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Responses of sensory hairs of the walking leg of the crayfish, *Procambarus clarkii*[*1),2)]

By

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(With 8 Text-figures)

Some of the properties of sensory hairs on the cuticular surfaces of crustaceans and insects have been described by Wolbarsht (1960), Laverack (1962 a, b) and Mellon (1963).

The careful measurements made by Wolbarsht (1960) on insect mechanoreceptor hairs have disclosed a linear relationship between the magnitude of receptor potential and the impulse frequency in the bipolar sensory neurons associated with these structures. Wolbarsht has also presented evidence suggesting that the receptor potential is a consequence of increase in the permeability of the dendritic membrane.

The investigations of the crustacean sensory organ have been done on the tactile hairs on the walking leg of the crab by Laverack (1964), and on the thoracic carapace of the crayfish by Mellon (1963). Although those studies have been made on the functions of tactile hairs, the analytical experiment of the response mechanism with the quantitative control of the stimulation seems to be lacking.

In order to analyse more quantitatively the sensory mechanism of those tactile hairs on the walking leg of the crayfish, the response characteristics must be thoroughly measured, applying the stimuli of pressure, water current and vibration with quantitatively controlled mechanical means. This paper deals with the results obtained from the experiments designed to invesitgate these characteristics of the crayfish.

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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 method

The crayfish, *Procambarus clarkii* was used throughout the course of the present investigation. The walking leg was isolated from the animals. The cuticle was removed and the nerve was exposed. The nerve bundles supplying the sensory hairs were cut at carpo- or meropodite with fine scissors. The excised preparation was then pinned to the cork piece, and placed in van Harreveld's (1936) solution. For recording one of these nerve bundles, it was separated from the surrounding tissue and hooked and lifted up into the air by a fine Ag-AgCl electrode. Impulses were led into a pre- and main-amplifier of high-gain for conventional oscilloscopic display and recording.

The part of the preparation was placed in the bathing solution to be stimulated by water current and vibration. For application of tactile stimulation, a glass rod was connected to the coil of an electromagnetic speaker, and the tip of the rod (usually 0.5 mm in diameter) which was placed on the hair was moved by application of electrical current of known strength. The time and strength of stimulation were decided along with the duration and intensity of electric current which were applied to a magnet marker.

The van Harreveld's solution was caused to flow into the chamber through a stop cock connected to a constant level reservoir, and by regulating the stop cock, the velocity of the water current was changed in various ways. The velocity was determined by measuring the amount of water which flowed through a glass tube of 5 mm in diameter per minute. The time points of opening and closing of the cock were displayed on the second beam of the oscilloscope.

To apply a vibration stimulus, a thin metal plate was placed in the vicinity of the sensory organ, this plate was connected to the electromechanic transducer and it was vibrated by application of the square wave pulses of given frequencies.

The sensory nerve impulses in response to those stimuli were displayed on the first beam of the cathode-ray oscilloscope and the frequency and the strength shape of stimulation were displayed on the second beam. All experiments were done at room temperature.

Results

Response to touch stimulation

In a preliminary investigation of the sensory hairs which project from the dactylopodite of the walking leg, the action potentials of the sensory fibres in response to touch stimulation were recorded. More than two hairs occurred in a single pit. These hairs were 2–3mm in length and colourless. One sensory nerve fibre innervated only one hair. The innervation of sensory hairs on the walking leg was the same type as that of hairs on the surface of the carapace (Mellon 1963).

The articulation of the sensory hair with the exoskeleton had some stiffness. Each hair usually stood on the cuticle almost at a right angle. A small glass rod connected to the electromechanic transducer was made to come in contact with the tip of a sensory hair which was in its normal position. The hair was bent through the transducer by application of a single pulse. It responded to the bend with a burst of nerve impulses, the frequency of which depended on the speed of bend.

Although the receptor responded phasically when the touch stimulation was applied, there was a variation in the time course of the response. The adaptation of the sensory response of small spike potential occured after 100~130 msec, and the

adaptation of another sensory response of large spike potential occured after about 60 sec.

