The Radical-Pair Mechanism of Magnetoreception

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Abstract

Although it has been known for almost half a century that migratory birds can detect the direction of the Earth's magnetic field, the primary sensory mechanism behind this remarkable feat is still unclear. The leading hypothesis centers on radical pairs—magnetically sensitive chemical intermediates formed by photoexcitation of cryptochrome proteins in the retina. Our primary aim here is to explain the chemical and physical aspects of the radical-pair mechanism to biologists and the biological and chemical aspects to physicists. In doing so, we review the current state of knowledge on magnetoreception mechanisms. We dare to hope that this tutorial will stimulate new interdisciplinary experimental and theoretical work that will shed much-needed additional light on this fascinating problem in sensory biology.

Contents

INTRODUCTION

The Earth's magnetic field offers directional and positional information that animals can use for the purposes of navigation and orientation. Migratory birds detect the direction of the field and use it as input to a magnetic-compass sense (32, 134, 204, 205, 208), which relies on the inclination rather than the polarity of the field (204, 208). The combination of an inherited migratory direction (13) and a compass sense is enough to enable a young bird on its first autumn migration to find its way, for example, from northern Europe to Africa (13, 78, 130, 131, 140, 155). However, to locate the same breeding and wintering site year after year, as adult birds do (131), true navigation, which requires not only a compass but also a map, is needed (117, 131, 159). The map sense is clearly multifactorial (55, 62, 137), and different cues might be used during different stages of a homing task (54, 55, 62, 137). However, magnetic information could be a useful part of a map sense, especially over larger distances, and there is a growing body of evidence that night-migratory songbirds can determine their approximate position on Earth using geomagnetic information (52, 97–99). Thus, the behavioral responses of night-migratory birds to geomagnetic cues are reasonably well known, and many birds seem to have both a magnetic compass and a magnetic map (99, 134, 204, 215). In contrast, understanding the underlying biophysical mechanisms remains one of the most significant unsolved problems in sensory biology.

We can expect the avian magnetic map and compass senses to have mutually distinct properties and mechanisms in the same way that human-made devices for measuring the intensity and for detecting the direction of a magnetic field usually rely on different principles and different technology. In general, a direction sensor need not respond to magnetic intensity and vice versa. As we shall see, the leading hypothesis for geomagnetic sensing, on which we focus here, involves magnetically sensitive chemical intermediates known as radical pairs, and in birds, this mechanism seems to form the basis of the magnetic-compass sense. The only other contender, which we discuss in Appendix 1, is based on magnetic iron-containing nanoparticles. If it exists in birds, this mechanism probably forms the basis of the magnetic-map sense.

The notion that radical pairs could be involved in the magnetic-compass sense of migratory birds and other animals dates back to 1978. Schulten et al. (174) imagined the primary event to be a magnetically sensitive photochemical transformation with a radical pair as a transient reaction intermediate, in what has proven to be a remarkably far-sighted proposal. If the yield of the products of the chemical reaction depends on the direction of the geomagnetic field with respect to the reactant molecules, then one has the basis of a compass sensor (**Figure 1**). Given the transparency of biological tissue to static and low-frequency magnetic fields and the absence of any obvious magnetosensory organ, this process could, in principle, occur almost anywhere in a bird's body, although the eye is the most obvious location for a light-dependent detector.

At first sight, a radical-pair compass seems implausible: The interaction of the Earth's magnetic field (30–65 μT) with a single molecule is more than a million times smaller than its thermal energy, $k_B T$, under physiological conditions. $k_B T$ (Boltzmann's constant multiplied by temperature) is the energy associated with the ever-present random motions of molecules as they bump into one another, rotate, and vibrate. Normally, a significant impact on the rate or yield of a chemical transformation is impossible unless an amount of energy that is at least comparable to the energy associated with these motions is supplied. **Figure 2** may help to elucidate why radical-pair reactions are different in this respect (see also Appendix 2). Imagine we have a heavy stone block, and ask whether a fly would be able to tip it over by bumping into it (**Figure 2*a***). The answer, obviously, is no. But suppose we have supplied the energy necessary to poise the stone on its edge: Clearly, it would not be stable and would tend to fall to the left or the right if left to its own devices. But what if a fly landed on its right-hand side while the block is teetering in this way (**Figure 2*b***)? Even though the energy imparted by the fly would be minute, it could be enough to cause the block to fall to the right rather than the left. Tiny interactions can have profound effects but only if the system has previously been brought into an appropriate state far from equilibrium. In the present context, the nonequilibrium state is the radical pair, and the energy required to reach that state comes from a photon of light.[1]

Nevertheless, for more than two decades, Schulten et al.'s proposal was regarded as an interesting curiosity partly, we suspect, because biologists were daunted by the mathematical presentation of the 1978 article (174). However, there were two key developments during this time. First, behavioral experiments suggested that the magnetic compass of birds (206) and newts (156) is indeed

[1]In the hope of making the text accessible to a broad audience, we occasionally oversimplify arguments and gloss over complications. In such cases, a footnote often points the way to a more precise description.

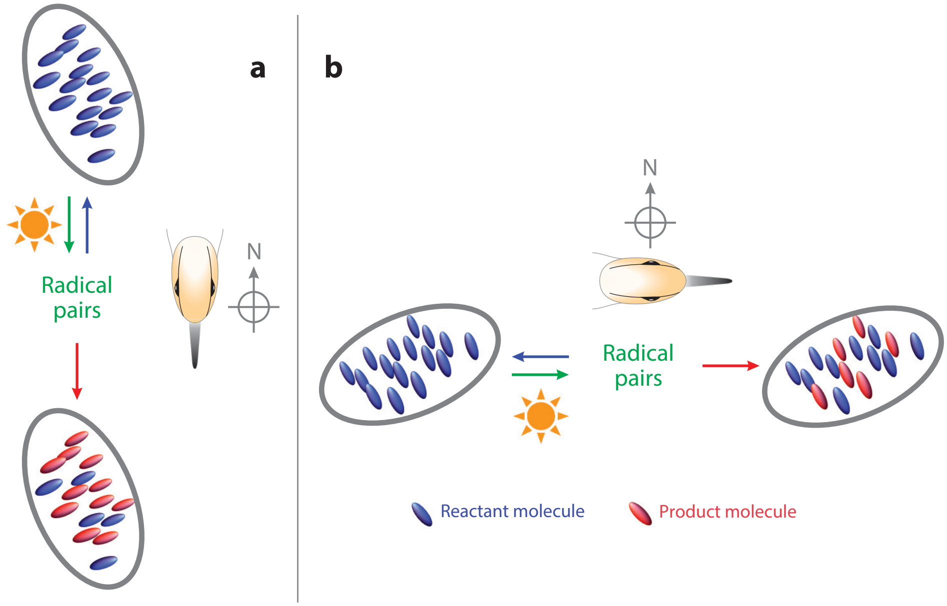

Figure 1

The principle of a radical-pair compass. Reactant molecules (*blue*) are photochemically converted into product molecules (*red*). This transformation occurs via radical-pair intermediates, which can either proceed forward to the products (*red arrows*) or return to the reactants (*blue arrows*). The reactants and therefore the radical pairs are aligned relative to one another and oriented within the bird's eye so that they experience a change in the direction of the Earth's magnetic field when the bird moves its head. If this change is to form the basis of a magnetic compass, it must affect the probability that the radical pairs proceed along the red and blue pathways. The figure shows, schematically, the case in which more efficient conversion of reactants to products occurs when the bird's head is (*a*) aligned with the north–south axis than when it is (*b*) aligned with the east–west axis.

light dependent, supporting the idea that magnetoreception could be based on a photochemical reaction. Second, the radical-pair mechanism—in its infancy in the 1970s—came of age (144, 171, 184). There are now hundreds of laboratory studies of (mostly organic) radical reactions on which relatively modest magnetic fields (1–100 mT) have been shown to have an effect (167, 184, 211). Theory and experiment have advanced in parallel to the extent that many experimental observations can now be interpreted quantitatively in terms of the physics and chemistry of the radicals. In addition, there are several other well-established radical-pair phenomena that share the same physical and chemical principles (23, 49, 60).[2] The radical-pair mechanism is unquestionably genuine. What is not yet proven is whether it lies at the heart of avian magnetoreception.

In 2000, Schulten's suggestion changed overnight from interesting curiosity to intriguing possibility when he, Adem, and Ritz wrote an article (162) that both made the 1978 proposal accessible to biologists and suggested a specific molecule in which appropriate radical pairs might be formed. This molecule, a protein called cryptochrome (1, 30), remains to this day the only candidate radical-pair magnetoreceptor (37, 115, 138). No other vertebrate photoreceptor molecule appears to form radical pairs when excited by light. Opsins, the visual receptor proteins, use light energy for a different purpose—to isomerize retinal—without the involvement of radicals (17).

