

Magnetoreception.

Magnetic field: **B**; electric field: **E**.

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
Crostatea
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.

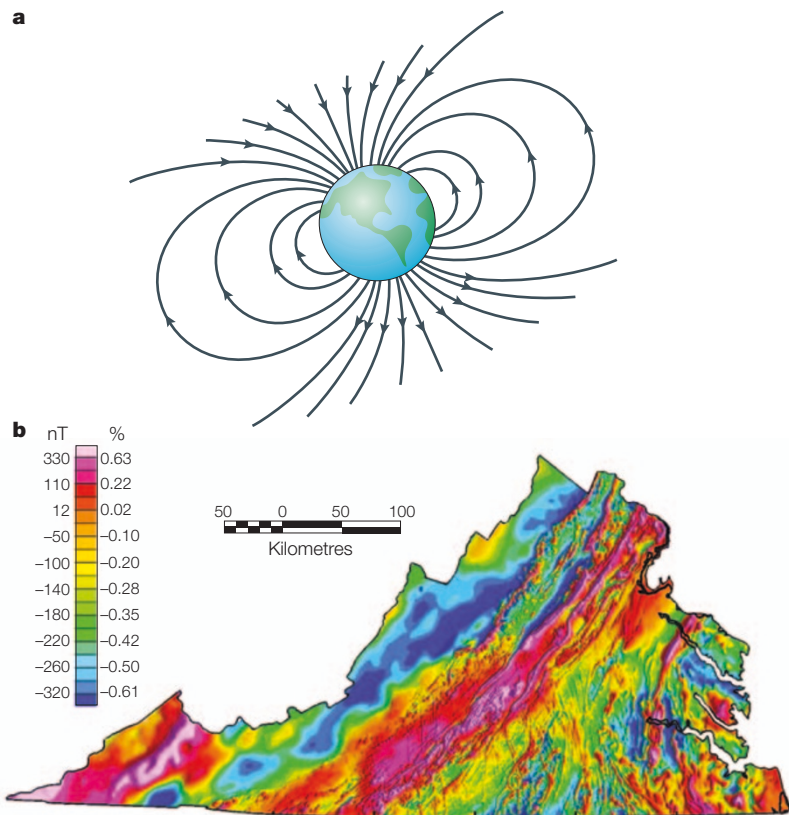


Figure 1 | Large-scale and fine-scale structure of the Earth's magnetic field.

a | Diagrammatic representation of the Earth's magnetic field. The general form of the Earth's field resembles the dipole field of a giant bar magnet, with field lines emerging from the southern hemisphere, wrapping around the globe, and re-entering the Earth at the northern hemisphere. The inclination angle (that formed between the field lines and the Earth) varies with latitude. At the magnetic equator, the field lines are parallel to the Earth's surface and the inclination angle is 0°. An animal migrating north from the magnetic equator to the magnetic pole encounters progressively steeper inclination angles along its journey. At the magnetic pole in the northern hemisphere, field lines are directed straight down into the Earth and the inclination angle is 90°. This variation in the inclination angle is used by some animals to assess geographic position^{11,16}. The strength or intensity of the Earth's field is weakest near the equator and strongest near the magnetic poles. Some animals can derive positional information from field intensity¹². **b** | Merged aeromagnetic anomaly map of the state of Virginia, USA. Although some long-distance migrants evidently extract positional information from the general dipole field^{10,13} shown in (a), fine-scale variations are more complex than the general regional patterns because concentrations of ferromagnetic minerals in the Earth's crust often generate local field anomalies. Although these variations are typically less than 1% of the total field, their gradients (that is, the variation per distance) can be significantly greater than the gradients due to the main dipole field, and can also be aligned in a different direction. The larger gradients might be easier for a short-distance migrant or homing animal to detect, but the complexity of local magnetic contours indicates that any navigational strategies that exploit magnetic topography over these smaller spatial scales are likely to be site-specific, difficult to generalize and learned rather than inherited. Colour scale shows deviations in the strength of the Earth's dipole field in Virginia (dipole field is ~52,000 nanoTesla (nT)¹²⁴) in both nanoTesla and percent. Image reproduced from REF. 125.

