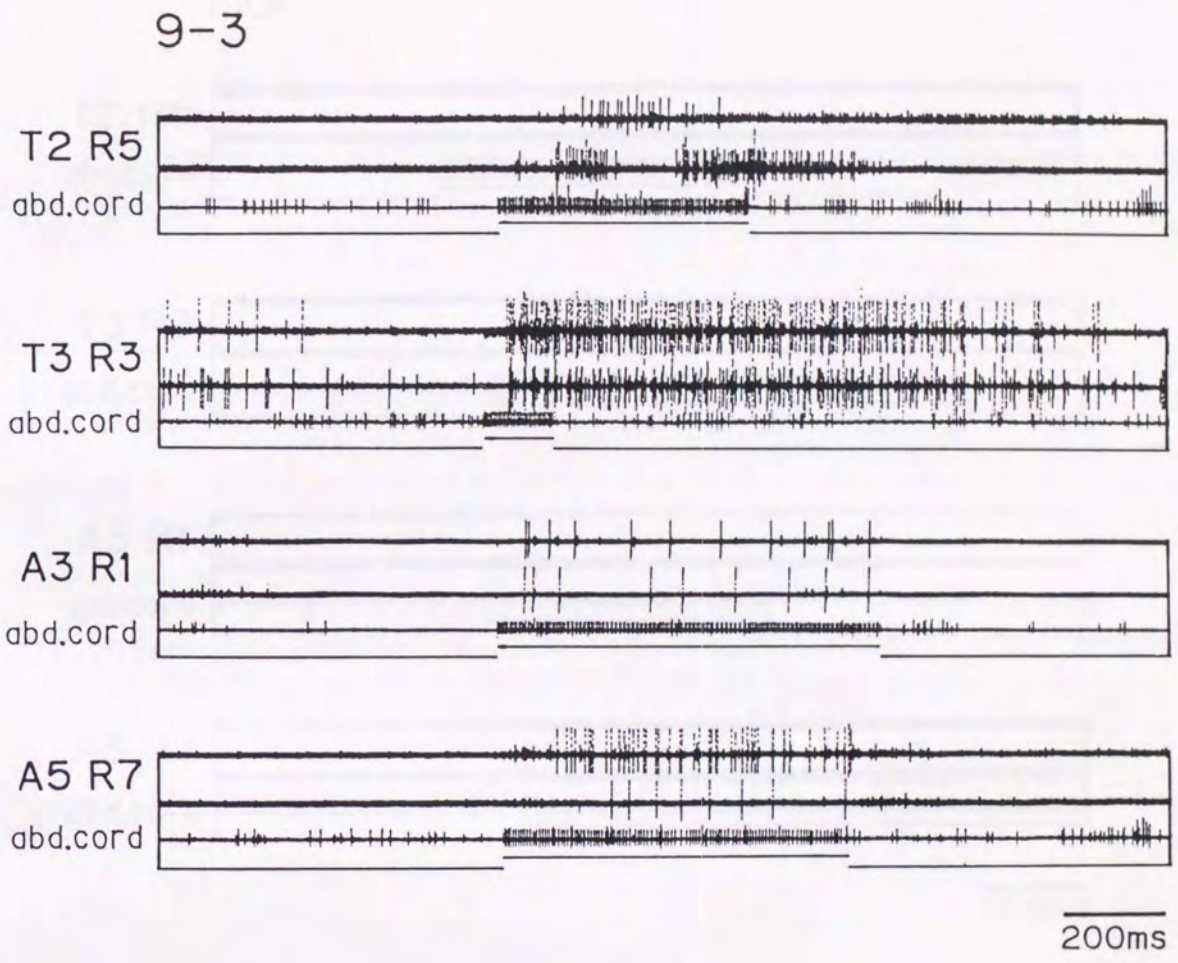

Fig. 19. Responses of motoneurons to the intracellular stimulation of the GIs, 9-3. Note that the magnitude of response of motoneurons in A5R7 on one side is different from that of motoneurons on the other side.

