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Impulse Transmission of the Statocyst Response to Vibration through the Supra-oesophageal Ganglion of the Crayfish*,[1),2)]

By

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(With 6 Text-Figures and 1 Table)

The function of the crustacean statocyst has been investigated by the analysis of the behavioural responses and the electrical response of the sensory fibres to various mechanical stimuli. In the lobster, *Homarus americanus*, the thread hair receptors and statolith hair receptors in the statocyst show the receptivities to change in angular acceleration and to change in orientation in the gravititional field independently (Cohen, Katsuki and Bullock, 1953, Cohen, 1953, 1955, 1960). The thread hair receptors, furthermore, are responsive to vibration conducted through the solid substrate (Cohen, 1960, Cohen and Dijkgraaf, 1961).

Similar dual-sensitivity to position change and to vibration has been observed in the statolith hair receptors of the crayfish, *Procambarus clarkii*, by the author (1965). The responses transmitted through the central nervous system (C.N.S.) were also recorded qualitatively from the circum-oesophageal commissures. These responses are elicited by a mechanical stimulus, such as position change, horizontal rotation, and vibration which includes that of sound at a low frequency and high intensity, applied to the statocysts. The anatomical results on the descending fibres related to the statocyst sensory fibres have been reported by Wiersma (1958) and Wiersma and Bush (1963). But further information on the electrical properties of these fibres is still insufficient. In the present experiments the author intended to analyze the types of the synaptic transmission pathways in the equilibrium reflex of the animal, with the aid of an external recording of the responses to vibration stimulus from isolated fibres of the circum-oesophageal commissures.

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

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Material and Methods

Preparation: The statocyst - C.N.S. (supra-oesophageal ganglion and circum-oesophageal commissures) preparations of the crayfish, *Procambarus clarkii*, were used for the experiments. The anterior region of the cephalothorax was dissected from the animal. The circum-oesophageal commissures exposed to the air were cut off as long as possible at the level of the oesophagus. The animal was then put into van Harreveld's fluid, and the connective tissues surrounding the C.N.S. were removed. The skeletal wall of the oral parts was cut off entirely, as a matter of convenience so that the circum-oesophageal commissures could be observed under the binocular microscope and in transmitted light. To avoid injury to the supra-oesophageal ganglion, both eye stalks were kept in intact. The first antennules were amputated at the peripheral level of the statocyst. The second antennae were also removed from the basal segment. The dissected preparation was then mounted on a small L-shaped plastic holder in a resin and bee wax mixture. The isolation procedure of the single fibres from the commissures is as follows. The preparation on the holder was transfered into a glass chamber filled with van Harreveld's fluid, which was kept at a temperature of 3°C. Under the binocular microscope, the hyaline membrane covering the circum-oesophageal commissure was stripped away, using a sharp forceps and scissors. The fibres of the commissure nerve were then separated free from injury, into small bundles or single fibres with two sharp forceps.

Stimulation: Vibration stimuli were applied directly to the statocysts by a moving coil type vibrator. The arrangements for stimulation and the recording system are shown in Figure 1. The small holder (H) with the preparation (P) is connected by a short iron rod to the vibrator (V) which is driven by a noiseless A.C. power amplifier (AP). Sinusoidal wave signals generated by an audio frequency oscillator (AFO) and rectangular pulse signals of short duration, less than 2 msec, generated by an electronic stimulator (ES) where led independently to the input of the power amplifier. The frequency and the amplitude of the vibrations were varied by operation of the oscillator and the stimulator.

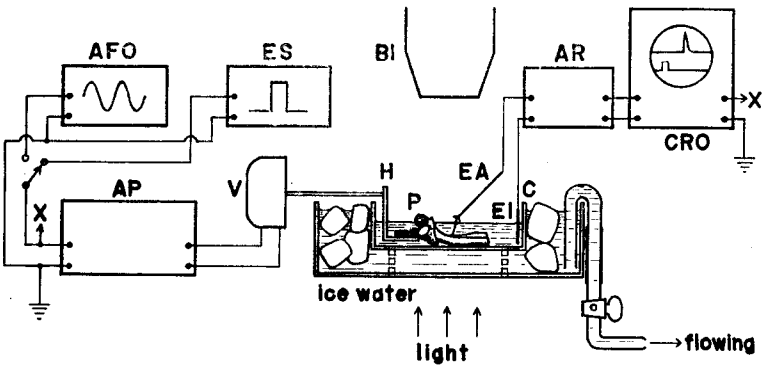

Fig. 1 Diagram of the experimental arrangements. AFO: audio frequency oscillator, AP: A.C. power amplifier, AR: recording A.C. amplifier, BI: binocular microscope, C: glass chamber filled with van Harreveld's fluid, CRO: cathode ray oscilloscope, EA: active electrode, EI: indifferent electrode, ES: electronic stimulator, H: holder, P: preparation, V: vibrator.

