Technical Papers

A Light Polarization Analyzer in the Compound Eye of Limulus

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Experiments currently in progress indicate that the lateral eye of limulus (*Xiphosura polyphemus* [L.]) can detect the plane of polarization of a stimulating light. The quantitative study of this phenomenon has advanced to a point that warrants a preliminary report. Furthermore, such an account seems desirable in view of recent widespread interest in the use of sky polarization as a light compass by the honeybee (*3, 4*; reviewed in *10*). The present limulus experiments may indeed, by contributing to the pertinent knowledge of the arthropod compound eye, provide a clue to the specific sensory mechanisms involved in this aspect of insect navigation (for a discussion of insect flight instruments, see *11*).

Single photoreceptor units of the limulus eye have been extensively used to study the general physiological properties of visual elements by Hartline and his coworkers (for review see *6*). Their results clearly show that the response to stimulation of these light receptors is closely comparable to that of other sensory units whose electrical response has been isolated (e.g. *1, 5, 7, 8*). In several types of such sensory elements, including the limulus visual unit, an initial series of transients associated with the onset of stimulation is succeeded by a steady rate of sensory nerve discharge which continues as long as the specific stimulus lasts. This uniform rate of discharge varies with the intensity of the stimulus. In a range of medium light intensities, the frequency of sensory impulses changes linearly with log I. For the present experiments it was this "steady state" discharge frequency that was utilized as a measure of stimulus effectiveness.

Lateral eyes of small limulus (cephalothorax about 50 mm in length) were prepared by excising the organ along with some of the surrounding carapace and a 20–30-mm length of intact optic nerve. Then, under a dissecting microscope, the ophthalmic artery which sheaths the optic nerve, was carefully cut away. Connective tissue and other extraneous material were also cleaned from the inner side of the retina. At this point the medial aspect of the whole structure appeared as in Fig. 1. Note that just anterior to the lateral eye the optic nerve divides, usually into two rami, one dorsal, the other ventral. In turn, each of these rami provides a nerve branch to the white body which constitutes the so-called rudimentary eye.

With the carapace around the eye fixed in a clamp, the nerve was extended and held under slight tension by lightly weighting a thread tied around its cut end. Next the nerve was split into successively finer bundles of fibers with the aid of glass needles. This was done with the nerve under sea water, which was the physiological saline used throughout. To record the electrical activity of the small bundles, they were lifted into air or mineral oil by a pair of recording electrodes held in a micromanipulator. The electrodes, made of fine silver wire, were connected to the input of a condenser-coupled three-stage amplifier, which in turn drove an oscilloscope and, through an audioamplifier, a loudspeaker.

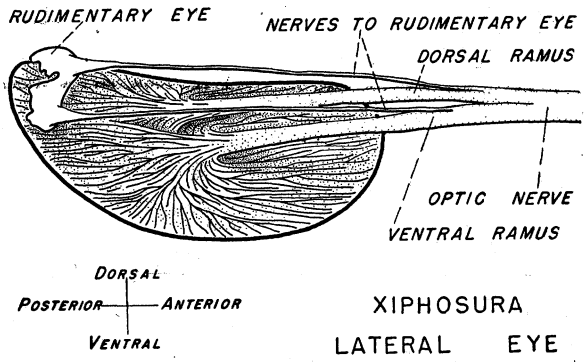

FIG. 1. Compound eye of a young limulus, medial aspect ×10. Blood vessels, digestive gland, and connective tissue dissected away. Branching nervous elements covering inner surface of retina are bundles of nerve fibers. Whether branching occurs in individual fibers is not known.

The sensory elements corresponding to any given bundle of optic nerve fibers were localized by exploring the ommatidia of the eye with a fine pin point of light directed onto the corneal surface. Then, by a combination of further fiber isolation and selective photic excitation, a physiologically unitary response could usually be obtained. Such a system was essentially a Hartline single-element receptor preparation.

When a successful isolation had been accomplished in this fashion, the visual unit was stimulated by a beam of linearly polarized light whose plane of polarization could be conveniently rotated. This was obtained with a Polaroid filter interpolated between the light source, a tungsten projection bulb in an ordinary microscope lamp, and the limulus eye. The light transmitted by the filter is rated at 99.8% polarization through most of the range within the visible spectrum.

The discharge rate of the single photoreceptor element was examined as a function of the stimulating light's plane of polarization. In general, it has been found that the sensory impulse frequency varies systematically with the change in the plane of polarization. Maximum and minimum response rates occurred with polarization planes 90° apart. Two complete cycles appeared during a 360° rotation of the polarizer.

