Spatial Orientation by Salamanders Using Plane-Polarized Light

Abstract. *Tiger salamanders* (Ambystoma tigrinum) *can perceive the plane of polarization in linearly polarized light and can learn to use that* e*-vector direction for spatial orientation in indoor orientation tests.*

The use of plane-polarized light for orientation in various invertebrates has been established (*1*); however, except for the work of Waterman and colleagues with fishes (*2*), polarized light reception has not been clearly demonstrated in vertebrates other than man. We present data which are interpreted to suggest that tiger salamanders can learn to perceive *e*-vector direction and use it as a clue for spatial orientation.

All experiments were performed with adult tiger salamanders (*Ambystoma tigrinum*) collected on 5 March 1971 in St. Joseph County, Indiana. The animals were kept in a greenhouse under natural lighting until training began. Different groups of animals were trained for a specified number of days (14 to 21 days). Animals were fed only once during training.

They were trained in a galvanized metal tank (122 cm long, 25 cm wide, and 31 cm high) filled to a depth of 9 cm with water. A "shore" of bricks (32 cm long and 25 cm wide) was provided at one end. Each day during training the animals were removed from the shore, placed in the water at the opposite end of the tank, and allowed to return to the shore.

The entire tank was enclosed with opaque black plastic curtains so that no light penetrated from outside. The ceiling was 81 cm above the water level, and polarized light was produced by superimposing a polarizing filter (HN-38; Polaroid Corp.) beneath frosted glass (8 mm thick) which was backlighted with a bulb. The polarization filter was located 22 cm above the shoreline. The plane of polarization (*e*-vector; Fig. 1A) was always perpendicular to the long axis of the tank during training (Fig. 1B). The light (1295 lux or 190.3 μw/cm^2) was turned on each day at 0600 for 14 hours (light-dark cycle, LD 14 : 10; 1295 : 0 lux). Intensity was measured with an ISCO spectroradiometer (Instrumentation Specialties Company) and was 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.

The test arena (a circular pale blue plastic pool 160 cm in diameter and 30 cm high) was filled to a depth of 9 cm with fresh tap water before each test and was completely enclosed by black plastic curtains. No known directional clues were present inside this arena. Observations were made through small holes cut in this plastic; all lights in the room were turned off except for the polarized light which, during testing, was 22 cm above the water surface and directly over the center of the arena (Fig. 1C). The polarization apparatus used in training was also employed in all testing, although the polarizing filter (and thus the *e*-vector) was rotated 90° from the compass bearing used in training.

Animals were placed individually into a release device (an opaque cylindrical cup 16 cm in diameter and 11 cm high) located at the center of the arena directly beneath the polarized light source. Each animal was held in the release device for 30 seconds before the cup was removed. The animal was then observed as it moved from the center and was scored at the first point at which it made contact with the pool wall.

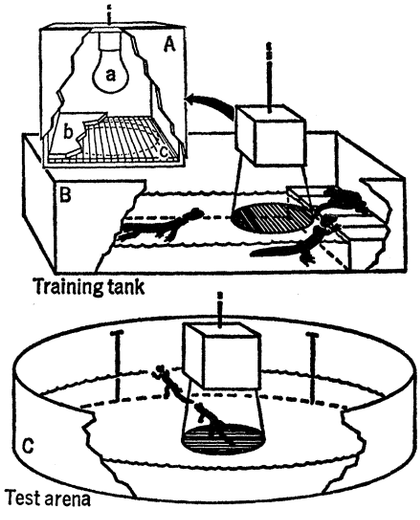

Fig. 1. (A) Schematic of the polarizing light source, viewed from the side; *a*, 25-watt tungsten bulb; *b*, frosted glass; *c*, HN-38 polarizing filter inserted between the frosted glass and an opaque shield in which a circular opening was cut (16 cm in diameter). (B) Schematic of the training apparatus. The relative positions of water, shoreline, and the polarizing light source are shown. The relative position of the *e*-vector is represented by the parallel lines and is perpendicular to the long axis of the tank (and thus parallel to the shoreline). (C) Schematic of the test arena with polarized light source in place. In testing, the *e*-vector (represented by parallel lines) has been rotated by 90° to reduce the possibility that some uncontrolled "geographical" clue for direction was present in both training and testing situations.

