# Terminal Projection of Descending Interneurones Controlling Uropod Movements of the Crayfish Procambarus clarkii Girard

Authors: Namba, Hisaaki, Nagayama, Toshiki, and Takahata, Masakazu

Source: Zoological Science, 12(5) : 523-534

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.12.523

ZOOLOGICAL SCIENCE 12: 523–534 (1995)


# Terminal Projection of Descending Interneurones Controlling Uropod Movements of the Crayfish *Procambarus clarkii* Girard

HISAAKI NAMBA, TOSHIKI NAGAYAMA and MASAKAZU TAKAHATA

*Animal Behaviour and Intelligence, Division of Biological Sciences, Graduate School of Science, Hokkaido University, 060 Sapporo, Japan*

**ABSTRACT**—Three types of descending interneurones (type A, B and C) that project into the terminal abdominal ganglion and control the movements of the uropod have been classified according to their physiological and morphological characters in the crayfish *Procambarus clarkii* (Girard) using intracellular recording and staining. They were activated by electrical stimulation of a small bundle of isolated fibres in a lateral portion of the ventral nerve cord containing the extension evoking fibres. The type A and B interneurones responded to each stimulus pulse with a spike in one-to-one fashion. The type A interneurones had antagonistic effects on uropod closer and opener motor neurones, while the type B interneurones coactivated both motor neurone types. The type C interneurones were activated indirectly by electrical stimulation and had antagonistic effects on the uropod motor neurones. The axons of the three types of interneurones descended in a dorso-lateral regions of the abdominal fifth-sixth connective, and entered the neuropil in the lateral dorsal tract. Type A and B interneurones were restricted to different regions of the bundle. Within the terminal abdominal ganglion, their branches were restricted to the dorsal neuropil, where numerous branches of premotor nonspiking local interneurones and motor neurones controlling the uropod movements project.

## INTRODUCTION

The intersegmental control of particular postures or movements has been widely described in both vertebrates and invertebrates [2, 4, 25, 35]. In arthropod motor systems, in particular, the neural mechanisms of descending control of various behavioural acts have been recently investigated physiologically [15, 24, 27]. Despite the simplicity of the nervous system of arthropods, however, only a few descending motor control interneurones have been identified [7, 16].

When a crayfish adopts a resting posture with the abdomen held passively extended, mechanical stimulation of the tailfan elicits an avoidance reaction in which the crayfish walks forward and closes both uropods [20]. While in a defensive posture or during active swimming with a strong extension of the abdomen, the same stimulus produces no obvious responses of the uropod. Electrical stimulation of a small part of the abdominal connective evokes fictive abdominal extension and an opening pattern of activity of the uropod motor neurones [24]. This pattern of the uropod movement was opposite to that elicited by sensory stimulation of the tailfan. Electrical stimulation of sensory afferents innervating the hairs on the surface of the uropod had, furthermore, almost no effect upon the uropod motor neurones during fictive abdominal extension. The activity of the uropod motor neurone is thus controlled by an interaction of the segmental sensory input and the intersegmental descending input depending on the behavioural context of the animal.

To understand the neural mechanisms responsible for the central modulation of uropod beheviour, it is essential to elucidate the neural connections of uropod motor neurones with the sensory neurones and descending interneurones. Previous studies have shown that two types of nonspiking local interneurones in the terminal abdominal ganglion [18] received both the sensory and descending inputs to form a parallel opposing circuit [24]. In this paper, we have focused on the descending interneurones with axons running in the loci that evoke fictive abdominal extension, and studied the physiological and anatomical characteristics of these interneurones to examine their possible connections with the nonspiking interneurones and uropod motor neurones. We have classified seven descending interneurones on the basis of the morphology of their arborizations in the terminal abdominal ganglion and output effects on the uropod motor neurones.

All interneurones except one are included in the fibres that evoke fictive abdominal extension upon electrical stimulation. The terminal branches of these interneurones are restricted to dorsal neuropil above the level of ventral medial tract (VMT) and ventral intermediate tract (VIT) in the lateral neuropil of the terminal abdominal ganglion in which fine branches of the nonspiking local interneurones and motor neurones also project, suggesting direct connections of descending interneurones with local interneurones and motor neurones.

## MATERIALS AND METHODS

Adult male and female crayfish *Procambarus clarkii* Girard, measuring 7 to 10 cm in body length (from rostrum to telson), were used in all experiments. They were obtained commercially (Sankyo

Accepted June 15, 1995
Received May 22, 1995

Lab, Tokyo) and maintained in laboratory tanks before use. There was no significant differences between the sexes in the relevant behavioral acts or electrophysiological responses.

The abdominal nerve cord from the second to the terminal (sixth) abdominal ganglia with nerve root was isolated from the abdomen. It was pinned out in a sylgard-lined dish filled with cooled van Harreveld's [32] solution.

Motor activity was recorded extracellularly by suction electrodes and stainless steel pin electrodes [1]. The activity of uropod closer and opener motor neurones was recorded from the second and third motor root in the terminal ganglion [21]. The activity of abdominal extensor and flexor motor neurones was recorded from the second root and the superficial branch of the third root respectively in the fourth ganglion.

To produce fictive abdominal extension, a small group of fibres running in a lateral region in a desheathed second-third or third-fourth abdominal connective was split and stimulated electrically (pulses of 0.02ms duration at 100Hz for 0.4-1sec) by a suction electrode with a tip of small diameter [24]. The correct position of the electrode was attained if repetitive stimulation evoked the following pattern of motor activity: the appearance of spikes in extensor motor neuron No. 6, an increase in the number of spikes in the extensor motor neuron excitors and/or the flexor motor neuron inhibitor, and a decrease in the number of spikes of extensor motor neuron inhibitor and/or the flexor motor neuron excitors. At the same time, uropod opening pattern was evoked in the uropod motor neurones: a decrease in the number of spikes in the closer, reductor motor neurone and an increase in the opener motor neurones [24]. The connective stimulation was carried out at the intensity just above the voltage eliciting the train of spikes of excitatory extensor motor neuron No. 6. The preparations that did not show the reciprocal extension pattern in the abdominal posture motor neurones or the reciprocal opening pattern in the uropod motor neurones were not used.

Intracellular recordings were made with glass microelectrodes filled with a 3% solution of Lucifer Yellow CH [28] with 1M lithium chloride. Electrode resistance was between 50–60 MOhm. Intracellular recordings were made from the abdominal fifth-sixth connective or the anterior neuropil of the terminal ganglion that was ipsilateral to the stimulation side. The recorded neurones were first examined for their response to the connective stimulation, then they were characterized in their motor effects upon the uropod motor neurones by the current injection through the recording electrode by means of a bridge circuit. All recordings were stored on a PCM data recorder (Biologic DTR-1801) for later analysis and display.

After physiological examination, Lucifer dye was injected into the interneurones with pulse of hyperpolarizing current (3 to 10 nA, 500 msec) at 1 Hz for 10 to 30 minutes. The ganglion was then dissected and fixed in 10% formalin for 20 min, dehydrated in an alcohol series and cleared in methyl salicylate. The ganglion was photographed under a fluorescence microscope at different focal planes for subsequent reconstruction of neuron morphology. Axonal position in the fifth-sixth abdominal connective was vertically scanned using a confocal laser scanning microscope (Sarastro 2000, Molecular Dynamics). The ganglion was then embedded in paraffin wax with a melting point of 58°C. Cross-sections were cut to a thickness of 20 $\mu$m. They were deparaffinized in xylene, mounted Bioleit (Oken Shouji Co., Tokyo), and photographed for reconstruction. Nomenclature for the tracts and neuropilar areas in the terminal ganglion is that of Kondoh and Hisada [12]. Tracts and neuropil structures are referred to by their abbreviations.

