Magnetoreception.

Magnetic field: **B**

Based on behavioral studies, it seems that at least 50 animal species use the Earth's magnetic field for orientation and migration:

All vertebrate groups
Mollusca
Crostacea
Insects
others..

Mainly studied: birds, Hymenoptera, Chelonia (turtles), salmon, sharks, cetaceans.

It could be a primitive mechanisms, also found in some bacteria, often used as a backup for other types of compass navigation (e.g., in pigeons or bees). Usually the celestial cues cause stronger orientating effects.

Potential information obtained:

DIRECTION: N/S polarity of the field

POSITION (more common): Inclination angle
Field intensity
etc.
(parameters vary as a function of position on terrestrial surface)

Some birds, turtles, salamanders, lobsters can discriminate with learning small parameter differences, in order to form magnetic maps of their habitat.

Magnetoreceptors.

Difficult to localize:

- magnetic fields freely cross biological tissues
- could be small and dispersed in a large tissue volume
- transduction could depend on a series of chemical reactions, not necessarily associated to a typical organ
- human experience does not help

Three mechanisms seem more likely.

a) Endogenous magnetite.
b) Chemical magnetoreception (chemical reactions modulated by terrestrial **B**).
c) Electromagnetic induction.

Functional implications: polarity and inclination.

All three mechanisms can indicate the direction and field elements useful to determine the position, but chemical magnetoreception does not seem fit to also indicate N/S polarity (and neither are the superparamagnet type magnets).

Magnetic polarity compass:	arthropods, salmons, some rodents.
Inclination compass:	e.g., birds, turtles and other reptiles (does not show polarity) [there may be problems around the magnetic equator for migrating birds].
Both types:	some salamander species.

Without detailed studies, nonetheless, one cannot conclude that these two types of compass depend on different mechanisms. E.g., CNS processing could strongly modify the receptor output.

Sensitivity: much higher with magnets; hence, a map based on field intensity seems difficult to obtain with magnetochemical or induction mechanisms.

A) Endogenous magnetite (Fe_3O_4) or greigite (Fe_3S_4).

Magnetotaxis in some marine bacteria.

Bacteria contain small magnets used to move towards the bottom**.**
They move forward and the magnete gives orientation towards the bottom (they are anaerobic).

Magnetite particles.

Found on the inner surface of the skull of birds, rodents, humans, often around the nasal cavity.

Often **single-domain** (~50 nm in diameter) permanently magnetized crystals. Could exert torque or pressure on mechanoreceptors or directly open ion channels.

Some animals have even smaller magnetite crystals (**superparamagnetic**), which have no permanent magnetic moment, but in presence of **B** can generate fields that affect nearby cristals.

However, it is necessary to demonstrate:

a) innervation (found in bees, but not vertebrates)

