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Instructions for use

Reflex Mechanism of Pinching a Soft or a Solid Body with the Claw of the Crayfish[1]

By

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(*With 8 Text-figures*)

Abstract: In order to make clear whether the crayfish can or not perceive the hardness of the body held with its claw, the reflex impulse discharges of excitatory and inhibitory axons of the dactylopodite opener muscle were analyzed. When the crayfish pinched nothing ("nothing case"), the active closing movement of the dactylopodite was accompanied with a strong burst in the opener excitor and a decrease in the impulse frequency of the opener inhibitor. The closing movement was inhibited by the opener muscle and a negative-feedback control system can be formed in this case. On the other hand, when it pinched a soft ("soft case") or a solid body ("solid case"), the reflex discharge patterns of both the excitor and inhibitor impulses were completely different from those in the "nothing case". An increase in the impulse frequencies of both the excitor and the inhibitor was observed in the "soft and solid cases". The rate of the increase was higher in the "solid case" than in the "soft case". The ratio of inhibitory impulse frequency to that of excitatory one (I/E) was calculated as an index for the determination of the degree of the muscle contraction. Remarkable differences were observed among the "nothing, soft and solid cases". Such a difference was not observed if the afferent nerve from the sensory hairs inside the claw facing to the object body was cut off and only the PD organ left intact as a sensory source (see Fig. 4). These facts indicate that the hardness of the body can be perceived by the sensory hairs and the crayfish pinches in different manner depending on the hardness of the body.

Introduction

When the crayfish pinches a solid body it immediately releases that body in natural behaviour, while when it pinches a soft body, it holds that firmly. This suggests that the crayfish can perceive the hardness or softness of the body held with its claw. In decapod crustacea, the presence of the proprioceptor which detects the deformation of leg segments was clarified by many investigators (Alexandrowitz and Whitear, 1957; Barnes, 1932; Burke, 1964). In the cheliped of the crayfish the propodite and dactylopodite organ (PD organ) was found by

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Wiersma (1959). According to Bush (1962a, b) the passive movement of the joint evokes a "resistance reflex" which tend to resist the movement by excitation of the muscle opposing to the movement. Murayama (1965) observed that the proprioceptive reflex produced by the passive and active movement of the PD joint of the crayfish is consistent with the result obtained by Bush (1962a, b) on the walking leg of the crab. If the Bush's mechanism were ture also in the cheliped reflex of the crayfish, the crayfish would not be able to pinch the body with its claw in different manner depending on the hardness of the body. Then the problems arise whether the crayfish really perceives the hardness of the body or not and what a function the PD organ has in the cheliped reflex when the crayfish pinches something with the claw.

On the other hand, the crayfish pinches a body with its claw, the input information from the sensory hairs touching that body will also play a role in the pinch reflex. There is no report on the feedback control from the sensory hairs to the opener and closer muscles of the cheliped. But it is considerable that the input information from the sensory hairs can exert a direct influence on the motor output information to these muscles, since touching the inside of the dactylopodite or the pollux of the propodite causes the claw to close.

The present work was performed in order to clarify the control mechanism of pinch reflex in the crayfish cheliped, according special attention to the cooperative function between the PD organ (an output monitor) and the sensory hairs on the animal surface (an input monitor).

Material and Methods

Preparation: The crayfish, *Procambarus clarki* Girard, kept in laboratory tanks was used throughout the experiment. The crayfish was cooled in a refrigerator at 0°–10°C for 3–5 hours before the experiment. Then it was strapped on the perspex plate with its drosal side down and put in a chamber filled with cooled van Harreveld's solution buffered at pH$=$7.2 with Na-bicarbonate (van Harreveld, 1936). A small piece of the shell of the ventral side was removed near the proximal part of the propodite, and the PD afferent nerve bundle, sensory hair nerve bundle (afferent nerve bundle from sensory hairs) and both the opener excitor and inhibitor axons innervated the opener muscle were exposed. The blood duct of the animal was tied with a fine silk thread under the binocular (24$\times$) as is shown in Figure 1. The routine of cooling the animal and of tying its blood duct made it possible to prevent autotomy without severing the apodemes of the coxo-basal levator muscle of its articulations and to keep the preparation fresh for 3 hours or more (Muramoto, 1965).

