Two Magnetoreception Pathways in a Migratory Salamander

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Male eastern red-spotted newts *(Notophthalmus viridescens)* under controlled laboratory conditions exhibit unimodal magnetic compass orientation either in a trained compass direction or in the direction of their home pond. If the vertical component of the magnetic field is inverted, newts exhibiting the simple-compass response undergo a 180° reversal in orientation, whereas newts orienting in the home direction are unaffected by this treatment. These results indicate that newts use an axial compass mechanism for simple-compass orientation similar to that found in migrating birds. However, a distinct magnetoreception pathway with polar response properties is involved in homing and is possibly linked in some way to the navigational map.

TERRESTRIAL VERTEBRATES EXHIBIT two forms of long-distance orientation behavior. (i) In simple-compass orientation a fixed directional heading is maintained, requiring only compass information (*1–3*). (ii) Navigation or homing, which has been demonstrated in amphibians (*4*), reptiles (*5*), and birds (*6*), requires a source of "map" information to determine the correct geographic position relative to home, as well as a compass to orient in the homeward direction (*7*).

Until now, navigation has not been elicited from any animal in the laboratory where potential cues can be easily and precisely manipulated. Moreover, it has not been possible to directly compare simple-compass orientation and navigation under the same experimental conditions. However, studies of the eastern red-spotted newt (*Notophthalmus viridescens*) (*2, 4*) have, to a large extent, overcome these problems. Both simple-compass orientation and homing are important components of the orientation behavior of this species. Like many other pond-dwelling vertebrates, newts learn a compass response perpendicular to their home shoreline (*y*-axis orientation) by using a variety of compass cues (*1, 2*). In addition, newts that have been displaced as much as 20 km from their home pond are able to navigate in the appropriate homeward direction (*4*). In the laboratory, eastern newts exhibit strong, replicable magnetic orientation and can be made to switch from *y*-axis orientation to navigation by manipulation of the water temperature before testing (*4*). Consequently, the functional properties of the magnetoreception systems involved in these behaviors can be directly compared.

Currently the most important clue to the physical process underlying magnetic field detection in terrestrial vertebrates is the axial sensitivity of migrating European robins (*Erithacus rubecula*) (Fig. 1, A and B) (*8*). In the present study, the technique of inverting the magnetic field's vertical component (described in the legend to Fig. 1) was used to investigate whether the simple-compass orientation and navigation of newts exhibit axial or polar response properties.

Groups of 40 to 60 adult male newts were collected from ponds located approximately 20 km to the east of the laboratory. The newts were transported to the laboratory (*9*), where they were housed in outdoor training tanks (Fig. 1C) aligned along the north-south or east-west geomagnetic axis. On the day of testing, the water temperature in the training tank was increased and then maintained between 33° and 34.5°C for the duration of the test. This increase in water temperature causes newts to exhibit strong, unimodal magnetic orientation (*2, 4*). The newts were removed individually from the training tank and tested in an enclosed, visually symmetrical arena inside the laboratory building (*10*). Each newt was tested only once. In each test, approximately equal numbers of newts were tested in four horizontal magnetic fields: the ambient field [magnetic north (mag N) at north] and three artificial fields (mag N rotated to east, south, or west); the artificial fields were produced by a double-wrapped cube-surface coil (*2, 11*). The distributions presented in Fig. 2 pool bearings from the four magnetic conditions. This method of analysis factors out geographical fixed nonmagnetic bias and retains only the component of orientation that is a consistent response to the magnetic fields (*2, 4*). Data were analyzed by statistical methods described elsewhere (*12*).

A procedure to determine whether the newt's simple-compass and navigational responses exhibit axial or polar sensitivity was performed by inverting the magnetic field's vertical component with a cube-surface coil wrapped with two strands of wire (*13*). In the control condition, the two strands were powered in opposite polarity, which did not alter the vertical component. In the experimental condition, the two strands were powered in the same polarity to produce an artificial field opposite to, and twice the strength of, the natural vertical component. Since the horizontal intensity of the field was not altered (and was the same in all four horizontal field alignments), the resultant fields in the experimental condition had the same total intensity as the control fields, but they had a positive (north up) rather than a negative (north down) inclination (Fig. 1B) (*14*).

