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The Function of the Thoracic Ganglion of the Crayfish, *Procambarus clarki*¹⁾

By

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(With 8 Text-figures)

It is well known that the function of the central nervous system is to integrate among intricate nerve networks, afferent informations from the peripheral sensory organs and to regulate efferent impulses to many organs through the similar networks. Electrophysiological studies on the synaptic transmission of the central nervous system have been extensively performed in the vertebrates by means of microelectrode (cf. Eccles, 1964).

On the contrary, electrophysiological studies on the synaptic transmission of the ganglions have been rather scanty in the invertebrate. They were started by Prosser (1934a, b) and Roeder (1941). Bullock (1948), Maynard (1953), Tauc (1955a), Wiersma (1957) and Watanabe (1963) investigated the characters of the synaptic transmission and the functions of the connective nerve networks in the ganglions of the squid, lobster, aplysia and crayfish. But there still remain many problems on the function and transmission of impulses in the simple central nervous system of the invertebrate. The transmission of impulses in the dorsal and abdominal ganglions were studied in the crayfish by Watanabe (1963), but the transmission in the thoracic ganglion has not been studied yet. This is mainly because the anatomical approach to this ganglion was very difficult.

In the present experiments, the sensory hairs of the walking leg of the crayfish were stimulated by water vibration, and relationships between the input and output signals were studied in the nerves entering and leaving the thoracic ganglion. Thus, the function of the thoracic ganglion was analysed.

Material and Method: The crayfish, *Procambarus clarki* was used. The whole exoskeleton was carefully removed from the cephalic carapace, thoracic groove and thoracic carapace, excepting a part of the exoskeleton which constitutes the basis of the second

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walking leg. The leg, together with the sensory hairs in the claw, was left intact but all the legs were removed. The sensory organs of the propodite and dactylopodite (P.D) and propodite and meropodite (P.M) were removed except the sensory hairs of the second walking leg. Thus, the sensory hairs of the walking leg and the afferent nerve fibres were isolated. On the other hand, the nerve bundles which innervated the walking leg from the thoracic ganglion were also left uninjured for recording of the impulses. Thus, a walking leg-thoracic ganglion preparation was made. The whole preparation was perfused with van Harreveld's solution (van Harreveld, 1936).

The sensory hairs of the walking leg was stimulated with water vibration. The method was described in a previous paper (Aoki, 1965). The responses elicited were recorded by means of a fine Ag-AgCl electrode (exploring electrode) and a silver plate (indifferent electrode). For the purpose of studying relationships between the input and output signals, impulses were recorded both in the homolateral afferent and efferent nerve fibres of the second thoracic ganglion. With the same purpose, impulses were recorded from the efferent nerve fibres and the preganglionic interneuronal fibres of the same side, from the pre- and postganglionic interneuronal fibres, and from the bilateral efferent nerve fibres. The impulses were recorded with a pair of Ag-AgCl electrodes. They were fed into a high-gain pre-amplifier and photographed on the cathode-ray oscilloscope. All experiments were done at room temperatures of 17°–22°C.

Results

There are five thoracic ganglions in the thoracic nerve cord of the crayfish. The nerve fibres from the first thoracic ganglion innervate the muscles of the cheliped and the nerve fibres from the other four thoracic ganglions (2nd, 3rd, 4th and 5th ganglions) innervate the muscles of the four walking legs respectively. These five thoracic ganglions are similar in the histological structure. These thoracic ganglions also resemble the structure of the abdominal ganglion (Wiersma, 1961). Each thoracic ganglion has a pair of afferent and efferent nerve bundles and the five ganglions are connected by the nerve fibres of interneurons. When the thoracic ganglion was sectioned horizontally, cells of 10–100 μ in diameter were observed in a layer of the dorsal side of the ganglion under the light microscope. In a cell layer of the ventral side, a mass of neuropils was observed. Next, recording the input and output signals in the thoracic ganglion, functional mechanism of the ganglion was studied, although indirectly.

