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Response to Light in *Paramecium**.1).2)

By

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(With 6 Text-figures)

The mechanism of excitation elicited by electric current or chemical agents has been studied in detail in *Paramecium* by many authors (Wichtermann, 1953). Sgonina (1939), on the other hand, described response to light in *Paramecium*. He reported that excitability to light was increased by photostimulus synchronized with electric current. However, Soest (1937) maintained that there was no evidence to show that *Paramecium* did in fact respond to light under conditions resembling those of Sgonina's experiments. These results have never been adequately examined with regard to the excitability to light in *Paramecium* and it is the purpose of the present investigation to do so.

Material and Method

All of the experiments were made with specimens of *Paramecium caudatum* cultivated in a vegetable powder infusion. In preparation for the experiments, the animals were transferred to a chamber and kept in the dark (2 lux) for more than an hour.

A tungsten or xenon lamp was used for the stimulating light, and the duration of the light was changed with a camera-shutter. The intensity of the stimulating light was measured with a luxmeter (Toshiba, No. 5) or a phototransistor (OCP-71) against the standard intensity. In order to obtain a monochromatic light, the light from the xenon lamp was passed through a monochromator (Olympus; useful ranges of wave length are from 2400 to 20000 Å). The radiation energy of the monochromatic light was measured with a thermopile-galvanometer system (Kokusai, HTC-50LA) to obtain equal intensity in each wave length. Light filters (Canon UV-filter, Toshiba IR-DIB, Conning 7-54 for exclusion of the visible rays) were also employed to select the wave length of the light. Observations of the response to the light were made with an optical microscope, and detailed observation was recorded with an 8 mm cine-camera (16 frames per sec).

All of the experiments were done in the dark room (averaged light intensity was kept at 2 lux) at room temperature (19°-25°C).

Results

* This paper is dedicated to Professor Atsuhiko Ichikawa, Zoological Institute, Hokkaido University, Sapporo, in honour of his sixtieth birthday, May 20, 1964.

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1) Phototaxis

The question of whether or not *Paramecium* shows phototaxis has not been clarified and one of the purposes of the present experiments was to analyze this response in detail.

A glass tube (1×150 cm) was filled with *Paramecium* suspension. The entire length of the tube was then illuminated from one end by tungsten lamp (2000 lux) which resulted in a gradient of intensity along the tube. The illumination in the glass tube was decreased with a gradient between both ends. Observation continued more than five hours after exposure to the light. It was ascertained that the animals did not respond to the applied light. A glass chamber (7×5×15 cm) was then filled with the *Paramecium* suspension and illuminated in the center by a spot light (10 lux). There was no movement of the *Paramecium* either toward or away from the light. From these results, it was concluded that *Paramecium* does not show phototaxis.

When *Paramecium* were kept in the dark (about 2 lux) for more than one hour, ciliary beating decreased but it was restored within 30 seconds following exposure to a weak illumination. Swimming velocities, measured 5 min. after the illumination, were 1100 μ /sec at 20 lux and 1500 μ /sec at 1000 lux. It was concluded that the weak light activated the ciliary beating previously inhibited by the dark adaptation of the animal.

2) Responses to the flash light

Paramecia were transferred into a chamber which was weakly illuminated (10 lux) with a tungsten lamp for more than an hour, to adapt them to the dark. A flash of light from a xenon stroboscopic photography lamp (Kako S-2) was employed for

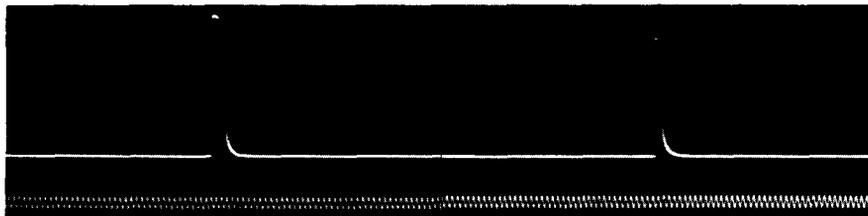


Fig. 1. Cathode ray oscilloscopic records showing the time course of the flash of light from the xenon stroboscopic photography lamp (Kako S-2), obtained with a phototransistor. Time mark: 1000 c.p.s.

stimulation (Fig. 1). The light intensity was 3.2×10^5 lux, and the duration was 4 msec.