Two kinds of preparations, i.e., the intact and the cut ones, were used to study the influence of the difference in input informations on the reflex responses of the opener muscle. In the cut preparation, sensory hair nerve bundles were cut off and only the PD afferent nerve bundle was remained intact to know an intrinsic role of the PD organ on the pinch reflex.

Histological examination of opener axons was made by the same procedure as before (Muramoto, 1965). Sectioned preparations of them were stained with eosin-hematoxylin.
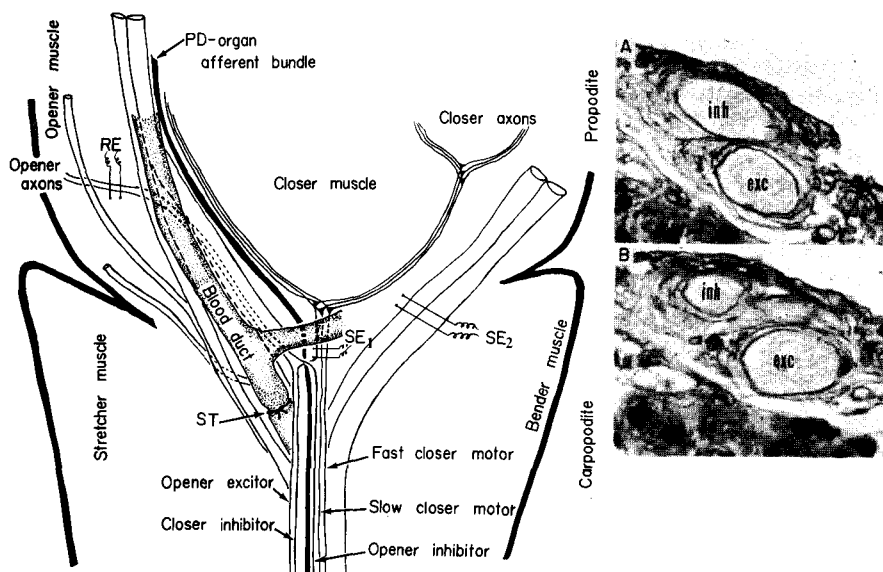
Fig. 1.   Diagram of the nerve distribution in the closer and opener muscles of the left cheliped and of the stimulating and recording electrodes position (left).   RE, the recording electrode position on the opener axons; SE1 and SE2, the stimulating electrodes on the closer axons and sensory hair nerve bundles respectively; ST, the position of tying the blood duct with a fine silk thread.

Cross-section of the opener axons at the propodite (A) and the carpopodite (B) near the opener muscle (right).   Eosin-hematoxyline stain.   Exc, opener excitor; Inh, opener inhibitor.

Stimulation and recordings: The action potentials of the opener axons were recorded during the reflex response, or the pinch reflex, when the crayfish pinches the soft body ("soft case") or the solid body ("solid case") with its claw by active closing movements. An elastic sponge (7 mm × 7 mm × 35 mm) was applied as the soft body and a round bakelite rod (8 mm in diameter) as the solid one.

Actively closing movement was produced by the electrical shocks (0.1 msec in duration 1–2 V in strength) applied to the closer axons (Fig. 1) with a square pulse generator. The reflex response of the opener muscle was recorded with an external electrode of the Ag-AgCl type.   Only the reflex response of the opener muscle was examined because the

