The Responses of Oculomotor Fibres in Statocyst-ectomized Crayfish, *Procambarus clarki*

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(*With 3 Text-figures*)

Introduction

Locomotory activity of the whole animal and/or the reflex movement of certain part or parts of the body are induced by one or more sensory inputs. When more than single sensory inputs participate with one motor output, each of them is usually able to modulate the output singly. In such a system, studying the input-output relationship is very interesting and useful for knowing the mechanism of central nervous system.

Crustacean eyestalk movement is controlled mainly by two sensory inputs, namely, visual and geotactic inputs. When the animal is tilted in light, both visual and geotactic organs contribute to eyestalk movement, but in darkness only the latter contributes. On the other hand, when the animal is in normal position, and the environment is moved, only the visual organ contributes. Contribution of each organ has been studied by several investigators (Cohen 1955, Schöne 1954, 1959, 1961, Hisada et al. 1969). However, they do not appear sufficient to clarify the mechanism of information treatment in the crayfish central nervous system.

Recently, the response of oculomotor nerve fibres to some sensory stimuli was studied by Wiersma and Oberjat (1968) and Hisada and Higuchi (1973) in the crayfish. They reported some types of oculomotor nerve fibres and both visual and geotactic contributions.

In this experiment, the responses of the oculomotor nerves, especially, the side down (SD) and the head down (HD) type fibres whose characteristics have been mentioned in the previous paper (Hisada and Higuchi, 1973) were studied in the animal with statocystectomy. In intact animal, it is difficult to observe the visual contribution in rotation of animal, for the geotactic contribution occupies so large part that the visual effect is greatly masked. Therefore, the response of the oculomotor fibres in the statocystectomized animal is studied to reveal more of the details of the visual contribution.

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Material and Method

Well grown adult crayfish of the species of *Procambarus clarki* of relatively large size was used for experiments. Removal of the statocyst was done by cutting the both antennules at their bases. The animal thus statocystectomized was kept for few days, till the injured part was sealed by healing and then subjected to the experiment.

The animal was clamped around the posterior carapace by a holder while two chelipeds were tied securely with an elastic thread, and the walking legs were left free. A ground electrode was provided by piercing the anterior carapace at one end just in front of the cervical groove between the anterior and posterior parts. The recording electrode of having the bare tip (tip diameter 3–5μ) of insulated insect needle was introduced through the soft membrane between the outer and inner eye segments. After establishing the recording of one oculomotor fibre activity, the electrode was left untouched for about 15 minutes to have firmer lodging in place. This made the pick up of the signal from one particular fibre possible for several hours even when the position of the eyestalk changed with rotation stimulus or the eyestalk moved violently in the eye withdrawal reflex.

Then the animal with the clamp was mounted on a specially constructed rotation device which allowed to rotate the animal around any desired body axis at various velocities. Rotation device also carried the striped drum which was used as the visual stimulus. Black and white stripes of the drum subtended an angle of 8.2° each at the crayfish eye. A point light source illuminating eye surface was also used as the visual stimulus.

Nerve impulses and stimulus parameters were led to the cathode-ray oscilloscope through a biological amplifier. Oscillographic records were taken by a long recording camera. Impulse frequencies were calculated from the recorded film, and if necessary, the statistical treatment was done with the aid of a computor.

Results

To investigate the influence of statocystectomy, it is, of course, desirable to compare the response of the same oculomotor nerve fibre of the same animal before and after the removal of the statocyst. But it is impossible to remove the statocyst while the position of the recording electrode is maintained, because the recording site is quite close to the statocyst to be removed, and, moreover, the animal moves violently during the operation. Consequently, the responses obtained in the statocystectomized animals were compared with the responses in other intact animals. It was fully expected that the response of the same fibre to the rotation of animal in the statocystectomized animal was quite different from it in intact animal, as Wiersma and Oberjat (1968) pointed out, so the type of fibres was defined by knowing the position of recording electrode where the particular type of the fibre was most likely encountered, and also by checking the detail of response to point light source, which was reported in the previous paper (Hisada and Higuchi 1973). For example, the fibre, which was found when the recording electrode was introduced into the proximal part of the eyestalk and which showed the excitatory and inhibitory response when the back and front of the eye was illuminated respectively, could be determined as a HD fibre. By the following reasons, it was unlikely to mistake the identities of the fibres. (1) The fibres rather readily picked up were usually HD, SD and horizontal optokinetic fibres, and other fibres were rarely encountered. (2) And horizontal optokinetic fibres did not change their characteristics in statocystectomized animal. (3) The response characteristics of HD and SD fibres to the visual stimulus had been thoroughly known and, thus, these two types of fibres can be readily identified.