and a migrating bird's heading can be altered by wind. Navigation can therefore be enhanced by an ability to determine position relative to a destination. For today's humans, this need is usually met through a GLOBAL POSITIONING SYSTEM (GPS), which provides users with their geographical position and continuously computes the direction to a goal. For some migratory animals, positional information inherent in the Earth's magnetic field provides a similar, although less precise, way of assessing geographical location.

GLOBAL POSITIONING SYSTEM (GPS). A network of artificial satellite transmitters that provide highly accurate position fixes for Earth-based, portable receivers.

Several geomagnetic parameters, such as inclination angle and field intensity, vary across the Earth's surface in ways that make them suitable for use in a position-finding sense^{6,7} (FIG. 1). Some animals, including certain birds⁸⁻¹⁰, sea turtles¹¹⁻¹⁴, salamanders^{15,16} and lobsters¹⁷, can discriminate small differences in at least some of these magnetic features. These animals exploit positional information in the Earth's field in several different ways, and at least a few are able to learn the magnetic topography of the areas in which they live and so acquire 'magnetic maps' that facilitate navigation towards specific locations (FIG. 2).

Because the parameters of the Earth's field that are useful for detecting directional and positional information differ, it is possible that some animals have two separate magnetosensory systems. Each might detect a different element of the Earth's field and each might also rely on separate receptors based on different biophysical mechanisms^{18,19}.

Possible mechanisms of magnetoreception

During the past three decades, a number of diverse mechanisms have been proposed that might provide the basis for detecting magnetic fields^{1,20}. However, the most recent research has focused on three possibilities: ELECTROMAGNETIC INDUCTION, magnetic field-dependent chemical reactions and BIOGENIC MAGNETITE. Each mechanism is discussed below.

Electromagnetic induction. A charged particle moving through a magnetic field experiences a force perpendicular to both its motion and the direction of the field. The magnitude of this LORENTZ FORCE is equal to the product of the magnetic field strength, the charge and velocity of the particles, and the sine of the angle between the motion and field vectors²¹. Therefore, if an electrically conductive bar moves through a magnetic field in any direction other than parallel to the field lines, positively and negatively charged particles migrate to opposite sides of the bar, resulting in a constant voltage that depends on the speed and direction of the bar's motion relative to the magnetic field. If the bar is immersed in a conductive medium that is stationary relative to the field, an electric circuit is formed and current flows through the medium and the bar.

This principle, known as electromagnetic induction, provides a possible explanation for how elasmobranch fish (sharks, skates and rays) detect the Earth's magnetic field^{22,23}. According to this hypothesis, jelly-filled canals on the fish, known as ampullae of Lorenzini, function as the conducting bars; the surrounding sea water functions as the motionless conducting medium, and the highly resistive and sensitive electroreceptors at the inner end of the ampullae detect the voltage drop of the induced current.

However, several factors significantly complicate these simple models. First, the electroreceptors of elasmobranchs cannot detect the steady fields that were originally thought to arise²⁴. Second, the water surrounding marine fish is seldom motionless under

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 magnetite 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 crystals.

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**.

