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ON THE PHYSIOLOGY OF LAND PLANARIANS

I. Phototaxis, with a Note on the Significance of the Eye Spots

(With 5 Text-Figures)

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CONTENTS

1. INTRODUCTION

There are only a few works on the physiology of land planarians including the work of COLE (1907) on the phototaxis of *Bipalium kewense*

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Moseley. This may be caused by the difficulties in obtaining material in mild climates but at Taihoku it is not difficult to obtain sufficient material for physiological researches. The results of COLE's experiments, that *B. kewense* reacts negatively to the light but is not sensitive, may probably come from its nature of crawling about in the twilight but *Bipalium sp.*, being strictly nocturnal is very sensitive to the light and its phototactic reaction differs from that of *B. kewense.*

2. MATERIAL AND METHOD

The material used was *Bipalium sp.*[1] collected from the University grounds. The body length of the material selected was within 8 cm. and 10 cm. when alive. Each specimen was kept in a non-alkaline glass vessel with a piece of wet filter paper. It was used within one day after collection and was kept in the dark room for at least three hours before the experiment. Although the experiments were done with freshly collected animals, it was also found that the specimens could be reared under laboratory conditions by keeping them in a large glass vessel with some grasses or wet filter paper to give a certain humidity, and by feeding them with one living *Limax* every day.

The apparatus used for observing the orientation of the animal in a horizontal light beam was essentially like that used by TALIAFERRO (1920). Two sets of horizontal beams of light were produced, the angle between them and the distances from the platform of the experiment were changed. Each beam of light was produced by a 250 watt gas-filled Matzuda lamp with concentrated filament, which was covered with a light-tight box and properly screened. A light filter of 2 cm. thickness, to avoid the thermal effect of light, was placed in front of the slit of the box, the source of light. The filter was filled with distilled water or different color solutions in the case of the experiments on color-reactions. A simple shutter was prepared between the screen and the source of light when the relations between the reaction time and

(1) Specimens of this species were sent recently to Prof. T. KABURAKI, of Tokyo Imperial University, and it is expected, the exact name will be determined by him before long.

the intensity of illumination were studied, so as to eliminate the unequal intensity of illumination when the current is turned on. A sheet of wet filter paper (30×30 cm^2.) was used as a creeping surface for the animals. It was wet with distilled water[2] and stretched on a glass plate on the horizontal platform. After each experiment the trail of the animal was recorded by following the mucus line left by the animal, and, on the other hand, searching movements in the air or on a horizontal plane were recorded in another note. The angle of orientation was measured by a graduator to half a degree. The experiments were performed in a dark room during a period from January to March, 1931. The room temperature was within 17–20°C.

3. PRELIMINARY EXPERIMENTS

There are two modes of locomotion in this species, one is ciliary gliding, the other is looping with muscular contractions. The former is the normal locomotion and the latter occurs when the animal is forced to move vigorously. In the case of ciliary gliding on a horizontal plane, the animal proceeds forward in a wavy path, holding the anterior end a little above, creeping with the sole, which exists only in the median ventral line and is about 1 mm. in breadth, while any other part of the ventral surface does not contact the plane.

a) Effect of starvation: The material which has been kept in a starved condition for three days reacts irregularly to light. Even if the animal can react normally in these conditions it fatigues earlier than is normal. On the contrary if the animal is fed, it reacts in an ordinary manner even after ten days in captivity.

b) Effect of fatigue: When the experiments are done successively on the same animal its reaction often becomes very irregular. For this reason one animal is not used more than two times in succession.

c) The above-mentioned effect of fatigue may come from two different causes, one is the fatigue from muscular movement or that of the motor system, and the other is the fatigue of the photosensory reaction.

(2) The reason why distilled water was used is to avoid any unnoticed effect of tap water, as chemicals effect phototaxis in several animals. (ROSE, 1929).

An experiment was done to ascertain this difference. One animal was exposed to about twenty minutes of continuous movement in the dark caused by several mechanical stimulations and the other one was put under a continuous exposure to a light stimulation at a distance of 150 cm. from the source of light for an equal length of time. These two animals were subjected to a light stimulation, the former reacted with apparently negative phototaxis, as is normal, but the latter did not react. These results seem to me to be caused by the existence of a constant amount of photosensory substance, as is proposed by several authors and is generally accepted. The reactions are supposed to be induced by the decomposition of this substance and this decomposed substance is re-synthesized in the dark.