Recording: Postganglionic responses which were elicited by the vibration stimuli and transmitted through the supra-oesophageal ganglion were recorded externally. This was accomplished by isolating the fibres. A single fibre or group of fibres was lifted free from injury into the air with an active Ag-wire electrode connected to a micromanipulator. Action potentials were led to the cathode ray oscilloscope through an A.C. amplifier (AR). Oscillographic records were taken by a long recording camera, and single frame pictures of the same responses were also taken by a synchronized fast sweep of the oscilloscope beam. This was done in order to measure the latencies in each of the descending fibres of the commissures.

In most of the experiments the temperature of van Harreveld's fluid was maintained at a constant value of 3°C. The room temperature was from 15° to 19°C.

Results

(1) *The synchronous postganglionic responses to vibration*

A group of fibres in which the postganglionic response could be observed was separated from the circum-oesophageal commissure and selectively contacted with the electrode. This group of fibres was then further separated into smaller fibre groups. Then the action potential was examined again in each of these fibre groups. Thus, the three large fibres and several other small fibres responsive to the vibration stimuli could be obtained from each commissure.

Responses of the large fibres

It was not so difficult to isolate each of the three large fibres because of the large size of their diameters (30–60μ), although they are considerably thinner than both the medial and the lateral giant fibres, and because of their location. The first large fibre, LF-1, is located at the inner part of the commissure near the medial giant fibre. The second large fibre, LF-2, is located between the two giant fibres. And the last, LF-3, is located in the ventral region near the surface of the commissure.

The types of responses to the vibration stimuli which were obtained from the three fibres are shown in Figure 2. No spontaneous activity was observed in any of the large fibres. Only when pulse stimuli were applied to the isolated preparation with intact statocysts, were the phasic spike trains of action potentials elicited in these fibres. The postganglionic response of each fibre was synchronous with the stimulation pulses. But if the frequency of stimulation was increased, while maintained at a constant intensity, such a synchronous response was no longer elicited and only irregular and scattered impulses were observed finally. The synchronous response was usually again recovered when the pulse frequency of stimulation was decreased to a certain frequency level. But it appeared that the two upper limits in the frequency ranges of the synchronous responses never agreed with each other. The impulse discharge rate of a single fibre stimulated at increasing frequency was regularly higher than that of the same fibre excited by vibration at decreasing frequency. This suggests that the synaptic region of the fibres in the

supra-oesophageal ganglion may be easily accommodated to repetitive stimuli at a high frequency. Moreover, if the recovery time from accommodation is prolonged, the frequency range of the response synchronizing with the square wave stimulation will become narrow.