Fig. 2.   The reflex response of the opener axons during the active pinch movement on the cut preparation where sensory hair nerve bundles were cut off.   Upper signal indicates the action potentials of the opener axons, and lower one the movement signal of the dactyl-opodite (one period of the trace like sinusoidal waves means the angular displacement of 5 degrees, as is described in the Text) and the signal of electrical stimulation.   The large spikes are inhibitor impulses and the small ones excitor impulses.   A, the crayfish pinches nothing ("nothing case"); B, it pinches the soft body ("soft case"); C, it pinches the solid body ("solid case"); a, the claw is going to close ("closing stage"); b, the claw is completely closed ("completely closed stage"); c, the claw begins to open by ceasing the electrical stimulation ("relesasing tage").
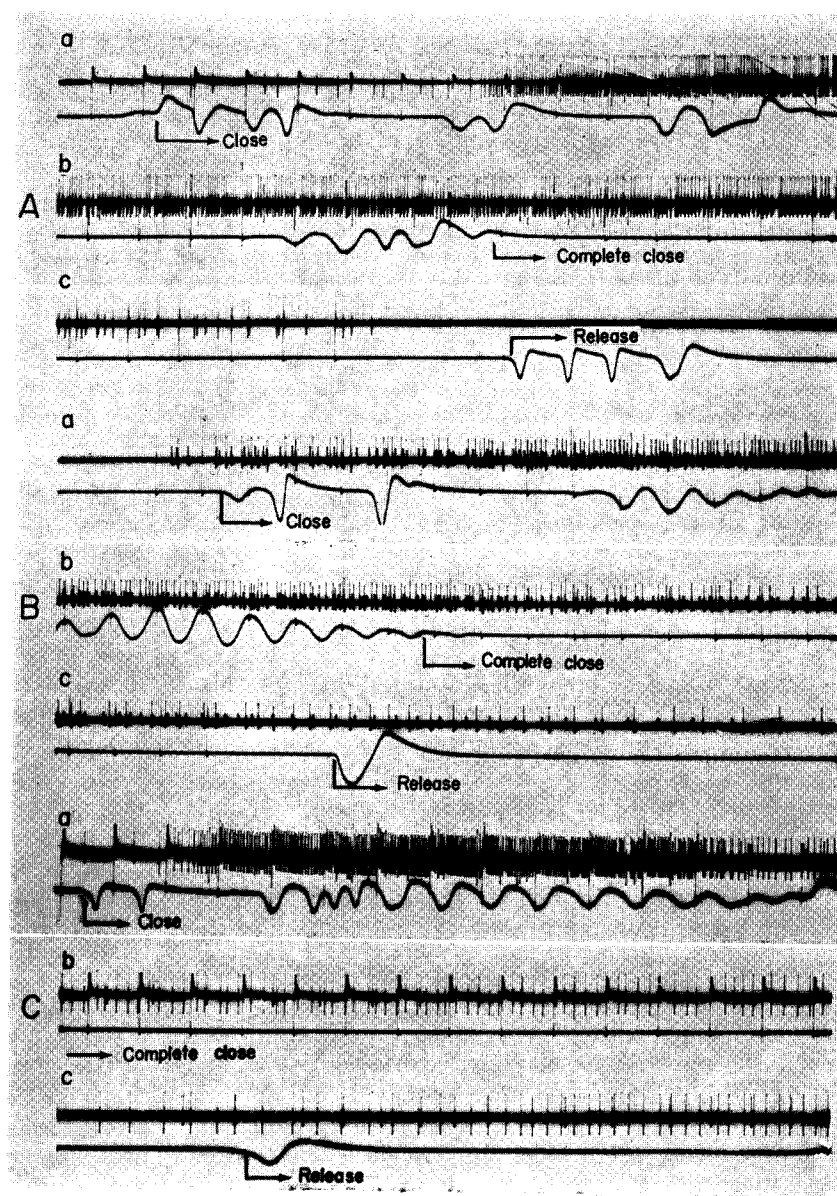
Fig. 2.
For legend see opposite page.

Erratum
Page 34 Fig.1 legend line 6 should read Cross-section of the opener axons at the proximal (A) and the distal part (B) of the opener muscle (right).

muscle has only two antagonistic motor axons which simplified the analysis. To compare the reflex response produced by the PD organ with that by the sensory hair nerve bundles, the reflex discharge produced by repeated electrical shocks to the sensory hair nerve bundles was examined as the control. An indifferent electrode for earth was provided in the saline of the chamber. An RC-coupled amplifier was used to record the response of opener axons and the action potentials were led into one channel of a dual beam oscilloscope (Ninhonkoden Type VC–5).

The displacement of the dactylopodite produced by electrical shocks was recorded in the following way: A 90° protracter made from cardbaord was slitted at 5° intervals. The right angle of this protracter was fastened to the dactylopodite with a clip. The light (6 V lamp was applied) through the slit fell on a phototransister, the output of which was led to another channel of the oscilloscope, together with the mark of electrical stimulation represented as a large spike in Figure 2. The trace like sine wave shown in Figure 2 was produced in accordance with the movement of the dactylopodite, and the duration required for one cycle means the displacement from a slit to the next slit (a flat line means that the dactylopodite did not move). The number of cycles is directly proportional to the amount of dactylopodite movement.