When the dark adapted animals were stimulated by the flashed light, there was a short latent period followed by a change in the direction of their movement resulting from a sudden change in direction of the ciliary beating. Hereafter this sudden change in the direction of the ciliary beating will be described as the 'response'. The swimming velocity of the animals and their excitability to light

was increased within 30 sec following the beginning of the response. When the illumination in the chamber was kept within 10 lux following stimulation, the swimming velocity and excitability to light again decreased.

A detailed examination was made to discover whether the response is evoked at the time when the stimulating light is on or when it is off. Light from the tungsten lamp was used as the stimulating light source (10^5 lux). The duration of the stimulating light was changed by means of a camera-shutter (Canon). Closer analysis of the response to the light was made on 8 mm microcinematographic records as is shown in Fig. 2. When the duration of the light

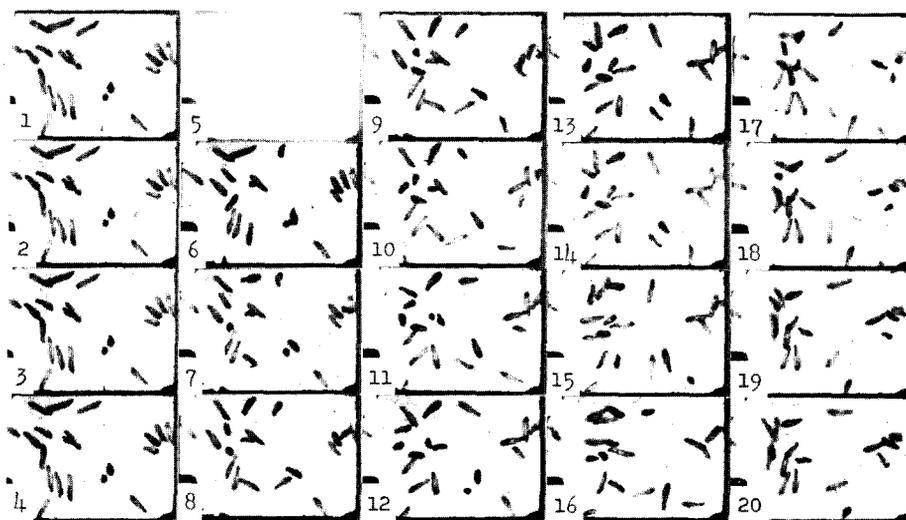


Fig. 2. Microcinematographs recorded with an 8 mm cinecamera. All the sequences were taken at 16 frames per second, but consecutive photographs were selected at an interval of 5 frames from original film and edited. 1-4: before stimulation. 5: during stimulation. 5-20: after stimulation. Note orientations of the animals.

exceeded one second or more, ciliary beating was augmented, but reversal of the ciliary beating never occurred while the light was on. When the stimulating light was turned off, however, there was a short latent period followed by reversal of the ciliary beating from which it was concluded that this response was evoked by turning the light off.

3) Latent period

To measure the latent period of the response, the light response in the dark adapted animal was recorded with a motor-driven 8 mm cine-camera (16 frames per sec). In the records obtained the observation error of the latent period was within about 30 msec. Minimal background illumination was used for the

cinematographic record prior to light stimulation. The intensity of the background illumination was kept to either 80 or 120 lux. The latent period of the response with each background illumination used was estimated from 50 measurements.

When the intensity of the background illumination was kept at 80 lux, the average time of latent period was 180 ± 30 msec. The average time of the latent period was 250 ± 30 msec, when the background illumination used was 120 lux. In *Paramecium*, the latent period of light response may be from 200 to 300 msec, since it varied with changes in the intensity of the background illumination when the intensity of the stimulating light was kept constant.

