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Reflex Response to Passive Movement of the Two Adjacent Joints (PD and CP Joints) in the Cheliped of the Crayfish[1]

By

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(*With 4 Text-figures and 1 Table*)

The function of the proprioceptor in the crustacean appendage has been reported by Burke (1954). According to him the proprioceptor acts as a movement receptor and a vibration receptor. A series of investigations on the proprioceptive reflex has been done by Bush (1962, 1965) on the crab, *Carcinus maenas*. He proved that this reflex was controlled by a "resistance reflex", analogous to the vertebrate stretch reseptor reflex and to the passive joint movement of the crab appendage (Bush, 1965). It became clear in the crayfish that the passive and active movement of the propodite-dactylopodite joint also elicits a similar "resistance reflex" (Murayama, 1965).

But the proprioceptive reflex of the bender and stretcher muscles of the crayfish propodite has not been reported. It is an attractive problem whether a reflex response similar to that of crab will also be observed in the axons of the stretcher and bender muscles of the crayfish since it is known that the pattern of inhibitory innervation differs considerably in the groups to which the crab and the crayfish belong (Wiersma and Ripley, 1952).

Movement of one segment of decapod leg causes the displacement of the adjacent segment. This suggests that it is based on the mechanical interaction among joints. In fact, Bush (1962) observed that the reflex responses to movement of the dactylopodite varied with the position of the propodite. But the influence of the position of the dactylopodite on the reflex response with movement of the propodite remained uncertain. It is usually observed that the movement of the propodite-dactylopodite (PD) joint of the appendage is accompanied with a displacement of the adjacent carpopodite-propodite (CP) joint. In this case, it is questionable whether the propodite-dactylopodite organ (PD organ) sensitive to the movement of the PD joint is related to the proprioceptive reflex to passive

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movement of the CP joint. It is not known whether such an interaction between the movement of the two joints is caused by a joint receptor or only but the mechanical connection of the two joints.

In the present paper an investigation on the reflex response of the stretcher and bender muscles to passive movement of the dactylopodite and propodite is described and the influence of the PD organ on the reflex response is analyzed.

Material and Methods

Preparation: *Procambarus clarki* kept in laboratory tanks was used throughout the experiment. Before the experiment the animal was cooled in a refrigerator for 2-3 hours. This procedure kept the animal fresh and prevented the autotomy of the appendage. A small piece of the shell was removed on the ventral side of the carpopodite and the animal body was turned ventral side up, then fixed on a perspex plate, and put into a chamber filled with van Harreveld's solution (van Harreveld, 1936) buffered at pH=7.2 with Na-bicarbonate. Under a binocular microscope (24×) the afferent nerve bundles and efferent axons of the cheliped were isolated near the base of the carpopodite.

In order to examine the efferent reflex response only through the CP organs a cut

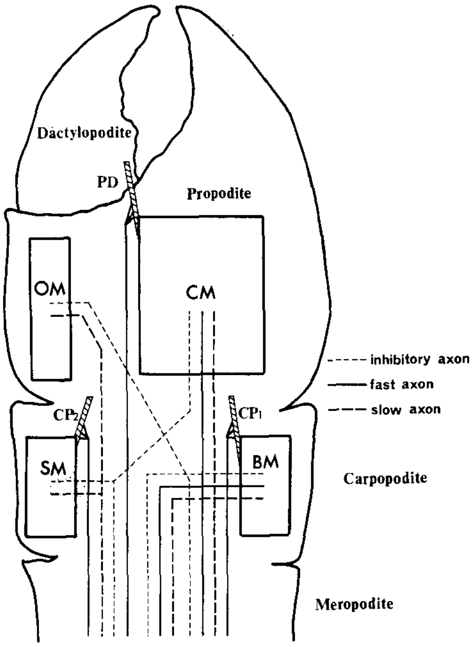


Fig. 1. Scheme of the innervation in the muscles of the cheliped and the arrangement of the joint receptors. PD, propodite-dactylopodite organ; CP1 and CP2, carpopodite-propodite organs; OM, opener muscle; CM, closer muscle: SM, stretcher muscle: BM; bender muscle

preparation was used in which the afferent nerve bundles other than the afferent nerve bundles of the CP1 and CP2 organs (Fig. 1) were severed at the proximal part of the carpopodite. The reflex response through both the PD and CP organs was also examined with the intact preparation in which all nerve bundles were left intact.

