THE BEHAVIOUR OF *DENDROCOELUM LACTEUM*

II. RESPONSES IN NON-DIRECTIONAL GRADIENTS

Bẏ PHILIP ULLYOTT

(From the Laboratory of the Freshwater Biological Association, Windermere, and the Zoological Laboratory, Cambridge)

(With Nine Text-figures)

INTRODUCTION

PHOBOTAXIS may be defined as the response of an animal by which it reaches, along a non-directional path, some region of particular stimulative intensity. In a previous paper it has been shown that earlier methods which were supposed to demonstrate phobotaxis did not eliminate the possibility of orientated movement (Ullyott, 1936). It was shown in *Dendrocoelum lacteum* that there was a very low threshold value of lateral light to which the animal could orientate itself, and that in most experiments designed to show non-orientated responses to light insufficient care had been taken to eliminate directional light factors in the environment to which the animals were subjected. As a consequence of this the animals were capable of responding in an orientated fashion, and the effects obtained could not, therefore, be ascribed to phobotaxis.

The essential condition which has to be fulfilled before an animal can orientate itself to some external stimulus is that sense organs situated in different parts should be differentially affected by the stimulus. In stimulation by light it is impossible not to excite photoreceptors differentially if there is any directional component in the light which is of supraliminal intensity. Further, it is very difficult to arrange the experimental conditions so that there is no differential component in the light incident on an animal, because reflected light of low intensity from surrounding objects can often exceed the threshold for stimulation.

In spite of the difficulty of obtaining experimental conditions in which there is no directional light component, the ease of control of light intensities, and the possibility of instantaneous application and removal of the stimulus in photic excitation, made it seem desirable to reinvestigate the problem of phobotaxis using light as the stimulus.

METHODS

Some of the apparatus used has already been described (Ullyott, 1936), and for the first series of experiments described in this paper was satisfactory. It was necessary in addition to subject the animals to a field in which there was no directional component, but one in which there was a uniformly graded series of light intensities.

It was also essential that in this "non-directional gradient" of light intensities there should be no "step" effect of a series of patches of increasing intensity, but that the transition from the highest to the lowest intensity should be gradual and uninterrupted.

Two pieces of apparatus were used in separate dark rooms in the cellars of the Laboratory of the Freshwater Biological Association at Wray Castle, Windermere. The general arrangement of these rooms was the same as that of the dark room which had been used for the earlier experiments, so only the special apparatus for obtaining the non-directional gradients need be described. Care was taken to avoid lateral reflections that could act as directional stimuli. One apparatus is represented
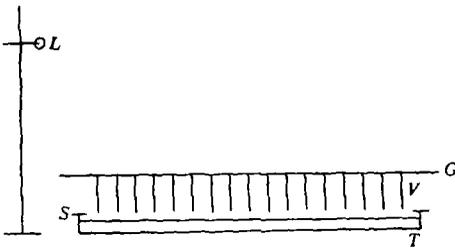

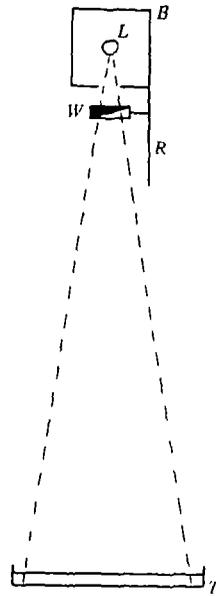Fig. 1. Trough for obtaining non-directional gradients. (For description see text.)

Fig. 2. Second type of trough for obtaining non-directional gradients. (For description see text.)

in Fig. 1. T is an experimental trough 35 cm. wide and 110 cm. long, with the bottom made from a sheet of plate glass. Above this trough a sheet of opal glass, G, equal in size to it, was held in a wooden framework. From the glass were suspended a number of vertical vanes V. These were made from the thinnest sheet tin, painted dead black, and they were arranged hanging parallel to each other at a distance of 3 cm. apart, and parallel to the ends of the trough. At the middle of one end of the trough was a vertical standard with a sliding clamp supporting an electric lamp. By varying the distance of this lamp from the surface of the opal glass, light gradients of different steepness could be obtained. A horizontal strip of black cardboard S was placed along the sides and edges of the trough, so that the light from above could not shine on the vertical surfaces and produce reflections. This arrangement gave the desired experimental conditions. The vanes prevented the animal from

receiving stimuli from any part of the light gradient, except the one immediately above it. At any particular point in the gradient the animal could not receive information about the direction in which greater intensities were to be found, or in which direction less.