Between aquatic tests, water inside the arena was stirred to provide randomized olfactory clues for succeeding animals in the event that such clues were being used to orient. Individuals were tested several times each but only once on a given day.

Three experimental and two control tests were performed. In the control experiments, the orientational responses of untrained salamanders under either a nonpolarized light source (Fig. 2A) or a linearly polarized light source (Fig. 2B) were compared. Under these two conditions both groups moved in random directions in the aquatic test arena, a result indicating that there is no spontaneous (nontrained) directional movement with respect to the *e*-vector in these animals. Movement of both groups was evaluated by the Raleigh test (*3*) [$r = .14$ (Fig. 2A); $r = .51$ (Fig. 2B)] and found not to deviate from random.

Following the control tests, one experimental group (13 animals) was trained to move perpendicular to the shoreline under the linearly polarized light source for 14 days and then tested in the aquatic arena under the same linearly polarized light source with the *e*-vector rotated 90° from its position in training (Fig. 2C). If the salamanders were using the plane of polarization to move in the test arena, they were expected to move perpendicular to the *e*-vector.

All individual tests except one, as well as the composite group score (Fig. 2C), were significantly different from random (pooled $\chi^2_{1,.05} = 19.69$); movement was in the predicted directions. A chi-square test for homogeneity indicated that the pooled test score of animals tested on different days was not significantly different from each subsample ($\chi^2_{3,.05} = .19$). This group of salamanders was tested four times at 1- to 2-day intervals.

A second group (14 animals) was trained similarly for 21 days before testing in the aquatic arena for a total of five times at 1-day intervals under the linearly polarized light source (Fig. 2D). No significant differences

between pooled tests and individual subsamples were indicated by a composite test for homogeneity ($\chi^2_{3,.05} = .79$). The directional responses of these animals were similar to those obtained in the first experimental group, that is, a strongly nonuniform response (pooled $\chi^2_{1,.05} = 20.63$) in directions perpendicular to the *e*-vector.

A third group (40 animals) was trained to swim to a shoreline under identical conditions for 18 days and then tested once only in the arena under the same light conditions as the preceding experiments but with the arena dry (Fig. 2E). Directional responses of these animals were also significantly different from uniform ($\chi^2_{1,.05} = 16.9$) and perpendicular to the *e*-vector.

We conclude that tiger salamanders can learn to use the *e*-vector of polarized light for spatial orientation. To date, only in fishes is there significant evidence that vertebrates other than man can perceive plane-polarized light (*2*). Although many invertebrates are capable of perceiving plane-polarized light, the mechanism of perception remains conjectural (*4*); however, recent research with crayfish has led to the development of new theories of perception for invertebrates (*2*). Much of the past difficulty arose from failure to separate responses due to perception of plane-polarized light (polarotaxis) from those due to brightness patterns produced by reflection or scattering of polarized light (phototaxis) (*1*). Many invertebrates are capable of perceiving, and responding to, both of these clues; however, it is now known that these animals can respond to each separately (*5*).

Although it is possible that our salamanders were utilizing brightness patterns in addition to the *e*-vector, this appears unlikely for two reasons: (i) The aquatic tests were performed in clear water, which would minimize differential scattering of polarized light and the concomitant production of brightness patterns. (ii) Ambystomatids typically are negatively phototactic (*6*) and, as such, their typical response should have been parallel (not perpendicular) to the *e*-vector since the region of maximum reflection (and thus brightness) is perpendicular to the *e*-vector.

The means of *e*-vector perception in these animals remains unclear although recent evidence points to an extraoptic site of reception (*7*).