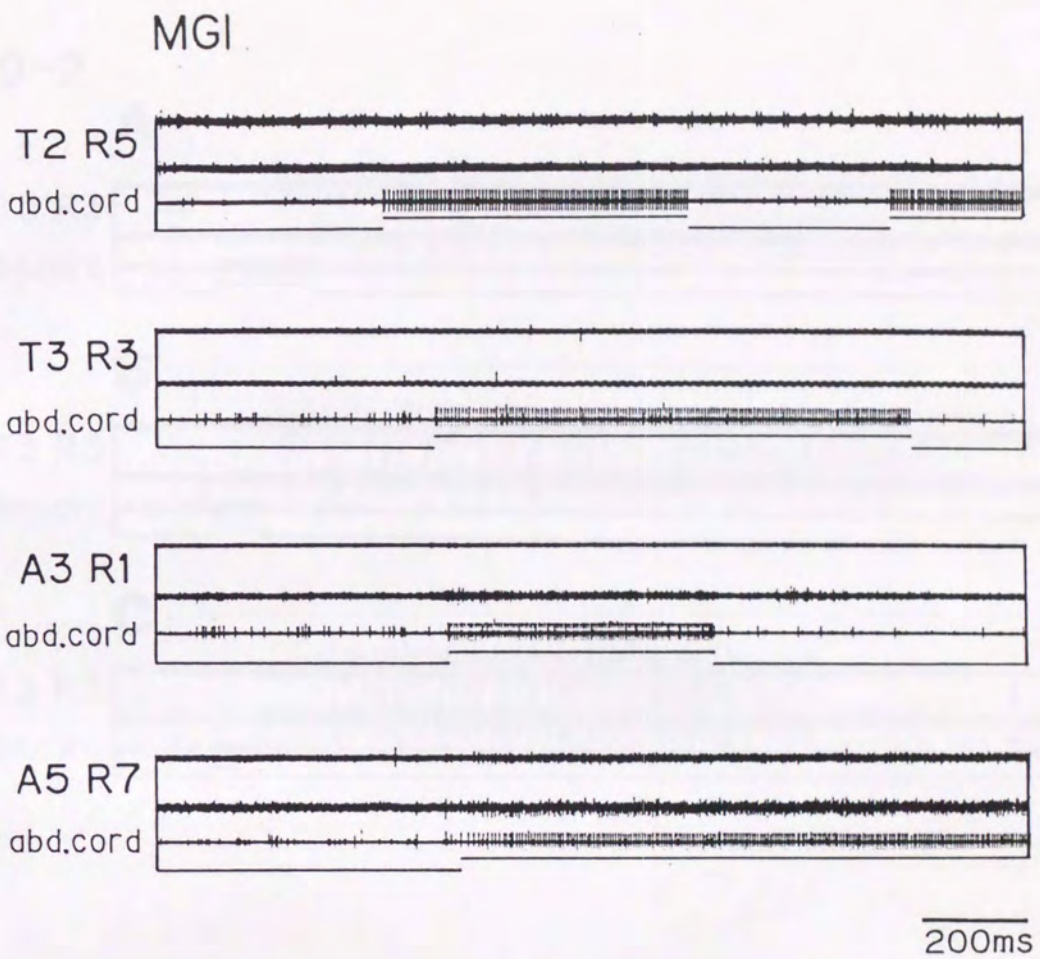

Fig. 20. Recordings from motor nerves during stimulation of MGI. Note that MGI elicited no response in these motor nerves.

