

*Invited Review***Morphological and physiological
bases of crayfish local circuit neurones****T. Nagayama, H. Namba and H. Aonuma**Animal Behaviour and Intelligence, Division of Biological Sciences, Graduate School of Science,
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Summary. As the subject of neuroethological studies, arthropods offer several advantages to elucidate the neural processes that generate and control behavioural acts. The relative simplicity of their neural organization and their identifiable neurones are particularly advantageous. The crayfish, *Procambarus clarkii*, uropods are paired appendages of the last abdominal segment that show bilateral closing movement in response to mechanical stimulation. This movement, the avoidance «dart» response, is mediated by a cascade and parallel organization of local circuit neurones in the terminal abdominal ganglion. Thirty intersegmental ascending interneurons and 20 spiking local interneurons have been identified both morphologically and physiologically. They receive exteroceptive inputs directly from the sensory afferents. Two complementary groups of unilateral nonspiking local interneurons have also been functionally identified. They exert fine control over the wide range of activity of motor neurones in a graded and sustained manner. These nonspiking interneurons form opposing and parallel pathways that are essential in modulating the pattern of movement of the uropods.

Key words: Neuroethology, Local interneurons, Nonspiking, Sensory, Motor

Introduction

Animal behaviour is expressed as a series of movements and postural changes. A wide variety of animal behaviour exists which attracts the attention of laymen and professional scientists alike. In recent years there have been great advances in our knowledge of the cellular mechanisms that underlie these behaviours, through neuroethological studies (e.g. Hoyle, 1975;

Huber and Markl, 1983; Camhi, 1984; Guthrie, 1987). The neurones that produce and control movement are functionally classified into three groups; sensory neurones, interneurons and motor neurones. There are vast numbers of sensory neurones that converge on the central nervous system, while there are only a small number of motor neurones that control the muscles to produce precise, delicate and adaptive movements. Emerging interest in the generation of behaviour patterns is: how is sensory information conveyed to, and organized in, the central nervous system; and how do interneurons process and integrate these sensory signals to organize coordinated movement? Moreover, how are these pathways modified, and what are their adaptive functions in different behavioural situations?

Arthropods, and in particular insects and crustaceans, offer several advantages to studies of neural operation underlying animal behaviour. Firstly, they frequently show reflex-like stereotyped behaviour (Wine and Krasne, 1972) so that behavioural sequences can be analyzed quantitatively. Secondly, the organization of their nervous system is relatively simple with each segmental ganglion containing from several hundreds to some thousands of central neurones (Kondoh and Hisada, 1986a). In contrast, vertebrates have some ten billion central neurones. Thirdly, in arthropods many neurones can be characterized and identified individually by intracellular recording and staining. These advantages make arthropods particularly suitable for cellular analyses of neural circuit design and organization.

In this paper, we will focus on the processing of mechanosensory signals that generate reflexive limb movements of the crayfish *Procambarus clarkii* (Girard). The initial step in a neuroethological approach are to observe the behaviour, to identify the peripheral and central neural elements involved, and to describe their activity patterns and connections.

Crayfish avoidance «dart» response

The crayfish uropods are the paired appendages of

the last (sixth) abdominal segment and are used for posture and locomotion during various behavioural acts, e.g. equilibrium reactions (Yoshino et al., 1980), escape swimming (Wine and Krasne, 1972) and avoidance reactions (Nagayama et al., 1986). When a crayfish adopts a stationary resting posture with its abdomen passively extended, a gentle mechanical stimulation to the exopodite evokes an avoidance «dart» response (Nagayama et al., 1986) in which the animal rapidly closes the uropods on both sides and walks forward away from the stimulus source (Fig. 1A). Opening and closing movements of the uropod are made by antagonistic opener and closer muscles that are innervated by motor neurones originating in the terminal abdominal ganglion (Larimer and Kennedy, 1969). Several motor neurones have been identified and their detailed structures have been revealed (Nagayama et al., 1983, 1984). On the surface of the uropods, there are different types of sensory hairs that detect water movements and mechanical contact (Plummer et al., 1983; Wine, 1984). These hairs are innervated by afferents in the second root nerve which projects to the terminal abdominal ganglion (Calabrese, 1976; Nagayama and Sato, 1993).

Both mechanical stimulation of the exopodites and electrical stimulation of the second root afferents elicit reciprocal activation of the antagonistic sets of motor neurones. The rate of discharge of an identified closer, reductor motor neurone No. 1 (Red MN No. 1; Nagayama et al., 1983) increases (Fig. 1B) while that of a slow opener motor neurones decreases (Fig. 1C). These motor neurones show long-lasting sustained changes in membrane potential. This reciprocal closing pattern of the uropod is formed by a local circuit within the terminal abdominal ganglion since this pattern remains essentially unaltered by cutting the abdominal 5th-6th connectives (Nagayama and Hisada, 1985). Neither the sensory afferents (Fig. 1D Left) nor the motor neurone dendrites (Fig. 1E Left) cross the midline of the ganglion. Furthermore, the terminal branches of the afferents project within the ventral half of the ganglion (Fig. 1D Right) while motor neurone dendrites project within the dorsal half of the ganglion (Fig. 1E Right). Sensory signals cannot be transmitted directly from the afferents to motor neurones on either the ipsilateral or contralateral side, but must be transmitted via interneurones in the terminal abdominal ganglion.

Using intracellular analyses, we have characterized three distinct groups of local circuit neurones (ascending, spiking local, and nonspiking interneurones) that receive sensory inputs from the exopodite and influence the activity of the uropod motor neurones. In this review, we will describe the morphological features and physiology of these three groups of interneurones, then discuss the possible functions of these local circuit neurones which are relevant to avoidance reactions.

Ascending interneurones

Ascending interneurones are intersegmental interneurones in which the soma originates in the terminal abdominal ganglion. Their axons project anteriorly through the abdominal connective. About 130 (65 neurones in each hemiganglion) of a total of 650 neurones in the terminal ganglion are thought to be ascending interneurones (Reichert et al., 1982; Kondoh and Hisada, 1986a) and 30 pairs (Fig. 2) have so far been identified as unique individuals by their characteristic shapes and physiological properties (Nagayama et al., 1993a). Each interneurone occurs in similar locations and extends branches in similar regions in the ganglion from crayfish to crayfish. Thus, single identified neurones can be studied in different preparations.

Ascending interneurones have somata in the ventral cortex of the terminal abdominal ganglion. Based upon the position of the somata, they are divided into three groups: rostral (n= 14 out of 30), medial (n= 3), and caudal (n= 13) groups. In all interneurones except RC-9 (Fig. 2), the primary neurite originates from the soma, crosses the midline and sends an axon anteriorly through the opposite connective. Nineteen interneurones extend predominant dendritic branches within the hemiganglion contralateral to their somata (=ipsilateral to the ascending axons). Some also have a limited number of small branches on the soma side. Five interneurones (CA-3, CI-4, RC-5, RC-9 and RC-10) have branches restricted mainly to the soma side, while 6 interneurones (CA-2, RC-6, RO-5, RO-6, RO-7 and NE-2) have extensive bilateral branches. On the side of their main branches, all interneurones except RO-4 receive excitatory mechanosensory inputs (see later section for detail). Bilateral interneurones also receive excitatory inputs from the exopodite on the opposite side, while unilateral interneurones often receive inhibitory inputs. Thus, directional information from exteroceptive inputs is encoded in various ways by ascending interneurones and conveyed to anterior neural centres on both sides.

