Proprioceptive Reflex of the PD Organ of *Procambarus clarkii* by Passive Movement and Vibration Stimulus*[1),2)]

By

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(With 10 Text-figures)

Since Burke (1954) discovered the PD organ in the leg of the crab *Carcinus maenas* which acts as the proprioceptive organ, morphological and physiological investigations have been made on such receptors existing in each joint. It was found that the organ consists of an elastic strand (Mendelson 1963) containing many sensory cells (Tonner 1933, Stuart 1953, Burke 1954, Pringle 1956, Alexandrowicz 1958). The microanatomical details of its neurons have been studied with the electron microscope by Whitear (1960).

Extensive physiological experiments have made it clear that the organ produces discharges of afferent impulses during stretching of the elastic strand and its afferent nerve comprises unidirectionally sensitive movement fibres (of phasic nature) in each direction, and position fibres (of tonic nature) for the opening or the closing of the claw (Wiersma, Furshapan and Florey 1953, Burke 1954, Cohen 1963). There is already a considerable amount of quantitative information about the mechanism of regulatory reflex arcs in the vertebrates (Skoglund 1956, Cohen 1958, Lippold, Redfearn and Vico 1958). But the reflex effect of discharge from the PD organ has not been investigated, except for the reports by Bush (1962a, b). By electrical recording from the muscles and the motor nerves in intact *Carcinus* and *Astacus*, Bush has shown that passive joint movement has a pronounced effect on the pattern of descending impulses, and that this reflex is mediated by the chordotonal organ. In the previous study (Muramoto and Murayama 1965) the reflex mechanism of the PD organ of the crayfish *Procambarus clarkii* was investigated by active and electrical stimulation.

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

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The present paper represents an investigation of the afferent and efferent reflex responses in the chelipeds of both sides to passive movements and to the vibration stimulus of the dactylopodite.

Material and Methods

The crayfish, *Procambarus clarkii* Girard, kept in laboratory tanks was used.

Histology: The cheliped was cut off at the joint between propodite and carpopodite. The PD nerve bundle and closer motor axons were exposed by removing the sheel. The isolated cheliped was fixed overnight in Bouin solution (saturated picric acid sol. 75 ml, 95% formalin 25 ml, 99.5% acetic acid glacial 5 ml). After fixation, the PD nerve bundle and closer motor axons were removed under the binocular miroscope (24×) and, because they were so small, they were mounted on a thin liver block (0.7×5×5mm) with albumen to keep them in the same position. This liver block with the nerve materials was sectioned by 9–10 μ in thickness and stained with eosin-hematoxylin.

Preparation: The cryafish was cooled in a refrigerator at 0° to 10°C for 2–3 hr. before examination. To prevent autotomy of the experimental appendage, the coxo-basal levator tendons were severed before operation in most of the experiments. Methylene blue