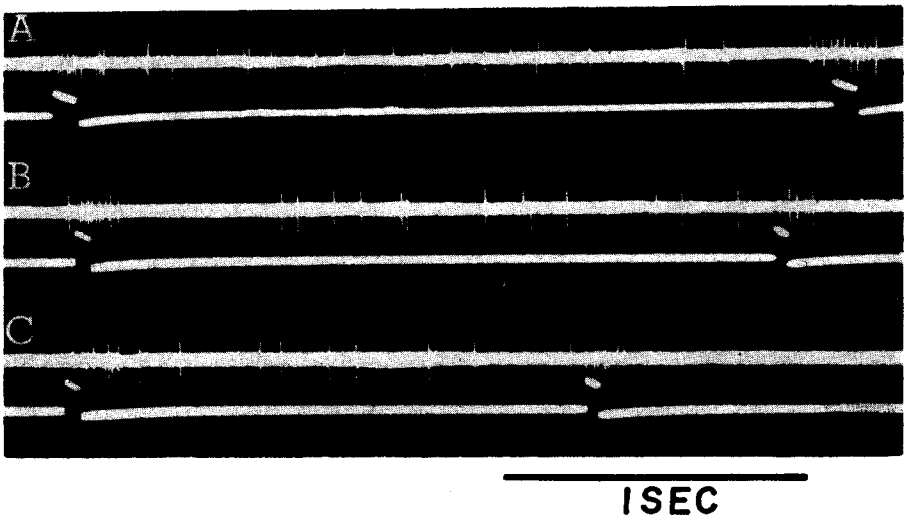

Fig. 1. The responses of slow- and fast-adapting units to touch stimulation. The stimulus strength is represented as the electrical potential difference (in volts) applied to the mehcanical stimulator. A: 9.0 V; B: 5.5 V; and C: 4.0 V. The lower signal in each record is of the touch stimulus.

Fig. 1 from A to C shows the results when the stimulus intensity was gradually decreased. The responses are independent on the intensity. The impulse frequencies in A (the strongest bend in the experiment), B and C are 17, 14 and 10 c.p.s. respectively. Such impulses of high frequency (200–300c.p.s.) which Adrian *et al.* (1931) described could not be observed in the present experiments.

Response to water current stimulation

A constant water current of 0.9cm/sec was used for stimulation of sensory hairs of the walking leg. The current direction was changed in the four ways; anterior to posterior (A–P), positerior to anterior (P–A), dorsal to ventral (D–V) and ventral to dorsal (V–D).

The records of A,B, C and D in Fig. 2 show the response patterns of sensory impulses in the four types of stimulation. The intensity of stimulation was the same in all the four cases. A–P current had a pronounced effect on the impulses as compared with P–A, V–D and D–V currents.

Fig. 3 shows the relation between the time and the frequency of impulses when the stimulation was applied in the four directions described above. The A–P

stimulation has the most remarkable effect on the response frequency in the figure. Therefore the stimulation of the water current was used in the anterior to posterior direction in the following experiments.