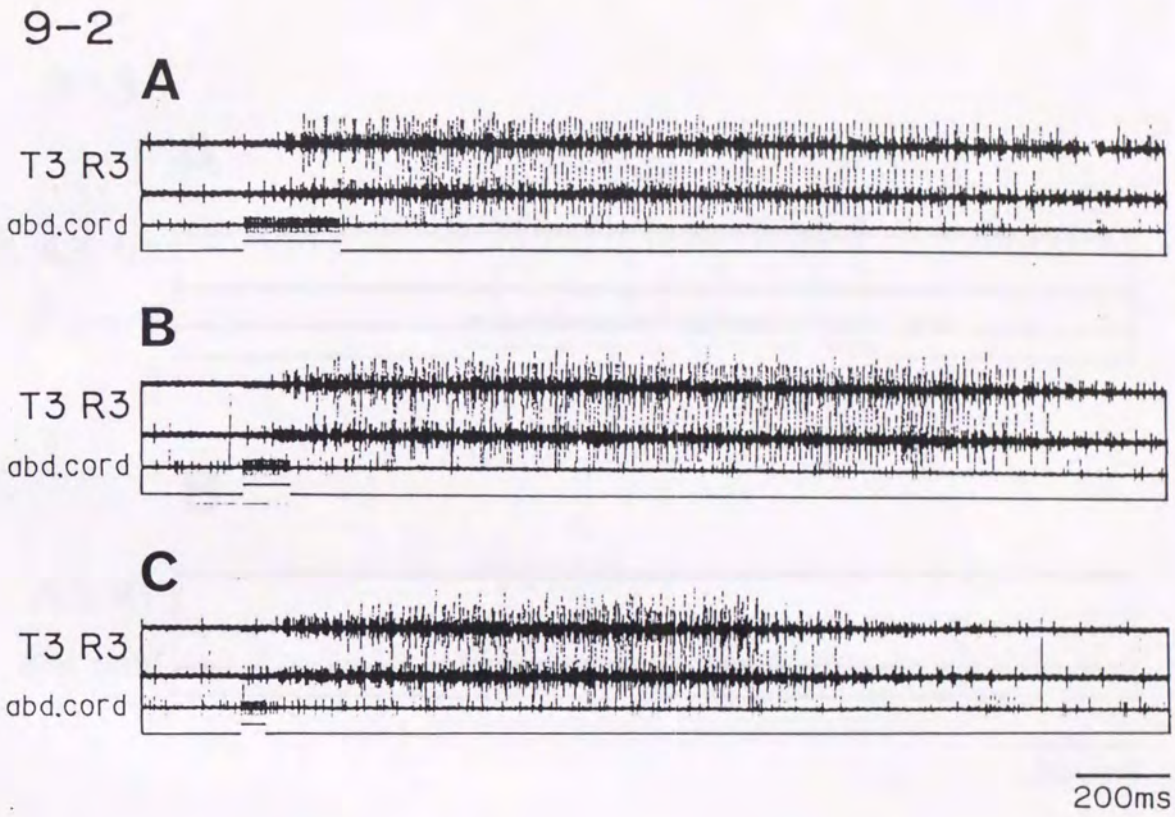

Fig. 21. Effect of stimulus duration of 9-2 on the activity of motoneurons in T3R3. The duration of stimulation was 200 msec in (A), 100 msec in (B) and 50 msec in (C).

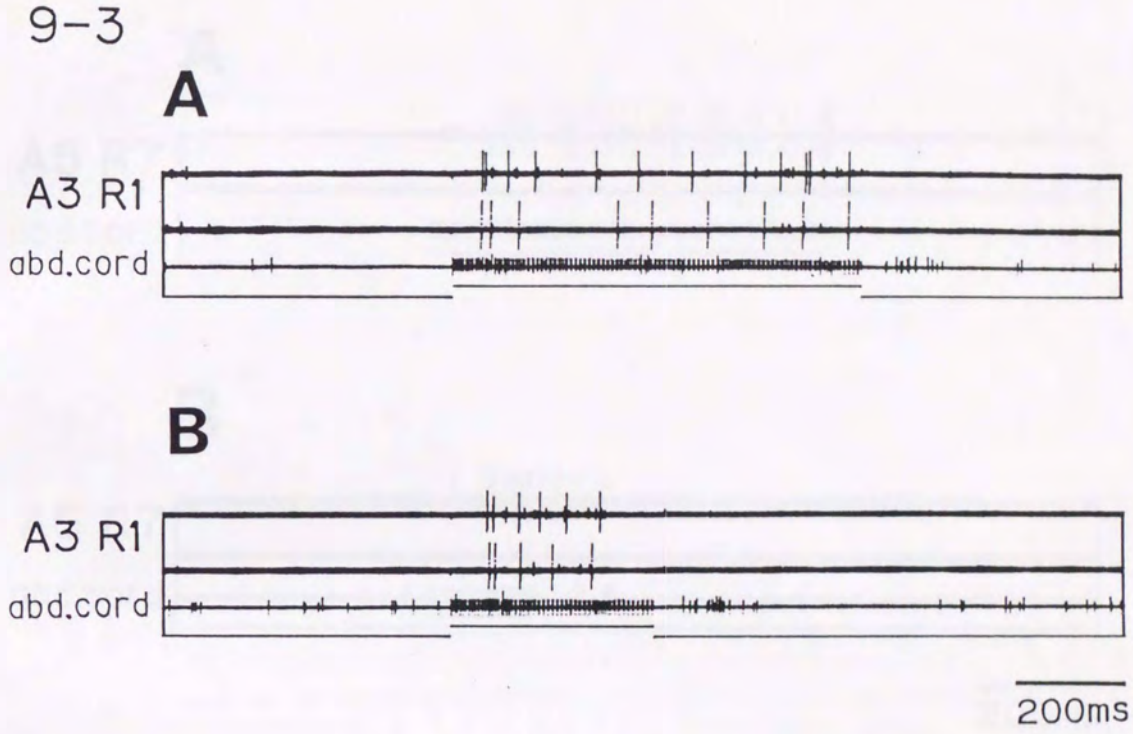

Fig. 22. Effect of stimulus duration of 9-3 on the activity of motoneurons in A3R1. The duration of stimulation was 700 msec in (A) and 350 msec in (B).

