

# NEURONAL MECHANISMS UNDERLYING THE FACILITATORY CONTROL OF UROPOD STEERING BEHAVIOUR DURING TREADMILL WALKING IN CRAYFISH

## II. MODULATION OF UROPOD MOTONEURONE EXCITATION BY LEG PROPRIOCEPTION

MAKI MURAYAMA\* AND MASAKAZU TAKAHATA

*Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

\*e-mail: makim@bio.sci.hokudai.ac.jp

*Accepted 5 February; published on WWW 20 April 1998*

### Summary

The synaptic activities underlying the uropod steering behaviour of crayfish evoked by tilting the substratum beneath the legs have been studied intracellularly in unanaesthetized animals standing or walking on a treadmill. The uropod motoneurons showed little or no synaptic response when the treadmill was tilted while the animal was in a quiescent state and the membrane potential was at its resting value. When the same stimulus was given while the animal was walking or in an active stance on the treadmill, the motoneurons showed transient much-enhanced excitatory or inhibitory responses to tilt, depending on the tilt direction. These responses were superimposed on a sustained level of background excitation so that the spike activity of the motoneurons either increased or decreased. Premotor nonspiking

interneurons also showed little or no synaptic response to the tilt stimulus while the animal was resting, but greatly enhanced responses, in either a depolarizing or a hyperpolarizing direction, while the animal was walking or in the active-standing state. The results indicate that the proprioceptor inputs converging onto the uropod motoneurons, either directly or through premotor nonspiking interneurons, are gated not only in the uropod motor system in the terminal abdominal ganglion but also at as yet unidentified sites upstream in anterior ganglia, thus suggesting multiple gate control of the descending proprioceptor pathway.

Key words: crayfish, *Procambarus clarkii*, posture control, uropod, steering, walking, multiple gate control.

### Introduction

Behavioural acts are elicited as a result of synaptic interactions between the specific trigger input, including sensory stimuli and motor commands, and the background excitation level of the motor system, which is affected both by stimulus conditions and by the behavioural context (Pearson and Rowell, 1977; Reichert and Rowell, 1989; Mori *et al.* 1991). Thus, the uropod steering reflex, a postural reflex of crustaceans elicited by a change in body orientation (Davis, 1968; Schöne *et al.* 1976; Yoshino *et al.* 1980), is significantly facilitated during walking (Takahata *et al.* 1984) when it is functionally most important (Grillner, 1985; Orlovsky, 1991). In the preceding paper (Murayama and Takahata, 1998), we analyzed intracellularly how the background excitation level of the uropod motor system is altered during walking. We found that the background excitation of motoneurons was maintained at an intermediate level by antagonistic synaptic inputs from the walking motor system mediated, at least partly, by premotor nonspiking interneurons.

The intermediate level of background excitation of motoneurons suggests that they can be both further depolarized to increase their spike activity and suppressed to

decrease it, thus increasing the degree of freedom in movements. However, the synaptic events associated with this modulation of the background excitation of motoneurons by sensory stimuli remains to be investigated. Moreover, the finding that the membrane potential of motoneurons is continuously kept at a depolarized level poses the problem that, although they can effectively accept suppressive inputs to decrease their excitability, they face difficulties in further depolarizing the membrane in response to excitatory inputs because of the decreased driving force for excitatory ionic movements (Hille, 1992), the decreased excitability due to prolonged depolarization (Cannone and Bush, 1980) and the desensitizing effect due to continuous release of neurotransmitters from presynaptic cells (Katz and Thesleff, 1957). Excitatory synaptic summation is also affected significantly by the shunting effect resulting from the adjacent arrangement of relevant synapses on the dendrite (Koch *et al.* 1983; Shepherd and Brayton, 1987).

In the present paper, we report the results of intracellular analyses of synaptic mechanisms underlying the steering response of the uropod motor system in crayfish to substratum

tilt during walking and standing on a treadmill, using a whole-animal preparation. The motor pattern for steering by the uropods was found to be generated both by excitatory synaptic inputs that further depolarized motoneurons and by suppressive inputs that hyperpolarized them from the background excitation level. Synaptic inputs from the leg proprioceptive system were more enhanced when the animal was engaged in walking or in the active-standing state than when it was at rest. The results suggest that the descending input pathway from the leg proprioceptors to the uropod motor system is controlled by multiple gates, as in the case of the descending statocyst pathway (Takahata and Murayama, 1992).

### Materials and methods

Experimental animals, design and procedures were all similar to those reported in the preceding paper (Murayama and Takahata, 1998). The treadmill could be tilted in the roll plane, while the animal was walking on it with the body kept upright, by means of a stepping motor (Nippon Pulse Motor, PJ55-B1) controlled by a personal computer (NEC, PC-8801). This enabled us to tilt the treadmill at the same time as it was moving longitudinally to make the animal walk. The rotation axis of the treadmill was coupled rigidly to a variable potentiometer through which the tilt angle was monitored during experiments. In this study, the substratum was always tilted by  $15^\circ$  in either the side-up or side-down direction with reference to the side of the cell from which intracellular recordings were being made. 'Substratum' traces in the figures represent the side-up tilt by upward deflection and the side-down tilt by downward deflection. All substratum traces start from the horizontal plane except in Fig. 2B.

Electrical stimulation of a sensory nerve bundle was carried out using a single pulse (0.1 ms in duration) generated by an electronic stimulator (Nihon-Kohden, SEN-3301) equipped with a constant-current isolator (Nihon-Kohden, SS201J). The synaptic response evoked by nerve stimulation was averaged over eight trials using home-made software running on a personal computer (Apple, Macintosh IIcx), which was also used to analyze the amplitude distribution in the membrane potential fluctuation.

### Results

#### *Synaptic responses of motoneurons to substratum tilt*

Uropod motoneurons showed little or no synaptic response to substratum (treadmill) tilt when the animal was in the quiescent state. Typical responses of a uropod closer motoneurone are illustrated in Fig. 1A–C, with its morphology (Fig. 1D). The substratum was first tilted from the horizontal position by  $15^\circ$  in a direction such that the side of the impaled cell was lowered, kept there for approximately 5 s and then returned to its initial position. When the animal was at rest, the first tilt elicited a small depolarizing response, whereas the return tilt evoked no visible response (Fig. 1A). Membrane

depolarization in general can be caused by either synaptic excitation or disinhibition. To clarify this point, the extents of the changes in membrane potential were compared, revealing that the membrane potential of the motoneurone fluctuated to a greater degree during the first tilt than before stimulation (Fig. 1Ci). This finding indicates the presence of excitatory synaptic inputs from leg proprioceptors to uropod motoneurons during the tilt stimulus. A similar membrane potential fluctuation was observed, however, before and during the return tilt in the same motoneurone (Fig. 1Cii).

When the animal was engaged in walking, or was in the active-standing state during which the activity was increased tonically but no rhythmic activity was observed in walking leg muscles, the same motoneurone showed a large excitatory response to the first tilt, leading to spike discharges, and a suppressive response to the return tilt (Fig. 1B). It should be noted here that, during walking, the membrane potential is kept at a depolarized level (broken line), and the extracellular recording from the axon bundle of motoneurons shows elevated spike activities before the stimulation. The excitatory response superimposed on this background activity was much larger than that observed at rest, and the suppressive input that was apparently absent in the quiescent state hyperpolarized the motoneurone in the active state from the sustained depolarization level towards its original resting potential level.

The enhancement of excitatory synaptic inputs from the leg proprioceptor system is in contrast to the reduction in the influence of local segmental sensory input during walking and in the active-standing state (Fig. 1E). When the animal was at rest, an opener motoneurone showed a large compound excitatory synaptic potential with spikes superimposed in response to electrical stimulation of the second root sensory nerve bundle, which consists of mechanosensory afferents from cuticle hairs on the telson and uropod on the same side as the motoneurone. Only a small response to the same stimulus, however, was superimposed on the sustained depolarization seen when the animal was engaged in walking (upper trace in Fig. 1E). This result indicates that an excitatory synaptic input cannot always make effective summation with the prolonged depolarization in the motoneurone.

