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Response Pattern of Single Fibres in the Circum-oesophageal Connective to Illumination of the Compound Eye of the Crayfish*,[1],[2]

By

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(*With 14 Text-figures and 1 Table*)

In general, the optic ganglion exists between the photoreceptor layer of the compound eye and the supra-oesophageal ganglion in crustaceans or insects. In many investigations, it has been established that a visual nerve response caused in the photoreceptor is, as the first step, integrated in the optic ganglion. Electrical response of single neurones in the optic ganglion has been studied mainly on the optic lobe of some insects. Thus, the unit responses to photic stimulation of the compound eye were classified as 'on' and/or 'off' excitatory and 'on' and/or 'off' inhibitory ones (Burtt and Catton, 1959, 1960, Ishikawa, 1962). On the other hand, although the properties of unit responses in the crustacean optic ganglion were not yet investigated, 'on' and/or 'off' responses were recorded from the optic peduncle, which carries the finally integrated visual information in the optic ganglion (Waterman and Wiersma, 1963, Wiersma and Yamaguchi, 1965, Sato and Yamaguchi, 1965). However, no inhibitory response was observed from the crustacean optic ganglion. This means that the degree of sensory integration at the level of the optic ganglion differs between the insect and the crustacean.

Following the integration of visual informations in the optic ganglion, they will be, as the second step, integrated in the supra-oesophageal ganglion. A little account of the modulation site of visual information in the supra-esophageal ganglion was presented. Recently, Blest and Collett (1965) recorded the single unit discharge from the medial protocerebrum of the insect and identified several types of the excitatory or inhibitory responses to 'on' and 'off' of the illumination of the eyes. According to their result, the visual nerve integration in the supra-

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oesophageal ganglion seems to be more complicated than that in the optic ganglion. In the crustacean supra-oesophageal ganglion, the discharge pattern of single neurones in response to visual stimulation has not yet been recorded directly. Some light-initiated impulses in the circum-oesophageal connective of the crayfish, which directly carries the descending discharges from the supra-oesophageal ganglion, were recorded by several researchers (Prosser, 1934, Wiersma, Ripley and Christensen, 1955, Wiersma, 1958, Sato and Yamaguchi, 1965). However, properties of all responses of the single visual units in the connective were not analysed in detail. The object of the present experiment is to clarify the integration mechanism of visual information in the supra-oesophageal ganglion of the crayfish. Accordingly, single unit activities in response to 'on' and 'off' components of light stimulation were observed in the circum-oesophageal connective. The advantage of using this connective is that by using it the finally integrated impulses in the supra-oesophageal ganglion are obtained. A preliminary report has already been given elsewhere (Sato and Katagiri, 1965).

Material and Methods

Preparation: The crayfish, *Procambarus clarkii* was used in all experiments. Over 100 animals, both the male and female, were used. The animal was first separated at the middle part of the cephalothorax by a pair of scissors. After removal of most of the cephalic carapace, the stomach attached to the oesophagus and the green glands were taken out. Then, a pair of circum-oesophageal connectives was clearly exposed, which was severed just above the sub-oesophageal ganglion. In most of the experiments, two pairs of antennae were entirely removed near their roots. A small part of the carapace of the ventral side was left in place in order to fix both the compound eyes and the supra-oesophageal ganglion. The optic peduncles were exposed only when the impulses from them were led out. The preparation was dipped in the freshwater-crustacean physiological saline (van Harreveld, 1936). The solution was continually cooled in order to keep the preparation fresh as long as possible. The circum-oesophageal connective was carefully split into single fibres or fine bundles of fibres under a binocular microscope, after removal of a thin membrane surrounding the connective. A physiologically stable condition of the preparation could be kept for less than half an hour, since the blood supply to the central nervous system was stopped.

Light stimulation: All of the experiments were performed in a laboratory dark room kept at 0.3 lux. Three different stimulating light sources were employed. The first source was a glow modulator tube (Sylvania 1B59/R1130B). The property and usage of this tube was described in a previous paper (Sato and Yamaguchi, 1965). The tube was used for the continuous illumination or repetitive light flashes (5 msec duration of one flash). The intensity of illumination at the corneal surface was about 15 luxes. This was measured with a luxmeter (Toshiba No. 5) and a phototransistor (OCP-71). The second light source was a tunsten lamp of 100 W, A.C. 100 V. This was employed to give a continuous illumination of strong intensity (about 4×10^3 luxes at the corneal surface). The third light source was a stroboscopic photograph xenon lamp (Toshiba, SS-4B and SX-2B), with which variously repeated flashes (0.4 msec duration of one flash) were applied. The flashed light had a strong intensity of about 4×10^5 luxes on the corneal surface. In most of the experiments, a lens system was used to focus the light on the cornea.

Recording: Most of the extracellular recordings of the nerve impulses were taken with a pair of fine silver wire electrodes (Ag-AgCl type). Sometimes, the metal microelectrodes were used, which were made by an electrolytic sharpening and then by insulating with enamel excepting the tip. These electrodes were impaled into the connective trunk to get single fibre activities. But this method did not always succeed in finding single nerve responses, because the injury current was frequently observed. A high gain C-R coupled amplifier was used for recording the nerve impulses, and a D.C. amplifier for recording the signals of light stimuli from the phototransitor. The amplified signals were displayed on a dual beam oscilloscope and recorded with a continuous recording camera.

All experiments were done at room temperature ranging from 13 to 24°C.

