USE OF A SPECIALIZED MAGNETORECEPTION SYSTEM FOR HOMING BY THE EASTERN RED-SPOTTED NEWT *NOTOPHTHALMUS VIRIDESCENS*

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Summary

Laboratory experiments were carried out to investigate the effects of varying the wavelength of light on the use of an earth-strength magnetic field for shoreward orientation and for the compass component of homing. In the earlier shoreward orientation experiments, newts tested under full-spectrum and short-wavelength (i.e. 400 and 450 nm) light exhibited shoreward magnetic compass orientation. Under long-wavelength (i.e. 550 and 600 nm) light, newts exhibited magnetic compass orientation that was rotated 90° counterclockwise to the shoreward direction. This wavelength-dependent shift in magnetic compass orientation was shown to be due to a direct effect of light on the underlying magnetoreception mechanism. In homing experiments, newts tested under full-spectrum and short-wavelength light exhibited homeward magnetic compass orientation. Under long-wavelength light, newts were randomly distributed with respect to the magnetic field. The different effects of long-wavelength light on shoreward orientation and homing confirmed earlier evidence that different magnetoreception systems mediate these two forms of orientation behaviour. The properties of the newt's homing response are consistent with the use of a hybrid magnetoreception system receiving inputs from the light-dependent magnetic compass and from a non-light-dependent intensity (or inclination) detector which, unlike the compass, is sensitive to the polarity of the magnetic field.

Introduction

Map-based homing (also referred to as true navigation) is the ability of an organism to return to its point of origin ('home'), after a displacement into unfamiliar territory, without reference to familiar landmarks, goal-emanating cues or directional information obtained during the displacement. Map-based homing has only been demonstrated in vertebrates (e.g. Walcott and Schmidt-Koenig, 1973; Rodda, 1984*a,b*, 1985; Wallraff, 1990; J. B. Phillips, K. Adler and S. C. Borland, in preparation) and requires both a geographic-position sense that is derived from spatial information available at the release site (the 'map') and a directional sense (the 'compass'). While vertebrates are known to use a variety of compass mechanisms (e.g. Ferguson, 1971; Taylor and Auburn, 1978; Wiltschko, 1983; Phillips, 1986*a*), the sensory basis of the map, or geographic-position

sense, remains controversial and poorly understood (e.g. Papi, 1990; Schmidt-Koenig, 1987; Walcott, 1991).

Interest in the possibility that spatial gradients in the geomagnetic field could provide one or more coordinates of a bicoordinate or multicoordinate map sense (the 'magnetic map' hypothesis; Gould, 1980; Moore, 1980; Walcott, 1980) has stemmed from evidence that temporal and spatial variations in the geomagnetic field of less than 1% are associated with changes in the direction and/or scatter of homing orientation (e.g. in the homing pigeon *Columba livia*: Keeton *et al.* 1974; Larkin and Keeton, 1976; Walcott, 1978; Kiepenheuer, 1982, 1986; Wagner, 1983; Kowalski *et al.* 1988; Lednor and Walcott, 1988; Schmidt-Koenig and Ganzhorn, 1991; in the American alligator *Alligator mississippiensi*: Rodda, 1984*a*). Such small changes in the magnetic field are unlikely to affect the magnetic compass (Wiltschko and Wiltschko, 1972) or, as a consequence, to affect map information derived from route-based positional information that requires only a compass. In contrast, a bicoordinate (or perhaps multicoordinate) map derived, at least in part, from subtle geographic gradients in the magnetic field (i.e. gradients in magnetic parameters such as inclination and/or total intensity) could be strongly affected by natural variation in the magnetic field. This is because the normal geographic variation in these magnetic field parameters is only about 0.01–$0.03\%\ km^{-1}$. If a magnetic map is used for homing, therefore, extremely small spatial or temporal irregularities in the magnetic field could generate large errors in estimates of home direction.

Critical tests of the magnetic map hypothesis have been difficult to carry out, largely because homing experiments must typically be carried out under field conditions where the apparent effects of spatial and temporal variation in the magnetic field on homing are difficult to interpret. Under such conditions, changes in homing orientation that appear to be associated with natural magnetic variation could be caused by some factor other than the magnetic field (Walcott, 1991) or could result from an effect of the magnetic field on some component of the navigational system other than the map (e.g. the magnetic compass, Southern, 1978, or the time compensation mechanism for the sun compass, Papi *et al.* 1983; but see Rodda, 1984*a*).

In order to avoid the difficulties of interpretation that arise when experiments are carried out under field conditions, we have carried out laboratory experiments to examine the role of the earth's magnetic field in homing by the eastern red-spotted newt *Notophthalmus viridescens*. The eastern newt is the only organism in which long-distance map-based homing can be studied in the laboratory (Phillips, 1987; J. B. Phillips, K. Adler and S. C. Borland, in preparation), where critical stimulus parameters can be precisely and independently manipulated (Phillips, 1986*a*, and this paper). Homing ability appears to be well developed in this group of salamanders. Western newts (*Taricha rivularis*) have been shown to return to traditional breeding sites after being displaced by up to 12 km (Twitty *et al.* 1966).