[2] Chemically induced dynamic electron and nuclear polarization, and the magnetic isotope effect.

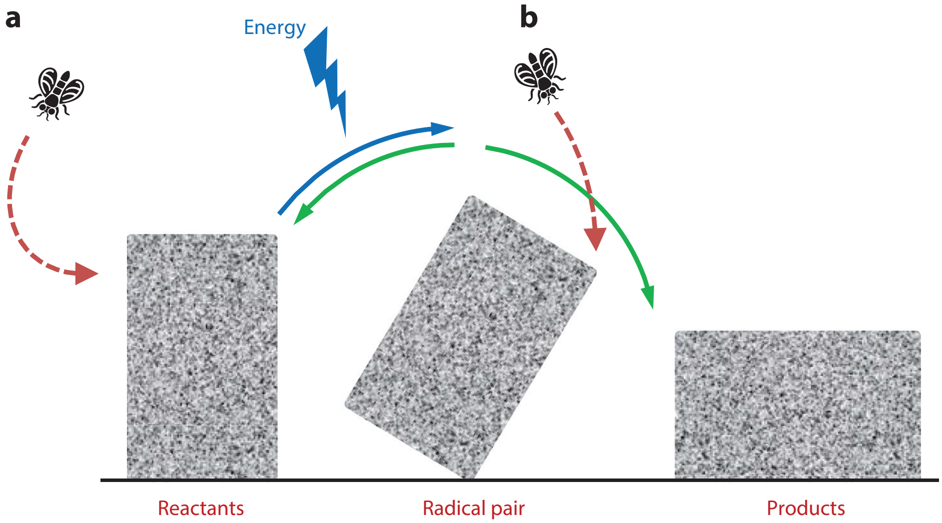

Figure 2

The radical-pair mechanism—an analogy. Insight into why the outcome of a radical-pair reaction can be significantly affected by extremely small magnetic interactions can be obtained from this mechanical analogy. (*a*) A fly bumping into the side of a heavy block of stone would have a negligible probability of tipping it over. (*b*) However, if the stone is first prepared in a highly nonequilibrium state (*blue arrow*), the tiny amount of energy imparted by the insect could profoundly alter the chance that it falls forward rather than reverting to its initial position (*green arrows*). Adapted from Reference 79 with permission.

Chlorophylls, the only other biomolecules known to form photoinduced radical pairs in vivo [in the primary steps of photosynthetic charge separation (125)], do not occur in birds.

In the following pages, we explain the physical and chemical basis of the radical-pair mechanism, concentrating on the properties required to detect the direction of the Earth's magnetic field. This is followed by a discussion of photoinduced radical pairs in cryptochromes and a summary of the evidence for their involvement in compass magnetoreception. We then examine some of the biological requirements for magnetic-compass sensing and discuss the effects of time-dependent magnetic fields, which have been used as a behavioral test for the involvement of radical pairs. We end with some theoretical considerations and a brief review of the magnetite hypothesis of magnetoreception.

THE RADICAL-PAIR MECHANISM

Radicals, Radical Pairs, and Electron Spin

A radical is a molecule that contains an odd number of electrons. A radical pair consists of two radicals that have been created simultaneously, usually by a chemical reaction. For example, consider methane (CH_4), a molecule in which a carbon atom is bonded to four hydrogen atoms in a tetrahedral arrangement. It has a total of ten electrons, six contributed by the carbon and one from each of the hydrogens. Two of the electrons surround the carbon nucleus; the other eight are involved in forming the carbon-hydrogen bonds, two electrons per bond. If one of the bonds is broken in such a way that both of the resulting fragments are uncharged, the result is a radical pair composed of a methyl radical, $CH_3^\bullet$, and a hydrogen radical, $H^\bullet$, otherwise known as a hydrogen atom. The dots indicate the odd electrons, one per radical.

Radicals are magnetic because the electron (in common with the proton and the neutron) has a property known as spin or, more accurately, spin angular momentum. Envisaging the electron as a small spherical object spinning around its axis, like a miniature planet, is tempting. As the electron is charged and moving, one could imagine that it would generate a magnetic field just like an electrical current in a loop of wire. However, spin is a quantum mechanical property, and quantum objects do not behave classically. Spin is best regarded as an attribute that some particles have and some do not, like mass or charge.[3]

To sum up, the electron is a microscopic magnet: It possesses a magnetic moment. Regarding the $CH_3^\bullet$ radical with its nine electrons, eight are present in pairs such that their magnetic moments exactly cancel. The magnetism of the radical therefore stems from the ninth, unpaired, electron. In the same way, $H^\bullet$ is magnetic because of its unique electron. The magnitude of the spin angular momentum of a quantum object is determined by its spin quantum number, S. For the electron, $S = 1/2$. Quantum mechanics stipulates that a radical pair (e.g., $[CH_3^\bullet \quad H^\bullet]$) can have a spin quantum number of only 0 or 1. Roughly speaking, the spins of the two unpaired electrons can be either parallel to one another (↑↑, giving $S = 1$) or antiparallel (↑↓, giving $S = 0$). There are three ways in which S can equal 1 but only one way in which the radical pair can have no net spin.[4] The two forms of the radical pair are therefore known as triplet ($S = 1$) and singlet ($S = 0$). In this respect, radicals and radical pairs differ from most molecules—referred to as closed shell molecules—which have only paired electrons and can therefore only be singlets.

Breaking a chemical bond in such a way that the two electrons end up in different molecular fragments is not the only way radical pairs can be formed. A common alternative is electron transfer, in which an electron is passed from one closed shell molecule to another: $A + B \rightarrow [A^{\bullet +} B^{\bullet -}]$. An important feature of such reactions of organic molecules is that they usually conserve spin.[5] Because A and B are singlets, the radical pair $[A^{\bullet +} B^{\bullet -}]$ must be formed in a singlet state. The same goes for $CH_4 \rightarrow [CH_3^\bullet \quad H^\bullet]$. The formation of radical pairs is said to be spin selective. Similarly, the reverse process, $[A^{\bullet +} B^{\bullet -}] \rightarrow A + B$, cannot occur for triplet radical pairs. Conservation of electron spin in radical-pair reactions is a fundamental requirement for a chemical magnetic-field effect.

Hyperfine Interactions

Two additional properties of radical pairs are discussed here and in the following subsection. Almost all radicals have internal magnetic interactions, known as hyperfine interactions. Many atomic nuclei have spin (a consequence of the spins of their protons and neutrons) and therefore magnetic moments that can interact with an unpaired electron. Normally, only the nuclei of isotopes that have even numbers of protons and neutrons (e.g., ^{12}C, ^{16}O, and ^{32}S) have no spin (**Table 1**). Some others (e.g., ^{13}C, ^{15}N, and ^{17}O) containing odd numbers of protons

[3] Classical arguments, analogies, and pictures are used to shed light on quantum behavior. Although we hope these will be helpful, they should not be taken too literally. Electron and nuclear spins do not obey Newtonian mechanics, often behave counterintuitively, and can only accurately be described mathematically, using quantum mechanics. The reader should bear in mind that many of the "hand-waving" explanations we offer here can lead to predictions that are misleading, confusing, or just plain wrong.

[4] In general, there are $2S + 1$ ways an object can have spin quantum number S. In the absence of a magnetic field, they all have the same energy and correspond (roughly) to $2S + 1$ different projections of a vector representing the spin angular momentum onto the same arbitrary axis.

[5] Spin is conserved in a chemical transformation when the magnetic moment arising from electron spin interacts weakly with the magnetic field generated by the orbital motion of the electron within the radical. This interaction, known as spin-orbit coupling, is usually small unless the radicals have high symmetry (e.g., linear) or contain heavy (e.g., transition metal) atoms.