Touch stimulation was given to the carpo-meropodite articular part or mouth part with a fine glass rod (1.5 mm in diameter). When the signal of touch stimulation was required, the fine glass rod was connected to the vibrator of modified dynamic speaker, and the change in coil current was led directly to other channel of the oscilloscope.

Room temperature during the experiments was 21°–25°C.

Results

As is clear from the histological sections shown in Figure 1, A and B, the opener muscle of cheliped of the crayfish was doubly innervated by an excitor and an inhibitor axon. The excitor (26.0–28.3 μ in diameter) was thicker than the inhibitor (17.2–27.8 μ in diameter), and the difference in the size of both axons became more obvious at the distal part of the opener muscle as shown in Figure 1, B. It was easy to distinguish the impulse of the excitor from that of the inhibitor since the excitor has spikes of larger amplitude on the extracellular record. However spike potentials larger than those of the excitor were recorded in some cases under different condition of electrode contact as shown in Figures 7, B and 8, A and C.

Pinch reflex to actively closing movement

Pinch behaviour with the claw can be devided into three stages; the "actively closing stage", the "completely closed stage" and the "releasing stage" when electrical stimulation is stopped. The patterns of reflex efferent discharges elicited in the opener axons were found to be characteristic of the three stages mentioned above.

The reflex burst of the opener axons decreased gradually during the "closing stage"; it continued to decrease to the resting level after termination of the stimulation as is represented in Figure 2. The discharge frequencies of the excitor and of the inhibitor during the three stages of pinch reflex are shown in Figure 3. Each

datum was obtained from the mean values of five experiments in both the intact and the cut preparations. A tremendous variation could be observed in the frequencies of reflex discharge of the excitor and the inhibitor depending on whether the claw pinches nothing, a soft body or a solid body. In the intact preparation, the inhibitor impulse frequency was higher than the excitor one in whole cases. During the "clsoing stage" and the "releasing stage", the excitor frequency showed the almost similar values and had no relation with what the crayfish pinched with its claw, but the inhibitor frequency varied in each case. The variation of inhibitor frequency was very obvious at the "closing stage" compared with that at the "completely closed stage" and the "releasing stage".

In cut preparation, the inhibitor and the excitor frequency increased compared with that in the intact preparation, especially the increase of the excitor frequency was clear.

The reflex response was always elicited simultaneously in both excitor and inhibitor axons. The difference in the excitation of the muscle may depend on
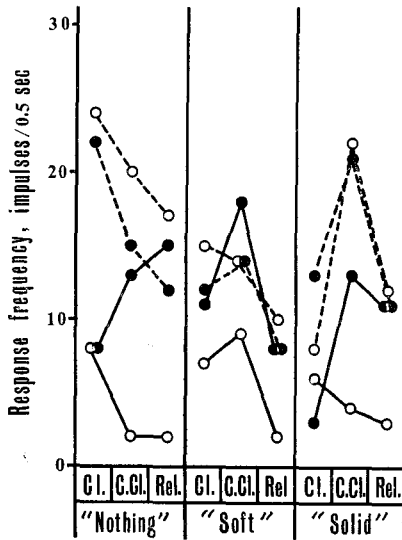

Fig. 3. Fig. 4.

Fig. 3. The response frequency of the opener excitor (white cricles) and inhibitor (black circles) produced by actively closing movement in the cut preparation (broken lines curve) and in the intact preparation (solid lines curve). Nothing, when the crayfish pinched nothing with its claw; Soft, when it pinched a soft body; Solid, it pinched a solid body; Cl, closing stage; C. Cl, completely closed stage; Rel, releasing stage.
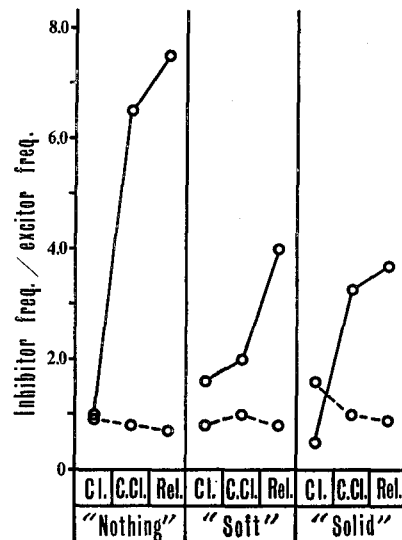
Fig. 4. Ratio of the inhibitor impulse frequency to the excitor impulse frequency for the opener muscle (I/E ratio) calculated from the values shown in Figure 3. The solid line curves are I/E ratios in the intact preparation and the broken line curves those in the cut preparation.