Most studies of the map component of homing have investigated the effects of alteration or elimination of potential sources of positional information that could be used for homing (see earlier references). When examining the involvement of the earth's magnetic field in homing, however, characterization of the underlying receptor mechanism(s) provides an alternative and complementary approach. This is because the

high sensitivity that would be necessary to derive map information from the subtle geographic gradients in the magnetic field is likely to require a different type of magnetoreception mechanism from that used solely to determine compass direction (e.g. Kirschvink and Walker, 1985; and see below). As a consequence, if the magnetic field is involved in the map component of homing, the magnetoreception mechanism used to detect the magnetic field and, therefore, the functional properties of the newts' response to the magnetic field during homing, may differ from that of newts exhibiting a compass response (e.g. shoreward orientation) that does not require map information.

Newts held in a water-filled outdoor tank with an artificial shore at one end will exhibit either shoreward magnetic compass orientation or homing depending on the time of year and the temperature conditions to which they are exposed prior to testing (Phillips, 1987). In a previous study (Phillips, 1986*a*), newts that were orienting towards the shore were found to utilize an inclination or dip-angle magnetic compass similar to that used by migrating birds (Wiltschko and Wiltschko, 1972; Beason, 1989). As predicted for this type of compass, an inversion of the vertical component of the magnetic field (which produces a reversal of the dip angle without affecting the horizontal polarity) caused shoreward-orienting newts to shift their direction of orientation by approximately 180°. In contrast, newts that were homing were unaffected by an inversion of the vertical component of the magnetic field (Phillips, 1986*a*), i.e. they appeared to respond to the horizontal polarity, rather than the dip angle, of the magnetic field. These data suggest that a separate magnetoreception system may be involved in homing.

More recent studies have demonstrated that the magnetic compass used by newts for shoreward orientation is light-dependent (Phillips and Borland, 1992*a–c*) as suggested by earlier neurophysiological studies of birds (Semm *et al*. 1984; Semm and Demaine, 1986). The shoreward magnetic compass response of newts was found to undergo a 90° rotation under long-wavelength visible light (Phillips and Borland, 1992*b,c*). Phillips and Borland (1992*c*) demonstrated that this 90° rotation resulted from a direct effect of light on the underlying magnetoreception mechanism and proposed a model to explain how a change in the wavelength of light could alter the directional response of a light-dependent magnetoreception mechanism.

The present experiments were carried out to compare the effects of variation in the wavelength of light on the use of the magnetic field for shoreward compass orientation and homing. Our findings, which show that these two forms of orientation behaviour exhibit different patterns of wavelength-dependence, provide further evidence for the involvement of a specialized magnetoreception system in homing.

Materials and methods

Experimental subjects

Adult male eastern red-spotted newts *Notophthalmus viridescens* were used in these experiments. Male newts displaced to a testing facility from a variety of directions have been shown to be able to orient in the direction of their home pond (Phillips, 1987; J. B. Phillips, K. Adler and S. C. Borland, in preparation). In the homing experiments, newts were seined from a group of ponds located approximately 50 km south-southwest of the

testing facility, which is located on the Indiana University campus in Bloomington, Indiana, USA. Except when male newts were in reproductive condition, they were held prior to training in 120 l water-filled all-glass aquaria in the laboratory building and fed salmon pellets (Rangen Inc.) three times per week. When freshly collected males were in reproductive condition, they were held for several days in an aquarium with only moist gravel or shallow (i.e. less than 1 cm) water in the bottom until they began to transform into the terrestrial form (i.e. cornified skin, reduced tail fin). This treatment was necessary because male newts in reproductive condition had difficulty moving in the test arena because their smooth skin and wide flat tails tended to stick to the Plexiglas surface (see description of test arena below).

Training tanks

Training tanks consisted of water-filled 120 l all-glass aquaria (90 cm×30 cm×45 cm) located outdoors 13–15 m from the laboratory building. The training tanks contained an artificial shore consisting of a sheet of opaque Plexiglas which sloped upwards at one end of the tank. Shelter was provided at the shallow end of the tank (Phillips and Borland, 1992b). Water was circulated up from beneath the Plexiglas floor of the training tank at the shallow end by means of a pair of air stones. The water flowed towards the deep end of the tank through a Plexiglas grille, which prevented the newts from leaving the water, and returned beneath the floor through a grid of small holes at the deep end. The sides of the tanks were enclosed in clear 'bubble plastic' (Consolidated Plastics) to provide insulation for year-round testing. The tops of the tanks were covered with Pyrex glass, which is transparent to both visible and near-ultraviolet light. The glass covering the half of the tank above the deep end was frosted to diffuse the incoming light and to help eliminate shadows. Finally, the outermost layer on the top and sides of the training tank consisted of 2–4 layers of aluminium window screening to decrease the intensity of sunlight, which otherwise caused overheating during the summer months.

Water temperature plays a crucial role in eliciting magnetic compass orientation in newts (Phillips, 1986b, 1987). In the present experiments, training tank water temperature was controlled by circulating water from a 6000 l underground cistern located 6–9 m from each training tank. Water from the cistern was circulated through a glass heat exchanger located beneath the Plexiglas shore of the tank. The water was pumped from the cistern to the training tank by means of a pneumatic pump located in a small wooden pump house above each cistern. The air supply for the pneumatic pumps was produced by an air compressor located in a small shed approximately 45 m from the laboratory building.

Three training tanks were used in these experiments. The tanks were located to the east, south and west of the laboratory building with the shore end of each tank towards the building (i.e. the shore directions were west, north and east, respectively). Groups of newts were placed in tanks in which the shoreward direction differed from the home direction to distinguish shoreward orientation from homing (see Phillips, 1987).