## RESULTS

Electrical stimulation of a small group of fibres in a lateral region of the second-third abdominal connective elicited fictive abdominal extension and evoked extracellular spikes in the lateral region of the fifth-sixth abdominal connective. At the same time, this stimulation elicited reciprocal activation of the uropod motor neurones: tonic spikes of the closer motor neurone was decreased and those of the opener motor neurones were increased (Fig. 1A). This reciprocal pattern of the uropod motor neurones disappeared after a partial lesion of the lateral fibres (100–120 $\mu$m in diameter) in fifth-sixth abdominal connective. After cutting the lateral fibres and disappearance of extracellular spikes in fifth-sixth connective (Fig. 1B: 4th trace), the connective stimulation did not elicit any changes in the activity of the uropod motor neurones (Fig. 1B: 2nd and 3rd traces), though the extension excitor motor neurones still discharged (Fig. 1B: top trace).

Nickel backfilling from the cut ends of the lateral fibre in the fifth-sixth abdominal connective showed projections of descending interneurones in the terminal ganglion (Fig. 1C). The number of axons that could be counted was about thirty. Since no soma-like structures were recognized on the ventral surface of the terminal ganglion, all the axons included in this area appear to belong to descending neurones. The majority of descending interneurones projected ipsilaterally and fine branches were given off laterally and medially. Several interneurones had processes which cross the midline.

### *Classification of descending interneurones*

This study is based on recording and staining of the axons and terminal arbors of 33 descending interneurones which are classified into seven interneurone types. Each type of interneurone was filled with dye at least twice, except for A3 interneurone showing distinguishable shape.

Thirty-one of the 33 descending interneurones characterized in this study elicited spikes one-to-one in response to connective stimulation at 100 Hz (Fig. 2A and B). Spikes of descending interneurones were usually over 40 mV in amplitude and showed a rapid rising phase with no synaptic potential (Fig. 2B). These descending interneurones affected the activity of uropod motor neurones when they produced a train of spikes by current injection. Fourteen out of 31 interneurones inhibited the tonic discharge of the closer motor neurones and reciprocally excited the opener motor neurones (Fig. 2C). These descending interneurones were designated type A interneurones and further divided into three subtypes, A1, A2 and A3, according to the morphology of their axons, including overall shape, number and projection of main branches, and the position of axons in the abdominal fifth-sixth connective. Another 17 interneurones, designated type B, increased the spontaneous discharge of both the opener and closer motor neurones (Fig. 2D). They were divided into three subtypes, B1, B2 and B3 by their morphological criteria.

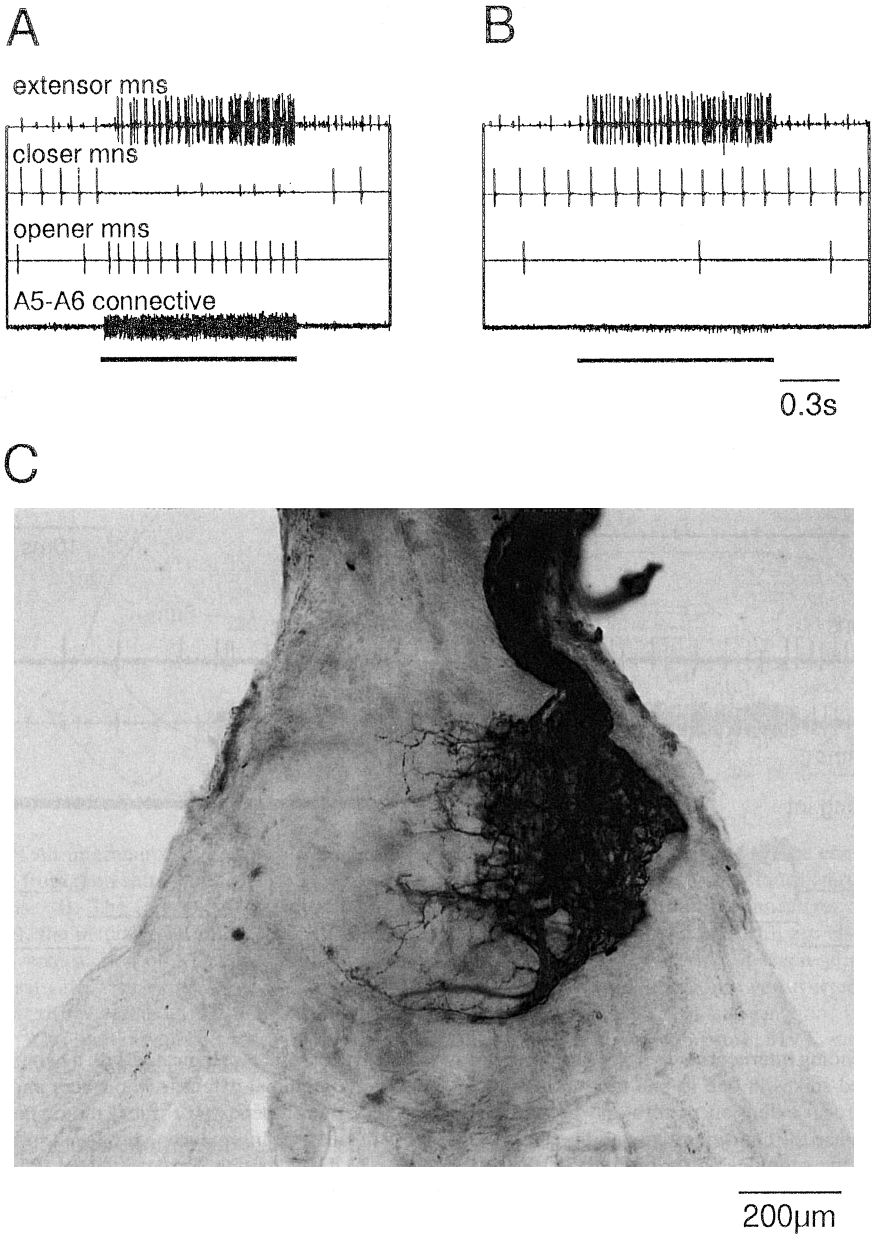


FIG. 1. A, B. Effect of transection of lateral fibres of abdominal fifth-sixth connective on uropod motor activity. A. Before cutting lateral connective, extracellular spikes were recorded from lateral region of abdominal fifth-sixth (A5-A6) connective (bottom trace) and reciprocal opening pattern on the uropod closer and opener motor neurones (2nd and 3rd traces) were elicited in response to the stimulation of extension evoking fibres in third-fourth connective (bar). B. After cutting lateral connective, reciprocal opening pattern on the uropod motor neurones (2nd and 3rd traces) were disappeared. C. Photograph of silver intensified whole mount preparations which were backfilled with $NiCl_2$ from the cut ends of lateral fibres in fifth-sixth connective. The cut ends of the fibres were exposed to a 250 mM $NiCl_2$ for 2 hours at room temperature. The development of nickel and silver intensification were done routinely described in Kondoh and Hisada [12]. Note that some descending interneurones crossed at the medial and posterior region of neuropil. Viewed ventrally, with anterior to the top.

The remaining two interneurones also responded with spikes to the connective stimulation but their spikes did not consistently follow each pulse of stimulation (see Fig. 9). They had antagonistic effects on the uropod closer and opener motor neurones, and were designated type C.

*Type A descending interneurones*

Three descending interneurones that had reciprocal opening effects on the uropod motor neurones belonged to this type.