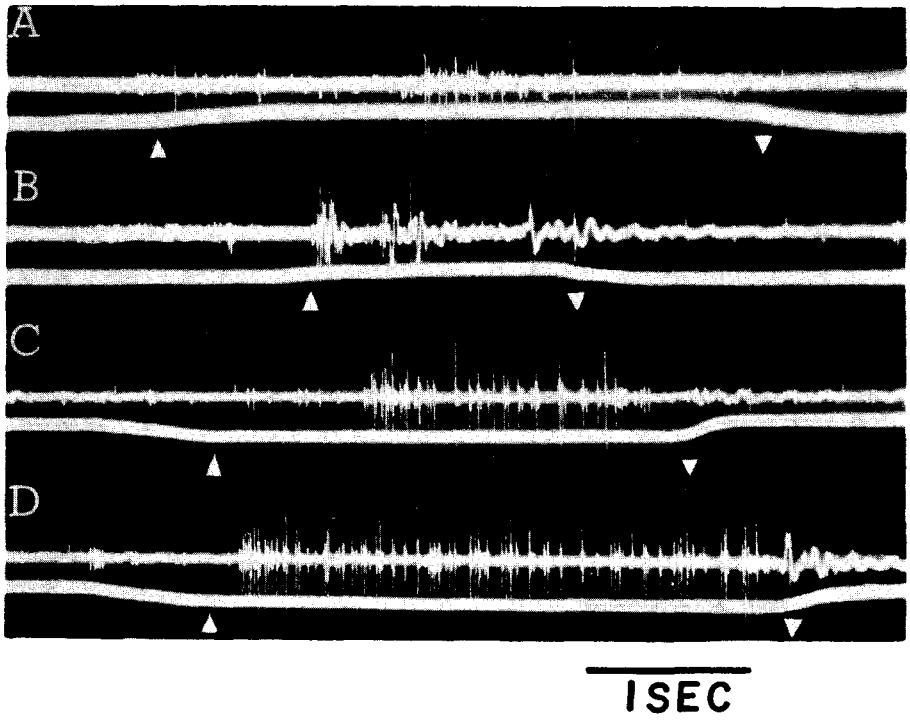

Fig. 2. Responses of the sensory hairs on the walking leg to water current stimulation in the four different directions (the upper signal in each record). A: V→D (from ventral to dorsal); B: D→V (from dorsal to ventral); C: P→A (from posterior to anterior); D: A→P (from anterior to posterior). The lower signal in each record is the stimulus signal. The white triangles represent the times of begining and end of the stimulation respectively.

There was no response until the speed of flow reached about 0.50 cm/sec. The response from the sensory hairs increased with increasing rate of the water flow from 0.5cm/sec to 3.0cm/sec. Fig. 4 shows the burst of impulses on the hair nerve in response to the stimulation of water currents of B and C (1.02cm/sec and 2cm/sec). But the burst of impulses decreased when the speed of the water current became above 3.5 cm/sec. The response frequency was between about 3/sec and 15/sec, depending on the preparation.

The "after effect" from number of impulses was not so pronounced as the "on response". It is not certain whether the "after effect" could be correlated to the recovery of the sensory hairs to their original positions.

Fig. 5 shows the record of the burst of nerve impulses from the sensory hairs when the water current speed was changed. When the speed was increased, the response frequency was increased, and then when the water current was kept constant, the activity of the sensory hairs was decreased. An adaptation occurred during the constant water current and the frequency of impulses remained also constant.

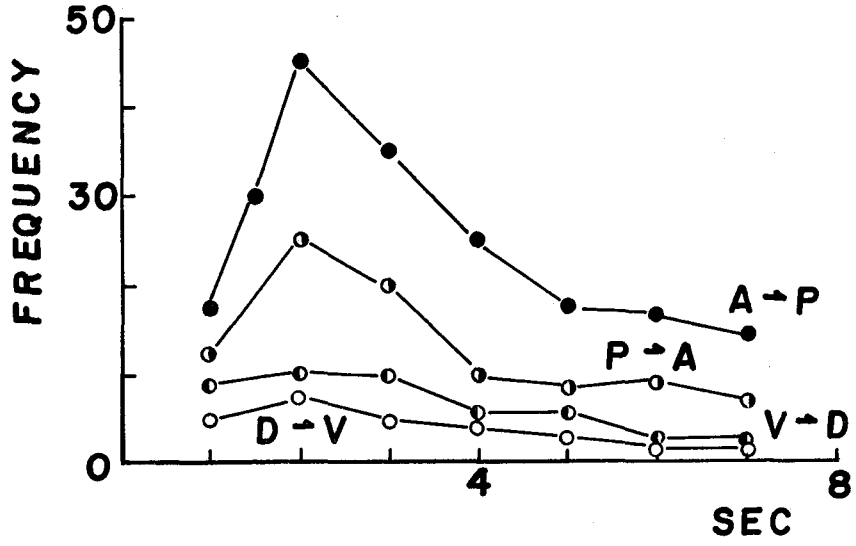

Fig. 3 The effects of water flow on hair sensory organs in the four different directions are represented in the four graphs. V→D and D→V flow were virtually ineffective. A→P and P→A flow were effective. The ordinates are impulse frequency on the sensory nerve and the abscissae are times in seconds.

The sensory hairs were also sensitive to the change in the speed of water current.

Fig. 5 A shows the record of the effect of change of the water current from 0.55 cm/sec to 0.75 cm/sec on the afferent impulses, and B shows the record when the speed of water current was changed from 1.0 cm/sec to 1.2 cm/sec. When the speed of water current changed gradually, the sensory hairs were sensitive to the change in the speed. The effect was basically the change in angular velocity at which the sensory hair was bent.

