THE SHADOW REACTION OF *DIADEMA ANTILLARUM* PHILIPPI

I. THE SPINE RESPONSE AND ITS RELATION TO THE STIMULUS

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*(Received 21 December 1959)*

**INTRODUCTION**

Though many animals respond to shadows some do so without clearly defined photoreceptors, and in such cases detailed studies of the mechanism involved are rare. Instances of this kind have been investigated by von Buddenbrock (1930) and Föhl (1932) who studied, in particular, *Balanus* and *Helix*, respectively.

The echinoid *Diadema*, previously examined by von Uexküll (1900) and Millott (1954), is another example, responding to changes in illumination by movements of its spines, podia and pedicellariae. Of these, spine movements are the clearest and most consistent, and we have studied them exclusively. They are reflexes, the pathways of which pass through the radial nerves.

Most of the body surface is light-sensitive, though morphologically defined photoreceptors have not yet been found and sensitivity is co-extensive with the nervous system, much of which is epidermal. This suggests that relatively unspecialized elements may be directly excited by light, and this has been strengthened by a direct demonstration of photosensitivity in the radial nerves (Yoshida & Millott, 1959).

In reactions to shadows, both the illumination and its change must be considered, since both are environmental agents. We therefore set out to discover what effects varying the intensity and duration of both lighting and shade exert upon the character of the spine response.

**METHODS**

Urchins from Madeira were used and they were kept in aquaria.

The urchin was cut into five pieces, each with a radial nerve cord at the centre. After removing viscera, each piece was carefully washed with fresh sea water and the spines were removed by cutting as closely as possible to the test, leaving one in a position slightly aboral to the ambitus. The piece was mounted horizontally in aerated sea water contained in the experimental tank already described (Millott & Yoshida, 1957), and left to recover and adapt for at least 45 min. before use. The inside of the tank was painted matt black, apart from two narrow slits on either side which allowed a light beam to throw a shadow of the spine tip on to a ground-glass screen. Spine movement was recorded photographically in the way already described.

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Light for stimulation was obtained from a 6 V. 30 W. tungsten filament lamp run on a.c. except where shadows shorter than 0.3 sec. were used, when it was operated on d.c. to avoid flicker effects. The voltage was kept constant by means of a variable resistor and voltmeter. Intensity was controlled by interposing neutral filters.

To minimize the spread of stray light and the complicating effects of spatial summation, the areas illuminated were kept as small as possible by projecting a beam through the objective lens of a microscope, as already described (Yoshida & Millott, 1959). The beam was focused on to the centre of the radial nerve.

However, when brief shadows were used a slight modification was required to produce them by a shutter rotating in a vertical plane (see below), so that the stimulating light had to be mounted horizontally and its beam deflected into the optical system, by means of a 45°-prism.

Shadows longer than 0.3 sec. were produced by manually operated shutters, the exact duration of shading being recorded photographically and measured by an adjacent time trace.

Various means of producing shorter shadows were tried. Of these the most consistently satisfactory is shown in Fig. 1. The axle A, rotating on ball bearings,
The shadow reaction of Diadema antillarum Philippi. I

The shadow reaction of Diadema antillarum Philippi. I

This carries a kymograph drum B and wheel D to which is bolted the calibrated shaft C. This carries the opaque shutters and sliding weights \( W_1 \) and \( W_2 \). The beam from \( L \) was interrupted by the shutter each time the shaft and its attachments moved under its own weight from the constant position ensured by a stop in the wheel, indicated in the figure by \( F \). Different durations of shadow were obtained by altering the position of the shaft, the weights and the length of the shutters. The speed with which the shutter passed over the light beam was determined for each setting of the apparatus by recording the speed of rotation, using the trace from a 100-cycle vibrator \( (V) \) writing on the drum surface.

Each record showed spine movement accompanied by a time trace (at 1 sec. intervals) and an automatic signal showing when the light beams were cut off. In recording the initial part of the reaction, the paper was run at 0.5 in./sec., which gave reasonable accuracy in calculating the reaction time and duration of shading. For the sake of economy, this speed was slowed down to 0.1 in./sec., 6–8 sec. after the beginning of the shadow. Examples of the recording are shown in Fig. 2.