Testing facility

The Animal Orientation Research Facility at Indiana University was designed specifically for studies of magnetoreception and magnetotactic orientation (Phillips and

Borland, 1992*b*). Experiments were carried out in a 6.5 m×6.5 m testing room in which the humidity was elevated to nearly 100 % saturation and the temperature was maintained at 29–31 ˚C for shoreward orientation tests and at 24–27 ˚C for homing tests. The newts' orientation was observed in an enclosed terrestrial arena (72 cm diameter). The floor of the arena consisted of Plexiglas marked with a circular grid which was used to record the newts' directional responses. The Plexiglas was supported by a 1.25 cm thick sheet of plate glass to prevent sagging. The central area of the arena floor on which the newts moved was machined to form a conical surface 41 cm in diameter that sloped up towards the edge at an angle of approximately 4 ˚. The conical surface was designed to mask any variation in the level of the arena floor which had been shown to bias the orientation response of newts in earlier tests (Phillips, 1986*b*). Despite the 4 ˚ slope, however, slight elevations of one edge of the conical surface (i.e. elevations of less than 0.1 ˚) produced strong orientation biases that masked any response to the magnetic field. The ability of newts to respond to such small slopes has been confirmed in an independent series of experiments (J. Schaefer, J. B. Phillips and S. C. Borland, unpublished data). Only in tests in which this response to the slope of the arena was eliminated could the newts' magnetic compass response be studied.

In later tests, to facilitate levelling the arena surface, the Plexiglas arena surface was replaced with a conical surface made of borosilicate glass (Continental Optics Corp.). The top edge of the glass was machined to a flat surface that varied by less than 0.00025 cm. In addition, the arena was equipped with precision electronic inclinometers (Lucas Shaevitz LSO) that could detect changes in the level of the arena surface of as little as 0.001 ˚.

For testing, newts were placed individually in a release device consisting of a vertical Plexiglas cylinder (7.5 cm inside diameter) located in the centre of the arena. The release cylinder could be lowered until it was flush with the arena floor by means of a hydraulic mechanism controlled by the observer in the adjacent room. The floor of the release device consisted of a stationary vertically aligned cylindrical chamber (7.49 cm diameter × 20 cm high), the top of which was level with the arena floor. Water from a temperature-controlled waterbath was circulated through the cylindrical chamber to warm the floor of the arena in the centre of the release device. The water temperature was increased (in increments of 1–2 ˚C to a maximum of 37 ˚C) after trials in which a newt failed to move from the centre of the arena. The circulating waterbath was turned off during trials to eliminate any vibration that might bias the newts' orientation.

A newt's movements were monitored by means of its silhouette visible through the floor of the arena and reflected in a 45 ˚ mirror located underneath. A video camera, pointed at the mirror from a location 3 m from the centre of the arena, allowed an observer in the adjacent room to follow the newt's movements on a video monitor. The arena was illuminated from above by means of a 150 W xenon arc lamp. The arc lamp was located in the adjacent room 6 m from the centre of the test arena to minimize electromagnetic disturbance. Light from the arc lamp was projected through a 10 cm diameter polyvinylchloride pipe and reflected down into the arena by means of a front surface mirror. The mirror was positioned above two 75 cm diameter frosted Pyrex glass diffusers centred above the arena.

Shoreward magnetic compass orientation and homing were examined under 400, 450, 550 and 600 nm light. The wavelength and intensity of light were controlled by placing interference and neutral-density filters in a filter carriage immediately in front of the arc lamp. The intensity of the light stimuli was measured by placing a calibrated photodiode (United Detector Technologies PIN 10DP/SB) at the centre of the arena floor. The quantal flux at 450, 550 and 600 nm was adjusted to 12.65 ± 0.10 log quanta cm^{-2} s^{-1}. At 400 nm, it was possible only to obtain a quantal flux of 12.35 ± 0.10 log quanta cm^{-2} s^{-1}. The same quantal flux was used at each wavelength in the shoreward orientation and homing experiments. In each test, controls tested under full-spectrum light were alternated with experimental subjects tested under a specific wavelength to ensure the comparability of data between tests.

Newts were tested in four horizontal magnetic field alignments (see below), i.e. the ambient magnetic field (magnetic north at north) and three artificial magnetic fields (magnetic north rotated to east, south or west). The rotated fields closely resembled the ambient field in inclination (±1–$2\,°$) and total intensity ($\pm5\,\%$). Rotation of the magnetic field was accomplished using the two-cube surface-coil system described by Phillips (1986*b*). In the present experiments, each of the cube coils was wrapped with two strands of wire. When current was flowing in the same direction in the two strands, the coil produced an artificial magnetic field. However, when the connections to one of the strands were reversed so that current in the two strands flowed in the opposite direction, there was no net effect on the magnetic field (Phillips, 1986*a*). The output of the power supplies (Lambda Electronics LQ-533) controlling the two coils remained the same in all four horizontal alignments of the magnetic field.

Testing procedures

Groups of newts were placed in a training tank at least 5 days prior to testing. Prior to the day of testing, the training tank water temperature was maintained between 12 and 22 °C, and generally varied by less than 2 °C within a 24 h period and by less than 3–4 °C during the entire period that the newts were in the tank.