Stimulation and recording. Movement of the examined joint was produced by a simple rack-and-pinion device arranged to make a maximum angular displacement of the joint of 50°. This device was connected to the dactylopodite or the propodite and the joint displacement was monitored by means of a phototransistor (Toshiba, OS14), the output of which was led to one channel of a dual beam oscilloscope (Nihonkoden Type VC-7). The speed of the displacement was about 10°–50° per 100 msec. The dactylopodite is usually left open at an angle of about 20° in the resting state and in the present experiment it was opened or closed in a range of about 20° from the resting level. On the other hand, the maximum angular movement of the propodite from the carpopodite was about 60°, but the propodite was bent or stretched within a range of 50°.

The action potentials of the axons supplying the stretcher and bender muscles were recorded by hanging the axons up on a recording electrode in the air just above the surface of the bathing solution. The electrode was of an Ag-AgCl type, held with a micromanipulator, and the action potentials were led to another channel of the oscilloscope through an AC amplifier.

Results

Procambarus clarki is a very suitable material to analyze the reflex response in the cheliped since the innervation by motor axons was clarified by van Harreveld and Wiersma (1937) as is shown in Figure 1. A slow motor axon and an inhibitory axon innervate the stretcher muscle, they are called a stretcher motor and a stretcher inhibitor, respectively, and a slow motor axon is also provided for the opener muscle and an inhibitory also provided for the closer muscle. A fast motor axon, a slow motor axon and an inhibitory axon innervate the bender muscle and they are called the fast bender motor, the slow bender motor and the bender inhibitor.

A propodite-dactylopodite (PD) organ sensitive to the displacement of the dactylopodite was shown to be present between the propodite-dactylopodite joint (Muramoto and Murayama, 1965). Two carpopodite-propodite organs (CP1 and CP2 organs) are spanned between the carpopodite-propodite (CP) joints. The distal end of CP1 is attached to the bender apodeme. CP1 is mainly sensitive to stretching of the CP joint. On the other hand, the distal end of CP2 is attached to the stretcher tendon and CP2 is responsive to bending of the CP joint (Wiersma, 1959).

Reflex response to passive movement of the dactylopodite: When the dactylopodite was passively opened, a burst of large impulses occurred in the bender inhibitor, as is shown in Figure 2, records A and B, and also in the stretcher slow motor, as is shown in Figure 2, records C and D. On the other hand, when the dectylopodite was passively closed, a strong burst of the smallest impulses was elicited in the stretcher inhibitor and also in the bender inhibitor, as is shown in Figure 2, records A and B. The frequency of impulses of the stretcher slow motor decreased but that of the bender fast and slow motors increased.

To know whether the contraction of the muscle was inhibited or not, the ratio of the impulse frequency of inhibition (I) to that of excitation (E), i.e., the I/E ratio was examined because it is a good index of controlled muscular activity. In various muscles of the crayfish cheliped, the I/E ratio necessary for "just complete" inhibition is called the critical ratio (Rc value). This value was found to be constant within the same neuromuscular system (Marmont and Wiersma, 1938; Wiersma and Ellis, 1942). The I/E ratio for passive movement of the dactylopodite and the propodite was calculated and is shown in Table 1. When the claw was passively opened, the impulse frequency of the stretcher slow motor (SE) was 17 impulses/sec and that of the stretcher inhibitor (SI) 3 impulses/sec and the I/E ratio (in the case of the stretcher SI/SE) was 0.18. This value is lower than the Rc value for the inhibitory system of the stretcher muscle because the Rc value is 0.65