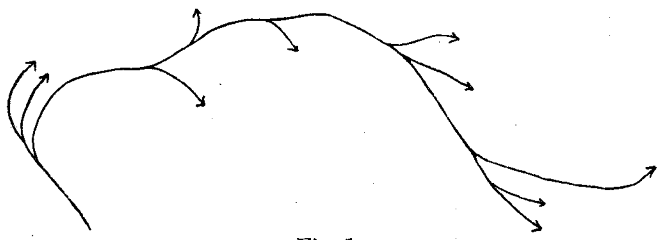

Fig. 1.
An example of the movement of a land planarian under a dark red light. Reproduced from the trace of the animal on filter paper, about 1/5.

d) There are some unbalanced specimens that easily react to one particular side, right or left. For example, one animal, which has been subjected previously to one light, reacts to a second light, which is perpendicular to the first, as follows: mean turning angle when the light is given from the right hand is 23° and mean turning angle when the light is given from the left hand is 50°. All data from such animals were discarded.

e) Movements under a dark red light: Movements were observed under a 10 watt red light at a distance of 80 cm. and there were no fixed type of movements but straight and curved movements occurred. The typical circular movement was observed in only one case out of more than ten animals. The amplitude of the wavy movements increased in the dark, and the searching movement in the air occurred frequently when the head was held higher in the air, even one-third of the total body length being lifted. Searching movements in the horizontal plane

also appeared more frequently. One example of these movements is shown in Fig. 1.

f) Thigmotaxis of the animal was examined under the dark red light, by using some glass rods as a contact object but it was proved that there is no thigmotaxis in the land planarians, as far as the lateral surface of body is concerned.

g) According to Cole (1907) the geotaxis of *Bipalium kewense* is so significant that by lifting very slightly the plane where the animal is creeping, it could be turned easily in any direction. In my experiment, however, the animal does not show such significant geotaxis.

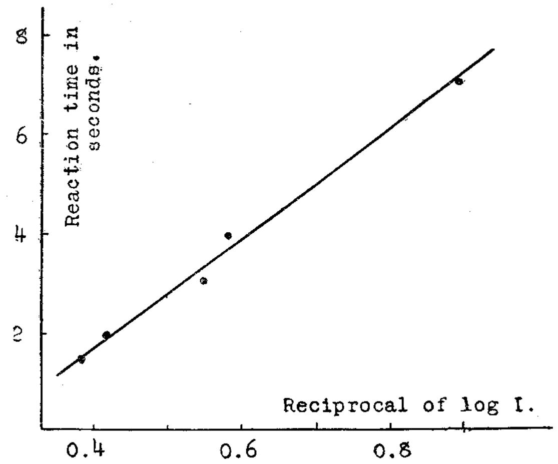

Fig. 2.
Relation between the reaction time and the reciprocal of the logarithm of the intensity. Each point represents the mean value of five animals; three readings for each animal for every intensity; room temperature 16°C.

4. REACTIONS TO SINGLE LIGHT SOURCE

a) Horizontal light beam

The animal reacts negatively even to the light of a one meter candle. The reaction time was measured in several intensities of illumination. There is a linear relationship between the reaction time and the reciprocal of the logarithm of the light intensity as shown in figure 2. This proves the fact that the photochemical effect (E) to produce the reaction in the animal is proportional to the product of the reaction time (t) and the logarithm of the intensity of the illumination (I), that is, $E=k. t. \log I$, where k is a constant, as studied by Hecht (1921). However, an attempt was made to analyse the effect of light into wave lengths and the amount of energy but conclusive results were not obtained, owing to the difficulties of fine determinations of the reaction time.

Generally, when the illumination is strong the reaction is clear and rapid, when the angle between the direction of the incident light beam and the direction of the animal is smaller, trial movements are correspondingly less frequent (Fig. 3). When that angle is maximum (180°) the animal is faced in the direction of the light beam, it often turns the head to and fro for several times, as shown in figure 3, c. In the case when the animal is placed perpendicular to the incident light beam and the shade of body is greatest, the largest wavy movement is seen, as shown in figure 3, d. With progression of forward movement, the amplitude of the wavy movement is gradually decreased and finally comes to the same state as in the case when it is put in a position parallel to the light beam, facing or away from the light (Fig. 3, a, b and c). There are no searching movements in the air when the light is sufficiently strong. When the animal has completely reacted to the light, the amplitude of the wavy movement is smaller according to the strength of the light. This is probably due to the fact that the sensory organ plays its function when it receives a definite amount of illumination.