Relationship between impulses from homolateral afferent and efferent nerves

The impulses recorded from the afferent nerve fibres were synchronous with the frequency of water vibration (Aoki, 1965). When the frequency of water vibration was low (below 40 c.p.s.), impulses recorded simultaneously from the afferent and efferent nerve fibres appeared corresponding to the stimulus frequency. The responses recorded from the homolateral afferent and efferent nerve fibres are shown in Fig. 1 (A and B). When the frequency of water vibration was increased from 20 to 100 c.p.s., the reflex discharges appeared for the initial 250 to 300 msec with the frequencies which corresponded to the stimulus and then disappeared.

Thus, a phenomenon of adaptation was observed. In these cases, there were found a delay of about 20 msec between the afferent and the efferent discharges, and a linear relation between the number of the afferent and the efferent nerve impulses. When the sensory hairs were stimulated at frequencies from 40 to 50 c.p.s., the responses from the efferent and afferent nerve fibres showed the "on-off" type (Fig. 1C, D). In the "on-off" response pattern, the number of the "on" impulses was remarkably larger than that of the "off" impulses, and the durations of the "on"-responses were longer than those of the "off" responses. But when stimulated at higher frequencies, only the "on" response was found in the afferent and efferent

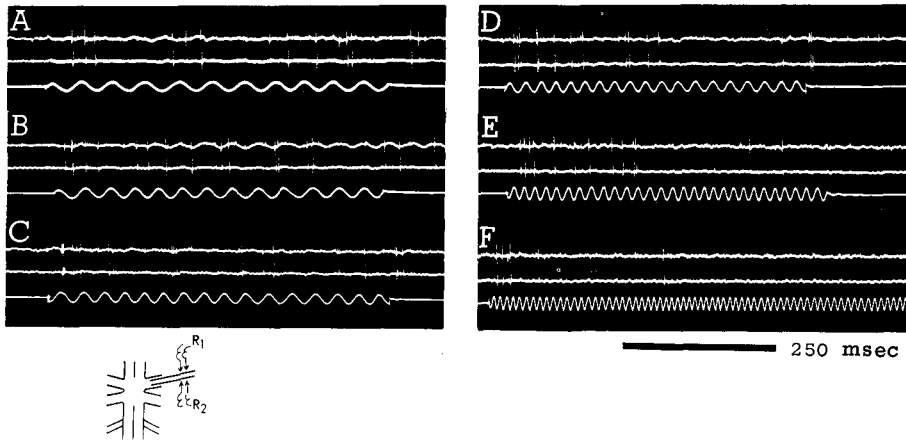


Fig. 1. Simultaneous records of impulse discharges of the afferent nerve (R_1) and the homolateral efferent nerve (R_2) in response to water vibration stimuli at various frequencies. Record A to F: Water vibration stimuli at frequencies of 20, 25, 40, 50, 70 and 100 c.p.s., respectively. In each record, the upper, the middle and the lower traces indicate the homolateral afferent discharges, the homolateral efferent discharges, and the stimulus signal, respectively.

nerve fibres (Fig. 1F). It seems that these phenomena are due to the structures of the dendrites of the sensory cells, and the sensory hairs as was described in a previous paper (Aoki, 1965). When impulses were recorded from the same afferent fibres as above and simultaneously from the different efferent fibres which belong to another nerve bundle, nerve discharges of the "on" or the "on-off" types appeared. The response of either type appeared depending upon the frequencies of vibration applied (Fig. 2A, B and C). Sato and Yamaguchi (1965) classified three different nerve fibres in the compound eye of the crayfish which transmitted on-, on-off- and off- impulses in the optic peduncle respectively. In the present experiment, there were also found nerve fibres which transmit "on" and "on-off" discharges in the efferent nerve fibres. The latent period of the reflex discharge in the efferent nerve fibres was found to be about 20 msec.