The synaptic response of motoneurons to substratum tilt was similar between walking and the active-standing state, except that it was enhanced during walking (Fig. 2). An opener motoneurone (shown in Fig. 2C) that transiently showed an excitatory response in the standing state to substratum tilting (Fig. 2A) responded more strongly and in a more prolonged way during walking: it showed a transient excitatory response to the return tilt from the side-down to the horizontal position and a more enhanced response to a side-up tilt from the horizontal position (Fig. 2B). The return tilt of the leg substratum in the standing state caused no noticeable response except for a few discrete hyperpolarizing synaptic potentials (Fig. 2A). In both cases, the response was superimposed on the sustained level of depolarization to cause a further increase in spike activity. Extracellular recordings from the opener and closer motoneurons on the same side

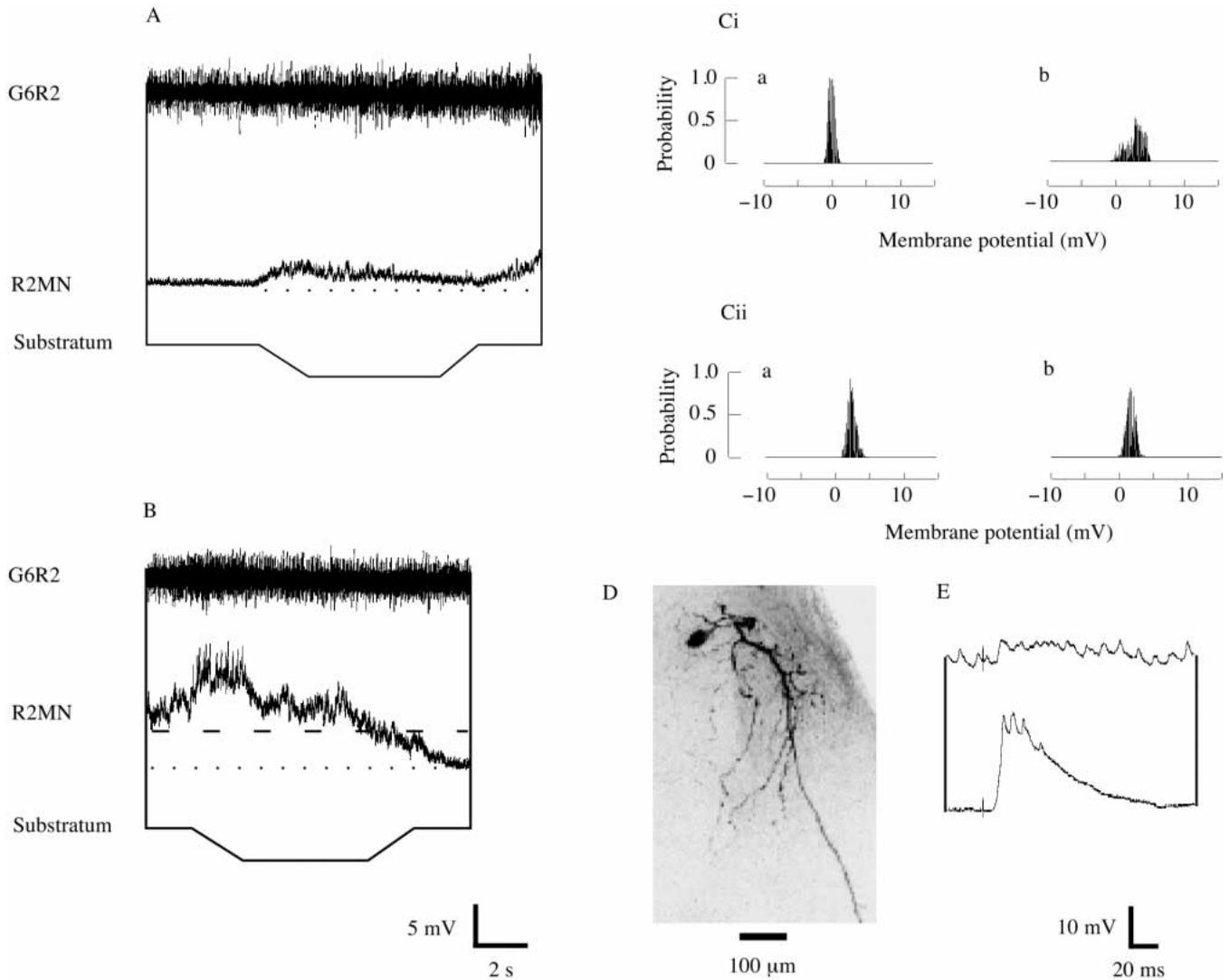


Fig. 1. Synaptic responses of a uropod motoneurone to tilting of the substratum. (A) The stimulus was applied when the animal was at rest. (B) The stimulus was applied to the same animal while it was in the active-standing state. The top trace (G6R2) monitors the spike activity of uropod closer motoneurones, the middle trace (R2MN) the intracellularly recorded synaptic activity of a closer motoneurone and the bottom trace (Substratum) the position of the substratum. In this and subsequent figures, downward and upward deflections of the 'substratum' trace indicate downward and upward tilts, respectively, of the side of the impaled neurone. The dotted line indicates the resting potential level at rest in A and B; the broken line in B indicates the level of the membrane potential during walking on the horizontal treadmill. Since the tilt stimulus itself often caused the general activity of the animal to rise slowly, the membrane potential did not return precisely to the resting level after stimulation in A and B. Such a general effect was, however, completely absent in the early phase of stimulation. (Ci) Amplitude distribution of the fluctuation in membrane potential before (a) and during (b) the first tilt stimulus at rest. (Cii) Amplitude distribution before (a) and during (b) the return tilt back to the horizontal position. The ordinates in Ci and Cii are normalized to the maximal values. Zero on the abscissa indicates the mean of the membrane potential fluctuation in the quiescent state. (D) The projected image of the motoneurone. The images in this and in the following figures were obtained by confocal laser scanning microscopy. (E) The synaptic response of an opener motoneurone evoked by stimulation of the second root sensory bundle of the terminal ganglion at rest (lower trace) and during walking (upper trace). The timing of stimulation in each case is indicated by an artefact. The vertical distance between the two traces corresponds to the sustained depolarization of the motoneurone during walking.

showed that they are activated in a reciprocal way: the spike activity of synergistic motoneurones increased while that of their antagonists decreased from the background level, both in the standing state (Fig. 2A) and during walking (Fig. 2B). It is suggested that the excitatory and suppressive inputs for generating the steering motor pattern are transmitted to the

uropod motor system in a similar way both during walking and in the standing state.

