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Proprioceptive Reflex Responses of the Efferent Axons to Passive and Active Movements in the Cheliped of the Crayfish*

By

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(With 8 Text-figures)

Studies on a series of proprioceptive organs in the joints of the chelipeds and walking legs of decapod *Crustacea* have been made physiologically by many investigators (Burke, 1954; Wiersma and Boettiger, 1959; Wiersma, 1959; Cohen, 1962; Mendelson, 1963; Muramoto and Murayama, 1965), and recent study of these organs using the electron microscope has revealed their fine structure (Whitear, 1960).

However, studies dealing with the reflex discharge mechanism from the proprioceptive organs to the efferent nerve via the central nervous system are quite rare. Bush (1962) studied the proprioceptive reflex response in the walking leg of *Carcinus* to passive movement of the dactylus and found that all the primary reflex responses were the proprioceptive motor and inhibitor 'resistance reflexes' for control and co-ordination of the walking leg. It is questionable whether similar proprioceptive reflexes are evoked also in the cheliped whose function is different from that of the walking leg in the freely living animal, and evoked by active movements of the joint through contractions of the appropriate muscles.

Burke (1954) reported that the effect of the active movement with a maintained contraction of the flexor differed from that due to a passive change of position in that there were continuous PD-sensory discharges in spite of the case of visible movement of the dactylus, and similar though less intense sensory discharges occurred even with isometric contraction of the flexor. These results indicate that there may be a significant difference, even in the reflex efferent responses to the active movements.

The present study was designed to clarify the mechanism of these proprioceptive reflex responses of the efferent axons through PD-afferent impulses to passive and active movements of the dactylopodite in the cheliped of the crayfish.

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* This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honour of his sixtieth birthday, June 21, 1966.

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Material and Methods

Preparation: The crayfish, *Procambarus clarkii* was used throughout. The animal was cooled in a refrigerator to reduce activity and to minimize the blood flow before preparation. This routine made it very easy to observe the axons without any elabroate prevention of blood flow. After cooling, the apodemes of the coxo-basal levator muscles were carefully severed at their articulations to prevent autotomy. A small part of the shell of the ventral side with the underlying muscle was removed near the base of the propodite, where the isolation of the required axons was easy in comparison with other parts, both efferent axons and the PD-afferent nerve bundle being separated from the main nerve. The animal was fixed with rubber bands ventral side up in plastic chamber filled with van Harrveld's solution (van Harreveld, 1936) the chelipeds being fixed in the naturally opened position. And then the required axons were exposed and isolated for recording and stimulation by removing carefully the connective tissue around the nerve under a binocular microscope (Fig. 1).

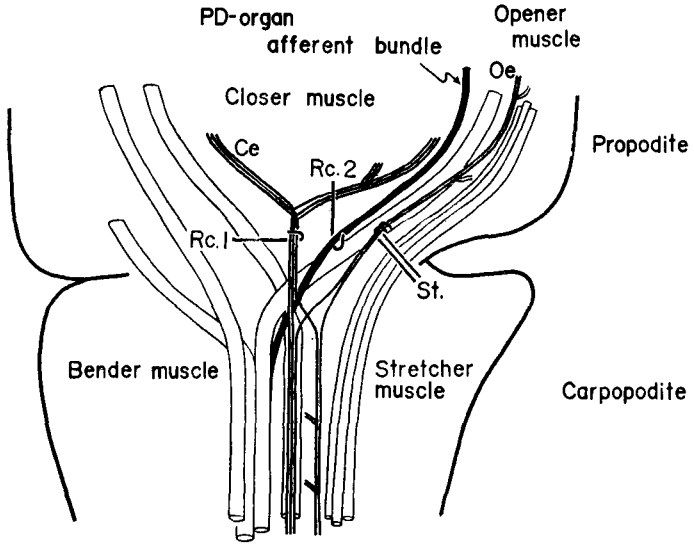

Fig. 1. Diagram of efferent axons innervating the closer (Ce) and opener (Oe) muscles and PD-organ afferent fibre bundle of the cheliped of the crayfish. Electrode system for recording (Rc. 1 and Rc. 2) and stimulation (St) used in the experiments is shown in Fig. 7

Stimulation and recording: In order to investigate a complete reflex loop (from the PD-afferent nerve to the efferent axons via the central nervous system), recordings were usually made simultaneously from the PD-afferent nerve bundle and the efferent axons (the fast, slow, and inhibitory axons) supplying the closer muscle or those (the slow and inhibitory axons) supplying the opener. When the opener efferent axons were stimulated, recordings were made from the closer efferent axons, and vice versa. In some cases, recordings were made simultaneously from the axons supplying both muscles(the opener and

closer muscles).

