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DIRECTIONAL DIFFERENCES IN THE COLOUR SENSITIVITY OF *DAPHNIA MAGNA*

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SUMMARY

1. The *Daphnia* compound eye movements can be driven by a flashing light.
2. The action spectrum for the threshold light intensity required to evoke this response depends on the orientation of the stimulus light beam with respect to the animal.
3. If the light falls on the eye through the top of the animal's head the action spectrum peaks at the low wavelength end of the spectrum, while if it falls on the eye through the side of the head the peak is in the yellow-green.
4. Eye movements cannot be evoked by illuminating any part of the animal except the compound eye so neither of these action spectra is due to a light receptor other than the compound eye.
5. Some anomalous action spectra in the literature on the behaviour of free-swimming *Daphnia* are accounted for.

INTRODUCTION

Viaud (1948) reviews a wide range of studies involving the effects of light colour on various aspects of *Daphnia* swimming behaviour. He concludes that the curves which result from plotting behavioural index against light colour fall into two main groups; those with a maximum response in the yellow-green region of the spectrum, and those with a maximum in the purple–ultraviolet (UV). The yellow-green curves occur when the response involves the orientation of the animal with respect to the direction of the light beam, and the purple near-ultraviolet curves if measures of swimming speed are involved. Following von Frisch & Kupelwieser (1913) he attributes orientation control to the single large compound eye, and hence thinks that all the processes giving yellow-green curves are mediated by the compound eye. This view is supported by the observation that *Daphnia* with their compound eyes extirpated still avoid strong ultraviolet light (Schultz, 1928; Baylor & Smith, 1957), suggesting that the purple-UV peaked responses are likely to be controlled by other light receptors, for example, the three ocellus naupliar eye or a generalized skin light sense. He favours the latter because Schultz (1928) found no disruption of the UV avoidance response when both compound and naupliar eyes were extirpated. Baylor & Smith (1957) question this last finding, but their negative result could be due to brain damage caused by the 'needle beam X-rays' they used to extirpate the naupliar eye.

Some experimental results, however, do not fit Viaud's hypothesis at all well. For example, he himself shows that swimming speeds of animals swimming towards a light follow a purple-UV type curve, while swimming speeds of animals swimming away from the light follow a yellow-green curve (Viaud, 1951). Further problems arise in the interpretation of an experiment by Robert, Scheffer & Médioni (1958) which attempts to relate the spectral response curves for eye movements to those for swimming behaviour. The response they measure is the angular amplitude of the rocking movement of the compound eye evoked by the onset of a light stimulus. Their results show that the spectral characteristics of this response can take the form either of the yellow-green peaked curve or the purple-UV peaked curve, depending on the precise orientation of the light stimulus relative to the eye. Several experiments involved illuminating the body but not the eye, and these all gave purple-UV peaked response curves. The remarkable conclusion from the work is that non-compound eye light receptors can, in some situations, control the movement of the compound eye. This is difficult to explain in terms either of the possible functional organization of the visual system, or of the neuroanatomy of the optomotor nerve connexions as described by Leder (1915).

Measuring the amplitude of single eye movements of *Daphnia* presents considerable problems because continual spontaneous movements occur in the absence of any applied stimuli. It is necessary to develop a more stable index of eye-movement response. The response chosen, hereafter called the following response, is the absolutely regular eye movements which result if an animal is stimulated with a regularly flashing light. These eye movements are exactly in phase with the stimulus, stereotyped, and very characteristic, making it relatively straightforward to determine the threshold light intensity required to produce the following response.

MATERIALS AND METHODS

Large female *Daphnia magna* were chosen for this experiment. Each animal was positioned lying on one side on a small glass window in the base of a shallow circular trough, the rest of which was painted matt black. The animal was held still with a loop of fine wire held in a small manipulator attached to the side of the trough, which was then transferred to the stage of a Union inverted microscope and filled with water to a depth of about 5 mm. The animal's eye was observed using only infra-red (IR) light. A filter passing only IR was interposed in the light path before the condenser, and an image converter substituted for one of the eyepieces. A Leitz Um × 50 long working objective produced a large image of the eye on the converter screen. No evidence exists of any response of *Daphnia* (either behavioural or physiological) to IR stimuli.