*A1 interneurone*. The A1 interneurone was characterized morphologically by three main branches, mb1, 2 and 3, that projected laterally. Two interneurones classified as A1 are

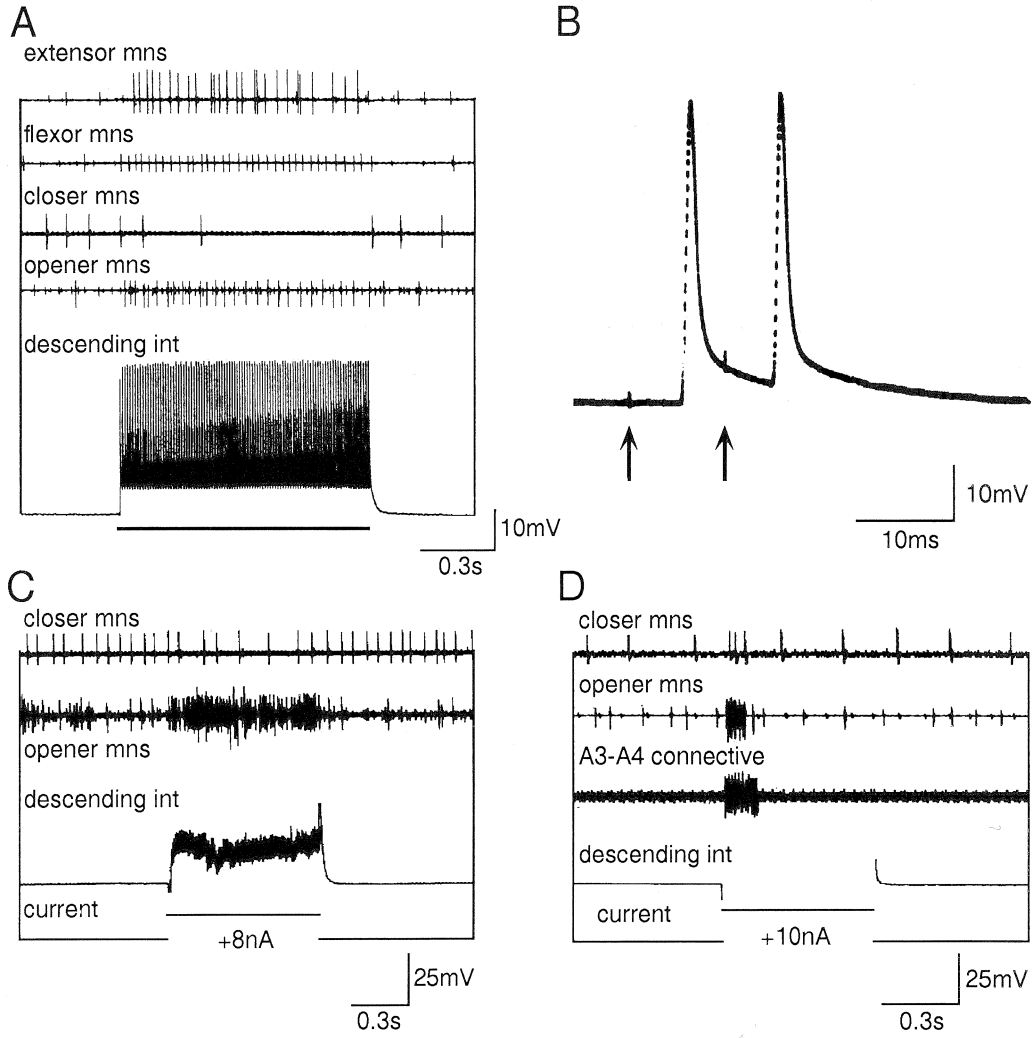


FIG. 2. Physiology of descending interneurones. A. During connective stimulation at 100 Hz (indicated with a bar), a descending interneurone (bottom trace) produced spikes in one to one fashion to stimulus pulses. Abdominal extensor (top trace) and flexor (2nd trace) motor neurones showed reciprocal extension pattern and uropod closer (3rd trace) and opener (4th trace) motor neurones showed reciprocal opening pattern in response to connective stimulation. See text for more detail. B. Interneurone produced spikes at a constant latency of 5.4msec in response to two stimulus pulses of interval 10msec (arrows). C. Depolarizing current injected into the interneurone elicited reciprocal opening effects on the uropod closer (top trace) and opener (2nd trace) motor neurones. D. Depolarizing current injected into another interneurone elicited coactivating effects on the closer (top trace) and opener (2nd trace) motor neurones. Extracellular spikes of descending interneurones that responded in one-to-one fashion to a stimulus pulse were recorded from a stimulus suction electrode placed on abdominal third-fourth (A3-A4) connective (3rd trace). Note that the descending interneurone spiked transiently during current injection.

drawn in Figure 3A to show the consistency of the morphology. By the rather weak depolarizing current injection (3-6nA), it produced spikes continuously during a passage of depolarizing current. It decreased the activity of the closer motor neurone and increased the activity of the opener motor neurones (see Fig. 2C). Current injection into A1 neurone also excited the extensor inhibitory motor neurone No. 5 in which spike activity was depressed during connective stimulation.

The descending axon (diameter=5 $\mu$m) is located at the most lateral edge of the fifth-sixth connective (Fig. 3B). After the axon enters the neuropil in the lateral dorsal tract (LDT), it gives off some short branches medially and a first main branch (mb1) laterally (Fig. 3C) in the anterior region of the neuropil. The descending axon further runs posteriorly to give off a second main branch (mb2) postero-laterally. The descending axon then runs slightly medially and bifurcates laterally and medially to form a third main branch, mb3. The axon ends at the level of root 1. All these branches are within the dorso-lateral neuropil above the level between ventral medial tract (VMT) and ventral intermediate tract (VIT) (Fig. 3C and D).

*A2 interneurone*. The A2 interneurone was characterized morphologically by a medially projecting main branch (mb1) in the anterior neuropil. Two interneurones classified as A2 are drawn in Figure 4A. In three of five preparations, the

