

REACTIONS OF LIMULUS TO ILLUMINATED FIELDS OF DIFFERENT AREA AND FLICKER FREQUENCY

BY ERNST WOLF AND GERTRUD ZERRAHN-WOLF

(From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratories, Harvard University, Cambridge)

(Accepted for publication, January 22, 1937)

For the reaction of various Arthropods to stimulation by intermittent light, the relation between illumination and critical flicker frequency has been well established (Sälzle (1932); Wolf (1933-34); Crozier, Wolf, and Zerrahn-Wolf (1936-37*a, b*); Bröcker, (1935)). Intermittent stimulation at flicker frequencies *below* fusion produces an increased reactivity compared with steady light. This was found for several insects (Mast and Dolley (1924, 1925); Wolf (1933); Zerrahn (1933)). It seems that under such conditions reaction depends upon the frequency and number of transitions of the retinal elements from one state of excitation into another (Wolf and Crozier (1932-33)). For vertebrates a pronounced effect of slow flicker as evidenced by the migration of retinal pigments has recently been described (Arey and Price (1936)). To obtain information about the effect of seen areas of different dimensions, illumination, and flicker frequency, and to establish quantitative relations, the honey bee has been used (Wolf and Zerrahn-Wolf (1934-35)). For the bee as a fast moving insect, the strong reaction to intermittent stimulation seems of great importance (Wolf and Zerrahn-Wolf (1936-37)). If flicker of low frequency was generally a strong stimulus for Arthropods, relations such as found with the bee should be found in other forms as well. In this study we used *Limulus*. *Limulus* has been proved to give rather precise phototropic responses (Loeb (1893); Northrop and Loeb (1922-23); Cole (1922-23)). At the same time we possess a good deal of information about the action of the optic nerve fibers in *Limulus* (Graham (1932); Hartline (1929-30); Hartline and Graham (1932)).

With the help of Fig. 1 the experimental arrangement can be understood. In a vertical piece of ply-wood two openings 17.7×17.7 cm. are cut. The distance between the centers of the two holes is 32 cm. Into each opening opal screens are fitted which are illuminated from behind. The areas of the illuminated fields can be varied by means of cardboard frames, reducing the original area. The ratios and areas of the test fields used are given in Table I. The frames

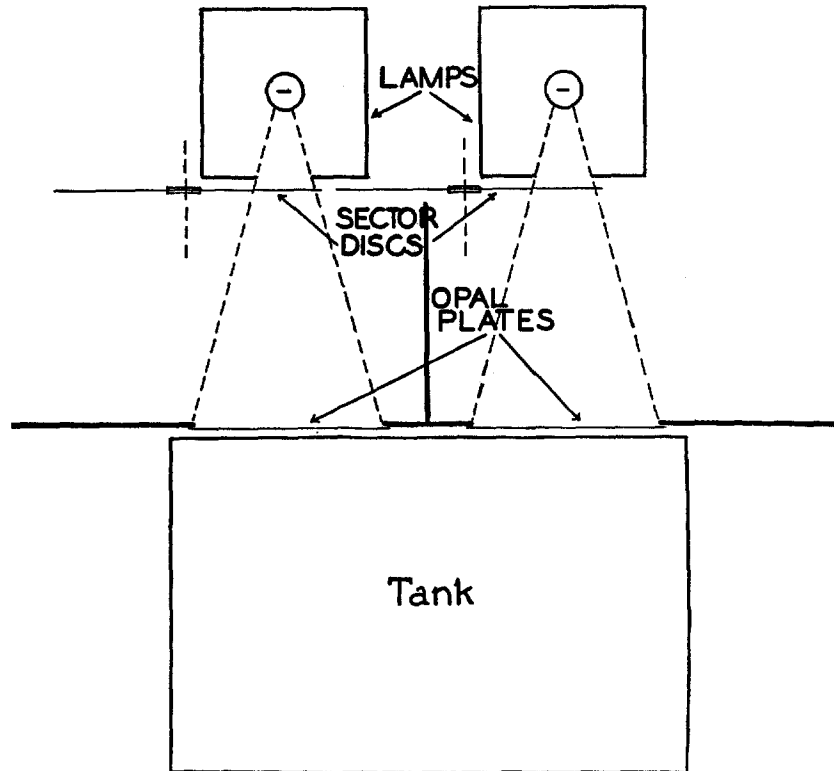


FIG. 1. Apparatus for testing the reaction of *Limulus* to two illuminated fields differing in area and in flicker frequency.

are made so that their lower edges coincide with the lower edge of the opening in the ply-wood; the illuminated fields are thus at "eye level" of our animals.