The lead was made monophasic by raising the nerve above the fluid surface with a hooked Ag-AgCl electrode (an active electrode) held in a micromanipulator. The indifferent electrode of Ag-AgCl type remained in the fluid and was connected with the earth. Nerve impulses were fed to the dual beam cathode ray oscilloscope after amplification with a RC amplifier. Displacement of the active electrode following the movement of the dactylopodite prevented the recording of impulses, so the efferent axons from which impulses were to be recorded were cut distally to the electrode to reduce the disturbance. However, the PD-afferent bundle was left intact and was slightly lifted out of the saline bath so as not to stretch or damage it.

The PD-organ was mechanically stimulated by moving the dactylopodite both passively and actively. The passive movement(at an angle of about 50° from the completely closed to "open" positions) was produced by pulling the thread connected with the tip of the dactylopodite. On the other hand, the active movement was produced by contraction of one of the opener or closer muscles produced by application of electrical stimuli to the appropriate motor axons. Using a square pulse generator as a stimulator, repetitive pulses (each pulse was usually 0.1 msec in duration) were fed to a pair of small stimulating electrodes of Ag-AgCl type. The axons to be stimulated were transected proximally to the stimulating electrodes and were also lifted out of the saline bath for stimulation.

Movement of the dactylopodite was registered as follows. The tip of the dactylopodite was connected with a small bottle filled with mercury through which a resistance string was stretched. This bottle was movable according to the dactylopodite movement, therefore the movement was transduced to the resistance change. This device functioned as an element of a bridge circuit, the out-put of which was displayed on one beam of the oscilloscope, together with the response of one of the two axon bundles.

Results

As was mentioned in the method, the axon bundle supplying the closer muscle consists of three axons: viz. the fast, slow, and inhibitory axons; and that supplying the opener consists of two axons: the slow and inhibitory axons. In this experiment, records of reflex discharges in the efferent nerve shown in the following figures were obtained from the axon bundle described above, not from an isolated single axon; for example, nerve impulses occurring in the slow motor and inhibitory axons for the opener were recorded simultaneously with a recording electrode. Therefore, it is necessary to distinguish in which axon the impulses occur. The axon can be identified from its impulse amplitude, because the spike amplitude of action potential recorded extracellularly is directly proportional to the fibre diameter (Eckert and Zacharová, 1957). In fact, this was experimentally proved correct by recording the spike evoked by stimulation of an isolated axon. In the closer efferen axon bundle, the spike amplitude of the fast axon was the greatest, the slow the second, and the inhibitory the smallest. But in the opener efferent axon bundle, as the sizes of the slow and inhibitory axons were not greatly different from each other, discrimination of axon was more difficult. It general, however, the size of the inhibitory axon was greater than that of the slow one.

The discharges in the PD-afferent bundle, seen in the following figures, do not show the responses of all the nerve fibres involved. The impulses of the large fibres were easily recorded, but not those of the small fibres. The nerve bundle, moreover, was slightly lifted out of the saline bath for recording. Therefore, it seems that only the impulse discharges occurring in the relatively large fibres could be picked up. Especially during closing and opening movements of the dactylopodite, a strong discharge appeared to occur. In contrast, when the dactylopodite was held stationary at a certain position, not so many discharges in the afferent fibres were produced. This seems to be because the nerve impulses from the position fibre is difficult to record as they are small in diameter (Bush, 1965), but not because they are small in number. Therefore, these results cannot clearly reveal the relationship between the input and output signals; only the general features can be observed.

