J. Exp. Biol. (1972), 57, 651–660
With 10 *text-figures*
Printed in Great Britain

PHOTOSENSITIVE NEURONES IN THE MARINE PULMONATE MOLLUSC *ONCHIDIUM VERRUCULATUM*

BY NOZOMU HISANO, HIDEKI TATEDA AND MASUTARO KUWABARA

Department of Biology, Faculty of Science, Kyushu University

(*Received* 17 *March* 1972)

INTRODUCTION

It has been amply demonstrated that the nerve cells of some animals respond to light, and that excitatory and inhibitory cells are involved in this response (Arvanitaki & Chalazonitis, 1949*a*, *b*, 1958; Kennedy, 1961, 1963; Yoshida & Millott, 1959). In the photo-excitative cell, depolarization of the membrane, appearance of spikes or increase of their frequency have been seen in response to light stimuli; whereas in the photo-inhibitive cell hyperpolarization of the membrane, disappearance of spikes or decrease of their frequency was noted. Arvanitaki and Chalazonitis found that some large ganglion cells of *Aplysia* were photosensitive, and they could distinguish 'primary' photosensitive neurones from others by using a minute spot of light. The light effect was mediated by two different pigments in primary neurones, i.e. carotenoid pigments for inhibition and haemoprotein pigments for excitation.

It is known that the marine pulmonate mollusc *Onchidium* has many ganglion cells which are as large as those of *Aplysia* (Hagiware & Saito, 1959), and in this animal it was found that some neurones were excited by light whereas others were inhibited. In order to analyse the mode of response of these neurones, microelectrode and micro-illumination methods were used in the present study.

MATERIALS AND METHODS

The experimental animals, *Onchidium verruculatum*, were collected from Kinko Bay in the southern part of Kyushu. Most of them were 3–6 cm in length, and they were kept in sea-water tanks in the laboratory at 15–16 °C.

The animal was pinned on to a dissecting plate, dorsal side up, and the sub- and supra-oesophageal ganglion complex was exposed and isolated. The isolated ganglion complex was pinned on to a paraffin block in a Lucite chamber of about 20 ml capacity and was continuously perfused with artificial sea water at a constant temperature. The connective tissue surrounding the ganglion was carefully removed with forceps under a binocular microscope. The composition of van't Hoff's artificial sea water used for the experiments was as follows: (g/l); NaCl, 27; $CaCl_2.2H_2O$, 1·25; $MgCl_2.6H_2O$, 7·2; $MgSO_4.7H_2O$, 27·8; $NaHCO_3$, 5.

Glass pipette microelectrodes filled with 3 M-KCl were used for intracellular recordings. A silver–silver chloride wire was placed in the sea water bathing the preparation as an indifferent electrode. Extracellular recordings were made from the

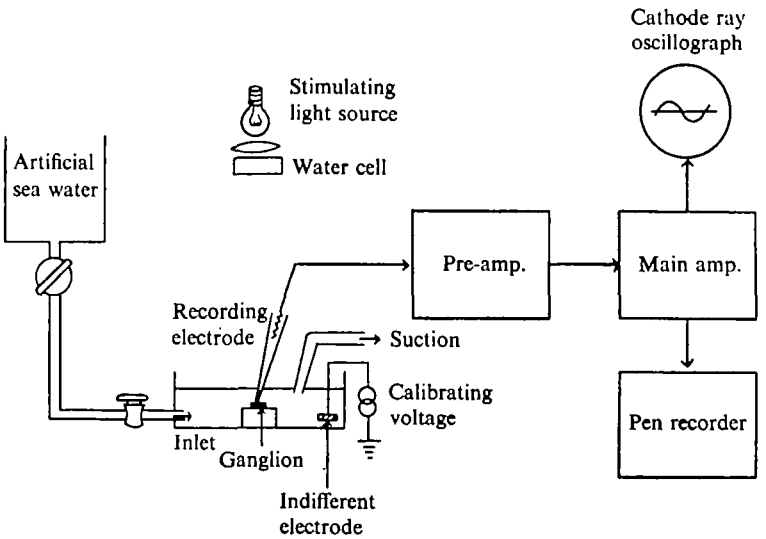

Fig. 1. The recording and stimulating apparatus. The apparatus for producing the minute spot of light is omitted. See text for description.

connective between the abdominal and right pleuro-parietal ganglia with a platinum wire connected to a high-gain pre-amplifier. All of the results were recorded with a pen-recorder.