The lamp supplying light for the coloured stimulus used was a type A1/20 projector lamp (240 V, 100 W), which was run from a smooth DC supply of adjustable voltage. A collimated beam from this lamp passed through an adjustable slit, and then a first order Barr and Stroud interference wedge. The resulting coloured light was focused down into one end of a 2 m length of 2 mm diameter Crofon plastic light guide. The other end of this guide, held 10 mm from the animal's eye in one of the two positions shown in Fig. 1, provided the stimulus. In condition 1 the guide points along the lon

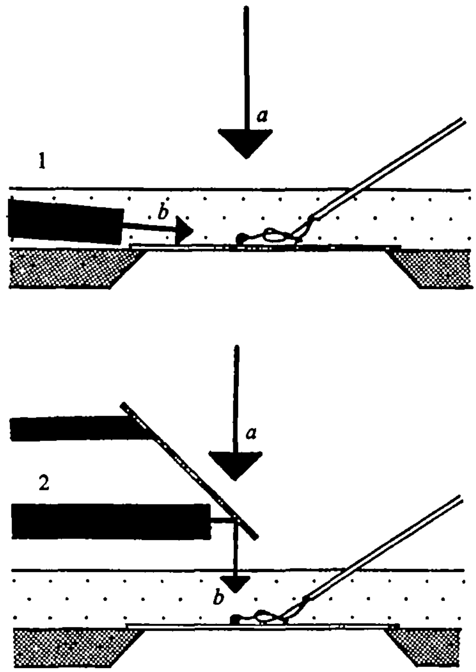

Fig. 1. The arrows labelled *a* show the direction of the infra-red observation light with respect to the experimental animal, while those labelled *b* give the path of the coloured stimulus light from the end of the stimulus light guide, which is shown in solid black. Condition 1 gives 'light from above', i.e. the light from the stimulus guide shines through the top of the animal's head. Condition 2 gives 'light from the side', the light from the stimulus guide shining through the side of the animal's head.

axis of the animal, corresponding to light coming from above for an animal in the normal upright swimming posture. In condition 2 the light is directed through the side of the animal's head. It is necessary to use a half reflecting plate in the optical system in condition 2 to avoid the blockage of the IR observation light by the light guide. The plate, made to the author's specification by Barr and Stroud, is a microscope slide coated on one side with a 50% reflecting aluminium layer, and bloomed on the other to avoid double reflexions. Its presence is allowed for in the photometric calculations. The terms, 'light from above' for condition 1, and 'light from the side' for condition 2 are used throughout.

The total light energy output from the stimulus guide was measured in watts for each of the five wavelength bands used in the experiment. This was done with a vacuum photocell (type 90 AV) calibrated against a standard lamp and an interference wedge and checked against a Hilger and Watts vacuum thermopile. Curves for lamp volts against light energy output for each wavelength interval were established, and the area of the light patch produced from the end of the stimulus guide was measured. Hence it was possible to calculate the illumination at the animal's eye, in W/m^2, for any given values of lamp volts and interference wedge setting. The stimulus was made to flash by means of a short rigid pendulum carrying a vane which occluded the light path for half of each swing. The period was 200 ms. The pendulum continued swinging freely for 5 min after being pushed.

Twenty animals were used for this experiment, ten for condition 1 and ten for condition 2. Each animal had its thresholds measured under each of five conditions of light colour:

A	400–450 nm	'Purple'
B	450–500 nm	'Blue'
C	500–550 nm	'Green'
D	550–600 nm	'Yellow'
E	600–650 nm	'Red'

To avoid any possible effects of fatigue or adaption the colours were not presented in the same order to each animal; a latin square design was used, four 5×5 squares being selected at random. Each block of five animals was tested consecutively, an experimental run taking about an hour. After immobilization, which inevitably involved a brief period of bright illumination, each animal was allowed a 4 min period of complete darkness before the first following response threshold was measured.