The majority of ascending interneurones have output effects on several motor neurones in both the terminal and anterior abdominal ganglia. They can be classified into six classes according to their pre-motor effects on the uropod motor neurones (Nagayama et al., 1993a). The CA interneurones coactively increase the activity of both the closer and opener motor neurones, while the CI interneurones suppress the activity of these motor neurones. Thus, they may control the tonus of the uropod muscles. The RC interneurones excite the closer motor neurones and inhibit the opener motor neurones, whereas the RO interneurones have the opposite effect. These interneurones can produce various patterns of uropod movement. One interneurone, VE-1, has inconsistent effects upon the motor neurones, while five, called NE interneurones, have no effect upon the motor neurones. These ascending interneurones could simultaneously affect the activity of the motor neurones

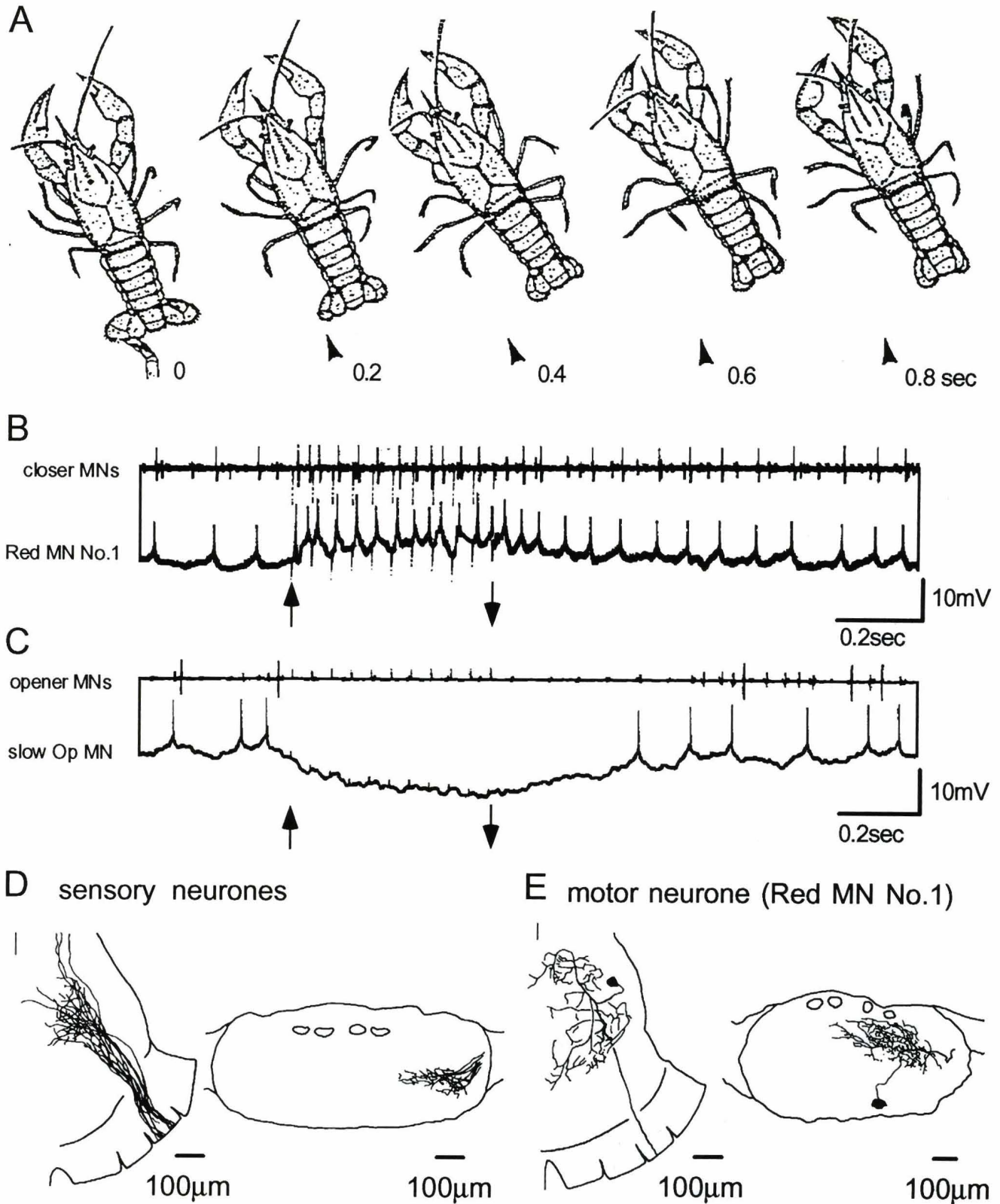


Fig. 1. **A.** Crayfish avoidance «dart» response. After touching right exopodite, crayfish walks forward with closing of bilateral uropods. **B-C.** Reciprocal motor pattern in response to electrical stimulation of 2nd root afferents innervating hairs on the surface of the exopodite. Identified closer, reductor motor neuron No. 1 is excited (B) and the antagonistic opener motor neuron is inhibited (C). **D-E.** Morphology and central projection of 2nd root afferents (D) and the closer, reductor motor neuron (E). A is based on Nagayama et al. (1986).

in the abdominal postural system (Aonuma et al., 1994). The coordination of the activity between uropods and abdominal muscles is a prerequisite to complete a series of sequential movements and postural changes in the avoidance reaction, i.e., an immediate uropod closing followed by forward locomotion. One of the most significant functions of the ascending interneurons would be, therefore, multisegmental integration of sensory signals.

Spiking local interneurons

Local interneurons are defined as neurones that have no axon-like structure and have their branches confined to the terminal abdominal ganglion, although the terminal ganglion is a fusion product of two embryonic

ganglia (Johnson, 1924). Histological studies have suggested that 50-60% of the total number of neurones in the terminal abdominal ganglion are local interneurons (Kondoh and Hisada, 1986a; Reichert et al., 1982). At least 150 neurones in the hemiganglion could, therefore, be local interneurons with either spiking or nonspiking properties (Takahata et al., 1981; Reichert et al., 1982; Nagayama and Hisada, 1985, 1987; Nagayama et al., 1993b).

In total, 20 types of spiking local interneurons have so far been identified in the terminal abdominal ganglion (Fig. 3). They are classified into three groups based on the position of somata in the ventral cortex of the ganglion: anterior ($n=6$ out of 20), medial ($n=6$), and posterior ($n=8$). All the interneurons have small-diameter soma (15-30 μm) and fine arborization in both