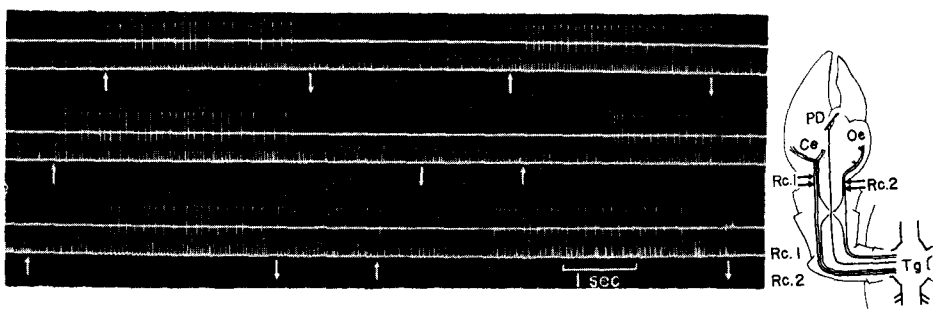

Fig. 2. Reflex responses of the closer (upper beam) and opener (lower beam) efferent a axons to passive movement of the dactylopodite. Closer impulses, larger: slow motor axon; smaller: inhibitory axon. Opener impulses, larger: slow motor axon; smaller: inhibitory axon. The variation in height of the impulses in lower beams is due to the fluid movement. The beginning of opening and closing of the dactylopodite is indicated by upward and downward arrows respectively. The figure on the right side of record shows the positions of recording electrodes. Rc. 1 and Rc. 2: recording electrodes. Ce and Oe: efferent axons supplying the closer and opener muscles respectively. PD: PD:organ. Tg: thoracic ganglion.

The reflex discharges by the passive movements: A type of reflex response elicited in the opener and closer axon bundles to passive movements of the dactylopodite is shown in Fig. 2. In the static state of the dactylopodite at the completely closed position before the movement the slow opener motor fibre kept discharging impulses at a frequency of about 7 impulses/sec. If the dactylopodite was opened passively, conspicuous discharges occurred not only in the slow closer motor but also in the opener inhibitor; the slow opener motor having ceased to discharge. On passive closing of the dactylopodite, on the other hand, discharges in the slow opener motor appeared, but the discharge in the slow closer motor suddenly stopped. The discharges in the opener inhibitor, gradually decrease in

frequency, and also ceased. The frequency of discharges in each fibre showed a tendency to increase gradually, as the speed of movement of the dactylopodite was increased. In this case, discharges in the closer inhibitor also occurred irregularly throughout, but this was independent of the movement of the dactylopodite and not noticeable. No discharge in the fast closer motor was observed at all. The results described above are in agreement with those obtained by Bush (1962) for the walking legs. However, such reflex responses of the efferent fibres as described above were not so common in the chelipeds.

Figs. 3 and 4 show the discharges in the PD-afferent fibre bundle and in the

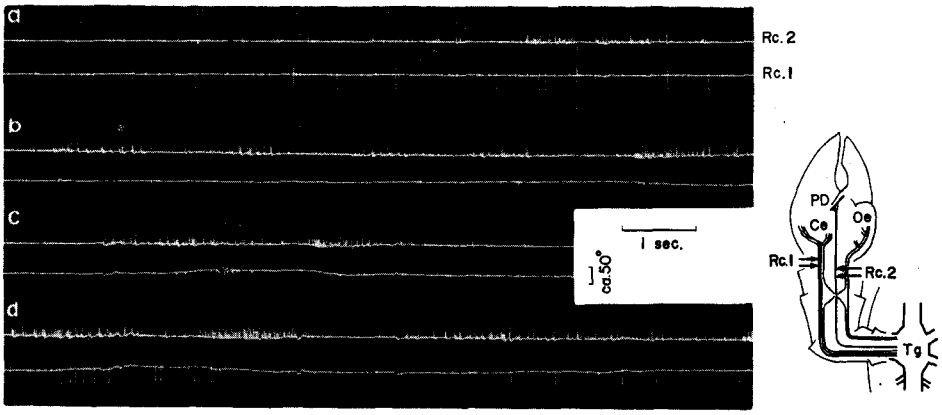

Fig. 3. Responses of the PD-organ afferent fibres (upper beam) and reflex responses of the closer efferent axons (lower to passive movement of the dactylopodite. Closer impulses, large: fast motor axon; middle: slow motor axon; and small: inhibitory axon. Movement of the dactylopodite is recorded on the lower beam together with responses of the closer efferents. Upward: opening, and downward: closing. The initial level indicates completely closed position of the dactylopodite. A vertical bar indicates calibration of the angular displacement of the dactylopodite.

