The eastern red-spotted newt is a terrestrial vertebrate known to exhibit two forms of orientation behavior based on the magnetic field of the Earth. One is a simple compass orientation (Phillips, 1986a), in which a fixed (shoreward) trained directional heading relative to the magnetic field is maintained. The other is a magnetic effect on navigation or homing (Phillips, 1987), which requires both some form of ‘map’ information to determine the correct geographic position relative to home and a compass to orient in the homeward direction. In a subsequent study (Phillips et al., 1995), newts were found to use true navigation, i.e. to return to the origin of a displacement (‘home’) without access to familiar landmarks or goal-emanating cues and without knowledge of the displacement route.

The compass component of homing could be derived from the same pathway (or mechanism) as the one involved in the simple compass orientation (Phillips, 1986b). Subsequent studies indicate that shoreward and homeward magnetic compass orientation are affected by the wavelength of light (short-wavelength and full-visible-spectrum versus long-wavelength light) in different ways (Phillips and Borland, 1992, 1994).

On the basis of their findings, Phillips and Borland (1992, 1994) suggested that a light-dependent magnetic compass was used for the shoreward orientation response, whereas the homing response was mediated by a ‘hybrid mechanism’. This latter response was proposed to combine information from the light-dependent compass and from a non-light-dependent system. However, the debate continues as to whether the light effect is a primary feature at the level of the biophysical magnetic compass transducer or an influence of light on the subsequent behavior of the animal (Kirschvink et al., 1997).

There is agreement that the sensitivity needed for magnetic navigation is incompatible with a response produced by an optically driven radical pair recombination system (Schulten, 1982), whereas it is within the theoretical range for a magnetite-based system (Kirschvink and Walker, 1985).
In the 35 years since Lowenstam (1962) proposed the hypothesis that ferromagnetic material could serve as a possible magnetic transducer, biogenic magnetite particles have been found in a wide variety of organisms (e.g. Gould et al., 1978; Kirschvink et al., 1985; Walcott et al., 1979; Wiltschko and Wiltschko, 1995), some of which have provided behavioral and neurophysiological evidence of Earth-strength magnetic field sensitivity (e.g. Beason, 1989, 1992; Beason and Semm, 1987; Semm and Beason, 1990; Walker et al., 1997). In parallel with these discoveries, numerous biophysical models have been developed indicating how small magnetite crystals might be transduced into neurological signals that could be used by an animal (Kirschvink et al., 1992a, 1993; Kirschvink and Gould, 1981; Kirschvink and Walker, 1985; Yorke, 1979). Given the properties of the homing response of newts, Phillips and Borland (1994) suggested that the non-light-dependent part of the 'hybrid mechanism' could be magnetite-based.

To assess this suggestion, the present experiments took advantage of the magnetometry facility at the California Institute of Technology. A group of newts that had been tested for homing ability by the Phillips group at Indiana University was used to test for any presence of ferromagnetic material. Our data show that the red-spotted newt contains magnetic particles with properties that are consistent with magnetite.

Materials and methods

Magnetic measurements were carried out on a group of 18 adult male newts Notophthalmus viridescens previously subjected to magnetic orientation experiments (the results of which will be presented elsewhere: J. B. Phillips and S. C. Borland, in preparation). These animals were collected in ponds to the southwest-southeast of Bloomington, Indiana, USA. They were anesthetized with MS-222 at least 24 h after testing at Indiana University, frozen in liquid nitrogen, and then shipped on dry ice to Caltech for magnetic measurements. The newts were kept frozen because they contain relatively high levels of the toxin tetrodotoxin in their skin (Brodie et al., 1974); indeed, a preliminary attempt to measure any magnetization carried by some newts of the same species suggested that this toxin might be released into the body after death and could interfere with any magnetization carrier present in the body; soaking them in liquid nitrogen would prevent this by suddenly blocking all the physiological processes. The experiments used ultrasensitive moment magnetometers (2G Enterprises) employing direct-current-biased superconducting quantum interference devices (SQUIDS). These devices are designed to measure the total ferromagnetic moment of samples placed within a Helmholtz-coil pickup loop (Fuller et al., 1985). To assess the characteristics of any ferromagnetic material they might contain, the frozen newts were subjected individually to several rock magnetic experiments: the natural remanent magnetization (NRM) vector was initially measured and then demagnetized progressively using alternating magnetic fields (Af demagnetization). The newts were then given an anhysteretic remanent magnetization (ARM) in progressively stronger biasing fields, the strongest of which was then Af-demagnetized. Next, they were given an isothermal remanent magnetization (IRM) in a peak pulse field of 100 mT, which was then Af-demagnetized. Finally, a complete IRM acquisition spectrum was conducted in log-spaced steps up to 1T. During the measurements, the newts were kept frozen by a flow of dry, cold-filtered air inside the magnetometer. The temperature was never allowed to rise above −40°C (223 K) to −50°C (233 K). All measurements were performed in magnetically shielded environments, dust-free in the case of IRM and ARM experiments. The newts were suspended on a thin nylon monofilament line, moved by a stepping motor between the loading and measurement regions of the magnetometer and, subsequently, the magnetization and demagnetization coils. As we cannot control the temperature precisely inside the magnetometer, we tentatively performed a rough ‘warming experiment’ on two newts to assess the presence or absence of very fine superparamagnetic (SPM) grains. Each sample was soaked in liquid nitrogen (−196°C; 77 K), then given an IRM with a 1 T pulse. Following this, the sample remanence was monitored every few seconds for at least the following 11 min as the sample was warming in an atmosphere with a temperature between −18 and 0°C (255 and 273 K). This procedure was then repeated once without the 1 T pulse and finally a second time with this pulse.

