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Extraocular Perception of Polarized Light by Orienting Salamanders*

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Summary. Spatial orientation corresponding to the bearing of the *e*-vector of linearly polarized light can be demonstrated in sighted and eyeless salamanders *(Ambystoma tigrinum)* trained under linearly polarized light. However, if opaque polyethylene plastic is inserted over the skull of these animals, whether they are sighted or eyeless, orientation is uniform within the test arena. Bidirectional oriented movement is restored in both groups, however, when transparent plastic is substituted in the same animals. A discussion of the possible mechanism for perception of polarized light by extraocular photoreceptors (EOPs) is given.

Zusammenfassung. Durch Dressur unter linear polarisiertem Licht wird bei *Ambystoma tigrinum* sowohl mit als auch ohne Augen eine Orientierung nach dem *e*-Vektor linear polarisierten Lichtes nachgewiesen. Wird jedoch über dem Schädel (unter der Haut) eine opake Polyäthylen-Scheibe eingeschoben, so findet sich weder bei geblendeten noch bei Tieren mit Augen eine Orientierung nach dem *e*-Vektor. Wird die opake Plastikscheibe durch eine transparente ersetzt, so tritt in jedem Fall die Orientierung (± 180°) wieder auf. Die möglichen Mechanismen der Wahrnehmung polarisierten Lichtes durch extraokulare Rezeptoren werden diskutiert.

Introduction

The fact that many invertebrates can perceive the plane (*e*-vector) of linearly polarized light and use it in spatial orientation is well known, beginning with von Frisch's original discovery in 1948 of this phenomenon in bees. Evidence for similar abilities in vertebrates is sparse; indeed, it is often stated that vertebrates generally cannot perceive polarized light. Some humans, however, can see an image ("Haidinger's brushes") presumably produced by selective absorption of linearly polarized light in the *fovea centralis* (references in von Frisch, 1967). Montgomery and Heinemann's (1952) conditioning studies with homing pigeons failed to demonstrate discrimination between stationary polaroid panels placed at 90° to each other, although Kreithen and Keeton (MS)

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[*] We dedicate this paper to the memory of our colleague and friend Hobart F. Landreth, a pioneer in the study of amphibian orientation, who lost his life in a canoeing accident on 4 March 1973.

showed that rotating polarizers can be told apart by pigeons. Groot (1965) suggested that salmon use polarization patterns to orient (polarotaxis), especially at twilight when the sun is below the horizon.

More recently, Waterman and co-workers have reported additional evidence for polarotaxis in two species of *Zenarchopterus*, west Pacific teleosts (Waterman and Forward, 1970, 1972; Forward, Horch and Waterman, 1972). In these field studies, a linearly dichroic polarizer was placed above a circular test container in which individual fish were observed; the experimental vessels were placed several meters below water or on land where the water surface in the vessels was open to air. In both situations, the mean direction of movement of the fish was modifiable if the bearing of the polaroid was suitably altered. Since the fish demonstrated unidirectional orientation, these studies clearly indicate that cues other than the imposed plane of polarization are also used; linearly polarized light cues by themselves provide ambiguous information since the two "ends" of the *e*-vector cannot be distinguished.

In lab studies with salamanders *(Ambystoma)*, we have reported bidirectional orientation in aquatic and terrestrial situations when a linear polarizer is placed over the light source during training and testing (Adler and Taylor, 1971; Taylor, 1971; Taylor and Adler, 1973). The directions of movement are related to the *e*-vector and can be predictably altered by changing the bearing of the plane of polarization. Additional evidence for this interpretation is given in this paper.

In none of these studies with lower vertebrates has the receptor been identified which is involved in responding to polarized light; yet such information is essential to an understanding of the mechanism of polarization analysis. We now report that the receptor for polarized light in salamanders is an extraocular site in the head and that the eyes apparently are not involved at all. We here use the term "extraocular" to replace "extraoptic" of our earlier papers, since it refers more precisely to any light receptors other than the eyes. However, "ocular" sometimes also refers to any eye-like structure, a meaning clearly not intended in our usage of this term.