b) behavioral response dependent on these structures

c) axon firing dependent on **B.**

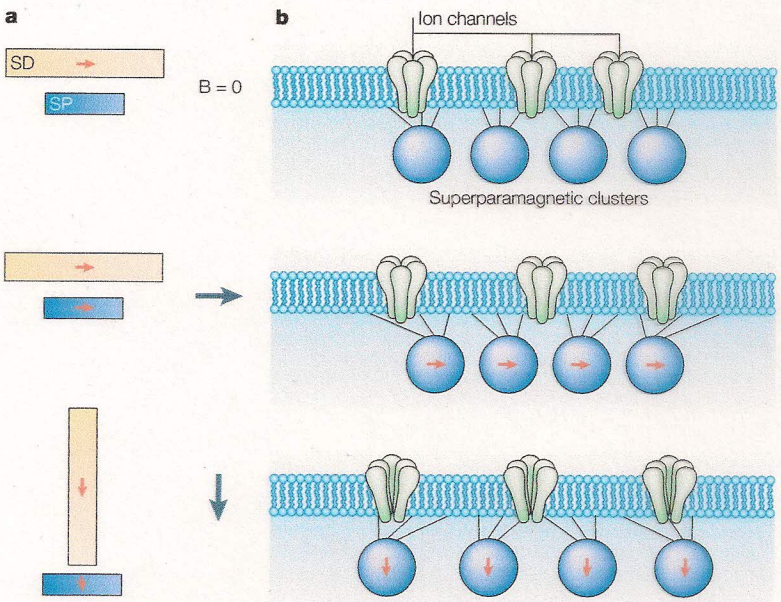


Figure 3 | **The different magnetic properties of single-domain and superparamagnetic crystals. a** | Single-domain (SD) and superparamagnetic (SP) magnetite crystals have different magnetic properties. Single-domain crystals have permanent magnetic moments (indicated by red arrows) even in the absence of an external magnetic field (B = 0). If an external field is present (black arrow) and the crystals are free to rotate, they will align with the external field. By contrast, superparamagnetic crystals have no magnetic moment in the absence of an external field. If an external field is present, however, the crystals develop a magnetic moment that tracks it, even though the crystal itself does not rotate. **b** | A hypothetical transduction mechanism based on interacting clusters of superparamagnetic crystals located in the membranes of neurons. Depending on the orientation of the external field, the clusters will either attract or repel each other, deforming the membrane and possibly opening or closing ion channels. For example, when the external field is parallel to the cell membrane, the fields in each crystal (red arrows) align in such a way that adjacent clusters attract each other like a row of bar magnets aligned end to end (middle panel). The membrane might, therefore, be slightly compressed. By contrast, a 90-degree change in the orientation of the external field (bottom panel) results in different interactions between clusters, because adjacent clusters now behave like a row of bar magnets aligned side by side. The resulting interactions might stretch the membrane and open ion channels. This model was inspired by the discovery of superparamagnetic crystals in pigeon nerve terminals[79]. Modified, with permission, from REF. 79 © (2003) Elsevier Science.

these crystals inside nerve terminals and arranged along the cell membrane[80]. However, in contrast to the single-domain magnetite detected in fish[82], the magnetite crystals in the beak of the pigeon are superparamagnetic[83,85].

An interesting similarity between fish and birds is that, in both cases, the anatomical site that contains the magnetite appears to be innervated by the ophthalmic branch of the trigeminal nerve[80,81,86]. Two further findings are consistent with the hypothesis that branches of the trigeminal nerve innervate magnetoreceptors in birds. First, cutting the ophthalmic branch permanently abolished a conditioned response of pigeons that had been trained to discriminate between the presence and absence of a small magnetic anomaly[87]. Second, electrophysiological recordings in birds indicate that specific neurons in the trigeminal ganglion, to which the ophthalmic nerve projects, respond to changes in vertical field intensity as small as about 0.5% of the Earth's field[9] (FIG. 4). These cells have been proposed to function in a magnetic map sense[52].

Although direct evidence that magnetite functions in magnetoreception remains limited, additional circumstantial evidence has been provided by pulse magnetization experiments. A strong magnetic field of brief duration can be used to alter the direction of magnetization in single-domain magnetite particles[88]. Recent analyses have also indicated that such a magnetic pulse might also disrupt superparamagnetic crystals under at least some conditions[83]. Pulse magnetization might, therefore, alter magnetite-based magnetoreceptors and so change the behaviour of animals that use such receptors to derive directional or positional information from the Earth's field.

In several studies, the application of strong magnetic pulses to birds and turtles either randomized the preferred orientation direction or else deflected it slightly relative to controls[19,89–92]. These results have generally been interpreted as evidence for magnetite-based magnetoreceptors, although other explanations cannot be ruled out entirely[19], particularly given that pulsed magnetic fields generate large transient electric fields[21].

Strong magnetic pulses might hypothetically alter magnetite-based receptors that are part of a compass sense, a map sense or both. However, findings in birds indicate that the effect might be on a map sense rather than a compass sense. Pulsed fields influenced the orientation of adult birds, which are thought to rely on map information for navigation, but failed to affect young birds, which complete their first migration by flying along a consistent compass heading[93]. At the same time, pulse magnetization also significantly altered the magnetic orientation behaviour of mole rats, which have a magnetic compass but are not thought to have a map sense[94]. These results highlight the possibility that magnetite-based receptors might have different functional roles in different animals.