excitatory factors in addition to the inhibitory ones. Then the ratio between frequencies of excitation and inhibition, i.e., the I/E ratio, was calculated and is shown in Figure 4. The I/E ratio at which the muscular contraction was just suppressed was termed as the Rc value and that in the opener muscle of *Procambarus* has been reported to be 0.41 by Wiersma and Ellis (1942). If the I/E ratio is less than the Rc value, the contraction of the opener muscle can not be suppressed. It was evident from the solid line curves in Figure 4 that in the intact preparation the I/E ratio varied in each stage of pinch behaviour and in each trial. When the crayfish pinched nothing, the I/E ratio was over the Rc value (0.41) during all stages. When it pinches a solid body, the I/E ratio was below Rc value in the "closing stage", but in "closing stage" of the "soft case" the value was beyond 1.0. This
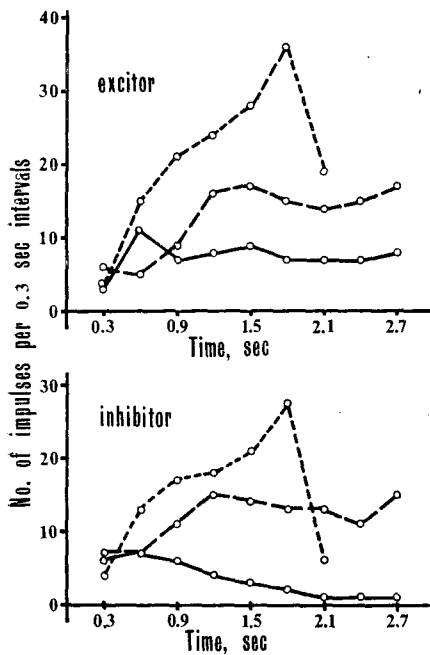

Fig. 5.

Fig. 6.

Fig. 5. Number of impulses of both the excitor (upper graph) and the inhibitor (lower graph) at every 0.3 sec interval during the closing stage in the intact preparation. The abscissa represents the time after beginning of the actively closing movement. Curve in short broken line (the upper curve) represents the values in the solid case, curve in long broken line (the middle curve) those in the soft case, and curve in solid line (the lower curve) those in the nothing case.

Fig. 6. Ratio of the inhibitor impulse frequency to the excitor one for the opener muscle (I/E ratio) at every 0.3 sec in the soft case (curve in long broken line), the solid case (curve in shrot borken line) and the nothing case (curve in solid line), calculated from Figure 5.
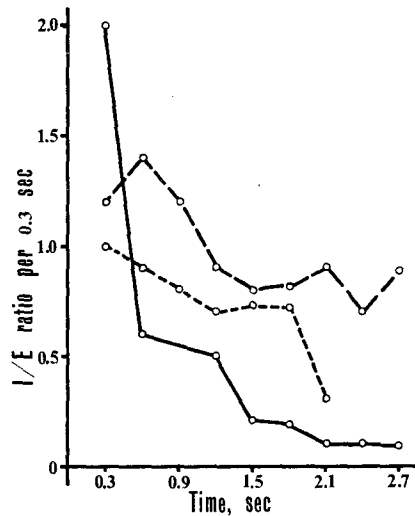
seems to suggest that the crayfish acts in different manner with respect to the hardness of the object as soon as it holds that body with its claw. It can be expected that the obvious variation in discharge frequency exists at the beginning of the closing movement. In the number of reflex impulses of the excitor and the inhibitor was calculated at every 0.3 sec, in order to know the time change of discharge frequency after the onset of closing movement, and the result is shown in Figure 5. It is clear that when the crayfish pinched something, the frequency of the reflex excitor and inhibitor impulses increased gradually until 1.2 sec in the "soft case" and 1.8 sec in the "solid case", and the rate of the increase is higher in the latter case than in the former. But in the "nothing case" no obvious increase in the number of excitor impulses was observed except at the very beginning of the closing movement while the number of inhibitor impulses decreased slowly. This reflex pattern in the "nothing case" would correspond with the result reproted by Bush (1962b) that passive movement of the joint elicits "resistance reflex". Also Murayama (1965) could not observe a signfiicant difference in the reflex efferent responses to active and passive movements. The difference in reflex patterns when the claw pinches nothing, soft or solid body should be based on the input information from the sensory hairs that are distributed on the surface of the dactylopodite and the propodite. The input information from the sensory hairs will elicit an increase in the number of impulses of the both axons.