1) HD fibre

In intact animal, as mentioned in the previous paper, HD fibre increased its discharge rate when the animal was rotated in head down direction around the transverse axis. The response profile to the 360° full turn was represented by trigonometric function, in which the maximal point was about at 90° turn (head down position) and the minimum was at about 270° turn (head up position). This response profile was very stable throughout all HD fibres observed both under overhead light and in the darkness, while the discharge rate in the darkness was lower than that under overhead light. On the other hand, HD fibres showed almost mirror image response profile in the rotation of opposite direction, namely the rotation to head up direction, while the total decrease in change of discharge rate was observed. The term "normal response pattern" hereafter applies to the response profile in intact animal.

Under overhead light, HD fibres in statocystectomized animal showed the response close to the normal pattern only for the rotation to head down direction. To the head up rotation, the response did not show any distinct point of maximum or minimum (Fig. 1). And moreover, the average firing rate was lower than the spontaneous level of the stationary animal. The latter indicates there is an inhibitory influence of the optokinetic components existing in the visual inputs of this type of the fibre. The difference seen between average frequencies of the head down and the head up responses of this fibre is clearly attributable to the optokinesis in preferred and non-preferred direction respectively. The optokinetic components consisting the HD fibre response can be demonstrated by giving a rotating striped drum to the stationary statocystectomized individual. The movement of the drum in the direction which was equivalent to the movement of animal in the head down direction in stationary visual surrounding, namely, from lower to upper in front of the animal eye increased the discharge rate and, on the contrary, the movement opposite to it which is equivalent to the head up rotation drastically reduced the discharge rate (Fig. 2).

In the darkness, the firing rate remained within the fluctuation range of the spontaneous discharge of the stationary animal for the rotation either in the head down and the head up direction.

As is in the case of the intact animal, the illumination of the part of the eye which corresponds to the visual field of certain interneurones of probably 038 or 014 of the sustaining type showed excitatory effects, namely increase in firing frequency, while the illumination of other part of eye, probably the visual field of 020 or 030 exerted the inhibitory influence.

Thus, in HD fibre, the apperance of sinusoidal change of firing frequency in the head down rotation and the disappearance in the head up rotation under overhead light were to be explained by the effect of illumination of parts of eye and vertical optokinesis (details in discussion).

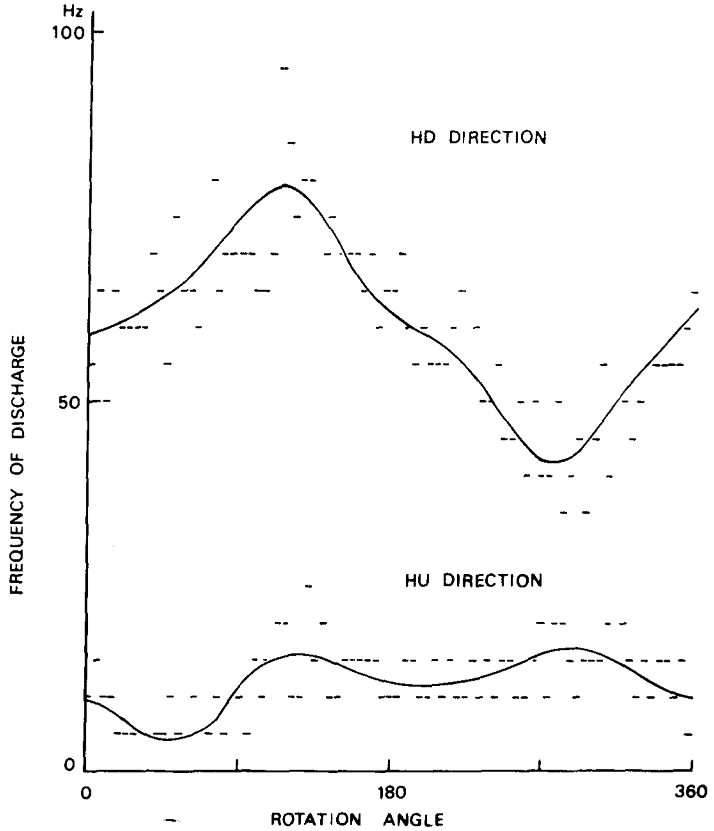

Fig. 1. The response pattern of HD fibre under overhead light in statocystectomized animal. The frequency of discharge in every 4° was plotted. Solid lines were drawn by computor assisted reconstruction. The response was clearly of sinusoidal function close to that of normal individual for the rotation to head down direction, but not to head up direction. In the darkness, the fibre showed only minor fluctuation around the spontaneous level (20–30 Hz) for the rotation to both head down and head up direction.