Table 1 Magnetic properties of isotopes of elements commonly found in organic radicals

Element	Isotope	Natural abundance	Number of protons	Number of neutrons	Magnetic field (mT)
Hydrogen	^{1}H	99.985%	1	0	2.44
	^{2}H	0.015%	1	1	0.61
Carbon	^{12}C	98.892%	6	6	0.00
	^{13}C	1.108%	6	7	0.61
Nitrogen	^{14}N	99.63%	7	7	0.29
	^{15}N	0.37%	7	8	0.25
Oxygen	^{16}O	99.8%	8	8	0.00
	^{17}O	0.037%	8	9	1.13
Phosphorus	^{31}P	100.0%	15	16	0.99
Sulfur	^{32}S	95.02%	16	16	0.00
	^{33}S	0.75%	16	17	0.42
	^{34}S	4.21%	16	18	0.00

Red and blue shading indicates elements for which the most common isotope does and does not, respectively, lead to hyperfine interactions. The final column gives, for each nuclide, the dipolar magnetic field (in mT) it generates at a distance of 0.1 nm.

or neutrons or both, do have magnetic moments but have such low natural isotopic abundance (1.1%, 0.37%, and 0.04%, respectively) that they can normally be ignored. The two most important magnetic isotopes in the present context are ^{1}H (1 proton) and ^{14}N (7 protons and 7 neutrons): Both are common in organic radicals and both have close to 100% natural abundance (**Table 1**).

As we shall see, Earth-strength magnetic fields cannot significantly affect a radical-pair reaction if there are no hyperfine interactions in either radical.[6] This is not a serious constraint: Almost every biologically relevant radical has one or more hydrogen and/or nitrogen atoms in the neighborhood of the unpaired electron.[7] It is important to realize that the unpaired electron in a radical usually interacts with several nuclei simultaneously, partly because it is delocalized (i.e., spread out over a portion of the molecule) and partly because electron-nuclear dipolar interactions can be significant at distances of up to ~0.5 nm. For example, **Figure 3*b*** shows the form of the molecular orbitals that contain the unpaired electrons in the flavin and tryptophan radicals (**Figure 3*a***) formed by photoinduced electron transfer in cryptochromes (see below). The unpaired electron has a significant probability of being near almost all of the carbon and nitrogen atoms that make up the aromatic isoalloxazine and indole groups of the flavin and tryptophan radicals. The ^{1}H and ^{14}N hyperfine interactions in the two radicals are represented in **Figure 3*c*** as surfaces centered on each of the hydrogen and nitrogen atoms: The larger and less spherical the surface, the stronger and more anisotropic[8] the hyperfine interaction. One might expect the hyperfine interactions of the nitrogens to be smaller than those of the hydrogens

[6]At least one hyperfine interaction is necessary to break the symmetry between the two electron spins.

[7]One exception, superoxide ($O_2^{\bullet-}$), a reduced form of dioxygen, is discussed below.

[8]The anisotropy of the hyperfine interactions, as discussed below, is the source of the directional information available from a radical-pair reaction.

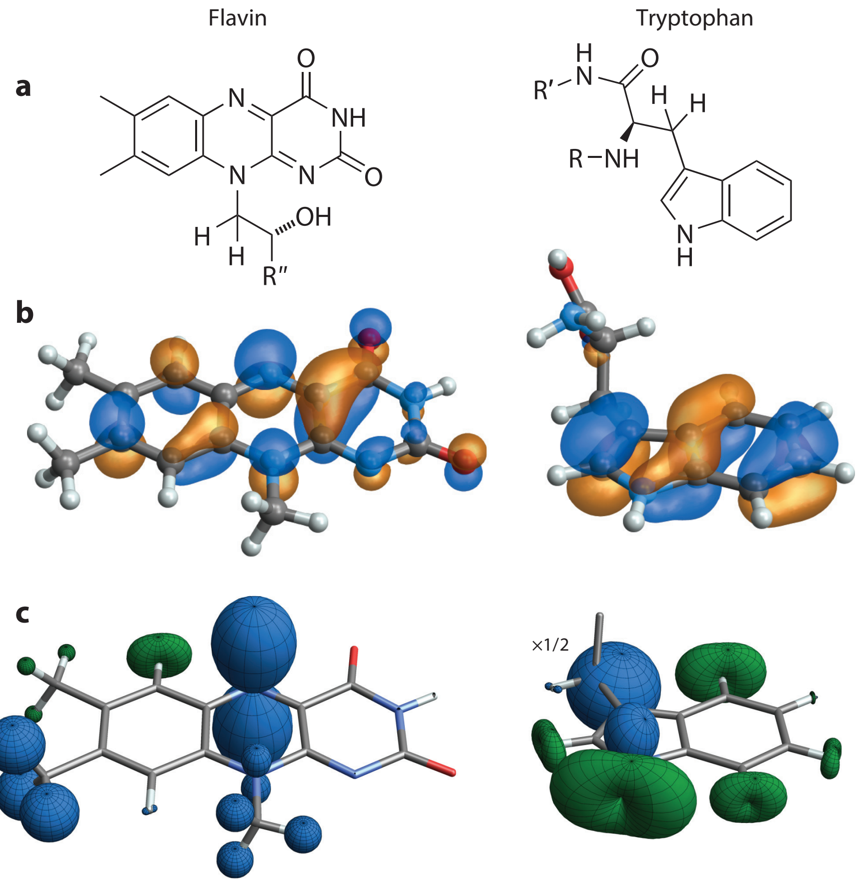

Figure 3

Molecular orbitals and hyperfine interactions in flavin and tryptophan radicals. (*a*) Structures of flavin adenine dinucleotide (FAD) and tryptophan. In the former, R″ denotes the adenosine diphosphate group and the rest of the ribityl chain. In the latter, R and R′ denote the peptide chains that flank a tryptophan residue in a protein. (*b*) Representations of the molecular orbitals that contain the unpaired electron in a flavin anion radical and a tryptophan cation radical. Blue and orange indicate parts of the wave function with opposite signs. In both cases, the electron is distributed (unevenly) over the whole of the aromatic part of the radical. The sidechain in FAD was replaced by a methyl group and the tryptophan was modeled as the free amino acid for the purpose of the calculation. (*c*) Representations of the hyperfine interactions of the hydrogen and nitrogen nuclei in a flavin anion radical and a tryptophan cation radical. The interaction of each nucleus with the electron spin is shown as a surface plot centered on the atom. The distance from the atom to its surface in any direction is proportional to the strength of the magnetic interaction in that direction. Blue and green indicate, respectively, positive and negative values of the hyperfine interaction. The large hyperfine interaction of one of the β-protons of the tryptophan radical has been scaled down by 50%. Nuclei with almost isotropic hyperfine interactions have near-spherical surfaces. The calculations in panels *b* and *c* were performed in Gaussian 03 (53) using density functional theory (166).

because the magnetic moment of ^{14}N is known experimentally to be approximately eight times smaller than that of ^{1}H (**Table 1**). However, the electron-spin density close to a nitrogen atom can be much greater than in the immediate vicinity of a hydrogen, hence the large hyperfine interaction of one of the nitrogens in the central ring of the flavin (**Figure 3*c***). To give an idea of the magnitude of typical hyperfine interactions, we have included in **Table 1** values of the magnetic field produced by different nuclei at a distance of 0.1 nm (roughly the length of a C−H or N−H bond).[9]

Some quantitative aspects of the energies, frequencies, and magnetic fields involved in the radical-pair mechanism are summarized in Appendix 2.

Singlet-Triplet Interconversion

The other important property of radical pairs is that singlets and triplets are rarely stationary states. When a radical pair is created as a singlet—for example, by electron transfer—it does not remain a singlet for long. As illustrated in **Figure 4*a***, the fraction of radical pairs in the singlet state[10] (and therefore, also, the fraction in the triplet state) oscillates in a complex fashion at frequencies—typically several megahertz—determined by the strengths of the hyperfine interactions. If there were no hyperfine interactions, there would be no oscillations, the radical pair would remain in the singlet state, and there would be no effect of an external magnetic field.[11] Roughly speaking, the sudden creation of a radical pair in a nonequilibrium state (e.g., the singlet state) is like hitting a piano with a hammer. The piano wires, each tuned to a different frequency, start to vibrate, and one hears a cacophony of superimposed frequencies. After a few seconds, however, the vibrations die away, and peace is restored. As we shall see, this approach to equilibrium has an important parallel in the behavior of radical pairs.

The oscillations in **Figure 4*a*** are actually a manifestation of the quantum mechanical spin coherence with which the singlet radical pair is formed. As we shall see, coherence plays a crucial role in the operation of the magnetic compass. In **Figure 5**, we attempt to give an idea of what coherence means in this context. When the system is in a singlet state (**Figure 5*a***), the two spins are exactly antiparallel to one another so that the total spin of this collection of radical pairs is zero, as appropriate for a state with $S = 0$. Although the relative orientation of the spins within each pair is exactly determined, all spatial orientations of the spin pairs are equally likely.

The situation is slightly more complicated for a triplet state (**Figure 5*b***). The two spins in each pair are now correlated such that they tend to point in a similar direction, but they are not constrained to be exactly parallel. The spin angular momentum averaged over all radical pairs is that appropriate for a state with $S = 1$.[12] Once again, the spin pairs have no preferred orientation in space. Finally, **Figure 5*c*** shows the noncoherent equilibrium state. Now there is no spin correlation, and the directions of the two spins in each pair are completely unrelated.