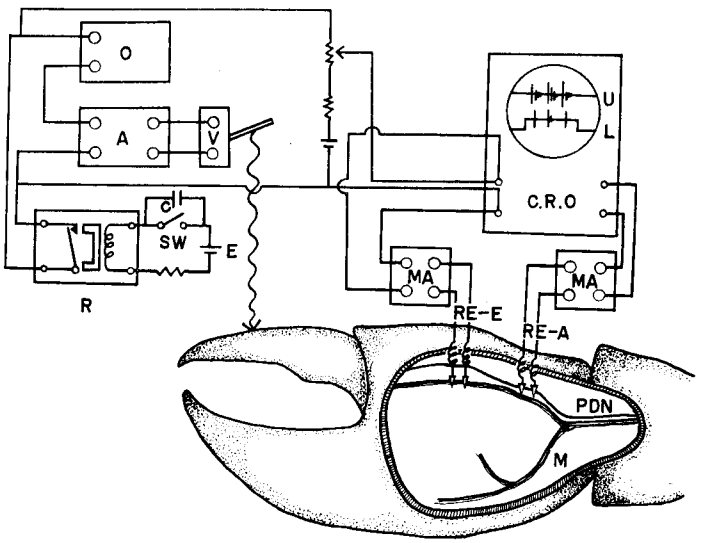

Fig. 1. Diagram of the experimental arrangement for stimulation of the PD organ and for recording the responses of the PD nerve. The PD nerve (PDN) and closer motor axons (M) were lifted above the fluid surface by the Ag-AgCl type electrode. The vibration stimulus was applied to the dactylopodite. The stimulus signal was displayed on the lower beam (L) of the cathode ray oscilloscope (C.R.O.) and the rectangular mark on the record represents the duration of stimulation. A, CR-amplifier; C, 400 μF condenser: E, 3V electric cell; R, relay: SW, switch; O, oscillator; U, upper beam indicating the action potentials of PD nerve.

staining was used in some experiments. After the PD nerve bundle and closer motor axons were exposed, the preparation was fixed with rubber bands to a perspexplate and immersed in a chamber filled with van Harrevelds' solution (van Harrevled 1936).

Stimulation and recording: Vibration stimulus was given as follows. A small glass rod, about 1cm long and 1mm wide, was connected vertically with colophonium wax to a vibrator of the moving coil type which was remodeled from a dynamic speaker. This was driven by oscillator through the amplifier. The stimulus signal was displayed on the one beam of the oscilloscope by a circuit which was opened and closed when the vibrator began and ceased to move respectively, with a 3V battery as electrical source. When the vibration stimuli were applied to the animal, the tip of the glass rod connected to the vibrator was put into contact with the top of the dactylopodite as shown in Fig. 1. To provide a signal of the displacement at the joint between dactylopodite and propodite, the tip of the dactylopodite was tied with a thread which was connected to the knob operating a potentio-divider. The displacement was converted into the change in divided potential and fed together with the nerve action potentials to the one beam of the oscilloscope.

For recording of the action potentials the PD nerve bundle was lifted above the fluid surface by one Ag-AgCl type electrode and the motor axons similarly by another electrode. Action potentials of both nerves were led into D.C. amplifiers and then recorded with the dual beam oscilloscope (Nihonkoden Type VC–5).

Solution and temperature: Van Harrveld's solution of the following composition was used throughout. Na; 79.3 mM, K; 2.9 mM, Ca; 4.7 mM, Mg; 0.6 mM, buffered to a pH of 7.2 by Na-bicarbonate. The cooled van Harrveld's solution at 10°C was stocked in a refrigerator and the temperature of the saline bath was maintained at 10°C by renewal of the cooled solution. The room temperature during experiments was 19°–25°C.

Results

Histology: As the anatomical and histological details of the PD organ of *Procambarus clarkii* have been previously presented (Muramoto and Murayama 1965), the histological structure of the PD nerve bundle and closer motor axons were investigated in the present study. The PD nerve bundle was about 50μ in diameter and consisted of 22 axons, comprising 9 large axons (5–12.5μ in diameter) and 13 small axons (less than 2.5μ in diameter) as shown in Fig. 2a. The bundle may consist of more than 22 axons, for all of them could not be counted because of their small size and the resemblance to the connective tissue.

Motor axons supplied to the closer muscle of the cheliped consist of the fast, the slow and the inhibitory axon (van Harreveld and Wiersma 1937, Nagahama 1950). Fig. 2b shows a microphotograph of these three axons of the histological preparation. They are different in diameter; the fast axon is 21.25–25.00μ, the slow axon 13.75–20.00μ, the inhibitory axon 10.00μ. The values were different from those measured under the intact condition without fixation (Murayama 1963).

Vibration stimuli: Burke (1954) has observed the response of the isolated PD organ with its afferent nerve of the walking leg of *Carcinus maenas* to the sinusoidal vibration stimulus at frequencies of above 100 c/s. In the study, the afferent response and the reflex efferent response to vibration stimuli of low frequencies were examined by recording the action potentials of both nerves. The frequency

of vibration stimuli applied was from 20 c/s to 80c/s. No visible movement was caused by the vibration stimuli.

