The Journal of Experimental Biology 210, 2199-2211
Published by The Company of Biologists 2007
doi:10.1242/jeb.002865

Behavioral context-dependent modulation of descending statocyst pathways during free walking, as revealed by optical telemetry in crayfish

N. Hama[1,*], Y. Tsuchida[2] and M. Takahata[1]

[1]*Animal Behavior and Intelligence, Division of Biological Science, Graduate School of Science and*
[2]*Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0810, Japan*

*Author for correspondence (e-mail: hnori@sci.hokudai.ac.jp)

Accepted 28 March 2007

Summary

Crustacean posture control is based on a complex interaction between the statocyst input and other sensory inputs as well as the animal's behavioral context. We examined the effects of behavioral condition on the activity of descending statocyst pathways using an optical telemetry system that allowed underwater recording of neuronal signals from freely behaving crayfish. A functionally identified statocyst-driven interneuron that directionally responded to body tilting without a footboard and to tilting of the footboard was found to show complicated responses depending upon the ongoing behavior of the animal when it freely walked around in water on the aquarium floor. The spike firing frequency of the interneuron increased significantly during walking. When the animal stood or walked on the tilted floor, the interneuron activity represented the tilt angle and direction if the abdomen was actively flexed, but not if it was extended. Two other statocyst-driven descending interneurons were found to be affected differently by the animal's behavioral condition: the spike activity of one interneuron increased during walking, but its directional response on the tilted floor was completely absent during abdominal posture movements, whereas that of another interneuron was enhanced during abdominal extension only, representing the tilt angle and direction. The results obtained in this study provide the first experimental demonstration that crustacean postural control under natural conditions is dependent on very fine aspects of the animal's locomotor behavioral context, suggesting far more complex control mechanisms than those expected from the experimental data obtained in isolated and fixed animals.

Key words: crayfish, statocyst, posture control, behavioral context, optical telemetry.

Introduction

Equilibrium responses for compensating and restoring the original body posture can be elicited independently by sensory signals of different modalities, i.e. equilibrium, somatosensory and visual inputs. The posture control in natural surroundings is ultimately based on a complex interaction between these inputs (Horak and Macpherson, 1996; Furudate et al., 1996). Furthermore, it has been shown in many animals, including vertebrates and invertebrates, that the activity of central neurons involved in postural control varies significantly depending on the animal's behavioral condition when the sensory stimuli are applied (Deliagina et al., 2000; Takahata and Murayama, 1992). The behavioral context-dependent sensory integration and sensori-motor signal transmission are not specific to postural control but quite general to the control of animal behavior (Delcomyn, 1998; Seki et al., 2003).

The variability in the central neuron responses to sensory stimuli has generally been studied by making physiological recordings from unanesthetized whole-animal preparations performing specific behavior that was restricted to the least extent. In lamprey, for example, extracellular electrodes were chronically implanted into the spinal cord to analyze the descending activity of the reticulospinal tract during restricted swimming (Deliagina et al., 2000). Intracellular recording and staining techniques were applied to crickets that were fixed for recording but could walk on a sphere-type treadmill while different kinds of auditory stimuli were present (Schildberger and Hörner, 1988). These studies revealed that the sensory responses of central neurons vary significantly depending on the animal's behavioral context. In crickets the gating of sensory responses of higher-order interneurons depends on the animal's walking activity, and it was concluded (Staudacher and Schildberger, 1998) that significant information about the properties of sensory processing in central neurons can only be gained from experiments in behaviorally relevant paradigms.

In crayfish, recent studies using intracellular techniques applied to unanesthetized whole-animal preparations have revealed that the control of body and appendage posture depends not only on the complex interaction between statocyst, visual and leg proprioceptor inputs (Okada and Yamaguchi,

1988; Okada et al., 1994; Furudate et al., 1996) but also on the behavioral condition of the animal (Murayama and Takahata, 1998a; Murayama and Takahata, 1998b; Hama and Takahata, 2003; Hama and Takahata, 2005). Since the recording in the behavioral experiments was made from fixed animals on a belt-driven treadmill or a movable substratum, however, it remains untested how the response characteristics of statocyst interneurons are affected by locomotor behavior in which postural control becomes most important for the animal.

One approach to the analysis of neuronal activities in freely behaving animals is telemetry, i.e. wireless transmission of neural signals from an animal to the oscilloscope and other recording devices (Fisher et al., 1996; Kudo et al., 1999; Ando et al., 2002). Whether the recording is made extracellularly from an animal tethered by chronically wired electrodes or intracellularly from an animal fixed on a treadmill to allow mimicked walking, the behavior of such animals has to be restricted to some extent. Valuable information on the neural activity in freely behaving crayfish can certainly be obtained by chronic wired recordings (e.g. Le Ray et al., 2005). There remains, however, a possibility of accidentally or inadvertently hampering the animal's intended behavior. The telemetry system, in particular the transmission device, has its own drawbacks, among which its volume and weight are the most critical, since they can potentially hinder the intended behavior of the animal. However, under telemetric recording, the animal can perform a far wider repertoire of behavior than in the tethered or on-treadmill condition. In the present study, we applied a newly developed optical telemetry system (Tsuchida et al., 2004) to study crayfish behaving freely on the tilted floor of a water-filled aquarium in order to analyze the activity of descending statocyst-motor pathways in different sensory and behavioral contexts.

Materials and methods

Animals and preparation

Experiments were carried out on male crayfish, *Procambarus clarkii* Girard (10–12 cm body length, 25–29 g mass), which were commercially obtained (ME Suisan, Miyagi, Japan) and kept in laboratory tanks. Before surgery, each animal was anesthetized in cooled water. A small portion of the dorsal carapace (5 mm×5 mm) was removed and then the left or right gastric muscle 1 (Katz and Tazaki, 1992) was cut away to reveal the circumesophageal commissures. The activity of statocyst-driven units was recorded from the left or right circumesophageal commissure. The extracellular electrodes, made from a pair of Teflon^{TM}-coated silver wires (125 μm in diameter) and hook-shaped polyethylene tubes, were placed at area 74 of the circumesophageal commissure (Wiersma, 1958), where the axon of interneuron C_1 is reported to be located (Takahata and Hisada, 1982), and fixed to the dorsal carapace by adhesive. The electrodes were then insulated using Vaseline^{TM}. To confirm that the spike activity of any statocyst-driven unit was recorded, the animal was manually tilted in the pitch and roll planes. After confirmation of the tilt-dependent spike activity, the removed cuticle was put back on the carapace again and fixed by adhesive. We could confirm that the tip of the recording electrode was staying at or near its original position during the experiment by examining the amplitude of the functionally identified unit on the oscilloscope. The experimental animal was rested for more than 1 h in water. Electromyographic (EMG) recordings were made from the mero-carpopodite flexor muscle of the second walking leg by anatomically placing a pair of Teflon^{TM}-coated silver wires (125 μm in diameter) on the muscle.

Optical telemetry and video recording

We used a dual-channel transmitter for underwater recording from freely behaving animals. The specifications of the transmitter and the methodological details have been described in a previous paper (Tsuchida et al., 2004). Briefly, the amplitude of electrical signals recorded from the animal was modulated to pulse duration using the pulse duration modulation (PDM) method, and then the PDM signal was further modulated to intervals of short pulses (2 or 4 μs duration) using the pulse interval modulation (PIM) method. The PIM signal drove an infrared emission diode to transmit neural information to four underwater receivers that de-modulated the infrared signals back to neuronal signals (Fig. 1A). De-modulated analog signals, i.e. the spike activity from the circumesophageal commissure and the leg muscle, were stored in a digital audio tape recorder (RD-135T; TEAC, Tokyo, Japan; DC-10 kHz) for later analyses. In some experiments, the recording was made in the air. The telemetry system could reliably transmit signals in those experiments.

Crayfish mounted with a transmitter and electrodes could freely walk in the experimental aquarium (40 cm width×60 cm depth×40 cm height) filled with water to a depth of 15 cm (Fig. 1A). The floor of the experimental aquarium was tilted by 10°. When the animal walked on it, the body was tilted depending on the orientation of the animal body in relation to the tilt direction of the floor. PIN (p-intrinsic-n)-photodiode receivers were placed at each corner of the square (30 cm×30 cm) shown in Fig. 1A. In this condition, stable recording could be obtained in the dark-blue square region (40 cm×40 cm). The behavior of crayfish was simultaneously video-recorded from above using a digital video camera (DCR-TRV9, Sony, Tokyo, Japan; 30 frames s^{-1}). The area covered by the camera image was 40 cm × 60 cm (Fig. 1B). Experiments were carried out in the dark, but the position and head direction of the animal were clearly determined. Video recording of the animal behavior from above was always done with periodically flashing, short-duration (100 ms) light signals (green LED) at 0.5 Hz. The electrical signals for controlling these light flashes were recorded together with the neural and muscle signals for synchronizing video data with electrophysiological ones.