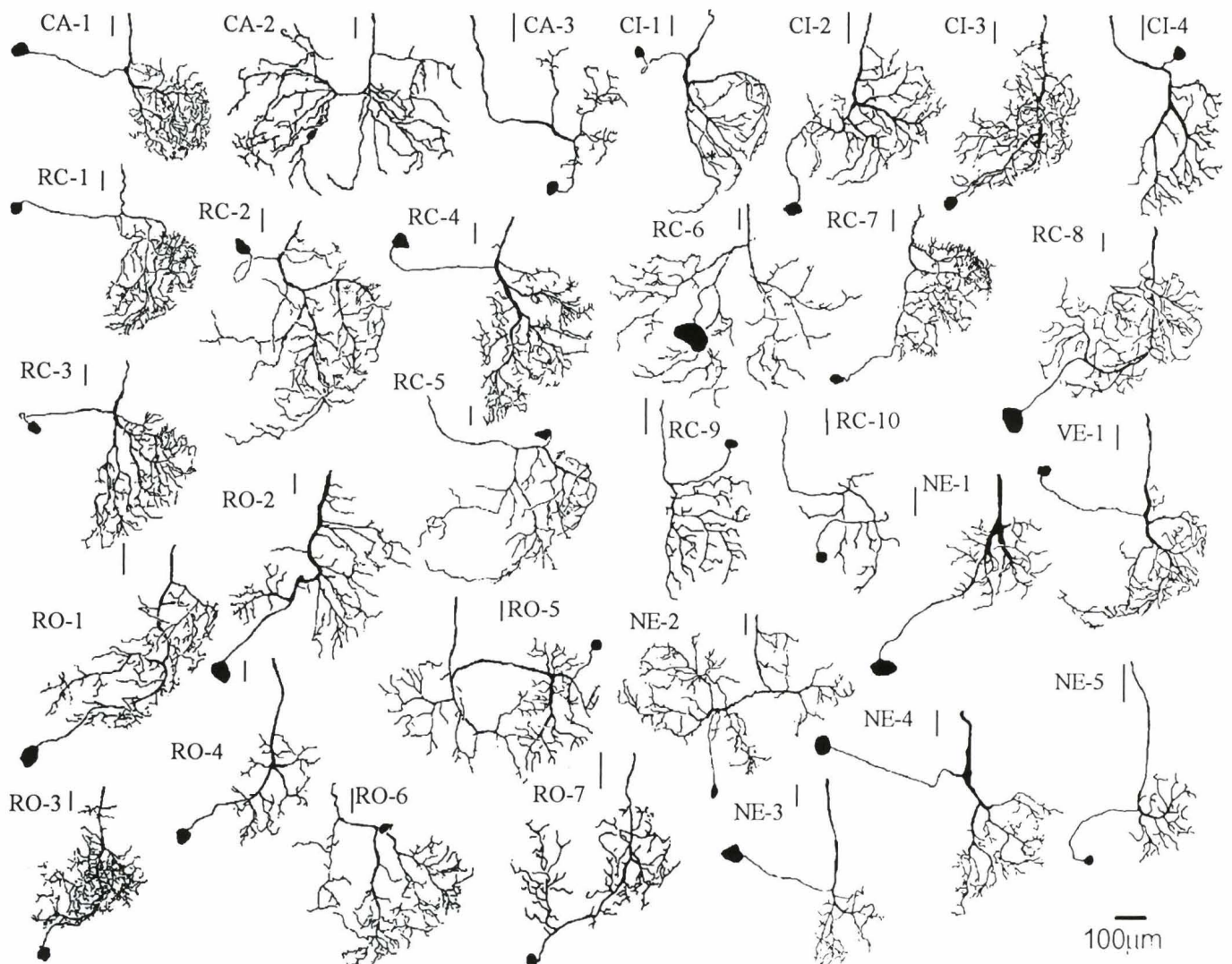


Fig. 2. Morphology of thirty identified ascending interneurons viewed from ventral side. Anterior is to the top. Each bar indicates midline. They are divided into six physiological (i.e., CA, CI, RC, RO, VE and NE) groups (based on Nagayama et al., 1993a) CA-3, CI-4, RC-9, RC-10, RO-7, and NE-5 are newly identified.

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the ventral and dorsal neuropil. The main branches of interneurons in both the anterior (sp-ant) and posterior (sp-pos) groups usually extend on the side contralateral to the soma while interneurons of the medial group (sp-med) have profuse bilateral branches.

Both the sp-ant and sp-pos interneurons receive excitatory sensory inputs from the afferents on the side ipsilateral to their main branches. They usually elicit a

reciprocal closing pattern of uropod movement by exciting closer motor neurones and inhibiting opener motor neurones. The sp-pos4 only inhibits the closer motor neurone. The sp-pos8 could be identical to the «C-cell» described by Kirk et al. (1986) that inhibits the telson-flexor motor giant, but which has no output effects upon uropod motor neurones.

Though all 6 interneurons in the medial group have

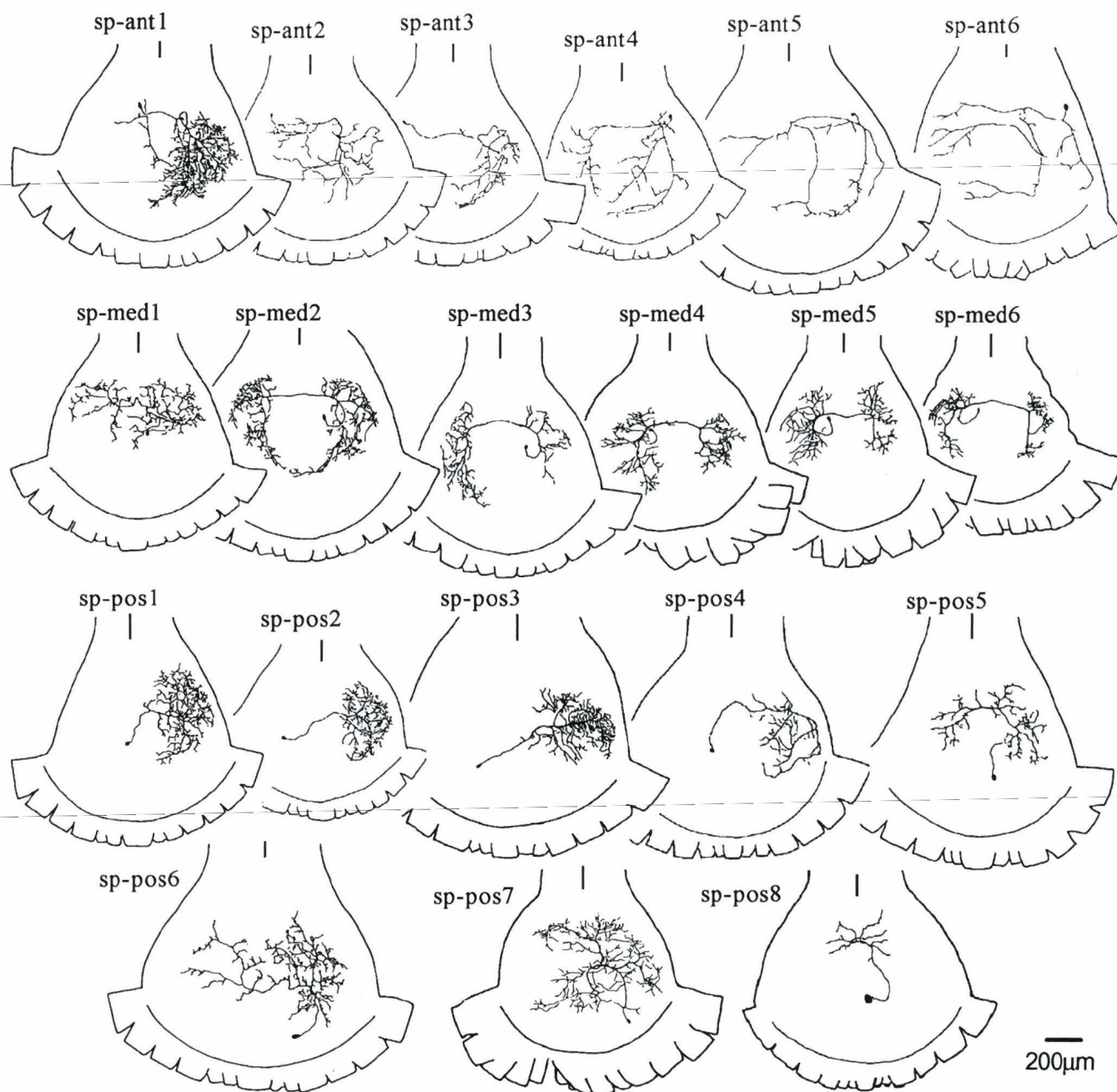


Fig. 3. Morphology of twenty identified spiking local interneurons viewed from ventral side. Anterior is to the top. They are divided into three anatomical (i.e., -ant, -med, and -pos) groups (based on Nagayama and Hisada, 1985; Nagayama et al., 1993b). sp-ant6 and SP-po8 are newly identified.

extensive branches on both the ipsilateral and contralateral sides, they are divided into two functional types. The interneurons, sp-med1, -med2, and -med3 receive excitatory sensory inputs from both exopodites and affect the uropod motor neurones on both sides. By contrast, sp-med4, -med5, and -med6 receive excitatory sensory inputs only from the exopodite on the soma side and usually receive inhibitory inputs from the afferents on the opposite side. These interneurons only affect the motor neurones on the contralateral side to the soma producing a reciprocal closing pattern of the uropod. By contrast, they have no significant effects upon the motor neurones on the soma side. Thus, they have functionally polarized bilateral branches: branches on the soma side as input sites and contralateral branches as output sites. Morphologically, sp-med4- sp-med6 are also polarized having smooth and short branches on the soma side and having branches with varicosities on the opposite side (Reichert et al., 1982; Nagayama and Hisada, 1985). They could transmit mechanosensory inputs to the contralateral side by crossing the midline and be involved in the formation of the uropod motor pattern on the opposite side.

Nonspiking local interneurons

Nonspiking local interneurons do not produce spikes either at resting potential or during current injection of either depolarization or hyperpolarization. Such neurones that function without spikes have been reported in many vertebrate and invertebrate sensory systems. For example, some interneurons in the vertebrate retina (Werblin, 1979) and invertebrate optic ganglion (Waldrop and Glantz, 1985; Wang-Bennett and Glantz, 1987) function without spikes. Some types of interneurons in the vertebrate olfactory bulb also function without spikes (Shepherd, 1981). Furthermore, certain motor neurones in the arthropod also function by means of graded potentials (Raper 1979; Graubard et al., 1983; Nagayama et al., 1983). Purely nonspiking neurones are, however, mainly found in arthropod motor systems (Pearson and Fournier, 1975; Burrows and Siegler, 1976; Siegler and Burrows, 1979; Heitler and Pearson, 1980; Takahata et al., 1981; Nagayama et al., 1984; Paul and Mulloney, 1985; Büschges, 1990).