The singlet-triplet oscillations shown in **Figure 4*a*** reflect the periodic changes in the relative orientation of the two electron spins brought about by their hyperfine interactions with magnetic nuclei. As we shall see, the rate at which the coherence/correlation is lost is a vital factor in

[9]There are two contributions to every hyperfine interaction. One depends on the probability that the electron exists at the position of the nucleus (the contact interaction). The other (the dipolar interaction) is an average over the distribution of the unpaired electron in the molecule weighted by $1/R^3$, where R is the distance from the nucleus.

[10]In the absence of chemical reactions that remove radical pairs, the singlet and triplet fractions are defined such that they sum to 1.0 at all times.

[11]This is not strictly true. The difference in the interaction of the two electrons with an external magnetic field can drive singlet-triplet interconversion. For organic radicals subject to Earth-strength magnetic fields, this effect—the Δg mechanism—is normally negligible. See Appendix 2.

[12]The average spin angular momentum of a particle with spin quantum number S is $\sqrt{S(S+1)}\,\hbar$, where $\hbar$ is Planck's constant (h) divided by 2π. Thus, a collection of triplet radical pairs has on average a spin angular momentum of $\sqrt{2}\,\hbar$. This allows us to see why, for a triplet state, the two spins cannot simply always be parallel. Because each electron has $S = \frac{1}{2}$, and therefore angular momentum $\frac{\sqrt{3}}{2}\hbar$, the exactly parallel arrangement would give a total angular momentum of $\sqrt{3}\,\hbar$, which is clearly inconsistent with an average of $\sqrt{2}\,\hbar$.

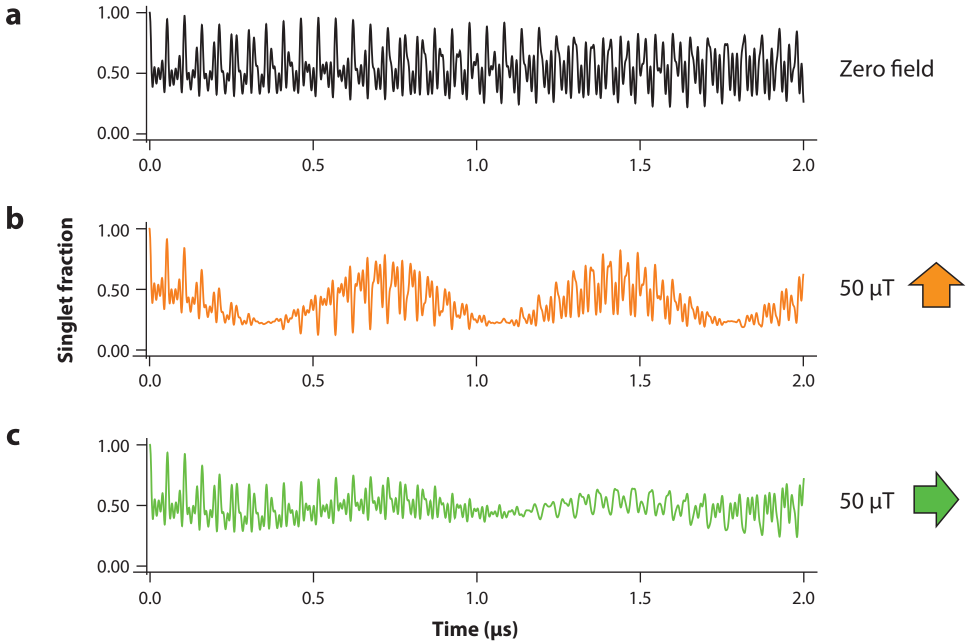

Figure 4

Interconversion of the singlet and triplet states of a simple radical pair. The fraction of radical pairs in the singlet state is plotted as a function of time ($0 \leq t \leq 2$ µs) starting with a singlet state at $t = 0$. (*a*) In the absence of an external magnetic field. (*b*) In the presence of a weak (50-µT) external magnetic field. (*c*) As panel *b* but with the magnetic field rotated by 90°. This model radical pair contains two nitrogen atoms (^{14}N) in one of the radicals with anisotropic (directionally dependent) hyperfine interactions (~1 mT). Chemical reactions of the radicals and spin relaxation of the electrons are not included.

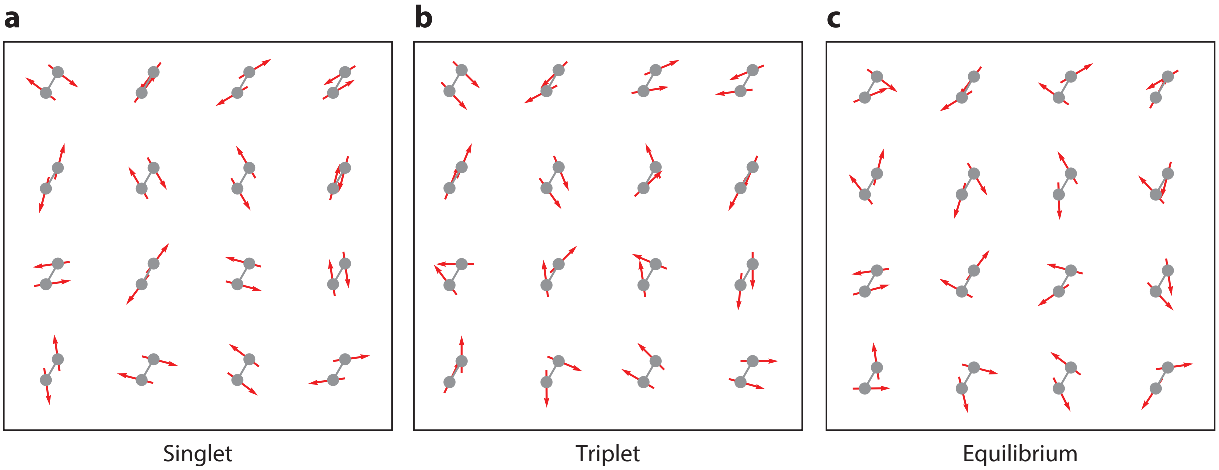

Figure 5

Electron-spin correlation. Each of the three panels shows 16 radical pairs. The gray disks represent the unpaired electrons, one on each radical, and the red arrows represent their spins. (*a*) A singlet state in which the two spins in each pair are perfectly anticorrelated. (*b*) A triplet state in which the two spins are correlated. (*c*) The equilibrium state in which the two spins are completely uncorrelated.

determining the response of the radical pair to an external magnetic field. But first, we need to see how the graph in **Figure 4*a*** changes when an external magnetic field is switched on.

Magnetic-Field Effects on Radical Pairs

Given that singlet-triplet interconversion (e.g., **Figure 4*a***) is driven by the internal magnetic fields produced by nuclear spins, it should not come as a surprise that externally applied magnetic fields also affect the spin dynamics. The interaction of an isolated electron spin and a magnetic field (the Zeeman interaction; see Appendix 2) causes the direction of the magnetic moment of the electron to oscillate. The frequency of this motion—the Larmor frequency—is proportional to the field strength with a conversion factor of 28 kHz per microtesla (see Appendix 2). Thus, for an Earth-strength field (e.g., 50 μT), the frequency is 1.4 MHz.

Figure 4*b* shows the singlet fraction for the same radical pair as in **Figure 4*a*** but now in the presence of a 50-μT magnetic field. The fast oscillations caused by the hyperfine fields are still visible but are now superimposed on a slower modulation with a period of ∼700 ns, corresponding to the 1.4-MHz Larmor frequency. The difference in the oscillation frequencies produced by the two interactions reflects their relative strengths: 50 μT for the external magnetic field and ∼1,000 μT (in this case) for the hyperfine interactions. Note that the external field does not have to be larger than, or even comparable to, the internal hyperfine fields to have a significant effect on the singlet fraction.

The next stage in the argument is to recognize that the effect of the external magnetic field depends on its direction with respect to the radicals. This is because almost all hyperfine interactions are anisotropic (**Figure 3*c***), usually as a result of the low symmetry of the molecular orbitals that contain the unpaired electron. Only if the probability of finding the electron in the immediate vicinity of the nucleus is the same in all directions (as in a hydrogen atom, for example) will the hyperfine interaction be isotropic. **Figure 4*c*** shows the singlet fraction recalculated with the magnetic field rotated by 90°. The fast and slow oscillations, coming from the hyperfine and Zeeman interactions, respectively, are still present but the details have changed because the hyperfine interactions are anisotropic. This anisotropy is what allows the radical pair to form the basis of a magnetic-direction (compass) sensor rather than simply a magnetic-intensity sensor. **Table 2** lists a few of the hyperfine interactions in photoinduced radicals that have been implicated in magnetic-field effects on cryptochrome.