Suppressive synaptic activities underlying a decrease in the spike activity of motoneurones were more difficult to demonstrate clearly than those underlying the increase in spike activity. The intracellular recordings obtained from one closer

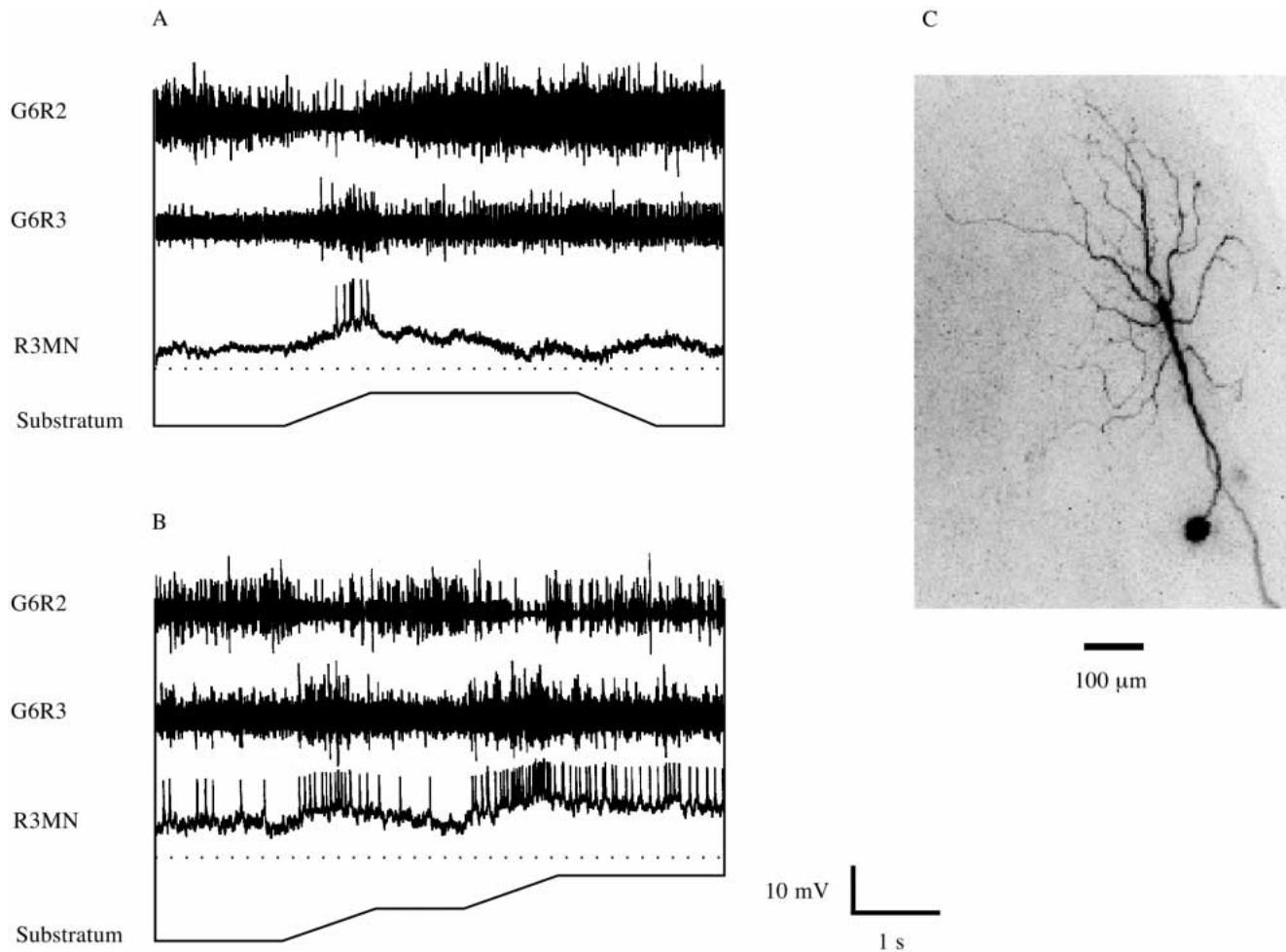


Fig. 2. Synaptic responses of a third root motoneurone to substratum tilt in the side-up direction. (A) The motoneurone (R3MN) transiently showed an excitatory response to side-up tilt from the horizontal plane, but no significant response to the return stimulus when the animal was in the active-standing state. Note the transient reciprocal activation of closer (G6R2) and opener (G6R3) motoneurons during the tilt stimulus (Substratum). Such activation is absent during the return stimulus. (B) During walking, the same motoneurone showed a similar but more prolonged response to the return stimulus from a downward-tilted position to the horizontal position and then to a further tilt stimulus from the horizontal position in the side-up direction. The dotted lines in A and B indicate the resting potential level. (C) The projected image of the motoneurone.

motoneurone (Fig. 3C) well illustrate the suppressive synaptic response to leg stimulation. When the substratum was tilted in the side-down direction from the horizontal position in the active-standing state, the motoneurone showed an excitatory synaptic response with spikes superimposed on it (Fig. 3A). This excitatory response was unusually prolonged (cf. Figs 1, 2), but the spike activity of closer motoneurons as a whole was as transient as usual. This motoneurone unambiguously received inhibitory synaptic inputs when the substratum was returned to the original horizontal position. It also showed an inhibitory synaptic response when the substratum was tilted in the side-up direction, followed by an excitatory response to the return tilt (Fig. 3B). A slight increase in spike activity was observed following the inhibition (Fig. 3A,B), which is indicative of a rebound response. These results clearly demonstrate that the decrease in the spike activity of uropod motoneurons during steering is based on inhibitory synaptic

inputs from the leg proprioceptor system rather than on a mere decrease in the excitatory input. The finding that the inhibitory input was absent during substratum tilting in the quiescent state, although the excitatory input was clearly seen (Fig. 1A), suggests that the inhibition is mediated indirectly *via* one or more synapses interposed in the descending pathway from the leg proprioceptors to the uropod motor system (see Discussion).

A motoneurone with an axon leaving the ganglion through its third root (Fig. 4C) exhibited synaptic responses that were in the opposite direction to those of other motoneurons leaving through the same root: it received inhibitory synaptic inputs during side-up tilting of the leg substratum and excitatory inputs during the return tilt, although the extracellular recording from the third root indicated that the overall spike activity increased more during the side-up than during the return tilt (Fig. 4A,B). When the animal was