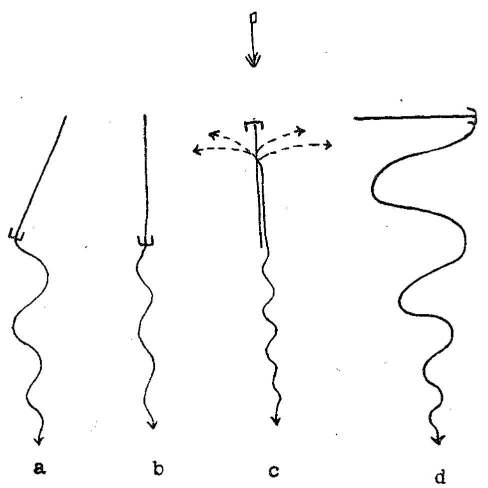

Fig. 3.

Diagram to show the reactions of land planarians in four different initial positions to one light beam. The direction of the light beam is shown by an arrow. The broken lines show searching movements. Further explanations in the text.

b) Reactions of animals to an inclined beam of light are essentially similar to those of the horizontal light but in the same intensity the amplitude of the wavy movements is smaller than in the latter case

and the searching movements in the air are not to be seen even in a fairly feeble light.

5. REACTION TO TWO HORIZONTAL BEAMS OF LIGHT

a) The case of equal intensity of illumination

When an animal is placed in a field of two light beams of equal intensity, it reacts negatively phototactic, and gets away toward the bisector of the angle between the two light beams. The intensity of the illumination was 200 meter candles. The experiments were made with beams of light which intersect each other by angles of 60°, 90°, and 120° respectively. Results are given in the Table I. Each datum is the mean value, with its probable error of total trials with from seven to ten animals, each of which was used twice.

TABLE I

Angle between two light beams	Orientation angle, measured from one light beam
60°	30.0° ± 0.4°
90°	44.9° ± 0.6°
120°	59.5° ± 0.5°

Whether the animals were subjected to the two lignt beams simultaneously or the second beam was added after they had reacted to the first, the actual data of orientation angles were the same.

b) The case of different intensities of illumination

Light A was 200 meter candles and was kept constant. Light B was diminished into one-half or one-fourth of light A by increasing the distances from the animal, that is, 100 or 50 meter candles respectively. The angle between A and B was kept constantly at 90°. The experiment was worked out with seven specimens in each case. The data are given in Table 2. The data are the mean values of 14 experiments and their probable errors of mean, each specimen being used twice.

TABLE II

Intensity of light		Ratio of intensity of light, A/B	Orientation angle, measured from axis of B
A	B		
200 m.c.	200 m.c.	1	$44.9° \pm 0.6°$
200 m.c.	200 m.c.	1/2	$52.7° \pm 0.3°$
200 m.c.	50 m.c.	1/4	$58.1° \pm 0.9°$

Fluctuation of individual datum is fairly large.

The experiment of COLE (1907) indicates that *Bipalium kewense* showed a slightly larger number of turnings away from the stronger light when the animal was exposed to two different illuminations from opposite directions. That is, they have only a slight ability to discriminate between light intensities. But in this case the animal is very sensitive to the light intensity and numerical treatment is possible.

NORTHROP and LOEB (1923) proposed a formula in the study of the reaction of *Limulus* to two light beams,

$$\tan\alpha = I_1/I_2,$$

where α is the orientation angle of the animal when it is subjected to two illuminations I_1 and I_2 at right angles. The result of my experiments do not support this theory, and corresponds more adequately to the formula which has been given by MOORE (1924) for the heliotropic reactions of *Cerianthus*. The formula is

$$k.\ \tan\alpha = \log(I_1/I_2),$$

where α is the orientation angle, I_1 and I_2 are intensities of illumination and k is constant. If this formula is right in our case, the values of k, calculated by inserting our observed data, must be fairly constant and it proved that values of k were 2.2 and 2.5 in this case.