Magnetic-Field Effects on the Products of Radical-Pair Reactions

To understand how the behavior shown in **Figure 4*b*,*c*** could form the basis of a chemical compass, consider the reaction scheme in **Figure 6*a***. We imagine a radical pair $[A^{\bullet+}B^{\bullet-}]$ formed instantaneously in a singlet state by a photoinduced electron transfer between two closed shell molecules, A and B, or two parts of the same closed shell molecule. $[A^{\bullet+}B^{\bullet-}]$ is envisaged as having two competing reaction pathways. The first is reverse electron transfer within the singlet radical pair, a process that regenerates the reactants A and B. The corresponding reaction of the triplet state is spin forbidden and does not occur. The second is the conversion of both singlet and triplet states of $[A^{\bullet+}B^{\bullet-}]$ to form a product C. This step is assumed to involve only one of the radicals and to proceed with the same rate constant for singlets and triplets. An example of such a reaction would be the addition of a hydrogen ion to one of the radicals (e.g., $B^{\bullet-} + H^{+} \rightarrow BH^{\bullet}$). Because the two electron spins interact very weakly, there is no reason why the protonation rate of $B^{\bullet-}$ should depend on whether the electron spins of $A^{\bullet+}$ and $B^{\bullet-}$ are parallel or antiparallel. While

Table 2 Selected hyperfine interaction parameters for the $FAD^{\bullet -}$ and $TrpH^{\bullet +}$ radicals

$FAD^{\bullet -}$		$TrpH^{\bullet +}$	
Nucleus	A_q (mT)	Nucleus	A_q (mT)
N5	1.757	N1	1.081
	−0.087		−0.053
	−0.100		−0.064
N10	0.605	H1	−0.007
	−0.014		−0.705
	−0.024		−1.083
H6	−0.198	H4	−0.188
	−0.434		−0.536
	−0.530		−0.740

The tabulated values (A_q, $q = x, y, z$) for each nucleus give the principal values of its hyperfine tensor. Note that many of these interactions are stronger than the geomagnetic field strength (0.05 mT) and that there are another 8–10 nuclear spins in each radical with significant hyperfine interactions. The small, but nonzero, values of two of the three hyperfine components of the two nitrogens, N5 and N10 (in the central ring of the flavin ring system), are partly responsible for the favorable properties of this radical as a compass magnetoreceptor (74, 108).
Calculated by Ilya Kuprov, Department of Chemistry, University of Southampton (166) using density functional theory in Gaussian 03 (53).

these reactions proceed, the remaining $[A^{\bullet +}B^{\bullet -}]$ pairs oscillate coherently between their singlet and triplet states (**Figure 6*a***).

There are thus two competing pathways for the removal of $[A^{\bullet +}B^{\bullet -}]$: from the singlet state with rate constant k_S, and from the singlet and triplet states with rate constant k_C (**Figure 6*a***). The proportions of radical pairs that go back to AB or forward to C depend not only on the two rate constants but also on the extent and frequency of the singlet-triplet interconversion. If the Zeeman interaction with the external field increases the average triplet fraction (with a corresponding decrease in the singlet fraction), then more radical pairs will react to form C and fewer will revert to AB (because only the singlet can go back to the reactants). The ultimate yield of C once all radical pairs have reacted therefore depends on the presence and direction of the external magnetic field. This is the origin of the magnetic-field effect. Although the oscillations in the spin state of the radical pair are crucial for the existence of the magnetic-field effect, it is the final yield of the product C, once all radical pairs have disappeared, that would provide the bird with information about the direction of the magnetic field. **Figure 6*b*** may help to elucidate the importance of competing reaction pathways.

To make this more concrete, we present the simulations in **Figure 7**. Each of the four panels contains three traces. One is the singlet fraction, as in **Figure 4**, for infinitely long-lived radicals. The second is the same singlet fraction but now with the reactions shown in **Figure 6*a*** included. The result is to cause the singlet fraction to decay toward zero as the radical pairs disappear along the two competing pathways. The third trace in each panel shows the buildup of the reaction product, C. As in **Figure 4**, the calculations were performed for two orthogonal directions of a 50-μT magnetic field. In **Figure 7*a*,*b***, the reaction steps are slow, so that the radicals react, and C accumulates, over a period of a couple of microseconds. This allows plenty of time for the 1.4-MHz oscillation to affect the singlet fraction and hence the yield of C. As can be seen, the final amount of C is different in the two cases. The yield of C is lower in **Figure 7*b*** than in **Figure 7*a*** because the average singlet fraction is larger in **Figure 7*b***, meaning that more radical pairs return to AB and correspondingly fewer go on to C.

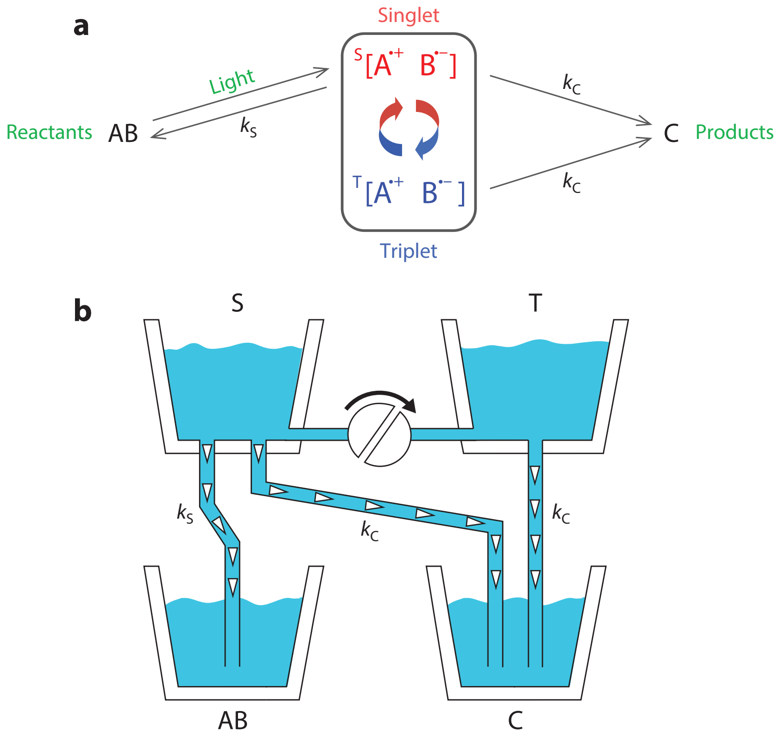

Figure 6

A simple radical-pair reaction scheme. (*a*) Reactant molecules (AB) are converted into products (C) via reversible formation of a radical pair ($[A^{\bullet +}\ B^{\bullet -}]$). k_S and k_C are the rate constants of, respectively, the spin-selective back reaction and the non-spin-selective forward reactions of $[A^{\bullet +}\ B^{\bullet -}]$. The curved arrows indicate the coherent interconversion of the singlet (S) and triplet (T) states of $[A^{\bullet +}\ B^{\bullet -}]$. This scheme differs from that normally used to discuss magnetoreception: Following Ritz et al. (162), most authors have envisaged a spin-selective reaction of the triplet radical pair instead of the spin-independent product formation shown here. The former requires there to be a triplet product state that is energetically accessible from the radical pair. As no such species exist in cryptochrome, we base our discussion on the more plausible scheme shown here [which satisfactorily accounts for the observed magnetic-field effects on cryptochrome photochemistry in vitro (120)]. (*b*) The origin of the magnetic-field effect on the yield of the reaction product C may be seen from this analogy. Two bathtubs, labeled S and T, are connected by a tube with a valve. At any time, the amounts of water in S and T correspond to the fractions of singlet and triplet radical pairs, respectively. T has an outlet at the bottom, which empties into a bucket labeled C, whereas S has two outlets, one of which goes to C and the other to a second bucket, denoted AB. We start with S full of water; T, AB, and C empty; and the valve partly open. Water flows from S to T and at the same time falls into the buckets until the bathtubs are empty. The amount of water ending up in the two buckets (the final yields of AB and C) depends on the diameter of the tubes (analogous to the values of k_S and k_C) and how far we turned the valve. If the valve is fully open so that water flows quickly from S to T, there would be two efficient routes to C (from S and from T). If, instead, the valve is partly closed, less water reaches T and the only efficient route to C is directly from S. At the end, there is therefore less water in C than when the valve is completely open. The setting of the valve in this picture is intended to represent the effect of the magnetic field. Clearly, this analogy has its limitations: Among other things, it fails to capture the oscillations in the singlet and triplet fractions (**Figure 4**).