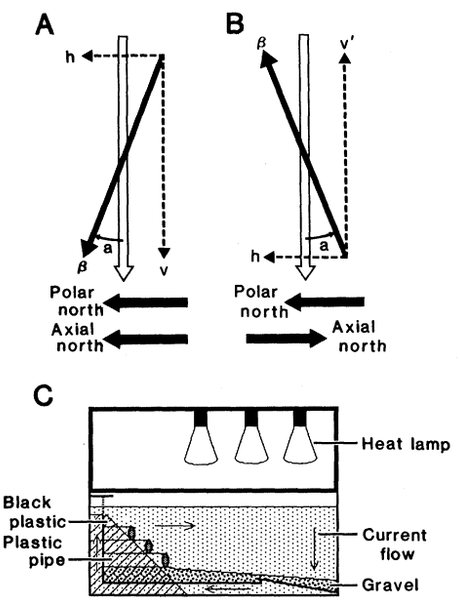

Fig. 1. (A and B) Axial and polar magnetic responses. A navigator's compass used by humans detects the horizontal polarity (h) of the magnetic field (β), which defines "polar" north. In contrast, migratory birds use the slope or inclination of the magnetic field lines to distinguish "axial" north (*8*). Axial north is defined as the direction in which the magnetic field lines form the smallest angle (a) with the gravity vector (open arrow). (A) For any given horizontal component (h), when the vertical component of the magnetic field (v) points downward, polar and axial norths coincide. (B) Inversion of the vertical component (v′) reverses the inclination but does not change the horizontal polarity of the magnetic field. Thus, when the vertical component is inverted, axial and polar norths are in opposite directions. (C) Training tanks were 110-liter, all-glass aquariums with one end partially enclosed in black plastic. Three rows of progressively shorter plastic tubes (5 cm in diameter) provided an artificial shoreline at the enclosed end. The bottom of the tank was covered with coarse gravel that sloped up toward the shore end. Heat lamps (150 W) located above the tank increased the water temperature. An air-driven circulation system prevented a thermal gradient from forming and produced a current flowing from the shallow to the deep end of the tank.

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Earlier studies showed that newts tested after residing for several days in water at stable temperatures exhibited *y*-axis orientation, whereas newts that experienced a period of widely fluctuating water temperatures before testing oriented in the direction of their home pond (*15*). Newts exhibiting *y*-axis orientation (Fig. 2, A and C) responded to an inversion of the magnetic field's vertical component by reversing their orientation relative to controls (Fig. 2, B and D). In contrast, newts orienting in the home direction (Fig. 2E) were not affected by the inversion of the vertical component (Fig. 2F). Similar results were also obtained in experiments in which a different technique was used to elicit unimodal orientation in newts (*16*). Although newts collected from ponds in only one direction from the laboratory were used in this study, previous work has shown that newts collected from ponds located in other directions exhibit appropriate homeward orientation (*4*). Therefore, the response shown in Fig. 2, E and F, appears to be true navigation. These results indicate that newts exhibiting simple-compass orientation respond only to the inclination of the magnetic field (axial sensitivity), whereas newts that are homing respond to the horizontal polarity (polar sensitivity).

The axial sensitivity of the newt's *y*-axis response is consistent with the Wiltschko's findings in European robins (*8*). Migrating birds are thought to maintain fixed compass headings during most of their journey (*3*), so they can rely solely on a compass mechanism to determine the path of migration. Thus, terrestrial vertebrates appear to use an axially sensitive receptor system in a variety of contexts that require only compass information. The polar sensitivity of the homing response suggests that newts also have a second pathway (or a second magnetoreception mechanism) for magnetic information used for navigation.

The possibility that terrestrial vertebrates have two magnetoreception pathways is intriguing because it has been suggested that both map and compass information may be derived from the magnetic field (*17*). A magnetic map would require detection of the subtle geographic variation in at least two parameters of the magnetic field (horizontal intensity, vertical intensity, total intensity, inclination, and declination), as well as the ability to use this information to derive a bicoordinate "fix" on the position relative to home. Because the average geographic variation in the magnetic field is only 3 to 5 nT/km and the earth's field is approximately 50,000 nT, deriving map information from the magnetic field would place quite different demands on the processing of magnetic information than would determining compass direction (*18*). Furthermore, depending on the type of receptor mechanism involved, precise intensity measurement needed for a map fix may require an independent estimate of the magnetic field's directionality. Thus, if vertebrates have a magnetic map, they are likely to have a separate magnetoreception pathway (or, possibly, a second magnetoreception mechanism) for measurement of magnetic intensity.

However, in this study, newts almost certainly derived map information before testing. Map information was not available in the test arena because all potential map cues except the magnetic field were excluded, and the magnetic field was sufficiently variable in the test arena that detection of the natural geographic variation in the earth's field would have been impossible (*19*). Thus, newts in the test arena were carrying out the compass step of homing, presumably having derived map information earlier while residing in the training tanks. It would appear, therefore, that newts use two different pathways for processing magnetic compass information: a pathway that responds only to the axis of the magnetic field for shoreward simple-compass orientation and one that responds to the magnetic field's polarity for the compass component of navigation. This apparent redundancy indicates that the response properties required for these two types of behavior differ, as would be expected if the receptor mechanism used for the compass component of homing is also involved in deriving map information.