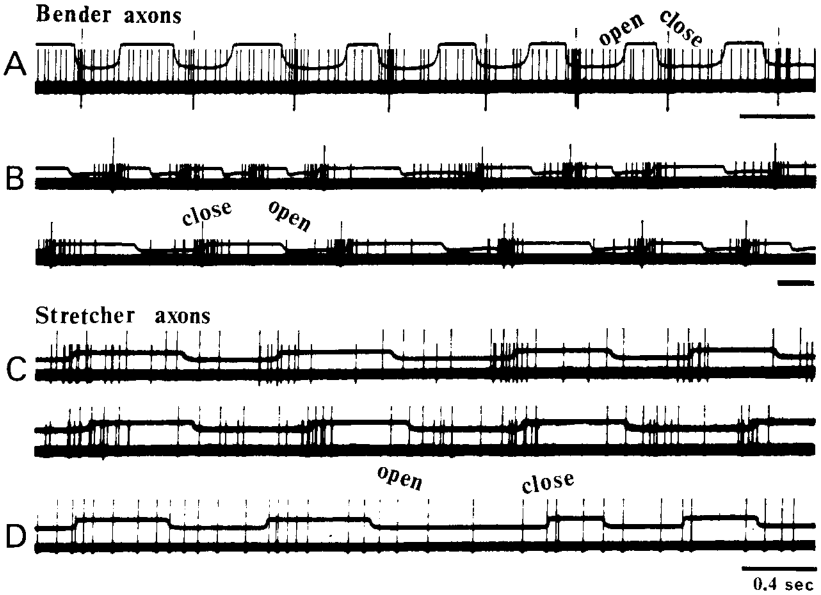
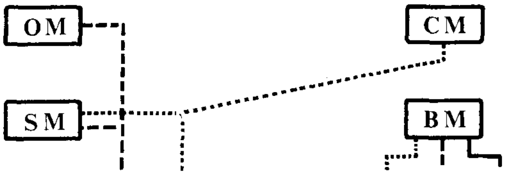


Fig. 2. Reflex discharge responses of efferent axons innervating the bender and stretcher muscles to passive movement of the dactylopodite. The intact preparation (A and C) and the cut preparation (B and D) were used. Passive opening elicits a burst of impulses in a stretcher slow motor (the large impulses in C and D) and passive closing elicits a burst in a bender inhibitor (the smallest impulses in A and B), in a bender slow motor (the large impulses in A and B), in a bender fast motor (the largest impulses in A and B) and in a stretcher slow motor (the large impulses in C and D). Movement direction of the dactylopodite is indicated by the words "open" or "close". The speed of movement is about 10°/100 msec.

or 0.41 according to the results of Wiersma and Ellis (1942), the activity of this muscle in response to passive opening movement was insufficiently inhibited. In the bender muscle, the I/E ratio for the fast and slow motors, represented as BI/SE and BI/FE respectively, was 15 which is higher than the Rc value which was determined as 0.70 and 1.25 for a slow bender and for a fast bender respectively by Wiersma and Ellis (1942). The activity of the bender muscle in response to the same passive movement was inhibited completely.



	SE	SI	SI/SE	BI	SE	FE	BI/SE	BI/FE
OPEN	+ 17 (13)	+ 3 (5)	0.18 (0.38)	− 15 (6)	− 1 (0)	− 1 (0)	15 (∞)	15 (∞)
CLOSE	− 13 (13)	+ 32 (0)	2.46 (0)	+ 27 (28)	+ 7 (9)	+ 2 (2)	3.86 (3.11)	13.5 (14.0)
BEND	+ 31 (25)	− 0 (0)	0 (0)	− 10 (2)	− 0 (2)	− 0 (0)	∞ (1.00)	∞ (∞)
STRETCH	− 1 (1)	+ 27 (27)	27 (27)	+ 38 (19)	+ 8 (15)	+ 5 (2)	4.75 (1.26)	7.6 (9.5)

Table 1. Scheme of innervation of the efferent axons in the stretcher and bender muscles and the frequency (impulses/sec) of reflex response of these axons to passive movement of the dactylopodite (open or close) and of the propodite (bend or stretch) in the intact preparation (shown with figures) and the cut preparation (shown with figures in parentheses). The increase (+) and the decrease (—) in the frequency of response to antagonistic movement are shown and the inhibitor frequency to excitor frequency ratio (SI/SE for a stretcher slow motor) is also shown. A black line represents a fast motor axon, a long broken line represents a slow motor axon and a short broken line a inhibitory axon. SE, excitatory frequency of a slow motor; SI, inhibitory frequency of a stretcher in inhibitor: BI, frequency of a bender inhibitor; FE, frequency of a fast motor; OM, opener muscle; SM, stretcher muscle; CM, closer muscle; BM, bender muscle

On the other hand, when the dactylopodite was passively closed the I/E ratio of the stretcher inhibitor system was 2.46 which is higher than the Rc value. Therefore the activity of the stretcher muscle in response to passive movement was also completely inhibited. The I/E ratios of the bender inhibitory system were BI/SE=3.86 for a slow bender and BI/FE=13.5 for a fast bender respectively, as is shown in Table 1. Though these valuses were higher than the Rc value, the bender muscle would be suspected to be in a rather excited condition in response to passive closing movement. The reason will be discussed in the discussion section.