Relationships between impulses from homolateral efferent nerves and preganglionic interneurons

Relationships between the impulses from the homolateral efferent nerve fibres and from the nerve fibres of the preganglionic interneurons were investigated. When the sensory hairs were stimulated with water vibration at frequencies of 20–40 c.p.s. three types of reflex discharges appeared in the efferent nerve fibres and in the preganglionic interneuronal fibres (Fig. 3).

The 1st type was the "on" response. The reflex discharges both from the preganglionic interneuronal fibres and the efferent nerve fibres were the "on" type

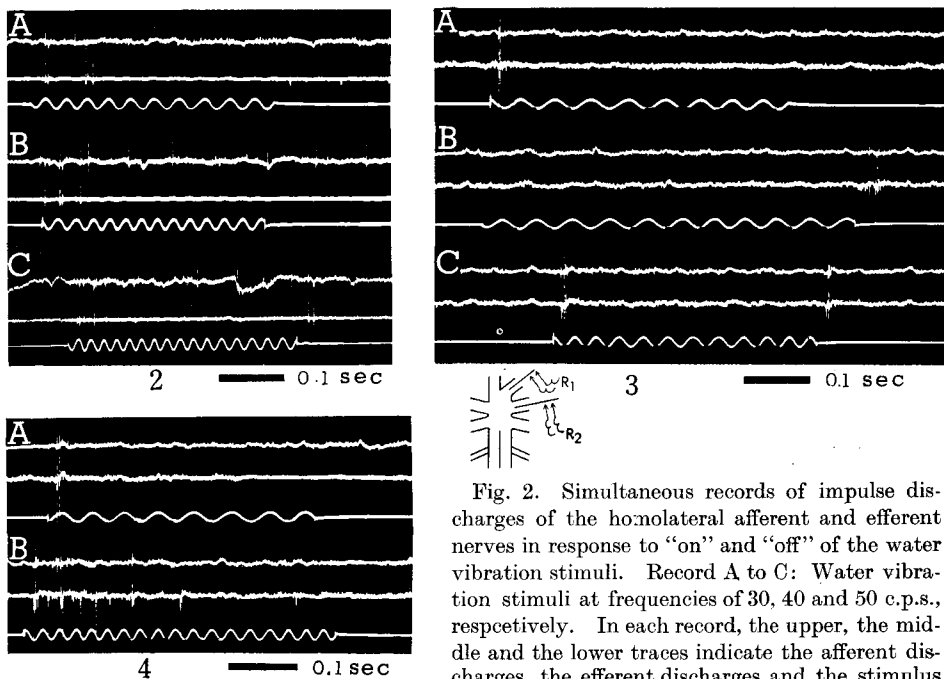


Fig. 2. Simultaneous records of impulse discharges of the homolateral afferent and efferent nerves in response to "on" and "off" of the water vibration stimuli. Record A to C: Water vibration stimuli at frequencies of 30, 40 and 50 c.p.s., respectively. In each record, the upper, the middle and the lower traces indicate the afferent discharges, the efferent discharges and the stimulus signal, respectively.

Fig. 3. Three types of ganglionic responses to the water vibration stimuli. Record A, B and C: The water vibration stimuli at frequencies of 20, 20 and 40 c.p.s., respectively. The upper signal is the transmitted impulses of the homolateral ganglionic interneurone through the ganglion (R_1) the middle one is the transmitted impulses of efferent nerve through the ganglion (R_2) and the lower one is the stimulation signal in each record. Fig. 4. "On" types of ganglionic responses to the water vibration stimuli. Record A and B: Water vibration stimuli at the frequencies of 20 and 40 c.p.s., respectively. In each record, the upper, the middle and the lower traces indicate the ganglionic interneurone discharge, the efferent nerve discharge through the ganglion, and the stimulus signal, respectively.

impulses when the hairs were stimulated at frequencies of 20–40 c.p.s. (Fig. 3A). There were found a delay of about 20 msec in these discharges. Impulses both from the preganglionic interneuronal fibres and from the efferent nerve fibres corresponded very well, namely they appeared with a ratio of one to one.