The majority of nonspiking interneurons have a unilateral structure with their branches restricted to within the hemiganglion ipsilateral to the soma (Fig. 4a-h). Three interneurons have bilateral branches (Fig. 4i-k).

Unilateral nonspiking interneurons have somata of small diameter (10-25 μm) located in a ventral ridge of the ganglion and have numerous fine branches projecting in both the ventral and dorsal neuropil. They are further classified into two major groups, posterolateral (PL) and anterolateral (AL) interneurons, by their gross morphology and somatic position (Nagayama and Hisada, 1987). The PL interneurons have somata that are located in a posterior region and are connected to thickened neurites by an unbranched fine process

(Fig. 4a-d). Secondary neurites have branches projecting predominantly toward an anterior region of the ganglion. The AL interneurons are further divided into two subgroups depending upon the position of somata. The AL-I interneurons have their somata in an anterolateral region of the ganglion (Fig. 4e-f), while the AL-II interneurons have their somata in a medial region (Fig. 4g-h). Both the AL-I and AL-II interneurons have an arched configuration and have secondary and fine branches extending both anteromedially and posterolaterally. Ultrastructural studies have shown that both input and output synapses are closely opposed within a single small neurite (Kondoh and Hisada, 1986c). Besides this gross classification, it is, however, difficult to identify individual nonspiking interneurons in each group. The nonspiking interneurons of similar morphology but different physiology are closely packed together to form a cluster in the ganglion (Hisada et al., 1984). For example, the gross morphology, soma position, and number and patterning of branches of AL interneurons illustrated in Figs. 4e,f are similar. Their output effects of identified motor neurone are, however, different: one interneuron (Fig. 4e) excites the closer motor neurone while another interneuron (Fig. 4f) inhibits the same motor neurone by the passage of depolarizing current.

In contrast with ascending or spiking local interneurons that almost exclusively receive excitatory inputs from mechanosensory afferents, about one half of the nonspiking interneurons of both the PL and the AL groups receive inhibitory inputs. The pattern of inputs from the afferents to the nonspiking interneurons is closely related to the output effects of interneurons on the closer motor neurone (Nagayama and Hisada, 1987; Namba et al., 1994). The PL interneurons that receive excitatory inputs mainly increase the activity of the closer motor neurone when depolarized, while those that receive inhibitory inputs decrease the activity of the same motor neurone. The polarity of output effects of the AL interneurons also vary. The AL interneurons that receive excitatory inputs decrease the activity of the closer motor neuron when depolarized, while the AL interneurons that receive inhibitory inputs excite the same motor neurone. We could, therefore, functionally identify four sets of unilateral nonspiking interneurons, that is, excitatory, disinhibitory, inhibitory and disfacilitatory types. Their functional properties will be described in more detail in the last section.

Three nonspiking interneurons have bilateral arborizations connected by an unbranched transverse process. Each has been identified as an unique individual (Reichert et al., 1983; Nagayama and Hisada, 1988;). Their somata are located in the ventral surface around the midline. Their cell bodies are relatively large (20-30 μm) compared to those of unilateral nonspiking interneurons. The local directionally-selective interneuron (LDS) is characterized by a large diameter (15-20 μm) transverse process that leads to extensive bilateral branches (Fig. 4i). The branches of the soma

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side are smooth while those on the contralateral side have numerous varicosities. Ultrastructurally, input synapses are mainly distributed on the soma branches while both input and output synapses are intermingled on branches on the contralateral side that suggest a functional division of branches on both sides (Kondoh and Hisada, 1986b). LDS receives excitatory sensory inputs only from the afferents on the soma side. This interneurone has no measurable outputs to the uropod motor neurones but affects intersegmental ascending

interneurones on the contralateral side. LDS could, therefore, mediate inhibition for some ascending interneurones that received IPSPs from the exopodite on the soma side (see Fig. 9). Another two bilateral nonspiking interneurones have asymmetric bilateral arborization that are connected by a transverse process of about 5 μm in diameter (Fig. 4j,k). In contrast with LDS, their main branches on both sides have a rather uniform appearance without any difference in shape (e.g. distribution of varicosities). These bilateral inter-

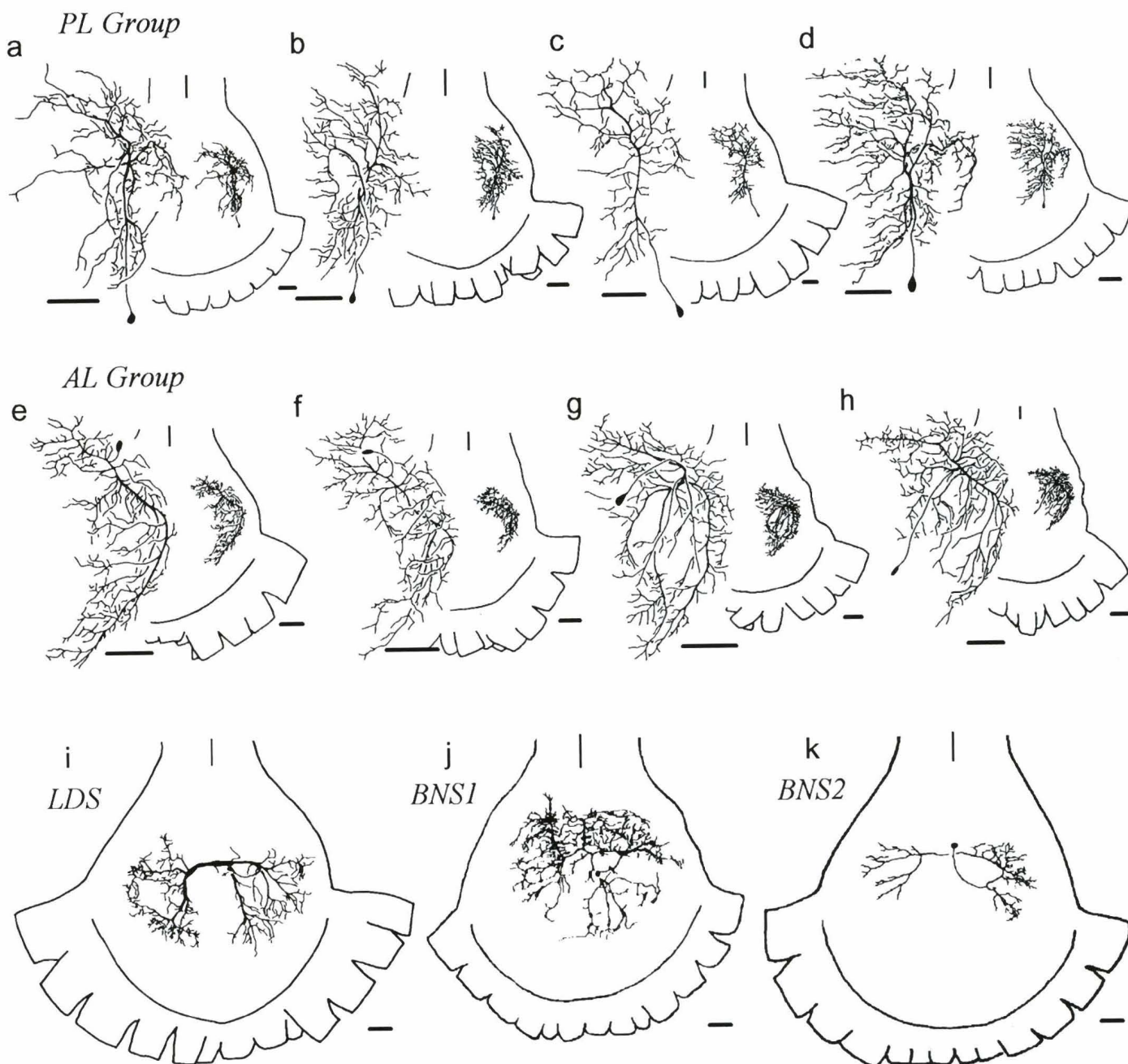


Fig. 4. Morphology of nonspiking interneurons. Unilateral interneurons are further subdivided into PL (a-d) and AL (e-h) types. Three bilateral interneurons (i-k) are identifiable (based on Nagayma and Hisada, 1987, 1988). Calibration bar is 100 μm .