Methods

All experimental animals (tiger salamanders, *Ambystoma tigrinum*) were collected as metamorphosed adults on 5 March 1971 in St. Joseph Co., Indiana, and were kept in a greenhouse under natural lighting until training began. They were trained indoors in an elongate galvanized metal tank (122 cm long, 25 cm wide, 31 cm high) which was filled to a depth of 9 cm with water. A shore of bricks was provided at one end. The entire tank, including the area directly above it, was surrounded with opaque black plastic curtains so that no light penetrated from the outside; the ceiling was 81 cm above the water level.

Polarized light was produced by placing a linear dichroic polarizing filter (HN-38, Polaroid Corp.) beneath a piece of frosted glass which was backlighted with a 25 W tungsten bulb. The bulb was situated behind the center of the polaroid; the latter was suspended directly above the shore-line at a height of 22 cm. A circular aperture was placed over the filter so that the light pattern seen by the salamanders was 16 cm in diameter. The plane of polarization (*e*-vector) of the filter was perpendicular to the long axis of the training tank (thus, parallel to the shoreline) at all times. The light (intensity, 1295 lux or 190.3 $\mu W/cm^2$ measured at water surface) was turned on each day at 0600 for 14 hours (or *LD 14*:10 [*1295*:0 lux]). Light intensity was measured using an ISCO spectroradiometer (Instrumentation Specialties Co., model SR); intensities were summed from 400 to 750 nm; no differences in intensity were noted directly beneath the polarization filter when it was rotated 90° from its original axis.

Animals were trained in this apparatus for 21 days, beginning 17 July. Once each day during training every animal was removed from the shore, placed in the water at the opposite end of the tank and allowed to return to the shore. Salamanders were fed crickets and raw calves liver once, on 15 July, but never during training or testing.

All training and testing was conducted in the same room under constant temperature (22° C). The test arena was a plastic wading pool (160 cm diameter, 30 cm high) filled to a depth of 13 cm with fresh, clear tap water; the pool liner was pale blue in color. The arena was completely enclosed by an opaque plastic curtain which hung from the ceiling 237 cm above the level of the water. Observations were made through tiny holes cut in the plastic; all lights in the room were turned off except for the polarized light source which, during testing, was located at a height of 22 cm above the water and directly over the center of the arena. The identical polarized light source used in training was employed also in all testing, although the polarization filter (and thus the *e*-vector) was rotated 90° from the compass bearing used in training; training and testing apparatuses are diagramed in Taylor and Adler (1973).

Animals were placed individually in the center of the arena, directly beneath the polarized light source. The release device was an opaque cylindrical cup attached to one end of a one-meter stick. Each animal was held in the release device for 30 sec before the cup was removed from the arena. The animal was then observed as it moved from the center of the arena and was scored at the first point where it contacted the wall of the pool. Between tests, the water inside the arena was stirred vigorously to provide confusing olfactory cues for succeeding animals in the event they were using odors by which to orient.

Certain animals were blinded by pushing the eyes gently from beneath and removing them with scissors; no anaesthetics or cold treatment were used since these are known to affect endogenous rhythms. Most sockets bled profusely but healed over in a few days. In other animals an incision was made transversely behind the eyes and pieces of opaque or clear polyethylene plastic were inserted under the skin to cover the skull, similar to techniques described previously (Adler, 1971). Such pieces covered the entire head between the eye sockets and from immediately behind the nostrils to the posterior end of the skull. Between tests animals were kept in the training apparatus under the original conditions, except that the clear plastic was left in place in those individuals where plastic had been inserted beneath the skin of the head during testing. None of the operated or normal animals died during testing.