efferent axons to the closer muscle elicited by passive movement of the dactylopodite. Three kinds of impulses elicited in the closer efferent axons, viz. the fast, slow, and inhibitory axons, were observed, see Fig. 3, a. These discharges could be readily evoked by mechanical stimulation of the ventral surface of the abdomen, the mouth part, or the telson, and they are also observed during spontaneous movement of the animal. On opening of the dactylopodite, phasic discharges in the fast closer motor occurred following the discharges in the slow one (Fig. 3, c). Such a response in the fast closer motor, being relatively easily elicited by mechanical stimulus to the body surface as mentioned above, was only rarely evoked by the PD-afferent impulses. Besides, the discharge in the slow closer motor occurred not only on opening but also in the initial phase of closing with two or three

impulses (Fig. 3, b-d).

Fig. 4, shows the response of the slow closer motor which was produced only during opening. The frequency of the discharges in the slow opener motor was, in general, proportional to the speed of the dactylopodite movement (Figs. 3 and 4). In a few cases, however, the frequency of impulse discharges in the slow closer motor was different in each case, though the dactylopodite was opened at almost the same speed. In other cases, on opening at sufficient speed to evoke discharges, no impulse occurred in the slow closer motor. On the other hand, in many cases no discharge in the closer inhibitor was produced by movement of the dactylopodite (Fig. 4), except the results of Fig. 3. In this case, its discharge pattern was irregular, but it was more prominent on closing and at the stationary position of the dactylopodite (Fig. 3, b-d).

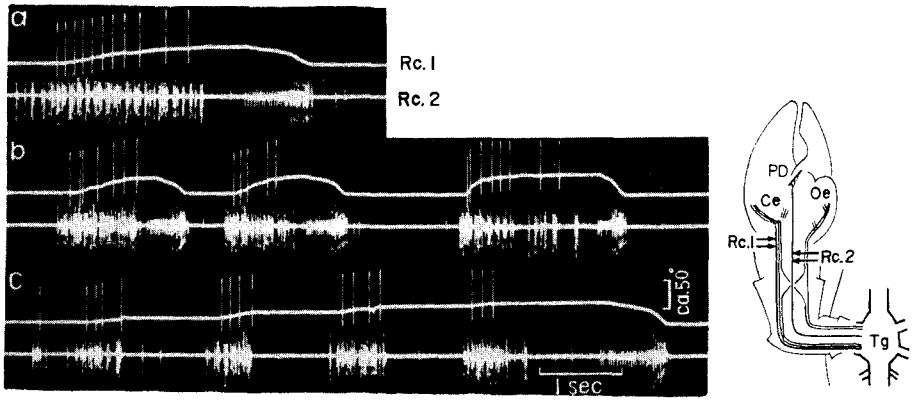

Fig. 4. Responses of the PD-organ afferent fibres (lower beam) and reflex responses of the closer efferent axons (upper beam) to passive movement of the dactylopodite. Closer impulses, slow motor axon only. Movement of the dactylopodite is indicated in the upper beam. Upward: opening; downward: closing; and the initial level; the completely closed position.

Responses obtained from the PD-afferent bundle and the opener efferent axons to movement of the dactylopodite are shown in Fig. 5. Passive opening produced conspicuous discharges in the opener inhibitor, the frequency of which increased according to the movement speed of the dactylopodite. In these records, a relationship between the discharges in the PD-afferent fibres and the reflex discharges in the efferent axons can be clearly observed. This opener inhibitor discharge also appeared at low frequencies on closing. On the other hand, discharges in the slow opener motor were produced at low frequency with the discharges in the opener inhibitor on closing (Fig. 5, a and b), but they were not always produced (Fig. 5, c).