For illumination of the whole ganglion the focused beam of a tungsten lamp (6 V, 30 W) was used. The light beam was passed through a water cell 3 cm thick in order to remove infra-red radiation. A minute light spot of less than 500 μm diameter was focused on to a single cell or a small area of a ganglion by converging the light beam through an objective lens of a microscope (Fig. 1).

EXPERIMENTAL RESULTS

Distribution and response pattern of the photosensitive neurones

The sub- and supra-oesophageal ganglion complex of *Onchidium verruculatum* consists of seven ganglia. All of the large neurones in these ganglia were examined to find whether or not they were photosensitive. Photosensitive neurones were first located by whole-ganglion illumination. When a photosensitive neurone was found, it was tested by minute light spot illumination to determine whether it was a 'primary' or a 'secondary' photosensitive neurone.

A primary neurone responded to light whenever the neurone itself was illuminated. The secondary neurone did not respond to light when it alone was illuminated, but responded to light when the primary neurone innervating it was illuminated. The results are summarized in Fig. 2 and Table 1. The symbols E and I indicate the photo-excitative and photo-inhibitive neurones, respectively, while the symbols p and s indicate the primary and secondary neurones, respectively. A discussion of the details of the synaptic connexion between the primary and secondary neurones will be presented later.

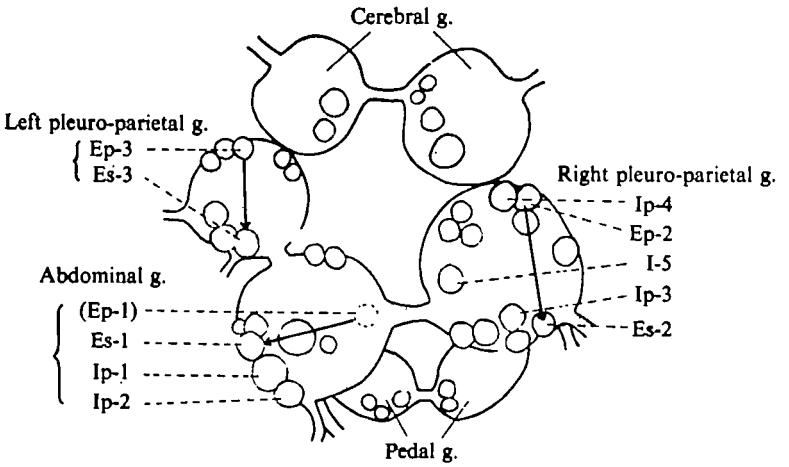

Fig. 2. Location of the photosensitive neurones. Giant neurones and ganglia are drawn schematically. E, Photo-excitative neurone; I, photo-inhibitive neurone; p, primary neurone; s, secondary neurone. An arrow shows a synaptic connexion between the primary and the secondary neurone. See text for details.

Table 1. *Distribution of the photosensitive neurones*

Ganglion	Giant cell	Excitatory	Inhibitory
Cerebral ganglion	5	0	0
Left pleuro-parietal ganglion	8	2 (Ep-3, Es-3)	0
Abdominal ganglion	10	2 (Ep-1*, Es-1)	2 (Ip-1†, Ip-2†)
Right pleuro-parietal ganglion	14	2 (Ep-2, Es-2)	3 (Ip-3, Ip-4, I-5)
Pedal ganglion	5	0	0

* Not distinguished
† White cell
For the abbreviations see Fig. 2.