Experiments were performed in a dark room the temperature of which was fairly steady, so that in any series of experiments the temperature of the sea water did not vary by more than 1°C.

The spine response

Responses appear consistently in pieces of test prepared as described above, and if adequate time for recovery is given the spines are motionless until stimulated, so that both beginning and end of a reaction are easily determined. Though spontaneous movements may occur, they are relatively rare.

Movement follows stimulation after a well-defined interval and lasts for a few seconds, or even a minute, declining gradually in vigour. Whether stimulation be general or localized, it affects most, if not all, of the spines so that extensive co-ordination is involved. The interpretation of such movement is difficult, so to simplify matters we directed attention to one spine and removed the remainder.

With the records obtained, reaction time, amplitude, frequency and duration of the contractions can readily be measured and compared. The reaction time is defined as the interval from the beginning of shading until the first signs of a response; the frequency as the number of beats recorded during a standard period after the beginning of stimulation; and the duration as the period from the beginning of shading until all contractions have finished. The amplitude is the total swing of the spine as it appeared on the ground-glass screen, expressed in arbitrary units. In comparing reactions we have used both the amplitude of the first contraction (initial amplitude) and that of the largest contraction occurring after 10 sec. from the time of shading (later amplitude).

These criteria can be used to compare reactions because they are reasonably constant in response to a constant stimulus, so that records taken within 1–2 hr. are often so similar as to be almost superimposable (Fig. 2).

However, as the preparation ages the vigour of response may decline gradually (Fig. 3), particularly in the later part of the reaction, but the reaction time stays
fairly constant, even when the response has become so weak that only few jerks ensue after a stimulus which earlier called forth a vigorous reaction. This means that when determining the effect of environmental factors on the vigour of the response, control experiments had to be carried out frequently.

Fig. 2. Reactions to a shadow of infinite duration and a constant intensity of 100% (see p. 369), shown by one preparation, to illustrate the constancy of the response. Records of three separate reactions, recorded at approximately hourly intervals, have been superimposed in the left half, which represents the more critical part of the reactions whose characteristics are compared. The interruption of field illumination is shown by the change in level of the line above the time trace. Time in seconds.

Fig. 3. The decline in vigour of the response. Abscissae, time of day. A, duration. Ordinates, time in seconds. B, frequency of contraction. Ordinates, number of beats in 10 sec. interval indicated alongside curve. C, amplitude of contraction. Ordinates, arbitrary units. D, reaction time in seconds.

Although spine responses follow shading of both the radial nerve and the skin, we have preferred to study the effect of the former because in the case of the outside surface the reaction times which follow shading at various places along a plane parallel to the ambitus vary considerably, as shown in Fig. 4, and a shift of only 2·0 mm. (from the ambulacral margin to the adjoining interambulacrum), implies very different reaction times. The variation is much less in the radial nerve, the
The shadow reaction of Diadema antillarum Philippi. I

367

mean and its standard deviation in different positions ranges from $1.22 \pm 0.04$ to $1.32 \pm 0.06$ sec.

It may be noted in passing that the variation in reaction time at various points on the outside surface corresponds with the relative sensitivity of these areas, previously determined by a different method (Millott, 1954); the most sensitive areas, having the shortest reaction time, are found at the ambulacral margin and a gradient in sensitivity and reaction time exists as follows:

ambulacral margin → ambulacral centre → inter-ambulacrum.

![Diagram of reaction times](image)

Fig. 4. The reaction times of the responses elicited by shading various positions internally and externally. Each curve corresponds to one preparation. Ordinates, reaction time in seconds, the vertical bar showing the range of variation at each point. Abscissae, the numbers show the approximate distance in mm. of the area stimulated from a position 0 which lies alongside the spine, as shown in the accompanying diagram where the ambulacral areas appear stippled.