Results

I. *The electroretinogram and the optic peduncle response.*

The relation between the electroretinogram (ERG) and the nerve impulses in the optic peduncle was examined with application of flickering light to the eye. One of the silver wire recording electrodes was placed on the cornea, and the other on the proximal end of the eyestalk. Various frequencies of light flashes (5 msec duration and 15 luxes in intensity) were used, which were emitted from the glow modulator tube. A series of the slow action potentials (negative at the cornea) appeared in the form of the ERG's following repetitive light stimuli. The amplitude of the action potentials was gradually diminished with increase in flash numbers up to a stimulus frequency of 10 c/sec. At frequencies of more than 20 c/sec, a single action potential was initially elicited, and afterwards, successive action potentials followed it but they were suddenly decreased in amplitude. In Fig. 1, the change in amplitude of the ERG's to flickering light is shown. It is well known that the electroretinogram of the compound eye has a certain fusion frequency for flickering light (Crozier and Wolf, 1939, Autrum, 1950). In the present experiments, a series of the small action potential waves following the initial action potential fused at stimulus frequencies of higher than 35–40 c/sec. In other words, the flicker fusion frequency of the crayfish was 35–40 c/sec (in 10 animals), at a light intensity of 15 luxes. With a frequency of more than 40 c/sec, only one single large ERG was elicited, which was not followed by any other ERG in spite of the progress of stimuli.

On the other hand, while the above-mentioned ERG's were produced, the impulses elicited in the optic peduncle showed the following characteristics. As was described in a previous paper (Sato and Yamaguchi, 1965), the optic peduncle responded to repetitive light stimulation with the periodic discharges which showed one flash to one burst correspondence up to 30 c/sec. With further increase in the stimulus frequency, the optic peduncle showed the sustained discharges during stimulation. However, in this case, most of the ERG's showed large or perfect diminution of their amplitudes in spite of the prolonged sitmuli (Fig. 1, C, D).

Hartline *et al.* (1952) recorded the simultaneous responses of an isolated ommatidium and its optic nerve of *Limulus*, in which no optic ganglion exists between the photoreceptor and the supra-oesophageal ganglion. According to their results, the optic nerve discharged a prolonged train of impulses even after cessation of the ERG elicited by a brief light flash. The present findings suggest that the optic peduncle of the crayfish whose activity resulted from the synaptic integration in the optic ganglion was subjected to maintained excitation by only a single large ERG, which was elicited by repetitive light stimulation of relatively high frequency.

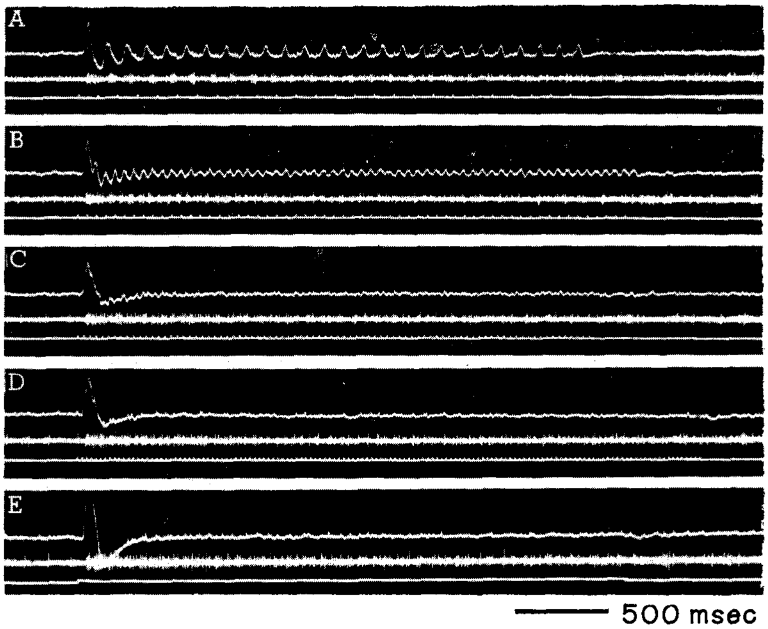

Fig. 1. Responses recorded simultaneously from the cornea and the optic peduncle to light stimulation of the compound eye. Upper trace in each record: electroretinograms. Middle trace: a train of impulse discharges of the optic peduncle. Lower trace: signals of light stimuli (deflections upward). Records A-D: the response to repetitive light flashes at frequencies of 10, 20, 30, and 40 c/sec respectively. Record E: the response to continuous illumination. Stimulation light: glow modulator tube.

Unit responses of the optic peduncle fibres to stimulation from a fixed light source had three types, 'on-off', 'on', and 'off' responses. In the 'on' responses, two groups were found, one of which showed 'on' burst with a sustained discharge during illumination and another of which showed only 'on' burst. The typical response patterns of 'on-off' and 'on' units are shown in Fig. 2, A, B. The 'off' response is of similar pattern with that of the 'off' response of the 'on-off' unit.

Initial peak frequency reached about 100 impulses/sec. These frequencies were higher than those of the discharges in the circum-oesophageal connective fibres, which carry the integrated visual information from the supra-oesophageal ganglion. A survey of the integration of the visual impulses in the supra-oesophageal ganglion will be described in a subsequent section.

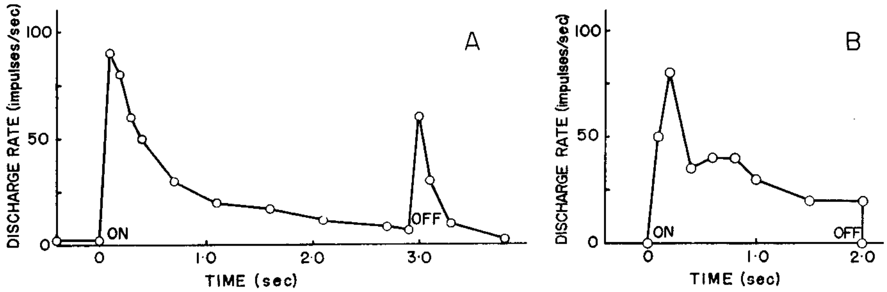

Fig. 2. Discharge rate of an 'on-off' unit (A) and an 'on' unit (B) in the optic peduncle plotted against time.