There was found a difference between the responses from the preganglionic interneuronal fibres and from the homolateral efferent nerve fibres (Fig. 4). When stimulated at frequency of 40 c.p.s., the preganglionic interneuronal fibres showed "on" discharges (Fig. 4B). A similar discharge type was observed when stimulated, at 20 c.p.s. (Fig. 4A). Besides, the efferent nerve fibres similarly showed "on" discharges which continued longer. The durations of these impulses were about 100 msec (Fig. 4). The reflex discharges recorded in these two nerve fibres were well coincided each other when stimulated at 20 c.p.s. But when stimulated at 40 c.p.s., the reflex discharge recorded from the preganglionic interneuronal fibres ceased much earlier than the efferent nerve discharge, although good coincidence was preserved.

The second type was the "off" response. When the sensory hairs were stimulated by water vibration, these nerve fibres did not show any response at the onset of the stimulation, but they showed response only at the cessation of the stimulation (Fig. 3B). When stimulated at the frequency of 20 c.p.s., both the preganglionic interneuronal fibres and the efferent nerve fibres showed "off" discharge of about 50 msec. As is shown in Fig. 5B, the preganglionic interneuronal fibres showed "off" discharge, while the efferent nerve fibres produced impulses which corresponded well with the frequency of the stimulation. The latent periods of the "off" responses recorded in the preganglionic interneuronal fibres and in the efferent nerve fibres were about 30 msec after the cessation of the stimulation (Fig. 5).

The 3rd type was the "on-off" response. When the sensory hairs were stimulated by water vibration, the preganglionic interneuronal fibres and the efferent nerve fibres produced impulses at the beginning and at the end of the stimulation (Fig. 3c). When stimulated at the frequency of 60 c.p.s. these nerve fibres showed longer reflex discharges than those produced at the frequency of 30 c.p.s. The preganglionic interneuronal fibres showed the "on-off" responses, in each of which there appeared a few impulses. The reflex discharges from the efferent nerve fibres continued for about 10 to 200 msec at the onset of the stimulation. A large number of the "off" impulses was produced in this nerve fibre (Fig. 6A, B). When stimulated at the frequency beyond 50 c.p.s., the preganglionic interneuronal fibres and the efferent nerve fibres produced impulses corresponding to the frequency of stimulation (Fig. 6C). When stimulated at higher frequencies, the reflex discharges appeared at higher frequencies. The latent period of the impulse discharges in these nerve fibres was found to be about 20 msec.

Relationship between impulses of preganglionic and postganglionic interneurons

When the sensory hairs were stimulated by water vibration at the frequencies of 20, 30 and 40 c.p.s., there were found one to one relationship between the frequencies of stimulation and those of the reflex discharges recorded in the pre- and postganglionic interneurons and a delay between the appearances of impulses in the two interneurons (Fig. 7). Spontaneous activity was also observed in the pre- and postganglionic interneurons. When the sensory hairs were stimulated by