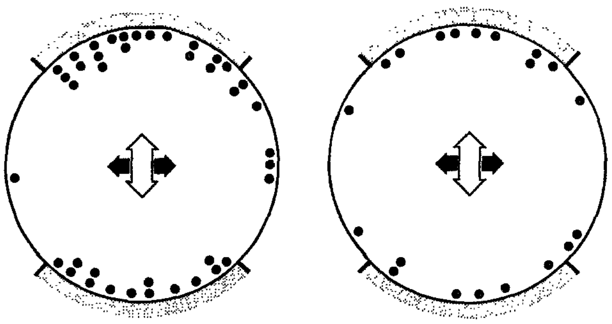
Training lasted from 17 July to 6 August 1971; all operations were performed on 4 August. Tests were done on 3, 4, and 6 August.

Table 1. Directional behavior of salamanders under linearly polarized light. The condition of the experimental animals, the testing date and the number of animals used in each test are indicated. The number of animals observed to move in the two expected directions and the chi-square statistic (df = 1) are given, along with the level of significance. In all instances where the chi-square value showed a significant deviation from a uniform distribution, the observed movement of animals was in the expected two quadrants around the test arena (see Fig. 1)

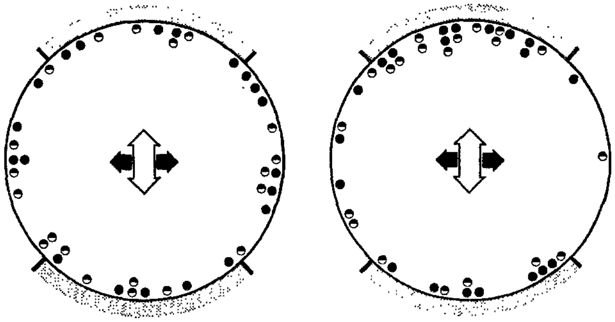
Condition	Date tested	N	Obs.	χ^2	Significance
Sighted	3 August	40	33	16.80	<0.005
Sighted, skin cut	6 August	20	15	5.00	<0.05
Sighted, opaque plastic	4 August	20	10	0.00	n.s.
Sighted, opaque plastic	6 August	20	9	0.20	n.s.
Sighted, clear plastic	4 August	20	16	7.20	<0.01
Sighted, clear plastic	6 August	20	15	5.00	<0.05
Blinded	4 August	19	16	8.89	<0.005
Blinded, skin cut	6 August	16	13	6.25	<0.025
Blinded, opaque plastic	4 August	19	8	0.47	n.s.
Blinded, opaque plastic	6 August	16	6	1.00	n.s.
Blinded, clear plastic	4 August	19	16	8.89	<0.005
Blinded, clear plastic	6 August	16	12	4.00	<0.05

Results

Results may be divided into those experiments where salamanders had both eyes intact and those where eyes were removed (Table 1). All normal, sighted animals (unoperated) moved in the trained directions (Fig. 1A, B, D) perpendicular to the *e*-vector of the linearly polarized light, except for that group possessing opaque plastic over the skulls. It was expected that salamanders would move in two opposite directions since the two "ends" of the *e*-vector cannot be distinguished; technically, a vector is unidirectional, but since the time average of electromagnetic vectors is zero, a net vectorial direction cannot be specified (except for the propagation direction). To assess the effects of the surgery which was necessary in order to insert the plastic, a control group was tested in which the skin was cut in the normal manner but no plastic was inserted; these animals moved in the expected directions (Fig. 1B). A second set of controls was run to assess the effect of the plastic beneath the skin of the head; in these animals a piece of transparent plastic was inserted but these animals also moved in the expected directions (Fig. 1D, 2 tests). Only those animals with opaque plastic over their skulls failed to move in the expected directions; their movement did not deviate from a uniform distribution within the test arena (Fig. 1C, 2 tests) even though their eyes were fully intact and undisturbed.