2) SD fibre

The response of SD fibre in the statocystectomized animal was very different from that in the intact animal. In the intact animal, SD fibre increased its discharge rate when the animal was rotated in the direction in which the side of the recording eye moved downward around the longitudinal axis.

In the darkness, SD fibre in the statocystectomized animal also showed only

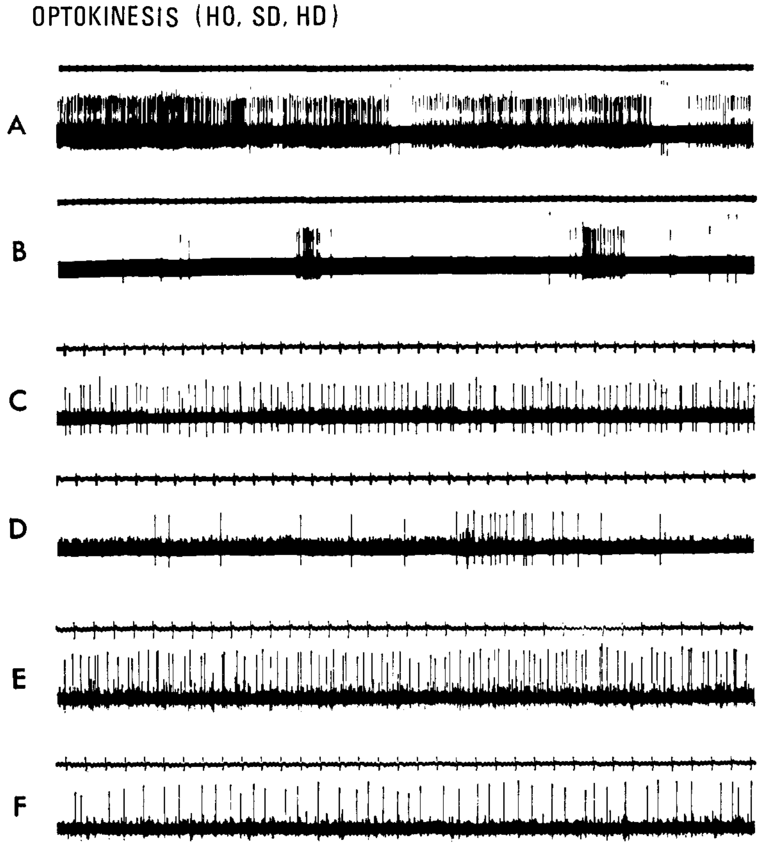

Fig. 2. The optokinetic response of horizontal optokinetic fibre (A: preferred direction, B: non-preferred direction), SD fibre (C: equivalent to side down rotation, D: side up rotation) and HD fibre (equivalent to head down rotation, F: head up rotation). In horizontal optokinetic fibre and SD fibre, 'flip back' phenomenon, a sudden cessation or a burst, was apparent.

minor fluctuation around the spontaneous level for the rotation of the animal to both side down and side up direction. However, under overhead light, with stationary striped drum, the fibre showed the similar response to horizontal optokinetic fibre (Fig. 3). The horizontal optokinetic fibre are known to responds when the animal is rotated around the dorsoventral axis, and two type of the fibre have been found. One is the fibre which responds when either the animal or the surrounding is rotated in a direction such as the visual objects move from back to front of the recording eye, and the other responds in opposite rotation. The direction of rotation in which the increase of discharge frequency was observed