The values of the "head angle" (H) of CROZIER's formula (1927),

$$\tan\frac{H}{2} = \frac{I_1 - I_2\tan\theta}{I_1\tan\theta - I_2}$$

where θ is an angle of orientation, were calculated and they were 132.3° and 133.3°. From these data it may be said that this formula is converted to the formula of MOORE, in this case, as shown by CROZIER.

Thus it is concluded that the tangent of the orientation angle of the animal, when exposed to the illuminations intersecting at a right angle, is proportional to the logarithm of the ratio of the intensities of the illuminations.

6. NOTE ON THE SIGNIFICANCE OF THE EYE SPOTS

COLE (1907) studied phototaxis and the function of the eye spots of *Bipalium kewense*, and considered that an individual eye spot is too

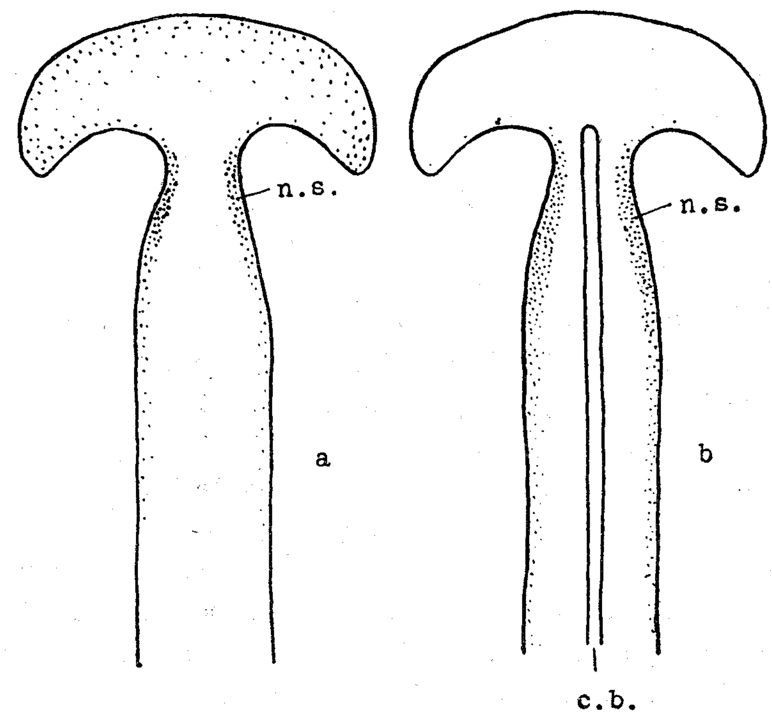

Fig. 4.

Diagrams showing the distribution of the eye spots in *Bipalium sp.*. Dorsal view (a) and ventral view (b) of about one-third of the anterior part of the body. A small dot shows an individual eye spot; n. s., the so-called neck spots; c. b.: a creeping ciliary band.

small to form an image or even to detect the direction of light, but as a whole system they can react to light from all directions. I took this problem, therefore, to ascertain more about the eye spots and their correlation with the phototaxis of *Bipalium sp.*, which has many eye spots on definite portions of the body wall.

Concerning the distribution of the eye spots, v. GRAFF summarized the results in BRONN's "Tier-Reiches". In *Bipalium sp.*, the eye spots are assembled on the dorsal side of the head portion and the side wall of the neck and the succeeding anterior part of the body. The distribution of the eye spots is as shown in the Fig. 4, a and b. They are distributed most densely at the neck from the dorsal portion at the base of the head to its ventro-posterior part on each side of the body surface. Along the margin of the dorsal side of the head there are fairly numerous eye spots, especially on both lateral margins. Behind the neck spots there are only a few on the dorsal side, as shown in Fig. 4, a, while on the ventral side the eye spots are not found on the head, but a considerable number are on the neck portion farther back often extending two-thirds of the body length, although their numbers diminish gradually as they approach the caudal end. It is curious that the eye spots are distributed on the ventral parts of the side wall, where the light is less than on the dosal side.