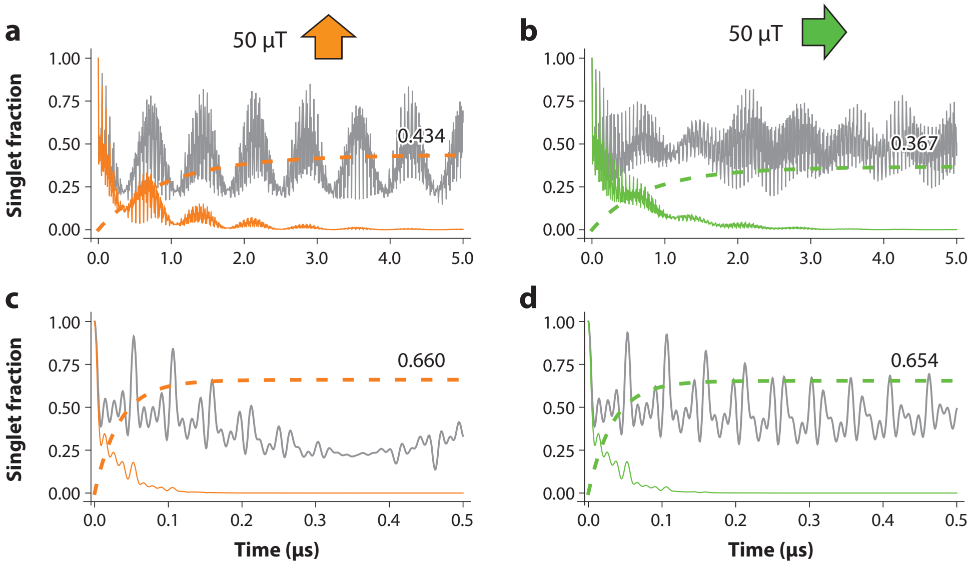

Figure 7

Time dependence of a reacting radical pair and its reaction product. Solid gray/colored lines show the fraction of singlet radical pairs in the absence/presence of chemical reactions. Dashed colored lines show the yield of the product C. The numbers at the right of each panel are the final yields of C. The hyperfine interactions used in the calculation are the same as for **Figure 4**. The reaction scheme is shown in **Figure 6*a***. Panels *a* and *c* are identical to *b* and *d*, respectively, apart from a 90° shift in the direction of the 50-μT external magnetic field. Panels *a* and *b*: slow reactions, $k_S = 2 \times 10^6$ s^{-1}; $k_C = 5 \times 10^5$ s^{-1}; $0 \le t \le 5.0$ μs. Panels *c* and *d*: fast reactions, $k_S = 2 \times 10^7$ s^{-1}; $k_C = 2 \times 10^7$ s^{-1}; $0 \le t \le 0.5$ μs.

Compare this with panels *c* and *d* of **Figure 7**, which differ from panels *a* and *b* only in the values of the rate constants. k_S and k_C are now approximately an order of magnitude larger so that the radicals disappear and C is formed in approximately a hundred nanoseconds instead of a few microseconds. With such a short lifetime, the radicals are gone before the 1.4-MHz oscillation can have a significant effect. As a result, the decay of the singlet fraction and the buildup of C are almost independent of the direction of the field.

Figure 7 does not show what happens when k_S and k_C are much smaller than $\sim 10^6$ s^{-1} so that the radical pair lives for 10 μs or longer. The reason is that there is hardly any change from **Figure 7*a*,*b***. As long as the lifetime exceeds the Larmor period (700 ns for a 50-μT field), we can expect to get the maximum possible effect of the magnetic field, at least in this simple case. A more complex case, in which longer lifetimes can be beneficial, is discussed below (74).

It is important that the values of k_S and k_C are not too dissimilar as well as not being too large. If $k_S \gg k_C$ or $k_S \ll k_C$, essentially 100% of the radical pairs would, respectively, return to the reactants or go forward to the product so that a weak external magnetic field would have little influence on the product yield. There must be an effective competition between the two reaction channels.

To summarize, for the yield of C to depend on the direction of a 50-μT field, the radical pairs must persist for a time that is not much shorter than, and ideally as long as, the Larmor period (700 ns). In general, this is not a serious problem. Electron-transfer rates in proteins (for example) cover an enormous range, from picoseconds to seconds, depending on the separation of the electron donor and acceptor and the relevant free energies (129).

Notice that the radical-pair magnetoreception hypothesis as we have presented it here is entirely iron-free: There is no requirement for permanently magnetized particles of iron oxide or other magnetic materials. Indeed, the presence of paramagnetic ions (e.g., iron, copper, manganese) in the vicinity of the radicals could have the counterproductive effect of inducing spin relaxation and thus destroying spin coherence (see the next subsection, Spin Relaxation). Nevertheless, speculative alternatives to a pure radical-pair mechanism have been proposed in which nearby magnetic nanoparticles locally amplify the Earth's magnetic field or otherwise enhance the response of the radical pair (14, 25, 33, 160). Currently, there is little evidence for the existence of such structures as compass magnetoreceptors.

Finally, there has been an experimental demonstration that a radical-pair reaction could act as a chemical compass. Using a carotenoid-porphyrin-fullerene model system, Maeda et al. (119) showed that the lifetime of the radical pair formed by photoinduced intramolecular electron transfer (from the carotenoid to the fullerene via the porphyrin) can be altered through the application of a 40–50-μT magnetic field. Both by aligning the molecules and by exploiting the photoselection effects of polarized light, it was further shown that the yield of radical pairs depends on the direction of a (somewhat stronger) magnetic field, thus establishing, as a proof of principle, the feasibility of a chemical compass sensor (119).

Spin Relaxation

Having seen how magnetic interactions can alter the product yields of radical-pair reactions, we can now understand why it is irrelevant that these interactions are absolutely tiny compared to the thermal energy, $k_B T$ (Boltzmann's constant multiplied by temperature; see Appendix 2). Radical-pair reactions are unusual in that their chemistry is controlled by electron spins that are not thermally equilibrated. Because the spin system of the radical pair interacts rather weakly with its surroundings, it is, to a large degree, thermally isolated from the rest of the world (a bit like a hot drink in a vacuum flask) and so takes a relatively long time—perhaps a microsecond or more—to come to equilibrium, allowing time for very weak interactions to influence the quantum spin dynamics and therefore the reactivity of the radicals.

As well as the restrictions on the rate constants k_S and k_C, there is another kinetic condition that must be satisfied if the radical pair is to function as a compass: Its electron-spin relaxation[13] must not be too fast. This turns out to be a rather more stringent requirement. From the moment of its formation in a spin-correlated (i.e., nonequilibrium) state, a radical pair will tend to relax toward the equilibrium state in which all correlation is lost and all oscillations (**Figures 4** and **7**) have died away. Once this happens, an external magnetic field can no longer alter the singlet fraction and can have no further effect on the yield of the reaction product. It is therefore crucial that the spins do not reach equilibrium before they react. Put another way, the coherence lifetime should not be much shorter than the radical-pair lifetime, which, as we have seen, should ideally be at least ~700 ns.

In general, spins are relaxed by the fluctuating local magnetic fields that arise from the modulation of spin interactions by molecular motion. These fields bring the spins into thermal contact, and eventually into equilibrium, with their surroundings (80). In the present context, electron-spin relaxation is likely to be dominated by the modulation of hyperfine interactions by molecular rotations and vibrations (107). Probably the most important motions are fluctuations in the positions

[13] Some authors have used the term relaxation to denote the chemical reactions that deplete the radical-pair population. We use it here to mean exclusively spin relaxation.

and conformations of the radicals. To get sufficiently slow relaxation (slower than ~700 ns), these motions should be fast ($>10^9$ s^{-1}) and of relatively low amplitude[14] (169).