4) Strength-duration curve

A series of experiments was carried out to analyze the relation between the

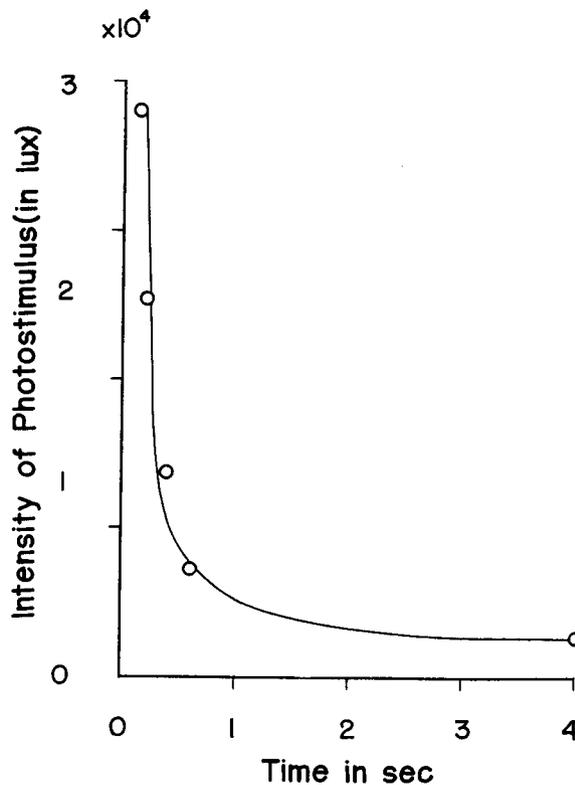


Fig. 3. Strength-duration curve for establishment of the ciliary reversal. When the number of animals responding reached 80 % or more the data were plotted as "positive". Ordinate: intensity of the stimulating light in 10^4 lux. Abscissa: duration of the stimulating light in sec.

intensity and duration of the stimulating light. Light from the tungsten lamp was used. More than 80 % of the tested animals responded and the plot of the data obtained is shown in Fig. 3.

The results show an exponential curve which is similar to the relationship between the duration and intensity of electrical stimulation applied to the cell with a microelectrode (Okumura and Yamaguchi, 1960).

5) Effects of changes in the intensity of both the stimulating and the background illumination

The effects of both the stimulating and the background illumination on the response were analyzed. The results shown in Fig. 4 were obtained by

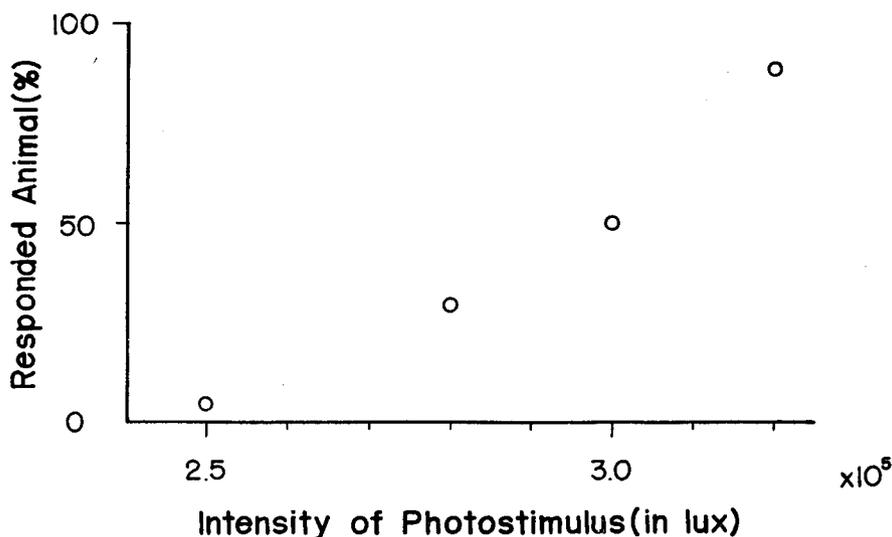


Fig. 4. Percentage of the animal response evoked by various intensities of stimulating light. Background illumination was kept constant at 10 lux. Ordinate: % of animals responding. Abscissa: intensity of the stimulating light in 10^5 lux.