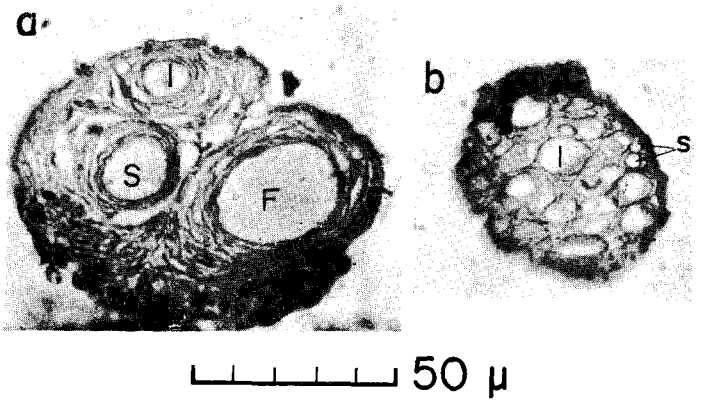

Fig. 2. Microphotographs of sections of the PD nerve bundle (a) and three motor axons (b). s, small sensory axon (less than 2.5μ in diameter); l, large sensory axon (5–12.5μ in diameter); F, fast axon (21.25–25.00 μ in diameter); S, slow axon (13,75–20,00μ in diameter); I, inhibitory axon (10μ in diameter).

The PD organ of *Procambarus clarkii* was very sensitive to mechanical shocks conducted from the experimental table or the floor and the afferent nerve was easily fired at high frequency. By a brief vibrational shock, serial impulses were evoked on the afferent nerve without fail and they disappeared in less than 40 msec after 7–10 impulses. Their amplitude decreased gradually. The afferent nerve impulses were five times as many as those of spontaneous discharge (37 impulses/sec). The nerve showed little or no adaptation to continuous vibration (see Fig. 3a). The rate of firing in the afferent nerve during vibration stimuli (from 20 to 80 c/s) was regular and dependent upon the frequency of the stimuli.

Three types of reflex responses (A, B and C) were observed in the efferent nerve. Type A; When the vibration stimulus was given to the dactylopodite, the afferent nerve was fired at high frequency and the reflex efferent impulses were proportional to the number of afferent serial impulses (Fig. 3a,b). Such reflex response was abolished by the vibration stimuli at frequencies of above 40 c/s. The reflex discharge appeared on the efferent nerve 32–46 msec after the afferent nerve discharge. Type B; Although the afferent nerve was fired regularly to vibration stimuli, the rate of reflex discharge to the efferent one was irregular and independent of the rate of afferent firing (Fig. 3c). The reflex response was produced on the efferent nerve from 25–300 msec after the afferent discharge. Complicated reflex routes may exist in the central nervous system. Type C; When a train of spontaneous impulses at constant intervals appeared on the efferent nerve, the spontaneous activity was not affected by the vibration stimuli. This seems to

be due to an inhibition in the centre.

Joint movement: The reflex responses in the same cheliped were investigated from the action potentials of the afferent (PD nerve bundle) and the efferent nerve (closer motor axons) to passive movement of the dactylopodite. And the effect of change in movement velocity on these responses was also examined.