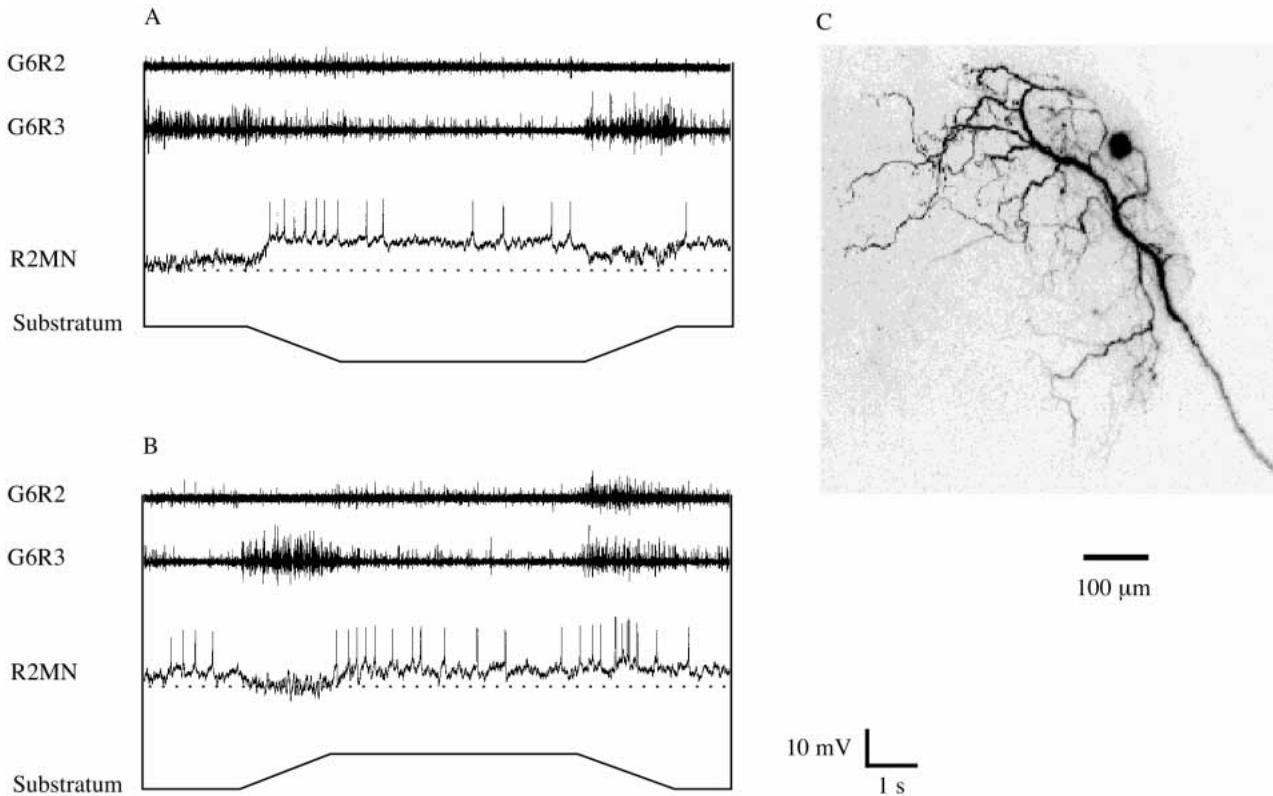


Fig. 3. Synaptic responses of a second root motoneurone to substratum tilting in the side-up and side-down directions in the active-standing state. (A) The motoneurone (R2MN) showed an excitatory response to the tilt in the side-down direction (Substratum). The closer (G6R2) and opener (G6R3) motoneurons are activated reciprocally. R2MN showed an inhibitory response to the return tilt to the horizontal position. The motoneurons are activated reciprocally in the reverse direction to their previous response. (B) The same motoneurone showed an inhibitory response to the side-up tilt stimulus and an excitatory response to the return stimulus. The dotted lines in A and B indicate the resting potential level. (C) The projected image of the motoneurone.

standing, the inhibitory inputs were more pronounced than during walking, whereas the excitatory inputs were less pronounced (Fig. 4A,B). It seems possible that this motoneurone is either one of the peripheral inhibitors that are known to innervate the opener muscles of the uropod (Larimer and Kennedy, 1969) or one of the closer motoneurons that exit through the third root (Higuchi, 1991). Although the response directionality is reversed, the transient response characteristics of this motoneurone clearly demonstrate the phasic nature of the effective leg proprioceptor inputs to the uropod motor system in both synaptic excitation and inhibition.

#### *Synaptic responses of premotor nonspiking interneurons to substratum tilt*

Premotor nonspiking interneurons showed synaptic activities that were similar to, but more variable than, those of motoneurons in response to substratum tilting. In the quiescent state, they typically showed little or no synaptic response to the stimulation (Fig. 5A), but they received greatly enhanced synaptic inputs from the leg proprioceptor system during walking (Fig. 5B) or in the active-standing state. Although nonspiking interneurons unambiguously responded to substratum tilting during walking in most cases, they

occasionally showed no discernible response to the same stimulation. The variability in the synaptic response of nonspiking interneurons suggests that the transmission of leg proprioceptive information is critically affected by some unknown processes occurring in the upstream synaptic pathway.

The synaptic interaction in nonspiking interneurons between inputs from the leg proprioceptors and those from the walking system was additive: when the animal began to walk, the interneurone shown in Fig. 6E received sustained hyperpolarizing input (Fig. 6A). In the course of walking, the sustained level was gradually changed, and tilt-evoked inputs in either the depolarizing or hyperpolarizing direction could be superimposed on this level. It was noted that the hyperpolarizing synaptic input to the interneurone associated with substratum tilting was as transient as that observed in motoneurons, but that the synaptic response during the return tilt was more prolonged than in motoneurons (Fig. 6D). When the animal was in the active-standing state, the synaptic response to substratum tilting in either direction became more prolonged than during walking (Fig. 6C). This finding is apparently inconsistent with the fact that motoneurons responded only transiently to substratum tilting during walking (Figs 2, 4). However, this nonspiking interneurone is not the

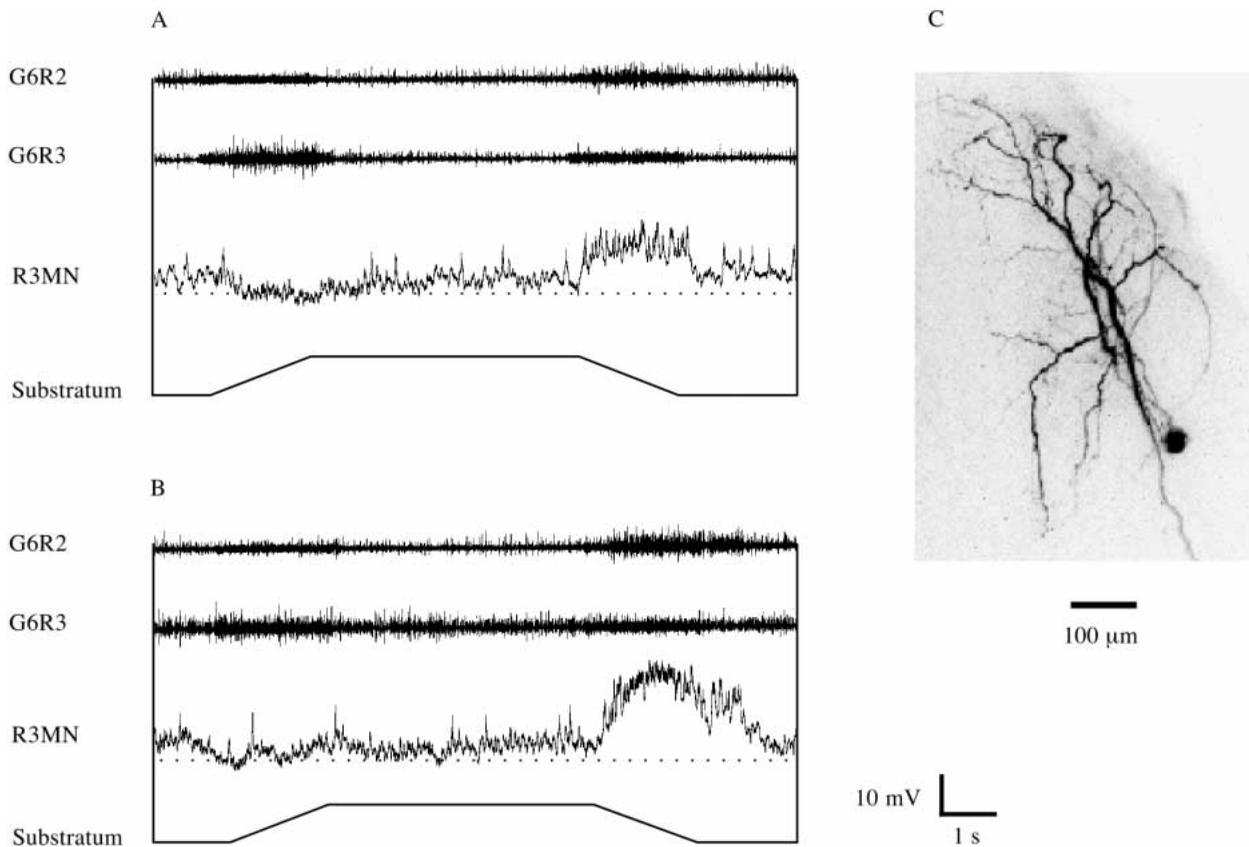


Fig. 4. Anomalous synaptic responses of a motoneurone exiting from the third root. (A) The side-up stimulus was applied while the animal was standing. The motoneurone (R3MN) showed an inhibitory response to the stimulus (Substratum), although extracellular spike activity in motoneurons of the whole third root (G6R3) showed an overall increase in activity while activity in the second root was decreased reciprocally (G6R2). In response to the return stimulus, the motoneurone showed an excitatory response while spike activity in the whole third root was reduced. (B) The same stimulus was applied during walking. The inhibitory response to the tilt stimulus was greater in the standing state (A) than during walking, whereas the excitatory response to the return stimulus was enhanced during walking (B). The dotted lines in A and B indicate the resting potential level. (C) The projected image of the motoneurone.