Validity of telemetric recording

We reported that the newly developed optical telemetry system can reliably transmit neural signals within a rectangle (30 cm × 40 cm) in freshwater (Tsuchida et al., 2004). The

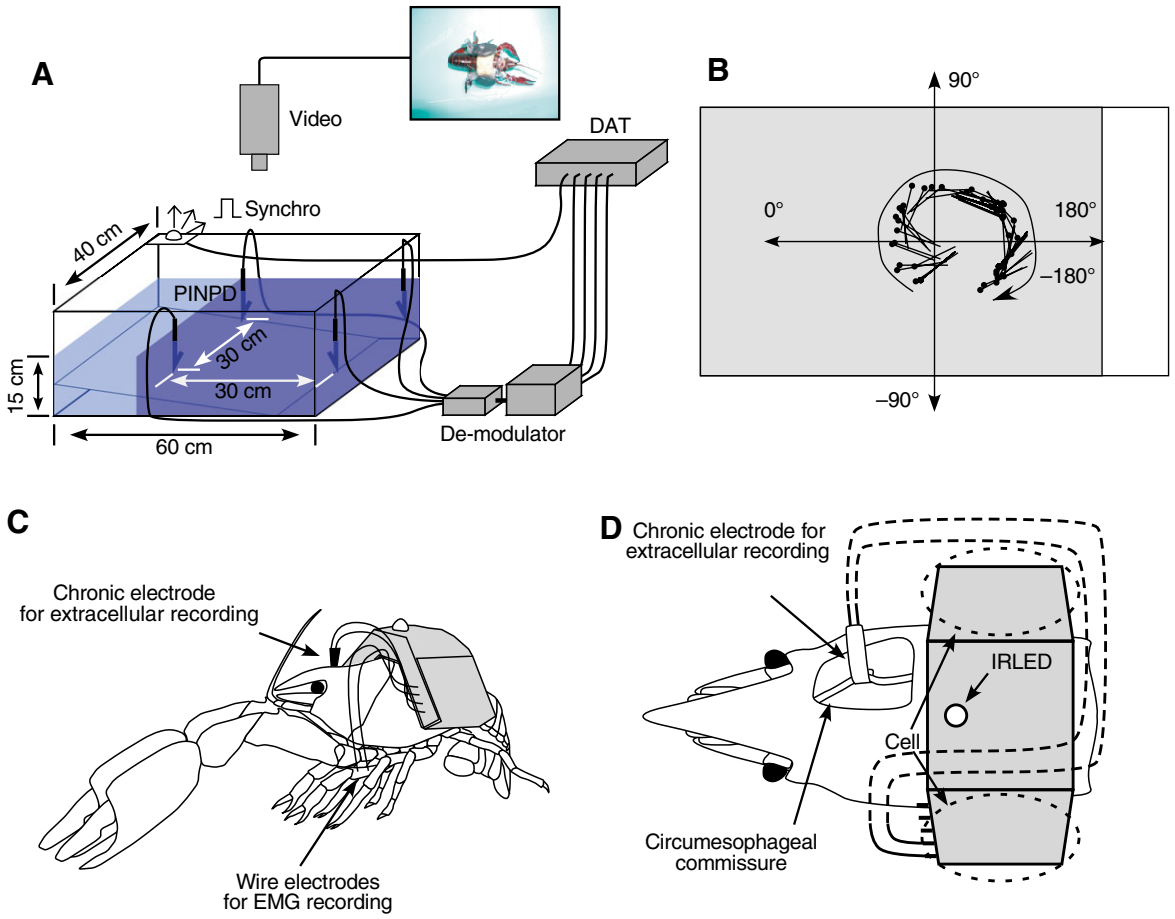

Fig. 1. Experimental set-up. (A) Arrangement of the telemetry and video-recording systems. The crayfish was placed in an experimental aquarium filled with water to a depth of 15 cm. The floor was tilted by 10°. Four PIN-photodiodes (PINPDs) as receivers were placed at the corners of a square of 30 cm×30 cm. An LED was driven by synchronous signals that were simultaneously fed, together with nerve and EMG signals, to a DAT recorder. Due to the optical signal decay along the distance from the transmitter, the recording was reliable only in a limited area. The area illustrated in dark blue indicates the area in which the telemetric transmission was secure. The light blue indicates water in the aquarium. (B) The angular coordinate adopted in the present study to describe the direction of animal body orientation. The head direction and behavior of crayfish were video-recorded from above. (C) The optical transmitter mounted on the animal. Wire electrodes for electromyographic (EMG) recording from the mero-carpopodite flexor muscle and the chronic electrode for extracellular recording from the circumesophageal commissure were connected to the dual-channel transmitter. (D) Schematic drawing of the chronic electrode for extracellular recording from the circumesophageal commissure. Wire connection from the electrode to the transmitter is shown by broken lines. The commissure was hooked up lightly by the electrode to secure the recording during free movements. Two broken ovals are lithium cells for the transmitter. They were omitted from the illustration in C for the sake of clarity. IRLED; infrared LED.

frequency range was narrower than in wired transmission so that small-sized spikes could hardly be discriminated in the wirelessly transmitted record due to its low signal-to-noise ratio. The system, however, had a wide-enough frequency range to transmit medium- and large-sized neuronal spikes recorded extracellularly in the present study. Hence, the problem we had to consider in applying the telemetry system to the current experiment was whether or not the transmitter and the electrode mounted on the animal (Fig. 1C,D) hampered its normal behavior. We video-recorded the free walking behavior of experimental animals with the transmitter mounted and unmounted on their back. Although we did not quantitatively analyze the walking behavior in this study, qualitative comparison of the walking behavior in the two conditions indicated that the mounted transmitter did not affect the animals' behavior in any serious way.

Data processing

Physiological data were digitized at 40 kHz using an A/D converter (PowerLab; ADInstruments, Colorado Springs, OH, USA) and software (Chart v.4.2; ADInstruments). All spikes were sorted depending on their amplitude and duration at the half amplitude (Schmidt, 1984) using additional software (Spike Histogram Extension for Chart v.4.2; ADInstruments). All spikes sorted automatically were manually re-sorted using our homemade software depending on their form. The position and head direction of the animal were plotted at the rate of 15 frames s^{-1} (0.5 s interval; Fig. 1B).

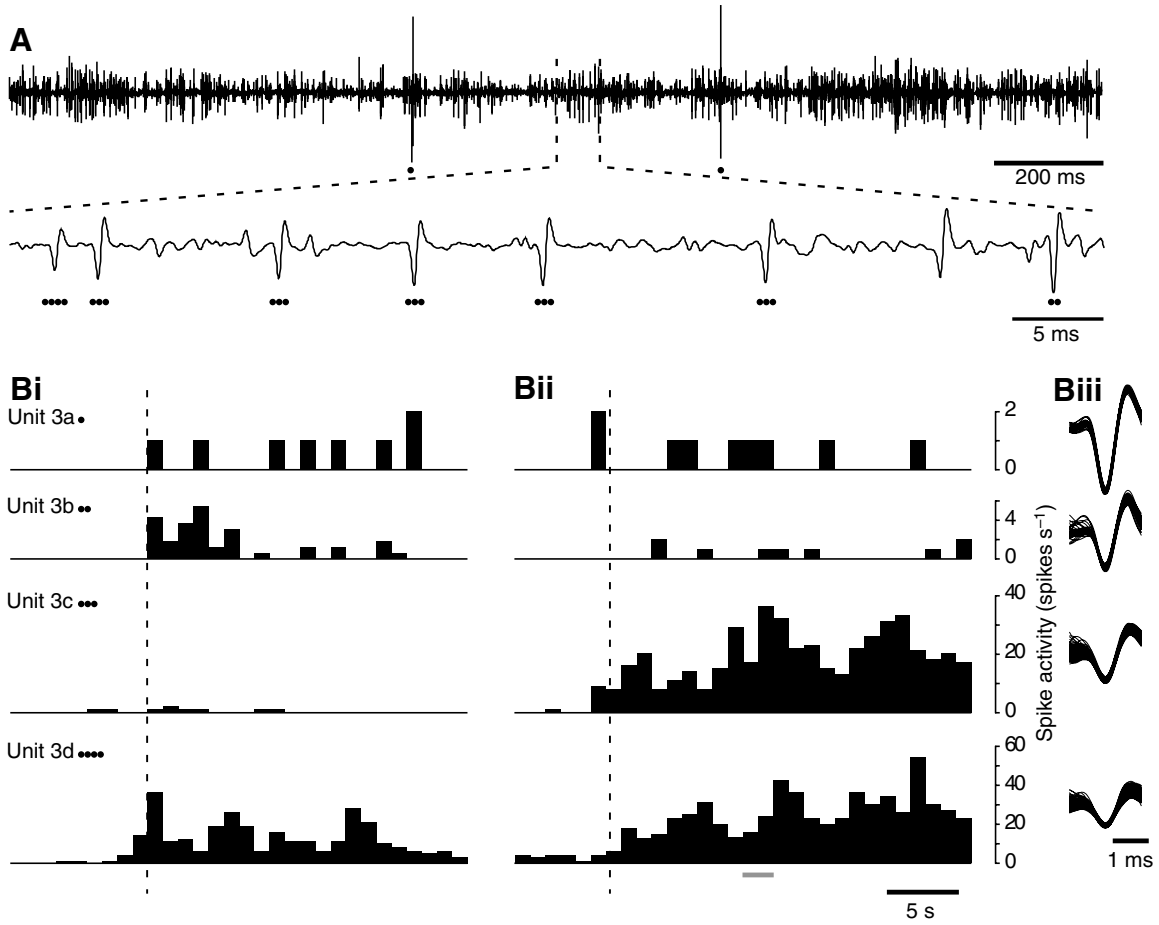

Fig. 2. Unit activities identified from extracellular recordings of the whole circumesophageal commissure using discriminating software. (A) Raw recording data during walking (upper trace); these data are partially expanded in the lower trace. Four units could be discriminated in this example, as indicated by four different symbols. (B) Activity of each unit during forward walking (i) and backward walking (ii). The histograms were based on 1 s time bins. Units 3a and 3b showed almost the same activity during forward and backward walking. Units 3c and 3d showed higher activities during backward walking than during forward walking. The vertical broken lines indicate the time of onset of walking behavior. The thick gray bar at the bottom indicates the expanded part of the data shown in A. The insets on the right (iii) show superimposition of each unit. They are expanded in the time scale as indicated but are the same size in the voltage scale as in A except that unit 3a was reduced to half of A in the voltage scale.