In front of the wooden wall is a tank 64×45 cm. filled with sea water as high as the upper margin of the illuminated fields. The bottom of the tank is slate. Since the experiments are performed in a dark room, influences by outside light or reflections from the walls are minimal.

The opal screens are each illuminated by a 200 watt concentrated filament lamp. The light sources are set back 75 cm. from the screens to secure an even illumination. The brightness of the lamps is determined and adjusted in such a way that the illuminations of both fields are equal. For intermittent stimulation by light, sector discs can be inserted in front of the openings of the lamp houses. The axles for the discs are connected by a gear and chain drive which permits keeping the same flicker ratio of the two fields at any desired flicker frequency. The sector wheels are cut so that with every revolution 180° permits light to pass through and 180° leaves the animals in darkness. The open half-circle can be split into sectors: 1 × 180°, 2 × 90°, 3 × 60°, 4 × 45°, and 5 × 36°. The discs are driven by a 1800 R.P.M., D.C. motor, the speed of which can be controlled by a rheostat. Between the motor and the sector wheels a reduction gear box is inserted so that, by adjusting the rheostat, flicker fre-

TABLE I
Field Sizes Used for Comparison of Effects of Areas of Flickered Illumination

Size of field	Length of side	Area
	<i>cm.</i>	<i>cm.²</i>
1/5	7.9	62.7
1/4	8.8	78.3
1/3	10.2	104.4
2/5	11.2	125.3
1/2	12.5	156.6
3/5	13.7	188.0
2/3	14.5	208.9
3/4	15.3	234.2
4/5	15.8	250.6
1/1	17.7	313.3

quencies between 2 and 20 per second can be obtained. We adjusted the flicker frequencies in such a way that the faster of the two fields gave flicker frequencies between 10 and 20 a second, by which we obtained the sharpest responses of our animals. Since it was shown for other Arthropods that the critical frequency for flicker fusion is a function of illumination and that the highest frequency of flicker which can be reacted to at all lies between 53 and 62 per second, we are certain that with the intensities and frequencies used we were far below the fusion point.

For test young *Limulus* were used varying in length from 4 to 8 cm. Young animals freshly collected are in most cases positively phototropic. They are used for test as soon as they have been brought to the Laboratory. If there are too many animals available at one moment, they are kept in a tank with running sea water of low temperature, half shaded in the supply house. If left in the

dark room over a longer period, all animals become negatively phototropic just as older ones do. A change in the sign of phototropism can also occur as a result of handling the animals (Cole (1922-23)). With a sufficient number available it is possible, however, to have always as many positively phototropic animals as needed.

One at a time the animals are brought into the tank in dim red light, and they are held in a position on the middle line between the two flickering fields. To keep the *Limulus* in a position to start its run on a course on the bisecting line between the two fields, a slight pressure is exerted on the tip of its tail by means of a glass rod which has a piece of rubber tubing on its end. The animal begins leg movements as soon as the two fields are illuminated and the sector discs are set into motion. It is released at a moment when its body axis is exactly on the bisecting line. It now takes a course toward the two fields, and at about the middle of the tank turns to the right or to the left field toward which it proceeds with considerable speed. Each arrival at one or at the other field is counted. Each animal will give six to ten successive runs which are definite. Pretty soon, however, the animal will during its course turn far to the right or to the left or run away from the lights just as if its phototropism was changed from positive to negative. This could easily be due to the repeated handling during which the animal was brought back to the starting point. As soon as there is any uncertainty about the conduct of an animal, it is eliminated. Before setting the sector discs into motion, the animal is tested several times and its reaction is observed. If there is any tendency for response toward the field of one side which could be due to bodily asymmetry, the animal is at once excluded from tests with flickering fields. For each ratio of flicker frequencies and areas, about 60 to 70 tests are made. It was found that this number of runs is sufficient for quantitative treatment of the data. During each set of experiments carried out with a given flicker frequency and area ratio, the sector discs and diaphragms securing the respective areas were shifted from right to left and from left to right repeatedly so as to avoid any possible influence of the position of a field relative to the animals tested.