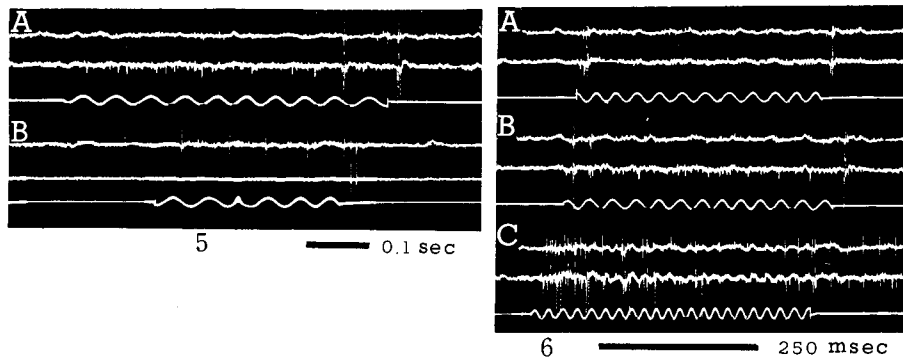


Fig. 5. "Off" type of ganglionic responses to the water vibration stimuli. Record A and B: Water vibration stimuli at frequencies of 20 c.p.s. In the record, A, the upper, the middle and the lower traces indicate the discharges of the homolateral preganglionic interneurone, the discharges of homolateral efferent nerve, and the stimulus signal and in the record B, the upper, the middle and the lower traces indicate the homolateral efferent nerve discharges, the homolateral preganglionic interneurone discharges and the stimulus signal. Fig. 6. "On-off" type of ganglionic responses to the water vibration stimuli. Record A, B and C: The water vibration stimuli at frequencies of 30, 30 and 50 c.p.s., respectively. In each record, the upper, the middle and the lower traces indicated the homolateral ganglionic interneurone discharges, the homolateral efferent nerve discharges and the stimulus signal, respectively.

water vibration, these interneurons showed impulses of the "on" and "on-off" types.

In the pre- and postganglionic interneurons, impulses of the "on" type increased in frequency with increasing stimulus frequency and they also increased in duration with increasing stimulus duration. On the contrary, the impulses of the "off" type were not influenced by the increase in frequency of the stimulation. When stimulated by water vibration at frequencies of 60–100 c.p.s., the impulses of a different type from in Figure 7A were recorded in the pre- and postganglionic interneurons (Fig. 7D, E). Even when the frequency of the stimulation was changed, the two nerve fibres produced only the "on" type discharges.

Relationship between impulses of bilateral efferent nerves

When the sensory hairs were stimulated by water vibration at the frequencies of 60–70 c.p.s., the efferent nerves on both sides produced only the “on” type discharges corresponding to the frequencies of stimulation (Fig. 8).

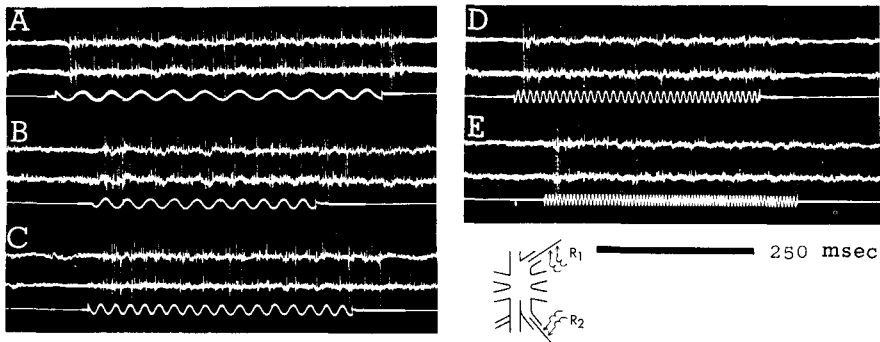


Fig. 7. Simultaneous records of impulse discharges of the preganglionic interneurone (R_1) and the postganglionic interneurone (R_2) in response to the water vibration stimuli at various frequencies. Record A to E: The water vibration stimuli at frequencies of 20, 30, 40, 80 and 100 c.p.s., respectively. In each record, the upper, the middle and the lower traces indicate the preganglionic interneurone discharge, the postganglionic interneurone discharges and the stimulus signal, respectively.