Results

We made successful unit recordings from more than 40 animals. Sixteen animals showed directional responses to body tilting in the air without a leg substratum, but only four of them showed sustained responses to body tilting during walking in the aquarium. Some animals responded only transiently to body tilting whereas others showed variable responses in the same behavioral context and tilted situation. Since our aim was to analyze the neural activity involved in the central mechanism of behavioral context-dependent posture control, for the present study we selected those four animals that reliably showed directional responses of statocyst-driven units during free walking. In one of them, the statocyst-driven unit became unresponsive during the experiment in the aquarium. We continued the experiment, however, to analyze the activity characteristics of non-statocyst units. The data obtained from the statocyst-driven unit of this animal in the early experimental stages were excluded from the current analyses.

Behavioral context-dependent unit activities

A total of 21 units, obtained from the selected four animals, were found to change their spike activity when they were engaged in locomotor behavior (Table 1). Four unit activities extracted from multi-unit recordings are illustrated in Fig. 2A. In two of them, named unit 3c and 3d, the activity during forward walking was different from that during backward walking (Fig. 2B): the spike discharge rate of unit 3c was 3.1±0.6 spikes s^{-1} (mean ± s.e.m.) during forward walking while it was significantly higher during backward walking (11.7±0.4 spikes s^{-1}; $P<0.01$ in the two-sided Mann–Whitney U-test). Unit 3d showed a similar tendency: the spike discharge rate was lower during forward walking (14.5±1.2 spikes s^{-1}) than during backward walking (18.7±0.5 spikes s^{-1}). Units 3a

Table 1. *Spike activities and responses to body titling of all units*

		Resting		Walking	
Unit no.	Response to body tilting	Extended/flexed	Resting/walking	Forward/backward	Extended/flexed
1a	No response	=	<<	<	=
1b	No response	=	<<	<<	<<
1c	No response	=	<<	=	>>
1d*	Ipsilateral side down/head-up	=	<<	<<	<<
1e	No response	=	<	<<	<<
1f	No response	>>	<<	=	=
2a	No response	=	<<	>>	>>
2b	No response	=	<<	=	>>
2c*	Ipsilateral side down/head-up	=	<<	<<	<<
2d	No response	=	<<	<<	<
2e	No response	<<	<<	<<	<
2f	No response	=	<<	<<	<<
3a	No response	<<	>>	=	>>
3b	No response	<<	>>	=	=
3c	No response	<<	<<	<<	<<
3d	No response	=	<<	<<	<<
4a**	Ipsilateral side down	<<	<<	<<	<<
4b***	Contralateral side down	<<	<<	<<	<<
4c	No response	=	<<	<<	<<
4d	No response	=	<<	<	<<
4e	No response	=	<	<<	<<

Activities of all units obtained from the four animals that showed reliable responses to body tilting and from another animal that showed reliable responses in the early stage of experiments but later became unreliable yet remained in the recording condition for analysis of non-statocyst units.

The third and fourth full columns summarize the activities during resting and walking, respectively. In the third column, the subcolumns compare the activity between abdominal extension and flexion and between resting and walking. In the fourth column, the subcolumns compare the activity between forward and backward walking and between abdominal extension and flexion. The symbols >> and << mean that the unit activity was significantly different in the respective direction (P<0.05; Mann–Whitney two-sided U-test). *C1 neuron, **Unit A, ***Unit B; <<, P<0.05, <, P<0.1.

Units 1d and 2c correspond to interneuron C_1 whereas units 4a and 4b were designated units A and B, respectively, in the text.

and 3b, in contrast, showed no significant change in their spike activity whether the animal walked forwards or backwards (Fig. 2B).

All four statocyst-driven units were found to change their spike activity depending on the animal's behavioral condition (Table 1). Their response characteristics are described in the following sections. Units 1d and 2c were judged to be the same unit on the basis of their responsiveness to the recorded-side-down and head-up tilting, their activity dependence on specific behavioral conditions and their relative amplitude in the multi-unit recording from the circumesophageal commissure.

Activity of an identifiable descending unit during body tilting

When the spike activity of the ventral nerve cord is recorded at the circumesophageal commissure, a medium-sized spike that is smaller in amplitude than the semi-giant spikes (Wilkens and Larimer, 1973) but characteristically larger than many other small units can be unambiguously recognized. This unit was designated interneuron C_1, and its directional responses to body tilting have been studied in detail under restricted conditions: it is characterized by tonic excitatory responses to head-up and recorded-side-down tilting in the air (Takahata and Hisada, 1982; Takahata and Hisada, 1985). In the present study, we first analyzed the response of this unit when the animal was engaged in locomotor behavior in unrestricted conditions. It showed a low level of spontaneous spike discharge in the upright body position when the animal was at rest. As reported earlier, the interneuron showed directional responses to body tilting in the air without a footboard or substratum (Fig. 3Ai). Thus, ipsilateral-side-down tilting (90°) increased the spike activity to 2.8±0.7 spikes s^{-1}, but the activity of interneuron C_1 was completely absent during contralateral-side-down tilting (Fig. 3Ai). Since only the statocyst was activated in this situation, the interneuron responses to body tilting were driven solely by the statocyst input.

Directional responses were also observed in the interneuron when the animal stood or walked on the substratum tilted by 10° (Fig. 3Bi). Compared with the firing rate observed when the animal was tilted in the air, the interneuron showed a higher firing rate (8.6±0.9 spikes s^{-1}) when the animal was on the substratum tilted in the same direction as in the body tilting in the air (Fig. 3Bii). Although the tilted angle of the substratum (10°) was smaller than that of the animal body in Fig. 3Bi, the firing rate was much greater. It should be noted here that the

experimental animal was not fixed to the upright body position nor to the substratum in this study. The animal therefore showed equilibrium reflexes of legs on the tilted substratum so that the animal body was less tilted than the substratum. Since the animal continuously changed its posture on the substratum, we could not measure the exact angle of body tilt on the substratum. The interneuron thus received a complex combination of statocyst and leg proprioceptor inputs during standing and walking on the tilted substratum. The larger response of the interneuron to substratum tilting than to body tilting suggested that the two modalities of sensory inputs would make facilitatory summation when the animal walks on the tilted substratum.

Activity of interneuron C_1 during resting and walking

We previously showed that the spontaneous spike activity and the sensory responses of many statocyst-driven descending interneurons were significantly modulated by free leg movements in the air, receiving central inputs from the locomotor center (Hama and Takahata, 2003). Interneuron C_1, however, was not affected by leg movements in the air (Fig. 4). When the animal was held upright in the air, the interneuron showed spontaneous spike discharges (0.4±0.4 spikes s^{-1}) (Fig. 3Ai and Fig. 4Bi). In response to body tilting in the ipsilateral-side-down direction, the interneuron increased its spike activity to 1.5±0.4 spikes s^{-1} (Fig. 4Ai). When the animal was engaged in free leg movements in the air, an increase was observed in muscle activity (upper trace in Fig. 4Aii) and in the spike activity of the circumesophageal commissure, but neither the spontaneous spike activity nor the directional response was affected (bottom trace in Fig. 4Aii). The animal body was kept tilted during the whole period of recording shown in Fig. 4Ai,ii. In one exceptional case, the interneuron showed an increase in the spontaneous spike discharge during active leg movements while the animal body was kept upright (Fig. 4Bii). We examined the interneuron C_1 activities associated with leg movements 39 times in two animals, and this exceptional activity was observed in only one case. Visual observation and EMG recording could not discriminate any difference in the behavioral condition between those two cases shown in