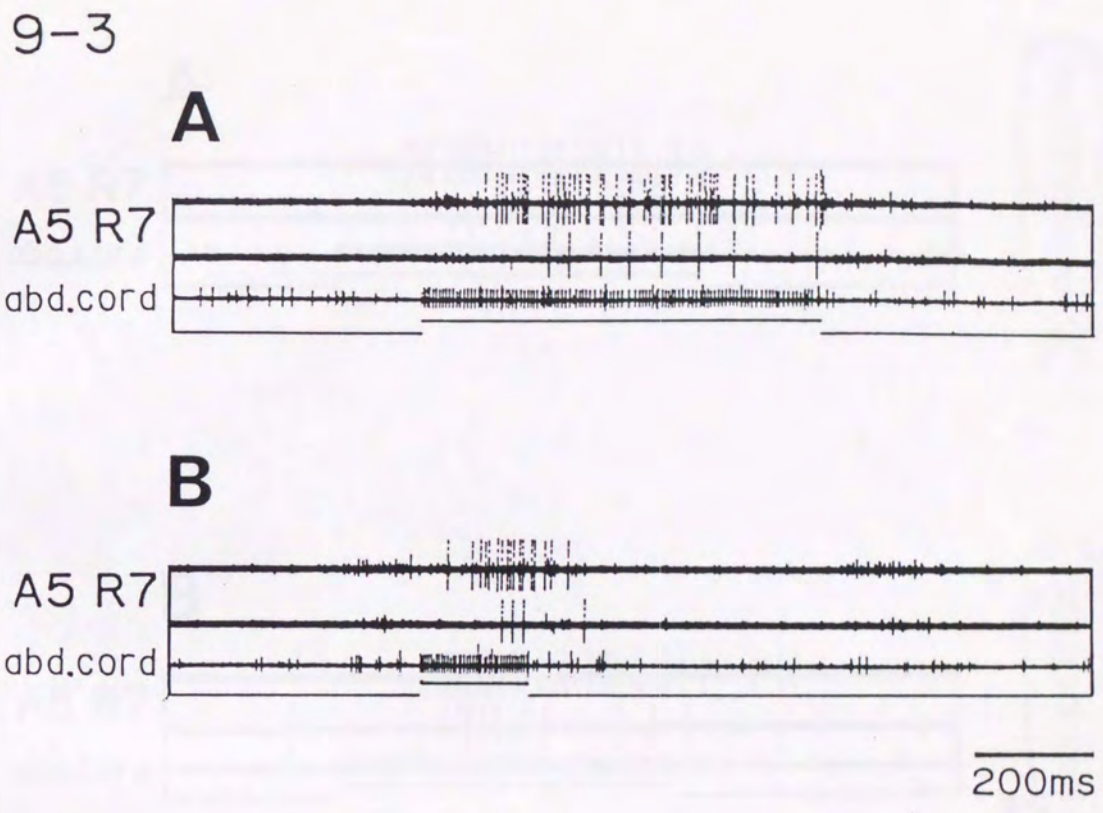

Fig. 23. Effect of stimulus duration of 9-3 on the activity of motoneurons in A5R7. The duration of stimulation was 650 msec in (A) and 170 msec in (B).