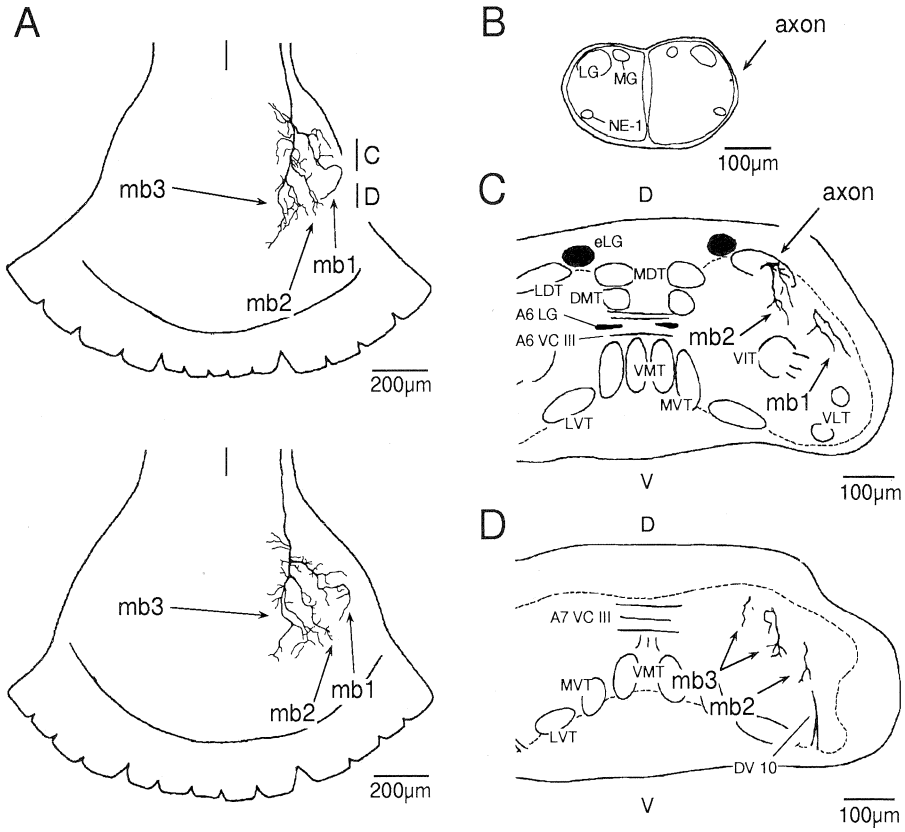


FIG. 3. Morphology of A1 interneurone. A. Two A1 neurones in different crayfish are drawn to show the consistency of morphology. Each neurone is viewed from the ventral surface with anterior to the top. The axon paths and main branch (mb1-3) arborizations are similar in both interneurones. B. The location of the descending axon in the abdominal fifth-sixth connective. The axon of the lateral giant interneurone (LG), the medial giant interneurone (MG) and the ascending interneurone, NE-1 [3] are also drown as landmarks. C, D. Drawings of transverse section of A1 neurone at the planes indicated in A. C is composite of seven adjacent 20 $\mu$m sections, D of five sections. D: dorsal side. V: ventral side. All subsequent figures of transverse sections are constructed in the same way. A6, sixth abdominal segment of the terminal ganglion; A7, seventh abdominal segment of the terminal ganglion; DMT, dorsal medial tract; DV, dorsoventral tract; LDT, lateral dorsal tract; MDT, medial dorsal tract; VC, ventral commissure; MVT, medial ventral tract; VIT, ventral intermediate tract; VLT, ventral intermediate tract; VMT, ventral medial tract.

A2 neurone produced spikes continuously during passage of depolarizing current. This interneurone did not have any obvious output effects on the abdominal posture motor neurones.

The descending axon (diameter=10 $\mu$m) is located at the most lateral and slightly dorsal edge of the fifth-sixth connective (Fig. 4B). The axon enters neuropil in LDT and gives off several branches medially and ventrally to form first main branch, mb1 in the anterior neuropil. The fine processes derived from mb1 are restricted in the neuropil above the level between dorsal medial tract (DMT) and dorsal intermediate tract (DIT) (Fig. 4C). The axon further descends posteriorly to send three large branches laterally and posteriorly (mb2) in the neuropil between VMT and VIT (Fig. 4D). The axon, then, curves medially and projects two large branches laterally to give off several fine branches dorsally and medially (mb3) in the most dorsal surface of the neuropil (Fig. 4E). The axon reaches the level of root 1. All terminal branches of A2 neurone were contained within the dorsal half of the neuropil.

*A3 interneurone*. The A3 interneurone was characterized by a branch crossing the midline in the medial neuropil (Fig. 5A). During a passage of depolarizing current, the A3 interneurone discharged spikes continuously and excited abdominal flexor excitors.

The axon (diameter=15 $\mu$m) is located in the lateral connective but slightly medial from the edge of the connective (Fig. 5B). After the axon enters the neuropil, it gives off two large branches postero-laterally and several short branches medially. The axon, then, projects posteriorly to give off a single fine branch medially that crosses the midline (asterisk in Fig. 5A). The terminal branches of this interneurone end above the level of root 1.

*Type B interneurones*

The second type of descending interneurone that responded in one-to-one fashion to connective stimulation had coactivating effects on the uropod motor neurones (Fig. 2D). Morphologically they were classified into three interneurone types.

*B1 interneurone*. The B1 interneurone was characterized morphologically by the limited extent of its terminal branches.

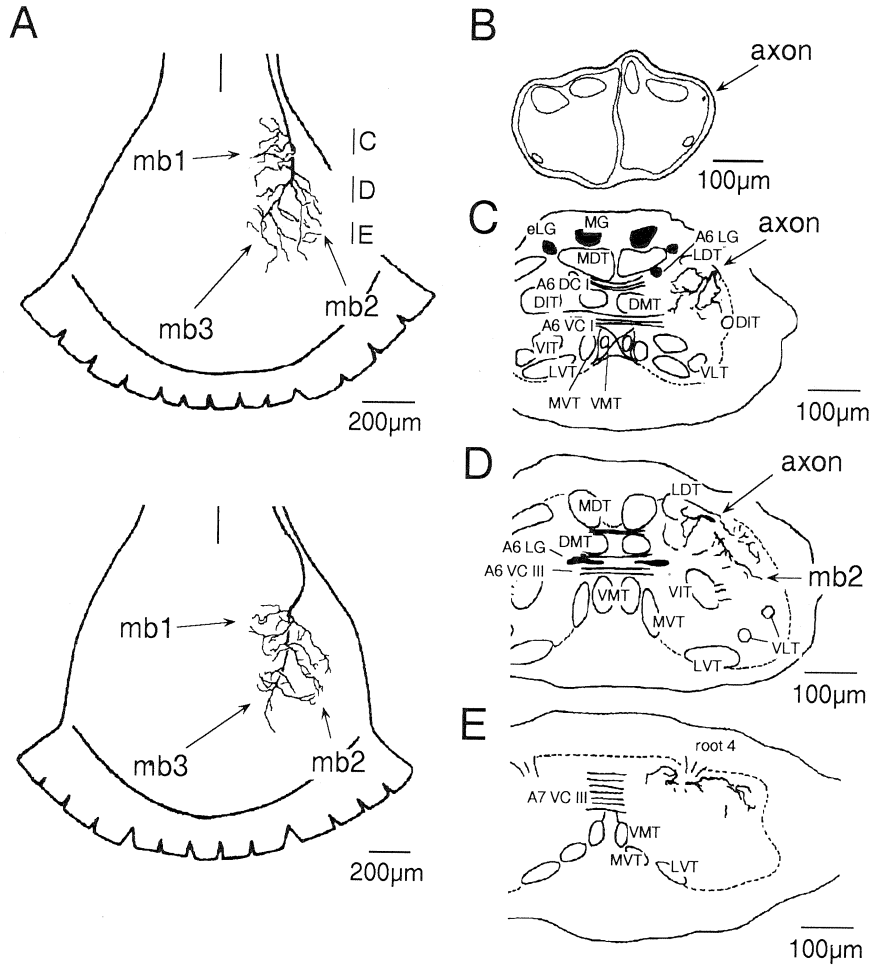


FIG. 4. Morphology of A2 interneurone. A. Three A2 interneurones in different crayfish are drawn to show the consistency of morphology. Each neurone is viewed from the ventral surface with anterior to the top. B. The location of the descending axon in the abdominal fifth-sixth connective. C-E. Drawings of transverse sections of A2 neurone at the planes indicated in A. C, D are composites of four sections, E of three sections. DC I, dorsal commissure I; DIT, dorsal intermediate tract.

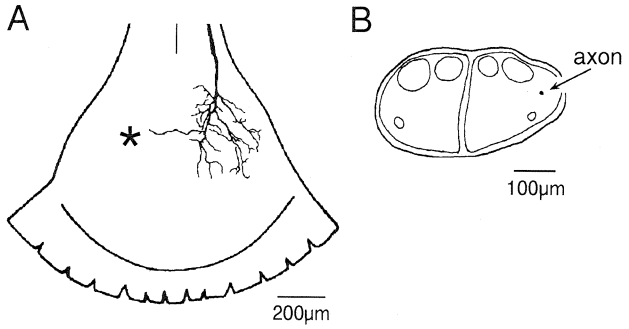


FIG. 5. A Morphology of A3 interneurone as viewed from the ventral surface with anterior to the top. One branch across the midline at the medial level of neuropil (asterisk). B The location of the descending axon in the abdominal fifth-sixth connective.