The second apparatus is shown in Fig. 2. The animal was kept in the trough *T*, above which was suspended a light-tight box *B*. Inside this box was a compact filament electric lamp *L*, which was allowed to shine through an optical wedge *W*, on to the animal in the trough. The usual precautions were taken to prevent the possibility of light being reflected from the sides of the trough. The optical wedge was a photographic plate transparent along one edge, but completely opaque along the opposite one, with an even gradation of tones between the two extremes. It was supported on the rod *R*, and by placing it nearer or farther from the lamp, the steepness of the gradient in the trough could be altered. This apparatus gave a rather more satisfactory gradient than the first one, and with it the results obtained with the earlier one were confirmed and enlarged. In all experiments discussed in this paper, one animal only was allowed to be in the experimental trough at any particular time.

EXPERIMENTAL DATA

(a) *Experiments using uniform fields of non-directional stimulus*

Experiments were done to analyse the effects of stimulation by non-directional light shining from above the animal, when all other sources of stimulation were excluded. In each experiment the observer entered the dark room through the double doors, and an electrical timing device already described was started before the light for stimulating the animal was switched on. The turning on of the light was made to coincide with one of the half-minute signals. The animal was put into the trough some 3 hours before the beginning of an experiment, and quite often it was found when the light was switched on that it was crawling along one of the edges of the dish, or along the angle between the glass plate which nearly covered the bottom of the trough, and the bottom of the trough itself. In these cases the experiment had to be abandoned for another 3 hours, and another attempt made when dark adaptation was complete again.

The only valid experiments were those in which the animal was found to be moving freely on the surface of the glass plate which covered the bottom of the trough. Under these circumstances the track of the animal was recorded from the first instant of illumination. It was anticipated that the dendrocoels might move at different speeds at different light intensities, for light frequently has an effect on the rate of movement of other animals. This was shown by the work of Garrey (1918) on the tonus effect of light in insects, and by that of v. Buddenbrock (1920) on *Helix*. Walter (1907) showed that *Planaria gonocephala* moved faster in the light, and Herms (1911) showed the same thing for blowfly larvae, and Muller (1925) for *Julus*. But in the present series of experiments no differences in the rate of movement could be detected over a range of light intensity from 0 to 2500 ergs/cm.^{2}/sec.

This range is very small, and it is quite certain that the intensity differences employed by the workers mentioned above were of quite a different order. They gave no exact figures, but daylight, which is very often of more than 500,000 ergs/cm.²/sec. illuminating intensity even in buildings, was used for the higher stimulating values. It is conceivable that the increases in the rate of movement which they described were at least partly due to temperature effects. The paths traversed by the worms were either copied on squared paper or a direct print of the mucous trail of the animal was obtained. These methods have been described in an earlier paper (Ullyott, 1936).
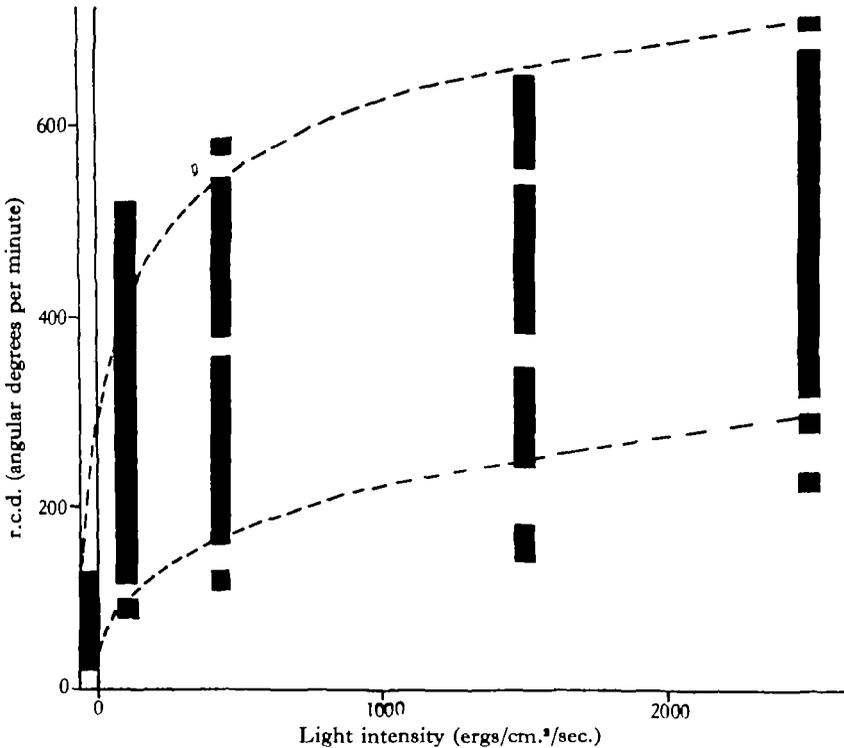