neurones receive sensory inputs from the afferents on both sides: BNS-1 receives excitatory inputs while BNS-2 receives inhibitory inputs. Furthermore, these bilateral interneurones affect the activity of uropod

motor neurones when depolarized. However, injection of a small amount of current could affect the motor neurones on the same side only (Nagayama and Hisada, 1988) suggesting independent functions of branches on

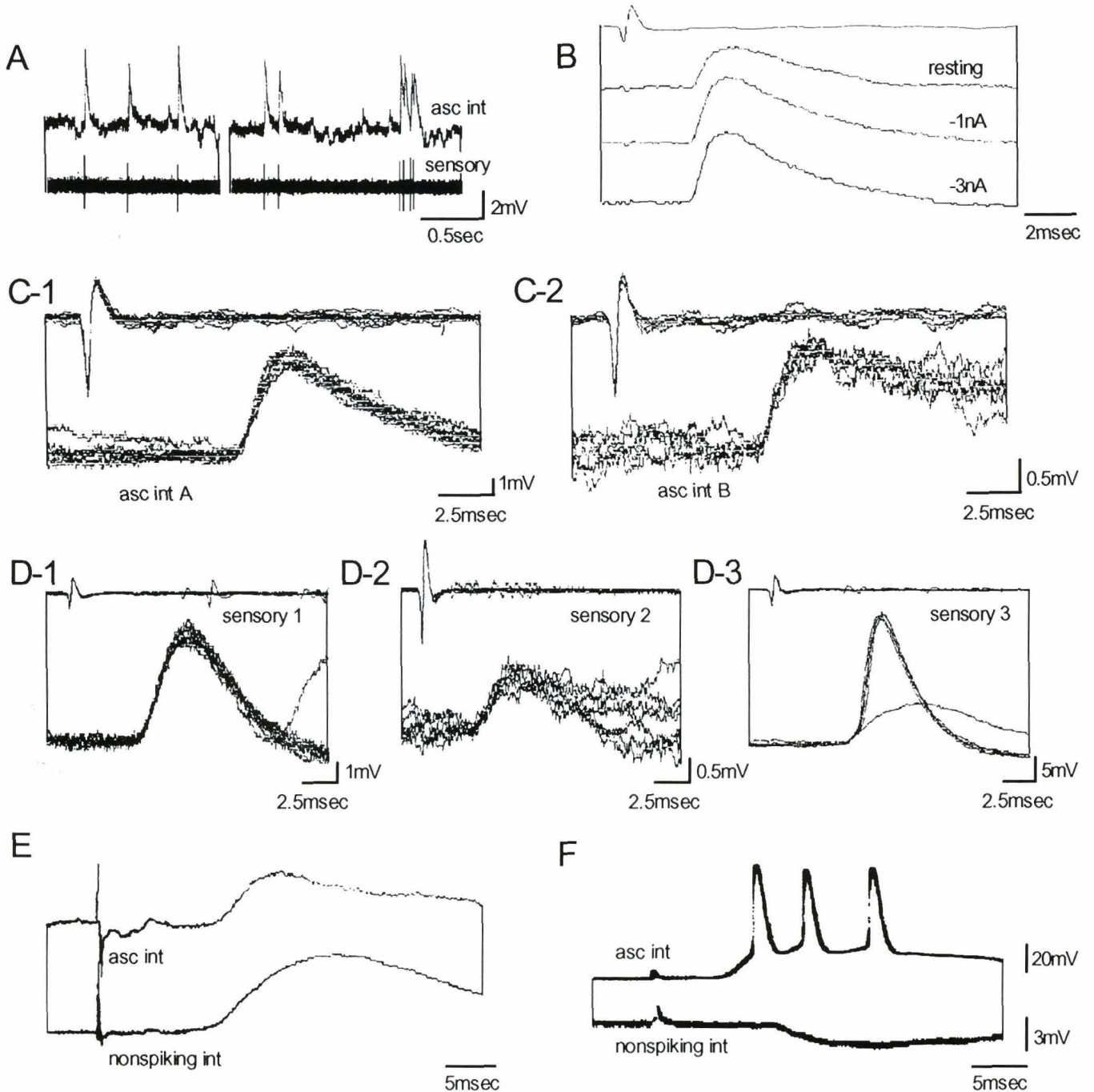


Fig. 5. Sensory processing of local circuit neurones. **A.** Response of ascending interneurone to hair stimulation. Each spike of hair afferent elicits discrete EPSP in the interneurone. **B.** Chemical nature of transmission. The amplitude of the EPSPs is increased by hyperpolarizing current. Each record is an average of 256 sweeps. **C.** Divergence of connections from a single sensory afferent to ascending interneurones. **D.** Convergence of inputs from several sensory afferents onto single ascending interneurone. **E.** Electrical stimulation of sensory nerve elicits EPSPs with the same latency in both the ascending and nonspiking interneurones. Records are an average of 64 sweeps. **F.** Electrical stimulation of sensory nerve elicits EPSP and spikes in the ascending interneurone and a long latency IPSP in another nonspiking interneurone. A-D from Nagayama and Sato, 1993.

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each side.

Sensory processing of interneurons

The movement of the uropods in response to sensory stimulation of the tailfan must be mediated through interactions among the above three groups of interneurons. To build up the pattern of connections between these interneurons, and to clarify the functional role of each group of interneurons, through the initial step to detect sensory inputs and the final step to organize motor outputs, are the next goals of this neuroethological approach.

Many thousands of mechanosensory afferents innervating the hairs on the surface of the tailfan detect waterborne movement or mechanical contact, and transmit exteroceptive information to the terminal abdominal ganglion (Calabrese, 1976; Wiese, 1976). Furthermore, the movement of the uropods itself is detected by the chordotonal organs located peripherally (Field et al., 1990) and is encoded by proprioceptive afferents (Nagayama and Newland, 1993; Newland and Nagayama, 1993).

Spikes of single afferents can be elicited by the localized stimulation of mechanosensory hairs. The responses of interneurons, especially those of the

ascending and spiking local interneurons to single afferent spikes can be analyzed in detail (Nagayama and Sato, 1993; Nagayama et al., 1993b). Each afferent spike is consistently followed in a one-to-one fashion by an excitatory postsynaptic potential in an interneuron (Fig. 5A). The amplitude of the EPSP can be increased by the injection of hyperpolarizing current into the interneuron (Fig. 5B). The central delay between the afferent spikes and the onset of the EPSP in the interneurons is generally between 0.7 and 1.5 msec. These physiological results indicate that these spiking interneurons receive exteroceptive inputs directly from the afferents. In other words, sensory afferents make chemically-mediated excitatory synapses with these interneurons. In general, each sensory afferent makes divergent outputs onto several interneurons (Fig. 5C) and each interneuron receives convergent inputs from several sensory afferents (Fig. 5D). In insect sensory systems, such as cricket cercal system or locust leg mechanosensory system, the organization of exteroceptive information has been analyzed in great detail (Bacon and Murphey, 1984; Burrows, 1992). Each elementary interneuron has its own specific receptive field by receiving selective direct excitatory inputs, and sometimes also inhibitory inputs via certain intercalating interneurons (e.g., Nagayama, 1989, 1990). Further studies are needed to