Three interneurones classified as B1 are drawn in Figure 6A to show the consistency of the morphology. In contrast with type A interneurones, large intensity of depolarizing current ($19 \pm 3$ nA, n=7) was required to produce spikes. Furthermore, the spike discharge of this interneurone was transient and did not continue during a passage of depolarizing current. In four of eight preparations, the B1 interneurone inhibited the flexor excitor motor neurones and excited the flexor inhibitor motor neurone, and others had no effects on the abdominal posture motor neurones.

The axon (diameter=8 $\mu$m) is located at the dorsolateral edge of the fifth-sixth connective (Fig. 6B). The axon enters the neuropil in LDT to give off several fine branches and bifurcates in the medial region of the neuropil to form two main branches, mb1 and mb2. The first branch, mb1 proceeds medially along the dorsal edge of the neuropil, while mb2 projects laterally (Fig. 6C).

*B2 interneurone.* The B2 interneurone produced spikes transiently during a passage of depolarizing current; rather weak current ($5 \pm 1$nA, n=4) was necessary to generate spikes. The B2 interneurone coactively excited both the extensor excitor and the flexor excitor motor neurones.

Two examples of morphology of B2 interneurone are

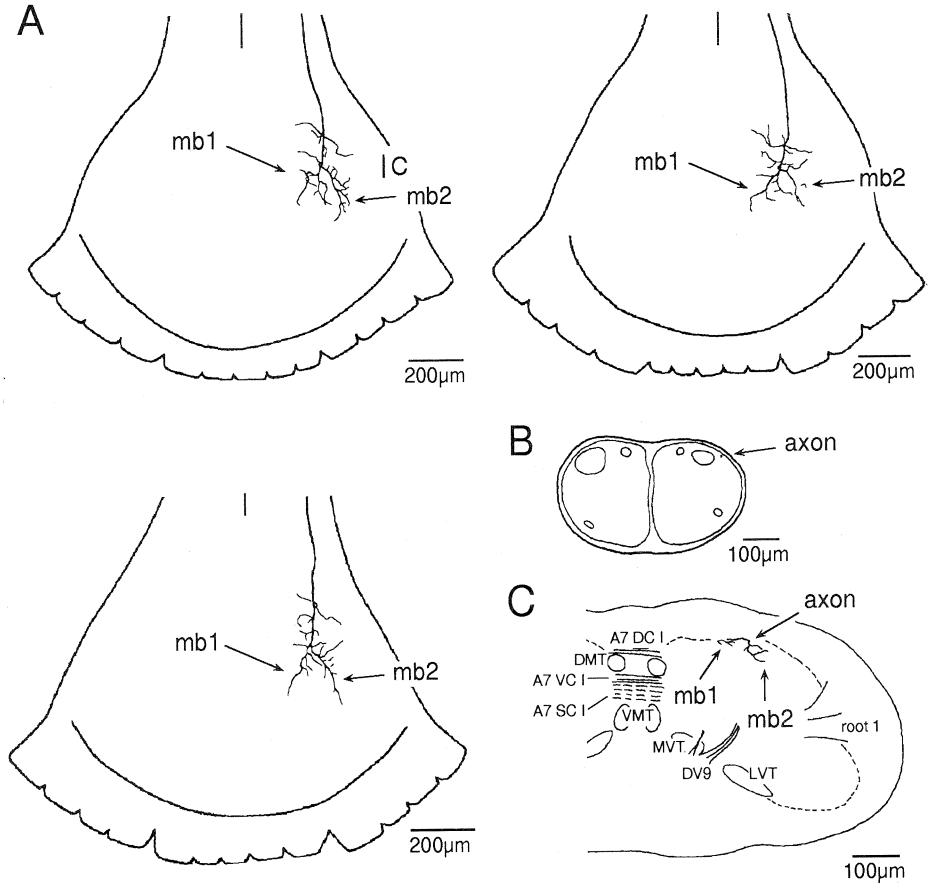


FIG. 6. Morphology of B1 interneurone. A. Three B1 neurones in different crayfish are drawn to show the consistency of morphology. Each neurone is viewed from the ventral surface with anterior to the top. B. The location of the descending axon in the abdominal fifth-sixth connective. C. Drawing of six adjacent transverse sections at the planes indicated in A. SC, sensory commissure.

illustrated in Figure 7A. The axon (diameter=12 $\mu$m) is located at the lateral edge of the connective (Fig. 7B). The axon enters the neuropil in the LDT and gives off a fine and long branch (mb1) medially. The axon, then, gives off a second main branch (mb2) posteriorly and ventro-laterally to the horizontal level between medial ventral tract (MVT) and ventral lateral tract (VLT) (Fig. 7C). The axon descends continuously and curves slightly medially to project the third main branch posteriorly (mb3) and the fourth main branch antero-medially (mb4). The axon runs further posteriorly to give off the fifth main branch (mb5) laterally and ends near the postero-medial edge of the neuropil.

*B3 interneurone*. The B3 interneurone was characterized morphologically by its main branch, mb3, ending around the posterior midline of the neuropil (Fig. 8A). In three of five preparations, the B3 interneurone produced spikes transiently during passage of depolarizing current. This interneurone slightly excited extensor inhibitor and/or flexor excitor No. 3 or 4.

The axon (diameter=7 $\mu$m) is located at the lateral edge of the fifth-sixth connective (Fig. 8B) and enters the neuropil in the LDT. In the anterior region of the neuropil, the axon projects a first main branch (mb1) posteriorly. This branch curves laterally to project in the ventral neuropil (Fig. 8C and D). The descending axon, then, gives off a second main branch postero-laterally (mb2) to the level between VMT and VIT. Sending several fine branches medially and laterally, the axon (mb3) runs postero-medially to cross the midline in posterior ventral commissure of seventh abdominal segment of the terminal ganglion(not shown).

*Type C descending interneurone*

One descending interneurone, that was classified as C1, also generated spikes in response to the connective stimulation (Fig. 9A). This interneurone fired spikes at about 70 spikes/s in response to 100 Hz stimulation (Fig. 9A-1) and this discharge continued for 3sec after the end of the stimulation. In contrast with the above mentioned interneurones, each pulse of electrical stimulation did not consistently evoke a spike of the interneurone (Fig. 9A-2). The passage of weak depolarizing current (=5nA) elicited spikes continuously. This interneurone also inhibited the closer motor neurones (Fig. 9B: first trace) and excited the opener motor neurones (Fig. 9B: 2nd trace). This interneurone slightly excited extensor excitors and flexor inhibitor.