Compasses, maps and mechanisms

All three mechanisms that we have described seem to be capable of providing an animal with directional information that might be used in a magnetic compass sense. However, the information derived from the field is not the same in all cases. The induction model and some single-domain magnetite models are capable of detecting field polarity (that is, they can distinguish between magnetic north and south)[28,76]. By contrast, no current model based on chemical magnetoreception or superparamagnetism can do this[3,79].

Interestingly, there are two functionally different types of magnetic compass in animals. Polarity compasses, which are present in lobsters[95], salmon[96] and mole rats[94], determine north using the polarity of the horizontal field component. By contrast, the inclination compasses of birds[1,97] and sea turtles[98] evidently do not detect the polarity of the field (that is, north

Do well-organized magnetic organs exist?

Good evidence in the trout in 1997 (similar observations in pigeons, 2001-2003; some evidence in ants and bees):

a) nerve fibers ramified around the cells containing single-domain magnetite crystals (fibers studied with intracellular dyes)

b) these cells are located in a tissue layer under the olfactory receptors

c) behavioral response of the trouts to **B**

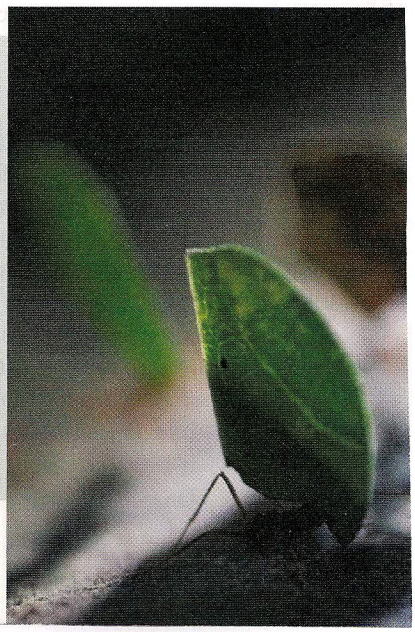
d) some fibers of the rosV nerve (olfactory branch of the trigeminus) respond to magnetic stimuli).

ANTENNE COME BUSSOLE

Ogni anno le formiche migratrici della foresta amazzonica intraprendono un viaggio in una direzione precisa: il loro cammino ha un angolo di 13 gradi rispetto all'asse magnetico nord-sud. Per orientarsi usano le antenne, che, come ha scoperto Jandira Ferreira de Oliveira del Centro brasiliano di ricerche in fisica di Rio de Janeiro, acquisiscono proprietà magnetiche grazie alle nanoparticelle di ossido di ferro e di silicato di alluminio che le formiche raccolgono abitualmente dal terreno. Grazie a osservazioni al microscopio ottico ed elettronico a trasmissione, de Oliveira ha stabilito che il materiale magnetico viene incorporato proprio nelle giunture delle antenne, come si legge nell'articolo pubblicato sul «Journal of the Royal Society Interface».

Successivamente, grazie a un modello teorico, gli scienziati hanno anche avuto conferma del fatto che la quantità di particelle raccolte dall'insetto è sufficiente a spiegare le proprietà magnetiche misurate nell'insetto. De Oliveira non è ancora certa della precisa localizzazione sulle antenne del recettore del magnetismo, ma ritiene che il migliore candidato sia l'organo di Johnston, che ha un ruolo importante nella percezione del movimento ma che potrebbe servire anche a rilevare i campi magnetici.

Federica Sgorbissa



B) Chemical magnetoreception.

1. It would seem unlikely, since electron displacements between atomic orbitals require a great energy. Moreover, thermal noise is much larger than terrestrial **B.**

2. However, there are reactions between couples of free radicals that can be affected by **B.**

For instance: Maeda et al.(Nature 453:387-390, 2008) show that the lifetime of photochemically generated radical pairs is modified by B < 50 μT (the intensity of Earth's B) and is sensitive to the field direction, which is essential for a compass working.

3. But what could the physiological sensor be?

Hypothesis of ***cryptochromes*** (photosensitive proteins implicated in circadian rhythms of plants and animals): retina, SCN, pineal gland (but do not appear to be the main photoperiodic receptors).