Fig. 3. The relationship between rate of change of direction and intensity of stimulation.

Although these records of the movement under different intensities of stimulation by non-directional light did not show any differences in the speed at which the animals were moving, it soon became clear that the light had a stimulative effect. Increase in intensity of the vertical light was responsible for an increase in the rate of change of direction in which they were moving. By *rate of change of direction* is meant the sum of all the deviations in the path of the animal during unit time. Both right-hand and left-hand deflections are counted as positive, and the result is expressed as angular degrees of deviation per minute. This rate of change of direction turned out to be most important from the point of view of phobotaxis, and since the expression occurs very frequently in the following pages it will be

represented by the letters r.c.d. The increase in r.c.d. with increase of intensity
of stimulation was reasonably constant for a particular intensity, so that figures
for the rate of change of direction are capable of furnishing quantitatively com-
parable results.

In order to have a standard for comparison, the r.c.d. was determined in absolute
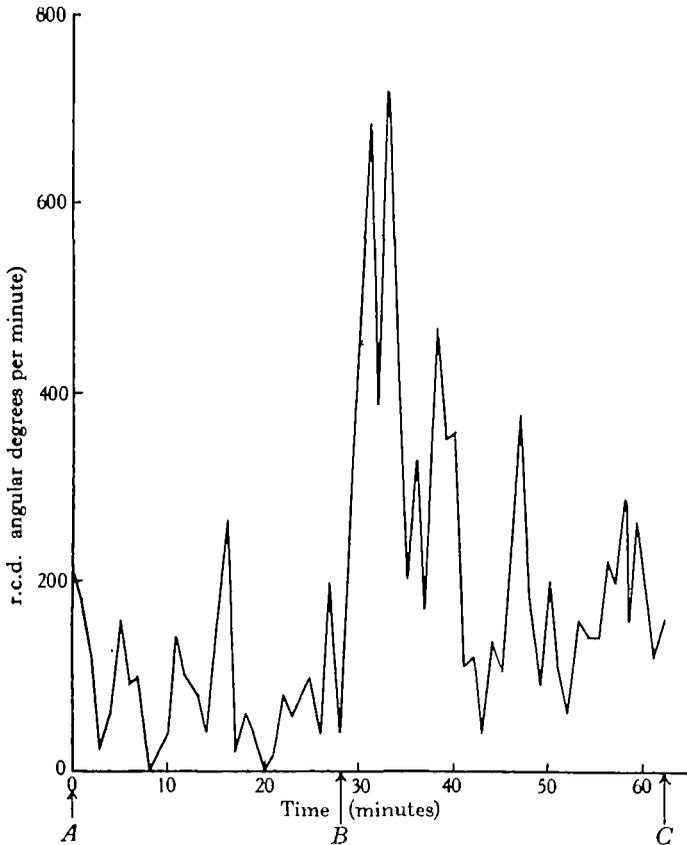darkness. This was done by the print method only. The time intervals were put

Fig. 4. The relationship between rate of change of direction and duration of stimulus. *AB*, r.c.d.
of the animal in darkness (basal r.c.d.). At *B* a light of intensity of 2500 regs./cm.²/sec. was switched
on. *BC*, adaptation to the stimulus. Individual record.

on to these tracings by marking in distances corresponding to the average distance
moved in unit time. This is not quite so accurate as the squared paper method,
which of course could not be used. From the records the r.c.d. was measured, and,
by taking an average of the results, a definite value was obtained. The r.c.d. for
total darkness will be called the basal r.c.d.