The procedure adopted was to turn the stimulus lamp to its full intensity with the power supply voltage control, and set the pendulum swinging. The eye, observed in the image converter, at once began the distinctive rocking movement. The lamp was steadily dimmed until the rocking movement was quite suddenly replaced by the typical slow, irregular eye movements of an animal in total darkness. At this point the DC voltage across the lamp was measured, enabling the threshold light intensity to be calculated as described above. The lamp was then turned out completely, and the interference wedge adjusted to the next colour required by the latin square design. The determination of the next threshold was begun exactly 2 min after the start of the procedure to determine the previous one. The testing room was kept in complete darkness for the whole course of each animal's trials. The rapid rate of testing was adopted so that the time taken to complete a 'balanced square' group of five animals should be small in relation to any diurnal sensitivity rhythms.

RESULTS

Threshold values for the light levels required to sustain the following response are tabulated in Table 1, and median values are plotted against light colour in Fig. 2. The large variations in absolute values between individuals for the same condition is typical of *Daphnia* responses to light stimuli, and necessitated the use of statistical techniques to extract the relevant information. The changes in threshold with light colour are statistically significant: $P < 0.001$ for condition 1, $P < 0.02$ for condition 2 (Friedman analysis of variance by ranks).

The apparent differences in threshold values for the two stimulus directions at each of the wavelength intervals, tabulated in Table 2, show that although there is no statistically reliable difference between the results for light from above and those for light from the side in the green and yellow regions of the spectrum, the two conditions do differ significantly on either side of this region (Mann–Whitney U test). Moreover, the animals are more sensitive to red light from the side, but to blue and purple light from above. Hence these results cannot be explained in terms of a dimunition of the total amount of light reaching the eye in one or other condition, for all such

Table 1. *Table of threshold light intensities for the following response for the various wavelength intervals tested*

For animals 1–10 the illumination is from above, while for animals 10–20 it is from the side. All the values are for the illuminations at the animal's eye, in microwatts per square metre. Since the stimulus light is flashed at 5 Hz, with equal periods of light and dark, average intensities are half those shown in this table.

Animal number	Light colour (wavelength interval) 400–450	450–500	500–550	550–600	600–650 nm
	Condition 1. Light from above				
1	7·04	133	60·4	281	412
2	2·01	16·1	8·05	211	531
3	11·1	2·01	84·5	50·3	199
4	105	2·01	1620	1005	927
5	7·04	24·1	26·1	56·3	403
6	74·4	74·4	288	423	461
7	930	7·44	91·4	356	419
8	372	115	964	1622	1690
9	271	54·1	312	355	541
10	40·5	81·2	372	250	386
	Condition 2. Light from the side				
11	311	346	517	18·0	414
12	552	593	56·9	96·6	290
13	372	642	787	345	331
14	725	900	725	648	286
15	725	104	145	328	427
16	115	281	74·8	81·1	35·0
17	398	1161	44·5	27·0	111
18	1750	1113	1511	286	254
19	684	127	38·2	74·9	175
20	843	763	2228	35·0	398

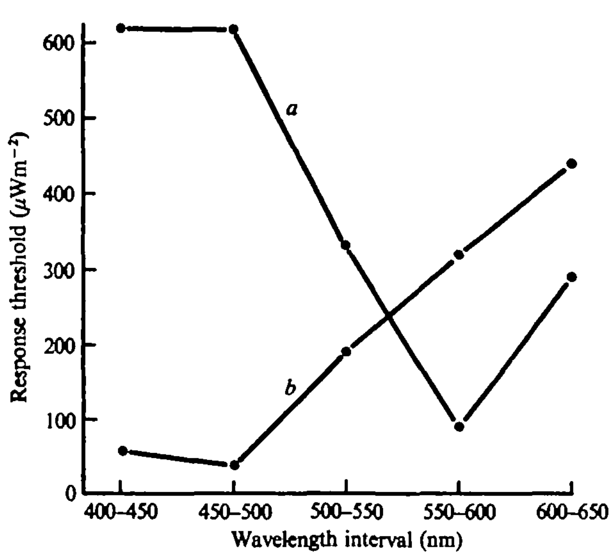

Fig. 2. Median threshold light intensities for the following response for various stimulus colours. Curve *a* gives the results for condition 2, light from the side. Curve *b* gives the results for condition 1, light from above.