application of the stimulating light at various intensities with a constant duration (4 msec), while the background illumination was fixed at 10 lux. More than 90 % of the stimulated animals responded to strong light (3.2×10^5 lux) but less than 10 % of the animals responded to weak light (2.5×10^5 lux). The relationship between the stimulus intensity and the number of animals responding was linear when the intensity of the background illumination was fixed at 10 lux.

The results shown in figure 4 were obtained by varying the intensity of the background illumination while keeping the stimulus intensity at 3.5×10^5 lux. The percentage of the animals responding varied exponentially with changes in the intensity of the background illumination. Excitability to light declined when the

background illumination was increased and the stimulating light was constant at 3.5×10^5 lux.

The wave length of the stimulating light used in these experiments varied from 2500 to 15000 Å and therefore it was important to examine the effects of the ultraviolet and infrared rays in this light. *Paramecia* were stimulated by infrared rays isolated by an infrared filter (Toshiba IR-DIB). None of the animals responded to the infrared rays. When the animals were stimulated by ultraviolet rays isolated with a Conning 7-54 filter, the number of animals responding reached 50% when the background illumination was kept at 10 lux (Fig. 5-c). The results obtained with ordinary light from which the ultraviolet rays were filtered with a UV-filter (Fig. 5-b) agreed with the results obtained from stimulation with light containing wave lengths from 2500 to 15000 Å (Fig. 5-a). According to these

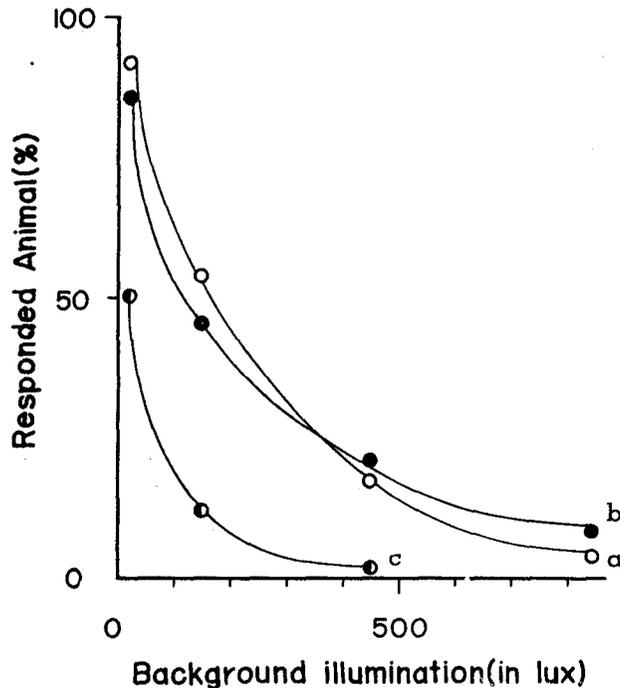


Fig. 5. Effect of background illumination on the response evoked by a stimulating light of 3.5×10^5 lux. Ordinate: % of animals responding. Abscissa: intensity of the background illumination in lux.

results, the response in *Paramecium* depended upon the difference between the relative intensities of the stimulating and the background illumination. The

number of animals responding to ordinary light may reach 100 % when the stimulus intensity is stronger than 3.5×10^5 lux.

6) Spectrum sensitivity curve of *Paramecium*

The spectrum sensitivity curve for *Paramecium* was determined. Monochromatic light were obtained by a monochromator (Olympus: useful wave length, 2400 to 20000 Å) and a xenon lamp (1 KW). Intensities of monochromatic light were measured against standard intensity with a thermopile-galvanometer system, in order to use equal intensity in each wave length. The standard intensity of the monochromatic light was defined to be that intensity having radiation energy equivalent to 5000 lux at 5500 Å. In the present experiment, the duration of the stimulating light was 1 sec with a background illumination of 10 lux.