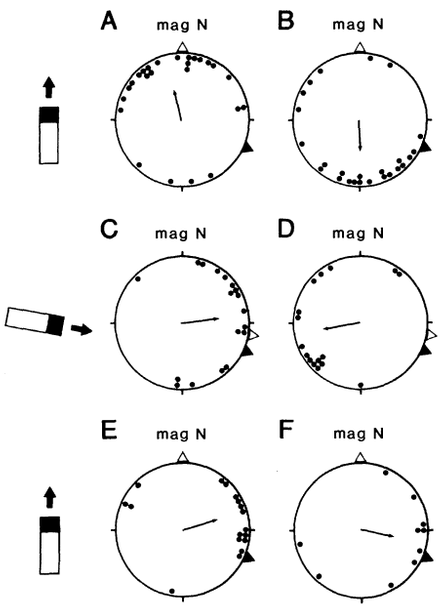

Fig. 2. Influence of inversion of the magnetic vertical component on simple-compass orientation and navigation. Each data point represents the magnetic bearing of a single newt. Distributions pool data from four horizontal alignments of the magnetic field. An arrow in the center of each distribution indicates the mean direction of orientation; the length of the arrow is proportional to the mean vector length (**r**). The open arrowhead on the edge of each circle is the magnetic bearing toward shore in the training tank; the closed arrowhead is the magnetic bearing from the laboratory to the home pond of the newts. Insets on the far left show the alignment of the training tank in each test (drawn with north at the top of the page); the shaded end indicates the artificial shoreline, and the arrow indicates the direction to the laboratory building. Distributions in the left column are control animals tested with a normal vertical component. Distributions in the right column are experimental animals tested with an inverted vertical component. (A to D) Newts tested after residing in water at stable temperatures. (A) Newts held in tanks aligned on the north-south axis and tested in the normal vertical field (controls) oriented at 345° [$\mathbf{r}$ = 0.48; n = 25; P = 0.003; Rayleigh test; (*12*)], and they were significantly oriented in the direction of shore [P(360° = expected direction) = 0.002; V test; (*12*)]. (B) Newts tested with the vertical component inverted were oriented at 178° [$\mathbf{r}$ = 0.45; n = 25; P = 0.006; P(360°), not significant (NS)]. The distribution of controls and experimentals is significantly different [$P < 0.05$; Watson U^2 test; (*12*)]. (C) Groups of newts held in tanks aligned on the east-west axis exhibited eastward orientation in the control condition [82°; $\mathbf{r}$ = 0.56; n = 18; P = 0.002; P(100°) = 0.0009], whereas experimental animals oriented to the west (D) [260°; $\mathbf{r}$ = 0.57; n = 16; P = 0.004; P(100°) = NS]. Again, the distribution of controls and experimentals is significantly different ($P < 0.05$). (E and F) Newts tested after exposure to fluctuating water temperatures. (E) Controls held in tanks aligned on the north-south axis oriented to the east (76°; $\mathbf{r}$ = 0.54; n = 17; P = 0.004), and they were significantly oriented in the direction of the ponds from which they were captured [P(113°) = 0.005]. (F) Newts tested with the vertical component inverted did not reverse their orientation relative to controls and continued to show significant homeward orientation [103°; $\mathbf{r}$ = 0.50; n = 10; P = 0.08; P(113°) = 0.02]. The distributions of controls and experimentals are not significantly different ($P > 0.05$). Moreover, the distribution of experimentals is significantly different from the distribution of the comparable group in tests in which the newts were exhibiting *y*-axis orientation (D versus F, $P < 0.05$).

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9. Newts were transported in airtight, 2-liter, plastic