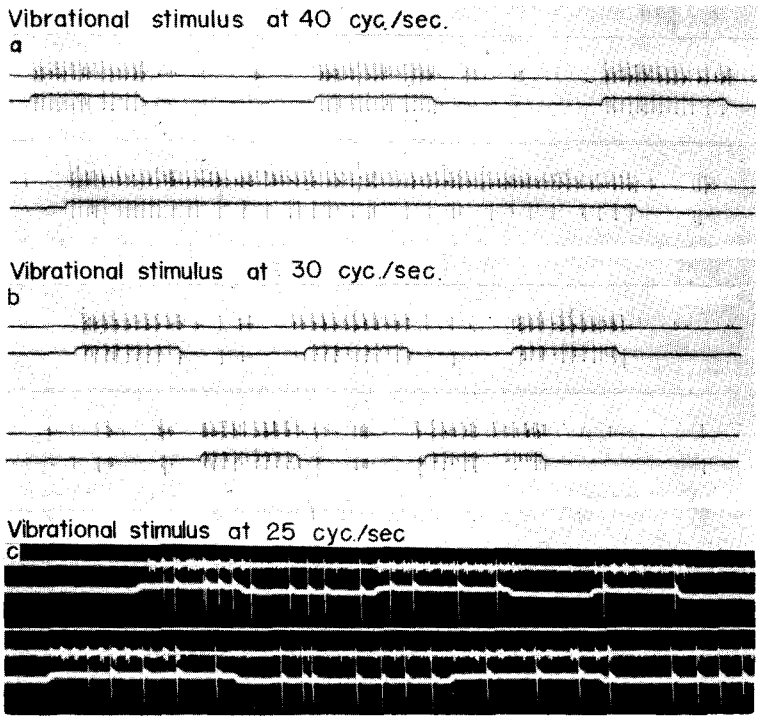

Fig. 3. Responses (type A and type B) of the afferent nerve to vibration stimulus at frequencies of 40, 30 and 25 c/s. Rectangular lower signal indicates the stimulus duration. Type A, the number of the reflex efferent impulses is proportional to the number of afferent serial impulses (a and b); Type B, the reflex efferent discharges are independent of the number of afferent impulses (c).

When the displacement of the joint was produced passively, a burst of discharge was recorded from the afferent nerve. The discharge frequency was affected by the velocity of movement as shown in Fig. 4. The afferent impulses caused by movement stimulation during 200 msec was plotted against the velocity (0°–48°/0.2 sec) in Fig. 5. The higher the velocity of movement is, the higher the afferent impulse fraquency. The discharge frequency is different depending on the movement direction of dactylopodite. It is expected that the units responding to flexion are more numerous than those to extension (Bush 1965b).

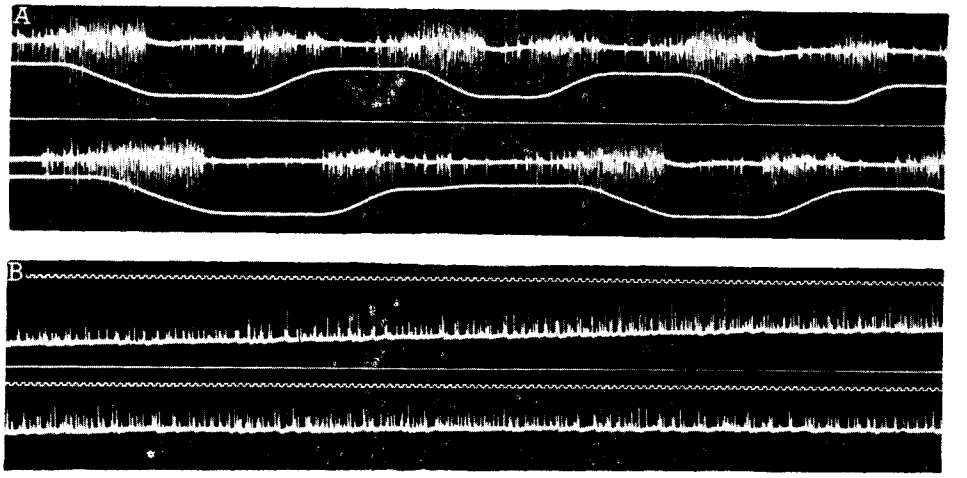

Fig. 4. The afferent responses to the rapid joint movement (A) and slow joint movement (B). (A) the upper signal, the afferent action potentials; the lower, the movement signal of closing upward and opening downward; the velocity of movement, about 48°/0.2 sec; (B) the upper signal, the time signal of 50c/s; the lower signal, the action potentials superimposed on the movement signal of closing and opening gradual upward and downward respectively; the velocity of movement, 8°/0.2 sec.