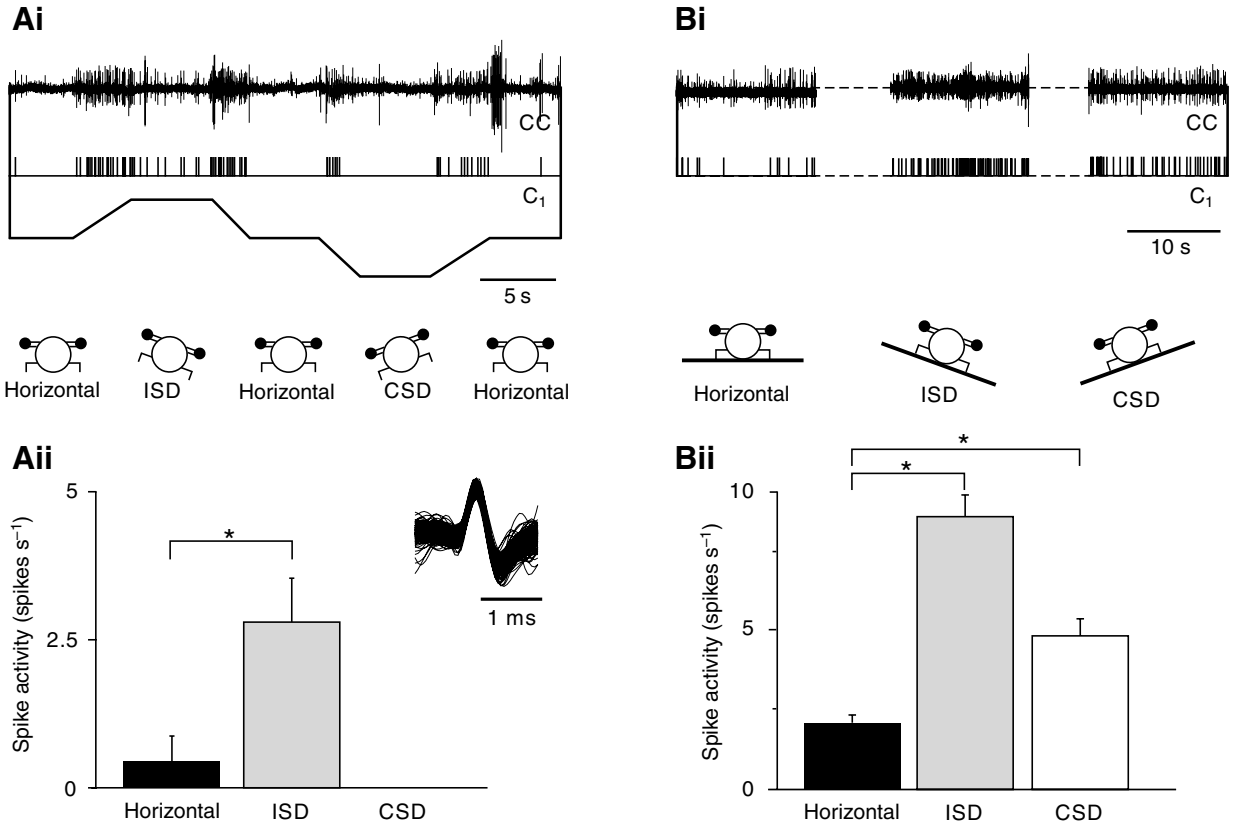

Fig. 3. Responses of interneuron C_1 to body and substratum tilting. (Ai) Body tilting by 90° in the air. The top trace shows the spike activity recorded extracellularly from the circumesophageal commissure (CC). The spike activity of interneuron C_1 was isolated electronically from the commissural recording and is shown in the middle trace. The bottom trace monitors animal body tilting, the upward and downward deflection indicating ipsilateral-side-down (ISD) and contralateral-side-down (CSD) tilting, in which the side of commissural recording was lowered and lifted, respectively. (Aii) Statistical comparison of interneuron activities between horizontal, ipsilaterally tilted and contralaterally tilted positions (*P<0.05; ANOVA). The inset shows superimposition of electronically isolated spikes in Ai. (Bi) Substratum tilting by 10°. The animal was standing on the tilted substratum. Since the animal was free to evoke postural reflexes, the exact angle of body tilt on the tilted substratum was unknown. The recording for each positioning was made intermittently whenever the freely behaving animal met the behavioral and orientation requirements. (Bii) Statistical comparison of the interneuron activities (*P<0.05; ANOVA).

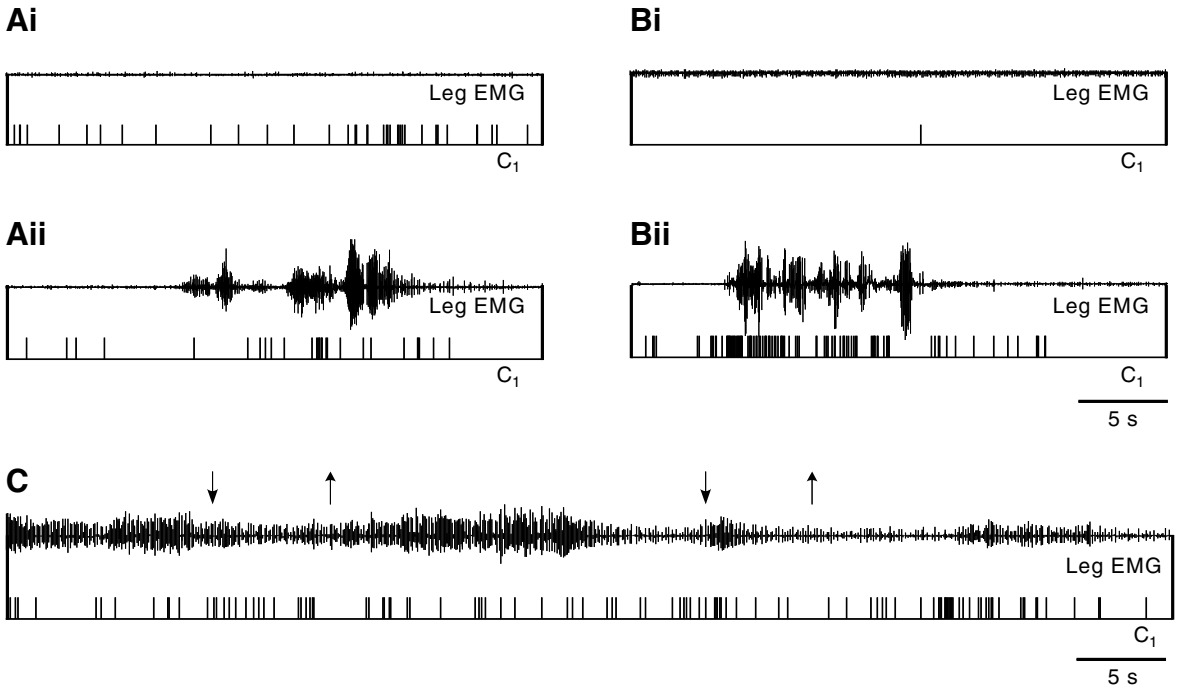

Fig. 4. Effects of leg and abdominal movements on interneuron C_1 activity. (A) Body tilting in the contralateral-side-down direction (i) without a leg substratum when the animal was at rest and (ii) with actively moving legs. The animal body was kept tilted during the recording shown in i and ii. In each part, the top trace monitors muscle activity whereas the bottom trace monitors interneuron C_1 activity. (B) An exceptional case in which the interneuron activity was affected by leg movements in the air. The spike activity during maintained tilt of the resting animal (i) was significantly enhanced when the animal actively moved its walking legs (ii). (C) Effects of abdominal posture movements on interneuron activity. Upward and downward arrows indicate the onset time of abdominal extension and flexion movements, respectively. No noticeable change was observed in interneuron activity during these movements.

Fig. 4Aii,Bii. The behavioral parameter that affected the interneuron activity during active leg movements in the upright body position therefore remains unknown. Our observation suggested that the interneuron activity was mostly invariable, independent of the animal's behavioral state, when the legs were maintained in the air without a substratum. Thus, the interneuron activity was not affected at all when the animal extended its abdomen endogenously in the air (downward arrows in Fig. 4C).

The leg and abdominal movements seen in the air and in the water on the aquarium floor are seldom observed in the locomotion of crayfish. In order to analyze the interneuron activity in the context of ongoing locomotor behavior under natural conditions, we applied the optical telemetry techniques to freely walking animals in the water. The spike activity of interneuron C_1 at the onset of and during endogenously initiated walking is illustrated in Fig. 5. When the animal was at rest on the substratum without sideward tilting, i.e. longitudinal axis of body directed to 0° or ±180°, few spike activities were observed in the circumesophageal commissure. The firing rate of the interneuron was also low (2.8±0.3 spikes s^{-1}; Fig. 5A). When the crayfish endogenously initiated walking on the substratum without sideward tilting (broken line in Fig. 5A), the spike activity of the circumesophageal commissure became high (top trace in Fig. 5A). This high level of spike activity resulted from increases in both ascending and descending spike discharges transmitting sensory and motor command signals, respectively. Interneuron C_1, which is one of the descending interneurons, also increased its spike activity and maintained a high discharge frequency during walking (bottom traces in Fig. 5A; Fig. 5B). Statistical analyses revealed that the interneuron showed a higher rate of spike discharge during walking on the substratum without sideward tilting than at rest (P<0.05; two-sided Mann–Whitney U-test). The spike activity of the interneuron during walking was not invariable, however, showing a certain degree of fluctuation since walking behavior activated internal and external mechanoreceptors of walking legs in a complex way.