Response to vibration stimulation

In order to clarify further the function of the sensory hairs, another type of mechanical stimuli, vibration stimuli, were applied to them, and the impulses on the sensory nerve were investigated. The experiments were performed to determine

their ability to follow the frequency of repetitive mechanical stimuli.

The responses of the sensory hairs were produced by stimulation at frequencies of higher than 5 c.p.s.

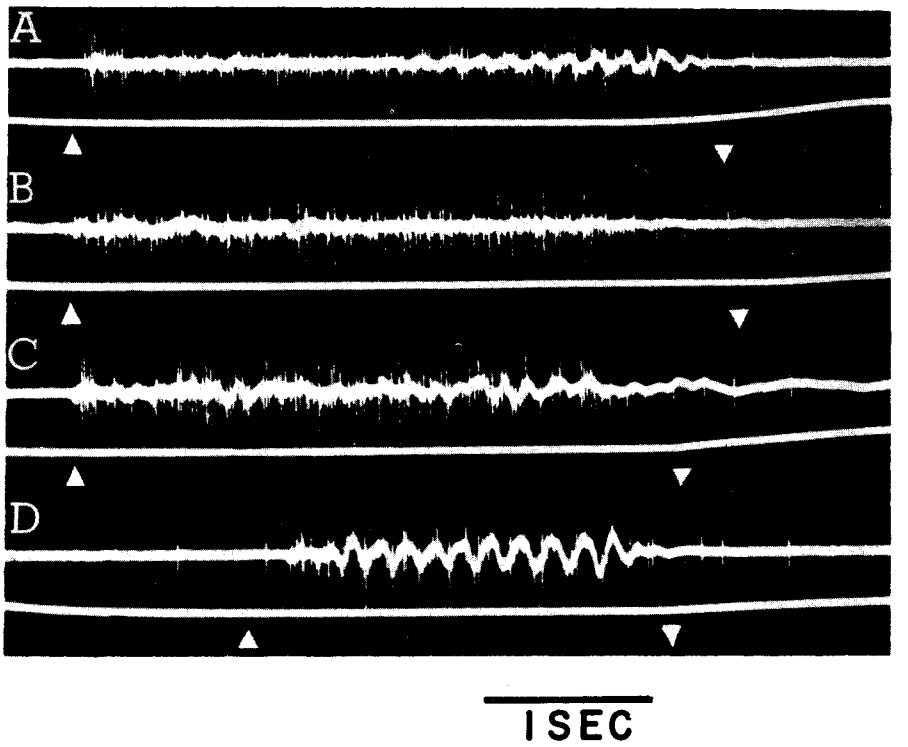

Fig. 4. Experimental results showing the response of the sensory hairs to water current. The water current starts at the stimulus artefact sign and it is continuous until the second artefact sign. A: shows the response to a current of about 0.55 cm/sec. B: at 1.02 cm/sec. C: at 2 cm/sec. D: at 3 cm/sec.

Fig. 6 shows the activity of the sensory hairs in response to vibration stimuli. The sensory hairs of the preparation were stimulated by the water wave at frequencies from 20 c.p.s. to 90 c.p.s. Between 50 and 60 c.p.s. the response to stimulation was synchronous with stimulation wave, and the frequency of impulses of the sensory nerve was the same with that of the stimulus wave. The synchronous response was not seen when the stimulation frequency was lower than 50 c.p.s. The response could not follow the stimulation frequency of higher than 70 c.p.s.

The "on effect" and "after effect" in the vibration stimulus was more pronounced than those in other types of stimulation. The number of impulses in the "on effect" increased in proportion to the increase in the frequency of wave stimulation.