The reflex time to the efferent nerve varied with the velocity of dactylopodite movement. The relationship between the reflex time and the velocity of movement is shown in Fig. 6. This is plotted from the result to passive opening of the dactylopodite, for the reflex discharge in response to the opening was more obvious than that to closing (Fig. 7). The faster the joint is opened passively, the shorter the reflex time becomes. It is very interesting that the afferent and efferent reflex responses depend upon the velocity of joint movement. The closer motor axons consist of three axons with different sizes as referred to before. The responses of each axon can be distinguished from those of the other two axons by the difference of the amplitude of their impulses (Bush 1962b); the amplitude of the fast axon is the largest and that of the inhibitory axon is the smallest (Fig. 7). The reflex response of each axon to dactylopodite movement is shown in Fig. 8. It is based on the result shown in Fig. 7. The afferent and efferent responses to the extension of the joint differ from those to the flexion. In this case, the reflex discharge to extension of the joint appeared on all three axons, but to flexion only the inhibitory axon was fired. In most of the preparations, the slow and fast axons do not always respond to movement of both directions. Cases with responses of all three axons were very rare and scarcely observed except for the case shown in Fig. 7. It could not be determined from one case whether there is a unidirectional effect on reflex discharges in the slow motor axon. The velocity of the joint movement acts upon the reflex response of the motor axons.

Co-ordination in the PD organs between both chelipeds: The interrelation between PD organs of both chelipeds was examined by recording action potentials of the afferent and efferent nerves of both sides. It was analysed whether the discharge of the afferent nerve in one cheliped produced the firing of afferent or efferent axons in the other chelped. One of the dactylopodites was fixed firmly and another was moved passively, and afterwards the former was moved, the latter being fixed.

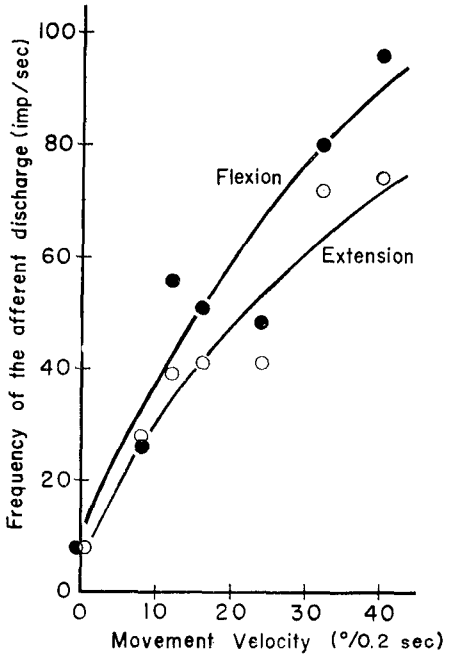

Fig. 5.

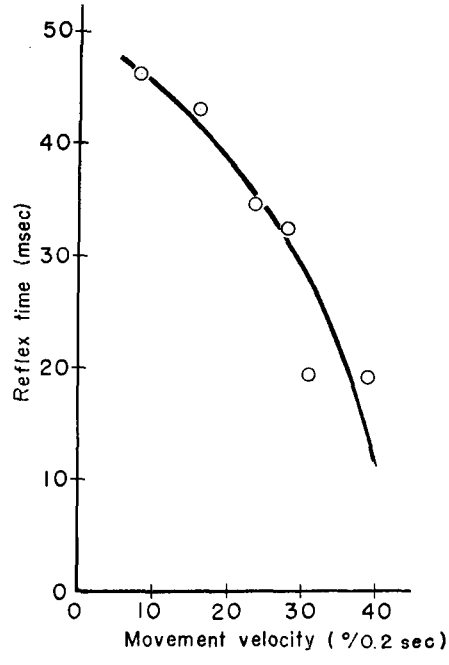

Fig. 6.

Fig. 5. Variation in the discharge frequency of afferent nerve with velocity of dactylopodite movement. Thick line, response to closing (flexion); dotted line, response to opening (extension).

Fig. 6. Variation in the reflex time plotted against velocity of dactylopodite movement to opening, for one preparation.