Using intracellular recording and staining techniques, we previously showed that some statocyst-driven descending interneurons enhanced their responsiveness to statocyst input when the animal actively moved its walking legs on a substratum (Hama and Takahata, 2003). Since we could not identify interneuron C_1, it remained unknown how the interneuron activity was affected during walking. In the present study, the extracellular recordings from freely behaving animals revealed that the spike firing frequency of the interneuron was 6.6±0.7 spikes s^{-1} when the animal walked on the substratum tilted in the contralateral-side-down direction, while it was significantly greater (8.5±0.4 spikes s^{-1}) when the animal walked on the substratum tilted ipsilaterally (P<0.05; two-sided Mann–Whitney U-test). More detailed analyses revealed that the

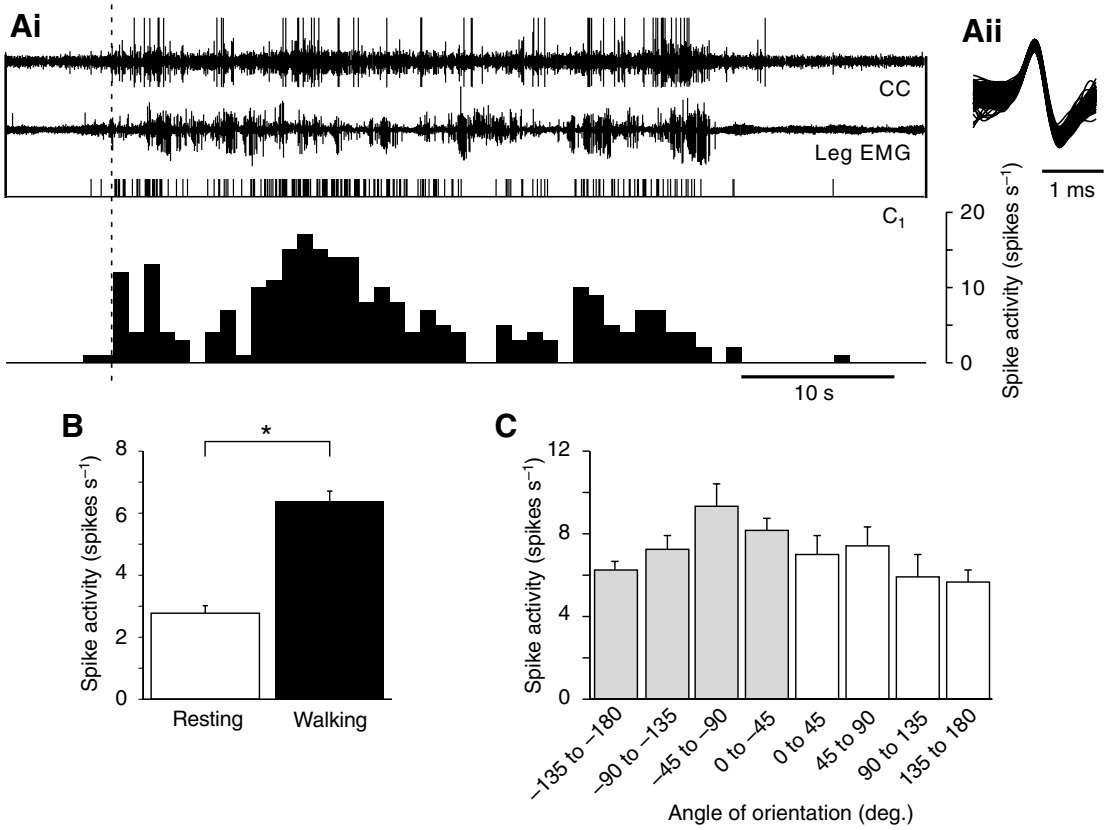

Fig. 5. Interneuron C_1 activity during free walking. (Ai) Recordings from an animal that spontaneously initiated walking (vertical broken line) on the horizontal floor of the aquarium. The top trace shows the extracellular recording from the circumesophageal commissure (CC); the second trace shows the EMG recording from the mero-carpopodite flexor muscle of the right second leg; the third trace shows interneuron C_1 activity, which is also represented in the form of a frequency histogram with the time bin of 1 s underneath. (ii) Superimposition of interneuron spikes discriminated from the commissural recording. For clarity, the superimposed record is enlarged in both the time and voltage scale. The former scale is provided in the figure while the latter factor was 3.0 relative to the raw data. (B) Statistical comparison of interneuron C_1 spike activity between the resting and walking conditions (*P<0.05; Mann–Whitney U-test). (C) Interneuron activity when the animal walked on the tilted floor in different directions. The angular coordinate is shown in Fig. 1B. When the animal walked in 0° and 180° directions, the floor was bilaterally symmetrical for the animal body. In the direction of 90° and –90°, the animal body was tilted in the contralateral-side-down and ipsilateral-side-down directions, respectively. The gray bars depict the animal walking on the substratum tilted in the contralateral-side-down direction.

interneuron retained directional responsiveness over the full range of body axis orientation (Fig. 5C). The recording electrode was placed on the left circumesophageal commissure in this experiment. When the longitudinal axis of the animal body was perpendicular to the tilt direction of the substratum, the side of commissural recording was either lowered (–90°) or lifted (90°) (see Materials and methods). There was no sideward tilting when the animal body was oriented in 0° and ±180° directions. The animal took a variety of intermediate orientation angles during free walking. The spike activity of interneuron C_1 tended to increase when the animal walked on the substratum tilted in the ipsilateral-side-down direction and decrease in the contralateral-side-down direction. However, no significant difference was observed between any tilt angles (P>0.05; ANOVA).

Effects of abdominal posture on interneuron activity

The equilibrium responses of crayfish are significantly modulated by behavioral context. Uropod steering in response to body rolling (Yoshino et al., 1980), for instance, is enhanced by abdominal extension during locomotion and suppressed during turning behavior (Takahata et al., 1984). However, as shown in Fig. 4C, the abdominal posture movement in the air itself did not affect the spike activity of interneuron C_1. We thought that the abdominal posture movement evoked in the air should differ in the leg proprioceptor activation from that evoked during locomotion on a substratum and therefore analyzed the interneuron activity changes associated with the abdominal movements during locomotion.

A typical record of interneuron C_1 activity during free walking on the tilted substratum is illustrated in Fig. 6A. The animal was at rest or pausing with its head up, the substratum being bilaterally symmetrical for the animal for 26 s after the recording was started (P_1 in Fig. 6B). It then showed forward walking, gradually changing the head direction rightwards, with the abdomen extended (Ab. Ex. FW). During this forward walking (27–64 s), the animal showed an asymmetrical posture

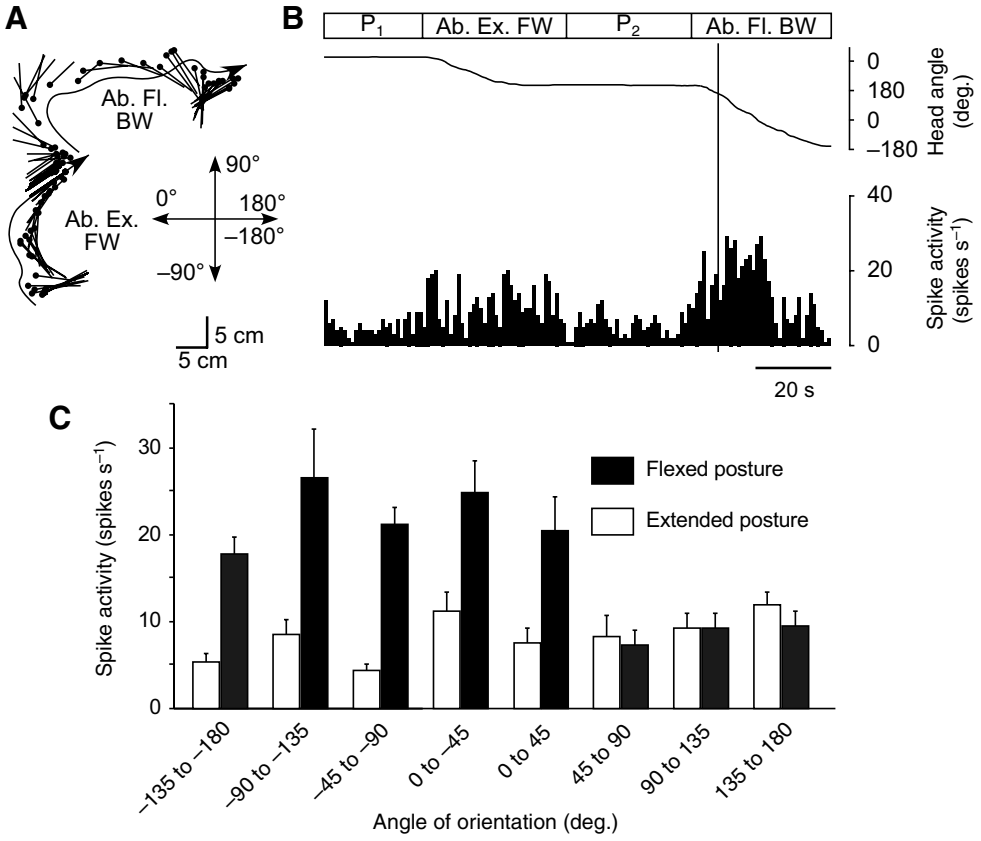

Fig. 6. Interneuron C_1 spike activity dependent on the abdominal posture movements during free walking. (A) Tracing of two bouts of walking on the tilted floor. The body position and head orientation are plotted at 1 s intervals. The filled circles indicate the head while the straight lines indicate the longitudinal body axis of the animal. In the first bout of walking, the animal walked forwards with the abdomen extended (Ab. Ex. FW). The animal then turned left and started the second walking in the backward direction with the abdomen flexed (Ab. Fl. BW). Smooth-line arrows in the figure indicate approximate displacement of the animal. (B) Spike activity of interneuron C_1 during the walking shown in A. Represented in the form of a frequency histogram with the time bin of 1 s, the record shows that the interneuron activity was enhanced when the animal started abdominal flexion (indicated by a vertical line). The crayfish behavior is shown in the top bar: P, pause; Ab. Ex. FW, forward walk with abdomen extended; Ab. Fl. BW, backward walk with abdomen flexed. Head orientation is monitored in the top trace. (C) Spike activities of interneuron C_1 in different orientation angles. Filled and open bars indicate the spike activity during abdominal flexion and extension, respectively.