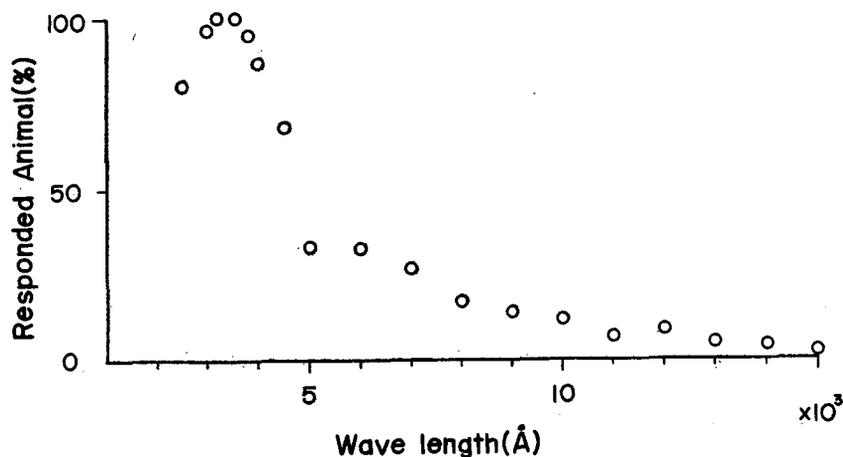


Fig. 6. Spectrum sensitivity curve of *Paramecium*. Ordinate: % of animals responding. Abscissa: wave length in Å. The radiation energy of monochromatic light was kept constant in each wave length.

Figure 6 shows the spectrum sensitivity curve of *Paramecium* stimulated by monochromatic light. At the wave lengths of infrared light (from 7700 Å), 20 % of the stimulated animals responded. A stimulating light of 15000 Å elicited no response in the animals. When the *Paramecia* were stimulated by light between 5000 and 7000 Å, about 30 % of the stimulated animals responded. Stimulation between 4000 and 4500 Å was more effective eliciting the response than that of 4500 Å or longer. At 4500 Å 77 % of the animals responded and at 4000 Å 86 % responded. *Paramecium* shows the most excitability to light at the wave lengths of ultraviolet rays. Between 3000 and 3300 Å, the number of animals responding reached 100 %. However, the percentage of the animals responding began to

decrease when the wave length of the stimulating light was less than 3000 Å, i.e. 85 % of the animals responded at 2500 Å.

Augmentation of ciliary activity was produced by light with a wave length of 4000 Å or longer, but the response was elicited at the time when the light was turned off. If the wave length of the stimulating light was shorter than 4000 Å, the response was elicited when the light was on. It is known that organisms absorb short wave length light more effectively than long wave length light. According to these results, it was concluded that the response was produced at the time the stimulating light was on when a large amount of radiation energy was absorbed from the light and that the response was evoked by turning off the light when the absorbed radiation energy was small.

7) Effects of ultraviolet rays or infrared rays on the response

It was confirmed that *Paramecium* stimulated by ultraviolet rays responded to the light when it was on, while animals stimulated by infrared rays showed no response. In order to analyze these results, the responses of *Paramecium* which was stimulated by weak ultraviolet rays or strong infrared rays were observed. For ultraviolet ray stimulation, light from a xenon lamp (1 KW) was passed through the monochromator (2400 to 20000 Å), and a infrared ray lamp (Toshiba, R-127) and a light filter for infrared rays (Toshiba IR-DIB) were employed to obtain the infrared rays (7700 to 20000 Å).

When *Paramecium* was stimulated by the strong ultraviolet rays, the "on-response" was elicited. However, the weak ultraviolet rays elicited the "off-response". The application of infrared rays to the animals produced an augmentation of ciliary activity, but strong intensities of infrared rays evoked the "off-response".