The striking characteristic of morphology of C1 is a

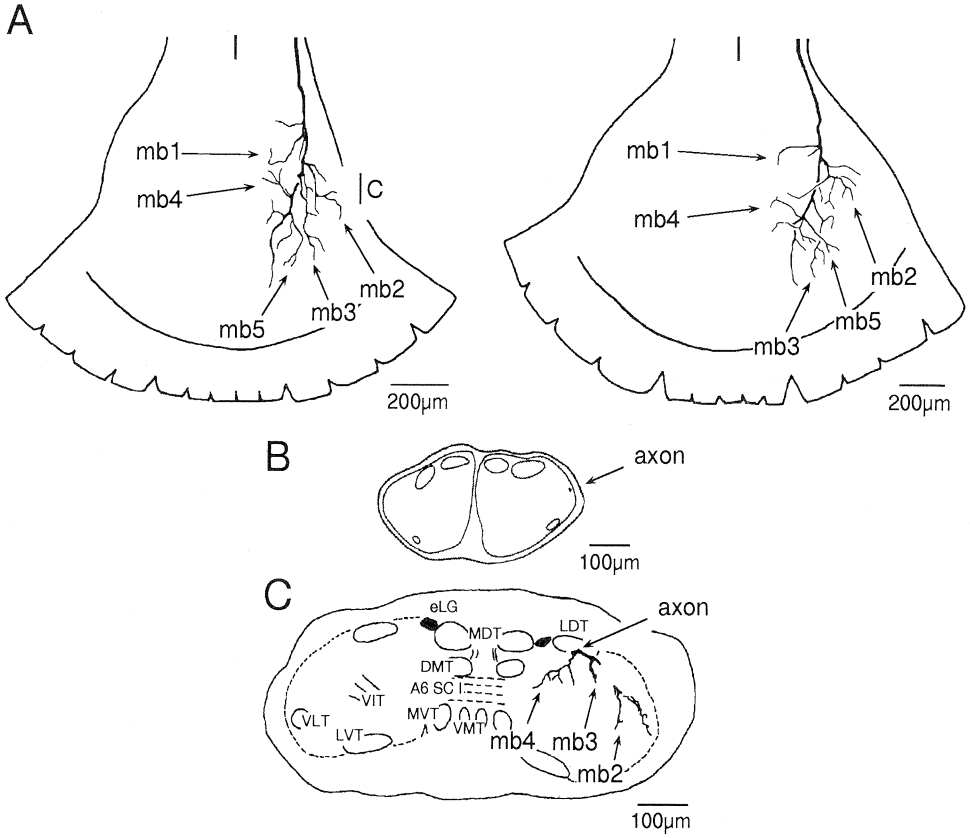


FIG. 7. Morphology of B2 interneurone. A. Two B2 neurones in different crayfish are drawn to show the consistency of morphology. Each neurone is viewed from the ventral surface with anterior to the top. B. The location of the descending axon in the abdominal fifth-sixth connective. C. Drawing of five adjacent transverse sections at the planes indicated in A.

terminal branch that crosses the midline (Fig. 9C). The descending axon is located in the lateral region of the connective (Fig. 9D), enters the neuropil and projects posteriorly to give off several fine branches medially and laterally. At the level of root 1, the axon turns medially to cross the midline. In the contralateral neuropil, the axon turns anteriorly to give off fine branches (Fig. 9C). Since the connective stimulation elicited reciprocal activation of the uropod motor neurones on the contralateral side, this interneurone could mediate bilateral coordination.

*Axonal position of descending interneurones in connective*

The axons of descending interneurones studied in this report were limited to the dorso-lateral edge, i.e., area 77 and 81 [33] of the fifth-sixth abdominal connective (Fig. 10A). The axons of type A interneurones that had reciprocal opening effects on the uropod motor neurones located at the medial level of the lateral edge in area 81 (Fig. 10B-1). The axonal position of type B interneurones was segregated from that of type A interneurones. The axon of B1 was located in the border between area 77 and 81 (Fig. 10B-2: stippled circles) and that of B2 and B3 was located in the more ventral level in area 81 (Fig. 10B-2: stippled triangles and squares) where the axon of C1 also positioned (Fig. 10B-1: star).

## DISCUSSION

*Classification of descending interneurones*

In this study, three types of descending interneurones have been characterized by their effects on the uropod motor neurones and their projection pattern in the terminal abdominal ganglion. The position of the axons of type A interneurones is separated from that of type B interneurones in the abdominal nerve cord (Fig. 10). The different axonal positions of interneurone also correlate with the different projection endings in the terminal ganglion. The axon of the B1 interneurone whose axonal terminal does not reach the level of root 1 in the terminal ganglion (Fig. 5A) is positioned more dorsally, whereas those of B2, B3 and C1 whose projections go across the level of root 1 (Figs. 7A, 8A and 9C) are located more ventrally (Fig. 10B). Morphologically different types of interneurones were found to have different physiological properties: most of the type A interneurones (11/14) produced spikes continuously due the current injection, whereas most of the type B (14/17) produced spikes transiently.

In intersegmental interneurones, it is difficult to stain the whole structure. The dendritic structure and soma location of the descending interneurones in more anterior ganglia remained unknown. Since extracellular action potentials at

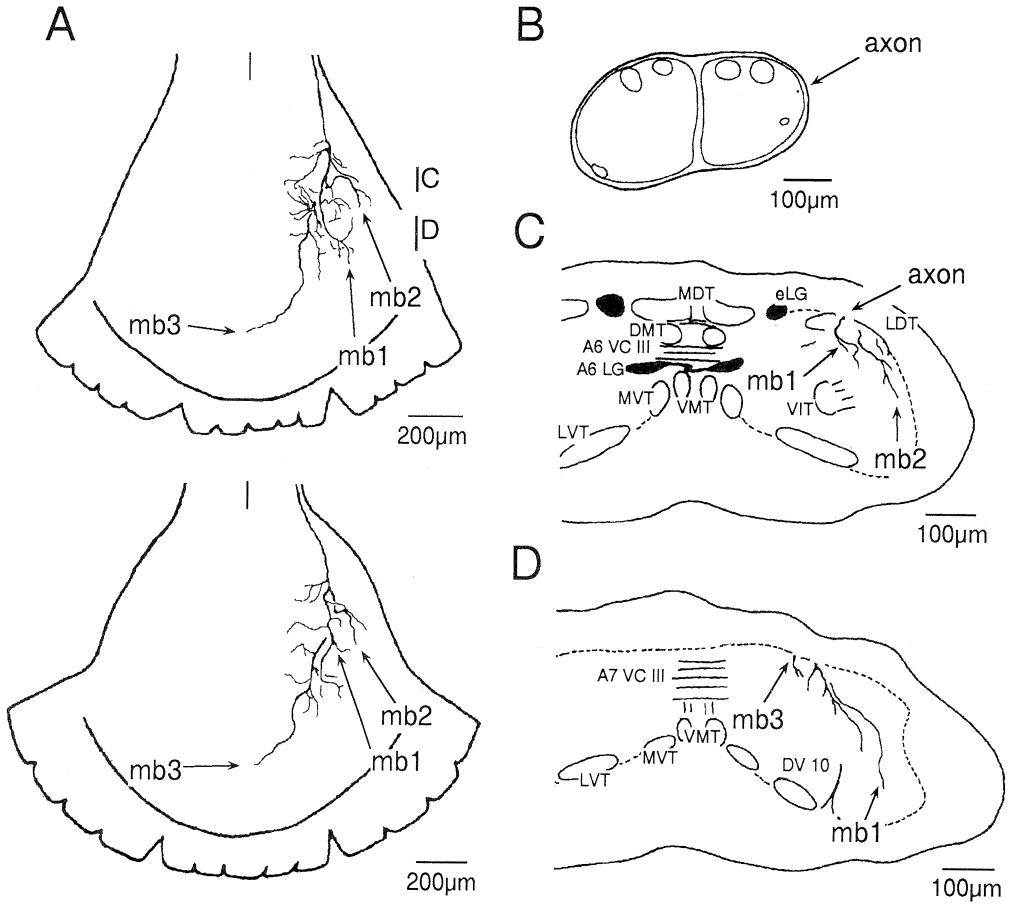


FIG. 8. Morphology of B3 interneurone. A. Two B3 neurones in different crayfish are drawn to show the consistency of morphology. Each neurone is viewed from the ventral surface with anterior to the top. B. The location of the descending axon in the abdominal fifth-sixth connective. C, D. Drawings of transverse section of B3 at the planes indicated in A. C is composite of five adjacent transverse sections, D of seven sections.

the site of the lateral region of the circumoesophageal connective consistently followed stimulus pulses applied to the abdominal third-fourth connective (data not shown), at least, some type A and type B interneurones would originate in the brain. Morphological characterization in the anterior ganglia of these descending interneurones is helpful to know the input site and their role in the control of behaviour.