In the "nothing case", the I/E ratio during the clsoing stage was very high at the beginning of the movement, declined abruptly, and at last kept at a constant value (0.3) (Fig. 6). The I/E values during the "closing stage" were higher in the "soft case" than in the "solid case" (Fig. 7). The difference in I/E values between these cases will be based on the variation in input information. Namely, when the cryafish pinches a soft body, the sensory information from the PD organ will be larger than that from the sensory hairs, and vice versa for the solid body.

The importance of the input information from the sensory hairs will be obvious in the I/E ratio in the intact preparation (the curve in solid line of Fig. 4) is compared with that in the cut preparation (the curves in borken line of Fig. 4). When the sensory hair nerve bundles were cut off (in the cut preparation), no clear variation in the I/E ratio was observed in both the "soft and solid cases". This proves that the input information from the sensory hairs is indispensable for the crayfish to perceive differences in hardness of the body held with its claw.

Reflex response to the electrical stimulation of sensory hair nerve bundles

The following experiment was performed in order to know precisely the reflex control by the input information from the sensory hairs, since the preceeding result made it clear that this input is essential to the pinch reflex. The electrical shok was given to the sensory hair nerve bundles which innervated the part of propodite facing the dactylopodite as shown in Figure 1. All afferent nerve bundles other than sensory hair nerve bundles were cut off to prevent any interference from the other systems.

The reflex discharge occurred in the opener axons with a single shock to the sensory hair nerve bundles (Fig. 7). This response was composed of two parts. The first burst of 3–8 impulses occurred 80 msec after the shock, and 60–70 msec later the second burst appeared and continued for a short time (Fig. 7, A) or long time (Fig. 7, B). The duration of the second burst did not depend upon the strength and the frequency of the stimulation. In this reflex, the inhibitor impulse frequency was 65 impulses/sec and the excitor impulse frequency was 79 impulses/sec. The I/E ratio (0.82) was twice the Rc value. It is certain that the input information from the sensory hairs can elicit the strong burst in the both axons as suspected in the above section.

Reflex response to touch stimulation

It is naturally expected that the reflex discharge of the opener axons is caused by the input information from the receptors which are located in other parts of the body than the cheliped. For example, the closing reflex of the claw can be inhibited by touching the carpo-meropodite articular part or the mouth part.
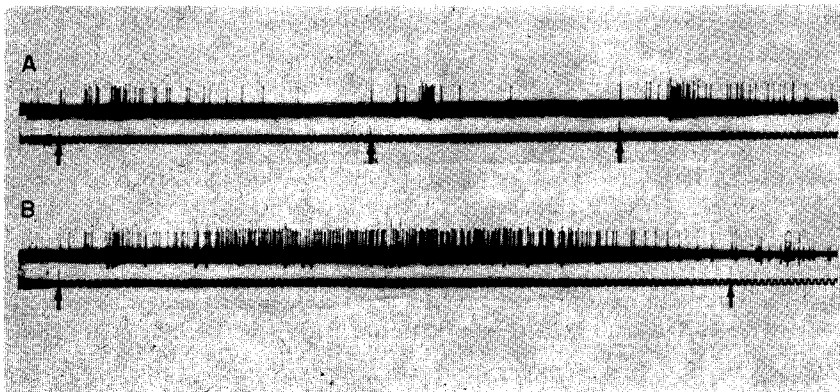

Fig. 7. The reflex discharges (upper signal) produced by electrical stimulation to the sensory hair nerve bundles. This response continues for a short time (less than 50 msec in A) or for a long time more than 1.8 sec (B). The arrow indicates the artifact from the electrical shock. The lower trace is the time signal of 50 Hz.