All of our tests on the perception of polarized light have been performed indoors; thus we cannot present direct evidence as to any adaptive value which this ability might have for salamanders. However, knowledge of the natural history of these animals and of certain physical properties of polarized light in nature suggest potential utility. The ability of amphibians to use a sun compass in spatial orientation is well documented (*8*). Since the *e*-vector of linearly polarized light bears a fixed relationship to the sun's position, it can be used for direction-finding under clear-sky conditions when the sun is not in view, as demonstrated for several invertebrates (*9*). However, use of polarized light has several additional advantages. Polarization of the sky is maximal after sunset (*1*), a time when many amphibians are either moving from place to place or possibly taking up a bearing preparatory to movement later in the evening. Also, animals living in photic aquatic zones are exposed to patterns of polarized light nearly as intense as those to which terrestrial counterparts are exposed (*10*). If oceanic physical measurements are indicative of those in fresh water, down to a depth of about 6 m the water's surface acts as a wide-angle lens, effectively providing a 180° aerial field view to an underwater observer. Because of refraction, the entire celestial hemisphere is constricted to a circular area subtending an angle of about 96° centered on the vertical. Thus, such atmospherically produced polarization observed underwater at this depth is an extension of the sun compass as observed terrestrially, except that altitudinal distortion occurs because of refraction and surface roughness. Beyond the angle of total reflection at this depth and in all directions at depths of 15 m and below, another component of polarized light exists, that produced by the aquatic medium itself. Since both atmospherically and aquatically produced plane polarization of light bears a systematic relationship to the position of the sun, these clues—if perceived—could be useful in orientation when the sun cannot be perceived directly. Indeed, because of absorption and scattering of radiant energy in an aquatic medium, the sun's disk is perceivable as such only at the surface, yet its radiance distribution and, especially, polarization patterns are perceivable at

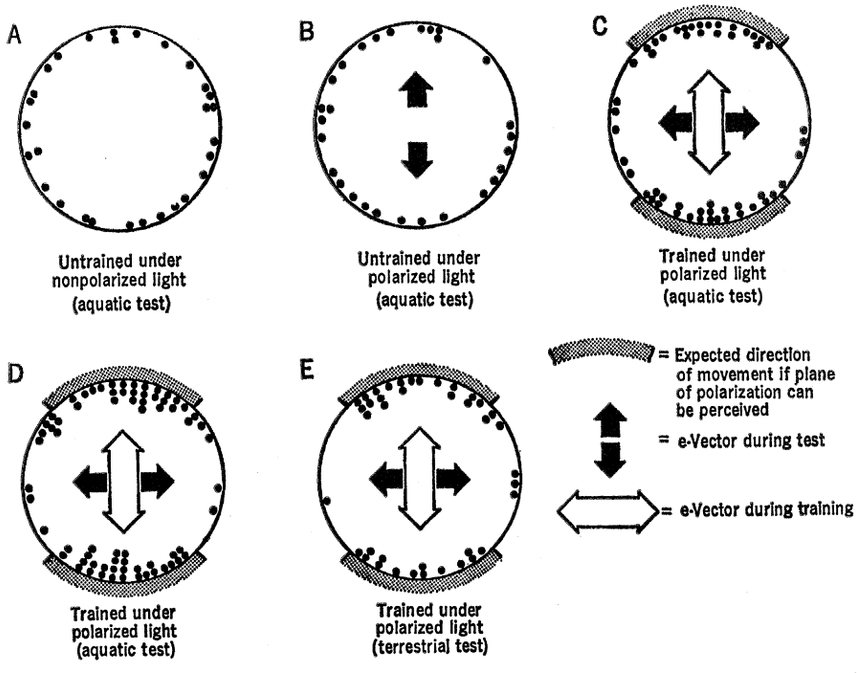

Fig. 2. Orientation responses of tiger salamanders under plane-polarized light. Each dot represents one animal, and the position of the dot represents the point at which the salamander made contact with the arena wall. Those quadrants where the salamanders are expected to score if they can perceive the plane of polarization (*e*-vector) are indicated by stippling. The plane of polarization is indicated by the solid arrows (test position) and by open arrows (training position). The relative position of the *e*-vector in testing is rotated 90° from that used in training. For the purposes of this study it is the relative bearing of the *e*-vector in these two situations that is critical and not the absolute geographical headings.

depths in seawater in excess of 200 m (*11*). Although salamanders are rarely found at depths below 10 m, the ability to perceive polarized light may have several distinct advantages. Amphibians in temperate zones typically migrate overland to shallow breeding pools after sundown and often move about on the pond bottom during the day. Especially in wooded areas or others where direct view of the sun is obscured by landmarks during much of the day, the ability to perceive plane-polarized light would provide a relatively continuous knowledge of the sun's position throughout the day and even for a time after sunset.