Reflex response to passive movement of the PD joint was observed in axons innervating the muscles of the carpopodite. To know whether the response is coupled by a mechanical factor or whether it is caused only by the afferent input information from the PD organ, the efferent reflex response was analyzed using the cut preparation in which the afferent nerve from the PD organ was cut off. The frequency of discharge response in the efferent nerve is summarized in Table 1. The impulse frequency of a stretcher inhibitor in the cut preparation was remarkably reduced in response to passive closing of the dactylopodite as compared with that in the intact preparation. However the frequency of bender axons was the same in both preparations. The input information from the PD organ elicited a burst of impulses only in the stretcher inhibitor which was common with the opener muscle but had almost no effect on the reflex discharge response of the other axons, the branches of which are not sent to the opener and closer muscles.

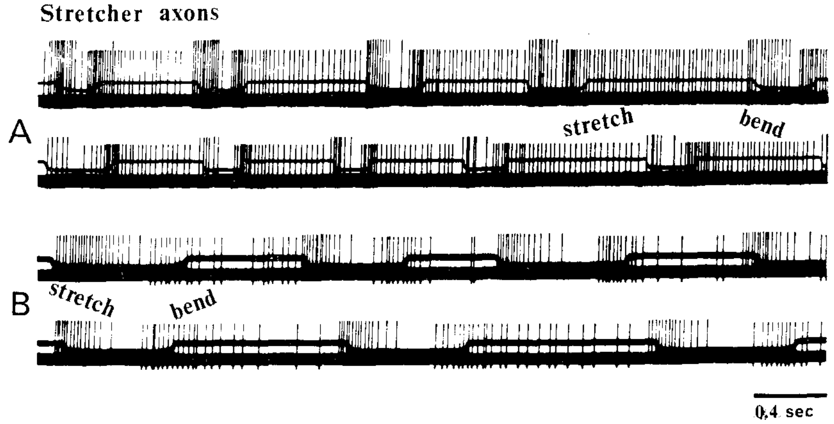


Fig. 3. Efferent reflex responses of the stretcher muscle to passive movement of the propodite in the intact preparation (A) in the cut preparation (B). Large spikes are impulses of a stretcher slow motor and small ones are impulses of a stretcher inhibitor, and the direction of the movement is shown by the words "stretch" or "bend". The speed of the movement is about 15°/100 msec.

Reflex response to passive movement of the propodite: When the propodite was passively bent but the PD joint was kept in completely closed position, a strong impulse burst was observed in the stretcher motor and in the bender inhibitor, shown as large impulses in Figure 3 and as the smallest impulses in Figure 4, respectively. But the passive stretching (re-duction of the propodite) elicited a strong burst in the stretcher inhibitor (the small impulses in Fig. 3) and in the bender inhibitor (the smallest impulses in Fig. 4). Fast and slow bender motors fired also in response to passive stretching movement.

In the case of passive bending movement of the propodite, SI/SE of the stretcher inhibitor system was 0, lower than Rc, and BI/FE of the bender inhibitor system was infinity (∞), much higher than Rc. The activities of the stretcher and bender muscles may be antagonistic, the former excited and the latter inhibited. In the case of passive stretching movement, SI/SE of the stretcher inhibitor system was 27 which is higher than the Rc value, so this stretcher muscle was completely inhibited. The I/E ratio of the bender inhibitor system was 4.75 for a slow motor (BI/SE in Table 1) and 7.6 for a fast motor (BI/FE in Table 1). In the case of passive stretching movement these ratios were higher than the Rc value but much lower than the I/E ratios in the case of passive bending movement. This may suggest that the contraction of the muscle is inhibited by passive stretching movement but activated by passive bending movement.