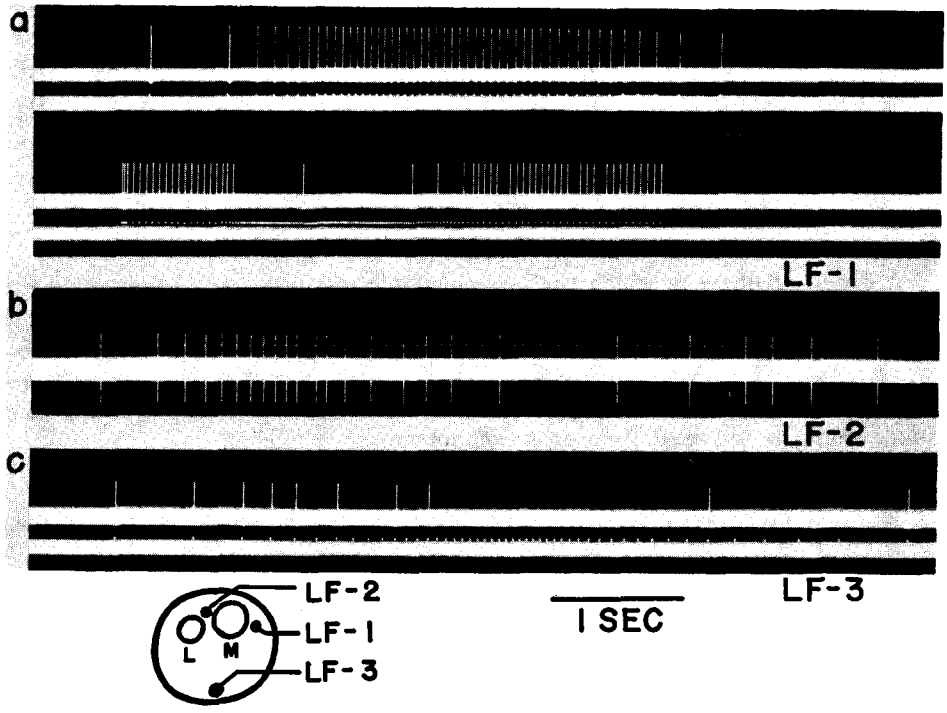

Fig. 2 Oscillograph records of the postganglionic responses of the isolated three large fibres in the circum-oesophageal commissure to the vibration stimuli applied to the crayfish statocysts. The vibration stimuli: square wave of changing frequency applied at a constant intensity. a: the impulses train recorded from the LF–1 large fibre, b: from the LF–2 large fibre, c: from the LF–3 large fibre. In each record the lower signal is of the stimulus. The location of each of these fibres is shown in the inset diagram of the cross section of the commissure nerve. L and M: the lateral and medial giant fibres respectively.

The upper limits in the frequency of the synchronous response to pulse stimuli and the latencies from stimulus to response of the same fibres are illustrated in Table 1. The upper limits of impulse frequency obtained by increasing the frequency of vibration are different for each of the three large fibres. The first fibre, LF-1, was responsive to stimuli at a higher frequency, higher than 30 c.p.s., and it showed a typical phasic response type. The second large fibre, LF-2, was capable of response to vibration at a middle frequency range, lower than about 10 c.p.s. In the last

one, LF-3, no synchronous response was elicited by stimuli at frequencies of lower than about 8 c.p.s.

If one of the statocysts of the preparation was removed and stimulation was applied to the remaining one, no postganglionic response was obtained in either the contralateral LF-2 or LF-3 large fibres. While the response was always observed in the contralateral LF-1 large fibre. In the LF-1 large fibre, no modification on the discharge type was affected by such an operation. These facts explain that the LF-1 large fibre synapses heterolaterally with the sensory fibres of the intact statocyst, and that LF-2 and LF-3 large fibres synapse only homolaterally with sensory fibres in the supra-oesophageal ganglion. On the other hand, the latencies of measured impulses were compared among the three large fibres. When the preparation with the statocyst intact was stimulated at a low frequency, the minimum latency could be obtained in each fibre. The values were 6.4, 13.4 and 16.9 msec in the LF-1, LF-2 and LF-3 large fibres respectively. The latencies of all the descending fibres were usually prolonged gradually by the repetitive pulse stimuli at a frequency of about 5 c.p.s.

Table 1. The upper limit frequencies of the synchronous responses to the square wave vibration stimuli and the latencies of impulses transmitted through the supra-oesophageal ganglion, obtained from the isolated three large fibres and the other thinner fibers of the circum-oesophageal commissures of the crayfish.

Fibre	Frequency of spontaneous activity (c.p.s.)	Upper limit frequency of synchronous response (c.p.s)	Minimal latency (msec)	Synaptic delay (msec)
Large fibre				
LF−1	—	30.0-33.8	6.4	4.0- 9.3
LF−2	—	7.6-13.8	13.4	11.0-11.8
LF−3	—	6.4- 8.5	16.9	14.5-24.4
Small fibre				
1	—	15.2	10.8	8.4-11.4
2	—	5.5	11.8	9.4-11.1
3	—	18.0	12.3	9.9-12.3
4	—	4.4	14.9	12.5-13.4
5	—	8.7	19.0	16.6-17.2
6	—	3.2	19.7	17.3-20.3
7	—	2.1	21.2	18.8-20.9
8	5.0	30.4		
9	1.3	19.0		
10	1.5	8.9		
...	...	....	....	.....
The minimum and maximum		2.1-30.4	10.8-25.8	8.4-31.6