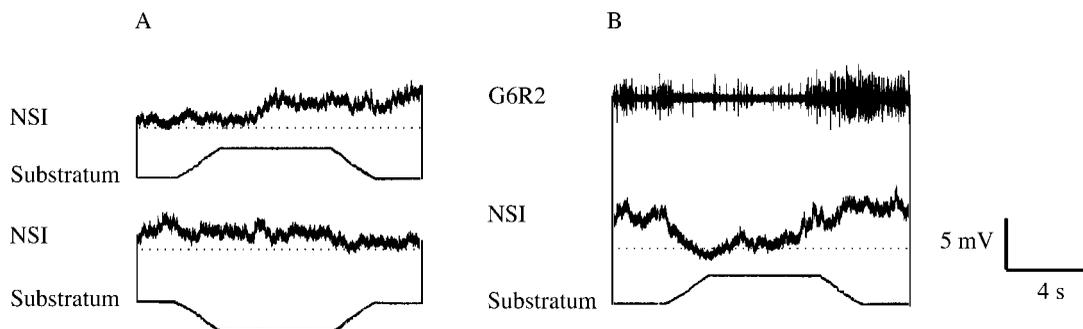


Fig. 5. State-dependent synaptic responses of a premotor nonspiking interneurone to substratum tilting. (A) The interneurone (NSI) showed no discernible response to the stimulus in either direction when the animal was at rest. Upward deflection of the substratum position monitor (Substratum) indicates the side-up direction. (B) During walking, the interneurone showed a hyperpolarizing response to side-up tilt superimposed on the continuously depolarized membrane potential and associated with a decrease in the spike activity of closer motoneurons (G6R2). The dotted lines indicate the mean resting potential level in the quiescent state.

only one that controls the activity of motoneurons. It seems that the synaptic response pattern of motoneurons is generated as the consensus of the activities of many premotor nonspiking

interneurons which show a certain degree of variability in their individual responses.

When constant depolarizing current pulses (6 nA) were

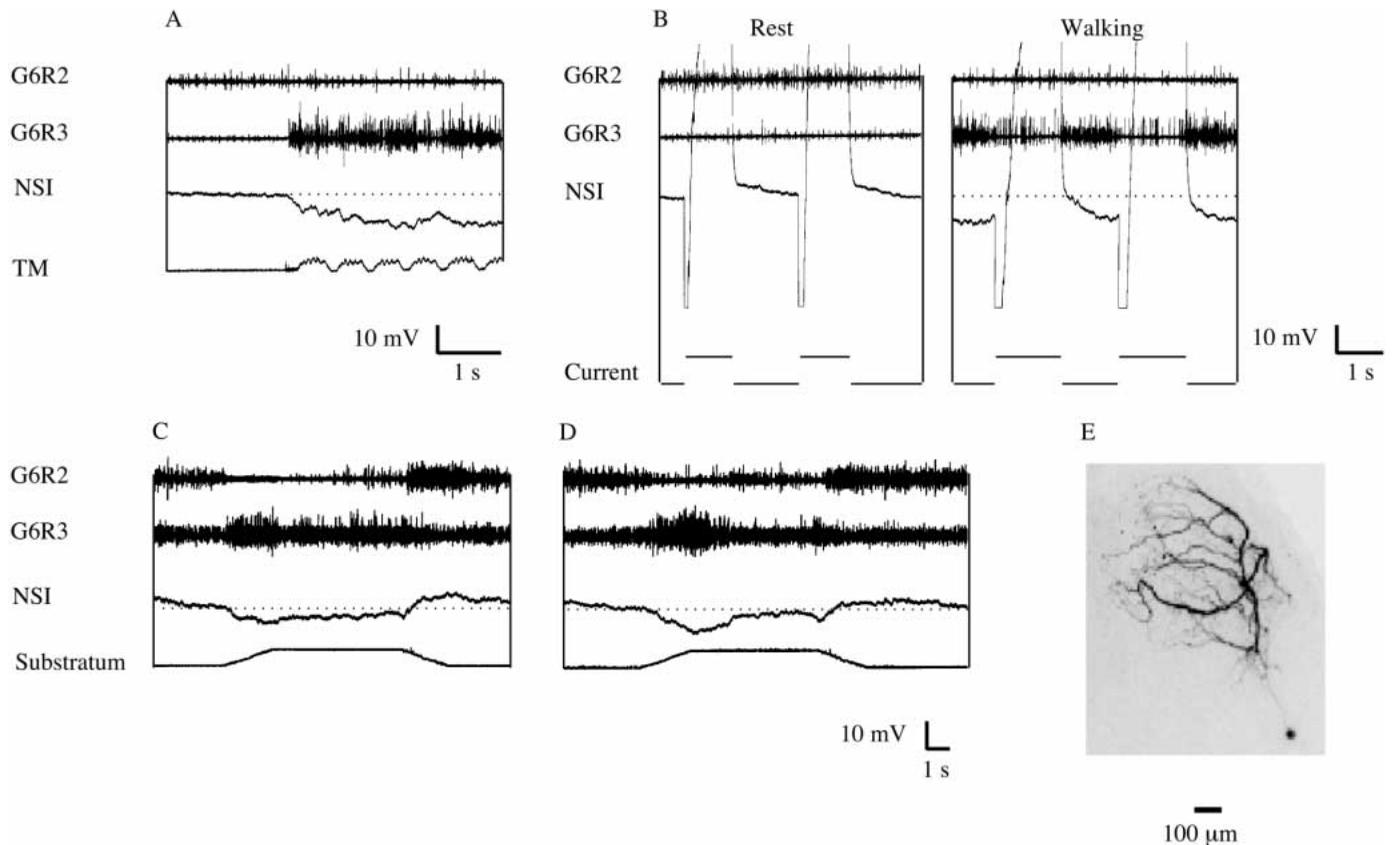


Fig. 6. Synaptic responses to the substratum tilting stimulus of a premotor nonspiking interneurone involved in disinhibition of motoneurons. (A) The interneurone (NSI) showed a sustained hyperpolarization during walking. Rotation of the treadmill is monitored by the bottom trace (TM). G6R2, spike activity of the second root; G6R3, spike activity of the third root. (B) When depolarizing current pulses (6 nA) were injected into the interneurone at rest (Current), no output effect was observed in motoneurone activities. When pulses of the same intensity were injected into the cell during walking, the increased spike activity was suppressed. (C) The interneurone showed a hyperpolarizing response to the side-up stimulus and a depolarizing response to the return stimulus (Substratum) when the animal was in the active-standing state. (D) When the animal was walking, the interneurone showed stronger transient responses to the same stimuli. In both cases, closer (G6R2) and opener (G6R3) motoneurons were activated in a reciprocal way by the stimuli. The dotted lines in A–D indicate the resting potential level. (E) The projected image of the interneurone.

injected into the interneurone, no effect was observed on the spike activity of motoneurons in the quiescent state, but an obvious decrease was observed during walking (Fig. 6B) and in the active-standing state. This result indicates that the inhibitory output of this interneurone to motoneurons becomes effective only when their background activity is elevated to a certain level. This recording does not show whether the synaptic output is transmitted to motoneurons, but remains ineffective because of the absence of background spike activities, or whether it is not transmitted to motoneurons at all as long as the animal remains in the quiescent state. However, the finding that motoneurons show no visible inhibitory synaptic response in the quiescent state (Fig. 1A) favours the latter possibility.