II. *Spontaneous activity in the circum-oesophageal connective.*

Wiersma *et al.* (1955, 1958) described that each one of a pair of circum-oesophageal connectives was composed of approximately 1,500–2,000 fibres. In the present experiments, the cross-section of the connective was made by means of a histological method. However, it was observed that the connective had approximately 1,000 fibres of various sizes in diameter. There are two giant fibres in each connective, one of which is the medial giant fibre (averaging 160μ in diameter), and the other the lateral giant fibre (80μ). The remaining fibre constitution is as follows; (1) large-sized fibres of 30–70μ (3%), (2) medium-sized fibres of 10–30μ (16%), (3) small-sized fibres of less than 10μ (81%). All these fibres are descending fibres carrying impulses from the supra-oesophageal ganglion to the sub-oesophageal ganglion and ascending fibres carrying the impulses in the reversed direction.

When an intact nerve trunk of the circum-oesophageal connective was placed on the recording electrodes, a complicated background of spontaneous impulses appeared. These were completely due to activity of the descending fibres, since the connective was severed just above the sub-oesophageal ganglion. From the view-point of impulse size and its frequency, the spontaneous impulses were divided into three groups; (1) large-sized impulses, (2) medium-sized impulses, (3) small-sized impulses. Most of the impulses in the first two groups represented a firing rate of below 10 c/sec, but the impulses in the last group, showing the size of noise-level in the base line represented a higher firing rate of about 20–30c/sec. The spontaneous activities showed the various patterns of both regular and irregular impulses.

When light stimulation was applied to the compound eye, the nerve trunk of the connective showed the response impulses in addition to the spontaneous ones. The fibres producing both large and medium impulses were activated by light stimulation, but the fibres producing small impulses were never activated. In Fig. 3, these mass responses to light stimuli in the circum-oesophageal connective are shown.

Fig. 3. Mass responses recorded simultaneously from the both trunks of the circum-oesophageal connectives to prolonged illumination of one eye. Upper trace: response of the ipsilateral connective. Middle trace: response of the contralateral connective. Lower trace: stimulus signal. Spontaneous discharges consist of three groups; large, medium, and small impulses. The fibres showing the first two groups are responsive to illumination. Appearance of the small impulses is not clear, because of the small amplitude of noise-level in the base line.

Besides the activity of the two impulse groups, another activity of the largest impulses to light stimulation was sometimes observed. From measurements of

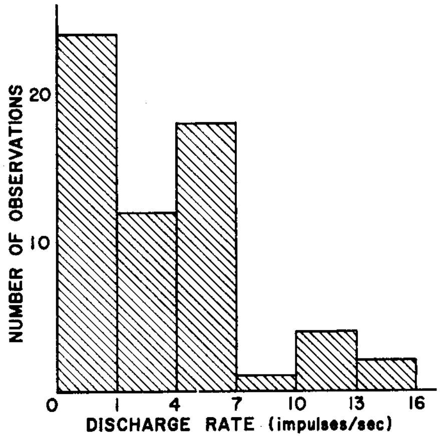

Fig. 4. Distribution of the spontaneous firing rates of fibres in the connectives with light-initiated response. Single unit responses were examined, sampling from many connectives.

fibre diameters under the binocular microscope, it was estimated that large and medium size groups in the light-initiated impulses corresponded to activities of large fibres (30–70μ in diameter) and medium fibres (10–30μ), respectively, and the largest light-initiated impulse was due to activity of the giant fibre.

Separating the connective into a single fibre, the response of single unit to light stimulus was investigated. Distribution of a spontaneous firing rates of fibres activated by illumination was determined (Fig. 4). Of 49 fibres observed, the value of discharge rates ranged from silent to 16 impulses/sec, and in the fibres of about 90 per cent it was less than 7 impulses/sec. In a few connectives isolated from the animals, the number of fibres showing light-induced responses was estimated in one connective. These were less than 20 fibres.

III. *Types of unit responses.*

The response pattern of single units in the cricum-oesophageal connectives to illumination of the eye was studied, using an isolated single fibre or a separated fine bundle of fibres. As was mentioned in the above section, some of the connective fibres which responded to light had spontaneous impulses in darkness, but the remaining fibres had scarcely the background of spontaneous impulses. Between these two groups, the property of impulse discharges to light stimulation was different. Therefore, the following results were roughly divided into the two groups; (A) fibres giving silent or rare spontaneous discharges of less than 1 impulse/sec, (B) fibres giving apparent spontaneous discharges of more than 1 impulse/sec.

(*A*) *Unit responses of fibres with silent spontaneous activity in darkness.*

Since most of the preparations were not maintained under stable conditions for more than half an hour, it was impossible for all of the fibres responding to illumination to be completely examined from each circum-oesophageal connective. Accordingly, the visual activity of a single fibre or a fine bundle of fibres was investigated. The sample was picked up at random from among the approximately 40 connectives.

(*a*) *'On' and 'off' excitatory untis:* These units responded to both 'on' and 'off' components of illumination. A typical record of the units is shown in Fig. 5, A. On application of steadily continuous illumination, a train of impulses which persisted for about 1 sec was elicited by an 'on' component. The initial peak frequency reached 10–30 impulses/sec and then it suddenly decreased. When the illumination was stopped, a few impulses were again elicited. On the other hand, under the repetitive light flashes (one flash, 5 msec duration) at frequency of 10–50 c/sec, the discharge pattern of 'on' and 'off' impulses was similar to that elicited by prolonged illumination. 'On' impulses elicited only at the initial part of stimuli did not show one flash to one burst correspondence, but the frequency of 'on' impulses was slightly enhanced with increasing stimulus frequency.

All the responses of these units were observed only in the large fibres of 30–70μ in diameter.

With a single isolated medial or lateral giant fibre, the effect of visual stimulation was investigated. Of 23 medial giant fibres isolated, all produced no response to 'on' and 'off' light stimulations. On the other hand, 4 fibres of 15 lateral giant fibres isolated, responded to 'on' and 'off' stimulation. The responding feature of the lateral giant fibre was, in some degree, different from that of the above-mentioned large fibre. When the prolonged illumination was applied, one or two impulses were evoked by both 'on' and 'off' components of stimulation (Fig. 5, C). Sometimes failure of either 'on' or 'off' impulses occurred, indicating that excitability of the giant fibre was rather labile. With repetition of relatively prolonged light stimulus, the giant fibre response was perfectly blocked. This implies that the activity of the fibre shows a fast light adaptation. When the light flash was repeatedly applied, a few impulses occurred only at the start and the end of repetitive stimuli as in the case of a single prolonged illumination.