In the first set of experiments the ratio of areas was kept 1:1 throughout (313.3 cm.²:313.3 cm.²), and the ratio of flicker frequen-

TABLE II

With equal areas equally illuminated having different frequencies of flicker below that critical for fusion (F_1 and F_2) in the ratios given (F_1/F_2), the number of times is given that animals went to F_1 and to F_2 and proceeded to the midregion (M) between the two flickering fields. The ratios of the numbers for F_1 and F_2 are given as N_1/N_2 .

F_1/F_2	Field	No. of animals						Total	N_1/N_2
		1	2	3	4	5	6		
0.20	1	1	2	0	2	1	1	7	0.149
	5	7	7	12	13	5	3	47	
	<i>M</i>	3	2	0	0	1	0	6	
0.25	1	1	3	2	2	3	—	11	0.250
	4	8	2	12	11	11	—	44	
	<i>M</i>	1	1	2	1	0	—	5	
0.33	1	0	2	2	5	3	1	13	0.368
	3	3	5	8	7	10	5	38	
	<i>M</i>	0	2	0	3	3	1	9	
0.40	2	2	3	1	4	3	2	15	0.385
	5	4	10	5	10	7	3	39	
	<i>M</i>	1	0	1	3	1	1	7	
0.50	1	2	6	4	3	1	—	17	0.472
	2	4	13	6	6	7	—	36	
	<i>M</i>	0	3	2	1	1	—	7	
0.60	3	3	8	2	4	5	—	22	0.611
	5	7	10	4	7	8	—	36	
	<i>M</i>	0	0	2	1	4	—	7	
0.66	2	5	6	4	5	—	—	20	0.690
	3	9	6	6	8	—	—	29	
	<i>M</i>	6	5	2	4	—	—	17	
0.75	3	5	4	5	6	1	—	21	0.759
	4	6	6	7	8	2	—	29	
	<i>M</i>	1	5	5	4	3	—	18	
0.80	4	5	5	5	3	3	—	21	0.840
	5	7	5	4	4	5	—	25	
	<i>M</i>	7	2	3	3	7	—	22	

cies was varied. It soon appeared that the number of animals going to the faster flickering field is always greater than to the slower field. In fact the ratios of the numbers of animals counted at each field are proportional to the ratios of the flicker frequencies (Table II).

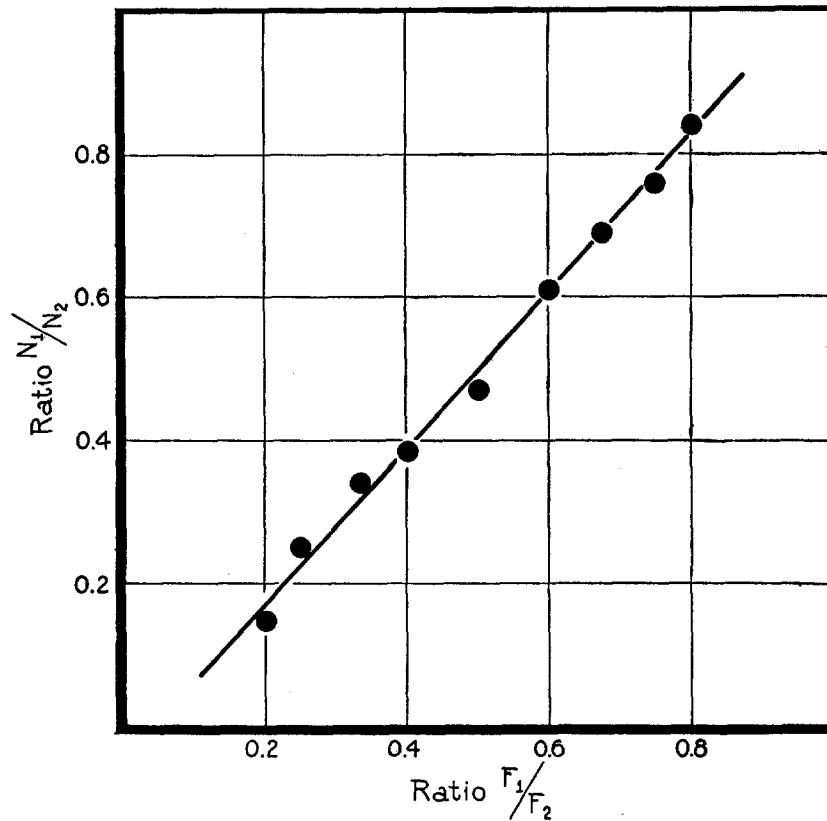


FIG. 2. Relations between flicker frequencies of two illuminated fields of equal areas and the number of animals reacting to the fields. The slope of the line is slightly above one.