Excitatory effect

Primary photo-excitative neurones were found in the left pleuro-parietal ganglion, abdominal ganglion, and right pleuro-parietal ganglion. Each of those ganglia had at least one primary photo-excitative neurone. Among these primary neurones, the one (Ep-2) in the right pleuro-parietal ganglion was a 'giant' neurone and was as large as 300–400 μm in diameter. The one (Ep-3) in the left pleuro-parietal ganglion was always smaller than Ep-2, and its size and location in the ganglion varied from preparation to preparation. Ep-1 has not been located precisely, but the neurone (Es-1) was excited only when a specific region (marked by a circle drawn with a broken line in Fig. 2) was illuminated by the minute spot of light. This fact shows the existence of a primary photo-excitative neurone (Ep-1). The neurone Ep-1 may be very small compared with the giant cells, or may be deep in the interior of the ganglion.

The response patterns of these neurones recorded from one preparation are shown in Fig. 3. The response patterns of Ep-2 and Ep-3 differ slightly from that of Es-1. The neurone Es-1 showed almost no discharge immediately after the cessation of the

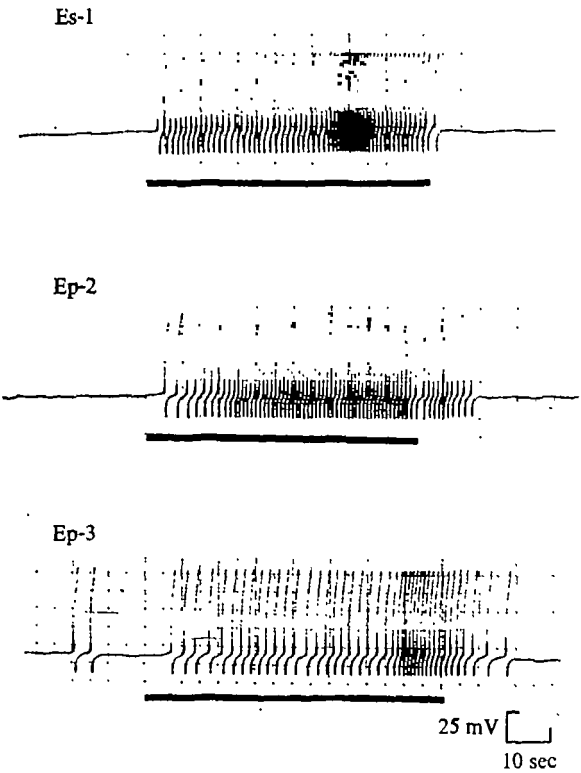

Fig. 3. Response patterns of the photo-excitative neurones recorded from one preparation. Duration of illumination in this and subsequent records is signalled by a black bar under each record.

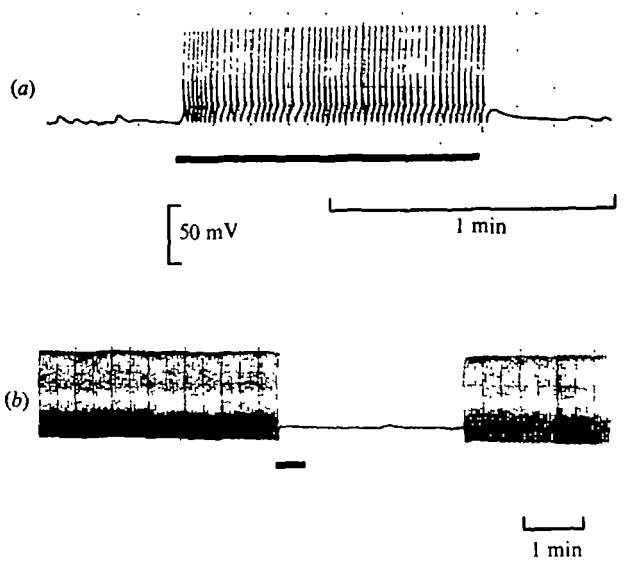

Fig. 4. Reversal of the light effect on Es-1. In the upper record, just after the insertion of a recording electrode, and in the lower record, 2 h later.