Results

A natural magnetic remanence was detected in all the newts examined, as indicated in Table 1. The NRM ranges from 0.58×10⁻¹¹ to 25.4×10⁻¹¹ A m², with an average of 4.40×10⁻¹¹ A m², whereas the background noise levels were approximately 1.1×10⁻¹² A m² (mean ± s.d., N=18). The NRM directional analysis relative to the anterior of the head shows no particular direction of alignment among the newts (P>0.30, test of uniformity applied to the distribution of the 18 NRM directions; Fisher et al., 1987). Experiments utilizing the alternating-field demagnetization of the NRM were designed to detect whether different subsets of magnetic particles within each newt were aligned in particular directions; these give a qualitative indication of the net directions carried in each newt by grains in different coercivity ranges (Fig. 1). This distribution is also statistically random using a positive test of uniformity (Fisher et al., 1987), except for three newts. For these latter, however, although the previous statistical test gives a probability of uniform distribution lower than 5 %, the scatter in the grain distribution is high (see, for example, newt X in Fig. 1). Thus, in each newt, the magnetic grain magnetizations are not more or less aligned along a specific direction relative to the body of the newt; instead, their directions are scattered rather randomly. However, the methods used in these experiments did not make it possible to determine whether the net direction of grains within a particular coercivity range was the same in different individuals.
### Table 1. Intensity and direction of the natural remanent magnetization measured in individual Notophthalmus viridescens

<table>
<thead>
<tr>
<th>Newt</th>
<th>NRM $\times 10^{11}$ (A m$^{-2}$)</th>
<th>$D$ (degrees)</th>
<th>$I$ (degrees)</th>
<th>$\alpha_{95}$</th>
<th>$k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.47±0.05</td>
<td>347.9</td>
<td>32.0</td>
<td>1.1</td>
<td>1166.6</td>
</tr>
<tr>
<td>C</td>
<td>25.44±0.10</td>
<td>136.5</td>
<td>−35.7</td>
<td>0.9</td>
<td>1946.1</td>
</tr>
<tr>
<td>E</td>
<td>5.92±0.03</td>
<td>284.4</td>
<td>23.8</td>
<td>0.8</td>
<td>2763.8</td>
</tr>
<tr>
<td>F</td>
<td>1.94±0.06</td>
<td>305.5</td>
<td>26.2</td>
<td>1.3</td>
<td>976.7</td>
</tr>
<tr>
<td>G</td>
<td>6.26±0.08</td>
<td>146.8</td>
<td>−1.5</td>
<td>0.5</td>
<td>8438.3</td>
</tr>
<tr>
<td>H</td>
<td>7.25±0.04</td>
<td>47.3</td>
<td>28.2</td>
<td>0.9</td>
<td>2309.2</td>
</tr>
<tr>
<td>J</td>
<td>2.79±0.12</td>
<td>298.5</td>
<td>−18.6</td>
<td>2.2</td>
<td>982.6</td>
</tr>
<tr>
<td>L</td>
<td>1.88±0.03</td>
<td>357.9</td>
<td>5.6</td>
<td>1.6</td>
<td>671.5</td>
</tr>
<tr>
<td>M</td>
<td>1.65±0.02</td>
<td>92.6</td>
<td>−1.3</td>
<td>1.6</td>
<td>564.0</td>
</tr>
<tr>
<td>N</td>
<td>1.94±0.01</td>
<td>128.3</td>
<td>−2.3</td>
<td>1.3</td>
<td>1296.5</td>
</tr>
<tr>
<td>O</td>
<td>0.58±0.03</td>
<td>291.3</td>
<td>−11.5</td>
<td>2.2</td>
<td>244.7</td>
</tr>
<tr>
<td>P</td>
<td>1.37±0.01</td>
<td>97.2</td>
<td>−11.3</td>
<td>1.7</td>
<td>410.3</td>
</tr>
<tr>
<td>Q</td>
<td>8.89±0.03</td>
<td>40.6</td>
<td>39.5</td>
<td>0.7</td>
<td>3978.5</td>
</tr>
<tr>
<td>T</td>
<td>1.02±0.03</td>
<td>14.9</td>
<td>−9.6</td>
<td>1.1</td>
<td>1579.5</td>
</tr>
<tr>
<td>W</td>
<td>1.05±0.03</td>
<td>4.3</td>
<td>−39.1</td>
<td>1.9</td>
<td>343.1</td>
</tr>
<tr>
<td>X</td>
<td>4.76±0.08</td>
<td>133.8</td>
<td>66.6</td>
<td>1.0</td>
<td>1506.0</td>
</tr>
<tr>
<td>Y</td>
<td>1.30±0.03</td>
<td>247.3</td>
<td>14.6</td>
<td>2.0</td>
<td>437.3</td>
</tr>
<tr>
<td>Z</td>
<td>3.66±0.06</td>
<td>105.0</td>
<td>53.3</td>
<td>2.0</td>
<td>471.1</td>
</tr>
</tbody>
</table>