A, meridional gradient. The curve marked 'inside' shows the effect of stimulating internally by shading positions lying along the radial nerve, that marked 'outside' showing the effect of shading positions on the outside surface, which lie along the margin of the ambulacrum. B, the effect of shading the outside surface in various positions which lie across the ambulacrum, in a plane parallel to the ambitus.

The effect of illumination

To show this we determined the effect of varying the duration and intensity of illumination, while the degree of shading was kept constant by cutting off the light completely and leaving the preparation in darkness, at least until the reaction had subsided.

It was necessary to guard against possible effects of sensory adaptation by inter-spersing experiments in which the field illumination was dim between those in which more light was used.

(a) Duration

The effect of this was determined by illuminating preparations at a constant intensity for periods between 1 and 300 sec., after which the field illumination was
cut off. The preparation then remained in darkness for not less than 5 min., after which another experiment was begun. Such intervals had been found adequate to allow full recovery of responsiveness.

The results from one preparation are shown in Fig. 5, where it will be seen that the reaction time decreases steadily as the duration of lighting is increased. In contrast, the frequency of the contractions and the duration of the reaction increase steadily as the illumination is prolonged. The changes are complete after 1-1½ min. illumination, beyond which no further significant increase in responsiveness appears. The amplitude of both initial and later contractions shows a similar tendency.

![Fig. 5](image)

Fig. 5. The effect of the duration of lighting on one preparation. Abscissae, duration of lighting in seconds. Ordinates: A, reaction time in seconds. B, amplitude in arbitrary units. Filled circles, initial contraction; open circles, later contraction (see p. 365). C, number of beats recorded in the 20 sec. period indicated alongside each curve. D, duration of reaction in seconds.

(b) **Intensity**

A complementary series of experiments was performed in which the light was cut off after a 5 min. exposure to different intensities with a range of $10^3$. The results from one preparation are shown in Fig. 6.

The effects on reaction time, amplitude, frequency and duration, produced by increasing the intensity, are essentially similar to those produced by prolonging the lighting.
The shadow reaction of *Diadema antillarum* Philippi. I

These experiments also revealed that the reactions of the primary spines vary with intensity in the same way as those of the spines of lower orders, so that possible differences in the effectors are not important here.

![Fig. 6. The effect of intensity of lighting on one preparation. Abscissae, logarithmic scale in arbitrary units. Ordinates: A, reaction time in seconds. B, amplitude in arbitrary units. Filled circles, initial contraction; open circles, later contraction. C, number of beats observed in the 10 sec. period indicated alongside each curve. D, duration of the reaction in seconds. The height of each shaded area represents the 10 sec. period during which the reaction subsided. The number of reactions whose duration is recorded, is represented by the relative width of the areas shaded.]

The effect of shading

The effect of a shadow may now be examined in the same way, its intensity being considered as the decrease in intensity of field illumination and its duration as the interval between the instant it is reduced and the time that the preparation is reilluminated. The field illumination was kept constant, preparations being illuminated at the same intensity for 5 min.

(a) Duration

Here the field illumination was cut off completely for periods varying between 26 msec. and about 1 min., a period of about 1 min. being the time taken for completion of the reaction.
Where the duration of shading is shorter than that of the reaction the increase in intensity due to the light re-admitted may superimpose an ‘on’ effect. The animal being relatively insensitive to such changes (Millott & Yoshida, 1959), this danger was avoided by working with an intensity of field illumination well below the threshold for such responses.

Fig. 7. The effect of shadows up to 120 msec. duration compared with that of a control shadow of infinite duration. From a single preparation. Abscissae, duration of shadow in milliseconds. The results of the control are shown alongside. Ordinates: A, reaction time in seconds. B, amplitude in arbitrary units. Filled circles initial contraction; open circles, later contraction. C, number of beats recorded during the 10 sec. period indicated alongside each curve. D, duration of the reaction in seconds.

Typical results, reproduced in Figs. 7 and 8, show that there is little or no effect on the initial part of the reaction, but more on the later parts.

Thus the amplitude of the first contraction is unaffected and the reaction time is affected only near the threshold, i.e. below 40 msec. and then but slightly and erratically.