In general, opening of the dactylopodite elicited discharges both in the slow closer motor and in the opener inhibitor. However, in comparsion with Bush's

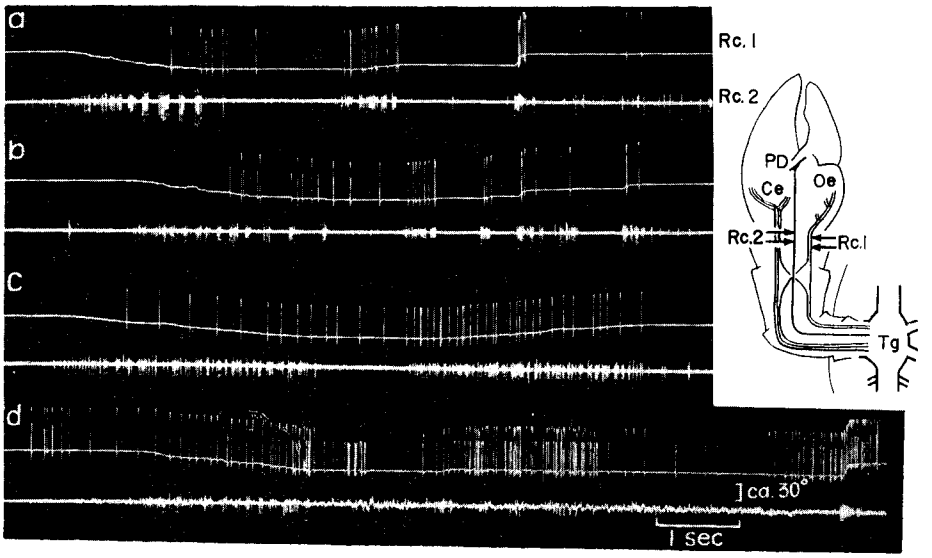

Fig. 5. Responses of the PD-afferent fibres (lower beam) and reflex responses of the opener efferent axons (uppr beam) to passive movement of the dactylopodite. Opener impulses, larger: inhibitory axon; smaller: slow motor axon. Movement of the dactylopodite is recorded in the upper beam. Upward: opening; downward: closing; and the initial level: opened position at about 30°.

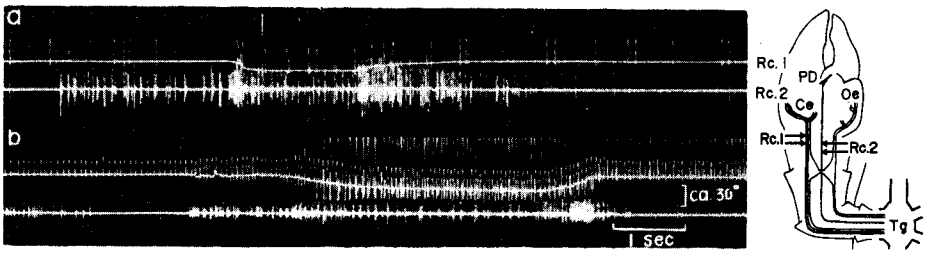

Fig. 6. Responses of the PD-afferent fibres (lower beam) and reflex responses of the closer efferent axons (upper beam) to passive movement, obtained from the preparation whose excitability was highly increased. Closer impulses, large: fast motor axon; middle: slow motor axon; and small: inhibitory axon. Movement of the dactylopodite is recorded on the upper beam, upward: closing; and downward: opening.

results and the first part of the present results, conspicuous discharges in the slow opener motor did not occur on closing. These results indicate that the effect of the reflex discharges makes the dactylopodite close, but not open more readily; the opener inhibitor discharges occur on opening in order to avoid over-opening of the dactylopodite, but no discharge to prevent closing is observed.