of bilateral uropods, i.e. the exopodite on the lifted side was closed whereas that on the lowered side opened. After a pausing period (65–97 s; P_2), the animal began backward walking, gradually changing the head direction rightwards, with its abdomen flexed (solid line in Fig. 6B; Ab. Fl. BW). The spike activity of interneuron C_1 was higher during backward and forward walking than at rest. The interneuron activity was much higher during abdominal flexion than during extension. When the animal was just standing and not walking, no significant change was observed in the interneuron activity between abdominal flexion and extension. During abdominal extension in walking, the general activity of the interneuron and its directional responsiveness to tilting were both significantly lower than those during abdominal flexion (Fig. 6C). In this experiment, interneuron activity was recorded from the circumesophageal commissure on the left side. It increased when the animal walked on the substratum tilted in the directions of 0–135° and 0–45°, i.e. in the ipsilateral-side-down or head-up directions. By contrast, interneuron activity decreased when the animal walked on the substratum tilted in the contralateral-side-down or head-down directions (45–180° and –135° to –180°). The interneuron activity became highest when the animal body was oriented in the directions of –90° to –135° (26.4±5.5 spikes s^{-1}) and 0° to –45° (24.8±3.6 spikes s^{-1}). When the animal was engaged in abdominal extension during walking, interneuron activity showed a drastic change: the general activity became significantly lower than that recorded during abdominal flexion and the maximal response to substratum tilting was observed in the directions of 135–185° and 0° to –45°. As described above, the body orientation in the 0° to –45° direction corresponds to head-up tilting of the animal body while the orientation in the 135–180° direction corresponds to head-down tilting.

Activities of other statocyst-driven descending interneurons

The abdominal extension that enhances the uropod steering behavior in response to body rolling was found to suppress the response of interneuron C_1 to body tilting. We looked for other statocyst-driven descending interneurons that would increase their responsiveness to body tilting during abdominal extension in walking. Since the type of units that could be recorded depended on the electrode positioning relative to the commissure, we intentionally changed the positioning angle and depth of the electrode to obtain units other than interneuron C_1. In the experiment illustrated in Fig. 7, two descending units were found to respond directionally to body tilting. One of them (unit A) increased its firing frequency when the animal body was tilted in the ipsilateral-side-down direction, whereas the other (unit B) was activated when tilted in the contralateral-side-down direction (Fig. 7A). Thus, the

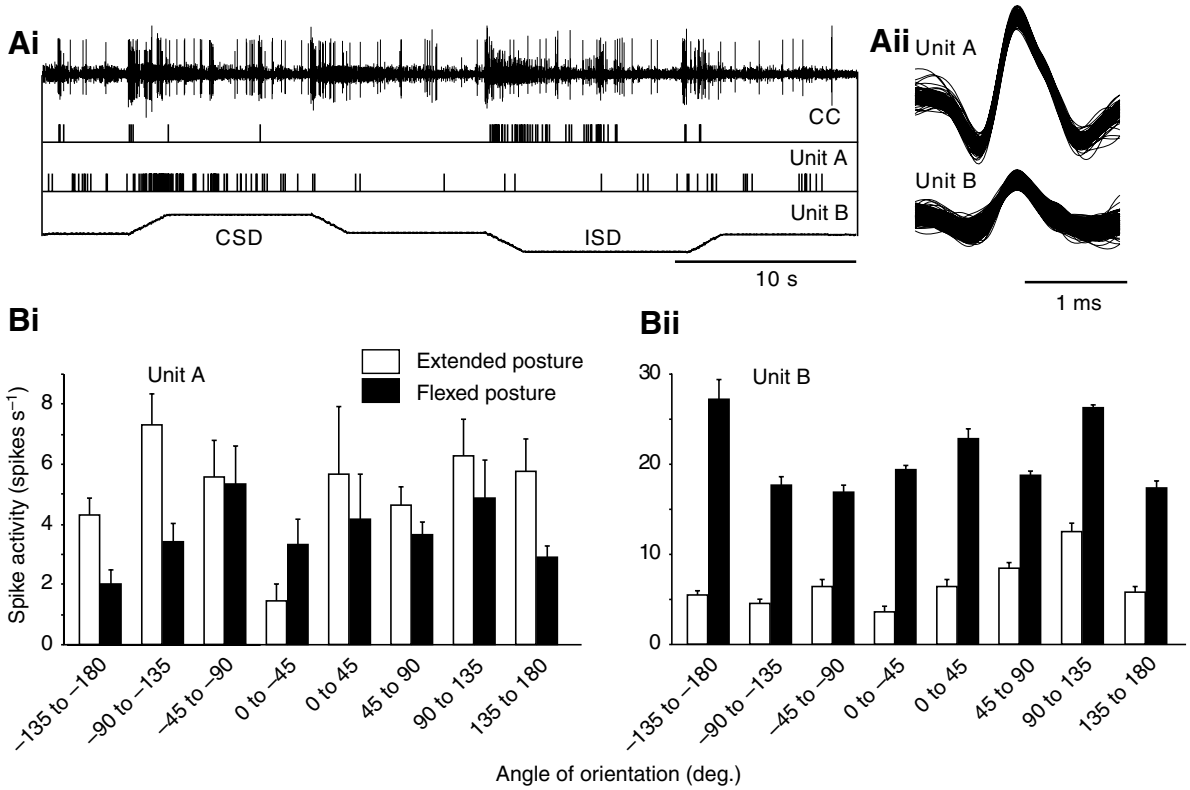

Fig. 7. Effects of abdominal posture on descending interneurons other than interneuron C_1. (Ai) Two statocyst-driven descending units in the circumesophageal commissure. The original recording is shown at the top (CC), and two unit activities (A and B) discriminated from the record are shown in the second and third traces. The bottom trace monitors body tilt angle. Unit A responded directionally to ipsilateral-side-down tilting (ISD) whereas unit B responded to contralateral-side-down tilting (CSD). (ii) Superimposition of interneuron spikes from the two isolated units. For clarity, the superimposed records are enlarged in both the time and voltage scale. The former scale is provided in the figure while the latter factor was 5.0 relative to the raw data for both units. (B) Spike activities during walking in different orientation angles with the abdomen extended (open bars) and flexed (filled bars). Unit A (i) showed no directional responses when the animal extended or flexed its abdomen whereas unit B (ii) could represent directional information when the animal extended its abdomen. Unit B showed no directional responses during abdominal flexion.

response directionality of unit A in body rolling was similar to that of interneuron C_1, but head-up and head-down tilting elicited no directional response in units A and B (data not shown). We analyzed the effects of abdominal posture movements on the responsiveness of these two units to the body tilt during walking. In the experiment illustrated in Fig. 7, the recording was made from the left circumesophageal commissure. When the animal body was oriented in the direction of –45° to –135°, the substratum was tilted in the ipsilateral-side-down direction. The orientation in the direction of 45°–135° indicated that the animal walked on the substratum tilted in the ipsilateral-side-up direction. Unit A clearly responded to body tilting when the animal was at rest (Fig. 7A). This directional response, however, was not observed during walking with the abdomen either flexed or extended actively (Fig. 7Bi).

Unit B, on the other hand, showed different responses from those of unit A and interneuron C_1. During walking with the abdomen extended, spike activity became maximal when the animal body was oriented in the direction of 90–135°. It then decreased as the orientation angle changed to –45° to –135° (Fig. 7Bii). The directional response of unit B during walking with the abdomen extended was larger than that observed at rest. By contrast, the general activity was enhanced, but the directional response to body tilting was not observed in unit B when the animal walked with its abdomen flexed. These findings suggest that the facilitation of uropod steering response during walking with abdominal extension is partly served by descending interneurons other than interneuron C_1, such as unit B.

Discussion

The responsiveness of central neurons to sensory stimuli varies dramatically depending on the ongoing behavior of the experimental animal (e.g. Schildberger and Hörner, 1988; Staudacher and Schildberger, 1998; Staudacher, 2001). The behavioral context-dependent modulation of sensori-motor pathways becomes particularly critical in postural control since it is during locomotion that the postural control mechanism is most needed. In the present study, we applied our optical telemetry technique (Tsuchida et al., 2004) to freely behaving

crayfish and recorded statocyst-related central neuron activity in order to analyze how the descending statocyst-motor pathway is modulated during locomotion under natural conditions. The results suggest that crustacean posture control is based on neuronal mechanisms far more complex than those expected from the experimental data obtained in animals fixed in the air (Takahata and Hisada, 1985) or on a treadmill apparatus (Murayama and Takahata, 1996).