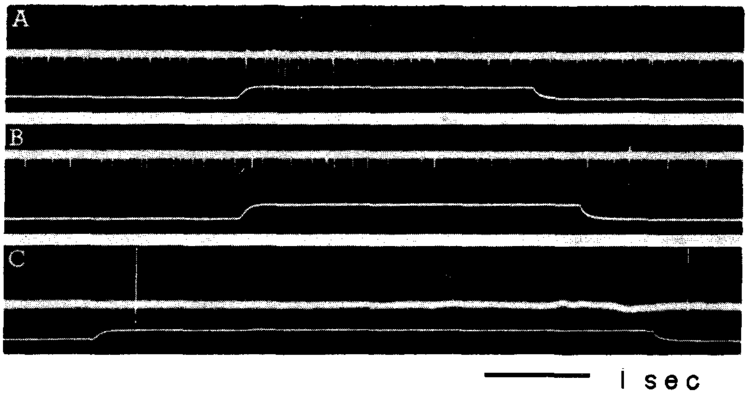

Fig. 5. Light-initiated responses recorded from the three single units with no spontaneous activity. Record A: 'on' and 'off' excitatory unit of the large fibre. Record B: 'off' excitatory unit of the medium fibre. Record C: 'on' and 'off' excitatory unit of the lateral giant fibre. Other smaller spike potentials are seen in record A and B. Stimulation light: 100W tungsten lamp.

(*b*) *'On' excitatory units:* These types of units were divided into the two groups according to properties of firing and fibre size. In the first group, the impulses lasting approximately 1 sec were elicited only by the 'on' component of light stimulation with an initial peak frequency of about 10–30 impulses/sec. Excepting the initial part of stimulation, the impulses disappeared during illumination and on cessation of it. 'On' excitatory impulses of these units were almost similar to those of the 'on' and 'off' excitatory units which were mentioned above. In the second group, 'on' impulses with the initial peak frequency of about 20–40 impulses/sec were followed by continuous discharges with gradual decrease in their frequency during illumination. In this response pattern, no 'off' impulse was evoked in addition, but an after-burst occasionally appeared. The response feature of the second type is shown in Fig. 6 and the relation between the discharge rate and the time course of illumination is shown in Fig. 7. Except for the fact that there was no spontaneous activity, the response pattern of the second type was almost similar to that of the 'on' excitatory units, having the spontaneous activity.

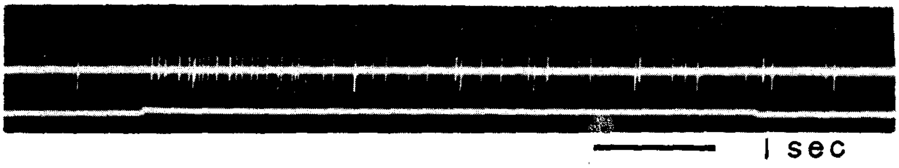

Fig. 6. Response of an 'on' excitatory unit of medium fibre which has no spontaneous activity. The discharges consist of the initial 'on' impulses and the sustained impulses during illumination. Another spontaneous larger spike potentials are seen. Stimulation light: glow modulator tube.

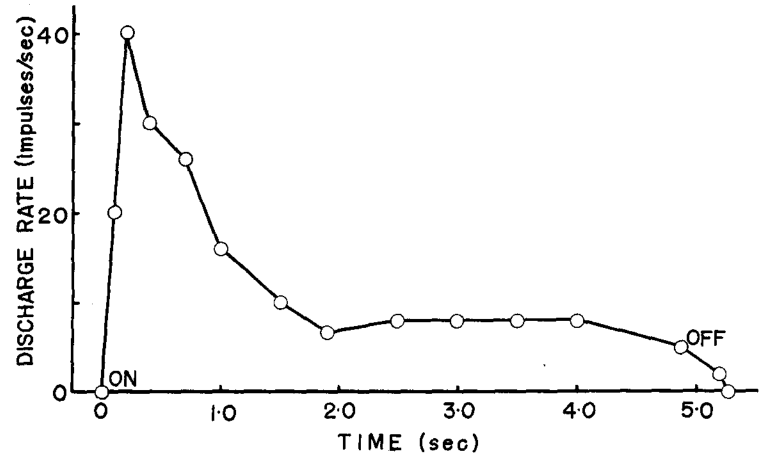

Fig. 7. Discharge rate of the 'on' excitatory unit in Fig. 6, plotted against time.

This phenomenon will be described in (B) of this section. The visual responses of the first and the second type were obtained with the large fibres of 30–70μ and the median fibres of 10–20μ respectively, which were already classified by the fibre constitution of the circum-eosophageal connective (see section II). When repetitive light flashes were applied, the response of the second type showed a gradual increase in impulse frequency during a period of stimulation up to a stimulus frequency of 40 c/sec, in the same manner as is shown in Fig. 10.

(*c*) *'Off' excitatory units:* When prolonged illumination was applied, the response, which showed only 'off' discharge with a few impulses on cessation of it, was encountered in some fibres (Fig. 5, B and Fig. 11, B). This response is called the 'off' excitatory unit. 'On' components of illumination were ineffective in the production of the visual impulse. Owing to the dark-adapted state of the eye, the latent period or the impulse number of 'off' discharge was more or less variable. It was found that responses of these units were recorded from the large fibres of 30–70μ in diameter but not from fibres smaller than 30μ.