Passive opening or closing of the dactylopodite of the one side elicited strong discharges of the afferent nerve on the same side, but there appeared no burst of discharge in the afferent nerve on the other (fixed) side (Fig. 9). However, co-ordination was observed between the efferent nerves of both chelipeds. By passive movement of the left dactylopodite the efferent nerve on the same side was caused to discharge and the efferent nerve on the other side was fired more than 12 sec after the discharge of the efferent nerve (Fig. 10). The delayed efferent impulses

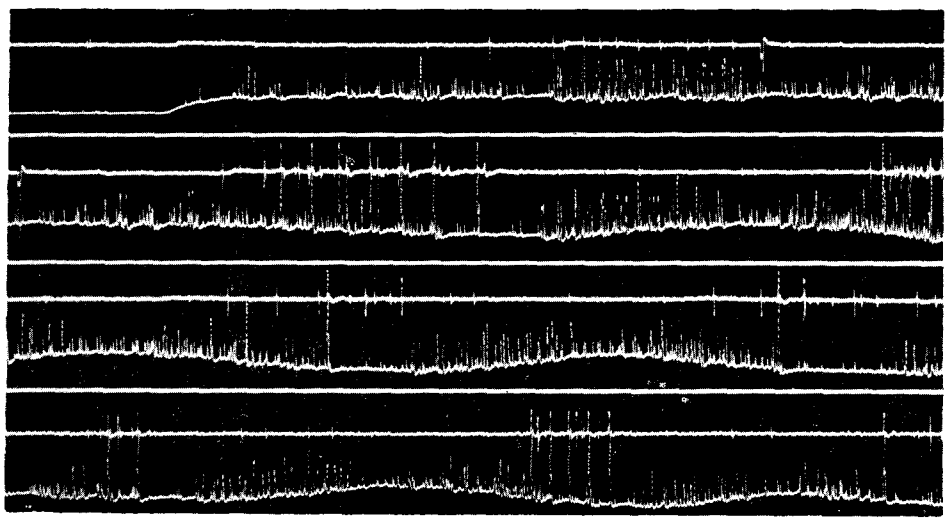

Fig. 7. The afferent and efferent responses to passive movement of the dectylopodite. Upper signal, efferent impulses (largest, fast axon; medial, slow axon; smallest, inhibitory axon); lower signal, action potentials of the afferent nerve superimposed on the movement signal closing and opening (gradual upward and downward respectively); movement velocity, 16°/0.2sec.

of the right side are the reflex response from the centre, for the right cheliped is so firmly fixed that the afferent discharge from the right PD organ does not occur.

Discussion

Histology: 22 axons could be counted in a PD nerve bundle, but many more small axons may exist. Murayama (1963) measured the diameter of the motor axons (fast axon 45.0–52.5μ, slow axon 25.0–37.5μ and inhibitory axon 7.3–30μ). These values are different from those measured in the present experiment. This is due to the difference of preparation, namely the intact or fixed condition.

Vibration stimuli: Burke (1954) reported that for the PD organ of the walking leg of *Carcinus maenas*, the rate of afferent firing during vibration stimuli at frequencies from 100c/s to 1000c/s was irregular, the afferent response did not follow the stimulus frequency and no response could be obtained above 1000c/s. In the present study, the rate of afferent firing by vibration stimuli of low frequency (20 to 80 c/s) was regular and dependent upon the frequency of stimuli. It is considered that the PD organ has a function as a vibration receptor of low frequency.

The reflex responses to vibration stimuli were also very obvious. The rate of reflex efferent discharge to the vibration stimuli at frequencies below 40 c/s was

dependent upon the afferent discharge frequency, but that to stimulation at higher frequency had no relation to the afferent discharge frequency. This means that the PD organ is sensitive to the vibration of low frequencies, but not to that of high frequencies. Such an independent efferent response will appear with no significant sensory information. It is very interesting that the reflex time by vibration stimuli at frequencies of below 40 c/s is shorter than that by electrical stimulation of the afferent nerve (Muramoto and Murayama 1965) or joint movement at slow velocity. The PD organ may be more sensitive to low frequency vibration than to joint movement.