Behavioral context-dependent unit activities in the circumesophageal commissure

The current method of optical telemetry revealed that many units descending from the brain to the thoracic and abdominal ganglia changed their spike activity depending on the behavioral condition of the animal (Fig. 2). Since we confined detailed analyses to the statocyst descending system in this study, our description of other units inevitably remains fragmentary and episodic (Table 1). Nevertheless, we could find several interesting aspects in the behavior-dependent control of unit activities descending from the brain for the first time by applying newly developed telemetry techniques to freely behaving animals. First, some units reliably increased their spike discharge rate when the animal engaged in specific behavior. Their activity even preceded the initiation of limb movements (unit 3c in Fig. 2B). These descending unit activities presumably represent the motor command (Edwards et al., 1999; Esch et al., 2002) for initiating or maintaining specific locomotor movements, and we are currently analyzing these activities by intracellular as well as extracellular techniques. Second, not all descending units changed their spike activities depending on the animal's behavioral condition. We could not identify those units that showed invariable spike activities all the time, but some of them are thought to be descending sensory interneurons since we previously reported those interneurons that kept constant responsiveness regardless of the animal's behavioral and sensory conditions (Hama and Takahata, 2003). Finally, some descending units showed great variability not only in their spontaneous spike discharge but also in their responsiveness to specific sensory stimuli depending on the animal's behavioral condition, as discussed in the following sections.

It is interesting to note here that both the activation of motor-related units (3c and 3d in Fig. 2) and the modulation of sensory responsiveness in statocyst-driven units (Figs 3–7) are not associated with general activity changes but are invoked in a specific behavioral context. This finding confirms the classical hypothesis (Sperry, 1950; von Holst and Mittelstaedt, 1950) that the motor center responsible for specific behavior generates not only motor commands for initiating and controlling the behavior but also efference copy signals to prepare the animal's sensory and motor systems for the behavior, as in the saccadic suppression of vertebrates (Thiele et al., 2002) and the escape behavior of crayfish (Wine and Mistick, 1977). It remains to be studied further whether the motor-related activities of units 3c and 3d in Fig. 2 reflect the motor command signals directed towards lower-level motor circuits or the efference copy signals to be distributed in the nervous system. The modulation of sensory responsiveness during locomotor behavior in the statocyst-driven units discussed below (Figs 3–7), by contrast, is thought to be primarily based on the efference copy signals originated from the locomotor center in the brain.

Modulation of descending statocyst pathways by sensory and behavioral conditions

Descending statocyst pathways were first found to involve four interneurons, each showing different directional sensitivities (Takahata and Hisada, 1982), and later to involve 14 other interneurons in the circumesophageal commissure (Hama and Takahata, 2003). Using intracellular recording techniques applied to fixed animals, we demonstrated that the synaptic responses of each descending statocyst interneuron to statocyst stimulation were differently modulated in the brain by leg movements in different conditions (Hama and Takahata, 2003). Thus, some interneurons showed enhanced responses due to synaptic summation of inputs from the statocyst and leg movement system regardless of whether a substrate was provided or not, whereas others showed more effective summation when a substrate was provided during leg movements than when it was not. In one interneuron, the synaptic response to statocyst stimulation was never affected by leg movements either on a substrate or in the air. Of these three groups, our present finding suggests that interneuron C_1 belongs to the second group, but there is a slight difference between the previously studied interneurons and interneuron C_1 in that C_1 activity was not only enhanced but also suppressed depending on the behavioral condition of the animal (Fig. 6). Response modification by sensory and behavioral conditions has been extensively reported in many sensori-motor systems of invertebrates (Staudacher, 2001; Frost et al., 2003) and vertebrates (Deliagina et al., 2000; Seki et al., 2003). In most cases, however, the activity of single neurons is either enhanced or suppressed. Interneuron C_1 therefore shows quite a novel type of response variability that is unprecedentedly complex.

The spike responses of interneuron C_1 to body rolling were not affected at all by active leg movements or abdominal posture movements in the air (Fig. 4), confirming the previous result obtained by conventional extracellular recordings from fixed animals (Takahata and Hisada, 1985). When the animal walked in the water on the horizontal aquarium floor, which was bilaterally horizontal, spontaneous activity was significantly greater than at rest (Fig. 4B). Interneuron C_1 activity was thus affected differently by leg movements in the air and on a substratum. It is difficult at this time to infer any difference in the central nervous activity during leg movements in the air or water and during walking on a substratum, since no experimental data are available in this regard. The situation, however, can be reduced to the leg movements with and without a specific load or disturbance. Although there are a variety of mechanoreceptors, including position detectors (Mill, 1975) and stress detectors (Marchand et al., 1995), in the

walking legs of crayfish, the nerve signals from these mechanoreceptors to the brain are filtered by centrally generated signals through presynaptic inhibition during locomotion (Cattaert et al., 1990; Cattaert et al., 1992; El Manira et al., 1991). This situation implies that the sensory signals themselves can remain constant in different load conditions. It is therefore suggested that the different effects on the interneuron activity of leg movements in different conditions are due, at least partly, to a difference in this centrally programmed peripheral filtering or cancellation. The possibility still remains, needless to say, that different leg sensory signals due to different load conditions directly affect interneuron activity.

Interneuron C_1 activity was also affected differently by abdominal posture movements, even when the animal was engaged in the same walking on the aquarium floor (Fig. 6). Other interneurons were also found to be affected by abdominal movements but in different ways from interneuron C_1 (Fig. 7). Since the crustacean abdomen is equipped with muscle receptor organs (MROs) that monitor the stretch of abdominal extensor muscles (Wiersma et al., 1953), the different effects of abdominal movements on interneuron activity can be due to either central signals that command the abdominal movements (Larimer and Moore, 2003) or peripheral signals from the MROs. Further study is needed to clarify to what extent each signal contributes to enhance or suppress the descending interneuron activity during abdominal movements and walking.

Neuronal mechanisms underlying behavioral context-dependent posture control

In some sensori-motor systems, the pathway transmitting specific sensory information to the motor system operates independently of the animal's behavioral condition. For example, in the locust flight control system, the sensory signal from ocelli for steering control is transmitted to pre-motor interneurons invariably regardless of whether the animal is engaged in flight behavior or not (Reichert and Rowell, 1985). The steering control of the flight behavior is based on synaptic summation of the ocelli input in the pre-motor interneurons and the central input from the flight pattern generator, so that the steering posture is taken at a certain phase of the rhythmical flight behavior (Reichert and Rowell, 1986). Similar reflex gating (Delcomyn, 1998) has been reported in many other sensori-motor systems (Staudacher, 2001; Frost et al., 2003). Gating of sensory information by other sensory inputs or by behavioral context is also common in the vertebrate brain (Deliagina et al., 2000; Seki et al., 2003).

In the uropod steering of crayfish with walking legs off the substratum, it has been shown that the statocyst pathways descending from the brain are not only gated by abdominal posture movements in the terminal abdominal ganglion to activate the uropod motor system; in anterior abdominal ganglia, they also activate other descending pathways that originate there and run in parallel with the original pathways to converge onto the uropod motor system in the terminal ganglion, thus constituting a multiple gate control system (Takahata and Murayama, 1992; Fraser and Takahata, 2002). The statocyst sensory signal is multiplied by a cascade of abdominal interneurons to enhance the synaptic response of the uropod motor system (Murayama and Takahata, 1998a; Murayama and Takahata, 1998b). The present study using optical telemetry techniques applied to freely behaving animals in water has clarified another aspect of the crustacean postural control system: the behavioral context-dependent changes in the statocyst sensory signal pathway. Responses of a functionally identified descending statocyst interneuron to body tilting were affected in different ways by leg movements and abdominal posture movements depending on the sensory and behavioral conditions (Figs 5, 6). We also showed that different descending interneurons were affected differently by the same sensory or behavioral condition (Fig. 7).

The present findings do not negate the importance of multiple gate control in the postural control of crayfish since the spike activities of descending statocyst interneurons can elicit only subthreshold synaptic potentials in the uropod motor neurons (Takahata, 1990). However, modulated by behavioral context, they will not be capable by themselves of eliciting spike activities in motor neurons. They have to make synaptic summation with central inputs from the locomotor system (Murayama and Takahata, 1998a). Although our previous study demonstrated that the sensory signal carried by interneuron C_1 and gated to the uropod motor neurons was invariable in the fixed animal with legs off the substratum (Takahata and Hisada, 1985), it was not the case in the freely behaving animal. In a particular behavioral context, i.e. during abdominal extension, interneuron C_1 activity does not represent the angle of body tilt that is reliably represented by the same interneuron during abdominal flexion (Fig. 6). The responses of other interneurons to tilting were affected differently from those of interneuron C_1 (Fig. 7). The posture of the animal body during walking is thus controlled by different interneurons, the combination of which changes every moment depending on the behavioral context of the animal. The reflex gating of sensori-motor pathways is undoubtedly one aspect of postural control in the freely behaving animal, but the gating is organized in multiple ways, and, furthermore, complex mechanisms modulate the sensory information before the gating mechanism converts it into motor output signals. Do the interneurons that are affected differently by sensory and behavioral conditions all converge onto the uropod motor system so that their signals are non-selectively gated to the uropod motor system? Alternatively, is the sensory information that is currently relevant selected by another upstream mechanism to be gated by central signals from the locomotor system to activate the motor system? Further study is needed to clarify these questions.

This work was supported in part by a Grant-in-Aid for Scientific Research (No.17370024) to M.T. from the Japan Society for the Promotion of Science. N.H. was supported by COE No.14COE001-01.

References

Ando, N., Shimoyama, I. and Kanzaki, R. (2002). A dual-channel FM transmitter for acquisition of flight muscle activities from the freely flying hawkmoth, *Agrius convolvuli*. *J. Neurosci. Methods* **115**, 181-187.

Cattaert, D., El Manira, A., Marchand, A. and Clarac, F. (1990). Central control of the sensory afferent terminals from a leg chordotonal organ in crayfish in vitro preparation. *Neurosci. Lett.* **108**, 81-87.