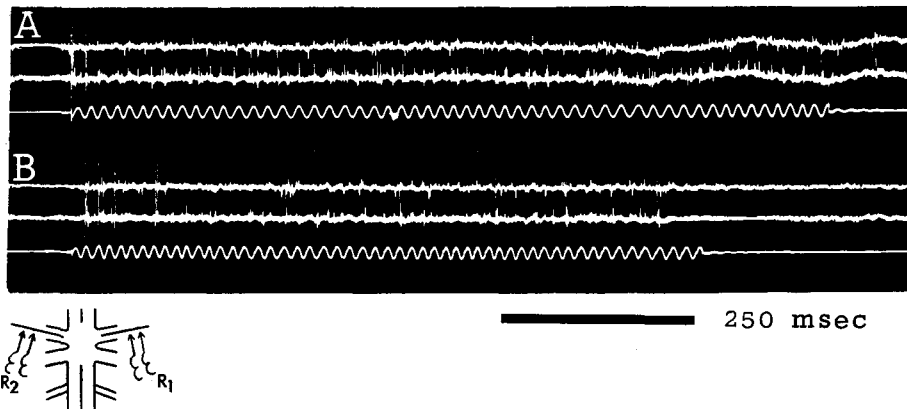


Fig. 8. Simultaneous records of impulse discharges of the bilateral efferent nerves (R_1) and (R_2) in response to the water vibration stimuli. Record A and B: The water vibration stimuli were applied at frequencies of 60 and 70 c.p.s. respectively. In each record, the upper, the middle and the lower traces indicate the right efferent (R_1) discharges, the left efferent (R_2) discharges, and the stimuli signal, respectively.

There was found a difference in number of the reflex discharges in the efferent nerve fibres of the two sides when stimulated at frequencies below 40 c.p.s. but one to one relationship was found between these two reflex discharges. The durations of these "on" discharges were found to be quite long, and the latent period was about 20 to 24 msec in these fibres.

Discussion

Mechanisms of pattern formation of the output signals in response to the incoming signals were investigated in the central nervous system of the invertebrates in detail by Burns (1957), Maynard (1961a) and Watanabe (1963). As a method to analyse such pattern formation, responses were recorded in the efferent and the afferent nerve fibres of the second thoracic ganglion *in vivo*.

In the present experiment, the reflex discharges were recorded in the efferent nerve fibres of the second thoracic ganglion. Three kinds of responses, the "on", "on-off" and "off" types were obtained in the efferent nerve fibres when the frequencies of stimulation were beyond 50 c.p.s. When mechanical stimuli were applied to the P.D. organ of the cheliped, in the efferent nerve fibres of the first thoracic ganglion reflex discharges appeared which were indifferent to the stimulus frequency (Muramoto, 1965). Since Bush (1965) recorded the same types of responses in the efferent nerve fibres of the second thoracic ganglion of the crab (*Carcinus maenas*), it is inferred that the difference between the results in the present experiments and in the experiments by Muramoto (1965) is mainly due to the difference in function between the first and second thoracic ganglia, and also to the difference in the kinds of organs to which the mechanical stimuli are applied.

The reflex discharges recorded in the pre- and postganglionic interneurons of the second thoracic ganglion corresponded well within a limit of the frequencies of stimulation (20–100 c.p.s.). These results conform well with the finding that all the homolateral legs show the same walking movements, and show that these pre- and postganglionic interneurons send the same information to each of the other thoracic ganglia. The reflex discharges which were recorded in bilateral efferent nerve fibres of the second thoracic ganglion also corresponded well when the stimulus frequencies were beyond 60 c.p.s. Such a correspondence of the reflex discharges in these efferent nerve fibres may be necessary to keep the balance of the body of the crayfish. But in case of low frequencies below 40 c.p.s., there was not found a correspondence between the reflex discharges from the bilateral efferent nerve fibres in many cases. It is likely that the inconstancy may be related to the reciprocal movement of the legs of both sides in walking.