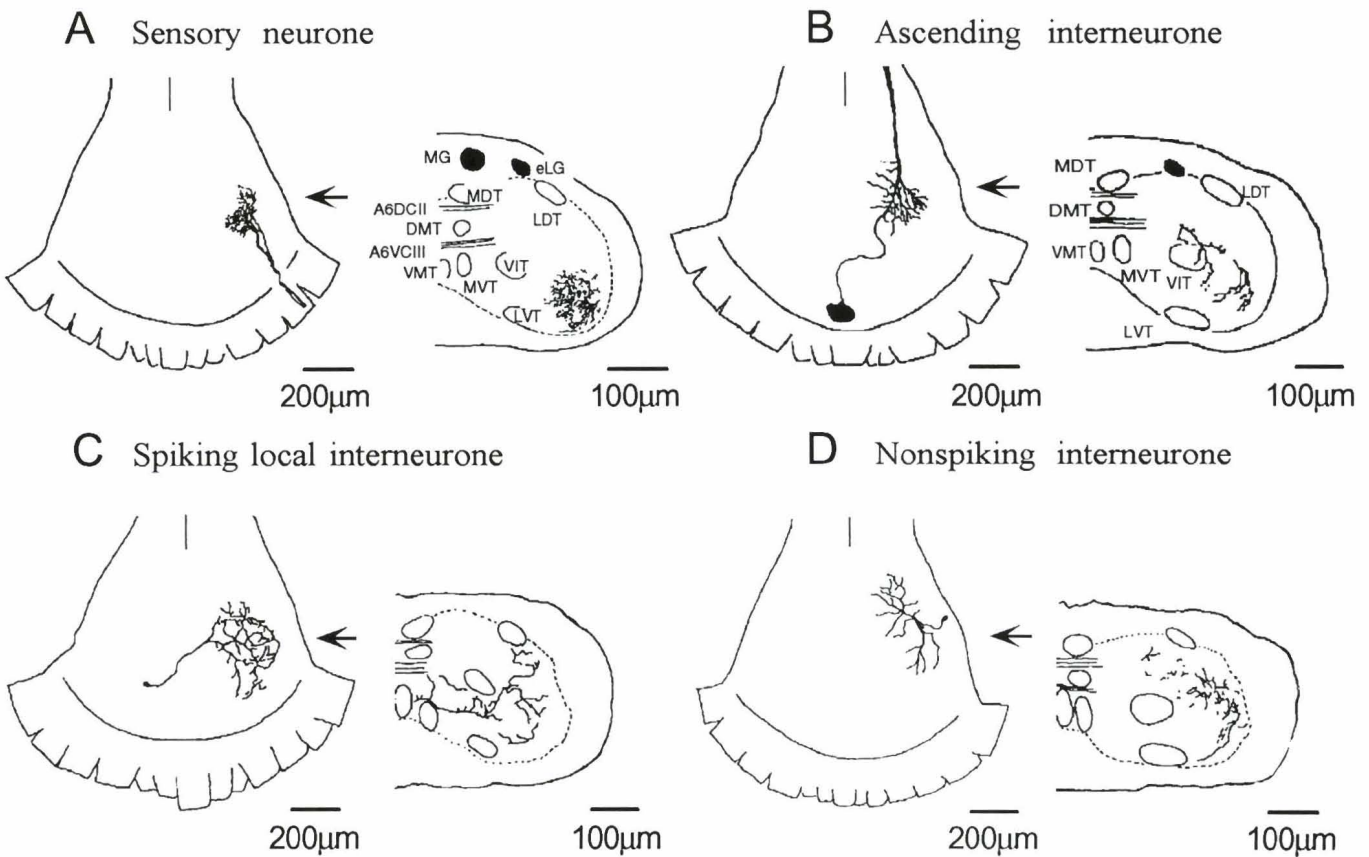


Fig. 6. Cental projections of hair afferents (A) ascending interneurone NE-1 (B), spiking local interneurone sp-pos2 (C) and nonspiking interneurone AL-I (D). Each drawing of transverse sections at the plane indicated by an arrow is a composite of 3-6 adjacent 10 μ m sections.

examine the detailed receptive fields of crayfish interneurones.

An examination of the connections from single afferents to nonspiking interneurones is now undertaken. Preliminary studies comparing the responses with ascending interneurones to the electrical stimulation of sensory nerves suggest the presence of monosynaptic connections between the sensory afferents and nonspiking interneurones, since the EPSP of some nonspiking interneurones is elicited almost simultaneously with the EPSP of an ascending interneurone (Fig. 5E). For the nonspiking interneurones which receive IPSPs, the start of membrane hyperpolarization is considerably slower (more than 3 msec) than that of the EPSP in the ascending interneurone (Fig. 5F). Thus, inhibitory inputs to nonspiking interneurones must be mediated via certain local circuit neurones.

These physiological results are supported by the anatomical observations shown in Figure 6. Several afferents innervating hairs on the surface of the

exopodite are stained sequentially (Fig. 6A). The anterior branches at the plane indicated with an arrow terminate in a ventro-lateral region of neuropil, below the level of the ventral intermediate tract (VIT). The ascending interneurone (Fig. 6B), spiking local interneurone (Fig. 6C) and nonspiking interneurone (Fig. 6D), which all receive excitatory inputs from the afferents, extend branches into the same area of the ganglion. The nonspiking interneurones that receive inhibitory inputs have no branches projecting to the most ventral region of neuropil (not shown).

Motor organization of nonspiking interneurones

Though each type of interneurones can affect the activity of the uropod motor neurones, the efficiency of the premotor effect of both the ascending and spiking local interneurones is considerably less than that of the nonspiking interneurones. To elicit a noticeable change in the activity of the motor neurones, a high-frequency

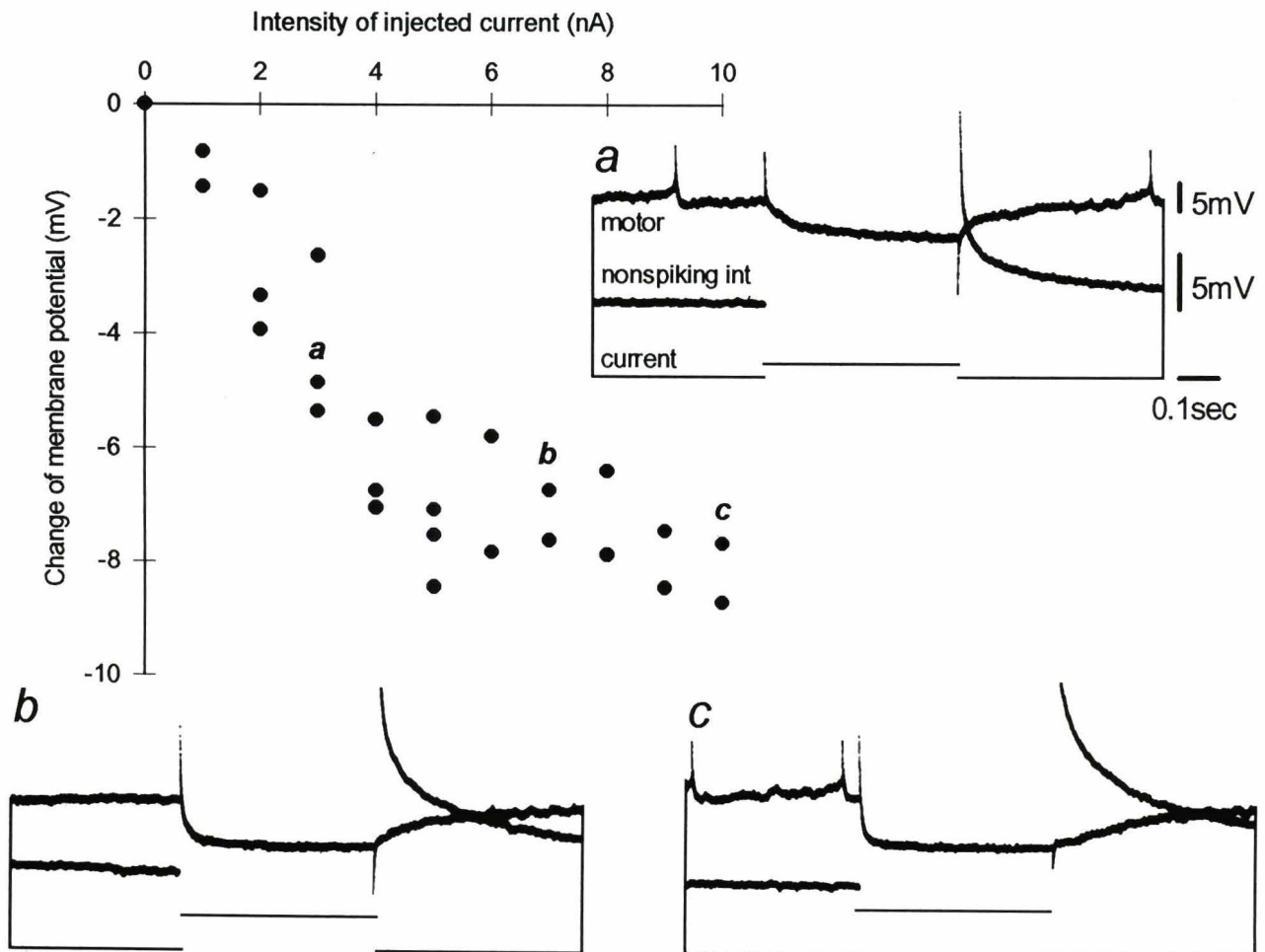


Fig. 7. Pre-motor effect of nonspiking interneurone with a graded and sustained manner. A passage of depolarizing current into the nonspiking interneurone (3nA in *a*, 7nA in *b*, and 10 nA in *c*) elicits sustained membrane hyperpolarization of the motor neurone. Inset shows relationship between the amplitude of membrane hyperpolarization of the motor neurone and the intensity of depolarizing current injected into the nonspiking interneurone.