Besides the responses already described, a more complex reflex mechanism may be involved in the ganglionic transmission. As shown in Fig. 5, d and Fig. 6, when the activity of the animal increased, though the reflex discharges in the efferent axons had a tendency to occur, spontaneous discharges in the efferent axons were dominant and reflex response by movement of the dactylopodite was hardly observed. On the contrary, there were many cases where no reflex response was evoked by any impulse of the PD-afferent fibres, produced normally by the movement of the dactylopodite. This result was independent of the preparation used but the responsiveness of the preparation varied with time. Even under this condition, tactile stimulus to various parts of the body, such as the antennae, the mouth, any part of the ventral surface, the telson, etc. could easily evoke response in the efferent axons (Fig. 3, a). Therefore, it may be considered that the fact that no reflex response occurred by the movement of the dactylopodite was not owing to

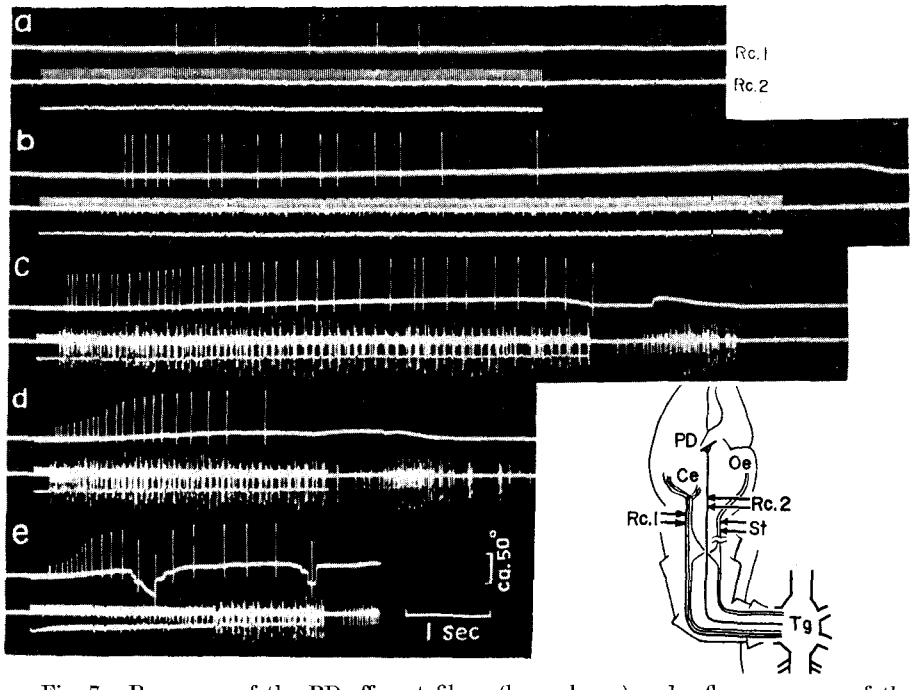

Fig. 7. Responses of the PD-afferent fibres (lower beam) and reflex responses of the closer efferent axons (upper beam) to various contractions of the opener muscle of the dactylopodite produced by stimulation of the opener efferent axons. Closer impulses, slow motor axon only. Frequency of stimulation, a: 40/sec, b: 50/sec; c: 70/sec; d: 80/sec; and e: 100/sec. Duration of the stimulus is indicated by the series of stimulus artifacts like a rattan blind. Movement of the dactylopodite is recorded on the upper beam. Upward: opening; and the initial level: completely closed position. The positions of stimulating electrodes (St) are shown in the figure on the right side.

fatigue or damage of any part of the reflex pathway, and that the reflex response from the PD-afferent was centrally inhibited in the ganglion.

The reflex discharges by active movements: The dactylopodite was moved actively at various speeds by contraction of the opener or closer muscles produced by repetitive stimuli at various frequencies to the efferent nerve supplying them, and then discharges in the PD-afferent and efferent fibres were recorded. The results reveal that, contrary to the author's expectation, there is no obvious difference in the reflex response between the active and the passive movements.

Active opening elicited striking discharges in the slow closer motor like those in passive opening (Fig. 7). The discharge frequencies at the initial phase of opening were 2 impulses/sec on the opening produced by 40 stimuli/sec to the efferent axons, 6/sec by 50/sec, 11/sec by 70/sec, and 13/sec by 80 and 100/sec. Contrasting with the results obtained by passive opening from the same animal (Fig. 4), it was observed that the slow closer motor on active opening continued to discharge impulses not only during movements of the dactylopodite but also at its stationary opened position.