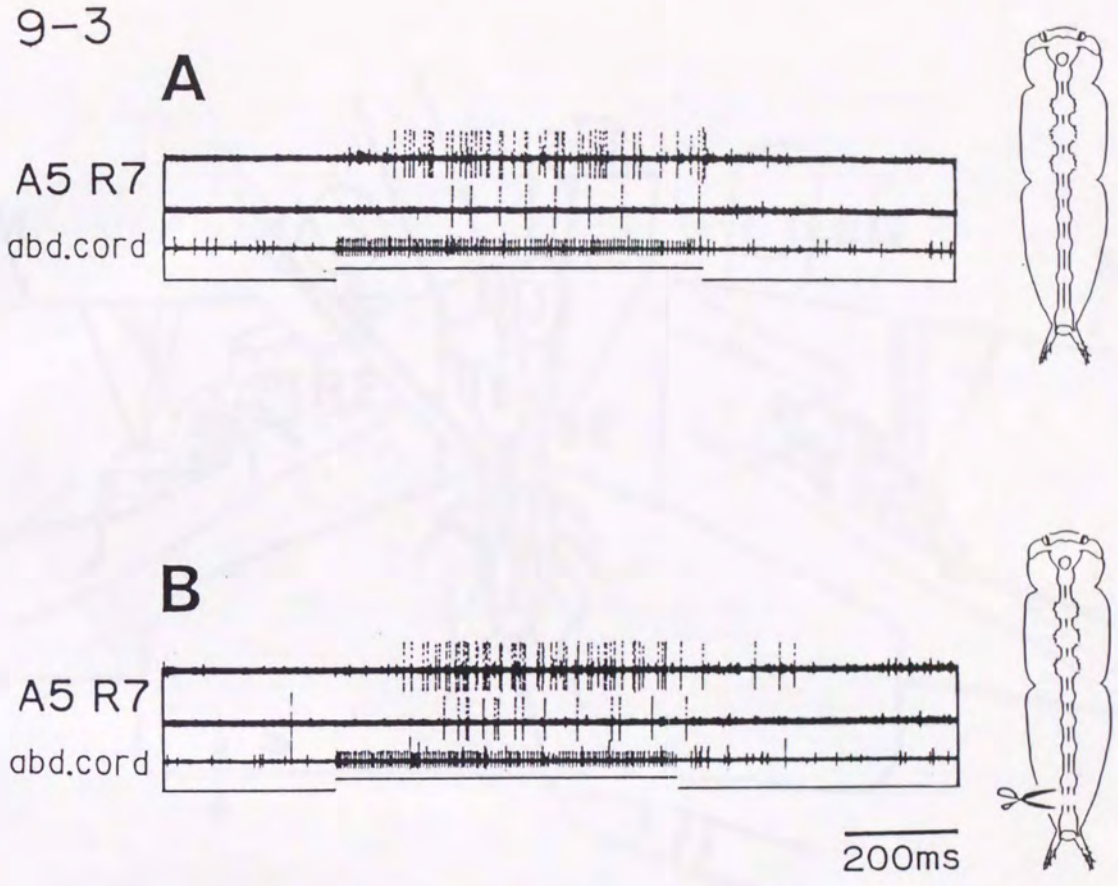

Fig. 24. Effect of the TAG isolation from the central nervous system on the activity in A5R7. (A) Response of motoneurons in A5R7 to intracellular stimulation of 9-3 in intact preparation. (B) Response of the motoneurons in the preparation isolated by cutting connectives between fourth abdominal ganglion and terminal abdominal ganglion.