Discussion

In protozoa, it has been well known that species which are in symbiosis with algae show positive phototaxis, but the phototaxis in species without algae has never been observed (Schulze, 1951). Sgonina (1939), on the other hand, reported that *Paramecium caudatum* did not show phototaxis, if only the light was applied. He also reported that *Paramecium* stimulated by light synchronized with an electric current become sensitive to the light, and showed phototaxis. Soest (1937), however, mentioned that *Paramecium caudatum* had never responded to the light under conditions resembling those in Sgonina's experiments. In the present experiment, the response to light in *Paramecium* was examined in detail, and no phototaxis was observed.

The ciliary beating of *Paramecium* declined with dark adaptation, but were augmented by exposure to weak light, and the swimming velocity of the animal increased. Heilbrunn (1951) also reported that the protoplasmic streaming of *Amoeba* was augmented by weak illumination. According to these results, it was concluded that weak illumination augmented the cell activities.

Alsop (1939) reported that the protoplasmic streaming of *Amoebae* ceased when they were strongly illuminated. Heilbrunn (1951) reported that the protoplasmic streaming of *Amoeba* or plant cells was effectively stopped by the ultraviolet rays. Contraction of the cell body in *Stentor* was also evoked by a strong flash of light (Okumura, unpublished data). In *Paramecium*, it was observed that a sudden change in the direction of ciliary beating was evoked by the flash of light. When ordinary light was used for stimulation, the "off-response" was evoked. The "off-response" was also evoked by weak ultraviolet rays, but the "on-response" began to be evoked by certain intensities of ultraviolet rays. In general, it has been well known that the light-absorption curves of proteins reach a peak in ultraviolet rays, and that the molecular structures of the protein was changed by absorption of the radiation energy of the light, especially of ultraviolet rays (Giese, 1945). According to the spectrum sensitivity curve of *Paramecium*, it had been considered that *Paramecium* structures effectively absorbed the applied ultraviolet rays. On the other hand, the excitation of the cell was due to a change in the permeability of the cell membrane when stimulated by various agents. The protein denaturation caused by irradiation of the ultraviolet rays is accompanied with some change in the protein structure (Sanders and Giese, 1959). If the radiation energy of the applied light is absorbed in the cell membrane and the molecular structure of the membrane is changed, the ionic permeability of the cell membrane may be changed to evoke the excitation. Therefore, the response evoked by the stimulating light in *Paramecium* is synchronized with the change in the permeability of the cell membrane that absorbed the radiation energy. The "off-response" was evoked by ordinary light or weak ultraviolet rays. The cell membrane can not absorb enough radiation energy from these lights, and therefore the change in the molecular structure in the cell membrane is small. The larger the radiation energy absorbed, the greater the change in the molecular structure in the cell membrane. The "on-response" was evoked by strong ultraviolet rays absorbed into the cell membrane. According to these results, the "on-response" is evoked by greater absorption of radiation energy, and the "off-response" is evoked by lesser absorption of radiation energy.

Summary

- 1) The responses to light in *Paramecium caudatum* were studied.
- 2) Phototaxis was not observed, when only light was applied to the animals.
- 3) When *Paramecium* was adapted to the dark, the swimming velocity of the animal slowed as a consequence of declining ciliary activity. When weak light was applied to the dark adapted animal, ciliary activity was augmented, and swimming velocity was recovered.
- 4) When the dark adapted animals were stimulated by a flash of light, a sudden change of direction occurred in the ciliary beating. The photo-response was evoked when the illumination of ordinary light was turned off. Excitability to

light in this case was affected by the relative difference in the intensities of the stimulating light and the background illumination.

5) Maximum values in the spectral sensitivity curve were between 3300 and 3000 Å.

6) It was concluded that the response to light in *Paramecium* was evoked by a change in membrane permeability due to the change in the molecular structure of the cell membrane which absorbed the radiation energy of the stimulating light. When the absorbed radiation energy is great, the "on"-response is evoked, and the "off"-response was evoked when the amount of absorbed radiation energy was small.

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