The following experiment will serve to illustrate the quantitative relation between the two factors, nerve

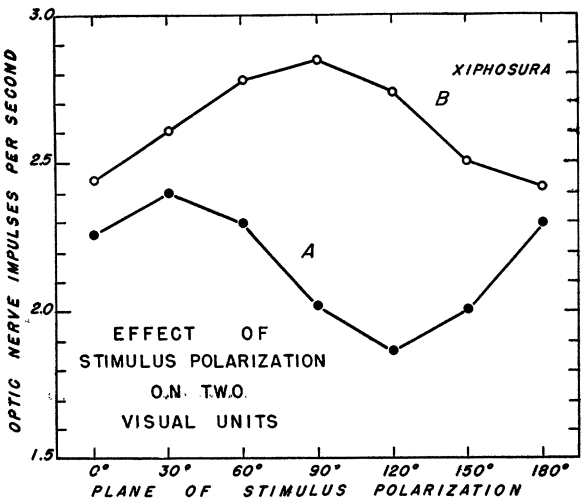

FIG. 2. Relation between optic nerve impulse frequency and polarization plane of the stimulating light for two different isolated photoreceptor units in the same limulus lateral eye. Each point is the mean of 10 frequency measurements. Retinal location of the units, A and B, is shown in Fig. 3.

impulse frequency and polarization plane of the stimulus. In making these actual rate measurements the intensity of the stimulating light was reduced, so that steady-state nerve discharge rates of 2–3 per sec were obtained. Their frequency was determined by measuring with a stop watch the time interval required for 25 discharges. Measurements were made for 7 planes of polarization, 30° apart, between 0° and 180°. To counterbalance drift in the physiological state of the preparation and to minimize the effect of its adaptation, 10 readings for each point were obtained by testing each position serially from 0° to 180°, then in reverse order from 180° to 0°, and so on until sufficient data were collected.

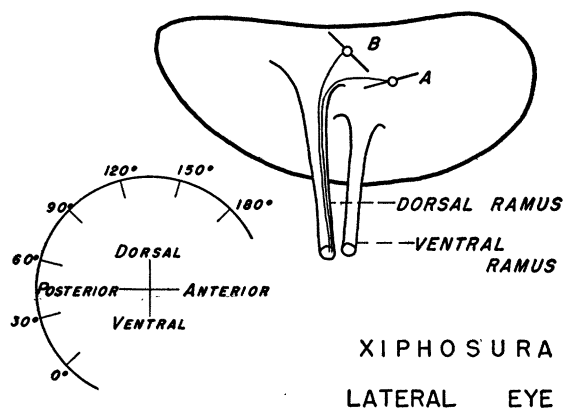

FIG. 3. Medial diagrammatic view of limulus lateral eye used to obtain data of Fig. 2. Optic nerve rami stretched mediad as during the experiment. Photoreceptor elements A and B are symbolized by circles, the alignment of their polarization analyzers by lines through the circles. Relative orientation of these ocular structures to the whole animal and to the scale measuring polarization angle shown at left.

Response curves secured in this way for two visual elements from the same limulus eye are plotted in Fig. 2. The location of the particular units concerned in the retina and in the optic nerve appears in Fig. 3. Note that the frequency of impulses in the optic nerve fibers varied 15% and 23%, respectively, for the two units, between the maxima and minima caused by rotating the polarization plane of the stimulating light. The resulting curves appear as smooth periodic functions with a wavelength of 180°.

Statistical analysis of the 70 raw scores used in plotting each of the mean curves in Fig. 2 shows that the differences in the means due to changes in the plane of polarization are highly significant. Analysis of variance in these data has been carried out and the reliability of the differences determined in the usual way from an F table. In both cases F values for the plane of polarization considerably exceed those required for significance at the 1% level.

Three independent lines of evidence have been examined to ascertain that the effect described is significant not only in a statistical sense but in a physiological sense as well. First, the possibility that polarization of the light source caused actual intensity fluctuations in the stimulating light was considered. This was checked initially by determining whether intensity changes detectable to the human eye were caused by rotation of the polarizing filter. Such changes were not present either in the total light transmitted by the polarizer or in the minute image of the light source focused on the limulus eye. As another check no significant changes appeared in the response curves when the stimulating light was rotated through 90° during an experiment. These facts would apparently eliminate the light source as a possible cause of the observed effect.

Second, the stimulating light and all other extraocular factors seem to be excluded by the following type of experiment. If the polarization analyzer effecting the response curves described is actually in the biological system, rotation of the eye through a given angle should produce a corresponding angular displacement in the response curves obtained in the usual way. The results of such a test are shown in Fig. 4. They demonstrate that within the limits of experimental error an identical displacement of the polarization curves was effected by a rotary change in the eye's position. Third, the data in Fig. 2 also suggest that instrumental artifacts are not responsible for the experimental results. They indicate that under similar experimental conditions two sensory elements, even though fairly close together on the same retina (Fig. 3), elicited response curves which were 60° out of phase.