Table 2. *Table of results of a series of Mann–Whitney tests applied to the data for both conditions at each wavelength interval*

Light colour (wavelength interval)	Mann–Whitney U	Significance level	Direction
400–450 nm	13	$0.02 > P > 0.002$	Animal more sensitive to light from above
450–500 nm	3	$P < 0.002$	Animal more sensitive to light from above
500–550 nm	39	No significant difference	
550–600 nm	29	No significant difference	
600–650 nm	12	$0.02 > P > 0.002$	Animal more sensitive to light from the side

hypotheses predict that the change in threshold between conditions should be in the same direction across the spectrum.

It would seem that the green-yellow peaked responses are associated with light falling on the compound eye through the side of the head and the purple-UV peaked curved with illumination through the top of the head. An attempt was made to see if any other structures were implicated in the following response mechanisms by reducing the stimulus light guide to a single fibre (diameter 0·3 mm) which was held directly against the animal's carapace with a micromanipulator, enabling very restricted regions of the animal to be stimulated. Green light was used for the initial investigation, being a colour to which both mechanisms gave a reasonable response. First the fibre was held against the carapace directly adjacent to the animal's eye. Flashing the light produced a strong following response. The end of the fibre was also placed in contact with the carapace near the naupliar eye, next to the heart, near the tail, on the second antenna, and near various parts of the gut. In no case did the brightest flashing light possible (0·2 W/m^2), which was 200 times brighter than the largest threshold recorded in the previous experiment, evoke a following response. This observation was repeated with several animals and light colours, and conflicts with the observations of Robert *et al.* (1958) on evoked eye movements when the body but not the eye was subjected to a flash of light. This discrepancy may arise because their apparatus, involving vertical opaque screens on the microscope stage to prevent light from the stimulus lamp reaching the animal's eye, excludes scattered or stray light much less effectively than the single fibre probe used here, and the very low threshold levels for the following response suggest that very little scattered light would be needed to evoke a response.

DISCUSSION

The implication of these results is that responses with action spectra peaking either in the purple-UV or in the yellow-green can be mediated in *Daphnia magna* by the compound eye, and that which class of response occurs is simply a function of the direction of illumination. If we assume that the action spectra for the eye movement following responses are reflected in action spectra for indexes derived from the swimming behaviour of a similarly illuminated animal it becomes possible to explain some puzzling results of experiments on swimming behaviour. For instance, Viaud's observation that animals swimming towards a light gave the purple-UV curve whi those swimming away from the light the yellow-green one (Viaud, 1951) is now just what would be expected; since animals swimming towards a light lean well forwards, thus the eye is illuminated through the top of the head; while animals swimming away from the light adopt an upright posture (often backing away from the light) and receive light through the side of the head. Viaud also reports that a survey of all experiments, in which the orientation of photokinesis of animals swimming in a vertical reference beam of light are affected by changes in a horizontal comparison beam, shows that this situation always results in action spectra with two maxima, one in the purple-UV and the other in the yellow-green. These two-peaked curves must represent a combination of characteristics of the upwards pointed purple-UV system and the sideways pointing yellow-green system, since the comparison beam was input to one system and the reference beam to the other.

The fact remains, however, that *Daphnia* with their compound eyes extirpated do avoid strong UV lights. It is necessary to postulate a low sensitivity extra-ocular UV receptor system as well as the dual system in the compound eye.

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