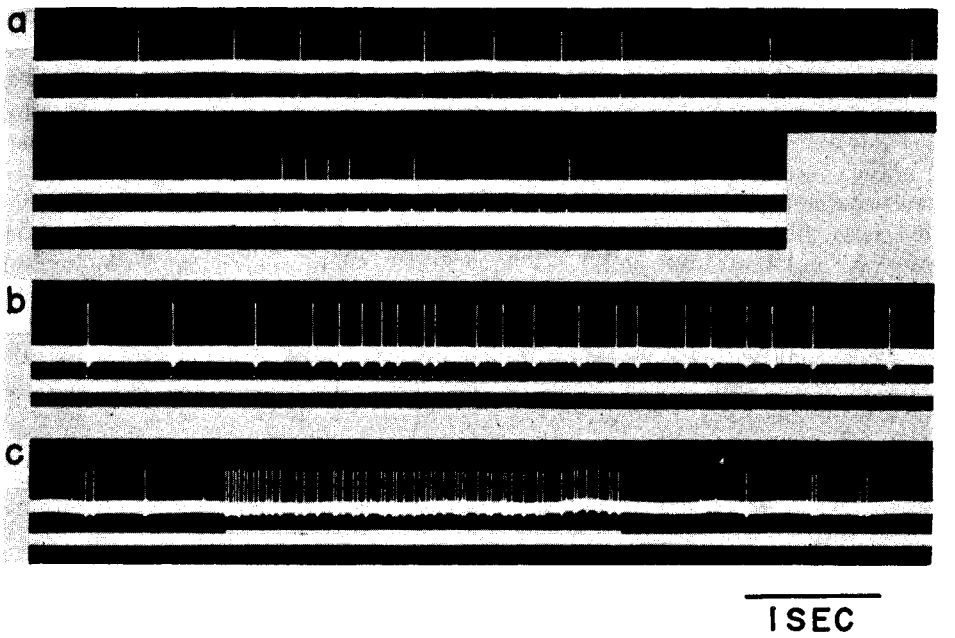

Fig. 3 Three examples of the oscillograph records of the postganglionic responses of the isolated small fibres in the circum-oesophageal commissure to the vibration stimuli of the square wave of changing frequency and of a constant intensity, except for the second record in which the stimulus intensity was increased. a: record from a single small fibre connected to the lateral giant fibre, b: from a single fibre located near the LF-3 large fibre, c: the single unit response of a fibre group, showing spontaneous activity, near the LF-3 large fibre. In each record the upper and the lower signal are respectively of the response and the stimuli.

Responses of the other fibre groups

The spike trains of postganglionic responses caused by vibration stimuli were observed in the other thinner groups (8–30μ) of the descending fibres in the commissure. The isolation of these fibres into single fibres was very difficult owing to their delicacy. It was confirmed that the number of the responding fibres was at least five in each commissure. They showed a specific response type to vibration.

Figure 3 shows three examples of the oscillographic records of the postganglionic responses obtained from isolated single fibres or from a small group of such fibres in the same commissure. The upper limits of the frequency ranges of the responses to the stimuli and of the latencies of the same fibres are also summarized in Table 1. Even for stimuli at a constant intensity of vibration great variations are seen at the upper limits of the frequency ranges of both the

synchronized responses and the latencies in these small fibres. The fibres which showed spontaneous discharge activity could also respond to the stimuli of a high frequency, higher than 30 c.p.s. On the contrary, the fibre which showed no spontaneous activity could hardly respond to the stimuli at frequency of even 2 c.p.s. The shortest latencies of the fibres were longer than 10 msec. They ranged from 10.8 to 25.8 msec in all the small fibres. The responses of the descending fibres with longer latencies tended to be unable to follow the stimulus frequency. This also may be applicable to the three large fibres.

(2) *Relation between the statocyst information and postganglionic response*

Concerning sensory information from the statocysts, the impulses of the primary sensory fibres in response to the vibration of the sinusoidal wave were perfectly synchronous in all the statolith hair receptors, as was reported in any previous paper (Sugawara, 1965). The discharge activity was of a non-adapting type and the frequency corresponded with the frequency of the stimulating sinusoidal vibration. The next investigation was carried out in order to find out up to what frequency of stimuli could the synchronous postganglionic responses be elicited by the sinusoidal vibration.