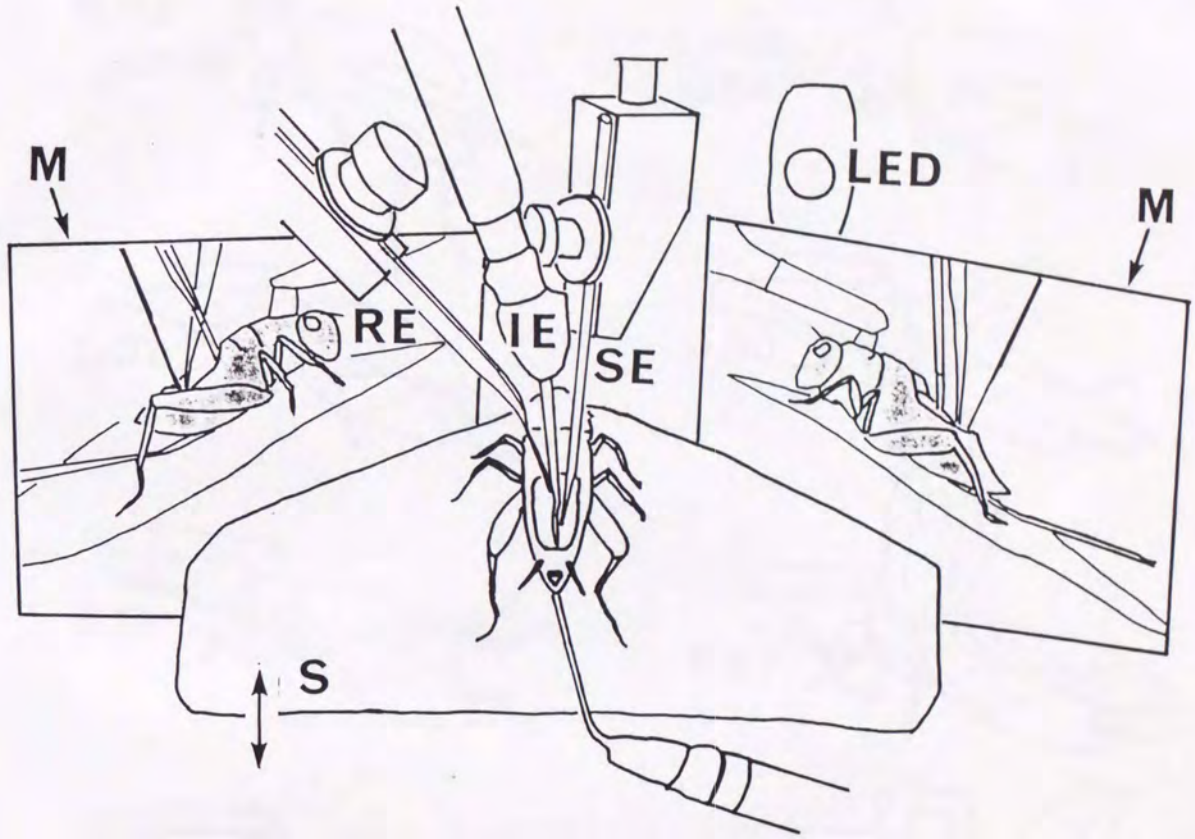

Fig. 25. Diagram of the experimental set up in a tethered-cricket. S, substratum; M, mirror; LED, light-emitting diode; SE, glass micro-electrode for intracellular stimulation; RE hook electrode for extracellular recording; IE, indifferent electrode. Dorsal and side views of the tethered cricket were recorded with a video recorder. The mirrors were employed to record simultaneously the dorsal and side views of the tethered cricket using a video recorder. For other explanations, see the Method in the text.