Shoreward orientation

Experiments examining shoreward orientation were carried out intermittently from late spring to early autumn of 1989, 1990 and 1991. At sunrise on the day that a group was to be tested, the circulation system was disconnected from the underground cistern and routed through a small (8 l) reservoir located in the pump house and containing 2–3 heaters totalling 2.5 kW. A thermistor probe placed in the training tank and connected to a temperature controller (Cole-Parmer, Dyna Sense 2158) was used to regulate water temperature. Immediately prior to testing, the temperature of the water in the training tank was rapidly elevated to 25 °C and then, after removing all but a single 1 kW heater, more slowly to 31.5 °C. Newts in the Bloomington area have been found living in natural bodies of water at temperatures as high as 34 °C. The water temperature was then maintained at $31.5\pm0.5\,°$C for the duration of the tests.

Homing

Homing orientation was studied in tests carried out during the late autumn and early winter (November–January) of 1990/91 and 1991/92, and during the late spring and early summer (May–July) of 1992 and 1993. For the homing experiments, the training tank water temperature was lowered to 1–5 °C on the night prior to testing (see Phillips, 1987, which contains a discussion of the biological significance of the response of eastern newts to variation in water temperature). To accomplish this, the water circulation system was disconnected from the cistern and antifreeze was added to the remaining water that circulated through the heat-exchange coils. Remote cooling coils from 2–4 refrigeration units (Grant model CZ2 or Lauda model IC-6) were placed in the small reservoir in the pump house and connected to the temperature controller, which was set to approximately 1–2 °C. A single 500 or 1000 W heater located in the small reservoir and regulated by the temperature controller prevented the training tank water from freezing when the air temperature was less than 0 °C. The time required to lower the training tank water temperature was 6–10 h depending on outside air temperature. On the following morning, beginning at or before dawn, the training tank water temperature was raised to 31.5 °C in the same manner as in the shoreward orientation experiments (see above).

Identical testing procedures were used in the shoreward orientation and homing experiments. Each newt to be tested was removed from the shallow end of the training tank by grasping it gently by the base of the tail. It was then placed in a small plastic transport box freshly rinsed with water from the training tank. The plastic box was placed inside a light-tight cloth bag and carried into the testing room. Upon entering the testing room, the newt was removed from the transport box in total darkness and gently placed in the release device from a constant direction. Newts that struggled violently or received rough handling at any stage of transportation to the test arena were not tested. After the observer had left the room, the arena was illuminated by opening a shutter in an outer room and, following a 60 s delay, the newt was released. The newt's directional response was measured at the point at which it first contacted a 20 cm diameter circle centred on the release device. Bearings obtained from newts that were startled by the release device (i.e. newts that exited immediately after the release device had been lowered and/or scored at the 20 cm radius circle in less than 1 min) were not used. Previous work has shown that such animals exhibit a randomly oriented escape response (see Phillips, 1986*b*). Furthermore, a trial was discontinued if the newt either did not leave the centre of the arena within 8–10 min (newts in the centre of the arena were not visible to the observer) or did not reach the 20 cm radius circle within 15 min.

Each newt was tested only once. Roughly equal numbers of newts were tested in each of the four field alignments (see above). This testing protocol made it possible to eliminate any consistent non-magnetic bias from the data when the magnetic bearings were pooled from newts tested in the four field alignments (Phillips, 1986*b*). A given test lasting 3–5 h generally yielded 4–10 bearings (i.e. 2–5 experimentals and 2–5 controls). Typically, an equal number of newts in each test failed to meet the time criterion described previously. To achieve the balanced design necessary to eliminate any non-

magnetic bias, data were pooled from a series of tests, each involving a new group of newts.

For data analysis, magnetic bearings from newts tested in the four horizontal alignments of the magnetic field were pooled with respect to the direction of shore in the training tank or with respect to the direction of the home pond. Data were analyzed according the procedures in Batschelet (1981). The Rayleigh test was used to test for a significant clustering of bearings; the 95 % confidence interval around the mean vector direction was used to test for orientation with respect to a predicted direction; the Watson U^2-test was used to test for differences between distributions.

Results

Shoreward orientation

Newts tested under short-wavelength (i.e. 400 and 450 nm) light were oriented towards shore (Fig. 1B) and their orientation was indistinguishable from that of full-spectrum controls (Fig. 1A) (statistics in Table 1). In contrast, newts tested under long-wavelength (i.e. 550 and 600 nm) light exhibited significant magnetic orientation (Fig. 1D), but in a direction that was rotated approximately 90° counterclockwise from the shore direction. The orientation of newts tested under long-wavelength light (Fig. 1D) was significantly different from that of the corresponding full-spectrum controls (Fig. 1C) and from that of newts tested under short-wavelength light (Fig. 1B).

Homing orientation

Newts tested under short-wavelength (i.e. 400 and 450 nm) light were homeward oriented (Fig. 2B) and their orientation did not differ significantly from that of full-spectrum controls (Fig. 2A), although the difference approached significance ($0.10 > P > 0.05$, Watson U^2-test) (statistics in Table 1) owing to an increase in scatter under 400 and 450 nm light. Under long-wavelength (i.e. 550 and 600 nm) light, newts in the homing experiments were randomly oriented with respect to the magnetic field (Fig. 2D) and their results differed significantly from those of full-spectrum controls (Fig. 2C) and from those of newts tested under short-wavelength light (Fig. 2B). When the magnetic bearings from the homing experiments were pooled relative to the shore directions of the training tanks in which the newts had been held prior to testing, there was no evidence of shoreward magnetic compass orientation by full-spectrum controls or by newts in either of the wavelength treatments ($P > 0.10$, Rayleigh test; data not shown).