train of spikes is required for both the ascending and spiking local interneurons (Nagayama et al., 1993a,b). On the other hand, small changes in the membrane potential of the nonspiking interneurons are sufficient for generating large changes in the activity of the motor neurones (Nagayama et al., 1984). For example, a membrane depolarization of the nonspiking interneurone by the passage of a weak depolarizing current is enough to cause a sustained change in the membrane potential (in this case, hyperpolarization) of the motor neurone (Fig. 7a). Injecting more current into the nonspiking

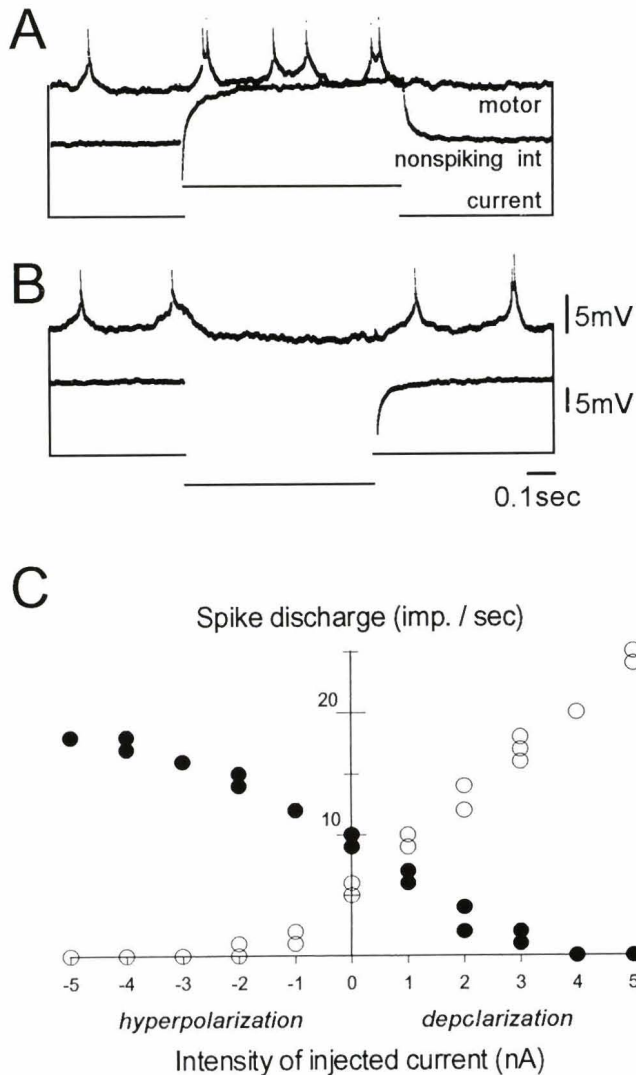


Fig. 8. Bidirectional effect of nonspiking interneurone. **A.** A passage of depolarizing current into the nonspiking interneurone excites the motor neurone. **B.** A passage of hyperpolarizing current into the same nonspiking interneurone suppresses the tonic spikes of the motor neurone with a sustained membrane hyperpolarization. **C.** Relationship between the rate of discharge of the motor neurone and the intensity of current into nonspiking interneurons. Open circle: interneurone with noninverting connection; Filled circle: interneurone with inverting connection.

interneurone elicits a progressively greater change in the membrane hyperpolarization of the motor neurone (Fig. 7b,c). The pre-motor effects of the nonspiking interneurons are mediated by the sustained and graded release of chemical transmitter. This manner of synaptic interaction makes it possible to exert a fine control over the postsynaptic motor neurones.

Some nonspiking interneurons, for example the PL groups, release chemical transmitter continuously at their resting potential, so that changes in the membrane of either polarity can affect the motor neurones (Burrows and Siegler, 1978; Heitler and Pearson, 1980; Nagayama et al., 1984; Nagayama and Hisada, 1987; Paul and Mulloney, 1985; Namba et al., 1994). For example, the passage of depolarizing current into the nonspiking interneurone shown in Figure 8A causes a membrane depolarization of a motor neurone with an increase in spike frequency. In contrast, when hyperpolarizing current is injected, the motor neurone shows a sustained membrane hyperpolarizing with a suppression of the tonic spikes (Fig. 8B). Thus, this interneurone has bidirectional effects upon the motor neurone. According to the output properties of nonspiking interneurons, connections of nonspiking interneurons onto motor neurones can be defined as one of either non-inverting (open circles in Fig. 8C) or inverting (filled circles in Fig. 8C), depending on whether the polarity of membrane potential change of motor neurones is inverted or not. The effect upon the motor neurones is linearly related to the intensity and polarity of current injected into the nonspiking interneurons (Fig. 8C).

Although unilateral nonspiking interneurons have strong postsynaptic effects upon the uropod motor neurones, the bilateral nonspiking interneurone, LDS, has no effect upon the same motor neurones. LDS receives excitatory mechanosensory inputs exclusively from its soma side and has inhibitory outputs on certain ascending interneurons on the opposite side (Reichert et al., 1983). Figure 9A shows the morphology of both LDS and the ascending interneurone RO-7 revealed by simultaneous intracellular staining. RO-7 has no effect upon LDS. In contrast, depolarizing current injected into LDS causes and inhibition of the spikes of RO-7 (Fig. 9B). The higher the intensity of current injected into LDS, the greater the inhibitory effect upon RO-7. This inhibitory interaction between LDS and RO-7 is mediated by chemical synaptic transmission (Fig. 9C). Depolarizing current into RO-7 increases the membrane hyperpolarization (Fig. 9C-d). On the other hand, the passage of 1 nA hyperpolarizing current into RO-7 decreases the membrane hyperpolarization (Fig. 9C-c). Injection of further hyperpolarizing current reverses the membrane potential change (Fig. 9C-a,b). Since LDS receives EPSP of large amplitude (more than 10 mV) in response to sensory stimulation on the soma side, LDS is sufficient to mediate lateral inhibition of ascending interneurons without generating spikes.