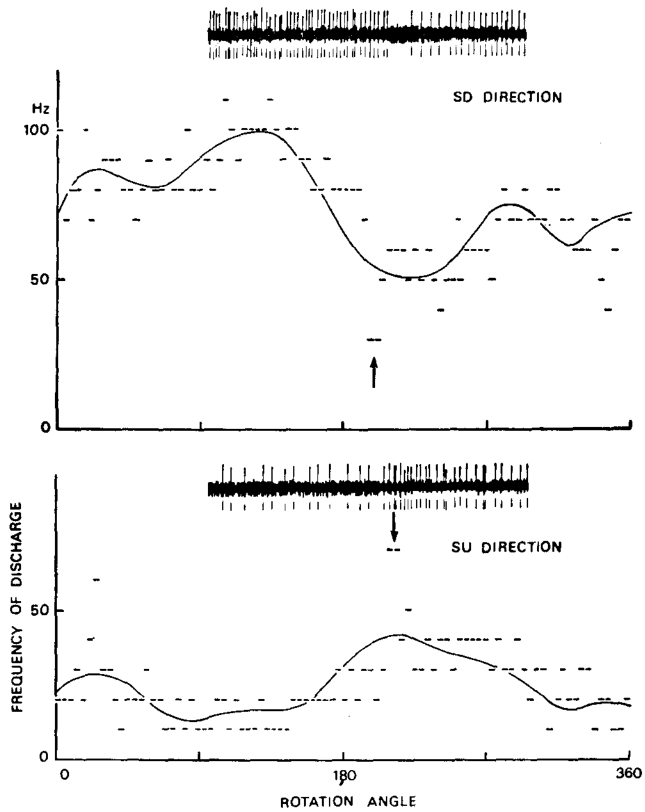

Fig. 3. The response pattern of SD fibre of statocystectomized animal under overhead light. The frequency of discharge in every 4° was plotted. Insets represent the actual recording which showed silence in side down rotation and a burst in side up rotation respectively. In the darkness, the fibre, like HD fibre, showed only minor fluctuation around the spontaneous level (20–30 Hz) for the rotation to both side down and side up direction.

is usually termed as the preferred direction. Both fibres show almost constant high discharge rate well above the spontaneous level in rotation of preferred direction, and quite low discharge rate in non-preferred rotation. These fibres also show a peculiar response known as the 'flip back'. The maintained high frequency during the rotation in the preferred direction suddenly becomes silent for a short while and then resumes it original frequency. Conversely, the silent fibre during the non-preferred rotation suddenly produce a short burst of impulses (Fig. 2). Combination of these two activities brings back the eyestalk, which is up to the moment following and tracking the movement of visual object, to the original position within a very short period of time, thus enabling the follow up movement renewed. Being different from HD fibre of the same animal, SD fibres of the statocystectomized animal in the side down rotation did not produce any sinusoidal profile in frequency change. The phase and amplitudes of each experiment appeared almost at random.

The effect of illumination on the eye did not change by statocystectomy, so illuminating ipsilateral eye (recording eye) and contralateral eye increased and decreased the discharge rate respectively. The mode of increase and decrease of discharge rate to the rotation of the striped drum was the same with that of HD fibre: the rotation which was equivalent to side down was excitatory and side up was inhibitory. The most remarkable point was the existence of 'flip back' phenomenon, which was smaller than that in horizontal optokinetic fibre (Fig. 2). The phenomenon was not observed in the same kind of experiment in the intact animal, and had been known only in the horizontal optokinetic fibre. In statocystectomized animal, thus, SD fibre showed considerably different response to both body rotation and striped drum rotation from that of the intact animal. Therefore, in SD fibre, more complex relationship between visual and geotactic contribution than that in HD fibre was expected (see discussion).

Discussion

The fact that both HD and SD fibres show only minor fluctuation of the frequency around the spontaneous level for the rotation stimulus in the darkness shows that the joint organs existing in the head and abdominal appendages are not well organized to give a sufficient information in regard of body position. The output of oculomotor nerve is relatively simple in characteristic when it is modulated only by the gravitational information. Contribution of the visual system in addition to the gravitational system and up more complex characteristics which are rather difficult to analyse. The modulation derived from visual system is usually in great part masked by the predominant contribution of the geotactic system. Therefore, the experiments comparing the responses of the normal and the statocystectomized animal are essential to analyse the visual contribution.

First, in the response of HD fibre, the visual information during the animal rotation is consisted of a) field information, namely the shift of light illumination from the excitatory to the inhibitory field or *vice versa*, and b) vertical optokinetic information. Under the overhead light, the excitatory field is most strongly illuminated when the animal is rotated to head down direction and reaches a maximum at about 90° and becomes least at around 270°. Therefore, even if derived only from the field effect, the HD fibre can show the similar maximal and minimal frequency points almost at the same places in the normal intact animal. At the same time, however, the vertical optokinesis is usually at work and should be considered in deciphering the response. When the animal is rotated in head down direction, the vertical optokinesis works as an excitatory input throughout the rotation, and thus raises the basic rate of discharge. The effect of illumination of the excitatory and the inhibitory fields will be superimposed on this basic high frequency response resulting in a conspicuous sinusoidal response profile. On the contrary, the optokinesis works as an inhibitory input when the animal is rotated in head up direction, and induces an extensive reduction of firing frequency throughout the rotation. In fact the frequency becomes so low that the possible excitable and inhibitory effects of field illumination are totally indetectable burried under the large fluctuation of the firing intervals.