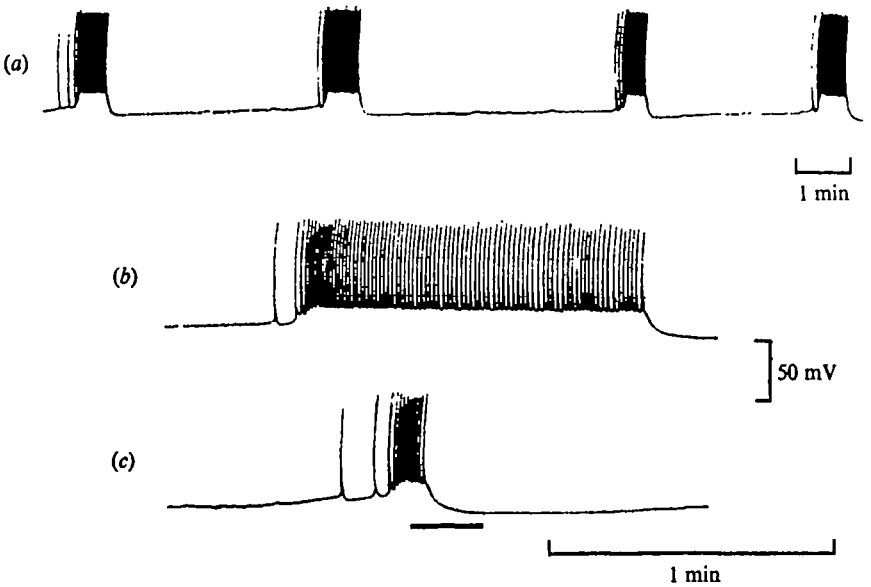

Fig. 5. Discharge pattern and response to light of Ip-1. Record *a*, spontaneous 'burst' discharge; *b*, spikes of one burst; *c*, inhibition of a burst. Record *b* and *c* have the same time-scale.

light stimulus, whereas Ep-2 and Ep-3 showed after-discharges lasting for 10 sec or more.

Although Es-1 was usually excited by light, its spontaneous discharge was sometimes inhibited by light. The upper record in Fig. 4 is the normal response of Es-1. The lower record shows the spontaneous discharge recorded 2 h later when the discharge was inhibited by light. It was found that the membrane potential in this state was slightly decreased (approximately 10 mV), compared with the resting membrane potential in the normal state. Occasionally, this inverse effect of light on the neurone Es-1 was seen after illumination had been repeated many times.

Inhibitory effect

Two white neurones (Ip-1, Ip-2) in the abdominal ganglion were as large as 300–500 μm in diameter and could easily be seen with the naked eye. The neurone Ip-1 showed two types of spontaneous discharge; one was intermittently generated rhythmic bursts of impulses and the other was continuous discharge. The effect of light on the former is shown in Figs. 5, 6. One burst normally occurred every 4–5 min (Fig. 5*a*) and consisted of 50–80 spikes (Fig. 5*b*). This burst was inhibited by a light stimulus (Fig. 5*c*). If a light stimulus was given before the occurrence of the expected first spike of the burst, the burst was usually inhibited completely (Fig. 6*b*); but, if the stimulus came after the occurrence of a number of spikes, inhibition did not appear immediately but occurred after a few seconds (Fig. 5*c*). If the bursts were blocked by a sequence of short light stimuli, the membrane was depolarized gradually; and eventually a small burst occurred during illumination (Fig. 6*b*, arrow 'A'). The membrane, however, was not repolarized completely to the resting level by light