The nonspiking interneurone illustrated in Fig. 7D also showed different synaptic responses to substratum tilting depending on whether the animal was in the active-standing state (Fig. 7A) or engaged in walking (Fig. 7B). It received depolarizing synaptic inputs in response to substratum tilting

in the side-up direction and transient hyperpolarizing inputs to the return tilt in the active-standing state (Fig. 7A). The depolarizing synaptic response was associated with a decrease in motoneurone activity in the closer and an increase in activity in the opener, whereas the hyperpolarizing response was associated with an increase in motoneurone activity in the closer and a decrease in activity in the opener. These synaptic inputs were superimposed on the prevailing membrane potential level of the interneurone. When the animal was engaged in walking, this interneurone showed a depolarizing synaptic response to substratum tilting that was more prolonged than that occurring in the active-standing state, but there was no hyperpolarizing response to the return tilt (Fig. 7B). This observation further supports the possibility that the transmission of leg proprioceptive information to premotor nonspiking interneurons is critically affected by some unknown upstream mechanisms (Fig. 6). Injection of depolarizing current pulses into the cell caused a decrease in the spike activity of closer motoneurons but had no effect on

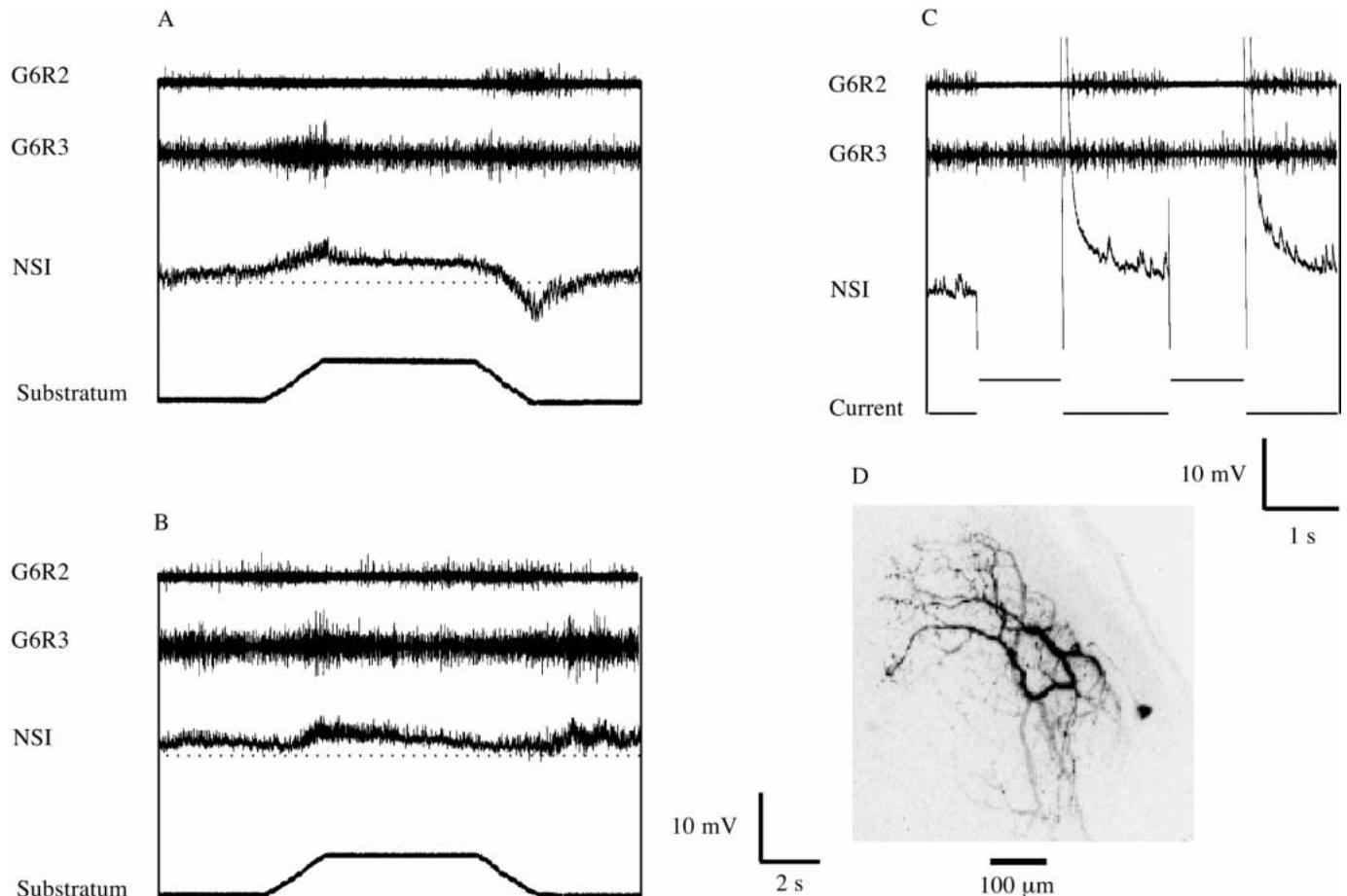


Fig. 7. Synaptic responses to the substratum tilting stimulus of a premotor nonspiking interneurone involved in the inhibition of motoneurones. (A) The interneurone (NSI) showed a depolarizing response to the side-up tilt and a hyperpolarizing response to the return stimulus (Substratum) when the animal was in the active-standing state. The closer (G6R2) and opener (G6R3) motoneurones were activated in a reciprocal way by the stimulus. (B) The same stimulus elicited less distinct responses in the same interneurone when the animal was engaged in walking. The dotted lines in A and B indicate the resting potential level. (C) When depolarizing current pulses (8 nA) were injected into the interneurone (Current), the spike activity of closer motoneurones (G6R2) was suppressed whereas that of the opener motoneurones (G6R3) was little, if at all, affected. (D) The projected image of the interneurone.

opener motoneurone activity (Fig. 7C). It is thus suggested that this interneurone is involved in the selective inhibition of closer motoneurone activities in uropod steering during locomotion.

### Discussion

The present study has demonstrated for the first time the synaptic activity of the uropod motor system that underlies the steering behaviour elicited by tilting of the substratum beneath the legs during walking in crayfish. It not only supports the previous results on the transient nature of the leg proprioceptive input (Schöne *et al.* 1976; Murayama and Takahata, 1996) and its gated control (Newland, 1989), but also reveals that the bilateral motor pattern of steering is generated by synaptic modulation of the background activity of motoneurones. A comparison of the synaptic responses in the quiescent state with those during walking or in the active-

standing state has also revealed a similarity in the organization of descending signal pathways for the statocysts and the leg proprioceptors, confirming the previous hypothesis that these pathways are controlled by multiple gates (Takahata and Murayama, 1992). The functional significance of the present findings is discussed in the following sections in relation to the neuronal mechanisms underlying facilitatory interactions between different types of behaviour.

#### *Synaptic modulation of the background excitation of motoneurones during steering behaviour*

Steering by the uropods in response to tilting consists of an opening of the uropod on one side and a closing of the opposite one from an initial position (Yoshino *et al.* 1980) and is actively maintained by continuous contraction of both the opener and closer muscles during walking and the abdominal posture movement (Takahata *et al.* 1984). It has been demonstrated by intracellular recordings from a whole-animal

preparation that both the opener and closer motoneurons receive sustained excitatory inputs during the abdominal posture movement (Takahata and Hisada, 1986), during walking and in the active-standing state (Murayama and Takahata, 1998). In the present study, we have shown that the steering behaviour of the uropods in response to tilting of the leg substratum is controlled by further increases or decreases in the background excitation of motoneurons, depending on the tilt direction. The closer motoneurons thus received depolarizing and hyperpolarizing synaptic inputs superimposed on the background depolarization when the substratum beneath the leg was tilted from the horizontal position in the side-down direction and hyperpolarizing inputs when the direction of tilt was reversed (Figs 1, 3). They received inputs in the opposite direction when the substratum was returned to the horizontal position.