Fig. 3 shows the average r.c.d. for the first 5 min. of stimulation, in a series of
experiments in which different intensities of light were used. The curve shows that
with increasing light intensity there is a progressive increase in r.c.d. above the

basal value. In these, as in all the experiments described in this paper, the experimental animals were kept in the dark for 3 hours and the stimulus applied instantaneously by switching on the light.

In an animal subjected to constant stimulation, the r.c.d. falls off as the time for which the stimulus has been acting becomes greater. If successive values are plotted, the result is of the general form shown in Fig. 4. In the graph, the section AB represents the animal's movements in the dark, and is therefore a record of its
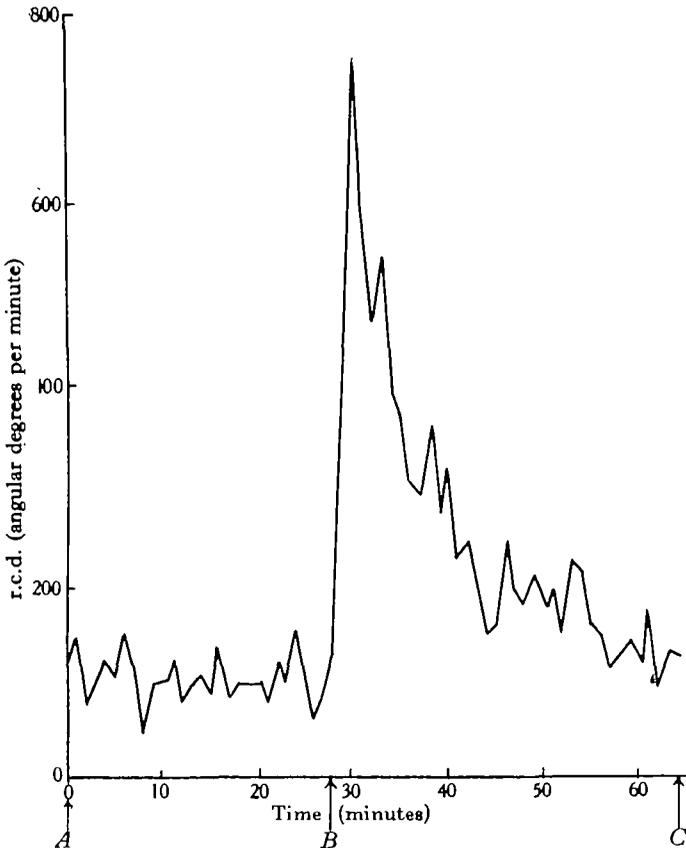
Fig. 5. As for Fig. 4, but each point on the curve represents the average of fourteen experiments.

basal r.c.d. At the point B the light was switched on, and immediately there was a very large increase of r.c.d. From B to C the values fell off irregularly as time went on, until a value again approaching the basal r.c.d. was reached after about 30 min. In a single set of readings with one animal it is possible to see that the falling off of r.c.d. with time is exponential, but the random variation from moment to moment is large compared with the rate of decrease. Fig. 5 is similar to Fig. 4, except that the points on it each represent the average of fourteen experiments. The form of the curve is much plainer.

This decrease of response under constant stimulation suggests that a process of adaptation is occurring. From the time relations it would seem that the adaptation must be of the same sort as that described by Hecht (1918, 1919) for *Mya*. Fig. 6 is a reproduction of part of a track corresponding to the section *BC* of Fig. 4: for the sake of clearness the part which corresponds to *AB* is not shown. Along the
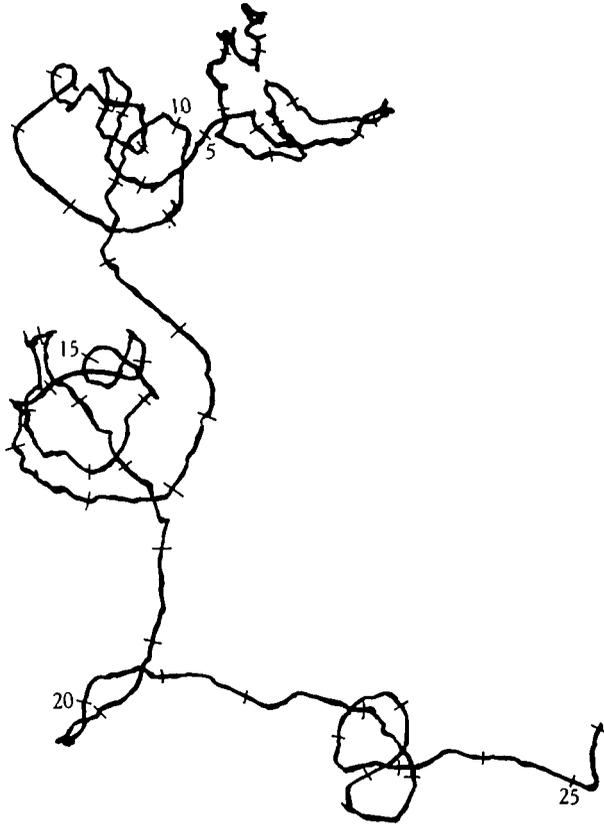
Fig. 6. Track corresponding to the part *BC* of Figs. 4 and 5. The transverse strokes represent half-minute time intervals, and the numbers the time elapsed (in minutes) since the beginning of stimulation. (Reduced to ⅓ of the original size.)