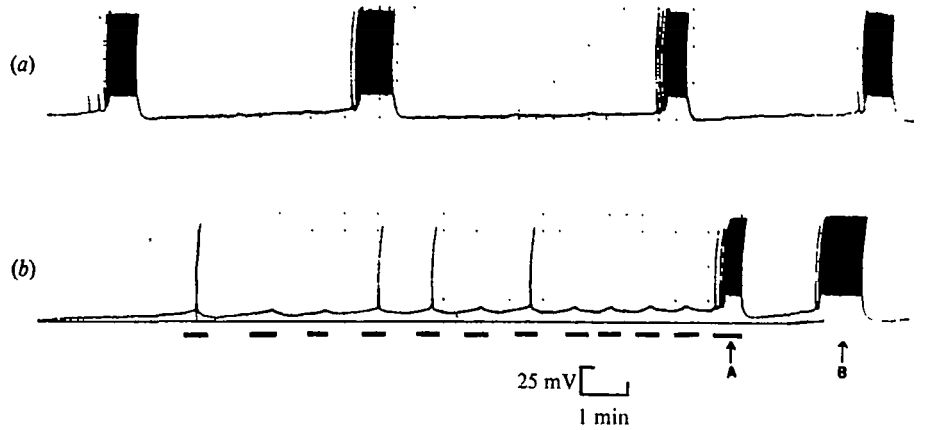

Fig. 6. The effect of repetitive inhibition on 'burst' discharge of Ip-1. Record *a*, spontaneous burst discharge; *b*, repetitive inhibition by light; arrow A indicates disappearance of the inhibitory effect of light; arrow B indicates post inhibitory rebound. For explanation see text.

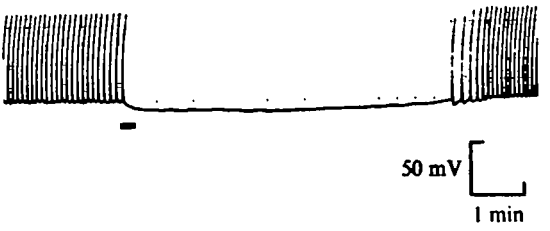

Fig. 7. Inhibitory effect of light on Ip-1 discharging continuously.

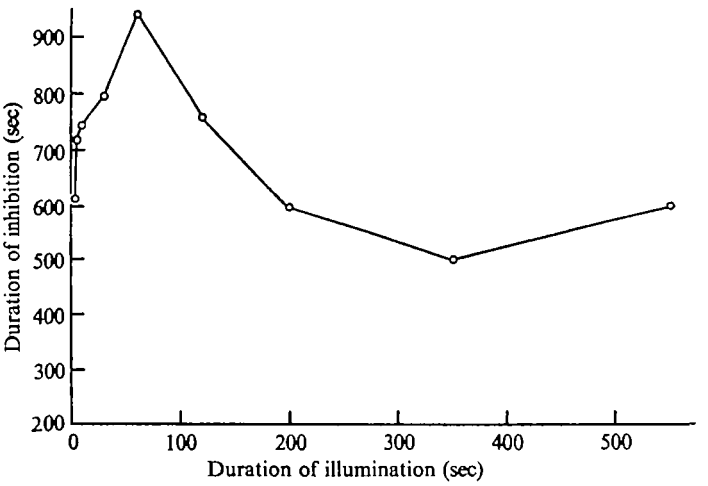

Fig. 8. The relation between duration of light stimulus and that of inhibition in Ip-1.

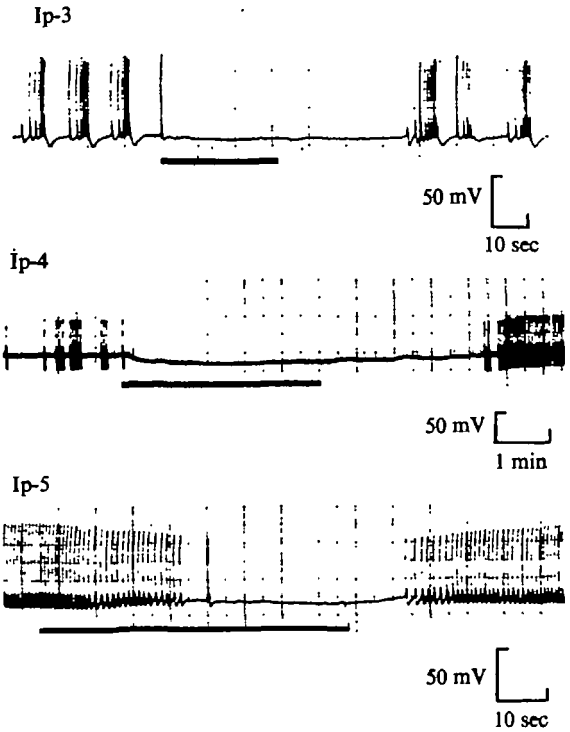

Fig. 9. Comparison of response patterns of photo-inhibitive neurones. These are recorded from different preparations.

stimuli and started to depolarize again when the stimuli were terminated. A larger than-normal burst occurred soon after the short burst (Fig. 6*b*, arrow 'B').