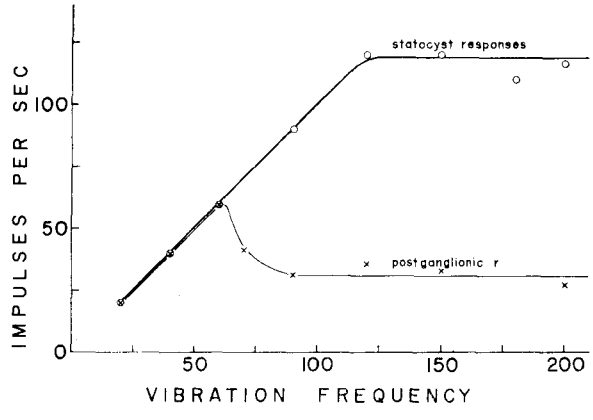

Fig. 4 The relationship between the stimulus frequency of sinusoidal wave vibrations and the response frequency in both the statocyst sensory fibres and the LF–1 large fibre of the circum-oesophageal commissure, at the same stimulus intensity. The maximum frequency of the postganglionic response of a single unit was plotted.

Figure 4 shows the relationship between the frequencies of the stimuli, and of the responses which were obtained from the statocyst sensory fibres and the circum-oesophageal commissure respectively, at the same stimulus intensity. The descending impulses in the commissure could not follow the stimulus frequency beyond 60 c.p.s., while the statocyst could respond synchronously to the stimuli of high frequency beyond 120 c.p.s. The frequency of the postganglionic discharges fell

gradually to the constant level of about 40 c.p.s. The descending fibre, showing the response type as mentioned above, was identified as the LF-1 large fibre. This identification was based on the discharge type of the highest frequency and on the position of the recording electrode on the commissure. When the stimulus intensity of both the sinusoidal and the pulse vibration was increased to a certain extent level, the postganglionic responses were synchronous with the stimuli of higher frequency.

(3) *Adaptation of the postganglionic response*

The postganglionic discharge activity was suppressed at high stimulus frequency. A similar result was observed when the duration of repetitive stimulation was prolonged, even if the stimulus frequency was kept at a constant level where the descending fibre in the commissure could respond synchronously.

Figure 5 shows the decrease with time in the discharge frequency of the responses of both of the circum-oesophageal commissures which were elicited by the application of the sinusoidal vibration at a frequency of 60 c.p.s. The burst of impulses was followed by an exponential decrease in the discharge frequency. About 5 sec after the initiation of the stimulus the rate of discharged impulses had returned to the spontaneous activity level.

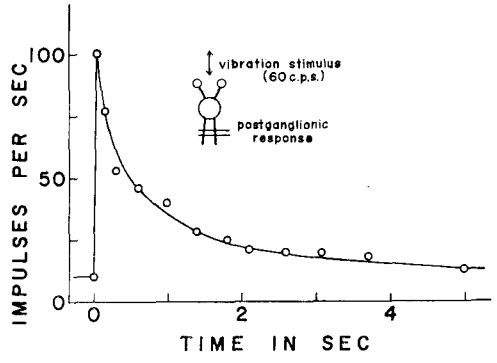

Fig. 5 The decrease with time in the discharge rate of the postanglionic responses to sinusoidal wave vibration at a frequency of 60 c.p.s. The record was taken from both the circum-oesophageal commissures.

(4) *Synaptic delay of the synchronous impulses*

Figure 6 shows the intensity-latency curves of both the sensory and postsynaptic nerves stimulated by the vibration applied to the statocyst. After 4 msec from the initiation of the statocyst response, the first spike of response transmitted through the supra-oesophageal ganglion appeared on the circum-oesophageal commissure. The latencies of these responses decreased exponentially with the increase in the intensity of the vibration stimuli, and they reached the two

independent minimal rates respectively. The postsynaptic fibre which had the shortest latency was identified as the LF-1 large fibre. The conduction velocity of this fibre was measured, and it ranged from 23 to 32 m/sec at 19°–20°C. This value is higher than that of the medial and lateral giant fibres (10–15 m/sec and 15–20 m/sec at 20°C respectively by Wiersma, 1947), in spite of the fact that the diameter of the former is less than that of the latter two. Therefore, the conduction times along both the sensory and postsynaptic fibres are very short and may be negligible in the present measurement of the synaptic delay in the supra-oesophageal ganglion. And it was confirmed that the time delay of 4 msec, as