If we plot the ratios of numbers of animals at each field, N_1/N_2 , against the ratio of flicker frequencies, F_1/F_2 , we obtain a rectilinear relationship (Fig. 2). The same has been found earlier in the honey bee (Wolf (1933); (Wolf and Zerrahn-Wolf (1934-35))). It is interesting, however, to note that the slope of the line in Fig. 2 is slightly

above 1. This indicates that the faster flickering field always has a slightly greater effect than the slower field.

Since it has been found that between two flickering fields of equal brightness and area but differing in flicker frequency the stimulating effect of the fields is proportional to the flicker frequencies, the question arises whether two fields differing in flicker frequency can be equalized in their stimulating effect by reducing the area of the faster flickering field. From experiments with the honey bee we know that such an equalization can be arrived at. It might be possible also to obtain it by reducing the brightness of the faster flickering field. Experiments with the bee showed, however, that the range of variation in this respect is very great before any effect can be noticed.

For tests with *Limulus* the area of one field is kept constant (17.7 cm. x 17.7 cm.), and a sector disc is inserted to provide a given flicker frequency. The area of the other field is made smaller, and its flicker frequency is adjusted so that the ratio of the two areas is inversely proportional to the respective flicker frequencies. If for *Limulus* the same is true as for the bee, we expect a ratio of 1 in the number of animals seeking either field. The results of our tests are given in Table III and in Fig. 3. By studying Fig. 3 we notice that the mean ratio, for which theoretically we would expect 1, is slightly above 1. The calculated mean = 1.028. If we indicate $3 \times P.E._1$ in the graph, all the points are within the limits of this band. The difference between the theoretical mean and the one experimentally found seems just barely significant since $P.E._M$ (mean ratio) = 0.0086. It seems therefore that in this series of tests, just as in the previous ones, we have a slightly greater stimulating effect of the faster flickering field.

With the new results for the eyes of *Limulus* another piece of evidence for the strong reaction of an Arthropod to flicker below fusion is given. Mast and Dolley (1924, 1925) pointed out that for the photic response of *Eristalis* the effect of intermittent stimulation of low frequency (between 10 and 25 per sec.) is considerably greater than with higher flicker frequencies or with continuous illumination. Quantitative evidence for reaction to flicker of low frequency was given in the honey bee (Wolf (1933); Wolf and Zerrahn-Wolf (1934-35)). In all the Arthropods tested the relations of area, intensity of

TABLE III

The ratio N_1/N_2 of animals going respectively to illuminated fields 1 and 2, flickered at frequencies F_1 and F_2 , is shown for different ratios of the areas of the fields. (M records, as in Table II, the number of journeys to the region between the two fields.)

Ratio of areas	Ratio F_1/F_2	Field	No. of animals						N_1/N_2	
			1	2	3	4	5	6		Total
1:5	5:1	1	5	5	4	7	6	—	27	0.964
		5	6	5	6	6	5	—	28	
		M	1	2	4	3	1	—	11	
1:4	4:1	1	5	4	4	5	7	4	29	1.036
		4	5	3	6	5	5	4	28	
		M	2	5	2	2	0	2	13	
1:3	3:1	1	4	6	3	5	6	5	29	1.074
		3	6	4	1	6	5	5	27	
		M	2	2	4	1	3	0	12	
2:5	5:2	2	6	3	6	8	3	6	32	1.032
		5	6	4	5	5	6	5	31	
		M	0	0	0	4	3	0	7	
1:2	2:1	1	8	3	7	5	5	6	34	1.030
		2	8	4	8	5	3	5	33	
		M	2	0	0	2	2	1	7	
3:5	5:3	3	6	4	5	8	6	—	29	1.074
		5	9	4	4	6	4	—	27	
		M	1	0	3	2	2	—	8	
2:3	3:2	2	6	3	4	5	2	7	27	0.964
		3	4	5	5	6	2	6	28	
		M	2	2	3	1	2	1	11	
3:4	4:3	3	6	5	4	5	4	—	24	1.043
		4	5	6	3	6	3	—	23	
		M	1	3	5	3	4	—	16	
4:5	5:4	4	6	4	7	5	6	—	28	1.037
		5	3	4	8	7	5	—	27	
		M	3	4	1	2	5	—	15	

light, and flicker frequency lead to the conclusion that by the visual fields certain numbers of retinal elements are stimulated which give rise to impulses to the central nervous system and thus cause definite action of the motor mechanism. In case of areas differing in size and illumination, it was found that the product of area and intensity has to be constant to give equal stimulating effects. With varying areas and flicker frequencies, equivalent stimulating effects are obtained if for two fields the areas are inversely proportional to their flicker frequencies. It therefore seems that for equality of stimulation it is