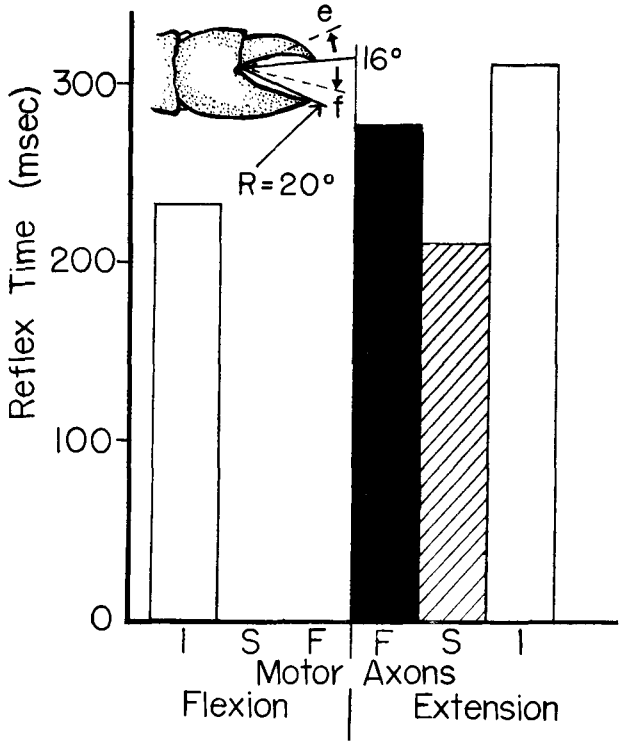

Fig. 8. Variation in the reflex time of the three motor axons to passive opening (extension) and passive closing (flexion) of the dactylopodite, for one preparation. Reflex time to opening (e) or closing (f) as much as 16° from the resting position at 20° is shown. Velocity of movement, about 16°/0.2 sec; F, fast motor axon; S, slow motor axon; I, inhibitory motor axon.

Joint movement: It has been reported that for the motor axons of four distal muscles in the walking legs of *Carcinus maenas* (Bush 1962b) and for the afferent nerve (Bush 1965a,b, Cohen 1963), the frequency of discharge increases

with speed of movement. In the PD organ of *Procambarus clarkii*, the afferent discharge frequency and the reflx time were also remarkably dependent upon the velocity of the movement. This may be based on the fact that the PD organ is rather more sensitive to the movement of the joint (phasic force) than to the position of the joint (tonic force), and that the more the velocity of movement increases, the shorter the reflex time becomes. In many preparations, the reflex responses to opening were more obvious than to closing. The afferent discharge frequency to closing was higher than to opening. Why such a difference exists between responses appearing on the antagonistic sensory pathways is an interesting problem yet to be solved: it could not be elucidated by the results of the present experiment. It may be considered that the sensory information of "claw open" is biologically more significant for the animal in catching and holding the prey.

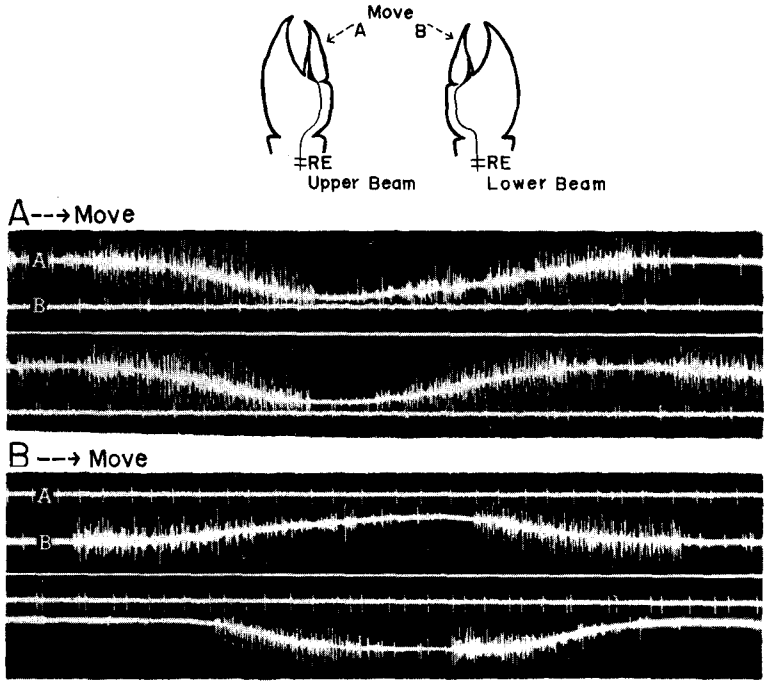

Fig. 9. The response of left (A) and right (B) afferent nerves to passive movement of either dactylopodite (A or B), opening (downward) and closing (upward). Upper signal, afferent discharge response of the left cheliped (A); lower signal, afferent response of the right cheliped (B); movement velocity, about 30°–40°/0.2 sec.