Discussion

Our previous work has shown that newts exhibiting shoreward compass orientation and homing respond differently to an inversion of the vertical component of the magnetic field (Phillips, 1986a; and see earlier discussion). These findings suggest that the magnetoreception mechanism(s) involved in homing differ from the mechanism involved in shoreward compass orientation (Phillips, 1986a). Subsequent studies have shown that
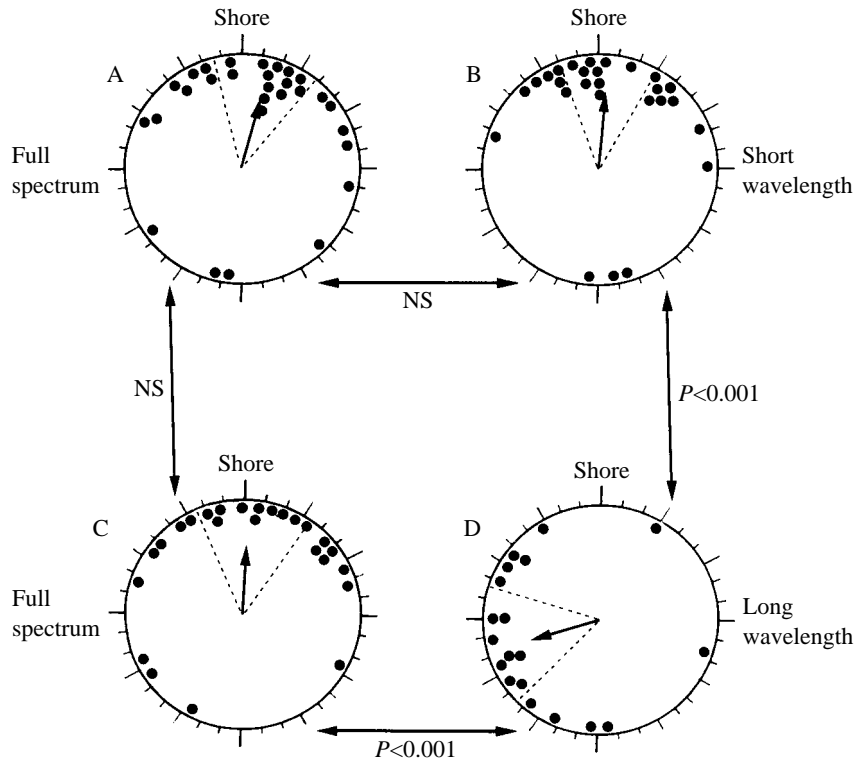
Fig. 1. Dependence of shoreward magnetic compass orientation on the wavelength of light (data from Phillips and Borland, 1992*b,c*; statistics in Table 1). Circular distributions on the left (A and C) show the magnetic bearings of controls tested under full-spectrum light, and on the right (B and D) the magnetic bearings of experimental newts tested under specific wavelengths of light. (A) Controls tested under full-spectrum light oriented towards shore. (B) Experimentals tested under 400 and 450 nm light also oriented towards shore and were indistinguishable from controls. (C) Full-spectrum controls were again oriented towards shore. (D) Newts tested under 550 and 600 nm light oriented approximately 90° counterclockwise to the shore direction and were significantly different from the full-spectrum controls and from newts tested under 400 and 450 nm light. Each dot represents the magnetic bearing of an individual newt that was tested only once in one of four symmetrical alignments of the magnetic field (see Materials and methods). The magnetic bearings are plotted with respect to the shore direction. Mean vectors (**r**) are indicated by arrows originating at the centre of the circular diagrams with the radii of the circles corresponding to **r**=1. Dashed lines indicate 95 % confidence intervals for the mean vector bearings. NS, not significant.

the magnetic compass used for shoreward orientation is light-dependent (Fig. 1; and see Phillips and Borland, 1992*a–c*). Because light-dependent magnetoreception mechanisms are not expected to respond to the polarity of the magnetic field (Leask, 1977; Schulten, 1982; K. Schulten, personal communication), evidence that the homing response of newts is sensitive to the polarity of the magnetic field (Phillips, 1986*a*) strengthens the case for a second (i.e. non-light-dependent) magnetoreception mechanism contributing to this response.

Interestingly, however, the results of the present study indicate that the newt's homing

Table 1.

Wavelength	Shoreward orientation MVB (degrees)	N	r	P_R	U^2	P_W	Homing MVB (degrees)	N	r	P_R	U^2	P_W
400 nm	2	9	0.48	NS			335	13	0.48	≤0.05		
Full spectrum	344	9	0.47	NS	0.061	NS	328	14	0.60	≤0.01	0.176	NS
400 nm bimodal	179–359		0.72	≤0.01								
Full spectrum bimodal	180–360		0.61	≤0.05	0.102	NS						
450 nm	7	18	0.68	≤0.001			23	14	0.34	NS		
Full spectrum	22	20	0.64	≤0.001	0.053	NS	19	14	0.75	≤0.001	0.161	NS
400 and 450 nm	5	27	0.61	≤0.001			355	27	0.37	≤0.05		
Full spectrum	13	29	0.56	≤0.001	0.060	NS	357	28	0.61	≤0.001	0.169	NS
550 nm	268	12	0.49	≤0.05			166	14	0.22	NS		
Full spectrum	14	16	0.50	≤0.01	0.211	≤0.05	21	14	0.79	≤0.001	0.376	≤0.01
600 nm	241	7	0.82	≤0.01			131	7	0.17	NS		
Full spectrum	350	9	0.66	≤0.01	0.245	≤0.05	18	8	0.41	NS	0.068	NS
550 and 600 nm	255	19	0.59	≤0.001			156	21	0.20	NS		
Full spectrum	4	25	0.55	≤0.001	0.405	≤0.001	21	22	0.65	≤0.001	0.331	≤0.01
400 and 450 nm	See above						See above					
550 and 600 nm	See above				0.609	≤0.001	See above				0.211	≤0.05