track time intervals have been marked, so that the total angular deviation per minute could be estimated. Fig. 7 is an enlarged part of Fig. 6 showing how the angular deviation is measured.

(b) Experiments with non-directional gradients

In each experiment in this series the animal was put into the trough 3 hours before the beginning of the experiment so that it should be completely dark adapted. The light was switched on at the end of that time and the track of the animal recorded. It was found that the behaviour of the animal depended on the steepness

of the light gradient. In gentle gradients, no matter where the animal might be initially, there was no evidence that it was possible for the animal to react in such a fashion that it was confined to some particular intensity zone. In fact, the animal wandered at random in a completely unorientated manner over the whole of the field presented to it.

However, if the gradient was steep the animals reacted phobotactically. If, when the light was switched on, the animal was at the dark end of the gradient, it never reached the light end, but remained confined to a relatively narrow zone at the dark end of the gradient. Yet the tracks of animals which were behaving in this way showed that there was no question of orientation, as there was in the case of Graber's Petri dish experiments (Ullyott, 1936), for the path was absolutely

Fig. 7. Part of Fig. 6 (original size) to show how the numerical value of the r.c.d. is obtained. The values in angular degrees for the successive half-minute intervals shown are 215, 185, 90, 150 and 160.

unorientated, and at many instants the animal was moving directly up the gradient towards regions of higher light intensity. If, when the light was turned on, at the beginning of an experiment the animal was at the end of the gradient where intensities were high, the first noticeable effect was that the r.c.d. became large. The animal wandered about the trough in an apparently perfectly random fashion, but it soon became clear that its average position was moving towards the end of the trough which had the lower light intensities. Eventually the animal reached the darker end of the trough, and remained confined to a relatively narrow zone there. In the case of animals which were at the light end of the trough at the beginning of the experiment, the tracks of animals which moved phobotactically from a region of high intensity to one of low intensity showed no signs of shock reactions. The shift of the position of the animal towards the end of the gradient was rather a gradual process, but in each case with the steep gradient, the animal was to be found at the darker end within 2 hours from the beginning of the experiment.

Similarly in the case of animals at the dark end of the trough at the beginning of the experiment, there were no shock reactions. Nevertheless phobotaxis does occur, and it remains to discover how.