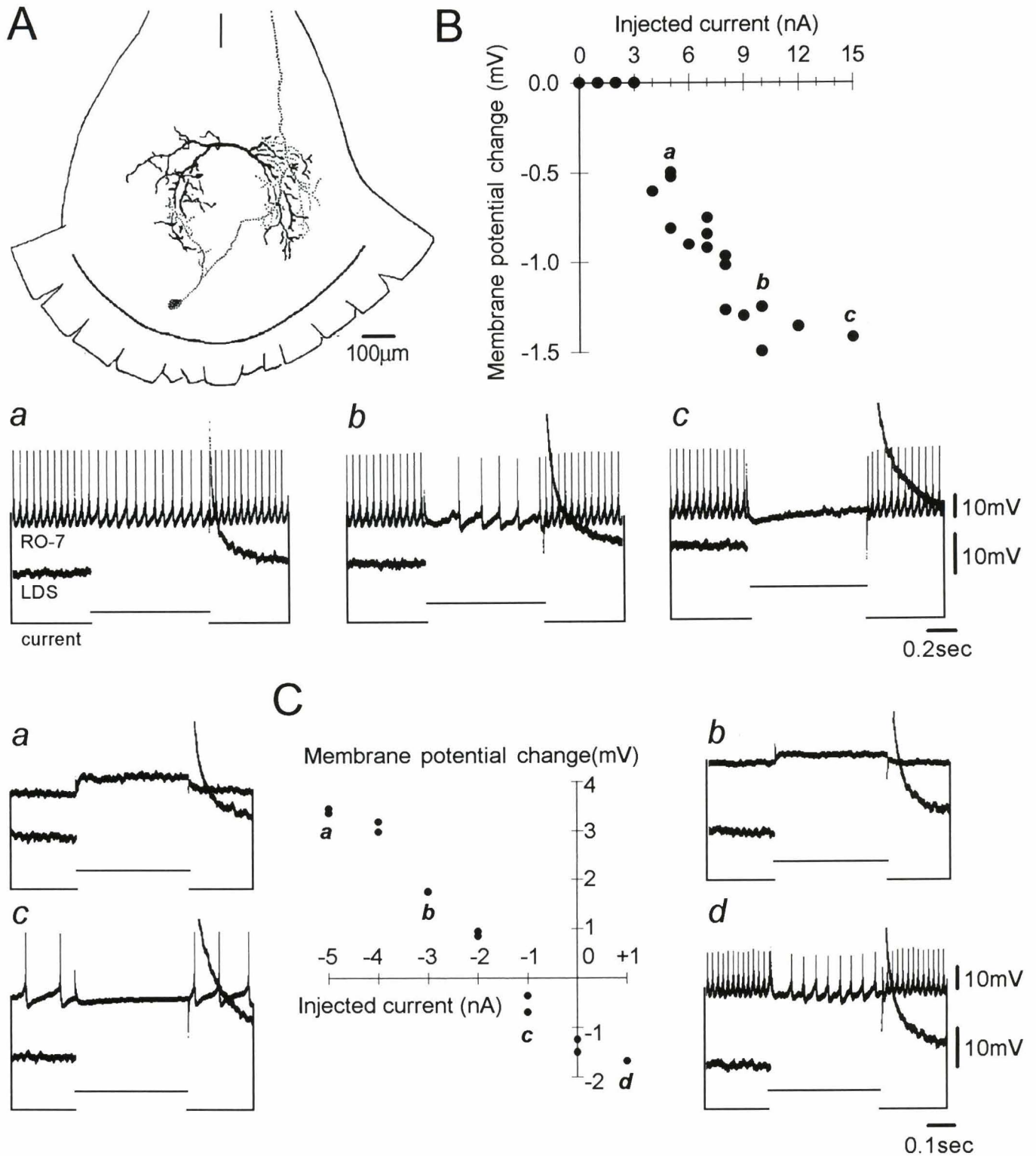


Fig. 9. Inhibitory effect of LDS upon the ascending interneurone RO-7. **A.** Gross morphology of presynaptic LDS (solid lines) and postsynaptic RO-7 (stippled lines). **B.** Graded inhibitory output of LDS. Depolarization of LDS (5nA in *a*, 10nA in *b*, and 15 nA in *c*) inhibits the tonic spikes of RO-7. Membrane of RO-7 is gradually hyperpolarized depending on the intensity of current injected into LDS. **C.** Chemical nature of transmission. The amplitude of membrane hyperpolarization of RO-7 is increased by depolarizing current, and is decreased by weak hyperpolarizing current and is reversed by further hyperpolarizing current. (-5nA in *a*, -3nA in *b*, -1nA in *c*, and +1nA in *d*).

Crayfish local circuit neurones

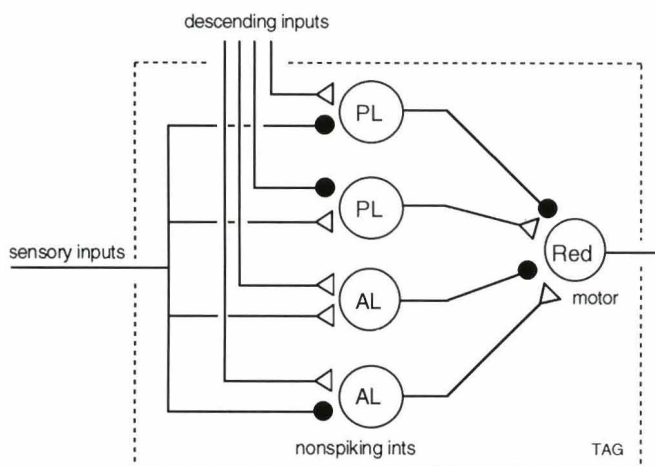


Fig. 10. Schematic neural circuit for the processing of sensory and descending inputs by two subgroups of nonspiking interneurons. Open triangles represent excitatory connections and filled circles represent inhibitory connections (based on Namba et al., 1994).

Parallel channels of local circuit neurones

These studies have shown that: (1) both the ascending and spiking local interneurons are usually silent at resting potential and mainly receive direct excitatory inputs from the sensory afferents; (2) their pre-motor effects upon the uropod motor neurones are not so strong when compared to those of nonspiking interneurons; and (3) spiking local interneurons of the locust methathoracic ganglion are thought to be primary sensory integrators for the leg adjustment system (Siegler and Burrows, 1983; Burrows, 1985). The spiking interneurons in the terminal ganglion of the crayfish must monitor and integrate exteroceptive sensory information. On the other hand, (1) many nonspiking interneurons receive inhibitory inputs from the sensory afferents with a long delay and (2) they can exert fine control over the wide range of activity of the motor neurones, and (3) some are tonically active at the resting level and can control the excitability of motor neurones in a continuous and graded manner. The nonspiking interneurons of the crayfish must therefore play a major role in motor organization. These distributed pathways through both digital and analog processing are another essential neural circuit for leg adjustment of the locust (Burrows, 1992).

The opposing parallel pathway through the two complementary groups of nonspiking interneurons, the AL and PL interneurons, in the reflex circuit (Fig. 10) is essential to modulate the motor pattern. The PL interneurons increase the activity of the closer motor neurones through either excitatory or disinhibitory pathway when they receive sensory input, while the pathways that include the AL interneurons decrease the activity of the same motor neurone through either disfacilitatory or inhibitory pathway (Nagayama and Hisada, 1987; Namba et al., 1994). Since the uropod

is closed during sensory stimulation, the pathways involving PL interneurons must be more dominant than the pathways involving AL interneurons. This hypothesis is supported by the observations that many PL interneurons of the disinhibitory pathway are tonically active to release transmitter continuously while the AL interneurons of the disfacilitatory pathway are usually inactive at resting posture. Even if these interneurons receive sensory inputs in the form of IPSPs, the AL interneurons are ineffective in affecting the motor neurones, but the PL interneurons can excite the motor neurone by disinhibition. At first sight, it is unnecessary that the AL interneurons should be included in this circuit. However, if other synaptic inputs converge onto nonspiking interneurons, and the level of activity between PL and AL interneurons is changed in advance, the pattern of the activity of the uropod motor neurones elicited by sensory inputs would be changed considerably. The performance of a particular behavioural act is often modulated depending upon the ongoing external inputs and/or the animal's own behavioural context (Rossignol et al., 1981; Skorupski and Sillar, 1986; Beall et al., 1990). In the avoidance reaction of the crayfish, stimulation of the exopodite fails to elicit any obvious responses when the crayfish shows defensive posture or active swimming with a strong extension of its abdomen (Nagayama et al., 1986). Abdominal extension is elicited by descending command fibres. Although nonspiking interneurons of both the AL and PL groups receive these descending inputs, the sign of inputs from both the sensory and descending signals to each subgroup of nonspiking interneurons is frequently opposed to neutralize their pre-motor effects (Fig. 10). This design of neural circuit, with opposing and parallel processing, has been recently demonstrated in many arthropods and vertebrates (Nagayama and Burrows, 1990; Appenteng, 1991; Büschges and Schmitz, 1991; Skorupski, 1992) and may represent a possible neural basis of behavioural flexibility. At present, each neural element subserving the avoidance «dart» response has been catalogued both morphologically and physiologically, thus preparing the way for the next step of research in constructing a detailed map of the local circuit. Further analyses to reveal the interactions between spiking and nonspiking interneurons will provide further insights into neural principles controlling animal behaviour.

Acknowledgements. We gratefully acknowledge support towards our work from Grants-in-Aid from the Ministry of Education, Science and Culture, Special Grant-in-Aid for Promotion of Education and Science in Hokkaido University provided by the Ministry of Education, Science and Culture, and Nissan Science Foundation to TN. We are grateful to Dr. P.L. Newland for his critical reading of this manuscript. We would also like to thank our colleagues and Profs. M. Hisada and M. Takahata for their support and comments on this work.

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