(*B*) *Unit responses of fibres with spontaneous activity in darkness.*

(*a*) *'On' and 'off' excitatory units:* Fibres showing spontaneous activity of more than one impulse/sec was different from those showing spontaneous activity of less than one impulse/sec. In general, these units had the activity of a relatively constant, spontaneous rhythm of 3–5 c/sec in darkness. According to the difference in both the response pattern and the fibre size, these units were classified into two groups. The first group was composed of large fibres of 30–70μ showing the following characteristic responses. With prolonged illumination, the response frequency of the 'on' component was increased to about two times (about 20 impulses/sec) of the frequency of spontaneous activity. Then, the initial peak frequency of light-induced impulses suddenly returned to the initial spontaneous firing level during illumination, and weak 'off' excitatory impulses were again elicited after cessation of illumination (Fig. 8). In Fig. 9, a temporal pattern of the response of the first group is illustrated. When repetitive light flashes at 10–50 c/sec were

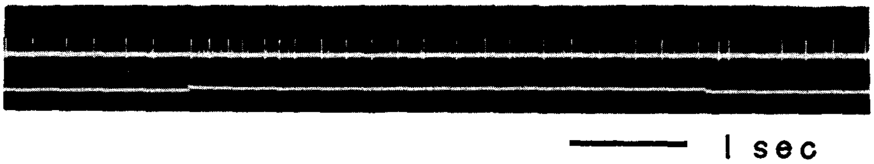

Fig. 8. Response of an 'on' and 'off' excitatory unit of large fibre which has spontaneous activity in darkness. Stimulation light: glow modulator tube.

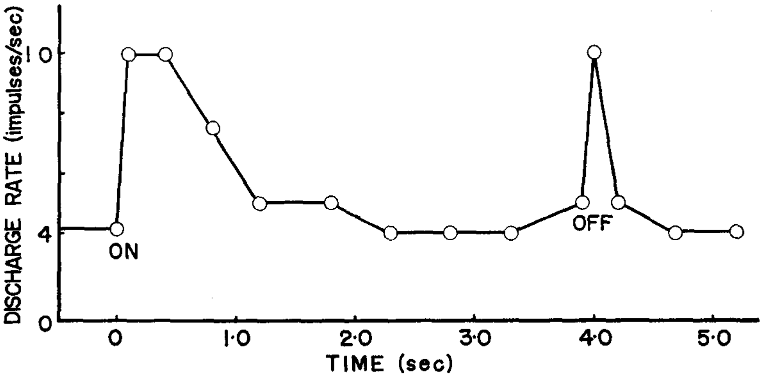

Fig. 9. Discharge rate of the 'on' and 'off' excitatory unit in Fig. 8, plotted against time.

applied, only a slight enhancement in the frequency of the initial discharge was observed.

The response of the second group of the 'on' and 'off' excitatory units was recorded from the median fibres of 10–20 μ in the connective and the following feature was observed. On application of prolonged illumination, the initial maximum discharge at a frequency of about 20–30 impulses/sec in response to the 'on' component was followed by sustained impulses during illumination, and on cessation of it, weak 'on' discharges occurred. When repetitive light stimulation was applied at 10–50c/sec, a gradual increase in the discharge rate of 'on' impulses was seen throughout the time course of flashes. At a flash frequency of more than 40c/sec, the response pattern reached a maximum, which was similar to that evoked by the steadily continuous illumination.

(*b*) *'On' excitatory units:* The response of these units was encountered only in the medium fibres of about 10–20μ in diameter. Property of the response to continuous light stimulation was almost the same as that of the above-mentioned (B)-(a) units, except for absence of the weak 'off' excitatory discharge. Under repetition of light flashes, the response frequency of both initial peak discharge and following sustained discharge was gradually increased, as the stimulus rate was increased (Fig. 10). This response built up to a maximum at 40 c/sec. The temporal discharge pattern of these units resembled to that of 'on' excitatory units giving no spontaneous firing (Fig. 6).

(*c*) *'On' inhibitory and 'off' excitatory units:* These units, which showed the inhibitory effect of light stimulation on their spontaneous visual activity were composed of only the large fibre of 30–70μ in diameter. Characteristics of these

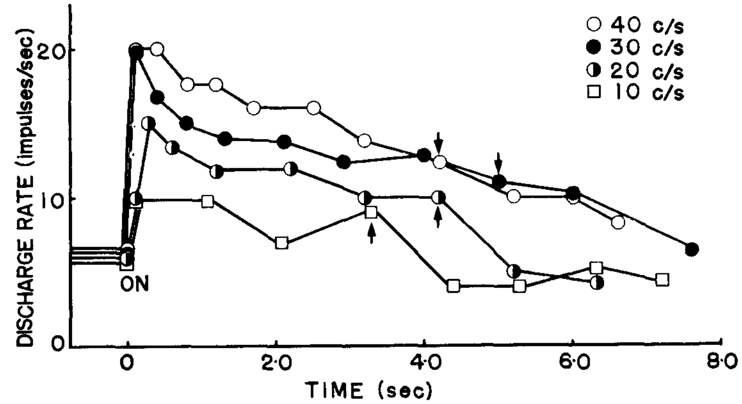

Fig. 10. Discharge rate plotted aginst time of impulses of an 'on' excitatory unit (the large fibre) to repetitive light flashes. This unit had regular spontaneous firings in darkness. The stimulus frequency is shown in the upper right corner. The arrow in each curve indicates the end of light stimulation.