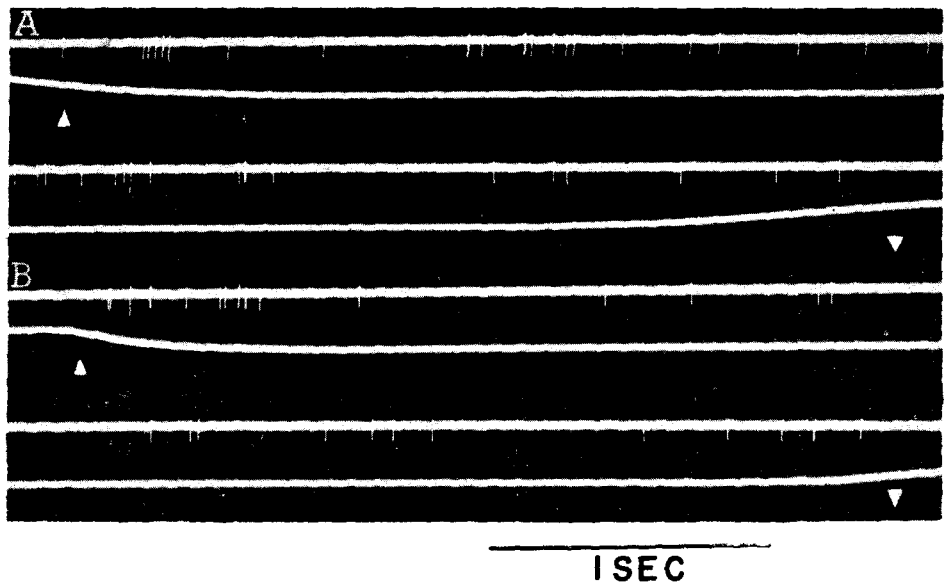

Fig. 5. Responses of the sensory hairs to the water current of a gradually increasing rate and the lower signal indicates an increasing rate of current when the deflection is downward. The white triangles represent the beginning and end of the stimulus respectively. A shows the response to change in the current speed from 0.55 cm/sec to 0.75 cm/sec. B shows the response to change in the current speed from 1.02 cm/sec to 1.20 cm/sec.

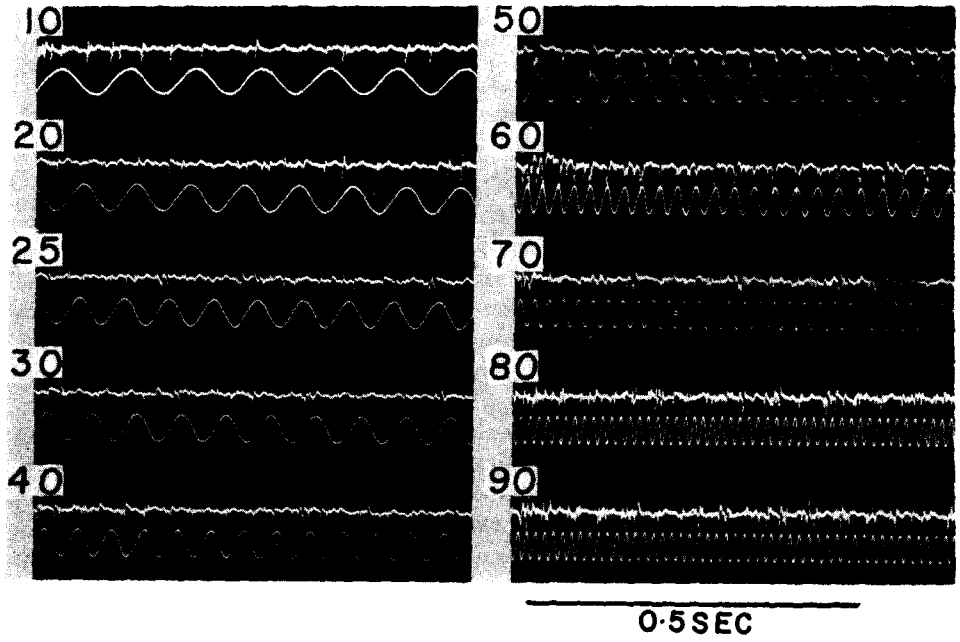

Fig. 6. Activity of a unit in response to water vibration. The upper signal in each record represents the impulses of a unit neuron. The lower signal in each record represents the vibration stimuli.