On the other hand, in some cases, active closing produced by a stimulus applied to the closer axons evoked discharges in the opener inhibitor at low

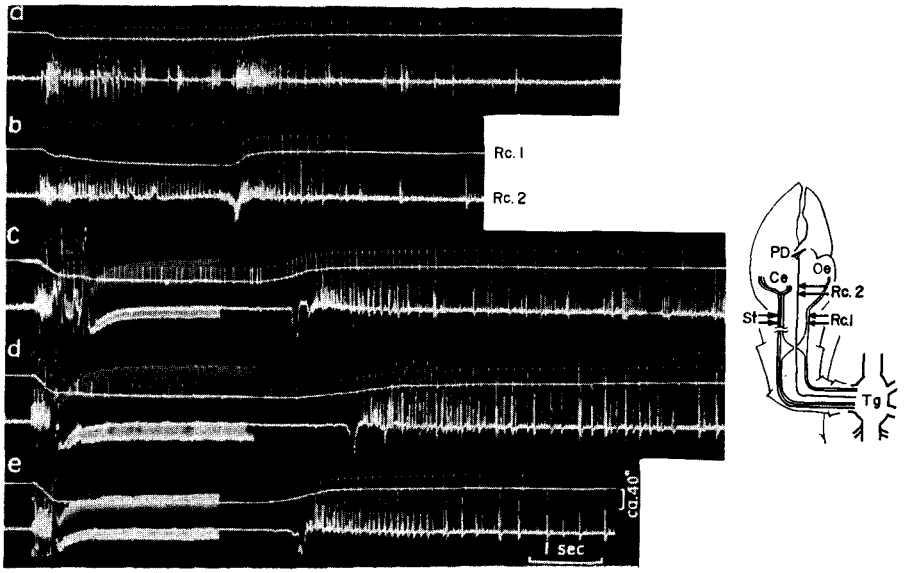

Fig. 8. Responses of the PD-afferent fibres (lower beam) and reflex responses of the opener efferent axons (upper beam) to active closing of the dactylopodite produced by stimulation of the closer efferent axons and to rebound opening. Opener impulses, inhibitory axon only. Frequency of the stimulation, a: 10/sec; b: 20/sec; c: 40/sec; d: 60/sec; and e: 80/sec. Movement of the dactylopodite is recorded on the upper beam. Downward: closing; and the initial level: opened position at about 40°.

frequencies not only during closing movement but also at the stationary closed position (Fig. 8). On rebound opening produced passively, striking tonic discharges occurred also in the opener inhibitor as in the case of the passive opening already mentioned. On rebound opening, the discharges in the opener inhibitor were maintained for a long period compared with passive movements. This may be due to a slow relaxation of the closer muscle, judging from the PD-afferent discharges. No discharge in the slow opener motor occurred here.

Discussion

Since the PD-organ is stretched between the dactylopodite and apodeme of the closer muscle, active movement of the dactylopodite should stimulate the PD-organ more effectively than passive movement, as was suggested in Burke's paper (1954). Therefore, it was expected that there would be some difference in the reflex efferent responses to active and passive movements, at least to active closing of the dactylopodite and isometric contraction of the closer muscle. In the above experiments, however, no significant difference in the reflex efferent responses could be observed.

Indeed, in the natural state, the animal can hold an object with the cheliped in different ways according to whether it is hard or soft, not in an all-or-nothing fashion. This mechanism is not yet clear, but the movement of the dactylopodite, in this case also, may be controlled by the reflex efferent impulses from the PD-afferent fibres through the central nervous system.