4. The nervous activity during magnetic orientation co-localizes with cryptochrome expression in **retinal ganglion cells** in migratory bird active during the night (*Sylvia*), but not during the day or in non migratory species.

5. **CRY1** and **CRY2**, UV-blue-green photoreceptor flavoproteins first identified in plants and likely to form radical pairs upon photoexcitation, as the closely related photolyases (and contrary to rhodopsin, which undergoes cis-trans isomerization of retinal, instead of electron transfer).

Also found in humans.

LETTERS

Chemical compass model of avian magnetoreception

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Approximately 50 species, including birds, mammals, reptiles, amphibians, fish, crustaceans and insects, are known to use the Earth's magnetic field for orientation and navigation[1]. Birds in particular have been intensively studied, but the biophysical mechanisms that underlie the avian magnetic compass are still poorly understood. One proposal, based on magnetically sensitive free radical reactions[2,3], is gaining support[4–11] despite the fact that no chemical reaction *in vitro* has been shown to respond to magnetic fields as weak as the Earth's (~50 μT) or to be sensitive to the direction of such a field. Here we use spectroscopic observation of a carotenoid–porphyrin–fullerene model system to demonstrate that the lifetime of a photochemically formed radical pair is changed by application of ≤50 μT magnetic fields, and to measure the anisotropic chemical response that is essential for its operation as a chemical compass sensor. These experiments establish the feasibility of chemical magnetoreception and give insight into the structural and dynamic design features required for optimal detection of the direction of the Earth's magnetic field.

Two principal mechanisms for animal magnetoreception have been put forward (reviewed in ref. 1), based on specialized deposits of magnetic iron minerals and magnetically sensitive photochemical reactions, respectively. The latter, the radical pair mechanism, is well established as the source of a variety of magnetic effects on free radical reactions *in vitro*[12,13]. It has been suggested that the avian compass mechanism relies on magneto-sensitive radical pairs formed by photoinduced intramolecular electron transfer reactions in an array of aligned photoreceptors located in the retina[2,3]. A promising candidate radical pair comprises the reduced flavin cofactor and an oxidized tryptophan residue in a cryptochrome flavoprotein[3,8,10]. Such a photochemical process could, in principle, account for two fundamental behavioural characteristics of the avian compass: its dependence on the wavelength of the ambient light[14] and the fact that birds respond to the inclination, rather than the polarity, of the geomagnetic field[15]. This proposal has been corroborated, in part, by the detection of cryptochromes in the retinae of migratory birds[5,6], and the finding that these proteins are expressed when the birds perform magnetic orientation[6], and by the observation of light-dependent, cryptochrome-mediated magnetic field effects on plant growth[11]. Theoretical work has also confirmed the principle and clarified some of the details[7–10]. Further compelling evidence for the involvement of radical pairs has come from the observation that weak radiofrequency magnetic fields, which can have profound effects on radical pair reactions *in vitro*[16], can disrupt the ability of birds to orient in the Earth's magnetic field[4,17].

Despite numerous studies[12], it has never been demonstrated that a static magnetic field as weak as that of the Earth can produce detectable changes in chemical reaction rates or product yields. Nor has a radical pair reaction been shown to respond to the direction of such a field, an essential requirement for a compass sensor. For this, it is essential that at least one of the radicals is immobilized so that its anisotropic magnetic interactions are preserved[2].

Here we demonstrate, as a proof-of-principle, that a photochemical reaction can act as a magnetic compass. The molecule selected for this purpose is a triad composed of linked carotenoid (C), porphyrin (P) and fullerene (F) groups (Fig. 1)[18]. Green-light irradiation efficiently produces the spin-correlated electronic singlet state of the radical pair (or biradical) $^{S}[C^{\bullet+}\text{–P–}F^{\bullet-}]$ by sequential intramolecular electron transfers (Fig. 1). $^{S}[C^{\bullet+}\text{–P–}F^{\bullet-}]$ undergoes reverse electron transfer, either directly to the ground state, with rate constant k_S, or to the excited triplet state ^{T}C–P–F, with rate constant k_T, having first converted to the triplet radical pair, $^{T}[C^{\bullet+}\text{–P–}F^{\bullet-}]$. This last process is controlled by the magnetic interactions of the two unpaired electrons and is the magnetic-field-sensitive step. As has been observed for related triads[19,20], an applied field alters the observed lifetime of $[C^{\bullet+}\text{–P–}F^{\bullet-}]$ by modifying the singlet–triplet character of its spin states, so changing the relative contributions of k_S and k_T to the overall kinetics.