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Maternal Influences on Mouse Embryos and Preservation of Mutant Strains by Freezing

Whittingham *et al.* (*1*), in suggesting that frozen mouse embryos may be used to preserve mutant strains, have made no allowance for maternal modifications. Even if prolonged viability is attainable, the ova-transfer substrain [distinguishable by the symbol e (*2*)] would not be expected to be identical to the parental strain from which the fertilized ova were obtained if influences exerted on the developing fetuses by their foster-mothers resulted in phenotypic modifications. Several types of experimental data support this contention.

The foster nursing experiments of Bittner (*3*) exemplify the early investigation of maternal influences. Law reported that foster nursing alone influenced the growth of transplantable leukemias in mice (*4*). Foster nursing alone was then found to affect both resistance and sensitivity to x-irradiation (*5*).

The technique for ova transplantation in mice (*6*) was developed at the Jackson Laboratory more than 30 years ago for the purpose of investigating intrauterine influences. Cloudman demonstrated that tumors of the genotype of the foster-mother grew progressively and killed recipients of the ova-transfer substrain, but not recipients of the ancestral strain, even though the tumors were of a different histocompatibility-2 (*H-2*) genotype from the recipients (*7*). Mice of the ova-transfer substrain C3HeB, exposed to a radiation dose lethal to some but not all animals and then inoculated with bone marrow of the $H\text{-}2^b$ or $H\text{-}2^d$ genotype, were found to exhibit changes specific for the *H-2* genotype of the foster-mother (*8*) and to have a shift in radiation sensitivity (*9*).

The ova-transfer substrains RIIIeB, DBA/2eB, C3HeB, and C3HeD and the foster-nursed substrain C3HfB were investigated for maternal alteration by using marrow transplantation into lethally irradiated recipients. At least one modification was demonstrable in each artificially derived substrain. No two ova-transfer substrains exhibited identical modifications. However, the genetic relationship between the transplanted ova and the foster-mother was different in each case; consequently, it was assumed that the type of alteration occurring was a function of the genetic disparity between the two strains. Maternal modifications of several types were documented. (i) RIIIeB mice were more sensitive to irradiation than either the ancestral strain or the strain of the foster-mother. They also had a reduced capacity to respond to tissue antigens of the genotype of the foster-mother, as shown when the immunogenicity of these antigens was quantitatively reduced by hybridization (*10*). (ii) DBA/2eB mice acquired an increased capacity to respond to certain tissue antigens, their maternal response being intermediate between that of the ancestral and foster lines (*11*). (iii) C3HeD mice also acquired an increased capacity to respond to certain tissue antigens. The foster-nursed substrain C3HfB, on the other hand, had a decreased capacity to respond to these antigens, apparently acquired from their foster great-great-grandmother C57BL (*12*). (iv) C3H and the derived substrains C3HfB, C3HeB, and C3HeD were tested for their capacity to recognize C3H as "self" or to be recognized as "self" by the other substrains. The two ova-transfer substrains, C3HeB and C3HeD, had acquired an increased antigenicity as compared with the foster-nursed substrain C3HfB (*13*). This change in antigenicity was detectable by marrow transplantation but not by reciprocal skin grafts between substrains.

The observed phenotypic alterations are permanent and vertically transmitted from mother to offspring for many generations, since none of the test mice had contact with allogeneic foster parents. From the available evidence it must be assumed that frozen ova transplanted into foster-mothers would be similarly modified by their fetal development in a foreign environment and thus be endowed with subtle differences characteristic for the substrain and absent in the ancestral strain. Since antigenicity and immunogenicity of hybrid tissues are also modified by their maternal environment, it was postulated that this phenomenon represents an immunologic adaptation that prevents rejection of the fetus by the mother (*14*). The same biological