Unfortunately, little is known either about spin relaxation in magnetic fields as weak as 50 μT or about the relaxation of radicals in cryptochromes. So far, there has been only one detailed study, in which molecular dynamics simulations of cryptochrome 1 from *Arabidopsis thaliana* (*At*Cry1) were used to assess the effect of spin relaxation on the performance of the protein as a compass sensor (89). It was concluded that (*a*) the optimal radical-pair lifetime for detecting the direction of the Earth's magnetic field is on the order of a microsecond; (*b*) the fluctuations in the positions and conformations of the radicals in isolated *At*Cry1 are incompatible with the long coherence times that have been postulated (56) to explain the disorientation of European robins in weak radiofrequency magnetic fields (see below); and (*c*) an avian cryptochrome in vivo would need to differ dynamically, and possibly also structurally, from isolated *At*Cry1 in order to have spin relaxation significantly slower than ~1 μs. Given the inevitability of spin relaxation and its potentially serious effect on the sensitivity of a radical-pair compass sensor, more work is needed on this aspect of the mechanism. With the exception of the Lau et al. (107) and the *At*Cry1 studies (89), the theoretical treatments of spin relaxation in the context of magnetic sensing have all employed phenomenological approaches that make no reference to the microscopic dynamics or magnetic interactions of realistic radicals (7, 26, 29, 56, 157, 198). An intriguing question is whether, as some of the phenomenological studies have indicated (7, 26, 29, 56, 198), spin relaxation processes can enhance rather than attenuate the anisotropy of the reaction product yield.

Finally, it is (just about) possible to get some insight into the effects of spin relaxation by returning to **Figure 2**. The impact of the fly on the balanced granite block is likely to be greater if the stone is not excessively influenced by its surroundings. For example, if the table on which the stone rests is wobbly, the outcome would probably be less sensitive to the antics of airborne insects.

Having outlined how a radical-pair reaction could form the basis of a magnetic-compass sensor, we now turn to cryptochrome and discuss the possible identities of the radicals $A^{\bullet-}$ and $B^{\bullet+}$ and the reaction product, C (**Figure 6*a***).

CRYPTOCHROMES—THE PROPOSED MAGNETIC SENSORY MOLECULES

Magnetic-Field Effects on Cryptochromes

Cryptochromes have a variety of known functions including entrainment of circadian rhythms and light-dependent regulation of plant growth and development (30, 116). They belong to the same family of proteins as photolyases (DNA repair enzymes) (22, 173, 200) and consist of a conserved photolyase homology region (PHR domain) with widely varying N- and/or C-terminal extensions. The PHR domain noncovalently binds a redox-active flavin adenine dinucleotide cofactor (FAD), which absorbs blue light when in its fully oxidized state. Cryptochromes are also assumed to bind, noncovalently, a second chromophore, either 8-hydroxy-5-deazariboflavin or 5,10-methenyltetrahydrofolate (115, 172). The second chromophore is not currently thought to be central to light-dependent magnetoreception. In contrast, photoreduction of FAD in many cryptochromes and photolyases is mediated by three consecutive electron transfers along a conserved triad of tryptophan (Trp) residues (16, 28, 59, 143, 217) to give a flavosemiquinone radical

[14]Fast motions more effectively average the variations in hyperfine interactions and so give rise to slower spin relaxation. The smaller the amplitude of the motions, the weaker are the local magnetic fields that cause the relaxation.

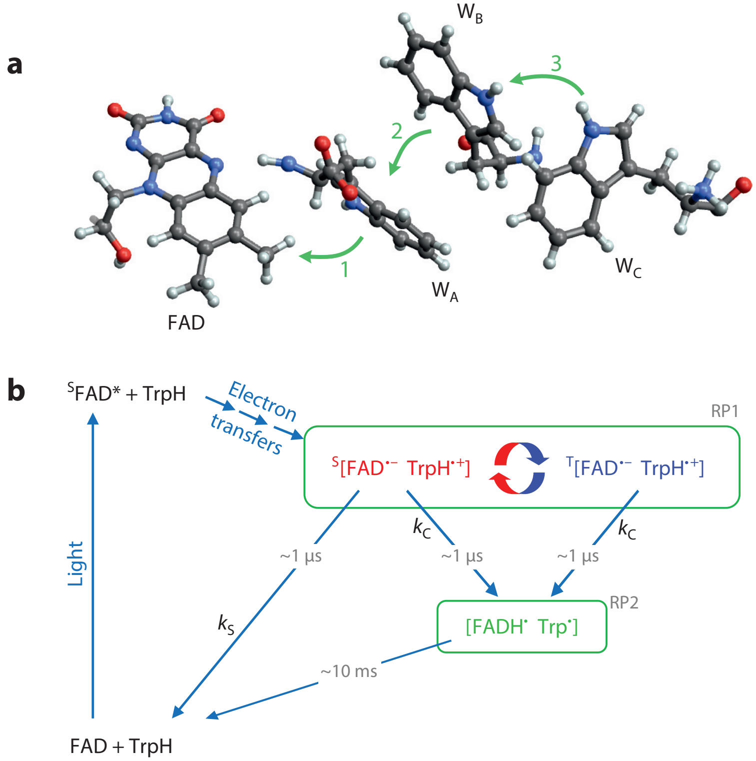

Figure 8

Radical-pair formation and reactions in cryptochrome. (*a*) The arrangement of the flavin adenine dinucleotide cofactor (FAD) and tryptophan (Trp) triad (W_A, W_B, W_C) in cryptochrome 1 from *Arabidopsis thaliana* as determined by molecular dynamics simulations starting from a modified crystal structure [Protein Data Bank ID: 1U3C (21)] containing FAD$^{•-}$ and $W_C^{•+}$. The three sequential electron transfers that follow photoexcitation of the FAD are indicated by the green arrows. The FAD sidechain is not shown beyond the second carbon atom. (*b*) A photochemical reaction scheme that accounts for the observed magnetic-field effects on the yields of long-lived radicals in *A. thaliana* cryptochrome and *Escherichia coli* photolyase. RP1 is the magnetically sensitive radical pair. RP2, formed from RP1 by a change in the protonation state of one or both radicals, does not generate magnetic-field effects. TrpH$^{•+}$ is the radical form of W_C, the terminal residue of the Trp triad. Approximate timescales of the various reaction steps are indicated. The lifetime of RP2 is ~10 ms in garden warbler (*Sylvia borin*) cryptochrome 1a and ~1 ms in *A. thaliana* cryptochrome 1. The curly arrows represent the singlet-triplet interconversion of RP1.

(FAD$^{•-}$) and a radical (TrpH$^{•+}$) derived from the terminal residue of the triad, approximately 2 nm distant from the flavin (**Figure 8*a***). Four different cryptochromes exist in the eyes of migratory birds (19, 115, 127, 139, 146, 149).

Studies of the isolated purified proteins have shown that [FAD$^{•-}$ TrpH$^{•+}$] in *A. thaliana* cryptochrome 1 and in *Escherichia coli* photolyase is magnetically sensitive according to the scheme in **Figure 8*b*** (120). Henceforth referred to as RP1, [FAD$^{•-}$ TrpH$^{•+}$] is formed in a singlet state by the spin-conserving transfer of an electron from the Trp triad to the photoexcited singlet state of the FAD (69, 120, 201). Singlet-triplet interconversion (**Figure 8*b***) ensues, accompanied by either spin-selective reverse electron transfer within the singlet state, which regenerates the ground state of the protein, or a spin-independent (de)protonation of one or both of the radicals (i.e., FAD$^{•-}$→ FADH$^•$ and/or TrpH$^{•+}$ → Trp$^•$) to give a secondary radical pair we call RP2. Both the recombination of the singlet state of RP1 and the conversion of RP1 to RP2 (corresponding to the k_S and k_C steps, respectively, in **Figure 6*a***) occur on a 1-μs timescale. Under the conditions of the experiments, RP2 (which corresponds to C in **Figure 6*a***) has a lifetime on the order of 1 to 10 ms and does not generate magnetic-field effects because the spin correlation it inherits from RP1 relaxes well before RP2 recombines. The amount of RP2 produced, once all of RP1 has disappeared, depends on the strength of an applied magnetic field (in the range of 1–25 mT) (120).

These experiments establish that cryptochrome could, in principle, be fit for purpose as a magnetoreceptor (37, 138). However, they raise far more questions than they answer. Do cryptochromes behave in a similar manner when interacting with other proteins (i.e., signaling partners), metabolites, and whatever structures might cause them to be aligned and immobilized[15] in a magnetoreceptor cell? Do any of the four known avian cryptochromes show magnetic-field effects? Are the responses of the protein to the direction of a 50-μT field large enough to form the basis of a compass sensor? Could a different radical pair be formed in or from cryptochrome, perhaps with a greater sensitivity to weak magnetic fields than RP1? Could RP2 be the state of the protein that initiates the signal transduction cascade (see the subsection titled Signaling below)? Some of these issues are discussed in the following paragraphs.