With each piece of experimental apparatus, the one using the optical wedge and the other with the vanes, two standard light gradients were used. Their characteristics of intensity are represented graphically in Fig. 8. With each piece of apparatus the less steep of the two gradients did not produce phobotactic movements towards the darker end of the gradient, but the steeper one in each case did so.
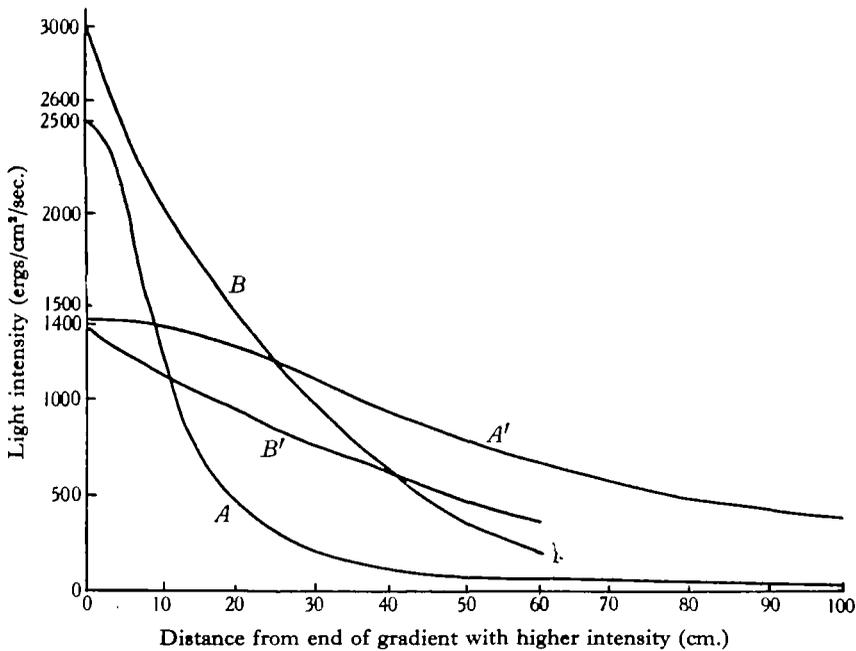
Fig. 8. Characteristics of different non-directional light gradients.. *A* and *A'* with the apparatus represented in Fig. 1. *B* and *B'* with the apparatus represented in Fig. 2. Phobotaxis occurred in gradients *A* and *B*, but not in *A'* and *B'*.

DISCUSSION

The experiments described in the last section showed that phobotactic responses are possible in a non-directional gradient of light intensities. By a path which was unorientated and apparently random in character, the animal reached a zone of low light intensity, and was able to maintain itself there. The form of the tracks made by the animals showed that shock reactions were not an essential part of the mechanism either by which the animal reached the zone of low intensity, or by which it was maintained in that zone. Similarly it was not possible to consider photokinesis as being responsible for the phenomena observed, for the animals were quite active when confined to the region of low light intensity, and were to be seen moving about in all directions. The fact that *Dendrocoelum* is not normally

quiescent in the dark is supported by ecological observations, for, in its natural environment, the animal is most active at night when the total light intensity is less than 1 erg/cm.²/sec.

A further consideration of the r.c.d. phenomena shows that they themselves provide a full explanation of phobotaxis. The facts are:

(1) An increase in stimulating intensity produces an increase in r.c.d.

(2) This initial increase in r.c.d. falls off under constant stimulation owing to adaptation.

(3) There is a basal r.c.d., which is an expression of the fact that turning movements occur even in absolute darkness or at complete adaptation.

In describing how these facts can provide an explanation of phobotactic movement, it is convenient to consider a particular case. Any animal in a gradient where directional stimuli are lacking will, sooner or later, be moving directly up the gradient parallel with its axis. The basal r.c.d. ensures that this will be so under any conditions.

In order to simplify the explanation, consideration will be restricted to movements of the animal parallel to the axis of the gradient, either up or down it. This assumes that the only expression of change of direction is a turn of 180°, but this assumption is valid because the total angular deflection of the path of the animal during the whole course of one of these phobotactic movements from a region of high light intensity to one of low intensity, is much greater than 360°. So in a general consideration of the type of behaviour in a given set of conditions, no importance is to be attached to the angle between the axis of the animal and the axis of the animal at any particular instant. Movements at right angles to the axis of the gradient, and components in this direction of oblique movements, can be neglected, for they represent conditions of unchanging intensity.

The behaviour of a fully adapted animal when exposed to a non-directional gradient can be considered. As it moves up the gradient the animal will be stimulated by the increasing light intensity. In any particular case the strength of stimulation will depend on the rate of increase of light intensity. This in turn will depend on the steepness of the light gradient and on the speed of the animal.

With *Dendrocoelum* the speed of the animal over the range of light intensities used was constant and can therefore be neglected, so that the case of a gradient steep enough to cause phobotaxis can be considered. Fig. 9 represents the ideal path of an animal under these conditions. If the animal is moving from *A* to *B* it is undergoing stimulation, and will soon perform a turning movement (*B*) on account of the increase in r.c.d. After this movement has been performed the animal will be going in the direction *BC*. Owing to the process of adaptation and to the decreasing stimulus, the r.c.d. along the track *BC* will be less than the r.c.d. along *AB*. Consequently *BC* is longer than *AB*, and the point *C* will be farther down the gradient than *A*, since the rate of movement remains unaltered. By the time the animal reaches *C* it will be at least partially adapted to the intensity at *C*, and after the turn (*C*) it will again move towards the light and be stimulated.