MVB, mean vector bearing of pooled distribution of magnetic bearings rotated so that shore or home direction is at 0°; r, mean vector length; P_R, probability Rayleigh test; U^2, Watson U^2 statistic; P_W, probability, Watson U^2 test, bimodal, Rayleigh test carried out on doubled angles; NS, $P>0.05$.

response is influenced by the wavelength of light (Fig. 2), although the pattern of wavelength-dependence differs from that of shoreward magnetic compass orientation (Fig. 1). This influence of light on the homing response would seem to be incompatible with the evidence that this response is also dependent on the polarity of the magnetic field (Phillips, 1986a), i.e. dependence on the wavelength of light is consistent with a light-dependent magnetoreception mechanism, while sensitivity to the polarity of the magnetic field is unlikely to be characteristic of such a system.

One possible explanation for the seemingly contradictory properties of the newts' homing response is that this system receives inputs from two different magnetoreception mechanisms. Neurophysiological evidence suggests that two magnetoreception mechanisms may be present in vertebrates. Recordings from the central nervous systems of birds and mammals have provided evidence for responses to directional magnetic stimuli in visual centres (e.g. optic tectum and nucleus of the basal optic root; Semm *et al.* 1984; Semm and Demaine, 1986; Olcese *et al.* 1988). These responses were reported to be eliminated in the absence of light and, therefore, appear to be consistent with the properties of the newt's light-dependent magnetic compass (Phillips and Borland, 1992a). In addition, Beason and Semm (1987) and Semm and Beason (1990) have reported responses to magnetic stimuli in the trigeminal nerve system of the bobolink that appear to be independent of visual input and are elicited by small (i.e. <1 %) changes in
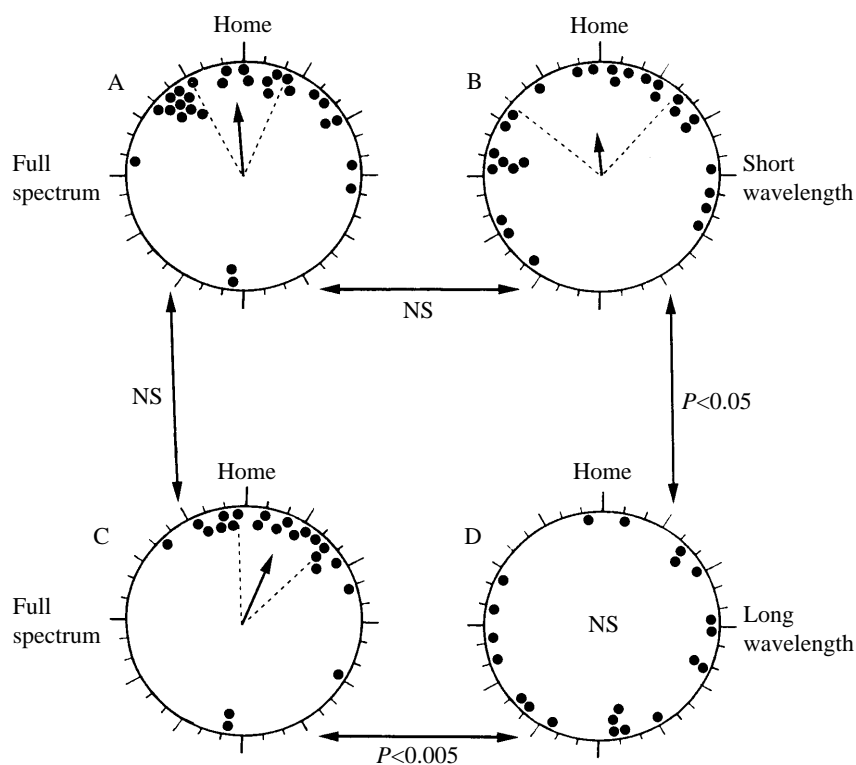
Fig. 2. Dependence of homeward magnetic compass orientation on the wavelength of light (statistics in Table 1). (A) Controls tested under full-spectrum light oriented towards the pond from which they had been collected. (B) Newts tested under 400 and 450 nm light were also homeward oriented and did not differ significantly from controls. (C) Full-spectrum controls were again oriented towards home. (D) Newts tested under 550 and 600 nm light were randomly distributed and differed signficantly both from the corresponding full-spectrum controls and from newts tested under 400 and 450 nm light. Data presented as in Fig. 1, except that magnetic bearings are plotted with respect to the magnetic direction of the home pond (see text).