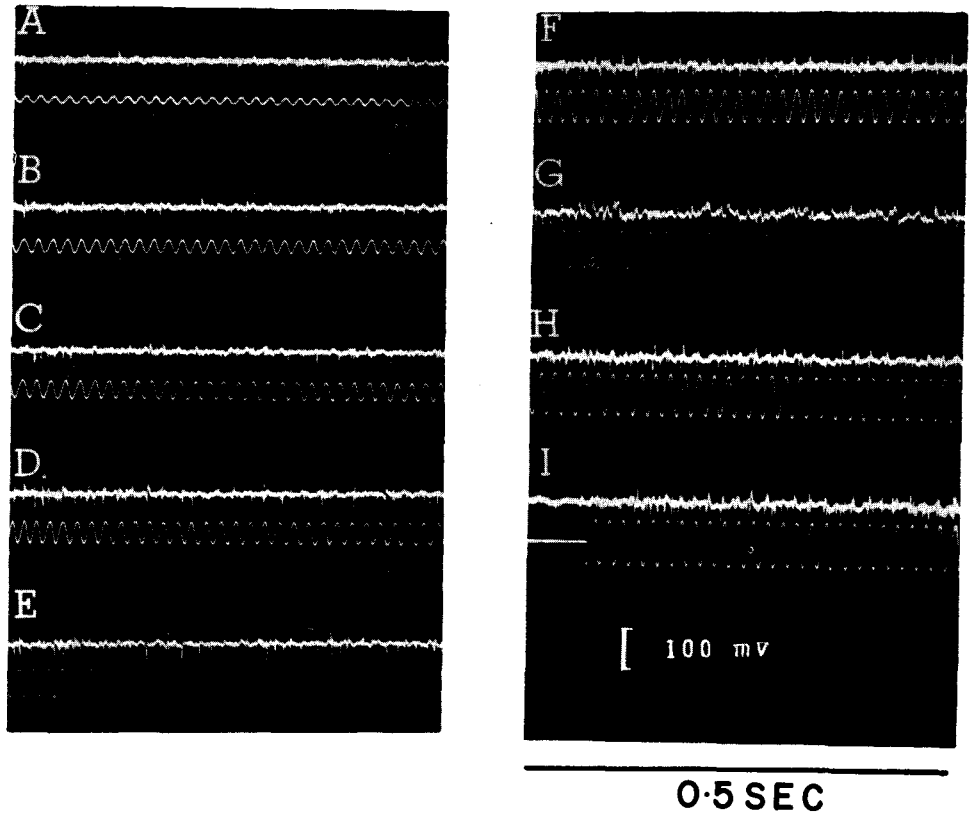

Fig. 7. Responses of a unit to vibration of various intensities. The frequency was kept at 60 c.p.s. The lower signal in each record represents the stimulus, and the intensity was changed from 20 mV to 120 mV.

The responses of sensory hairs to vibrations of various intensities at frequencies of 30, 50, 60 and 70 c.p.s., were investigated. The response of the hairs to stimulus at a frequency of 60 c.p.s. is shown in Fig. 7 for example. When the sensory hairs were stimulated by the vibration, the frequency of sensory impulses depended on both intensity and frequency of the stimulus vibration until the strength voltage of the oscillator was about 70 mV. But if the strength of the oscillator was high enough beyond 70 mV, the response frequency was nearly constant, and depended only on the stimulus frequency. Fig. 8 shows the relation between the impulse frequency and the stimulus strength at each stimulus frequency.

After the strength of the oscillator reached 70 mV at which the sensory hairs could be sufficiently deformed, there was no effect of change in the stimulus strength on the sensory impulses. When the sensory hair was deflected in the

posterior direction from the normal position, an impulse was evoked on one neuron. If the hair was kept in this new position, the threshold of the active neuron for vibrational stimuli was lower than it was in the normal position. Another neuron exhibited response to stimuli only when the hair was deflected in the opposite direction.