The reflex discharges recorded in the efferent nerve fibres and pre- and postganglionic interneurons of the second thoracic ganglion showed the "on" and "on-off" responses when the frequency of stimulation was higher than 50 c.p.s., and the reflex discharges recorded in the preganglionic interneurons showed the "off"

response when the frequency of stimulation was lower than 50 c.p.s. It seems that the frequency of stimulation may play an important role in the occurrence of the "on", "on-off" or "off" patterns in these reflex discharges. The "off" response appeared at the low frequencies which may be elicited when the sensory hairs slowly return from deflected positions towards a resting perpendicular position to the cuticular surface. The "on" response pattern was observed when the sensory hairs were stimulated at the high frequency stimulus (beyond 50 c.p.s.), and the "on" reflex discharges disappeared in about 100 msec after the onset of the stimulation (adaptation). The "on" component in the "on-off" response also disappeared in about 100 msec after the beginning of the stimulation (adaptation), and the "off" response ceased in about 30 msec after the end of the stimulation. When the discharges were recorded in the afferent nerve fibres of the sensory hairs in the thorax of the crayfish, touch stimulation produced the "on" and "on-off" responses (Mellon 1963). But in the present experiments these reflex types were recorded in each of the three efferent nerve fibres of the second thoracic ganglion. Therefore, it does not seem that the response types found in the pre- and postganglionic interneurons and the efferent nerve fibres of the second thoracic ganglion are determined only by the frequency of stimulation.

When recorded simultaneously in the preganglionic interneurons, and the homolateral efferent nerve fibres, and the pre- and postganglionic interneurons of the thoracic ganglion, the responses showed the "on" and "on-off" types. It seems that the efferent nerve fibres and pre- and postganglionic interneurons contain the "on" and the "on-off" fibres. When these responses are investigated in detail, the durations of the reflex discharge recorded in the efferent nerve fibres are longer than that of the reflex discharge recorded in the preganglionic interneuron. It seems that the difference of these reflex discharges are due to difference in the function of the synapses in the thoracic ganglion. Waterman and Wiersma (1963) and Sato and Yamaguichi (1965) recorded "on", "on-off" or "off" responses in single interneurons between the brain and the optic ganglions when light stimuli were applied to the compound eye of the crab, lobster and crayfish. Thus, they could classify three kinds of nerve fibres, the "on", "on-off" and "off" fibres. Besides, Wiersma (1961) found that one of these three types of responses appear depending upon the intensities of stimuli. Since microelectrode technique or isolation of a single fibre preparation was not tried in the present experiment, the three kinds of nerve fibres could not be classified. Therefore, from considering the above results, it is presumed that there exist the "on", "on-off" and "off" fibres in the efferent and the ganglionic interneurons and that the three types of responses appear in these nerve fibres depending upon the intensities of water stimulation.

Summary

By application of water vibration to the sensory hairs of the crayfish, the responses which appeared in the afferent and efferent nerve fibres and pre- and postganglionic interneurons of the second thoracic ganglion were recorded *in vivo*.

1. The relationship between the impulses of the afferent and efferent nerve fibres was studied. They corresponded to the frequency of water vibration, when the frequency of stimulation was below 40 c.p.s. The "on" and "on-off" responses were observed in the efferent nerve fibres when the frequency of stimulation was beyond 40 c.p.s.

2. There were found the responses of the three types, the "on", "on-off" and "off" in the reflex discharges recorded in the efferent nerve fibres and preganglionic interneurons, when the frequencies of stimulation were 20–100 c.p.s. The "off" response was recorded in the efferent nerve fibres and the preganglionic interneurons when the frequency of stimulation was below 40 c.p.s. The duration of the "off" response was about 50 msec.

3. The relationships between the impulses of the pre- and postganglionic interneurons were studied. These impulses mutually corresponded very well at frequencies of 20–100 c.p.s. These two interneurons showed the "on" response at frequencies beyond 60 c.p.s. and the "on-off" response at the frequencies below 60 c.p.s. but the "off" response was not observed.

4. There was found one to one relationship between the impulses of the bilateral efferent nerve fibres when stimulated at the frequencies of 60–70 c.p.s. However, such relationship was not found at the frequencies below about 40 c.p.s. and the number of impulses was larger in the homolateral efferent fibres than in the heterolateral fibres.

5. It is presumed that the three types of responses found in the present experiments appear in the "on", "on-off" and "off" fibres depending upon the intensities of water vibration.

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