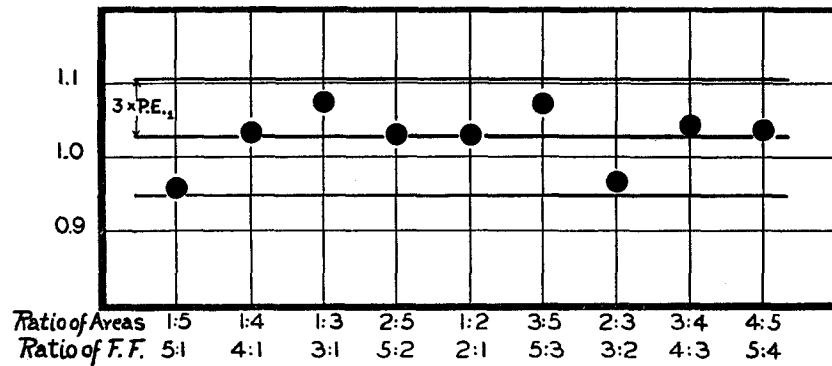


FIG. 3. Ratios of number of animals turning to one of two illuminated fields differing in area and in flicker frequency. The theoretical mean for equal effect should be 1. It was found to be 1.028, suggesting a slightly superior effect of flicker as such.

irrelevant whether a greater number of elements is stimulated by a large area which is weakly illuminated or has a low flicker frequency, or a smaller number of elements is stimulated by a smaller area of stronger illumination or high flicker frequency. For the coordination of motion during a tropistic response it seems that the same effect is obtained when few impulses due to low illumination or low flicker frequency are coming from a greater number of receptor elements or when more frequent impulses due to stronger illumination or faster flicker are sent out from a smaller number of receptor elements.

SUMMARY

In phototropic tests with young *Limulus*, the phototropic reactions to flickering fields were studied. If the two fields are equal in area and brightness but different in flicker frequency, the number of animals going to the two fields is proportional to their flicker frequencies. Equal stimulating effects of two fields differing in flicker frequency are obtained by reduction of the area of the faster flickering field. The areas for equal effect must be inversely proportional to their flicker frequencies. It seems that equal effects are dependent upon equality of the number of active excitation elements per unit of time.

CITATIONS

- Arey, L. B., and Price, R. G., *J. Exp. Zool.*, 1936, **74**, 303.
 Bröcker, H., *Zool. Jahrb., Abt. Allg. Zool.*, 1935, **55**, 399.
 Cole, W. H., *J. Gen. Physiol.*, 1922-23, **5**, 417.
 Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *J. Gen. Physiol.*, 1936-1937a, **20**, 363; *J. Gen. Physiol.*, 1936-37b, **20**, 393.
 Graham, C. H., *J. Cell. and Comp. Physiol.*, 1932, **2**, 295.
 Graham, C. H., and Hartline, H. K., *J. Gen. Physiol.*, 1934-35, **18**, 917.
 Hartline, H. K., *J. Gen. Physiol.*, 1929-30, **13**, 379.
 Hartline, H. K., and Graham, C. H., *J. Cell. and Comp. Physiol.*, 1932, **1**, 277.
 Loeb, J., *Arch. ges. Physiol.*, 1893, **54**, 81.
 Mast, S. O., and Dolley, W. L., Jr., *Am. J. Physiol.*, 1924, **71**, 163; 1925, **72**, 84.
 Northrop, J. H., and Loeb, J., *J. Gen. Physiol.*, 1922-23, **5**, 581.
 Sälzle, K., *Z. vergl. Physiol.*, 1932, **18**, 347.
 Wolf, E., *Z. vergl. Physiol.*, 1933, **20**, 152. *J. Gen. Physiol.*, 1933-34, **17**, 7. An analysis of the visual capacity of the bee's eye, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1935, **3**, 255.
 Wolf, E., and Crozier, W. J., *J. Gen. Physiol.*, 1932-33, **16**, 787.
 Wolf, E., and Zerrahn-Wolf, G., *J. Gen. Physiol.*, 1934-35, **18**, 853; 1936-37, **20**, 511.
 Zerrahn, G., *Z. vergl. Physiol.*, 1933, **20**, 117.