magnetic field intensity, suggesting that a second magnetoreception mechanism may be present that functions as a non-light-dependent magnetic intensity detector. The trigeminal nerve innervates the anterior region of the head, where particles of the mineral magnetite have been localized in a number of different vertebrate groups (Kirschvink *et al.* 1985). This raises the possibility that a magnetite-based receptor may underlie the responses recorded by Semm and Beason (1990). A magnetite-based receptor is capable of responding to the polarity of the magnetic field (Kirschvink and Walker, 1985), as do newts that are homing (Phillips, 1986*a*). In addition, a magnetite-based receptor could, in theory, provide the high level of sensitivity exhibited by the trigeminal nerve system (Yorke, 1979; Kirschvink and Walker, 1985; and see below). Such a high level of sensitivity would be necessary to derive map information from the geomagnetic field (Gould, 1980; Moore, 1980; Walcott, 1980). In contrast, a light-dependent magnetoreception mechanism is unlikely to exhibit such a high level of sensitivity

(Schulten, 1982; and personal communication). Thus, although the neurophysiological responses continue to be difficult to obtain reliably (P. Semm, personal communication), the evidence is consistent with the presence of two distinct magnetoreception mechanisms in some vertebrates.

If the magnetoreception system used for homing receives inputs from the light-dependent magnetic compass (Phillips and Borland, 1992*c*) and from a non-light-dependent (possibly magnetite-based) intensity detector (Semm and Beason, 1990; and see below), this type of 'hybrid' response might be expected to exhibit properties of both systems. Thus, the influence of light on homing orientation (Fig. 2) could be a consequence of input from the light-dependent magnetic compass (Phillips and Borland, 1992*c*), while sensitivity to the polarity of the magnetic field (Phillips, 1986*a*) could be a consequence of the input from a non-light-dependent (possibly magnetite-based) intensity detector. Moreover, the interaction of these two inputs could produce additional properties that are not characteristic of either magnetoreception mechanism, i.e. the random orientation under long-wavelength light (Fig. 2D; and see below).

The possibility that a hybrid magnetoreception system is involved in homing raises a number of questions. Why would a magnetoreception system that receives inputs both from a magnetic compass and from a magnetic intensity detector be required for homing? How would the inputs from these two magnetoreception mechanisms be expected to interact? Are the functional properties of the proposed hybrid system consistent with those exhibited by newts that are using the magnetic field for homing?

In order to begin to address these questions, it is necessary first to consider the likely properties of an intensity-sensitive magnetoreception mechanism. Yorke (1979) proposed a theoretical model of an intensity-sensitive magnetoreception system in which the variance in the alignment of weakly magnetic particles of magnetite provides a measure of the intensity of the magnetic field (see also Kirschvink and Walker, 1985). In this type of system, an increase in the intensity of an external magnetic field would produce a decrease in the variance of particle alignment and *vice versa*. Averaged across a large number of particles, a receptor that measures the variance in particle alignment could, in theory, provide sufficient sensitivity to detect the subtle geographic variation in the intensity of the geomagnetic field (Yorke, 1979; Kirschvink and Walker, 1985).

A potential problem with a detector that responds to variance in particle alignment is that many of the transduction mechanisms that could function in this type of receptor (e.g. hair cells, stretch receptors) exhibit at least some degree of directional sensitivity. As a consequence, this type of 'intensity' detector would be likely to be influenced by changes in *both* the intensity and direction of the magnetic field. If so, an accurate measurement of intensity would only be possible if the directional component of the response was eliminated or held constant.

One strategy that an animal could use to obtain a reliable measurement of magnetic field intensity would be to vary the alignment of the intensity detector systematically until it determined the maximum response. This strategy would be inefficient and/or inaccurate, however, if the detector had a relatively long time constant (see Yorke, 1979) and/or required the organism to be stationary to obtain an accurate reading. An alternative strategy would be to use an independent directionally sensitive magnetoreception

mechanism, i.e. the light-dependent magnetic compass, to align the intensity detector. We propose, therefore, that the magnetoreception system used for homing is a hybrid system in which the light-dependent magnetic compass is used to align a non-light-dependent intensity detector to obtain the precise measurements of magnetic intensity necessary to derive map information from the geomagnetic field. Although the argument for a hybrid magnetoreception system is based on the need to align an intensity detector accurately in order to measure geographic variation in the intensity of the magnetic field, a similar argument could be made for aligning the azimuth of an 'inclination detector' to measure the geographic variation in the inclination or dip-angle of the magnetic field.

If homing is mediated by the proposed hybrid magnetoreception system, a 90° shift in the directional information from the magnetic compass under long-wavelength light should prevent this system from operating. If, for example, the magnetic compass were used to align an intensity detector that is involved in the map component of homing, the 90° shift in the perceived direction of north under long-wavelength light (Phillips, 1992*c*) would cause the intensity detector to be aligned at right angles to the actual magnetic field axis and, thus, to give a 'null' reading of intensity. Conversely, if the intensity detector were to provide information about the polarity of the magnetic field that is used during the compass component of homing, under long-wavelength light the axis of the magnetic field would appear to be at right angles to the horizontal polarity. As a consequence, the horizontal polarity could not be used to distinguish between the two ends of the axis. Previous studies of magnetic compass orientation (Wiltschko and Wiltschko, 1972; Light *et al.* 1993) have shown that an inability to distinguish between the two ends of the magnetic axis can result in random (rather than bimodal) orientation. The random orientation of newts under long-wavelength light in the homing experiments (Fig. 2) is therefore consistent with the predictions of the proposed hybrid magnetoreception system.