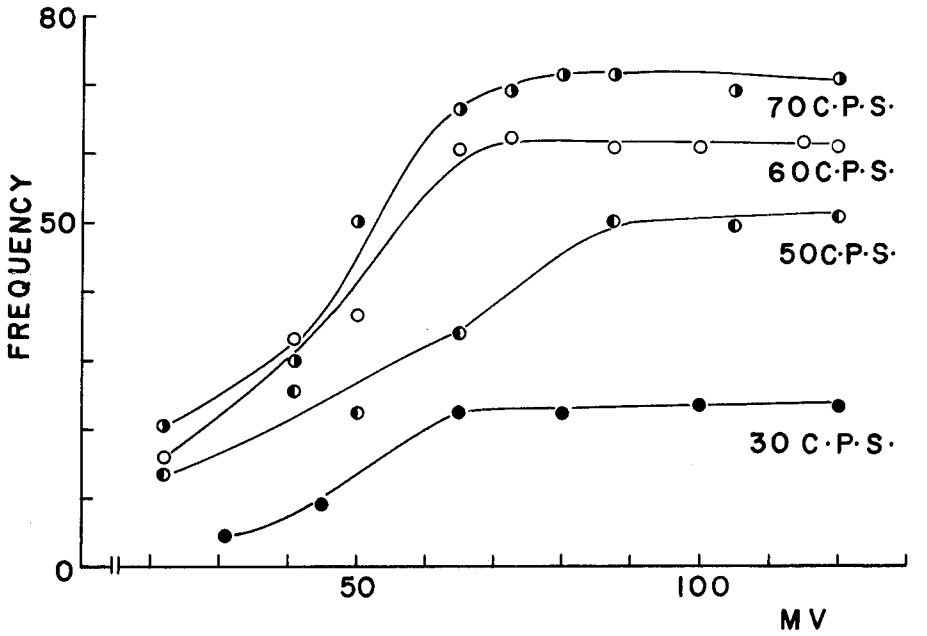

Fig. 8. The effects of stimulation intensity on sensory hairs at frequencies of 70, 60, 50 and 30 c.p.s. The ordinates are impulse frequencies on the sensory nerve, and the abscissae are stimulus voltages of the oscillator.

It can be said that the sensory hairs on the walking leg respond to the movement of fluid medium in the same way as the pit organ on the external surface of carapace of the crayfish (Laverack 1962 a,b).

Discussion

Comparing the above results with those of the peg receptor on thorax described by Mellon (1963), the innervation of the sensory neurons was similar to the innervation of the peg receptor, but the external shape of the hairs was morphologically different, and the receptor of the walking leg was hair-like in shape.

As was stated in the above results, the two units of different types of adaptation were observed. Gray and Malcolm (1951) reported the similar observation.

Loewenstein (1956) also suggested that there was functional difference between the two receptors having slow and fast adaptation. But the adaptation of the two units had no difference in the impulse pattern in the present result. It seems that between the two units there is no essential difference in the structure but there is a difference in the way the hair bends.

When the speed of the water current was changed, the response impulses were clearly elicited in the sensory nerve fibres. When the speed was kept constant, no response was observed. This means that the change in the angular velocity of bending given to the sensory hairs by the change in current speed is effective to produce the sensory impulses. If the sensory hairs were maintained in a constantly deformed state, no sensory impulse was produced on the hair nerve. The bend of the hair in a direction gives a stretch on one neuron. Therefore it seems that the mechanical excitation results from a deformation or stretch of the dendritic surface of one neuron when the sensory hair is bent.

In the result described above, when the vibration stimulation was applied to the sensory hairs, a maximum response was obtained at the frequency of 50–60 c.p.s. This shows that the frequency of elastic oscillation of the hair pad are between 50 and 60 c.p.s., and the hair pad can be oscillated synchronously with the stimulus vibration at that frequency.

The sensory hairs of the walking leg are a tactile receptor, a rheo-receptor and also a vibration receptor. They have the maximum response to the water current stimulation in the anterior to posterior direction. The results is that the hairs become also distant receptors and can detect the vibration source in the water. The animal can take the body orientation according to the current direction or to the vibration source. This was also confirmed by the observation of the rheotropism of the animal.

Summary

1. An electrophysiological investigation was made on the sensory hairs on the walking leg of the crayfish. Various kinds of mechanical stimuli of touch, water current and vibration were applied to the sensory hairs, controlling mechanical stimulation quantitatively. It was found that each receptor is innervated by a pair of sensory neurons. These neurons responded when the sensory hairs were deflected in the anterior-positerior and posterior-anterior direction.

2. In response to touch stimulation, there were two types of adaptation, slow and fast adaptation.

3. The sensory hairs responded to deflection by the water current. The action potentials were produced by the water current at a speed of more than 0.50 cm/sec.

4. The response frequency of the sensory hair nerve to vibration stimulation increased with the stimulation frequency. The response could not follow the stimulation frequency higher than 60 c.p.s. The synchronous response with the

stimulation frequency was found at 50–60 c.p.s. of the vibration.

5. Sensory hairs are not only the tactile receptor, but also they are sensitive to motion of the surrounding fluid and the animal can take its orientation perceiving changes in the speed and direction of water current.

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