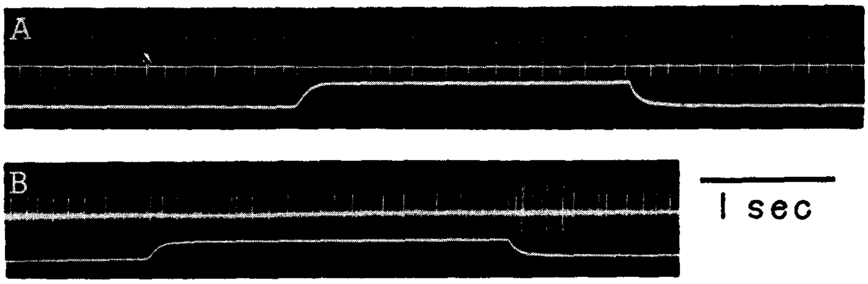

Fig. 11. Responses of an 'on' inhibitory unit (A) and an 'on' inhibitory and 'off' excitatory unit (B). These units of large fibres had regular spontaneous discharges in darkness. Another response of 'off' excitatory unit without spontaneous activity is seen in record B. Stimulation light: 100W tungsten lamp

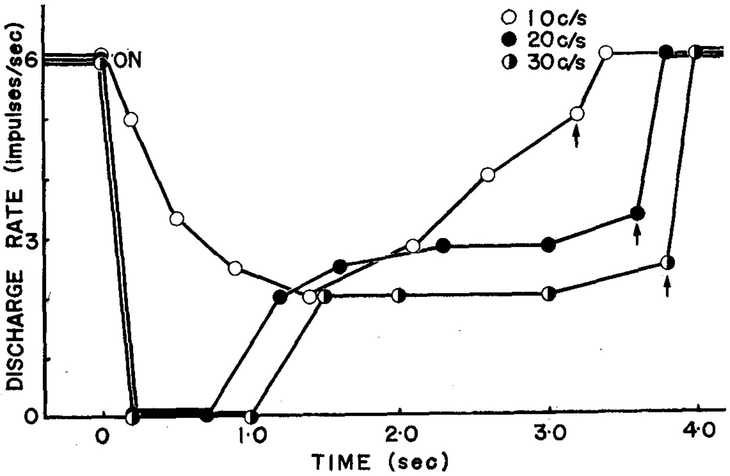

Fig. 12. Inhibitory effects of repetitive light flashes on the spontaneous firing, plotted against time. Response was recorded from an 'on' inhibitory unit of large fibre with spontaneous activity. The arrow in each curve indicates the end of light stimulation. Stimulus frequency is indicated in the upper right corner.

responses were that, under a prolonged illumination, the spontaneous rhythmic discharge of about 3–7 impulses/sec was inhibited at the initial period of illumination, and that, on cessation of it, a slight increase in the spontaneous discharge frequency also occurred, which means the weak 'off' excitatory discharge. In Fig. 11, B, the response of these units is shown. Under repetitive light flashes at a frequency of 10–40c/sec, the inhibitory effects on the spontaneous discharge were, to some extent, enhanced (Fig. 12). At frequency of above 40 c/sec, the inhibitory response reached a maximum. The shorter the dark adaptation of the eye, the lower the inhibitory effect.

(*d*) *'On' inhibitory units:* In these units, the property of the light-induced response was almost the same as that in the response of above-mentioned units of (B)-(c), except that the 'off' excitatory response was absent.

IV. *Distribution of unit types.*

In the preliminary experiments, light-initiated responses were recorded from the whole trunks of the circum-oesophageal connectives and it was found that there was difference between the large fibres (30–70μ) and the medium fibres (10–20μ) in respect to excitatory and inhibitory effects. Therefore, isolation into single fibres or fine bundles from the connectives containing a variety of fibre sizes was carried out distinguishing one fibre group from another. From a series of experiments for exploration of the large fibre group, the distribution of response units classified in section III were determined, and form another series of experiments, the distribution of response units of the medium fibre group were effected (Table 1). The response of the lateral giant fibres was omitted from this Table. One half of the large fibres explored (36) were the fibres with spontaneous activity in darkness and the remaining half were the fibres without spontaneous activity.

Table 1. Classification of unit responses in the circum-oesophageal connectives

Units	Large fibres		Medium fibres	
	No. of observation	(%)	No. of observations	(%)
Fibres without spontaneous activity				
(a) 'On' and 'off' excitatory	6	(17)	0	(0)
(b) 'On' excitatory	5	(14)	7	(44)
(c) 'Off' excitatory	6	(17)	0	(0)
Fibres with spontaneous activity				
(a) 'On' and 'off' excitatory	2	(5)	4	(25)
(b) 'On' excitatory	0	(0)	5	(31)
(c) 'On' inhibitory and 'off' excit.	13	(36)	0	(0)
(d) 'On' inhibitory	4	(11)	0	(0)
Total	36	(100)	16	(100)

A large proportion (47%) of the fibres with relatively regular spontaneous discharge showed inhibitory responses to the 'on' of the illumination.

As is shown in the Table, units of the inhibitory responses were nerver obtained from the medium fibres.

V. *Change in response pattern.*

Most of the response patterns described in section III were kept under a constant condition for a long period of time. However, in some cases, the interesting phenomena were observed. Some units of the large fibres were changeable in some degree under the following conditions: (1) the period of dark adaptation, (2) the duration of the repetitive light stimulation. When the duration of dark adaptation was inadequate (less than 3 sec), the response pattern of the 'on-off' and 'off' excitatory units, which did not have spontaneous discharges in darkness was changed; the impulse frequency of 'on' discharge was decreased, and the 'off' discharge was completely eliminated. The response pattern similarly changed was also seen under inadequate dark adaptation in the 'on' inhibitory and 'off' excitatory units or the 'on' inhibitory units. The 'on' inhibitory effect on spontaneous activity was diminished to some extent.