It might be argued that the data in Fig. 2 do not rule out the alternative hypothesis that newts were using a non-light-dependent magnetoreception system for homing and that the wavelength-dependence of the homing response (Fig. 2) was due to a non-specific effect on the motivation to home, i.e. the newts may have been motivated to home under short-wavelength light (Fig. 2B) but not under long-wavelength light (Fig. 2D). This alternative hypothesis, however, does not explain why different magnetic compass mechanisms would be used for the shoreward orientation and for the compass component of homing. Newts exhibit the compass component of homing in the indoor arena (Phillips, 1986*a*). Map information appears to be derived while the newts are held in the outdoor tanks prior to testing (J. B. Phillips, S. C. Borland and K. Adler, in preparation).

As discussed previously, Phillips (1986*a*) found that newts exhibiting shoreward magnetic orientation were sensitive to changes in the dip-angle or inclination of the magnetic field, but were unaffected by changes in the polarity of the magnetic field. In contrast, newts exhibiting the compass component of homing were unaffected by changes in inclination, but were sensitive to changes in polarity. These findings, in conjunction with evidence that the magnetoreception mechanism used for shoreward orientation is light-dependent (Phillips and Borland, 1992*c*), suggest that newts have two magnetoreception mechanisms. While an involvement of the magnetic field in both the

map and compass components of homing could account for the presence of two magnetoreception mechanisms (see earlier discussion), this would not explain why shoreward compass orientation and the compass component of homing (both of which require only compass information) are mediated by two different magnetoreception systems (Phillips, 1986*a*). Similarly, if the 'intensity detector' could serve as an autonomous source of directional information for the compass component of homing, this magnetoreception mechanism could presumably also mediate shoreward compass orientation.

In the proposed hybrid magnetoreception system, the properties of the newt's homing response result from an interaction between two magnetoreception mechanisms, i.e. the light-dependent magnetic compass and a non-light-dependent magnetic intensity detector. Although an interaction between the inputs from these two magnetoreception mechanisms may be necessary to derive map information from the magnetic field (see earlier discussion), there is no *a priori* reason to expect that the compass component of homing (Fig. 2) should also require an interaction of these two mechanisms. Such an interaction would not be surprising, however, if the magnetic field is involved in both the map and compass components of homing. As argued earlier, an input from the compass system may be used to align an intensity (or inclination) detector in order to obtain the precise measurements that would be necessary to derive map information. Map information, in turn, must feed back onto the magnetic compass system to enable the animal to orient in the correct homeward direction (i.e. to exhibit the compass component of homing). If this reciprocal exchange of information from the two receptor mechanisms occurs, the roles of the geomagnetic field in the map and compass components of homing are unlikely to be independent. As a consequence, both the map and compass components of homing would be likely to exhibit properties derived from both magnetoreception mechanisms.

If, as argued here, the proposed hybrid magnetoreception system functions in the map, as well as the compass, component of homing, newts should not be able to obtain map information when held in the outdoor tanks under long-wavelength light. Preliminary evidence from experiments now in progress supports this prediction (J. B. Phillips and S. C. Borland, unpublished data).

Evidence that newts utilize a specialized magnetoreception system for homing (Phillips, 1986*a*; and the present study) has important implications for understanding the sensory basis of the map. Recent experiments have shown that newts are able to home after being deprived of magnetic, visual, olfactory and inertial cues during displacement from their home pond (J. B. Phillips, K. Adler and S. C. Borland, in preparation). This ability to home in the absence of route-based directional information suggests that newts utilize a true bicoordinate or multicoordinate map. Evidence for the involvement of a specialized magnetoreception system in homing (Phillips, 1986*a*, and the present study), therefore, suggests that this map may be derived, at least in part, from the geomagnetic field.

The possible use of a magnetic map by an organism such as the eastern newt that moves over distances of at most 2–3 km (D. Gill, personal communication) raises a number of questions. For a magnetic map to operate over such short distances, it would have to be

derived from the local magnetic gradients in the vicinity of the home pond (J. B. Phillips, in preparation), which vary considerably in both steepness and direction at different sites (J. B. Phillips and S. C. Borland, unpublished observations). This local variation could explain why newts from some sites exhibit consistent errors in the direction of homing orientation after long-distance (30–60 km) displacement (J. B. Phillips and S. C. Borland, unpublished data).

Because newts move relatively slowly, the use of a magnetic map would require a strategy for measuring the geographic variation in the magnetic field that minimizes the error introduced by temporal variation in the magnetic field. For example, newts might obtain an accurate measurement of magnetic field parameters at a particular location by averaging over several hours during the night when the field is relatively stable, as suggested by data from juvenile American alligators (Rodda, 1984*a*).

In conclusion, evidence that the use of the magnetic field for shoreward compass orientation and homing are differentially affected by the wavelength of light (Figs 1 and 2) is consistent with earlier evidence for the involvement of a specialized magnetoreception mechanism in homing (Phillips, 1986*a*). Additional research will be necessary to determine whether homing involves only one or more than one magnetoreception mechanism (e.g. the hybrid system proposed here) and to identify the mechanism(s) involved. Ultimately, characterization of the underlying receptor mechanism(s) will contribute to a better understanding of the role of the geomagnetic field in the map component of homing.

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