Cattaert, D., El Manira, A. and Clarac, F. (1992). Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. *J. Neurophysiol.* **67**, 610-624.

Delcomyn, F. (1998). *Foundations of Neurobiology*. New York: Freeman.

Deliagina, T. G., Zelenin, P., Fagerstedt, P., Grillner, S. and Orlovsky, G. N. (2000). Activity of reticulospinal neurons during locomotion in the freely behaving lamprey. *J. Neurophysiol.* **83**, 853-863.

Edwards, D. H., Heitler, W. J. and Krasne, F. B. (1999). Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22**, 153-161.

El Manira, A., DiCaprio, R. A., Cattaert, D. and Clarac, F. (1991). Monosynaptic interjoint reflexes and their central modulation during fictive locomotion in crayfish. *Eur. J. Neurosci.* **3**, 1219-1231.

Esch, T., Mesce, K. A. and Kristan, W. B. (2002). Evidence for sequential decision making in the medicinal leech. *J. Neurosci.* **15**, 11045-11054.

Fisher, H., Kautz, H. and Kutsch, W. (1996). A radiotelemetric 2-channel unit for transmission of muscle potentials during free flight of the desert locust, *Schistocerca Gregaria*. *J. Neurosci. Methods* **64**, 39-45.

Fraser, P. J. and Takahata, M. (2002). Statocysts and statocyst control of motor pathways in crayfish and crabs. In *Crustacean Experimental Systems in Neurobiology* (ed. K. Wiese), pp. 89-108. Berlin: Springer.

Frost, W. M., Tian, L. M., Hoppe, T. A., Mongeluzi, D. L. and Wang, J. (2003). A cellular mechanism for prepulse inhibition. *Neuron* **40**, 991-1001.

Furudate, H., Okada, Y. and Yamaguchi, T. (1996). Responses of nonspiking giant interneurons to substrate tilt in the crayfish, with special reference to multisensory control in the compensatory eyestalk movement system. *J. Comp. Physiol. A* **179**, 635-643.

Hama, N. and Takahata, M. (2003). Effects of leg movements on the synaptic activity of descending statocyst interneurons in crayfish, *Procambarus clarkii*. *J. Comp. Physiol. A* **189**, 887-888.

Hama, N. and Takahata, M. (2005). Modification of statocyst input to local interneurons by behavioral condition in the crayfish brain. *J. Comp. Physiol. A* **191**, 747-759.

Horak, F. B. and Macpherson, J. M. (1996). Postural orientation and equilibrium. In *Handbook of Physiology: Section 12, Exercise: Regulation and Integration of Multiple Systems* (ed. L. Rowell and J. T. Shepherd), pp. 255-292. New York: Oxford University Press.

Katz, P. S. and Tazaki, K. (1992). Comparative and evolutionary aspects of the crustacean stomatogastric system. In *Dynamic Biological Networks* (ed. R. M. Harris-Warrick, E. Marder, A. I. Selverston and M. Moulins), pp. 221-261. Boston: MIT Press.

Kudo, Y., Satou, M., Kitamura, S., Iwata, M. and Takeuchi, Y. (1999). A newly designed underwater antenna and its application to underwater radio-telemetry for measuring electroencephalographic activity from rainbow trout freely swimming in natural environments. *Front. Med. Biol. Eng.* **9**, 285-294.

Larimer, J. L. and Moore, D. (2003). Neural basis of a simple behavior: abdominal positioning in crayfish. *Microsc. Res. Tech.* **60**, 346-359.

Le Ray, D., Combes, D., Déjean, C. and Cattaert, D. (2005). In vivo analysis of proprioceptive coding and its antidromic modulation in the freely behaving crayfish. *J. Neurophysiol.* **94**, 1013-1027.

Marchand, A. R., Leibrock, C. S., Auriac, M. C., Barnes, W. J. P. and Clarac, F. (1995). Morphology, physiology and in vivo activity of cuticular stress detector afferents in crayfish. *J. Comp. Physiol. A* **176**, 409-424.

Mill, P. J. (1975). Chordotonal organs of crustacean appendages. In *Structure and Function of Proprioceptors in the Invertebrates* (ed. P. J. Mill), pp. 243-297. London: Chapman & Hall.

Murayama, M. and Takahata, M. (1996). Sensory control mechanisms of the uropod equilibrium reflex during walking in the crayfish *Procambarus clarkii*. *J. Exp. Biol.* **199**, 521-528.

Murayama, M. and Takahata, M. (1998a). Neuronal mechanisms underlying the facilitatory control of uropod steering behaviour during treadmill walking in crayfish. I. Antagonistically regulated background excitability of uropod motoneurones. *J. Exp. Biol.* **201**, 1283-1294.

Murayama, M. and Takahata, M. (1998b). Free full text neuronal mechanisms underlying the facilitatory control of uropod steering behaviour during treadmill walking in crayfish. II. Modulation of uropod motoneurone excitation by leg proprioception. *J. Exp. Biol.* **201**, 1295-1305.

Okada, Y. and Yamaguchi, T. (1988). Nonspiking giant interneurons in the crayfish brain: morphological and physiological characteristics of the neurons postsynaptic to visual interneurons. *J. Comp. Physiol. A* **162**, 705-714.

Okada, Y., Furudate, H. and Yamaguchi, T. (1994). Multimodal responses of the nonspiking giant interneurons in the brain of the crayfish *Procambarus clarkii*. *J. Comp. Physiol. A* **174**, 411-419.

Reichert, H. and Rowell, C. H. F. (1985). Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. *J. Neurophysiol.* **53**, 1201-1218.

Reichert, H. and Rowell, C. H. F. (1986). Neuronal circuits controlling flight in the locust: how sensory information is processed for motor control. *Trends Neurosci.* **9**, 281-283.

Schildberger, K. and Hörner, M. (1988). The function of auditory neurons in cricket phonotaxis I. Influence of hyperpolarization of identified neurons on sound localization. *J. Comp. Physiol. A* **163**, 621-631.

Schmidt, E. M. (1984). Instruments for sorting neuroelectric data: a review. *J. Neurosci. Methods* **12**, 1-24.

Seki, K., Perlmutter, S. I. and Fetz, E. E. (2003). Sensory input to primate spinal cord is presynaptically inhibited during voluntary movement. *Nat. Neurosci.* **6**, 1309-1316.

Sperry, R. W. (1950). Neural basis of the spontaneous optokinetic response produced by visual inversion. *J. Comp. Physiol. Psychol.* **43**, 482-489.

Staudacher, E. M. (2001). Sensory responses of descending brain neurons in the walking cricket, *Gryllus bimaculatus*. *J. Comp. Physiol.* **187**, 1-17.

Staudacher, E. and Schildberger, K. (1998). Gating of sensory responses of descending brain neurons during walking in crickets. *J. Exp. Biol.* **201**, 559-572.

Takahata, M. (1990). The crayfish posture control system as a model for studying mechanisms underlying behavioral variability. In *Frontiers in Crustacean Neurobiology* (ed. K. Wiese, W. D. Krenz, J. Tauz, H. Reichert and B. Mulloney), pp. 301-308. Basel: Birkhäuser.

Takahata, M. and Hisada, M. (1982). Statocyst interneurons in the crayfish *Procambarus clarkii* Girard. I. Identification and response characteristics. *J. Comp. Physiol. A* **149**, 287-300.

Takahata, M. and Hisada, M. (1985). Interaction between motor systems controlling uropod steering and abdominal posture in crayfish. *J. Comp. Physiol. A* **157**, 547-554.

Takahata, M. and 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.

Takahata, M., Komatsu, H. and Hisada, M. (1984). Positional orientation determined by the behavioural context in *Procambarus clarkii* Girard (Decapoda: Macrura). *Behaviour* **88**, 240-265.

Thiele, A., Henning, P., Kubischik, M. and Hoffman, K. P. (2002). Neural mechanisms of saccadic suppression. *Science* **295**, 2460-2462.

Tsuchida, Y., Hama, N. and Takahata, M. (2004). An optical telemetry system for underwater recording of electromyogram and neuronal activity from non-tethered crayfish. *J. Neurosci. Methods* **137**, 103-109.

von Holst, E. and Mittelstaedt, H. (1950). Das Reafferenzprinzip (Wechselwirkungen zwischen Zentralnervensystem und Peripherie). *Naturwissenshaften* **37**, 464-476.

Wiersma, C. A. G. (1958). On the functional connections of single units in the central nervous system of crayfish, *Procambarus clarrkii* (Girard). *J. Comp. Neurol.* **110**, 421-471.

Wiersma, C. A. G., Furshpan, E. and Florey, E. (1953). Physiological and pharmacological observations on muscle receptor organs of the crayfish, *Cambarus clarkii* Girard. *J. Exp. Biol.* **30**, 136-150.

Wilkens, L. A. and Larimer, J. L. (1973). Sensory interneurons: some observations concerning the physiology and related structural significance of two cells in the crayfish brain. *Tissue Cell* **5**, 393-340.

Wine, J. J. and Mistick, D. C. (1977). Temporal organization of crayfish escape behavior: delayed recruitment of peripheral inhibition. *J. Neurophysiol.* **40**, 904-925.

Yoshino, M., Takahata, M. and Hisada, M. (1980). Statocyst control of uropod movement in response to body rolling in crayfish. *J. Comp. Physiol. A* **139**, 243-250.