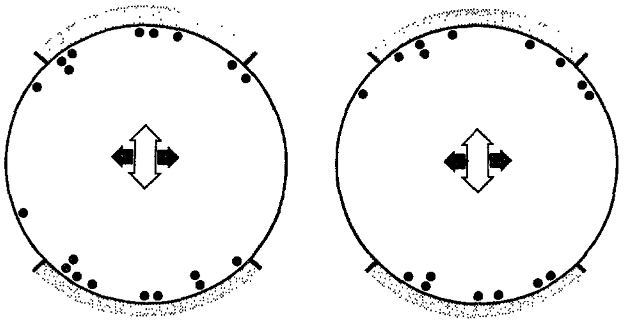
A: SIGHTED **B: SIGHTED, Skin Cut**



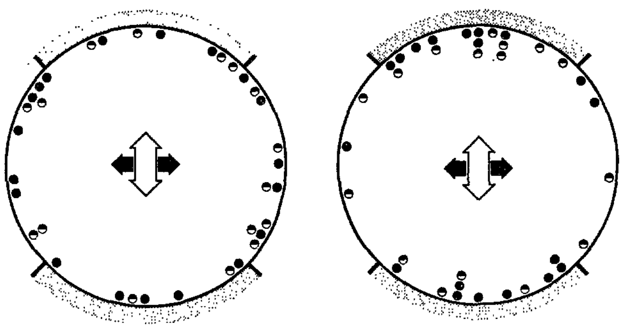
C: SIGHTED, Opaque Plastic **D: SIGHTED, Clear Plastic**

Fig. 1. Directional behavior of sighted and blinded salamanders under linearly polarized light. Animals were released individually in the center of the arena; the large circles represent the perimeter of the arena. The dots along the inside edge of each circle indicate where individual animals made first contact with the side and were scored; in composite diagrams, separate test and re-test are designated by solid and half-solid dots, respectively. Two quadrants, where the animals are expected to score if they can perceive the plane of polarization, are shaded along the edge; the hollow double-headed arrows indicate the expected direction of movement. The plane of polarization (e-vector) of the light source is indicated by a solid double-headed arrow. Note that all animals, whether blinded or not, can perceive the plane of polarization and use it to move non-uniformly in the arena, except for those where the head is covered with opaque plastic (C and G)

In the second set of experiments all animals were blinded, and the results paralleled the earlier tests with sighted animals. Salamanders which were simply blinded (Fig. 1E) or controls in which the skin was cut (Fig. 1F) or where clear plastic was inserted (Fig. 1H, 2 tests) all moved in the expected directions, perpendicular to the e-vector. Blinded animals which had opaque plastic over their skulls, however, did not show bidirectional oriented movement in the test arena; the direction of their activity did not deviate from uniform (Fig. 1G, 2 tests).



E: BLINDED **F: BLINDED, Skin Cut**



G: BLINDED, Opaque Plastic **H: BLINDED, Clear Plastic**

Fig. 1 E–H

Discussion

Taken together, these experiments are consistent with the hypothesis that the critical receptor for perception of the *e*-vector of linearly polarized light in *Ambystoma tigrinum* is an extraocular site located in the head. Apparently the eyes are not at all involved in this response, at least under the conditions of this particular set of experiments. The possibility that these animals were orienting to reflection patterns set up by the polarized light rather than directly to the *e*-vector itself is discussed elsewhere (Taylor and Adler, 1973); the apparatus and experimental design are such that this possibility is minimized if not eliminated.

If we assume that the critical receptor for polarized light in these animals is intracranial, it is obvious that passage of such light through the various layers of the skin and skull would have profound effects upon the characteristics of the light before it reached any receptor. Preliminary measurements have been made by a colleague, Michael Brines, using a sodium lamp polarimeter; quantitative results will be presented elsewhere (Brines, in preparation). Briefly, in *Ambystoma* the skin of the head is isotropic and greatly decreases the intensity of light transmitted. The cartilaginous skull also somewhat decreases intensity but has optical axes located along the body axes; it thus can be said to possess wave-plate characteristics. When the incident light is linearly polarized, transmission can vary from almost completely linearly polarized light to elliptically polarized light depending on the orientation of the skull's wave-plate axes to the incident linearly polarized light.