The observation that the range of membrane potential fluctuation increased in the depolarizing response of motoneurons to substratum tilting suggests that the depolarization is caused by synaptic excitation rather than by disinhibition (Fig. 1A, Ci). The increased activity of motoneurons during steering is, therefore, thought to be due to a summatory interaction between the excitatory proprioceptor input and the background excitation. The proprioceptor input is subthreshold by itself (Fig. 1A), but can make synaptic summation with the background excitation to exceed the firing threshold (Fig. 1B). Comparison of the synaptic responses to the same stimulus shows that the enhancement is greater during walking than in the quiescent state. In general, the sustained depolarization will decrease the ionic driving force in synaptic activation so that the membrane potential change induced by the same excitatory synaptic activity becomes smaller at more depolarizing levels (Hille, 1992). The sustained depolarization that is maintained by continuous release of neurotransmitters will also cause adaptation in excitability (Nakajima and Onodera, 1969; Blight and Llinas, 1980; Cannone and Bush, 1980) and desensitization of receptors to suppress further synaptic activity (Katz and Thesleff, 1957; Feltz and Trautmann, 1982). The segmental synaptic input from cuticular sensory hairs was found to decrease in amplitude during the sustained depolarization (Fig. 1E). The present finding, however, that the proprioceptor input can make effective summation with the sustained depolarization (Fig. 1B), in spite of these potentially unfavourable conditions, suggests that there is some synaptic mechanism for boosting the proprioceptor input during walking. The fact that the axons of leg proprioceptors terminate in the thoracic ganglion (El Manira *et al.* 1991) suggests that the neuronal pathway from leg proprioceptors to the uropod motor system is polysynaptic. It is possible, therefore, that the proprioceptor input is further gated upstream of the motoneurons, in which it is also gated (Fig. 1B). An alternative, but not exclusive, possibility is that the proprioceptor input is enhanced in motoneurons by the excitatory input from the walking system, just as in the case of heterosynaptic facilitation in the molluscan central nervous

system (Kandel and Tauc, 1965; Castellucci and Kandel, 1976; Braha *et al.* 1990). Further study is needed to examine these possibilities by tracing the proprioceptive signals on their way down from the thoracic ganglia to the uropod motor system in the terminal abdominal ganglion.

Both the excitatory and inhibitory responses of motoneurons to substratum tilting were limited to its moving phase (Figs 1, 3, 4), although they were more prolonged during walking than in the active-standing state (Fig. 2). It is well known that the leg proprioceptors generally consist of both position- and movement-sensitive receptors (Burke, 1954; Wiersma and Boettiger, 1959; Bush, 1965*a,b*; Mill, 1976). Electromyographic recordings from uropod muscles have revealed their dynamic, but not static, activation by substratum tilting, suggesting that the descending proprioceptor pathway exclusively carries movement information on the displacement of the leg position (Murayama and Takahata, 1996). The possibility remains, however, that the proprioceptor pathway carries both position and movement information but that only the movement information is extracted and transmitted to the motoneurons by some local processing mechanism in the terminal abdominal ganglion. The present finding that not only the uropod motoneurons but also the premotor nonspiking interneurons selectively receive transient inputs from leg proprioceptors (Figs 6, 7) makes this possibility unlikely. We conclude, therefore, that the steering motor pattern during walking is primarily generated by transient proprioceptor inputs that further excite or inhibit the motoneurons from their background level of excitation. Some of the observed synaptic responses to the tilt stimulus contained initial phasic and subsequent tonic components (Figs 2B, 3A, 6C, 7A). However, the tonic component remained subthreshold in motoneurons. The positional proprioceptor signals, if any, are thus not functional in generating spike activities in motoneurons during the steering response.

#### *Multiple gate control of the descending proprioceptor pathway*

The finding that motoneurons show excitatory synaptic responses to leg substratum tilting in the quiescent state (Fig. 1A) indicates that there is a neuronal pathway that can reliably transmit spike signals from leg proprioceptors to the uropod motor system without any additional excitatory inputs. Neuronal pathways involving multiple synapses would be reliable only if the synaptic efficacy were sufficiently high. Hence, we hypothesize that the descending proprioceptor pathway drives the motoneurons directly. Further study is needed to demonstrate the monosynaptic connection between the descending interneurons carrying proprioceptor signals and uropod motoneurons, but the present results clearly indicate that a certain amount of proprioceptive signalling is transmitted to the motoneurons irrespective of whether the animal is engaged in walking or is at rest. These signals are subthreshold, however, so that no spike activity can be elicited in the motoneurons by the tilt stimulus in the quiescent state (Fig. 1A). During walking, the excitatory input from leg

proprioceptors makes synaptic summation with the sustained depolarization in motoneurons to elicit spikes (Fig. 1B). The proprioceptive signals are thus gated in motoneurons by the sustained depolarizing signals associated with walking. It should be noted that the proprioceptive input superimposed on the sustained depolarization during walking is more enhanced than that observed in the quiescent state (Fig. 1A). Possible mechanisms, including the upstream gating of proprioceptor signals, are discussed above.

In contrast to the excitatory input, the inhibitory synaptic input is completely absent in the quiescent state (Fig. 1A), although it can be unambiguously observed during walking (Figs 1B, 3, 4). One possible reason for this difference is that the inhibitory input is present but can cause no membrane potential change because its equilibrium potential is approximately equal to the resting potential. However, the finding that the membrane potential fluctuation was unaffected during stimulation (Fig. 1Cii) strongly suggests the absence of any inhibitory synaptic input in the quiescent state. It is thus proposed that the neuronal pathway carrying the inhibitory synaptic input and probably also the excitatory pathway from the leg proprioceptor system are gated upstream of the motoneurons (Fig. 8) by a mechanism similar to that observed in the motoneurons themselves and based on synaptic summation. The exact site of this upstream gating is at present unknown. Since the premotor nonspiking interneurons do not generate spikes and have no threshold, they are unlikely to be the site of gating unless they are endowed with special abilities such as heterosynaptic facilitation (Kandel and Tauc, 1965; Castellucci and Kandel, 1976; Braha *et al.* 1990). One possible site of upstream gating is the proprioabdominal interneurons that have been identified in the descending statocyst pathway (Takahata and Murayama, 1992). These interneurons, with somata in the anterior abdominal ganglia and projecting directly to the terminal ganglion, can sum signals from the statocyst and the abdominal posture system to generate spikes only while the animal is engaged in abdominal posture movements. Further study is needed to test the working hypothesis proposed in Fig. 8 by examining this possibility in the descending proprioceptor pathway.

In summary, the full motor pattern of uropod steering, consisting of uropod opening on one side and closing on the opposite side from the initial configuration, can be generated by the tilt stimulus only when the background excitation of motoneurons is tonically increased to an intermediate level during walking, so that, depending on tilt direction, it can be further increased or decreased by leg proprioceptor inputs. This excitation level is attained by antagonistic inputs from premotor nonspiking interneurons (Murayama and Takahata, 1998). In the quiescent state, the motor output is absent or, at most, abortive, since the excitatory input remains subthreshold and the inhibitory input is absent. During walking, however, the leg proprioceptive input can sum with the increased background excitation in the motoneurons to elicit spikes. It is also enhanced by upstream gating mechanisms enabling it to drive the motoneurons effectively. This type of

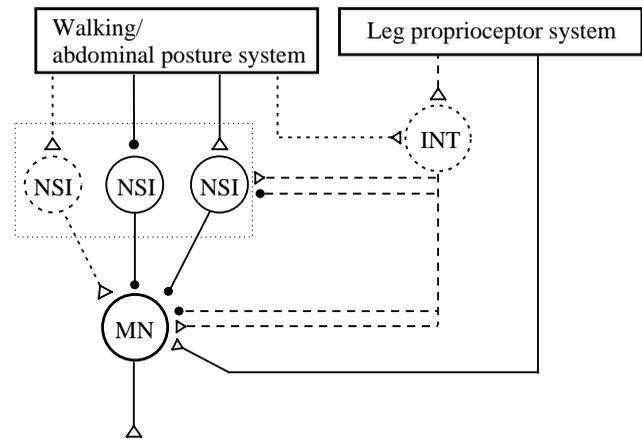


Fig. 8. Proposed synaptic pathways mediating descending signals from the walking generator system and the leg proprioceptor system to the uropod motor system. Since the walking system is functionally coupled with the abdominal posture system (Takahata *et al.* 1984), both systems are combined in this diagram. Open triangles, excitatory connections; closed circles, inhibitory connections. The dotted pathways and cells were demonstrated in a previous study (Takahata and Murayama, 1992). Results obtained in this study not only demonstrate that the signals from both systems converge onto motoneurons (MN) and premotor nonspiking interneurons (NSI) but also suggest that they converge onto a population of spiking interneurons (INT), including the proprioabdominal interneurons, located upstream from motoneurons and nonspiking interneurons, as indicated by broken lines. The input from these interneurons to the uropod motor system is either excitatory or inhibitory, depending on the direction of tilting.

organization is functionally relevant, since the postural reflexes become most important during locomotion (Grillner, 1985; Orlovsky, 1991). Facilitatory interaction between different but related behavioural acts has been described, particularly in relation to postural and locomotory behaviour patterns, in both vertebrates and invertebrates (Lind, 1959; Dawkins, 1976; Mori *et al.* 1991). Although further research is needed, the present series of experiments suggests the working hypothesis that a common mechanism, involving modulation of the background activity of the motor system by multiple-gate-controlled sensorimotor pathways, underlies the facilitatory interaction between different behaviour patterns in crayfish.