*Output of descending interneurones*

All the three types of descending interneurones currently examined were found to have functional interactions with uropod motor neurones, since electrical stimulation of the descending axons elicited specific pattern of uropod motor activity (Figs. 2, 9). Uropod motor neurones could be driven by the descending interneurones either monosynaptically or polysynaptically through nonspiking interneurones which effectively control uropod motor neurone activity without generating action potentials [30]. The axons of the descending interneurones studied in this report enter into the neuropil in the LDT and their projections occupy the dorsal region in the neuropil of the terminal abdominal ganglion above the level between VMT and VIT. This dorsal region of neuropil contains the arborization of motor neurones [12] and nonspiking local interneurones [11]. The present results thus indicate that the descending interneurones activate uropod motor neurones in the dorsal neuropil.

Spatial overlap of the interneurone axon terminals with the dendrites of uropod motor neurones and premotor nonspiking interneurones further suggests that the connection between descending interneurones and motor neurones is organized in parallel by monosynaptic and polysynaptic pathways as has been physiologically shown in the descending statocyst-motor pathway [29]. Further physiological investigation is needed to clarify the functional connection of the descending interneurones currently studied and uropod motor neurones.

*Functional roles of descending interneurones*

The interneurones examined in this study descend the abdominal nerve cord in its lateral region (Fig. 10). Electrical stimulation of a small bundle split from the lateral region of the nerve cord including these interneurones elicited motor activities in the abdominal posture system. Thus, when each interneurone was excited by current injection, it was expected that a similar motor activity should be elicited in the abdominal posture system. However, the effects of

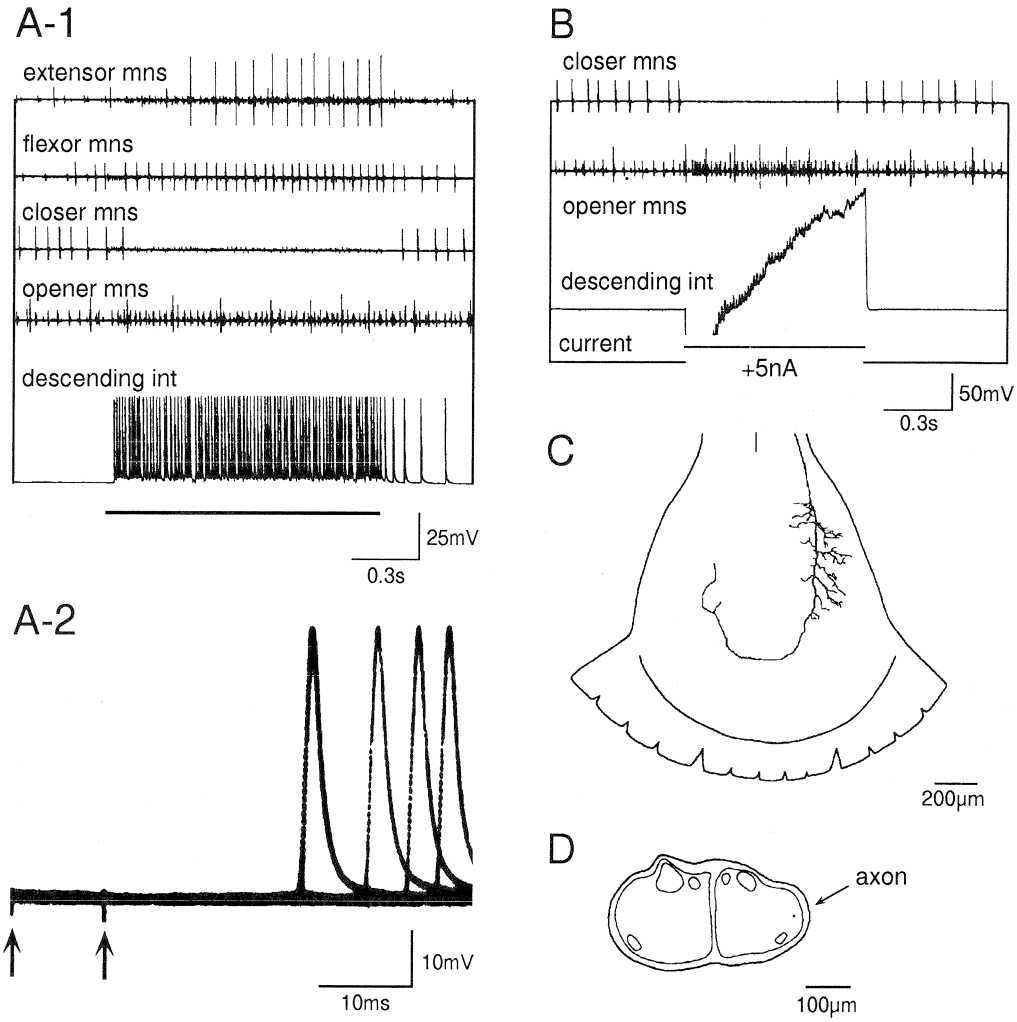


FIG. 9. Physiology and morphology of C1 interneurone. A. In response to connective stimulation, C1 interneurone produced spikes without one-to-one fashion. A-1. During connective stimulation at 100 Hz (bar), C1 interneurone produced spikes at about 70 spikes/s. A-2. Ten superimposed sweeps triggered from two connective stimulus pulses of interval 10msec (arrows) reveal that C1 neurone produced five spikes at various latencies ranging from 22–35msec. B. Depolarizing current injected into the C1 elicited reciprocal opening pattern of the uropod closer (top trace) and opener (2nd trace) motor neurones. C. Drawings from whole mount of C1 interneurone as viewed from the ventral surface with anterior to the top. D. The location of the descending axon in the abdominal fifth-sixth connective.

current injection into these interneurones on the posture motor neurones were always weaker than those on the uropod motor system. The effects were even variable among the same descending interneurones classified by their projection and output connection with uropod motor neurones.

There are several possible explanations for this observation. The axons in the lateral region have diameters ranging from 3 to 15 $\mu$m whereas those of penetrated neurones have diameters ranging from 5 to 15 $\mu$m. Thus, several axons in the lateral region which were too thin to be penetrated in our experiment might be the primary pathway for the abdominal posture control.

An alternative possibility is that each of the interneurones only makes weak connections with a part of abdominal posture motor neurones so that a whole ensemble of descending interneurone activity is required for eliciting the complete activity in the abdominal posture system [3]. During the electrical stimulation of the lateral bundle, the motor output in the abdominal system was more enhanced at higher stimulus frequencies (>50 Hz) and intensities. This observation indicates that the motor output of the abdominal posture system is based on the summation of individual descending neurone activities. It was shown that in the abdominal posture system, interneurones involved in the posture movements were regarded as the command elements [10] and that the motor pattern was formed by the synaptic interaction among functionally similar command elements [8, 14, 17]. Those descending interneurones studied in this study are therefore most likely to function as the elements of the abdominal posture command system. The characteristic motor pattern in the abdominal posture movement would result from spatially summed outputs of a number of interneurones including all the descending interneurones that