The foregoing data prove that at least under specific conditions, a polarization analyzer is present in the compound eye of *Xiphosura*. It may thus be concluded that for this photoreceptor significant differences in the "apparent brightness" of a stimulating light depend on the plane of its polarization. A number of critical experiments remain to be done before the biological importance of this phenomenon can be assessed.

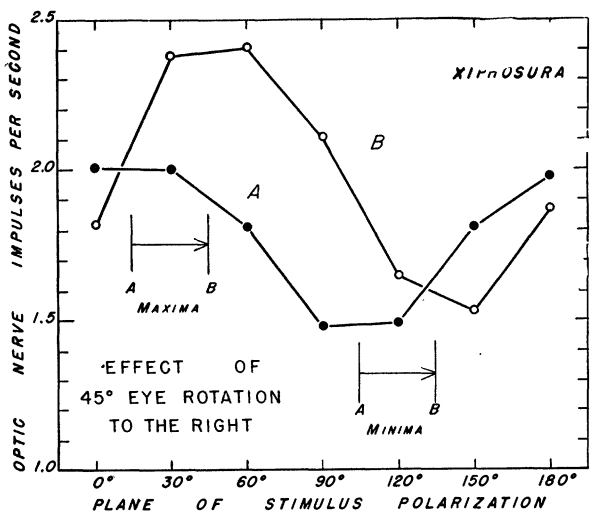

FIG. 4. Control data demonstrating that the polarization effect observed is a function of the biological system. After measurements were made as in previous experiments (A), the limulus eye itself was rotated clockwise through 45° and a second series of measurements taken. Corresponding displacements of both maxima and minima of the response curve occurred.

First, a quantitative study should be made to determine the extent of this effect through the full ranges of intensity and wavelength to which this visual system is sensitive. Then an analytical search should be instituted to discover the structures constituting the polarization analyzer involved. Some clues for such research should be sought in the studies of polarization optics of biological materials in general (*9*) and of eyes in particular (*2*). The dioptric properties of the limulus eye and possible birefringence of various of its elements, including the retinula cells, clearly should be investigated in this connection. Third, the behavior of *Xiphosura* should be examined to see whether polarized light has any normal functional significance for this arthropod. Finally the possible relationship of the present observations to von Frisch's work on bees, mentioned in the introduction, should be worked out. Particularly pertinent here would be the determination of the over-all pattern of sensitivity and orientation of the individual polarization analyzers in the whole retina. In the solution of the various structural and functional problems involved here, one may expect to find answers to certain of the specific questions asked, but one may also confidently expect in the process to learn much that will contribute toward our broad understanding of the compound eye.

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The Combined Action of Penicillin with Streptomycin or Chloromycetin on Enterococci *in Vitro*[1]

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Synergistic effects have been demonstrated with a number of chemotherapeutic agents (*1, 2, 6–10*). A synergistic effect of penicillin-streptomycin mixtures *in vitro* against certain staphylococci and a hemolytic streptococcus has been reported by Nichols (*8*). The combined action was greater than a simple additive effect of the drugs but the synergism was of low order. Clinical reports (*4, 11, 13*) indicate that combined therapy with penicillin and streptomycin is often successful in curing bacterial endocarditis due to enterococci, which ordinarily fails to respond to either drug alone, even when administered in high dose over a long period. With enterococci it has been shown (*5*) that mixtures of penicillin and streptomycin *in vitro* rapidly brought about death of the entire bacterial population, whereas streptomycin alone had no effect, and penicillin alone had mainly bacteriostatic properties. The experiments presented here may add to the understanding of this antibiotic synergism. In the course of our studies it was also noted that Chloromycetin[2] interfered with the action of penicillin on many strains of enterococci *in vitro*. This drug was therefore included in these experiments to compare streptomycin-penicillin synergism with apparent Chloromycetin-penicillin antagonism.

The bacteriological culture media used were Proteose-Peptone #3 agar (Difco) and a broth having the same base. Crystalline Sodium Penicillin G, Streptomycin sulfate, and Chloromycetin (Rx 117344) were dissolved in sterile saline. The Chloromycetin solution was sterilized by Seitz filtration. Final dilutions of the drugs were made in broth in a total volume of 20 ml. The bacterial inoculum consisted of 1 ml of an 18-hr broth culture containing 10^8-10^9 organisms/ml. All cultures were incubated at 37° C. At intervals aliquots were removed from the test mixtures, and the number of viable organisms determined by serial dilution and plate count. In other aliquots the penicillin was inactivated with penicillinase (Bacto-Penase), in order to permit penicillin-inhibited bacteria to grow. Absence of growth after the addition of penicillinase was interpreted as absence of

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[2] Commercial brand of chloramphenicol. Generous supplies of this drug were kindly made available by Dr. G. Rieveschl, Parke Davis and Company, Detroit, Michigan.