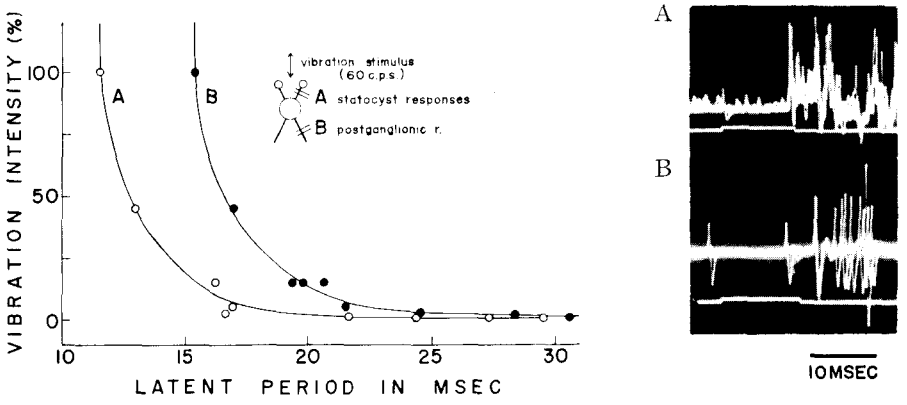

Fig. 6 The relationship between the stimulus intensity of the sinusoidal wave vibration and the latency of the responses of both the statocyst sensory fibres (A) and the LF–1 large fibre of the circum-oesophageal commissure (B). The oscillograph records on the right show the superimposed action potentials of the sensory nerve and of the postsynaptic fibres in the commissure. The lower rectangular signal in the record is that of the stimulus.

mentioned above, was the synaptic delay associated with the LF-1 large fibre. The statolith hair receptors were responsive to the sound stimuli of high intensity and of a frequency of lower than 300 c.p.s. In this case, the synaptic delay was about 27 msec as was reported previously by Sugawara (1965). Therefore, it is clear from the difference in the synaptic delays that each of the impulses elicited by the two different kinds of stimuli, the vibration at a low frequency and the audible sound, is transmitted in the independent synaptic pathways from the statolith hair receptors.

Discussion

Descending fibres in the circum-oesophageal commissures

From the results of the present experiments, it was revealed that the upper limits of the frequency ranges of the postganglionic impulses, evoked by the vibration stimuli of sinusoidal or square waves, and the latencies of the impulses varied widely

in all the descending fibres of the circum-oesophageal commissures. The variation in the minimal synaptic delay ranged from 4 to 24 msec. The fibres having longer synaptic delay could not follow the higher stimulus frequencies. These facts suggest the complicated synaptic transmissions of the statocyst responses. The three large fibres, LF-1, LF-2 and LF-3, isolated in the present studies seem to correspond respectively to the fibre type 4, 84 and 87 which have been classified by their locations in the circum-oesophageal commissure and by their functional relationship (Wiersma, 1958). Moreover, similar large fibres responsive to the statocyst movements have been observed at the thoracic and abdominal levels of the animal (Wiersma and Bush, 1963).

A large number of the descending fibres, including these large fibres studied in this paper, can be considered to be related to the locomotional functions. Especially the LF-1 large fibre, which crosses over heterolaterally in the supra-oesophageal ganglion, can transmit impulses of the highest frequency in response to the vibration stimuli, and the latency of which is the shortest of all the descending fibres. So, the synaptic transmission pathway of this fibre is confirmed to be much simpler than that of the other fibres. In proved large fibres can evoke the jumping movements of the animal; this was by the application of electrical stimuli to these fibres in an experiment by Rowell (1963). These large motoneurones were crossing heterolaterally in the supra-oesophageal ganglion. In the crayfish, it is considered that the impulses of the LF-1 large fibre have an important function in the bilateral co-ordination of its locomotion, especially in the equilibrium compensatory movements, at the supra-oesophageal ganglion and the caudal ganglia of the thoracic level. It is thought that the impulses of some of the other descending fibres modify the equilibrium compensatory movements of the animal.