We began by characterizing the effects of applied magnetic fields on the disappearance kinetics of the radical pair in isotropic solution. The transient absorption signal of $C^{\bullet+}$ in $[C^{\bullet+}\text{–P–}F^{\bullet-}]$ at 133 K (Fig. 2a, top), which has a lifetime of ~190 ns in zero field, was markedly increased to ~380 ns in an 8-mT field. The amplitude of the magnetic field effect decreased as the temperature increases and the difference signals were biphasic below ~200 K (Fig. 2a, bottom). Both of these properties are characteristic of a singlet-born radical pair with $k_T < k_S$, undergoing spin-lattice relaxation at a rate comparable to its recombination[21].

The magnetic field dependence of the $[C^{\bullet+}\text{–P–}F^{\bullet-}]$ transient absorption at 119 K (Fig. 2b) shows the biphasic magnetic field response expected for a long-lived radical pair[22]. The change in sign below ~1 mT is the 'low field effect': normally observed for the product yields of radical reactions in solution[12,13], it was manifested here as a change in the radical pair kinetics. The effect of the applied field on the radical pair absorption was opposite at 100 and 400 ns, as expected from the biphasic time dependence in Fig. 2a (bottom).

Finally, experiments performed in magnetic fields comparable to that of the Earth (Fig. 2c) revealed changes in radical pair absorption of up to ~1.5%. The biphasic time dependence observed at higher fields (Fig. 2a) was inverted here because of the low field effect (Fig. 2b). Thus, for $t > 400$ ns, $[C^{\bullet+}\text{–P–}F^{\bullet-}]$ recombined more rapidly in a ~50-μT field than it did in zero field, which was in turn faster than when the field exceeded 1 mT. This seems to be the first observation of a chemical effect of a magnetic field as weak as ~50 μT. The electron Zeeman interaction in such a magnetic field is more than a million times smaller than the thermal energy, $k_B T$, implying a negligible effect on the position of a chemical equilibrium or the kinetics of an activated reaction. However, such considerations are irrelevant for the interconversion of singlet and triplet states of radical pairs, a process that is activationless and far from equilibrium.

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Cryptochromes and neuronal-activity markers colocalize in the retina of migratory birds during magnetic orientation

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Migratory birds can use a magnetic compass for orientation during their migratory journeys covering thousands of kilometers. But how do they sense the reference direction provided by the Earth's magnetic field? Behavioral evidence and theoretical considerations have suggested that radical-pair processes in differently oriented, light-sensitive molecules of the retina could enable migratory birds to perceive the magnetic field as visual patterns. The cryptochromes (CRYs) have been suggested as the most likely candidate class of molecules, but do CRYs exist in the retina of migratory birds? Here, we show that at least one CRY1 and one CRY2 exist in the retina of migratory garden warblers and that garden-warbler CRY1 (gwCRY1) is cytosolic. We also show that gwCRY1 is concentrated in specific cells, particularly in ganglion cells and in large displaced ganglion cells, which also showed high levels of neuronal activity at night, when our garden warblers performed magnetic orientation. In addition, there seem to be striking differences in CRY1 expression between migratory and nonmigratory songbirds at night. The difference in CRY1 expression between migrants and nonmigrants is particularly pronounced in the large displaced ganglion cells known to project exclusively to a brain area where magnetically sensitive neurons have been reported. Consequently, cytosolic gwCRY1 is well placed to possibly be the primary magnetic-sensory molecule required for light-mediated magnetoreception.