The r.c.d. along *CD* is greater than along *BC*, and, if we assume that the animal

started off at *A* with the same degree of partial adaptation as it had at *C*, then *CD* is shorter than *BC*, and *D* will be at a point farther away from the intense end of the gradient than *B*. Similarly the point *F* will be still farther away, and so on.

By this process of alternate stimulation and adaptation the animal is gradually brought to the weaker end of the gradient. For the sake of clearness, simplifications were made at the beginning of this explanation. But it is plain that they do not in any way prejudice the truth of the explanation when applied to the animal making its normal random movements in every direction.

Adaptation is an essential factor in this process. If the r.c.d. were some function of the absolute light intensity, the behaviour of the animal could be compared satisfactorily with that of the molecule of a gas in a temperature gradient. In the case of any one particular molecule, there is no directional factor in its movement. It is true that on the average it will be found for greater lengths of time at the cooler end of the gradient, but it is just as likely to be moving towards the hotter end of the gradient as in the opposite direction.

It has been maintained that the stimulation to which the animal is exposed should depend on the steepness of the gradient and the speed of the animal. If the gradient is very gentle the animal will be able to adapt itself completely to successive intensities of stimulation as it passes from one to the next. That is to say, the animal should not be able to accomplish "phobotactic orientation" in a very gentle gradient. This is exactly what happens. In the experiments recorded on p. 272 the animals continued to wander about all over the trough in spite of the fact that some of them were left there for periods of more than 10 hours.

Fig. 9. Schematised path of an animal moving in a non-directional light gradient. (For explanation see text.)

The form of the track left by animals put into a steep gradient confirms the explanation of phobotaxis which has just been given. The tracks show clearly that there is no orientation, and that the response does not involve shock reactions. On the other hand, most of the tracks show the overlapping step-like character with respect to the long axis of the gradient which is to be expected if the explanation given above is correct. There is, in fact, every indication that the hypothesis put forward to explain the behaviour of *Dendrocoelum* is the right one. It remains to investigate how far one is justified in attempting to apply it to other cases.

Many examples of phobotactic behaviour involving shock reactions have been described, but it has been shown on pp. 272–3 that the shock reaction is not essential to the establishment of definite spatial relationships on the part of the organism to particular values along a gradient of stimulative intensity. This makes it desirable
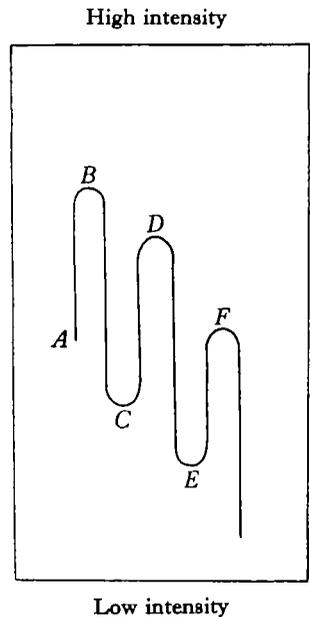
to reconsider the part played by shock reactions in phobotactic responses, and to investigate the relationship between the shock reaction and r.c.d. phenomena. It is not really surprising that shock reactions do not occur under experimental conditions similar to those described in this paper, for shock reactions are not likely to happen if there is a gradation between one intensity of light and another, with no sharp boundary between the two.

But it is true that responses to light of an apparently all-or-none kind are to be found in the animal kingdom, so, from one point of view, it appears at first sight that there is no reason why the shock reaction should not be of this kind. However, if the cases of this sort of response are examined, it is found that the authentic examples of "all-or-none" behaviour occur in sessile or semi-sessile organisms, such as the tube worms, ascidians, and lamellibranchs.

The literature on responses of animals to light indicates that, in free-living animals, the magnitude of the response bears some definite relationship to the intensity of stimulation, and is not of the all-or-none type. Yet explanations involving shock reactions have often been put forward to account for their phobotactic behaviour. The results obtained with *Dendrocoelum* in connection with r.c.d. form a convenient basis for reconsidering the relationship between the shock reaction and the more normal type of response. The curve represented in Fig. 3 shows that the r.c.d. of an animal is a function of the stimulus received, and that the r.c.d. increases rapidly with increasing stimulus. Also the curve in Fig. 5 shows that adaptation occurs, and that the r.c.d. under constant stimulation falls off with the time for which the stimulus has been acting, but initially is very high. These two sets of results point to the conclusion that the reaction would be very violent if the animal were to be stimulated suddenly by a high light intensity. Those factors which constitute a "shock reaction" seem to be precisely the ones which are represented in a violent and exaggerated r.c.d. movement.