Whether such a reflex obtained by touching an animal body was different from the pinch reflex or not was examined.

Two kinds of preparations (cut and intact preparations) were also used. By severing the PD organ afferent nerve and sensory hair nerve bundles (cut preparation), it was possible to observe the reflex response produced only by the touch stimulation of the body.

High frequency reflex bursts occurred in the opener axons with single touch stimulation to the mero-carpopodite (MC) articular part of the cut preparation (Fig. 8, A). The discharge frequency of the reflex (106 impulses/sec) was ten times higher than the spontaneous impulse frequency of these axons (10 impulses/sec). The inhibitor impulse frequency was 56 impulses/sec and the excitor impulse frequency was 50 impulses/sec. The I/E ratio was 1.1. But in the intact preparation, the inhibitor frequency was 79 impulses/sec, the excitor one 65 impulses/sec, and the I/E ratio 0.82. The I/E ratio in the touch stimulation showed a higher value in the cut preparation than in the intact one similar to the pinch reflex mentioned above.

The reflex efferent burst was produced by the actively opening movement produced by the touch stimulation of the mouth parts (jaw, maxilla, maxilliped or gill part). In the intact preparation, the frequency of the burst was 39 impulses/sec and the I/E ratio 0.54 (Fig. 8, C). But in the cut preparation, the frequency increased to 100 impulses/sec and the I/E ratio was 0.98 (Fig. 8, D). The inhibitor fired at a higher frequency in the cut preparation than in the intact one. In the
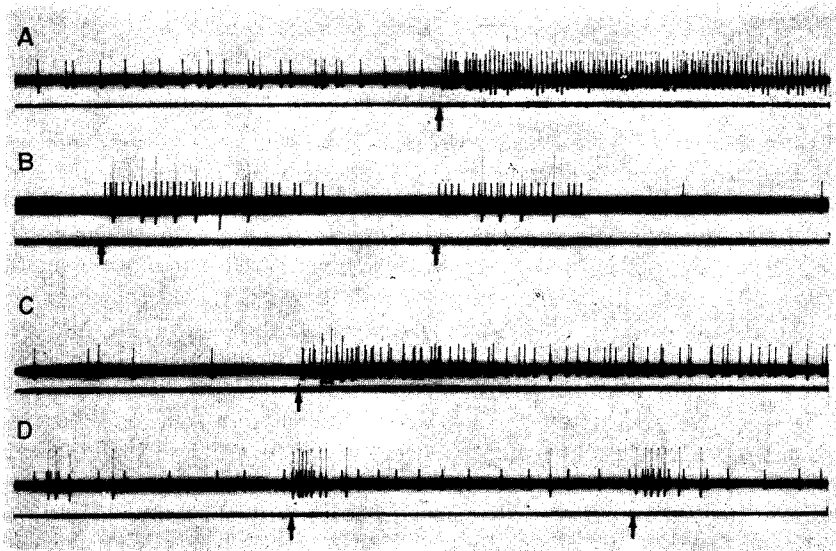

Fig. 8. The reflex discharges of the opener axons with touch stimulation. Large spike potentials are excitor impulses, small spike potentials inhibitor ones, and the largest spike potentials in A and C are unidentified (see the result section of the text). The arrow indicates the time of the touch stimulation. A, response to touch stimulation to the mero-carpopodite articular part in the intact preparation; B, response to touch stimulation to the mero-carpopodite articular part after autotomy; C, response to touch stimulation to the green gland in the intact preparation; D, response to touch stimulation to the mouth part in the cut preparation.

latter preparation where the touch stimulation was given to green gland, the duration of the reflex burst was 3 sec or more (Fig. 8, C). In the cut preparation, the duration of the reflex burst was short (0.1–0.15 sec) (Fig. 8, D). Also in touch stimulation, the information from the PD organ and sensory hairs seems to reduce the value of the I/E ratio and to prolong the duration of reflex discharge.

From these results mentioned above, it is suggested that the reflex efferent responses will be modified by the input information from parts of the body other than that from the PD organ and sensory haris, and the information from the other receptors will show a facilitation of the excitor and the inhibitor activity in the centre.