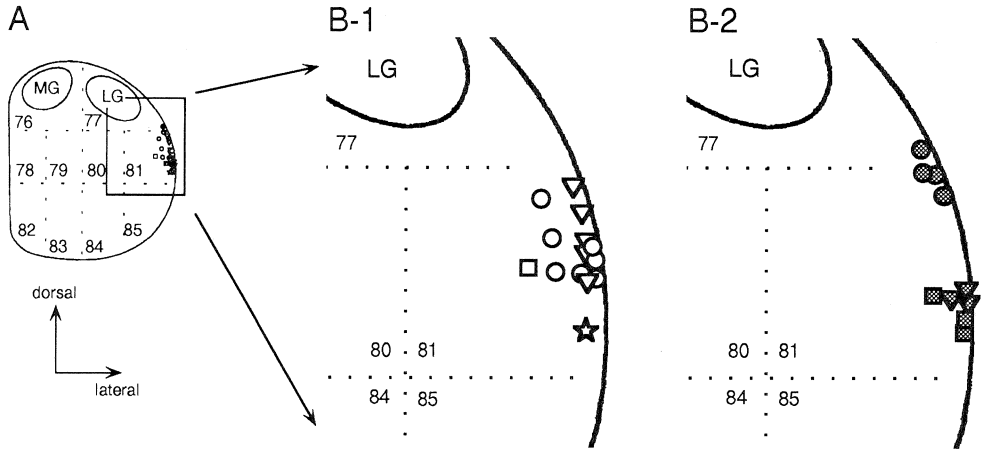


FIG. 10. Location of axons of descending interneurones. A. A cross-section of left hemiconnective, showing the relative position of the axons of seven types descending interneurones classified in this study. Dotted lines and numbers indicate the divisions of the hemiconnective by Wiersma and Hughes [33]. The axons of LG and MG are also drawn as landmarks. B. Partial expansion of the hemiconnective shown in A. B-1. Axonal location of A1, A2, A3 and C1 interneurones that had reciprocal opening effects on the uropod motor neurones. Open circles, axons of A1 interneurones obtained from seven crayfish; open triangles, A2 interneurones from five; open square, A3 interneurone; open star, C1 interneurone. B-2. Axonal location of type B interneurones that had coactivating effects on the uropod motor neurones. Stippled circles, axons of B1 interneurones obtained from four; stippled triangles, B2 from three; stippled squares, B3 from three crayfish.

were activated by the connective stimulation.

## ACKNOWLEDGMENTS

We thank Dr. P. L. Newland for his critical reading of this manuscript. MT was supported by a grant (05640758) from the Ministry of Education, Science and Culture.

## REFERENCES

1 Aonuma H, Nagayama T, Hisada M (1994) Output effect of identified ascending interneurons upon the abdominal postural system in the crayfish Procambarus clarkii (Girard). Zool Sci 11: 191–202
2 Cohen AH (1987) The structure and function of the intersegmental coordinating system in the lamprey spinal cord. J Comp Physiol A 160: 181–193
3 Davis WJ, Kennedy D (1972) Command interneurons controlling swimmeret movements in the lobster. II. interaction of effects in motoneurons. J Neurophysiol 35: 13–19
4 El Manira A, DiCaprio RA, Cattert D, Clarac F (1991) Monosynaptic interjoint reflexes and their central modulation during fictive locomotion in crayfish. Eur J Neurosci 3: 1219–1231
5 Evoy WH, Kennedy D, Wilson DM (1967) Discharge patterns of neurones supplying tonic abdominal flexor muscles in crayfish. J Exp Biol 46: 393–411
6 Fields HL, Evoy WH, Kennedy D (1967) Reflex role played by efferent control of an invertebrate stretch receptor. J Neurophysiol 30: 859–875
7 Griss C, Rowell CHF (1986) Three descending interneurons reporting deviation from course in the locust. I. anatomy. J Comp Physiol A 158: 765–774
8 Jellies J, Larimer JL (1985) Synaptic interactions between neurons involved in the production of abdominal posture in crayfish. J Comp Physiol 156: 861–873
9 Kennedy D, Takeda K (1965) Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. J Exp Biol 43: 211–227
10 Kupferman I, Weiss KR (1978) The command neuron concet. Behav Brain Sci 1: 3–39
11 Kondoh Y, Hisada M (1986a) Distribution and ultrastructure of synapses on a premotor local nonspiking interneuron of the crayfish. J Comp Neurol 254: 259–270
12 Kondoh Y, Hisada M (1986b) Neuroanatomy of the terminal (sixth abdominal) ganglion of the crayfish, Procambarus clarkii (Girard). Cell Tissue Res 243: 273–288
13 Kondoh Y, Hisada M (1987) The topological organization of primary afferents in the terminal ganglion of crayfish, Procambarus clarkii. Cell Tissue Res 247: 17–24
14 Larimer JL (1988) The command hypothesis: a new view using an old example. TINS 11: 506–510
15 Laurent G, Burrows M (1989) Distribution of intersegmental inputs to nonspiking local interneurons and motor neurons in the locust. J Neurosci 9: 3019–3029
16 Laurent G (1987) The morphology of a population of thoracic intersegmental interneurones in the locust. J Comp Neurol 256: 412–429
17 Miall RC, Larimer JL (1982) Interneurons involved in abdominal posture in crayfish: structure, function and command fiber responses. J Comp Physiol 148: 159–173
18 Nagayama T, Hisada M (1987) Opposing parallel connections through crayfish local nonspiking interneurons. J Comp Neurol 257: 347–358
19 Nagayama T, Takahata M, Hisada M (1984) Functional characteristic of local non-spiking interneurons as the pre-motor elements in crayfish. J Comp Physiol 154: 499–510
20 Nagayama T, Takahata M, Hisada M (1986) Behavioral transition of crayfish avoidance reaction in response to uropod stimulation. Exp Biol 46: 75–82
21 Nagayama T, Takahata M, Hisada M (1983) Local spikeless interaction of motoneuron dendrites in the crayfish Procambarus clarkii Girard. J Comp Physiol 152: 335–345
22 Nagayama, T., Isogai, Y., Namba H (1993) Physiology and morphology of spiking local interneurons in the terminal abdominal ganglion of the crayfish. J Comp Neurol 337: 584–599
23 Nagayama T, Isogai Y., Sato M, Hisada M (1993) Intersegmental ascending interneurones controlling uropod movements of the crayfish *Procambarus clarkii*. J Comp Neurol 332: 155–

174
24 Namba H, Nagayama T, Hisada M (1994) Descending control of nonspiking local interneurons in the terminal abdominal ganglion of the crayfish. J Neurophysiol 72: 235–247
25 Paul DH, B Mulloney (1986) Intersegmental coordination of swimmeret rhythms in isolated nerve cords of crayfish. J Comp Physiol 158A: 215–224
26 Reichert H (1993) Sensory inputs and flight orientation in locusts. Comp Biochem Physiol [A] 104: 647–657
27 Reichert H, Rowell CHF (1985) Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. J Neurophysiol 53: 1202–1218
28 Stewart WW (1978) Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalimide tracer. Cell 14: 741–759
29 Takahata M, Murayama M (1992) Multiple gate control of the descending statocyst-motor pathway in the crayfish *Procambarus clarkii* Girard. J Comp Physiol A 170: 463–477
30 Takahata M, Nagayama T, Hisada M (1981) Physiological and morphological characterization of anaxonic non-spiking interneurons in the crayfish motor control system. Brain Res 226: 309–314
31 Toga T, Takahata M, Hisada M (1990) An identified set of local nonspiking interneurones which control the activity of abdominal postural motoneurones in crayfish. J Exp Biol 148: 477–482
32 van Harreveld A (1936) A physiological solution for freshwater crustaceans. Proc Soc Exp Biol Med 34: 428–432
33 Wiersma CAG, Hughes GM (1961) On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambarus clarkii* (Girard). J Comp Neurol 116: 209–228
34 Williams BJ, Larimer JL (1981) Neural pathway of reflex-evoked behaviors and command system in the abdomen of the crayfish. J Comp Physiol 143: 27–42
35 Wolf H (1992) Reflex modulation in locusts walking on a treadwheel- intracellular recordings from motoneurons. J Comp Physiol A 170: 443–462