It should be pointed out here that when the change in direction in which the planarian is moving is more than 90°, the rate of movement decreases while the turning is being executed. This decrease in the rate of movement becomes more pronounced as the angular deflection of the turn becomes greater, until a point is reached when the animal almost ceases to creep while the turn is being made. This corresponds to a change in direction of about 180°. If a change of direction through a wide angle is to be made efficiently, a reduction of speed during the movement must necessarily take place. The true state of affairs appears to be this. A simple angular deflection of small amplitude, with no perceptible decrease in speed, represents one end of the scale of responses. The other is represented by the shock reaction. The important point is that a complete series of intermediate stages fills up the apparent gap between the two. The two extremes are represented by the opposite ends of the curve in Fig. 8. The continuity of these curves shows that the shock reaction due to sudden stimulation by light is not an all-or-none type of reaction as was assumed in previous work. It really is one extreme of a set of responses which are of the same kind, though different in degree, for different intensities of stimulation. The shock reaction appears to be simply an extreme case

of a general phenomenon, namely, the over-production of random movements during stimulation.

The fact that the so-called shock reaction can be brought into line with other responses to light, in this way, has an important bearing on all-or-none responses. It shows that almost all reactions to light can be expressed as functions of the stimulating intensity. There are, however, the few exceptions mentioned before, which appear to be all-or-none in character. It is significant that these specialised reactions occur in animals which are themselves specialised to a particular mode of life by sessile or semi-sessile habits; this way of living means that the possibilities of response are greatly reduced.

Shock reactions are not necessary for phobotactic movements, and, before a definition of phobotaxis can be attempted, the factors in the behaviour of *Dendrocoelum* which are of general significance must be emphasised. In a general way, the ultimate possibility of an animal reaching some particular intensity of stimulation by a non-orientated path when exposed to a non-directional gradient of stimulative intensities depends on these conditions. The behaviour of the animal moving up the gradient must be different from its behaviour when moving down. If the animal were to move at different speeds when subjected to increasing and decreasing intensities of the same range, then a directional factor parallel to the long axis of the gradient would be introduced into its movements, even if the r.c.d. were constant. It seems probable that there are cases where such a difference of speed could be important in phobotactic responses, but such a case has not yet been investigated. The example of *Dendrocoelum*, where no differences of speed exist, shows that the directional factor relative to the long axis of a non-directional gradient depends on an over-production of random movements (increase of r.c.d.) when the animal is stimulated. The over-production of random movements is a function of the stimulating intensity, and, under constant stimulation, diminishes as the animal becomes adapted to the stimulus.

Whether the phobotactic response depends, as in *Dendrocoelum*, on changes of r.c.d. only, or, as it may do in other cases, on changes of speed, it is clear that phobotaxis depends on the stimulative effect of changes of intensity during successive periods of time. Phobotaxis is, in fact, concerned with time-intensity stimulation of all the receptors simultaneously, not, as with orientated responses, with differential stimulation of receptors in different parts of the organism. It is therefore the sort of response which could be made by animals with sense organs of the simplest type.

SUMMARY

1. Stimulation by non-directional light produces in the animal an increase in random movements, that is to say an increase in the rate of change of direction (r.c.d.).

2. This r.c.d. can be expressed as a function of the stimulating intensity.

3. In total darkness there is a basal r.c.d.

4. In constant stimulation the r.c.d. decreases as the animal becomes adapted.

5. The "shock reaction" is not an essential part of the mechanism of phobotactic behaviour. Photophobotaxis occurs without it.

6. The r.c.d. phenomena and the adaptation associated with them are sufficient to explain the mechanism of photophobotaxis. Alternate adaptation and stimulation occur when the animal is moving down or up a non-directional gradient, as a result of its random movements.

7. This alternate stimulation and adaptation has an effect on the r.c.d. of such a kind that the animal is led automatically to the place of minimal intensity.

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