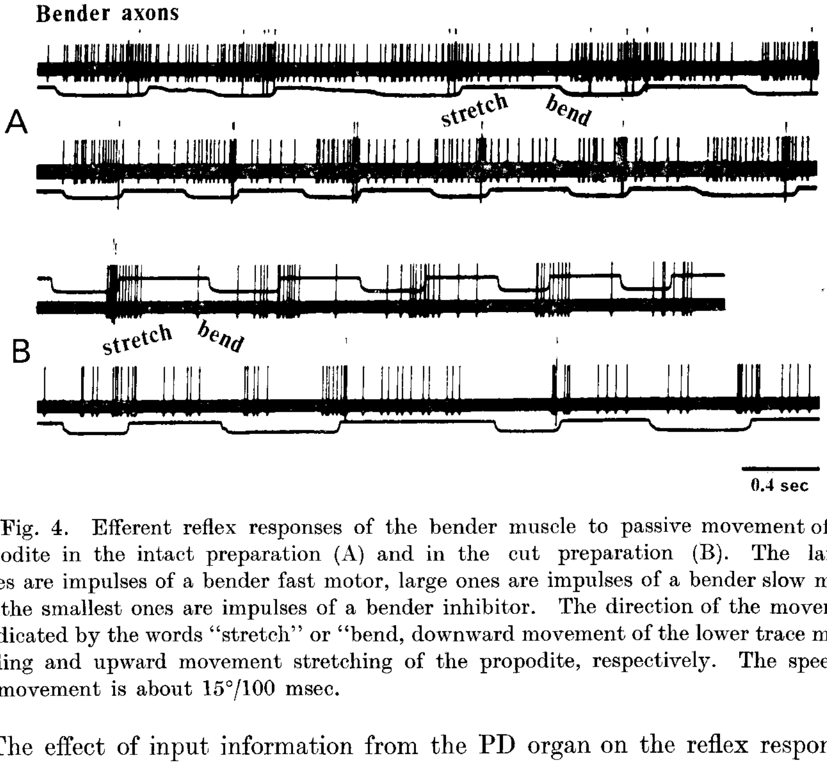


Fig. 4. Efferent reflex responses of the bender muscle to passive movement of the propodite in the intact preparation (A) and in the cut preparation (B). The largest spikes are impulses of a bender fast motor, large ones are impulses of a bender slow motor and the smallest ones are impulses of a bender inhibitor. The direction of the movement is indicated by the words "stretch" or "bend, downward movement of the lower trace means bending and upward movement stretching of the propodite, respectively. The speed of the movement is about 15°/100 msec.

The effect of input information from the PD organ on the reflex response to passive movement of the propodite was examined by comparing the reflex response in the intact preparation with that in the cut preparation. As is evident from Table 1, there is no characteristic difference between them. It can be said that the reflex response to passive movement of the propodite is not disturbed by input information from the PD organ. This is supported by the fact that the PD joint was kept in a completely closed position during the movement of the propodite and the afferent nerve from the PD organ was silent.

Co-ordinated movement between the PD joint and the CP joint: The "resistance reflex" response of the CP joint, i.e., passive bending caused the excitation of the stretcher muscle and the inhibition of the bender muscle, was observed. A similar "resistance reflex" response was also produced by the movement of the PD joint. Namely, passive opening of the PD joint caused stretching movement of the CP joint while passive closing of the PD joint caused bending movement of the CP joint. However the impulse frequency of the reflex response was different in the response to the movement of the dactylopodite and in the response to the movement of the propodite as is shown in Table 1. The "resistance reflex" response to movement of the propodite showed a more characteristic antagonistic response of the muscles than the response to movement of the dactylopodite. In such co-ordinated movement, the input information from the PD organ was found to be a very important factor in causing the "resistance reflex" response. When the afferent nerve bundle of the PD organ was cut off, passive movement of the PD joint elicited a burst of impulses only in the stretcher slow motor but no burst in the stretcher inhibitor. It may be said that the stretcher muscle shows an excited state in response to the movement in both directions but does not show the "antagonistic movement" for both stretching and bending. This means that the mechanical interaction based on the structural connection between the joint and the proprioceptor was effective for the "resistance reflex" response only in the case of the PD organ.

Discussion

Bush (1962) observed that in the crab, *Carcinus maenas*, passive stretching, i.e., re-duction of the propodite, elicits a strong response in the slow bender motor axon and in the stretcher inhibitor but passive bending, i.e., pro-duction of the propodite, elicits a response in the stretcher motor axon, that is, a "resistance reflex" response controls the movement of the legs. Though there is a difference in the innervation of the motor axons between the crab and crayfish (Wiersma and Ripley, 1952), a similar "resistance reflex" response was observed in the axons of the stretcher muscle of the crayfish. The excitation and the inhibition of this muscle depends on the Rc value determined by Wiersma and Ellis (1942). But the Rc value seemed to have little significance for the bender muscle because when the bender muscle was excited, the I/E ratios of the bender axons were always infinity (∞) and higher than the Rc value, but when the bender muscle was suspected to be inhibited the I/E ratios were also higher than the Rc value, that was, the I/E ratio for a slow bender motor (=BI/SE) was about 7 times higher than Rc and that for a fast bender motor (=BI/FE) was about 6 times higher than Rc.