Reflex-like burst was observed in the preparation after autotomy (Fig. 8, B). This reflex-like burst (64 impulses/sec) was caused by the single touch stimulation of the MC articular part and it was the same as that of the normal preparation. The excitor impulse frequency (50 impulses/sec) increased much more than the inhibitor one (14 impulses/sec) compared with frequencies before autotomy and the I/E ratio was 0.33. In this case, it must be considered that the reflex-like discharge was produced by neuro-neural transmission through a kind of ephapse (artifiical synapse). The possibility of this transmission will be mentioned later (see discussion).

Discussion

It has been shown that all joints within the walking legs of *Carcinus maenas* are subjected to the control of "resistance reflex" such that any passive movement of a joint elicits a reflex response in the motoneurones of the muscle stretched by the movement: this reflex tends, therefore, to resist the imposed movement (Bush, 1962a, b; 1965). Moreover, according to Murayama (1965) there is no signfiicant difference in the reflex efferent responses though it was expected that there would be some difference between the reflex efferent responses to active and passive movements. When the crayfish pinches something this reflex is also controlled by a mechanism similar to that mentioned above, and a more obvious burst will be elicited in the opener excitor for a soft body than for a solid one, because the displacement of the closing movement is larger in the former case than in the latter case. But it is clear that such an impulse burst was not observed in the present experiment. On the contrary the excitor impulse frequency was higher for the solid body than for the soft one and the I/E ratio was higher in the soft case than that in the solid case. This difference in the reflex pattern will be explained as follows. When the crayfish pinches nothing, the negative-feedback system will control the reflex through the PD organ, but when it pinches something with its claw, the input information from the sensory hairs in contact with the thing held with its claw will elicit impulse bursts in the inhibitor and the excitor axon. Also the inhibitor impulse frequency was higher than the excitor impulse frequency.

From the observation mentioned above, it seems that if the pinch reflex were

a negative-feedback system, the more the reflex response increases, the more it would be suppressed in the centre by the input information from the sesnsory hairs. Then it may be considered that the pinch reflex will be regulated by a positive feedback system rather than by a negative-feedback one. So the crayfish may be able to percieve the hardness of the body with its claw and hold it in different way depending to the hardness. But in this case it must be pointed out that the input information from the sensory hairs is indispensable for the pinch reflex.

It is natural to consider that Rc (0.41) might have no meaning for this pinch reflex since that value was measured as the ratio of the impulse frequency of the inhibitor to that of the excitor necessary for "just complete" inhibition when the mechanism of peripheral inhibition in the muscle of the crayfish was investigated by separate stimulation of the excitor and the inhibitor (Marmont and Wiersam, 1938; van Harreveld and Wiersma, 1937).

It remains uncertain whether input information other than those from the PD organ and the sensory hairs will influence the pinch reflex or not, and how much influence it will have. But it seems likely that this input information will elicit bursts of the inhibitor and the excitor and make it easy to facilitate the activity of both axons during the pinch reflex response, since a stronger burst in the both axons was observed in the intact preparation compared with the cut preparation where the PD afferent nerve bundle and sensory hair nerve bundles were removed. Anyway integrated input information from every part of the body may be useful for the pinch reflex based on a positive-feedback system.

Bush (1962a) has suggested that the proprioceptive reflex responses are mono-synaptic, afferent-efferent reflexes. This may be true in the case where the crayfish pinches nothing but the pinch reflex seems more likely to be polysynaptic. For an example, when the electrical shock was applied to the sensory hair nerve bundles, the reflex burst occurred in two parts on both the axons and the second burst was unrelated to the strength and the frequency of stimulation. This second burst is implied to take place at a higher level than the thoracic ganglion.

After autotomy, a reflex-like burst was observed with touch stimulation to the MC articular part. It may be considered that this burst is induced by neuro-eural tramsmission outside the central nervous system. It is certain that squid giant nerves in contact (Arvanitaki, 1942) and also crab leg nerves in contact (Jasper and Monnier, 1938) make artifiical junctions, and it seems that the reflex-like phenomenon in the present experiment is caused by accidental efferent-afferent nerve connections. In fact in several species of Crustacea it was also observed that, after the claw or limbs were severed close to the body, efferent discharges were elicited by touching the hairs or bending the limb and these discharges were sometimes observed for an hour or longer (Barnes, 1932). Nobody has observed the anatomical connection between the receptors and effectors in Crustacea, but there may exist connections such as the ephaps.

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