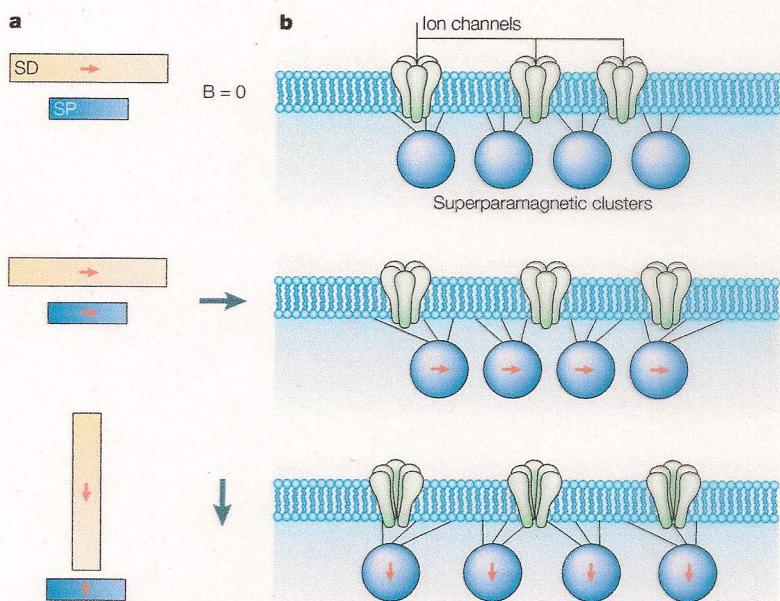


Figure 3 | The different magnetic properties of single-domain and superparamagnetic crystals. **a** | Single-domain (SD) and superparamagnetic (SP) magnetite crystals have different magnetic properties. Single-domain crystals have permanent magnetic moments (indicated by red arrows) even in the absence 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 terminals⁷⁹. Modified, with permission, from REF. 79 © (2003) Elsevier Science.

intensity as small as about 0.5% of the Earth's field⁹ (FIG. 4). These cells have been proposed to function in a magnetic map sense⁵².

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 particles⁸⁸. Recent analyses have also indicated that such a magnetic pulse might also disrupt superparamagnetic crystals under at least some conditions⁸³. 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 entirely¹⁹, particularly given that pulsed magnetic fields generate large transient electric fields²¹.

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 heading⁹³. 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 sense⁹⁴. 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 lobsters⁹⁵, salmon⁹⁶ and mole rats⁹⁴, determine north using the polarity of the horizontal field component. By contrast, the inclination compasses of birds^{1,97} and sea turtles⁹⁸ evidently do not detect the polarity of the field (that is, north

these crystals inside nerve terminals and arranged along the cell membrane⁸⁰. However, in contrast to the single-domain magnetite detected in fish⁸², 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 anomaly⁸⁷. 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

DIAM. PARAM. FERROM. ↓ NON. MAGN. bi. Gibi. MOLEC.

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 $B < 50 \mu\text{T}$ (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.