Furthermore the firing of a fast bender motor in this case would increase the activity of contraction of the crayfish bender muscle since a single impulse in the firing for the fast bender gave a strong twitch contraction of the muscle in the shrimp *Stenopus hispidus* (Marmont and Wiersma, 1938).

However the bender muscle may be difficult to excite compared with the stretcher muscle and the Rc of the bender inhibitor system may be higher than the Rc value determined by Wiersma and Ellis (1942). Generally it is believed that one of the antagonistic muscles is more active than the other. A similar relationship can be observed in the opener and closer muscles because it was easy to open the claw but difficult to close it and the opener muscle was more functional than the closer one.

The passive opening of the dactylopodite produced contraction of the stretcher muscle while it inhibited contraction of the bender muscle, but in response to passive closing of the dactylopodite the stretcher muscle was inhibited while the bender muscle was excited. This observation was in accordance with the result from visual inspection that when the crayfish opened its claw, it simultaneously stretched its CP joint. Such co-ordinated movement of both joints may be caused by two factors: the input information from the PD organ and the mechanical force. The input information from the PD organ had influence on the impulse burst in the strether inhibitor but no influence on the burst in other axons. It especially had no influence on the axons of the bender muscle. Since a motor axon and a inhibitory axon innervating the stretcher muscle were common to both the opener muscle and to closer muscle, respectively, as is shown in Table 1, the movement of the PD joint might elicit a reflex response in the axons of both the opener and closer muscles (Bush, 1962; Murayama, 1965). In the present experiments, no characteristic influence of the PD organ was observed in the reflex response of the axons other than the axons common to both the opener and closer muscles. This proves that the reflex loop between the PD organ and the muscles of the propodite can not make a synapse with a different reflex loop between the CP organ and the muscles of the carpopodite.

Besides the effect of the PD organ, a mechanical factor was expected to have a role in the co-ordinated movement of each segment of the appendage. In fact, a structural connection was found where one end of the muscle was attached to the skeleton of the appendage and the other end connected with the tendon, and the proximal part of the tendon attached to the wall of the adjacent leg segment. With such a structural connection the displacement of one leg segment might produce the mechanical displacement of the other segment. However it became clear that if the input information from the PD organ was cut off, the mechanical force could not cause the "resistance reflex" response of the axons of the muscles of the proximal segment. The proprioceptor in the distal segment of an appendage was found to be important for the "resistance reflex" response of the axons of the muscles of the proximal segment. The branches of a motor axon innervate the muscles of different segments of an appendage but it seems that they have no significant function in the co-ordinated movement of the each segment of the appendage.

Summary

The reflex response of the axons of the bender and stretcher muscles of the cheliped of *Procambarus clarki* to passive movement of the dactylopodite and the propodite was studied.

1. Passive closing of the dactylopodite elicited a strong burst of impulses in a stretcher inhibitor and in a bender inhibitor. The bender fast and slow motors also fired. On the other hand, passive opening elicited a burst of impulses in a stretcher slow motor but the impulse frequency of all axons innervating the bender muscle was decreased.
2. Passive movement of the propodite elicited the "resistance reflex" response in the axons of the stretcher muscle and probably also in the axons of the bender muscle.
3. The contraction of the stretcher muscle in response to passive movement was controlled by the I/E ratio: ratio of the frequency of inhibitory impulses (I) to that of excitatory impulses (E). When the I/E ratio was higher than the Rc value (the critical value of the I/E ratio just necessary for complete inhibition, as determined by Wiersma and Ellis (1942)), the muscular contraction was inhibited. But the bender muscle was not inhibited even by an I/E value higher than the Rc value.
4. Passive opening of the dactylopodite elicited the stretching (re-duction) of the propodite while passive closing of the dactylopodite elicited bending (pro-duction) of the propodite. Such a co-ordinated "resistance reflex" response of the muscles was controlled by the input information from the propodite-dactylopodite (PD) organ but the input information from the PD organ had no influence on any efferent axons other than the common axons to be opener and closer muscles.

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