Furthermore, the following interesting fact was observed in some cases. 'On' inhibitory effect on the spontaneous firing, which the above-mentioned two units showed, was changed into an 'on' excitatory effect. In this case, 'on' inhibitory effect which is shown in Fig. 11 was always seen at the early stage of stimulation. However, after the preparation was kept in a dark-adapted state for a long period (over 10 min), the 'on' excitatory effect occurred at the initial part of 'on' component of illumination before appearance of the 'on' inhibitory effect (Fig. 13, A). With further dark adaptation, this 'on' excitatory effect was increased

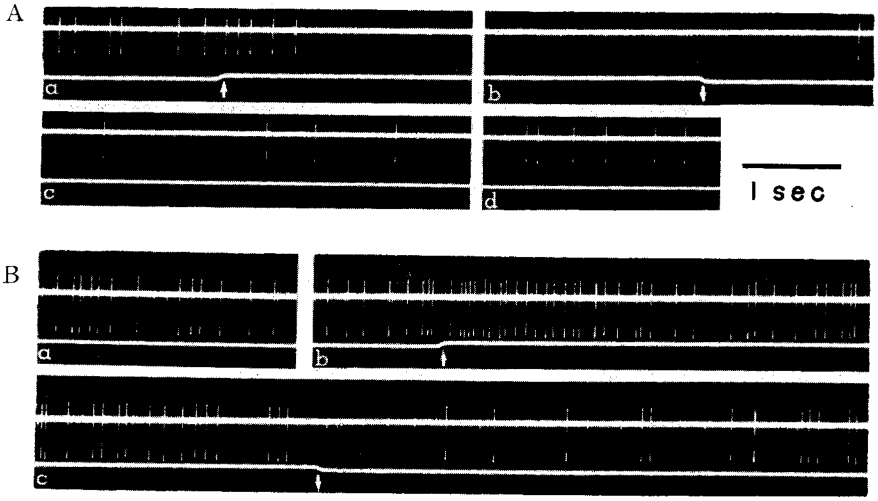

Fig. 13. Change of response pattern under dark adaptation of a long period. At the early stage of observation, the response of 'on' inhibitory unit as in Fig. 11, A, was seen. Record A: a, 'dark' discharge and appearance of 'on' excitatory response which was suddenly inhibited; b, the perfect inhibitory effect; c and d, the gradual recovery of spontaneous discharge. Record B: a, 'dark' discharge; b, 'on' excitatory response which attained gradually to 'dark' discharge rate; c, the appearance of inhibitory effect. Record A and B were taken from the same fibre. Arrows in each record indicate the start and end of illumination.

and the following inhibitory effect was decreased and appeared only late (Fig. 13, B). This phenomena seem to be induced by a change in physiological conditions in the central nervous system during dark adaptation.

It was found in some cases that the effect of repetitive light stimulation changed the response pattern. In Fig. 14, an example of these cases is shown. The discharge which had unstable impulses to 'on' and 'off' illumination was changed into the maintained discharge during illumination by repeated stimulation. This suggests that activity of the central nervous system, paticularly of the visual centre was facilitated by repetition of continuous illumination.

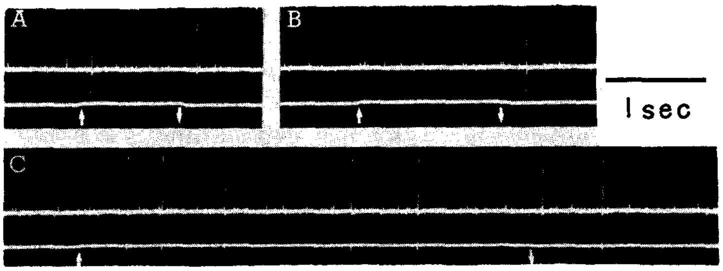

Fig. 14. Change in the response pattern during repeated stimuli. Record A and B: unstable response to 'on' and 'off' of illumination. Record C: maintained response during illumination. Arrows under all lower traces indicate the start and end of illumination.

Discussion

In the present experiments, the simplified preparation which was composed of both the compound eyes including the optic ganglia and the central nervous system was used. Most of the sensory impulses, therefore, originated only in the visual system and they transmitted to the supra-oesophageal ganglion. Excitability of this ganglion may be always affected by not only stimulus information from many sorts of sensory organs but also intrinsic spontaneous information from the lower ganglia. Ikeda and Wiersma (1964) and Biederman (1964) presented the evidence that the abdominal ganglion, particularly the sixth one was a pacemaker for initiation of the spontaneous ascending impulses, which were transmitted to the upper ganglia presumably including the supra-oesophageal ganglion. Since all of the ventral nerve cord except the circum-oesophageal connective was removed in the present experiments, these ascending impulses were entirely eliminated.

The present experiments revealed that the responses in the optic peduncle of the crayfish to light stimulation at a fixed distance consisted of three units; 'on–off', 'on', and 'off' units. The movement-sensitive unit in the optic peduncle is not activated by such stimulation (Waterman and Wiersma, 1963, Wiersma and Yamaguchi, 1965, Sato and Yamaguchi, 1965). All the visual responses of these three units showed an excitatory nature. And the response inhibited by light was not observed in the crayfish optic peduncle. In other crustaceans such as marine lobsters and crabs, the presence of such inhibitory response has not yet been reported (Waterman and Wiersma, 1963). On the other hand, in the optic ganglion of the insects, the inhibitory response was found (Burtt and Catton, 1960, Ishikawa, 1962). On the basis of sorts of the response patterns, it seems that there is, to a certain extent, a difference in the integration mechanism of the visual information between the crustacean optic ganglion and the insect optic ganglion. Probably this difference is due to the structure of the synaptic regions in the optic ganglion. Hartline and Graham (1932) studied the response of single units in the optic nerve of *Limulus,* in which there is no optic ganglion between the photoreceptor and the supra-oesophageal ganglion, and concluded that the single units showed uniform activities with only 'on' impulses. These data suggest that the postretinal fibres directly coming from the photoreceptor of the crustaceans or insects may show uniform visual activities in response to visual stimulation. These uniform visual impulses are, as the first step, integrated in the optic ganglion, and thus they result in the various types of unit responses.