Co-ordination between the PD organs of both chelipeds: Passive movement of either one of the dactylopodites elicited an impulse discharge in both efferent nerves.

This suggests that the information is trasmitted also to the contralateral fibres through the centre. The same effects from the contralateral nerve are observed on the tympanic organ of *Gampsocleis* (Suga and Kastuki 1961). The delayed reflex efferent response in the dactylopodite of the fixed cheliped can be explained along the lines of the inhibition reflex of the spinal frog.

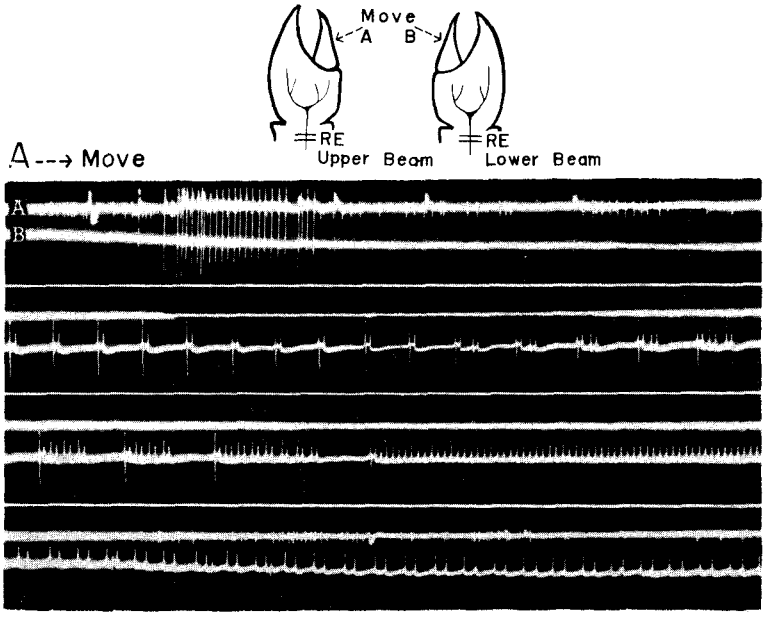

Fig. 10. The response of left (A) and right (B) efferent nerves to passive movement of the left (A) dactylopodite. Upper signal, efferent discharge response of the left (A) cheliped; lower signal, efferent discharge response of the right (B) cheliped; RE, recording electrode; movement velocity, 16°/0.2 sec.

Summary

1. The afferent nerve of the PD organ in the cheliped of *Procambarus clarkii* consists of at least 22 axons, some of which are small (less than 2.5μ in diameter) and some large (5–12.5μ in diameter).

2. The diameters of the three closer motor axons were measured in the histological preparation; the fast axon was 21.25–25.00μ, the slow axon 13.75–20.00 μ and the inhibitory axon 10μ.

3. The PD organ responded to vibration and a burst of impulses was produced in the afferent nerve and the reflex efferent nerve. The rate of discharge frequency of the afferent nerve was dependent upon the frequency of the vibration stimulus (20 to 80 c/s).

4. The reflex discharge appeared on the ipsilateral efferent nerve 32–46 msec after application of the vibration stimulus.

5. The frequency of discharge in the afferent nerve increased with the velocity of movement of the dactylopodite, and the response frequency during passive closing (flexion) was greater than that during passive opening (extension).

6. The reflex time required for the efferent response to opening became shorter according to the increase in the velocity of opening.

7. The response of the PD organ in one cheliped to movement of the joint caused a contralateral response on the efferent nerve in the other (fixed) cheliped, that is, a crossing-over of the sensory communication by effector inhibition was observed.

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