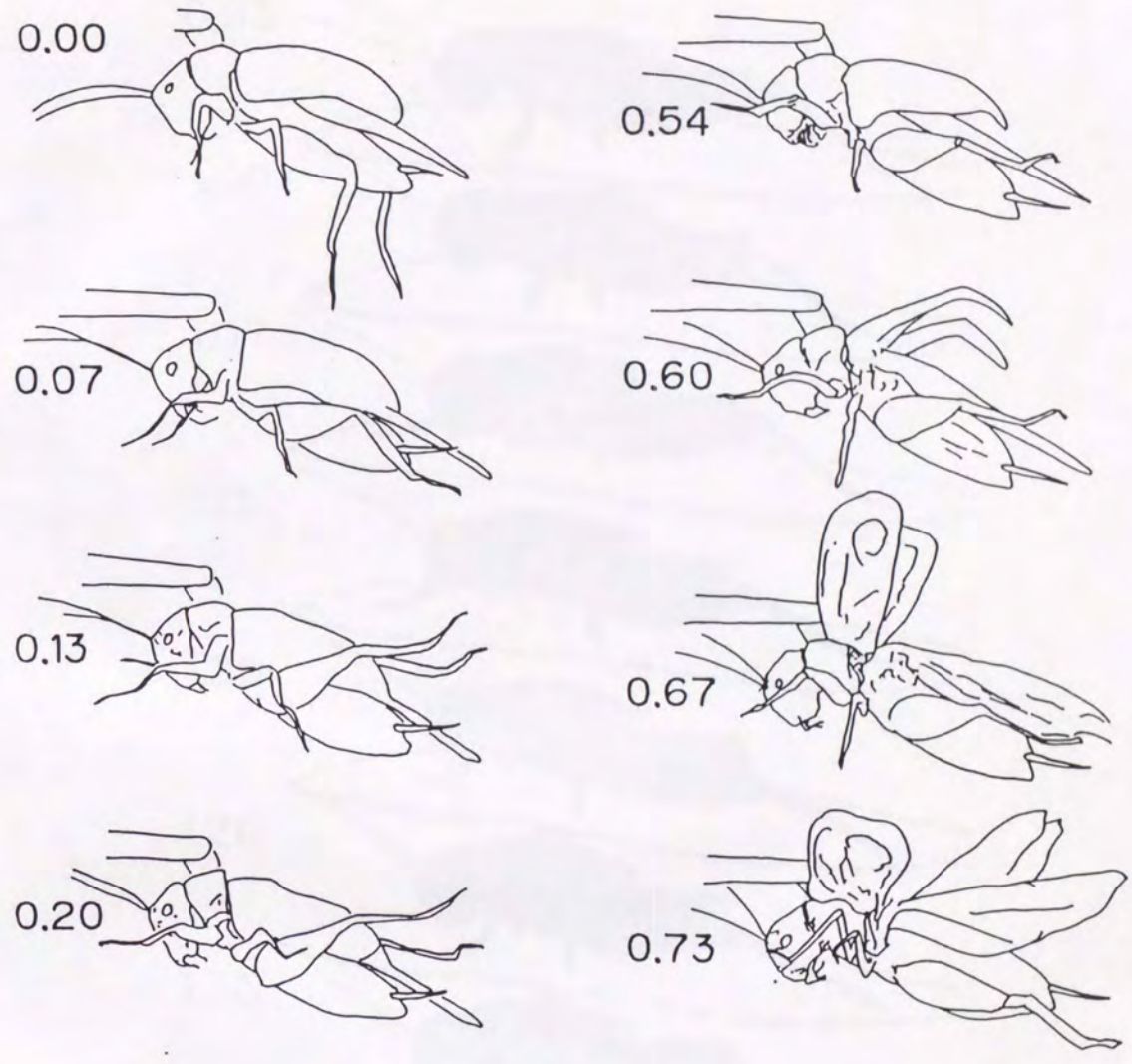

Fig. 26. Flight behavior evoked by wind stimulation on the cerci. This behavior occurred only when the cricket was suspended in the air. Each outline was drawn from a still picture which was played back from the tape. The number on the left side of each outline indicates the time after the onset of wind stimulation in second.

9-3

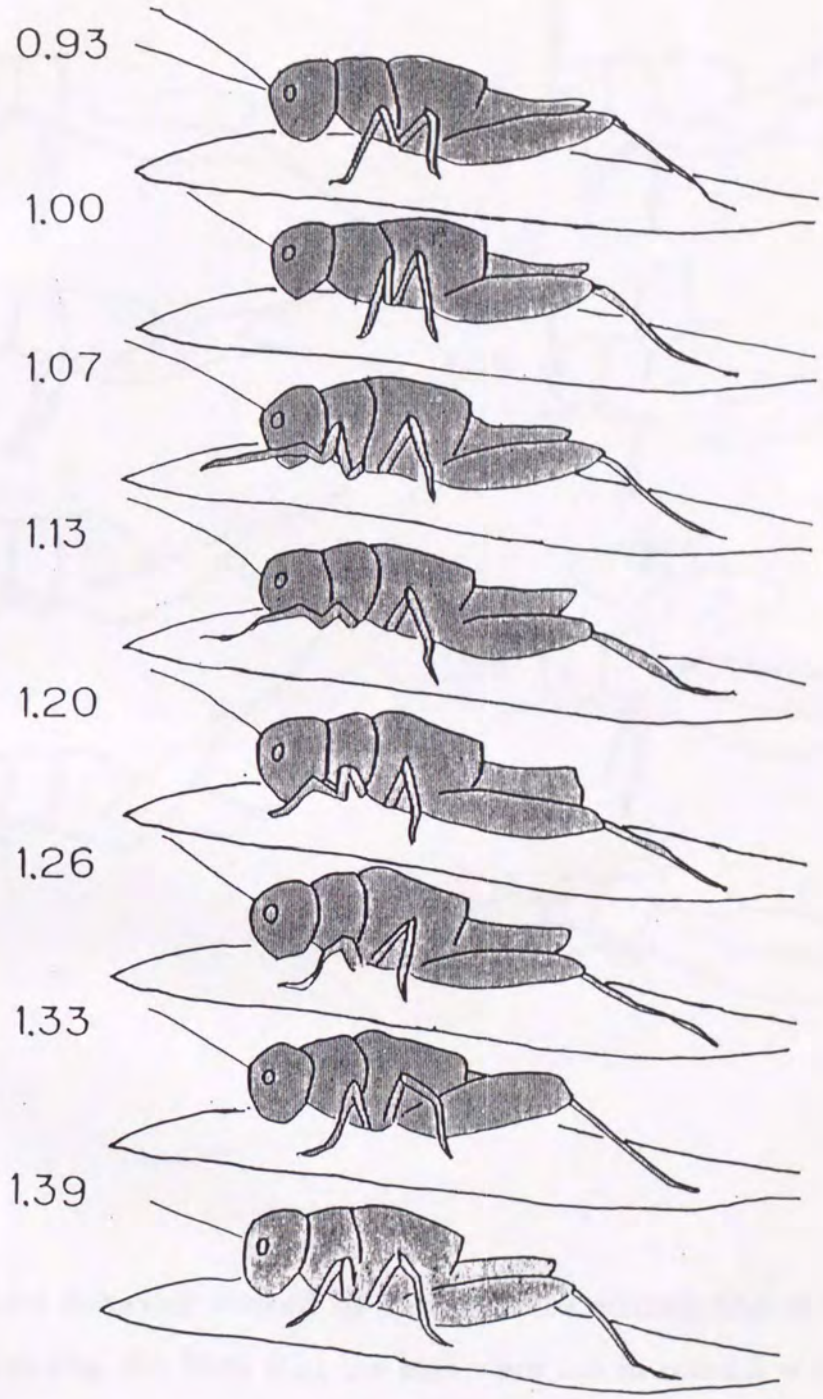

Fig. 27. Walking behavior evoked by intracellular stimulation of a GI, 9-3. In this case, the tarsi were in contact with a substratum. For other explanation, see Fig. 26.