In the present experiments, output responses of the supra-oesophageal ganglion of the crayfish to visual stimulation were recorded from the circum-oesophageal connective, and the characteristic response patterns detected were as follows: (1) The fibres with rare spontaneous activity in darkness showed the 'on' and/or 'off' excitatory responses. (2) The fibres with spontaneous firing in darkness showed, in addition the 'on' inhibitory and 'off' excitatory response and the 'on' inhibitory response alone. The fibres which showed 'on' inhibitory effect on the spontaneous discharge were to a large proportion of all types of the light-initiated responses. These facts indicate that (1) visual impulses initially integrated in the optic ganglion are further integrated in the supra-oesophageal ganglion and that (2) various types of the visual information, a part of which is not included in the optic peduncle, are transmitted to the lower ganglia. In the crayfish, inhibition of the spontaneous visual impulses was first found at the level of the circum-oesophageal connective. This finding means that the generation site of the inhibitory effect in the visual system of the crayfish is situated in the supra-oesophageal ganglion. On the other hand, in the insects, this effect is partially situated in the optic ganglion. From the view-point of the generation site of visual inhibitory response, the function of the crayfish optic ganglion seems to be more primitive than that of the insect optic ganglion.

Furthermore, the present experiments revealed that the circum-oesophageal connective was composed of various sizes of fibres, and that the lateral giant fibre, the large fibres (30–70μ) and the medium fibres (10–20μ) were activated by light stimulation. This indicates that the three groups of fibres in the connective make synaptic connection with the optic peduncle fibres. Sato and Yamaguchi (1965) found that all the fibres in the circum-oesophageal connective of the crayfish, which responded to illumination of the eye, descended through interneurones, because the synaptic delay of more than 50 msec was measured in the supra-oesophageal ganglion. Moreover, this finding was also comfirmed by recording the responses in the connective elicited by electrical stimulation of the optic peduncle (Sato and Katagiri, unpublished data). The data suggest that the functional relation between the fibres in the optic peduncle and the fibres in the circum-oesophageal connective is modulated by many interneurones in the supra-oesophageal ganglion. Since the descending fibres of the connective are final neurones from the supra-oesophageal ganglion, it is conceivable that the somata of these neurons receive complicated synaptic events and thus produce various combinations of the excitatory and inhibitory responses to 'on' and 'off' illumination.

The results from the simplified preparation showed that only the lateral giant fibre of two types of the giant fibres responded to illumination. It is well known that when backward swimming for escape in the crayfish occurs, either of the giant fibres fires (Wiersma, 1947). In the intact animals, visual stimulation alone is sufficient to elicit a nerve impulse of the giant fibres for escape swimming (Waterman, 1961). If the present experiments were done with intact animal, even the medial giant fibre may produce the similar impulse to light stimulus.

As was stated above, the results also show that all responding units in the connective did not always show uniform response under all conditions of the eye. The response in some of visual units could be changed under the duration of dark adaptation or duration of repeated light stimulation. As a most interesting fact, 'on' inhibitory effect on the spontaneous activity was changed into an 'on' excitatory effect. This apparently suggests that some somata of the fibres in the circum-oesophageal connective are converged from many interneuron and that they are integrated by two antagonistic synaptic events, excitatory and inhibitory. It is also considered that change in the impulse pattern depends on a physiological state of the supra-oesophageal ganglion.

The present experiments show no direct evidence for the integration site of the visual informations in the supra-oesophageal ganglion. Recently, Blest and Collett (1965) studied the light-induced response of single neurones in the medial region of the protocerebrum of the insect and identified several types of responses including excitatory and inhibitory responses, which were more complicated than those in the optic ganglion. Furthermore, Huber (1959, 1960) and Rowell (1963) have shown that the corpora pedunculata of the protocerebrum were a selector site of the motor patterns. These descriptions suggest that, since the visual route of the optic peduncle of the crayfish first reaches the corpora pedunculata, this command centre is modulated by light induced-impulses in the optic peduncle. Therefore, it may be concluded that the visual information in the optic peduncle is re-integrated at the corpora pedunculata in the supra-oesophageal ganglion to set up motor activities of the animal.

Summary

1. Electrical responses to light stimulation of the compound eye of the crayfish were recorded from the following visual pathways: (1) the retinular layer, (2) the optic peduncle, (3) the circum-oesophageal connective, and the relation among them was studied.

2. By application of repetitive light flashes (15 luxes), a series of action potentials on the retina was elicited, which were composed of a single large, slow action potential and successive small action potentials. At a stimulus frequency of more than 35–40c/sec, only the former potential appeared, since the latter potentials fused and fell to the resting level. On the other hand, a train of impulses in the optic peduncle was elicited during repetitive stimuli.

3. Unit responses of the optic peduncle fibres to stimulation from a fixed light source were of the three types. (1) 'on-off' (2) 'on', and (3) 'off' excitatory responses.

4. Visual response patterns of fibres in the connective without the spontaneous activity in darkness were classified into the three groups; (1) 'on' and 'off' excitatory response, (2) 'on' excitatory response, and (3) 'off' excitatory response. On the other hand, visual response patterns of fibres with the spontaneous activity were classified into the four groups; (a) 'on' and 'off' excitatory response, (b) 'on' excitatory response, (c) 'on' inhibitory and 'off' excitatory response, and (d) 'on' inhibitory response.

5. Large fibres of 30–70μ in the connective showed all of the responses except (b) response described above. Medium fibres of 10–20μ showed (2), (a) and (b) responses. Small fibres of less than 10μ did not respond to illumination. Only the lateral giant fibre of the two giant fibres sometimes responded to both 'on' and 'off' component of illumination.

6. Distribution of the response units of large fibres in the connective was as follows; (1) 'on-off' excitatory units (22%), (2) 'on' excitatory units (14%), (3) 'off' excitatory units (17%), (4) 'on' inhibitory and 'off' excitatory units (36%), and (5) 'on' inhibitory units (11%). On the other hand, the distribution of the response units of the medium fibres was as follows; (1) 'on-off' excitatory units (25%) and (2) 'on' excitatory units (75%).

7. It was observed that the response pattern of some units of the large fibres was, to some extent, changed by the following conditions; (1) duration of dark adaptation, and (2) duration of repetitive light stimulation.

8. Mechanism of integration of visual informations in the supra-oesophageal ganglion was discussed. It was concluded that the visual information transmitted through the optic ganglion to the supra-oesophageal ganglion was re-integrated to set up impulses for the animal locomotion.

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