NRM, intensity of the natural remanent magnetization; values are means ± s.d. (N=18).

$D$, $I$, the declination (azimuthal angle between the horizontal component of the NRM and the anterior end of the head of the newt, ranging from 0 to 360°, positive clockwise) and inclination (vertical angle between the horizontal and the NRM, ranging from −90 to +90° and defined as positive downward) of the NRM, respectively.

$\alpha_{95}$, $k$, 95% confidence cone and the precision parameter of the Fisher’s (1953) statistics, respectively.

The NRM shows no preferred direction of orientation among the newts (N=18, $P>0.30$; probability of uniform distribution; Fisher et al., 1987; non-significant preferred orientation= $P>0.05$).

The characteristics of the magnetometer did not allow even a rough estimate of the localization of grains in the body of the newt. However, the magnetic signal recorded along the body (obtained by measuring the magnetization for different longitudinal distances between the nose of the newt and the magnetometer sensors; data not shown) has a very consistent pattern among the newts and is definitely not compatible with the idea that the magnetic particles are clustered in a unique area (at least less than 0.5–1 mm long). Indeed, the magnetization carriers seem to be spread out in a large volume of the body of the newt. However, the anatomy of their distribution and the volume they occupy are both unknown. It was not possible, for example, to determine whether particles within a particular coercivity range were more localized in distribution, as might be the case if only a subset of particles contributing to the NRM were located in a specialized sensory structure.

There is a consistency in the characteristics of acquisition and alternating-field demagnetization for IRM and ARM among the newts: Fig. 2 displays the mean patterns with their standard deviation. The fact that the newts gain and lose magnetization in such experiments suggests the presence of ferromagnetic materials in the body of the newt. The magnetic characteristics given by IRM acquisition and alternating-field demagnetization are displayed in Table 2. The saturated IRM values are two orders of magnitude higher than the NRM, with a mean of 2.08±1.13 nA m$^{-2}$ (mean ± s.d., N=18), and with saturation arising between applied fields of 400 and 600 mT, but usually...
occurring at approximately 400 mT. The ARM acquisition (Fig. 2B) shows the same pattern as for the chiton teeth (Cisowski, 1981), which contain biogenic magnetite (Kirschvink and Lowenstam, 1979). All these results are consistent with single-domain-grained magnetite with a high intergrain interaction (Cisowski, 1981) and suggest that the ferromagnetic crystals are disposed in interacting clumps. However, the decrease in magnetization exhibited in our ‘warming experiment’ (Fig. 3) suggests that the newts also contain superparamagnetic grains, as do other organisms (Kirschvink and Gould, 1981; Kirschvink and Woodford, 1991).