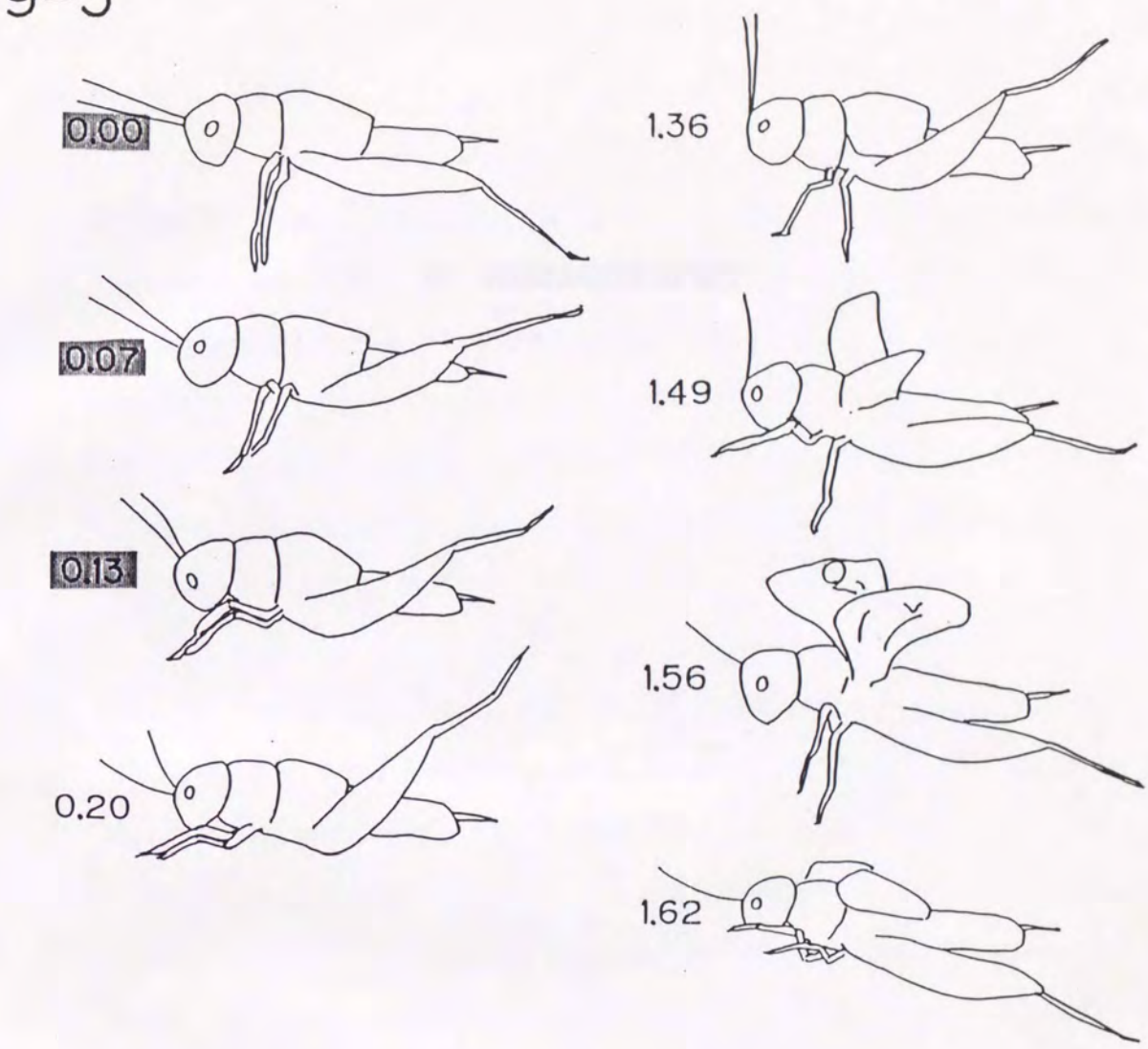

Fig. 28. Flight behavior evoked by intracellular stimulation of the same 9-3 as in Fig. 27. Note that the legs were not in contact with a substratum. The duration of stimulation was 150 msec; it corresponds approximately to three drawings with the numbers superimposed by stripped patterns.

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