It remains uncertain whether the proprioceptive reflex responses observed in the present study are monosynaptic or polysynaptic. Bush (1962) reported that 'the remarkable specificity and consistency of the proprioceptive reflex responses observed in the *Carcinus* walking legs suggested that they might represent monosynaptic, afferent-efferent reflexes". Indeed, in the present study on the *Procambarus* chelipeds also, the reflexes in the slow closer motor and those in the opener inhibitor are quite specific, but there are cases where reflex discharges in the fast closer and slow opener motors and those in the closer inhibitor are not so specific. Tactile stimulation to the body surface could more consistently elicit discharges in the efferent axons, perhaps through giant nerves. Moreover, the presynaptic pathway through the tangled fibre mass of neuropil cannot be exactly measured (Preston and Kennedy, 1960).

Afferent fibres which signal the position of the dactylopodite in the proprioceptive organ in Crustacea has been reported (Wiersma and Boettiger, 1959; Cohen, 1962; Bush, 1965). However, it is doubtful whether there is a real 'position fibre' or whether it is only a kind of movement fibre. In the present study, no remarkable reflex response was observed when the dactylopodite was kept stationary at given positions. A further experiment on this point is necessary using sinusoidal movement of the dactylopodite at various frequencies.

Some of the results above stated are in complete agreement with those Bush

(1962) obtained from experiments on his 'resistance responses', but in many cases the resistance responses are not obvious in the reflex responses to closing of the dactylopodite, though the closer motor and opener inhibitor responses to opening resist imposed movement. On the other hand, also according to Eckert's results (1959) for the *Astacus* claw, it can be observed that small discharges in the opener motor axon in addition to opener inhibitor discharges on passive opening occurs and passive closing elicits small discharges in the opener inhibitor with many impulses in the opener motor axon. The proprioceptive reflex responses in the cheliped are strongly affected by control of the nervous system as was shown in the present experiments.

Such a difference in the reflex responses between the cheliped and the walking leg seems to be due to a functional difference between them. In the walking legs, such resistance reflexes as a negative-feedback, or a servo-control mechanism would be used by the animal to support and balance the body and to move it. However, the function of the cheliped is to grasp prey; in this action, various different factors must be involved, compared with the action of the walking legs. It may be concluded that the proprioceptive reflex mechanism in the cheliped is 'essentially' similar to reflexes in the walking legs, but the reflex responses in the cheliped are often variously modified under the influence of other parts of the body, especially the central nervous system.

Summary

The mechanism of the proprioceptive reflexes in the claw of the crayfish, *Procambarus clarkii*, was studied recording the electrical response of the PD nerve and of the efferent nerve to passive and active movement of the dactylopodite.

1. Passive opening of the dactylopodite elicited conspicuous reflex impulse discharges in the slow closer motor axon and in the opener inhibitory axon. On the other hand, on passive closing impulse discharges in the slow opener motor axon occurred. These reflex responses of the efferent axons were not always common in the cheliped.

2. In many cases, the reflex responses of the efferent axons appeared to make it easier to close the dactylopodite, but not to open it. That is to say passive opening evoked strong discharges in the slow closer motor axon, but slight discharges in the fast closer axon, and passive opening also evoked discharges in the opener inhibitory axon. Reflex discharges in the slow opener motor axon and those in the closer inhibitor on closing were not consistent.

3. These reflex responses were often centrally inhibited and no reflex discharge in all the efferent axons was produced by any impulse of the PD-afferent fibres. When activity of the animal was increased, the spontaneous discharges in the efferent axons were more dominant and reflex response to the dactylopodite movement was hardly observed.

4. Reflex responses produced by active movements were almost the same as

those by passive movement, except for prolonged discharges in the opener inhibitory axon on rebound opening.

5. It is concluded that the proprioceptive reflex mechanism in the cheliped is essentially similar to that in the walking leg, but the reflex responses of the efferent axons in the cheliped are often greatly modified under the influence of the other afferent fibres in the central nervous system.

This work was begun while the author was at the Zoological Institute, Hokkaido University, and it was later extended at the Department of Physiology, Kyoto Prefectural University of Medicine. The author wishes to express his appreciation to Prof. Mituo Tamasige, Hokkaido University, for his kind guidance and encouragement through the course of these experiments and for improvement of the manuscript. He would also like to thank Prof. Yoshihiko Iwase, Kyoto Prefectural University of Medicine, for all the facilities he provided.

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