In order to determine the function of the eye spots, we performed two sets of experiments, namely, the reactions of pieces of the body and the reactions of whole animal under the illumination which is given to several different parts of the body. When only the head is illuminated by a horizontal light beam it reacts very rapidly, which is probably due to the distribution of the eye spots and the modes of locomotion already described. When the neck portion is exposed to a horizontal light it reacts as rapidly as though the entire animal was exposed to the light. Illumination of the ventro-posterior part of neck has a slight effect on the animal, and illumination on any other part without eye spots shows no effect in the behaviour of the animal. An animal, when the right or the left projection of the head is cut off (Fig. 5, a or b), reacts normally to a horizontal light, but occasionally turns towards the intact projection, exposing the wounded surface to the light. When both projections are cut, the animal curves up the wounded surface and seems

to react a little slower than is normal but after more than 15 minutes the animal can react almost normally. When the entire head is cut off (at c Fig. 5) it reacts a little more slowly than in the case of the head being intact, but no irregularity is found. In this case the rapidity of the reaction does not return for several minutes, as in the former case, and in such an animal without a head the speed of locomotion is very slow and the amplitude of the wavy movements diminishes extremely. A piece of the body which is decapitated and decaudated (at c and d of Fig. 5), reacts essentially simillar to the decapitated animal, but differs from the latter in its activity and in the stability of the pieces. According to the stability of the piece, such a piece creeps at first by holding up the cut surfaces and the side walls of the body but it frequently inclines to the right or left and consequently the side wall comes into contact with the creeping surface, and it often lies on that side. The other middle piece, cut from lines d and e of Fig. 5, and the caudal piece react no more to the light and this corresponds to the fact that eye spots are wanting in these regions.

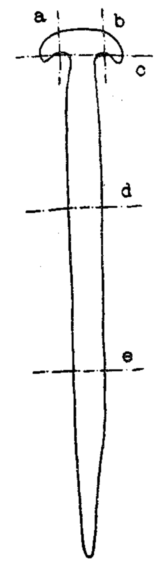

Fig. 5.
Diagram showing the position of cutting in the experiments.

From these results it is concluded that the number of the eye spots is an important factor, and the densest portion, as the neck region, is the most important portion in sensitivity in this animal. Both sides of the neck with eye spots are the main center of the photosensory system and the band of eye spots on the margin of the head, the hinder neck portion follows them in importance.

Now, it may be considered that the eye spots constitute a system of photosensation in *Bipalium sp.* and it consists of three districts, one on the dorsal side of the head and the other two on both sides of the body, centering on the neck portions. Thus we shall understand the phenomena more clearly.

7. UNSTABLE POSITIVE PHOTOTAXIS

In studying the reaction of the animal to horizontal light beams from two different sources we encountered a curious phenomenon in which the animal reacts positively to the light. When the animal is placed nearly on the bisector of the angle of the axes of two beams of light, facing their sources, it often proceeds forward with a large, wavy motion and several searching movements in a plane. Such reactions will occur frequently when the animal is starved or fatigued.

The phenomenon may be explained as follows; if an animal is placed in a certain place where the intensity of the illumination is stronger on the right hand than on the other, then it may turn to the left according to the theory of tropism. But if the illumination on the left side becomes stronger than on the right when it turned to the left, it must be driven to the right again by the photosensitive effect of the left side, thus the animal is forced to move on toward the lights, positively phototactic on the bisector and this is an unstable equilibrium of phototaxis. These explanations are supported by two facts; primarily, the percentage of occurance of this phenomenon increases with an increase of the angle between the axes of the two light beams, for instance, the percentages of occurrence are 0%, 5%, and 48% in the cases of angles of 60°, 90°, and 120° respectively; secondarily, it is acertained that the head angle of CROZIER's formula was about 130° in *Bipalium* and this is the limit of the angle in which the animal can orient itself freely to the light, therefore if the angle between the axes of two light beams is larger than this angle the animal cannot definitely orient itself.

8. SUMMARY

1. Modes of reaction of land planarians to several circumstances of illumination are described.

2. Some attempts have been made to analyse the nature of the negative phototaxis.

3. *Bipalium sp.* is very sensitive to the light and follows the law which has been deduced from other photosensitive animals.

4. Distribution of the eye spots in *Bipalium sp.* is described.

5. Some attempts have been made to analyse the meaning of this distribution and it was seen that the neck portion is the most sensitive and the main center of phototaxis.

6. A curious phenomenon of positive phototaxis is explained.

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