Transmission of the impulses

The statocyst nerve of the crayfish contains no ascending fibre and each sensory information converges directly to the synapses in the supra-eosophageal ganglion. It was previously known that when the animal was stimulated by position changes, horizontal rotations or loud acoustic stimuli of low frequency, the sensory responses were of various asynchronous impulse patterns in all the sensory fibres (Sugawara, 1965). And, the descending responses transmitted through the supra-eosophageal ganglion were decreased in impulse frequency. Actually when loud acoustic stimuli were applied to the statocyst, the postganglionic responses ceased, even though the asynchronous sensory responses of high frequency continued without adaptation. On the contrary, the sensory response patterns evoked by the vibration stimuli were almost synchronous in all the statocyst fibres; moreover the postganglionic responses to vibration stimuli remained synchronous with the stimulus frequencies. Thus, the sensory response patterns caused by the various kinds of stimuli differed remarkably in impulse frequencies. The supra-oesophageal ganglion can discriminate the kind of sensory information. This central discrimination mechanism seems to be the same as that of the thread hair receptors of the

crustacean statocyst (Cohen, 1960) and as that of the hair cells of the vertebrate labyrinth (Lowenstein and Roberts, 1951).

On the other hand, the postganglionic impulses evoked by the vibration and the loud acoustic stimuli of low frequency were also of independent response patterns, as described above. The discharge activity of each descending fibre was increased when the stimulus intensity was increased. It is considered that the synaptic junctions in the supra-eosophageal ganglion can transmit the phasic impulses into certain descending fibres only when the sensory impulses of all statocyst fibres are accurately synchronous. In other words, the spatial summation of the postsynaptic potentials can be produced by the synchronous sensory volleys in the supra-oesophageal ganglion, and the synaptic junctions in the transmission pathways can be facilitated. However, the postganglionic responses to the loud acoustic stimuli seem to be initiated by the temporal facilitation of the synaptic junctions. The durations of the postsynatpic potentials, observed in the abdominal ganglia of the crayfish, were of about 5 msec (Watanabe, 1958). If the durations of the postsynaptic potentials in the supra-oesophageal ganglion are similar to those of the abdominal ganglia, this seems to be enough to evoke the spike potentials in the descending fibres. This is because the intervals of the synchronous impulses from the statocyst, in response to the acoustic stimuli of low frequency, were usually only a few msec. But the phenomenon of the fast adaptation of the descending responses to the acoustic stimuli can not be explained by such a temporal facilitation mechanism. For the solution of this problem the following hypothesis can be considered: that some inhibitory system of the sensory fibres or negative feedback system of the supra-oesophageal ganglion is mediated in the transmission pathways of the impulses. It is thought that these systems are made to function by only the repetitive asynchronous sensory volleys. Actually each of these mechanisms has been observed, in the abdominal ganglia of the crayfish (Watanabe, 1963) and in the thoracic ganglia of the locust (Rowell, 1964). In the supra-oesophageal ganglion of the crayfish such complexity of the control system in the transmission of impulses is deduced from the normal behavioural functions of the statocysts as an equilibrium sensory organ.

Summary

The crayfish statocysts were stimulated by vibration stimuli of sinusoidal or square waves. The postganglionic responses were recorded from the isolated descending fibres in the circum-oesophageal commissures in order to analyze the synaptic transmission pathways in the supra-oesophageal ganglion.

1. The postganglionic responses were recorded in the three large fibres and in the other several small fibres. These responses were phasic. Moreover, in the case that the statocyst was stimulated at higher stimulus frequencies, the responses were always adapted soon, even if the statocyst was still responding synchronously.

2. Wide variations in both the synaptic delay and the upper frequency limit

of the synchronous postganglionic responses were found in these fibres. The frequency ranges of the synchronous responses were extended remarkably by the increase in the intensity of vibration of the square wave.

3. One large fibre was responsive to the vibration stimulus of the highest frequency; moreover, this fibre seems to synapse heterolaterally with each of the statocyst nerves. The minimal synaptic delay in this fibre was 4 msec, and that value was the shortest for all of the descending fibres. The other two large fibres were connected homolaterally to the statocyst nerve. and they responded to the stimuli of low frequencies. The minimal synaptic delays in these fibres were about 11 and 15 msec respectively.

4. In the small fibres group, the minimal synaptic delay ranged from about 8 to 24 msec. The fibres having longer synaptic delay could not follow the higher stimulus frequencies. This suggests that complicated synaptic transmission pathways are involved.

5. From the comparison of the response patterns of the sensory and descending fibres to the vibration stimuli, it was confimred that the spatial summation of the postganglionic potentials was produced by the synchronous sensory volleys in the supra-oesophageal ganglion, and thereby the synapses were facilitated.

6. The impulses of these descending fibres are considered to control the locomotion, especially the equilibrium compensatory movements, of the animal.

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