The inhibitory effect of light during continuous discharge of the neurone, Ip-1, is shown in Fig. 7. The relation between the duration of illumination and that of inhibition is shown in Fig. 8. Two characteristics of this inhibitory phenomenon are demonstrated. First, a few seconds of illumination caused prolonged inhibition. Such an effect of light was not seen with the excitatory response. Second, if the duration of illumination was longer than a certain period, which varied from preparation to preparation, the membrane was depolarized gradually during illumination, and eventually the inhibition was terminated. The mechanisms underlying this phenomenon will be discussed later.

The responses of three photo-inhibitive neurones in the right pleuro-parietal ganglion are shown in Fig. 9. The neurone Ip-3 showed burst-type spontaneous discharges which were inhibited by light stimuli. The response of Ip-4 was similar to that of Ip-1 in that spontaneous discharges were abolished. On the other hand, I-5 showed a different type of response. When this neurone was stimulated by light, the height of the spontaneously generated spikes diminished gradually until the discharge of spikes was completely inhibited. When spontaneous discharges occurred again after the cessation of the light stimulus, the spike height gradually increased. It was not determined whether the neurone I-5 was the primary neurone or not, because this neurone was rarely found.

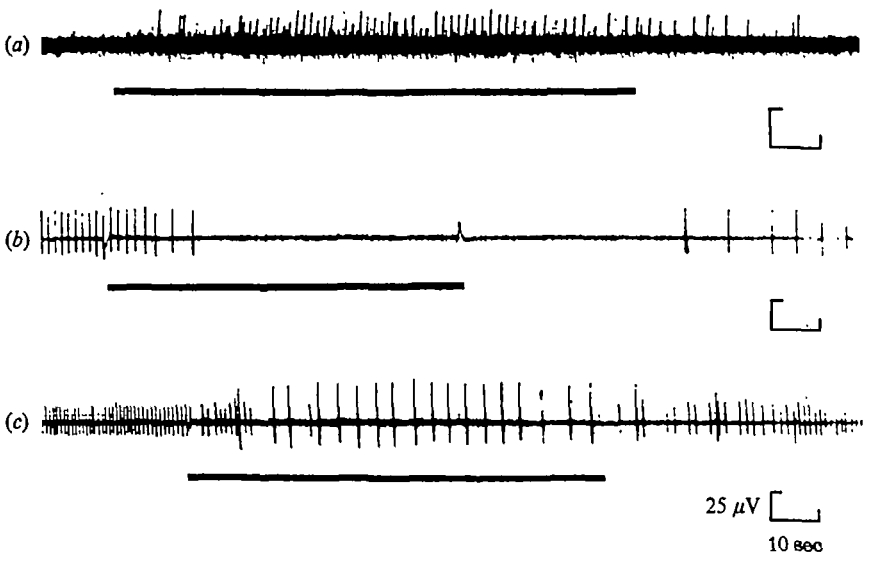

Fig. 10. Responses obtained with extracellular recording from the connective between the abdominal ganglion and the right pleuro-parietal ganglion in different preparations. Record *a*, photo-excitative spikes; *b*, photo-inhibitive spikes; *c*, the larger spike is photo-excitative and the smaller spike is photo-inhibitive.

Extracellular recording

The results of extracellular recordings from the connective between the abdominal and pleuro-parietal ganglia are shown in Fig. 10. Record (*a*) shows the discharge of a photo-excitative neurone and record (*b*) shows that of a photo-inhibitive neurone. Two kinds of spikes can be seen in record (*c*). The larger one is from a photo-excitative neurone and the smaller is from a photo-inhibitive neurone. Although it was impossible to detect the neurones from which these spikes originated, two types of effects of light on neural photo-receptors can be seen cleanly in this figure. Spikes from the photo-sensitive neurones were conducted through the connective between ganglia.