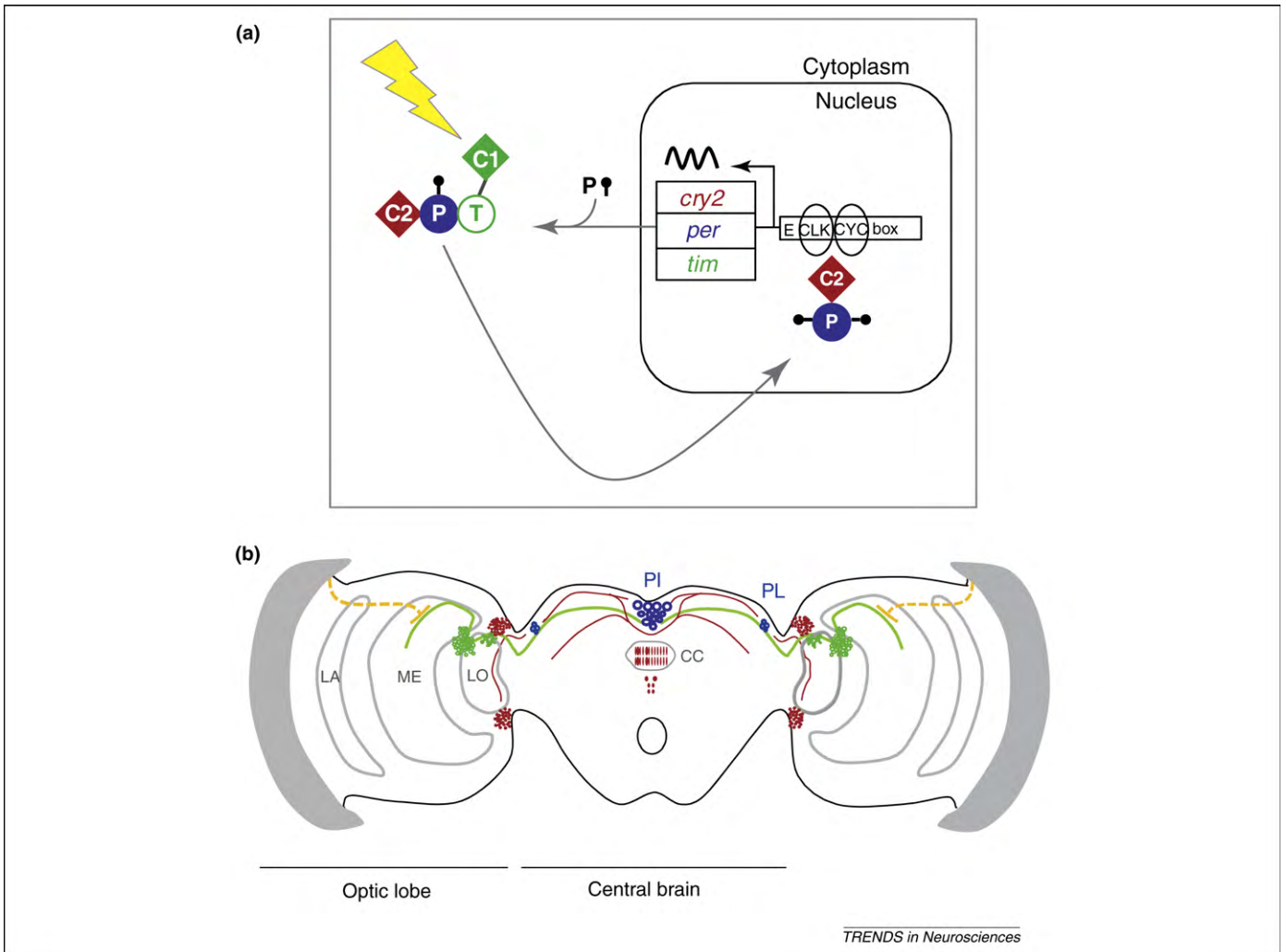


Figure 3. Monarch clockwork model, brain clocks and circuits. **(a)** Molecular clockwork model. The main gear of the clock mechanism is an autoregulatory transcription feedback loop in which CLOCK (CLK) and CYCLE (CYC) heterodimers drive the transcription of the *period* (*per*), *timeless* (*tim*), and *cryptochrome2* (*cry2*) genes through E box enhancer elements. TIM (T), PER (P), and CRY2 (C2) are translated and form complexes in the cytoplasm; 24 h later CRY2 is shuttled into the nucleus to inhibit CLK:CYC-mediated transcription. PER is progressively phosphorylated and probably helps to translocate CRY2 into the nucleus. CRYPTOCHROME1 (CRY1, C1) is a circadian photoreceptor which, upon light exposure (lightning bolt), causes TIM degradation, allowing light to gain access to the central clock mechanism for photic entrainment. The monarch clockwork, which has both *Drosophila*-like and mammalian-like aspects, was formulated from a combination of *in vitro* and *in vivo* approaches, including the use of the monarch DpN1 cell line, which contains a light-driven diurnal clock, and the use of *Drosophila* transgenesis, to augment the differential clockwork functions of the monarch CRY proteins [37]. Adapted, with permission, from Ref. [37]. **(b)** Schematic frontal view of brain clocks and circuits in the monarch butterfly brain. Cells expressing TIM, PER, CRY1 or CRY2 are highlighted in blue [26,37]. In these areas the clock proteins partially colocalize. All four clock proteins are colocalized in two of the four cells in the pars lateralis (PL) on each side of the brain; these four cells in total are the *bona fide* circadian clock cells in brain [26,37]. CRY1-positive cell bodies and projections are represented by green dots and green lines, respectively. Projections from the dorsal rim area photoreceptors are indicated by the dotted gold lines. Neurons and fibers expressing exclusively CRY2 are represented in red and within the central body are shown as red circles and red hatching. PI, pars intercerebralis; PL, pars lateralis; CC, central complex; LA, lamina; ME, medulla; LO, lobula. Adapted, with permission, from Ref. [40].