**Discussion**

This study indicates the presence of ferromagnetic material in the body of the eastern red-spotted newt Notophthalmus viridescens. The consistency of the magnetic properties of the group of newts makes it unlikely that the observed magnetizations could be the consequence of occasional external contamination. Although it was impossible to define the spatial disposition of the magnetic particles, our results suggest that they form clumps that could be situated in rather different areas of the body. This would not be unusual given that some animals, and even humans, have been shown to contain ferromagnetic particles in a variety of different tissues (e.g. Grassi-Schultheiss et al., 1997; Kirschvink et al., 1992b; Kirschvink and Walker, 1986; Wiltschko and Wiltschko, 1995). This suggests that a significant proportion of the particles may not be involved in magnetoreception, but instead may have a non-sensory function. At the moment, however, this non-sensory function has not been identified.
to resolve, the presence of such a material in the eastern red-spotted newt represents an important element when considering the navigation system of this animal. Indeed, the newts have been demonstrated to display true navigation after being deprived of magnetic, visual, olfactory and inertial cues during displacement from their home pond (Phillips et al., 1995). Given that in this case no route-based directional information is available to them, it appears that they are able to utilize at least a bicoordinate map. Following these findings, Phillips and Borland (1994) suggested that the geomagnetic field plays a role in this map. New results from a recent study (J. H. Fischer, M. Freake, S. C. Borland and J. B. Phillips, in preparation) yield direct experimental evidence for the use of one component of the magnetic field (i.e. inclination) in the map step of homing by newts. However, an important requirement of a system deriving map information from the geomagnetic field is a high level of sensitivity (Gould, 1980; Moore, 1980). The radical pair recombination mechanism that could be involved in part of the orientation behavior of the newt (Phillips and Borland, 1994) does not provide this kind of sensitivity (Schulten, 1982), whereas a system based on a ferromagnetic material such as magnetite theoretically could (Kirschvink and Walker, 1985). This latter system would also be compatible with the magnetic polar discrimination that the newts display when homing is elicited experimentally (Phillips, 1986b). It is thus of critical importance that the location and functional properties of the ferromagnetic material be unambiguously assessed, as this information is needed to further our understanding of the involvement of the geomagnetic field in the navigation system of the newt.

In conclusion, although magnetic sensitivity has been demonstrated in a large number of animals (for a review, see Wiltschko and Wiltschko, 1995), with the possible exception of the rainbow trout (Walker et al., 1997), the search for magnetosensitive cells has been frustrating. Our study reveals the existence of a ferromagnetic biomineral in the body of the eastern red-spotted newt. This is the first direct evidence for the presence of ferromagnetic particles in an amphibian, thus expanding the variety of animal species known to contain such materials. Its magnetic properties are consistent with an SD/SPM grain-sized material belonging to the maghemite–magnetite family, which is in accordance with the ‘hybrid mechanism’ hypothesis (Phillips and Borland, 1994). However, although theoretical considerations associated with our results and behavioral data now make magnetite the best candidate for the putative magnetoreceptors involved in the map component of homing of this amphibian, any full interpretation needs clear spatial and structural characterization of this ferromagnetic biomineral.

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Fig. 3. Variation of the remanence acquired at low temperature monitored during zero-field heating. Néel (1949) has shown that the relaxation time for a magnetic domain will increase exponentially as the temperature is lowered. Thus, some grains that are superparamagnetic (SPM) at room temperature (≈300 K) will behave as single domains (SDs) capable of retaining remanence at much lower temperature. Conversely, any net remanence held by these domains at low temperature will disappear as they warm in field-free space across their SPM/SD transition (their blocking temperature). For the first warming cycle, the sample was given an isothermal remanent magnetization (IRM) at low temperature, and the magnetization was then monitored over time (curve 1) as the sample warmed inside the field-free region of the magnetometer. Curve 2 was obtained after the sample had been cooled down, but not given an IRM. Curve 3 shows a subsequent warming cycle following the same procedure as for the first cycle. See text for more details. The progressive decrease in curves 1 and 3 compared with curve 2 suggests the presence of SPM ferromagnetic grains in the newt.

Our magnetometry results, together with the fact that ferromagnetic material found in animal and human tissue has mostly been identified as the iron oxide magnetite, suggest that SD/SPM magnetite particles are the most plausible magnetization carrier in the eastern red-spotted newt (Notophthalmus viridescens). This remains to be confirmed, however, by extraction and clear characterization (using, for example, transmission electron microscopy and electron diffraction).

This is the first time a ferromagnetic material has been detected in an amphibian. More interestingly, this amphibian has the ability to rely on the Earth’s magnetic field for orientation (e.g. Phillips, 1986b). Regardless of the debate about the compass mechanism, which still requires other kinds of biophysical, behavioral and neurophysiological data...
References