DISCUSSION

The experiments described above showed that the neural photo-sensitive cells of *Onchidium* consist of photo-excitative and photo-inhibitive neurones. Four types of the probable connexions between the primary and secondary neurones, Ep–Es, Ep–Is, Ip–Es and Ip–Is, may exist. The first type of synaptic connexion, Ep–Es, was found among photo-sensitive neurones. Primary photo-inhibitive neurones were found, but their connexions to secondary neurones were not detected. Since Arvanitaki & Chalazonitis (1961) showed that primary photo-excitative neurones inhibited secondary (or tertiary) neurones in *Aplysia*, further analysis will probably reveal more complicated neural connexions in photo-excitation and photo-inhibition in *Onchidium*.

The neurones Ip-1 and Ip-2 were primary photosensitive neurones and were nhibited by light stimuli. Hyperpolarization of the membrane by light stimulation was clearly observed when no spontaneous discharge was present.

The neurone Ip-2 usually showed no spontaneous discharge. On the other hand

Ip-1 showed two types of spontaneous activity. One of them was intermittent 'burst' discharges and the other was continuous discharge. What condition of the neurone determined the type of discharge could not be outlined clearly. The 'burst' discharge was seen just after insertion of the recording electrode, and the continuous discharge appeared later in most cases. In spite of the difference in discharge patterns, the activity of Ip-1 was always inhibited by light. The inhibitory effect of light was overcome by the excitatory effect during illumination of long duration or after repetitive short light stimuli (cf. Fig. 8). We tentatively propose the following mechanisms which may underly this phenomenon: (1) This could be due to the adaptation of a photosensitive substance which initially hyperpolarizes the membrane when it is excited by light; (2) there could be an accumulation of some substance which depolarizes the membrane, but which is consumed regularly in the dark. The inhibitive effect of light would then be overcome by the inherent depolarizing action of this substance; and (3) the excitatory process by a photo-excitative substance could overcome the inhibitory process by a photo-inhibitive substance in a neurone such as Ip-1 during long or repeated light stimuli. Probably some other mechanisms are also involved in this phenomenon.

Arvanitaki & Chalazonitis (1949*a*,*b*) found that photo-excitation was mediated by a haemoprotein pigment, and photo-inhibition took place through a carotenoid pigment in *Aplysia* neurones. The colour of the neurones in *Onchidium* is similar to that in *Aplysia*. The same pigments as those of *Aplysia* may therefore be concerned in the photo-response of the neurones in *Onchidium*.

One possible mechanism for the reversal of the light effect on the neurone Es-1 is a change in the resting potential of Ep-1 or Es-1, because the resting membrane potential of primary photo-excitative neurons was generally lower by 10–20 mV than that of primary photo-inhibitive neurones. At present, however, we cannot explain the reversal of the light effect on the neurone Es-1 in terms of the reversal potential of the photo-response in Ep-1 or Es-1. It is probable that the absorbancy change of the photosensitive substance in the primary photo-excitative neurone Ep-1 brought about the reversal of the light effect (Chalazonitis, 1964). More detailed spectral information is needed in this respect.

SUMMARY

1. Giant photosensitive neurones were found in the sub- and supra-oesophageal ganglia of the marine pulmonate mollusc *Onchidium verruculatum*.

2. Some were photo-excitative neurones; spikes appeared or their frequency increased in response to light stimuli. The others were photo-inhibitive neurones in which disappearance of spikes or decrease in their frequency was noted in response to light.

3. Photosensitive neurones were distinguished according to whether or not they were 'primary' neurones, i.e. responded directly to a minute light spot. Three primary neurones and three secondary neurones were detected among the photo-excitative neurones. There were four primary photo-inhibitive neurones. It was not determined whether a neurone was primary or not.

4. An inverse effect of light on one of the primary photo-excitative neurones,

Es-1, occasionally occurred after illumination of long duration. A light stimulus which had previously excited the neurone, inhibited subsequent spontaneous discharge.

5. The inhibitory effect of light on one of the primary photo-inhibitive neurones was overcome by some excitatory process during illumination of long duration or after repeated light stimuli.

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