Compared with HD fibre, SD fibre appeared to possess a quite interesting property. Both to the rotation of the body and to the rotation of the striped drum, SD fibre of the statocystectomized animal shows the 'flip back' response hitherto known only in the horizontal optokinetic fibres. This appears to suggest in SD fibre the integrative process of the visual information is constructed in a similar manner to that of the horizontal optokinetic fibre.

Another interesting point in the function of the SD fibre is that in contrast to the HD fibre where the visual inputs from the both eyes are connected in a synergistic way, the visual input from each eye is connected in reverse sign with each other to the SD motorneurone.

In SD fibre, as in HD fibre, the rotation in side down direction produces more profound effect than in side up direction, although the difference is not quite large as in the extent observed in HD fibre. This indicates the excitatory influence of the vertical optokinesis is raising the level of basic discharge frequency in the side down direction, while the inhibitory effect of the vertical optokinesis in the side up rotation reduces the frequency. However, the sinusoidal profile of the response can be observed in both the side down and the side up rotations suggesting the discharge rate in the side up rotation is still large enough to accommodate the modulation derived from the field effect.

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Summary

1. The oculomotor responses to the rotation of the animal body and to the visual stimuli were studied in the statocystectomized crayfish. Studies have been mainly performed on two major classes of oculomotor fibre, head down (HD) and side down (SD) fibres.

2. HD fibre of the statocystectomized animal showed the response close to the normal pattern only when the animal was rotated to head down direction under overhead light, while in other experimental conditions, namely, for the rotation to head up direction under overhead light and both head down and head up rotation in the darkness, the responses lacked sinusoidal trend in frequency change which is supposed to contribute to the compensatory eyestalk movement.

3. To the rotation of the striped drum, HD fibre in the statocystectomized animal showed the almost same response pattern with that of intact animal.

4. SD fibre, under overhead light, showed the similar directional response to horizontal optolinetic fibre when the animal was rotated to side down and side up direction. In the darkness, the fibre showed random fluctuation around the spontaneous level in discharge frequency.

5. SD fibre also showed the similar response to horizontal optokinetic fibre to the rotation of the striped drum.

6. The contributions of visual input to the response of HD and SD fibres were proved, and it was also suggested that the visual contribution of SD fibre was more complex than HD fibre.

References

Cohen, M. J. 1955. The function of receptors in the statocyst of the lobster *Homarus americanus*. J. Physiol. **130**: 9–34.

Higuchi, T. and M. Hisada 1973. Visual and geotactic contributions to oculomotor responses in the crayfish, *Procambarus clarki*. J. Fac. Sci. Hokkaido Univ. Ser. VI Zool. **18**: 495–506.

Hisada, M. and T. Higuchi 1973. Basic response pattern and classification of oculomotor nerve in the crayfish, *Procambarus clarki*. *Ibid.* **18**: 481–494.

——— and K. Sugawara 1969. Sensory output from crayfish statocyst. Zool. Mag. **78**: 11 (Japanese abstract in Proc. 39th Ann. Meet. Zool. Soc. Japan).

———, ——— and T. Higuchi 1969. Visual and geotactic control of compensatory eyecup movement in the crayfish, *Procambarus clarki*. J. Fac. Sci. Hokkaido Univ. Ser. VI Zool. **17**: 224–239.

Schöne, H. 1954. Statozystenfunktion und statische Lageorientierung bei dekapoden Krebsen. Z. vergl. Physiol. **36**: 241–260.

——— 1959. Die Lageorientierung mit Statolithenorganen und Augen. Ergeb. Biol. **21**: 161–209.

——— 1961. Complex behavior. In The Physiology of Crustacea, edited by T.H. Waterman, Vol. II: pp. 465–520, Acad. Press, New York.

Wiersma, C.A.G. and T. Oberjat 1968. The selective responsiveness of various crayfish oculomotor fibres to sensory stimuli. Comp. Biochem. Physiol. **26**: 1–16.

——— and T. Yamaguchi 1966. The neuronal components of the optic nerve of the crayfish as studied by single unit analysis. J. Comp. Neurol. **128**: 333–358.