The effect on the later part of the response involves the amplitude, frequency and duration, all of which increase with the duration of shading. The increase is not uniform, being greater as the shadow increases up to about 60 msec. (Fig. 7). Prolonging the shading further brings about relatively little change, except in the
The shadow reaction of Diadema antillarum Philippi. I

case of the frequency of the contractions occurring later in the reaction (after about 20 sec.), which increase more significantly. In all features, with the notable exception of the reaction time and initial amplitude, the reaction is smaller after light has been re-admitted, regardless of whether this occurs before or after the reaction has begun.

Fig. 8. The effect of shadows 0-3 sec. or longer in duration compared with that of a control shadow of infinite duration. A, effect on the number of beats in a single preparation. Abscissae, successive 10 sec. periods. Ordinates right, the number of beats recorded in each 10 sec. period. Ordinates left, the duration of the shadow. The vertical height of each shaded area represents the number of beats recorded in each 10 sec. interval. Each shaded area is displaced along the ordinate axis so as to show the duration of shadow which produced this effect. The average reaction time (R.T.) is shown by the broken line. B, effect on the duration of the reaction; abscissae, duration of shadow in seconds. Ordinates, duration of reaction in seconds. Open circles and broken line, filled circles and continuous line, represent two different preparations. The reaction time is shown by the corresponding vertical lines.

(b) Intensity.

To show the effect of intensity, neutral filters of different densities were interposed in the light beam after 5 min. and allowed to remain until the reaction subsided. The results are shown in Fig. 9, the intensity of shading being expressed as a percentage decrease in field illumination.

They differ from the preceding in that the reaction time decreased steadily as the depth of shading was increased. For other criteria, the shortage of material compelled us to confine our measurements to two preparations, which showed that the duration of the reaction and the frequency of the beats increased with the
Fig. 9. The effect of the shading intensity (p. 369) on one preparation. Abscissae, percentage decrease in intensity. Ordinates: A, reaction time in seconds. B, amplitude in arbitrary units. Filled circles, initial contraction; open circles, later contraction. C, number of beats recorded in the 10 sec. period shown alongside each curve. D, duration of the reaction in seconds.

intensity of shading; in addition, the amplitude of the later contractions was increased and in some cases that of the initial contractions also.

DISCUSSION

Very little is known of the neuromuscular organization of echinoids, so that the conclusions which can be drawn from an analysis of a gross response such as spine movement must necessarily be limited. As compared with an analysis by electrophysiological means the approach not only lacks precision but is hampered by the fact that one sees only the terminal event in a chain involving various responsive structures, receptive, conducting, interacting and contracting. But despite the resultant complexity, some significant relationships emerge between stimulus and response. These are summarized in Table 1.

The reaction time, frequency, amplitude and duration of the response are clearly related to both the intensity and duration of the preceding field illumination.

Their relationship with the shadow is different, for its intensity affects the reaction time (and in some cases the amplitude of the initial contractions) but its duration does not.
The shadow reaction of Diadema antillarum Philippi. I

Table 1. Effects of light and shade on various criteria
(+ = clear effect; − = no effect; ± = doubtful effect.)

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<th>Criterion</th>
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<th>Shadow</th>
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<td>Duration</td>
<td>Intensity</td>
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<td>Initial part of reaction</td>
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<td>Reaction time</td>
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<td>Number of beats</td>
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<td>Later part of reaction</td>
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Fig. 10. Comparison of the effects of lighting and shading on the reaction time. Curve $T$: duration of lighting, ——●—●—●, Curve $I$: intensity of lighting, ○———○, Curve $s$: intensity of shading, ○———○, Curve $t$: duration of shading, Δ···Δ. Abscissae: ($T$), in seconds; ($I$), in arbitrary logarithmic units; ($I$), percentage decrease in field intensity; ($t$), in milliseconds.

The relation between stimulus and reaction time is especially significant for here the different effects of illumination and decrease in illumination appear (Fig. 10). The duration of the field illumination affects the reaction time, that of the shadow does not, and so the expected relationship between intensity and duration of the stimulus does not appear in the case of the shadow.