containers that were half-filled with pond water and then placed inside a Styrofoam cooler.
10. A newt was placed in a release device located in the center of the arena floor in total darkness. When the lid of the arena was closed, a light centered above the release device was turned on. The newt was released after a delay of 30 seconds. Its movements were observed through the frosted Plexiglas floor of the arena by means of a mirror located underneath. Directional responses were measured to 5° accuracy at a distance of 10 cm from the center of the arena. A detailed description of arena design is provided elsewhere (*2*).
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14. The testing sequence was one animal in mag N = north (vertical component down), the next in mag N = east (vertical component up), then one in mag N = west (down), mag N = south (up), mag N = east (down), mag N = north (up), mag N = south (down), and mag N = west (up).
15. Newts exposed to a mean temperature of 18°C and fluctuations of less than ±5°C in the temperature of training tank water before the water temperature was increased for testing exhibited *y*-axis orientation [Fig. 2, A and C; (*2, 4*)]. However, newts exposed to diel fluctuations in water temperature of greater than 20°C (from 3° to 5°C at night to 25° to 27°C during the day) for one or more days before testing responded to the increase in water temperature by orienting in the direction of their home pond [Fig. 2E; (*4*)]. The adaptive significance of the newt's response to differing water conditions is discussed elsewhere (*2, 4*).
16. In two earlier test series, an abrupt drop in training tank water level just before testing elicited a unimodal magnetic response lasting about 30 minutes (*20*). Newts exposed to this treatment exhibited either *y*-axis or homeward orientation, with the type of orientation varying between tests; in these early tests, the factors responsible for the switch in behavior had not been identified. Newts orienting along the *y*-axis reversed their orientation when the vertical component of the magnetic field was inverted. Newts orienting relative to the home pond direction did not respond to an inversion of the vertical component.
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19. Within the test arena, the magnetic field varied by ±5% in intensity and direction because of the presence of iron in the surrounding building.
20. J. B. Phillips, unpublished data.
21. I thank V. Church, J. Plissner, T. Rourke, and J. Weed for assistance in collecting animals and conducting experiments; K. Adler, J. Crawford, E. Debski, F. Dyer, S. Emlen, T. Goldsmith, T. Lednor, G. Rodda, C. Walcott, J. Waldvogel, and J. Zeiger for helpful comments and suggestions. Supported by grants from NSF (BNS-7924525) and NIH (NS-19089) to K. Adler, and from NIMH (training grant 5T32MHI5793), U.S. Dept. of Agriculture (Hatch Funds to K. Adler), Sigma Xi (Cornell University and the National Society), and the Andrew D. White Scholarship Fund (Cornell).

20 November 1985; accepted 8 May 1986

Molecular Cloning of the Chicken Progesterone Receptor

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To define the functional domains of the progesterone receptor required for gene regulation, complementary DNA (cDNA) clones encoding the chicken progesterone receptor have been isolated from a chicken oviduct λgt11 cDNA expression library. Positive clones expressed antigenic determinants that cross-reacted with six monospecific antibodies derived from two independent sources. A 36–amino acid peptide sequence obtained by microsequencing of purified progesterone receptor was encoded by nucleotide sequences in the longest cDNA clone. Analysis of the amino acid sequence of the progesterone receptor deduced from the cDNA clones revealed a cysteine-rich region that was homologous to a region found in the estrogen and glucocorticoid receptors and to the avian erythroblastosis virus *gag-erb*-A fusion protein. Northern blot analysis with chicken progesterone receptor cDNA's indicated the existence of at least three messenger RNA species. These messages were found only in oviduct and could be induced by estrogens.

STEROID HORMONE REGULATION OF gene expression in eukaryotic cells is mediated by specific intracellular receptors (*1*) that have a high affinity for their respective steroid hormone ($K_d = 10^{-10} M$) and are present in low concentrations in target cells (*2*). In the chick oviduct, the interaction of progesterone with its receptor results in increased transcription of a defined set of genes coding for egg white proteins (*3*). In addition to its role as gene regulator, the progesterone receptor (PR) itself appears to be regulated by estrogen (*4*). The PR in chick oviduct consists of two hormone-binding moieties (*5*). Protein A, which has a molecular mass of 79 kD, binds to DNA with high affinity. Protein B, which has a molecular mass of 108 kD, binds less well to DNA but interacts with certain nonhistone chromosomal proteins (*2*). The progesterone-responsive ovalbumin gene has sequences in its 5′ flanking region that may be involved in the regulation of its expression by the PR-progesterone complex (*6*). However, direct analysis of the relation between PR proteins A and B and of their interactions with target gene elements has been hampered by their low concentration and the difficulty of purifying substantial quantities of receptor. Therefore, to define the functional domains of the PR required for such interactions with gene elements, we have cloned a complementary DNA (cDNA) encoding the chick progesterone receptor.

A panel of six monoclonal antibodies, each recognizing the chick PR, was used to screen a λgt11 cDNA library for expression of PR epitopes. Five of these antibodies, raised against the chick receptor, have been characterized (*7*). Each of the antibodies appears to react with a different antigenic determinant of the receptor. The additional monoclonal antibody was raised against human PR from T47D cells and cross-reacted with chick PR. We analyzed the antibodies by immunoblotting to determine their specificity for PR under conditions used to screen the expression products of the cDNA clones (Fig. 1A). Crude cytosolic extracts from chick oviduct were subjected to electrophoresis in 10% sodium dodecyl sulfate (SDS)–polyacrylamide gels and transferred to nitrocellulose filters. Filter strips containing resolved cytosolic proteins were then incubated with each of the monoclonal antibodies. All six antibodies reacted specifically with PR protein B, and all but two reacted equivalently with both protein A and protein B. Antibody PR22 had the highest apparent affinity for PR under these conditions and was chosen for primary screening of the cDNA library (*8*).

To screen large numbers of recombinants, we constructed a λgt11 cDNA expression library (*9*) with size-selected polyadenylated RNA (larger than 2 kb) from chick oviduct. The library contained approximately 6×10^6 members, 95% of which were recombinants. Approximately 3×10^6 phages

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