two distinct CRYPTOCHROME (CRY) proteins; monarchs have both a type 1 *Drosophila*-like CRY (designated CRY1) that functions as a circadian photoreceptor and a type 2 vertebrate-like CRY (designated CRY2) that functions as the major transcriptional repressor of the clockwork feedback loop [37]. The discovery of type 2 vertebrate-like CRYs in insects, which emerged from the initial finding of CRY2 in monarch butterflies [38], has changed our view of how circadian clocks of non-drosophilid insects work and has redefined our understanding of the evolution of animal CRYs [11,39]. Four cells in the dorsolateral region of the central brain (the pars lateralis; PL) house the major circadian clocks in the butterfly brain (Figure 3B); these are the only brain cells in which the transcriptional repressor CRY2 cycles into the nucleus at the appropriate time to regulate the molecular clock feedback loop [37].

The monarch butterfly could also use the CRY proteins as output molecules that define clock circuits in the brain (Figure 3B). Indeed, CRY1-staining fibers extend from the clock cells in the PL to the optic medulla, in which axons of the dorsal rim photoreceptors terminate, suggesting a brain clock modulation of polarized light input [26] (Figure 3B). Other CRY1-expressing fibers connect the PL with neurons of the pars intercerebralis (PI), another clock gene/protein-expressing region that could regulate diapause and aging [26]. CRY2-expressing neuronal fibers that could arise from the PI and/or the PL are also found in the central complex, where CRY2 shows circadian oscillations in its expression [37] (Figure 3B).

Based on all the evidence, it was assumed that the circadian clocks necessary for time-compensation of sun compass orientation reside in the PL [11]. However, this

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 navigation¹. 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 μ T) 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 μ T 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 light¹⁴ and the fact that birds respond to the inclination, rather than the polarity, of the geomagnetic field¹⁵. 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 orientation⁶, and by the observation of light-dependent, cryptochrome-mediated magnetic field effects on plant growth¹¹. 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 studies¹², 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 preserved².

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) $^1[C^{\bullet+}-P-F^{\bullet-}]$ by sequential intramolecular electron transfers (Fig. 1). $^1[C^{\bullet+}-P-F^{\bullet-}]$ undergoes reverse electron transfer, either directly to the ground state, with rate constant k_S , or to the excited triplet state $^3C-P-F$, with rate constant k_T , having first converted to the triplet radical pair, $^3[C^{\bullet+}-P-F^{\bullet-}]$. 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 $[C^{\bullet+}-P-F^{\bullet-}]$ 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 $[C^{\bullet+}-P-F^{\bullet-}]$ 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 recombination²¹.

The magnetic field dependence of the $[C^{\bullet+}-P-F^{\bullet-}]$ transient absorption at 119 K (Fig. 2b) shows the biphasic magnetic field response expected for a long-lived radical pair²². 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, $[C^{\bullet+}-P-F^{\bullet-}]$ recombined more rapidly in a ~50- μ T 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 μ T. 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 phototropins (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 $\mathbf{F} = q\mathbf{E} + q\mathbf{v}\times\mathbf{B}$

$$q\mathbf{v}\times\mathbf{B} = qvB(\sin\theta)$$

θ = angle between the direction of the field \mathbf{B} and of q motion.

Electromagnetic induction may be possible in electric fishes.

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

Electroreceptors would sense the ΔV produced by the induced current.

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

There are hints, but not conclusive evidences.