Of all of the intracranial structures that might be responsible for perception of polarized light, the pineal body *(epiphysis cerebri)* has properties which single it out for special consideration. It is located on the dorsal surface of the diencephalon, directly beneath that part of the skull which was covered with plastic in certain of our experiments. In salamanders as in probably most lower vertebrates, the pineal body has a photoreceptor-like ultrastructure resembling that of cones and rods in the lateral eyes (references in Adler, 1970). The photoreceptoral organelles of vertebrates, being of the ciliary line of evolution, differ markedly from the microvilli-possessing, rhabdomeric type characteristic of molluscs, annelids and arthropods (Eakin, 1968). Indeed, it is the special arrangement of these microvilli in certain invertebrates that is thought to account for the detection of linearly polarized light (Moody, 1964; Moody and Parriss, 1961; Waterman and Horch, 1966; Waterman, Fernández and Goldsmith, 1969). The number of photoreceptive elements in vertebrate extraocular sites (EOPs) is far less than in the corresponding retina of a given species and there is apparently no cornea or lens in amphibian EOPs. Furthermore, pineal photoreceptors differ in another, perhaps more important way from those of the lateral eyes – in a manner reminiscent of the camera-like eyes of the octopus. Unlike the optic cups which invaginate during development and thus invert the polarity of rods and cones, the elements of EOPs retain their original polarity so that their outer segments, composed of stacks of flattened discs, are turned toward the incident light (Eakin, 1968); this particular developmental sequence in analogous to that in molluscs.

How could pineal outer segments detect polarized light when similar structures in lateral eyes apparently cannot? As originally discovered by Schmidt (1934, 1935, 1936) and later expanded upon by Denton (1959), Liebman (1962), and Wald and co-workers (1962), the absorption of linearly polarized light in frog outer segments is independent of the bearing of the *e*-vector for light passing *axially* through the outer segment (that is, parallel to its long axis), which is the ordinary bearing for outer segments in the lateral eyes. If, however, the same light passes *transversely* through the same outer segment (that is, perpendicular to its long axis), the amount of absorption is directly related to the bearing of the *e*-vector, being maximal when the *e*-vector is parallel to the flattened, photopigment-bearing lamellae of the outer segments.

If these absorption properties exist also for the outer segments of pineal EOPs, then a possible mechanism for perception of polarized light by the pineal can be suggested which is consistent with our hypothesis that the lateral eyes of salamanders apparently cannot perceive such light. Although the geometry of outer segments in amphibial EOPs is not known in detail, it is known that many outer segments protrude horizontally into the lumen of the epiphysis (Oksche, 1965; Hendrickson and Kelly, 1969; Kelly, 1971) which would put them in such a position as to allow light to pass through them transversely; this is in contrast to the lateral eyes where such passage is ordinarily axial. Such a proposed mechanism could operate also if the light incident on the outer segments is elliptically, rather than linearly polarized. In this situation, absorption would presumably be maximal when the major semiaxis is parallel to the lamellae of the outer segments and would be expected to be directly proportional to the ellipticity or "slenderness" of the ellipse.

Recent studies have demonstrated the importance of EOPs for several behavioral and physiological activities in amphibians; the pineal body (or additionally its derivative in frogs, the frontal organ) has been implicated experimentally in several responses, including pigmentary adaptation (Bagnara and Hadley, 1970), sun-compass orientation (Taylor and Ferguson, 1970), and synchronization of circadian locomotor rhythms (Adler, 1969, 1970, 1971). The present study suggests to us that EOPs are able to perceive linearly polarized light for purposes of a directed locomotor response, a phenomenon termed polarotaxis (Waterman, 1966). Such an ability would presumably be adaptive underwater where polarization patterns exist which have a systematic relationship to the position of the sun, or during twilight when the sun cannot be directly perceived but when sky polarization is maximal; adaptive aspects are considered in more detail elsewhere (Taylor and Adler, 1973). In any event, because of the existence of EOPs in a wide variety of organisms, especially invertebrates, it may be fruitful to consider the possibility that EOPs may be involved in polarotactic responses in organisms other than salamanders. To date, such studies have not been made in investigations on perception of polarized light.

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