Since the description of animal magnetosensory capabilities in the 1960s (1–3), it has been convincingly shown that songbirds can use a magnetic compass for orientation during their migratory journeys (3–6), but the physiological mechanisms enabling migratory birds to sense the reference direction provided by the Earth's magnetic field still remain unknown. Two types of potential magnetoreception mechanisms have been suggested over the past decades: one mechanism that is based on magnetite particles and one mechanism that is based on photoreceptors forming radical-pair intermediates (for summary, see ref. 7). Although no direct physiological or molecular evidence has been reported, numerous orientation cage experiments with captive migratory songbirds have revealed several important characteristics of their magnetic compass.

The magnetic compass of migratory songbirds is an inclination compass; that is, it detects the axis but not the polarity of the magnetic field lines (4–5). Furthermore, magnetic orientation in migratory songbirds depends on the wavelength of the ambient light (8–11). Migratory songbirds are active and orient magnetically under dim blue and green light, whereas they are active but disoriented under dim red light (8–10). These findings strongly suggest that photoreceptor molecules in the eye are involved in magnetoreception and that these photoreceptor molecules should absorb in the blue and green range of the spectrum. The involvement of photoreceptors in the eye is further supported by the finding that birds with their right eye covered seem unable to perform magnetic orientation (12). A recent behavioral experiment (13) testing the magnetic orientation responses of European robins, *Erithacus rubecula* (a night-migrating songbird), exposed to oscillating magnetic fields provided strong indirect evidence that the magnetic-inclination compass of night-migrating songbirds is based on a radical-pair mechanism (7, 13).

Photoreceptor-based radical-pair mechanisms were suggested by Schulten *et al.* (14) and strongly elaborated on by Ritz *et al.* (7). They are based on the fact that radical-pair reactions will be modulated differently depending on the direction of the Earth's magnetic field relative to the orientation of the radical-pair-forming molecule (7, 15, 16). In short, the current hypothesis (7) further suggests that light in the blue-green range will excite photoreceptors forming radical pairs upon photoexcitation in the retina of the migratory bird. Because of the shape of the retina (half ball) and the presumed fixed orientation of the radical-pair-forming photoreceptors inside the cells, the magnetic field would modulate the radical-pair reaction and, thereby, the light sensitivity differently in different parts of the retinas, leading to perception of the magnetic field as visual patterns (7). Radical-pair-mediated magnetoreception would not be able to detect the polarity of the field lines, but only their axis, which is in line with the inclination-based nature of the songbird magnetic compass (4, 10).

Based on these theoretical considerations and behavioral evidence, the primary magnetic-sensory molecule in the retina of migratory songbirds should be a photopigment that is excited by light in the blue-green range and forms radical pairs upon photoexcitation. The cryptochromes (CRYs) (17–22) have been suggested as the most likely candidate class of molecules (7) because they are blue-green photoreceptors in plants (17, 19, 22) and because closely related 6,4-photolyases have been shown to form radical pairs upon photoexcitation (23). Other classes of photoreceptors, such as phototropsins (24) and chlorophylls (25), found in plants can also undergo radical-pair reactions. Rhodopsins should not be able to form radical pairs because photoexcitation leads to cis–trans isomerization of retinal rather than an electron transfer (e.g., ref. 26). Thus, CRYs are the only currently known class of molecules found in the retina of vertebrates that are likely to fulfill the physical and chemical characteristics that are required for function as the primary magnetic sensor (7).

Therefore, the aims of the present article are to (*i*) test whether CRYs exist in the retina of migratory birds performing a magnetic orientation task at night, (*ii*) elucidate their cellular location within the retina, (*iii*) test whether the CRY-containing cells show neuronal activity when migratory birds perform night-time magnetic orientation behavior, and (*iv*) compare the

Abbreviations: CRY, cryptochrome; gwCRY, garden-warbler CRY; INL, inner-nuclear layer.

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C) Electromagnetic induction

Lorentz force $\mathbf{F} = q\mathbf{v} \Lambda \mathbf{B} = qvB(\text{sen}\theta)$ (if $\mathbf{E} = 0$)

θ = angle between the direction of the field **B** and of q motion.

It may be possible in electric fishes.

Lorenzini ampullae could work as conductive bars and sea water as stationary conductive milieu.

Electroceptors would sense the ΔV produced by the induced current.

However: moving water produces fields, moreover electroceptors do not perceive stationary fields.

There are hints, but not conclusive evidences.