However, the duration of the shadow affects the later part of the reaction, which means that it differs from the initial part in its variability and susceptibility. This
might imply that an additional mechanism comes into play in the later reaction (see below).

Although these findings resemble those of von Buddenbrock (1930) and Föh (1932)—both of whom used a similar index, namely the terminal response of a reflex arc—there are significant differences. Also it must be remembered that the methods differ, for they used intact animals whereas we used spines isolated from other similar effectors, and the areas illuminated and shaded were far greater than those used here.

In *Helix* Föh found the intensity of field illumination and shading to affect the reaction time, vigour and duration of the response in essentially the same way as in *Diadema*, but the effect of the duration of shading was different in that it affected not only the vigour and duration of the reaction, but also the reaction time, provided that the duration of shading was within a critical range, viz. 240 msec.

The lack of an effect of duration of shading on the reaction time, except when the duration is near threshold, leads us to interpret our results in a different way.

The parallel effects of duration and intensity of field illumination are such as to suggest that it is here that light acts in conditioning a system which is ‘set’ thereby, and remains so until overt effects are released by the decrease in intensity, the duration of which has no effect on the early part of the response.

The fact that under the conditions in the experiments described above steady light produced no overt effects suggests that it may be exerting an inhibitory effect, release from which is provided by the shadow and that the reaction which ensues is roughly in proportion to the effect of the preceding illumination, as well as to the intensity of light remaining during shading, which continues to exert an inhibiting effect. Then it would be evident why the intensity of shading is important; for, being a measure of the decrease in intensity, it is also a measure of the intensity of the light remaining. Further, when light is re-admitted inhibition might again affect the reaction, diminishing or cutting it short according to the moment at which light is re-admitted. The significant timing would therefore be that of the re-admitted light and its ineffectiveness on the initial part of the reaction could thus be an expression of the latency of the inhibition.

This possible explanation at once recalls that usually adduced to explain the ‘off’ effect in eyes (Granit, 1947), which involves nervous interaction. In this connexion it is pertinent to recall the work of von Buddenbrock, who showed the effect of the intensity and duration of shading on the duration of the withdrawal reaction of *Balanus*, which he further states does not depend on the intensity of field illumination, though he shows that the duration of the threshold stimulus depends on it. He also showed the existence of spatial and temporal summation in the shadow response. Most significantly, this led him to argue cogently against much current opinion, notably that of Puetter and Hecht, and to emphasize the importance of the central nervous system. Föh, on the other hand, on a basis of a quantitative study of the relationship between duration and intensity of shading, made a comparison of the effect of a shadow with that of illumination in the formal
The shadow reaction of Diadema antillarum Philippi. I 375

scheme advanced by Hecht. As already mentioned, our different results have led us to reject Föh's approach.

To strengthen our suggestion concerning the participation of inhibition, it is desirable to demonstrate by direct experiment, the inhibitory action of light on the shadow response. This will be shown in a succeeding communication.

SUMMARY

1. Isolated pieces of test of Diadema bearing a single spine show responses to a constant shadow cast on the radial nerve which are consistent in reaction time, duration, amplitude and frequency of the contractions.

2. Variations in the intensity and duration of the lighting which precedes the shadow exert similar effects on the whole reaction, affecting all the above features.

3. Variations in the duration and intensity (% decrease in intensity of illumination) of the shadow differ in their effects. The intensity affects all the features of the whole reaction, but the duration affects only the later part.

4. The differing effects of light and shade suggest that the shadow response may be a rebound from inhibition due to light which, when re-admitted, diminishes any shadow reaction that may be in progress.

We are greatly indebted to the Zoological Society of London, especially to Dr H. G. Vever, for much assistance. We are similarly indebted to Dr D. Pye, of the Institute of Laryngology and Otology, and to Mr S. E. White of the College Science Workshop. Our thanks are also due to Miss M. E. Rablah of the Physics Department and to Drs Brindley, Denton and Pirenne, who kindly criticized the manuscript. The research was supported by a grant from the Medical Research Council.

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