This work was supported in part by Grants-in-Aid (07554071, 09440274) from MESSC Japan.

## References

- BLIGHT, A. R. AND LLINAS, R. (1980). The non-impulsive stretch-receptor complex of the crab: a study of depolarization-release coupling at a tonic sensorimotor synapse. *Phil. Trans. R. Soc. Lond. B* **290**, 219–276.
- BRAHA, O., DALE, N., HOCHNER, B., KLEIN, M., ABRAMS, T. W. AND KANDEL, E. R. (1990). Second messengers involved in the two processes of presynaptic facilitation that contribute to sensitization

- and dishabituation in *Aplysia* sensory neurons. *Proc. natn. Acad. Sci. U.S.A.* **87**, 2040–2044.
- BURKE, W. (1954). An organ for proprioception and vibration sense in *Carcinus maenas*. *J. exp. Biol.* **31**, 127–137.
- BUSH, B. M. H. (1965a). Proprioception by chordotonal organs in the mero-carpopodite and carpo-propodite joints of *Carcinus maenas* legs. *Comp. Biochem. Physiol.* **14**, 185–199.
- BUSH, B. M. H. (1965b). Proprioception by the coxo-basal chordotonal organ, CB, in legs of the crab, *Carcinus maenas*. *J. exp. Biol.* **42**, 285–297.
- CANNONE, A. J. AND BUSH, B. M. H. (1980). Reflexes mediated by non-impulsive afferent neurones of thoracic-coxal muscle receptor organs in the crab, *Carcinus maenas*. II. Reflex discharge evoked by current injection. *J. exp. Biol.* **86**, 305–331.
- CASTELLUCCI, V. F. AND KANDEL, E. R. (1976). Presynaptic facilitation as a mechanism for behavioral sensitization in *Aplysia*. *Science* **194**, 1176–1178.
- DAVIS, W. J. (1968). Lobster righting responses and their neural control. *Proc. R. Soc. Lond. B* **170**, 435–456.
- DAWKINS, R. (1976). Hierarchical organization and postural facilitation: Rules for grooming in flies. *Anim. Behav.* **24**, 739–755.
- EL MANIRA, A., CATTART, D. AND CLARAC, F. (1991). Monosynaptic connections mediate resistance reflex in crayfish (*Procambarus clarkii*) walking legs. *J. comp. Physiol.* **168**, 337–349.
- FELTZ, A. AND TRAUTMANN, A. (1982). Desensitization at the frog neuromuscular junction: a biphasic process. *J. Physiol., Lond.* **322**, 257–272.
- GRILLNER, S. (1985). Neurobiological bases of rhythmic motor acts in vertebrates. *Science* **228**, 143–149.
- HIGUCHI, T. (1991). Innervation pattern of some tonic muscles in the uropod of the crayfish, *Procambarus clarkii*. *Zool. Sci.* **8**, 193–196.
- HILLE, B. (1992). *Ionic Channels of Excitable Membranes*. Sunderland: Sinauer.
- KANDEL, E. R. AND TAUC, L. (1965). Heterosynaptic facilitation in neurones of the abdominal ganglion of *Aplysia depilans*. *J. Physiol., Lond.* **181**, 1–27.
- KATZ, B. AND THESLEFF, S. (1957). A study of ‘desensitization’ produced by acetylcholine at the motor endplate. *J. Physiol., Lond.* **138**, 63–80.
- KOCH, C., POGGIO, T. AND TORRE, V. (1983). Nonlinear interactions in a dendritic tree: Localization, timing and role in information processing. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2799–2802.
- LARIMER, J. L. AND KENNEDY, D. (1969). Innervation patterns of fast and slow muscles in the uropods of crayfish. *J. exp. Biol.* **51**, 119–133.
- LIND, H. (1959). The activation of an instinct caused by a ‘transitional action’. *Behaviour* **14**, 123–135.
- MILL, P. J. (1976). Chordotonal organs of crustacean appendages. In *Structure and Function of Proprioceptors in the Invertebrates* (ed. P. J. Mill), pp. 243–297. London: Chapman & Hall.
- MORI, S., SAKAMOTO, T. AND TAKAKUSAKI, K. (1991). Interaction of posture and locomotion in cats: Its automatic and volitional control aspects. In *Neurobiological Basis of Human Locomotion* (ed. M. Shimamura, S. Grillner and V. R. Edgerton), pp. 21–32. Tokyo: Japan Scientific Societies Press.
- 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. (1998). Neuronal mechanisms underlying the facilitatory control of uropod steering behaviour during treadmill walking in crayfish. I. Antagonistically regulated background excitability of uropod motoneurons. *J. exp. Biol.* **201**, 1283–1294.
- NAKAJIMA, S. AND ONODERA, K. (1969). Membrane properties of the stretch receptor neurones of crayfish with particular reference to mechanisms of sensory adaptation. *J. Physiol., Lond.* **200**, 161–185.
- NEWLAND, P. L. (1989). The uropod righting reaction of the crayfish *Procambarus clarkii* (Girard): an equilibrium response driven by two largely independent reflex pathways. *J. comp. Physiol.* **164**, 685–696.
- ORLOVSKY, G. N. (1991). Cerebellum and locomotion. In *Neurobiological Basis of Human Locomotion* (ed. M. Shimamura, S. Grillner and V. R. Edgerton), pp. 187–199. Tokyo: Japan Scientific Societies Press.
- PEARSON, K. G. AND ROWELL, C. H. F. (1977). Functions of tonic sensory input in insects. *Ann. N.Y. Acad. Sci.* **290**, 114–123.
- REICHERT, H. AND ROWELL, C. H. F. (1989). Invariance of oscillator interneurone activity during variable motor output by locusts. *J. exp. Biol.* **141**, 231–239.
- SCHÖNE, H., NEIL, D., STEIN, A. AND CARLSTEAD, M. (1976). Reactions of *Palinurus vulgaris* to substrate tilt (I). *J. comp. Physiol.* **107**, 113–128.
- SHEPHERD, G. M. AND BRAYTON, R. K. (1987). Logic operations are properties of computer-simulated interactions between excitable dendritic spines. *Neuroscience* **21**, 151–166.
- TAKAHATA, M. AND HISADA, M. (1986). Sustained membrane potential change of uropod motor neurons during the fictive abdominal posture movement in crayfish. *J. Neurophysiol.* **56**, 702–717.
- 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.
- TAKAHATA, M. AND MURAYAMA, M. (1992). Multiple gate control of the descending statocyst–motor pathway in the crayfish *Procambarus clarkii* Girard. *J. comp. Physiol.* **170**, 463–477.
- WIERSMA, C. A. G. AND BOETTIGER, E. G. (1959). Unidirectional movement fibres from a proprioceptive organ of the crab *Carcinus maenas*. *J. exp. Biol.* **36**, 102–112.
- YOSHINO, M., TAKAHATA, M. AND HISADA, M. (1980). Statocyst control of the uropod movement in response to body rolling in crayfish. *J. comp. Physiol.* **139**, 243–250.