Do cryptochromes have the same photochemistry in vivo? Not necessarily. Some mutations within the Trp triad [e.g., in *Arabidopsis* and *Drosophila* cryptochromes (40, 112, 151, 152)] prevent photoreduction of the FAD in vitro but do not affect biological activity in vivo, implying the existence of alternative electron-transfer pathways when the protein is in a cellular environment. However, because almost nothing is known about the structure or properties of avian cryptochromes, arguments based on the behavior of plant or insect proteins may be misleading, especially because a cryptochrome with a magnetic sensing function may have evolved differently from one that regulates plant growth or circadian timing.

Could a 50-μT magnetic field have a large enough effect on the cryptochrome photocycle? This question is unanswerable until something is known about the structure, binding partners, and signaling of the avian proteins. Simulations of the spin dynamics of cryptochrome-inspired radical pairs give a variety of values for the anisotropy of the reaction product yield, depending on the identities of the radicals, their lifetimes, and whether spin relaxation is included. The strongly anisotropic ^{14}N hyperfine interactions in the $FAD^{\bullet-}$ radical appear to make it almost ideal as a component of a magnetic direction sensor (108, 177) provided the counter radical has no or just a few small hyperfine interactions. Other things being equal, a strongly asymmetric distribution of hyperfine interactions gives larger magnetic-field effects than a more even share of interactions between the two radicals (38, 108, 165, 170). For example, with a lifetime of 1 μs and ignoring spin relaxation, a [$FAD^{\bullet-}$ $TrpH^{\bullet+}$] radical pair is approximately 100 times less sensitive to the direction of a 50-μT magnetic field than is a pair in which the tryptophan, which has many significant hyperfine interactions, has been replaced by a radical with no hyperfine interactions (108). This then leads to speculation about whether nature could have optimized the primary magnetic sensitivity of the compass by using an electron donor D to reduce the $TrpH^{\bullet+}$ radical in RP1 to give a radical pair [$FAD^{\bullet-}$ $D^{\bullet+}$] in which the $D^{\bullet+}$ radical is magnetically much simpler than $TrpH^{\bullet+}$. Ascorbic acid, the radical form of which has only a few small ^1H hyperfine interactions, has been discussed in this context (108).

Another aspect of the sensitivity of cryptochromes to weak magnetic fields has recently been explored. Kattnig et al. (89) have shown that the primary magnetic-field effects on flavin photoreactions can be amplified chemically by slow radical termination reactions (i.e., $FADH^{\bullet} \rightarrow FAD$

[15]The need for alignment and immobilization is discussed below.

and Trp$^\bullet$ → TrpH, **Figure 8*b***) under conditions of continuous photoexcitation and that the amplification factors are larger for weaker fields. There appears to be ample scope for evolution to have optimized the degree of amplification by tuning the rate constants of the above reactions in cryptochromes via the redox potentials of the flavin and tryptophan radicals and their accessibility to, and the local concentrations of, intracellular oxidants and reductants.

An alternative route to a potentially magnetically sensitive radical pair is via the fully photoreduced form of the flavin, $FADH^-$. Electron transfer from $FADH^-$ to an acceptor A could form a radical pair [$FADH^\bullet$ $A^{\bullet-}$] without further absorption of light. There is some evidence that seems to support this idea (119, 142, 147, 148, 165), and superoxide, $O_2^{\bullet-}$, has been discussed as a potential $A^{\bullet-}$ radical (76, 142, 183, 195) (see below).

Evidence for the Involvement of Cryptochromes

The evidence that cryptochromes are involved in the ability of animals to detect magnetic fields is now reasonably compelling; the evidence that it is actually the magnetoreceptor is sadly lacking.

Various cryptochrome-mediated magnetic-field–dependent behaviors have been reported for fruit flies: binary choices in T-mazes (48, 57, 58), circadian timing (44, 214), locomotor activity (44), negative geotaxis (45), and seizure response (122). These experiments, using transgenic flies, suggest that cryptochrome is essential for magnetic responses but cannot exclude that it plays a nonmagnetic role upstream or downstream of the magnetically sensitive entity. Nor do such investigations of field intensity effects necessarily have a bearing on how (or whether) flies use cryptochrome to detect the direction of a magnetic field. Although the observed magnetic behaviors were light dependent, the experiments do not even establish that cryptochrome is the photoreceptor molecule. Very recently, it has been reported that ocular cryptochrome 2 mediates directional magnetic responses in two cockroach species (9). Magnetic-field effects on cryptochrome-dependent, blue-light responses in the model plant *Arabidopsis* have also been reported (hypocotyl growth, anthocyanin accumulation, and degradation of cryptochrome 2) (2), but they could not be replicated in an independent study (65).

Other evidence is equivocal, circumstantial, or both. First, the avian compass seems to operate best under light in the wavelength range 400–565 nm (202, 206), which only roughly matches the visible absorption spectrum of fully oxidized FAD (400−500 nm). This suggests that if cryptochrome is the photoreceptor molecule for the compass sense, there must be one or more additional chromophores that absorb in the 500–565-nm range; one possibility is the neutral $FADH^\bullet$ radical (147, 148). Second, photochemically formed FAD and tryptophan radicals with millisecond lifetimes have been detected in a (migratory) garden warbler cryptochrome (114), but so far, there has been no convincing report of a magnetic-field effect on any bird cryptochrome; the difficulty of expressing animal cryptochromes with FAD correctly bound is no doubt partly responsible. Third, FAD–tryptophan radical pairs in cryptochromes exhibit long-lived (microsecond) electron-spin polarization in strong magnetic fields (>100 mT) (15, 16), but whether the spin coherence would persist for this long in weak fields is not clear. What is needed is a "killer experiment" to establish that cryptochrome really is the magnetic detector in vivo.

Alignment and Immobilization of Cryptochromes

A directional magnetic-field effect requires not only at least one anisotropic hyperfine interaction but also that at least one or preferably both radicals have restricted mobility (107). If both were tumbling end over end, as they would do in a nonviscous liquid, the directional effects would average to zero. Moreover, because a compass sensor is likely to require the correlated responses of many radical pairs to achieve an adequate signal-to-noise ratio, it is important that they are not only immobile but also at least partially aligned relative to one another (73, 107, 182). Although each member of a randomly oriented array of immobile radical pairs could sense the direction of an external field, the integrated signal from the whole array would be vanishingly small.

Cryptochromes can be aligned if they are associated with organized intracellular structural elements such as the cytoskeleton or, more likely, cell membranes. Cryptochromes are water-soluble proteins and do not associate directly with membranes. However, in subcellular fractionation experiments, they are found with high abundance in the membrane fraction (146 and P. Bolte, A. Gunther, and H. Mouritsen, unpublished data). This suggests that cryptochromes could bind to interaction partners that are themselves parts of cell membranes. The seemingly ideal location within the bird retina would be in the outer segments of the photoreceptor cells, where stacks of hundreds of parallel cell membranes are found, or in the inner segments, which contain a layer of parallel membrane cylinders (182). Indeed, bird cryptochromes have been found in photoreceptor cells (139, 146). Cryptochrome 1a seems to be located exclusively in the ultraviolet cones in chicken and night-migratory European robins (133), whereas cryptochrome 1b appears primarily in retinal ganglion cells (19, 139, 149). However, cell membranes are not rigid structures (96); they are dynamic on a variety of timescales, although membrane stacking may restrict the motion somewhat in the outer segments of the photoreceptor cells. Moreover, many transmembrane proteins can rotate around the axis perpendicular to the plane of the membrane. The effects these motions would have on the performance of a radical-pair sensor depend crucially on their timescale. Very slow motion is equivalent to static disorder and is not too serious (73, 107, 182). Faster motion is likely to be much more of a problem because of the efficient spin relaxation that can result (107). It is particularly interesting in this respect that rhodopsin proteins in the disks of the outer segments of mouse rod photoreceptors appear to be arranged in long, parallel tracks consisting of up to several hundred dimers (64), which do not easily rotate. If cryptochromes were associated with these structures, highly aligned and relatively rigidly fixed arrays of magnetoreceptor proteins could be achieved.

The requirement that the cryptochromes must be mutually aligned may not be as strict as originally thought (162). At first sight, it seems unlikely that a collection of immobile, randomly oriented molecules in a cell could serve as a compass sensor because the responses of different molecules to the direction of a magnetic field would differ and tend to cancel one another. However, the condition for a directional response at the cellular level is that the radical pairs are (at least partially) aligned with one another and not necessarily that the molecules from which they are formed are themselves ordered. Suppose that a ray of light entering the eye and striking the retina is linearly polarized.[16] The probability that a given cryptochrome molecule is photoexcited depends on its orientation with respect to the electric vector of the light, a well-known effect called photoselection (106). This means that radical pairs are more likely to be created in cryptochrome molecules with certain orientations. Even though there may be little (or no) rotational order among the proteins themselves, it is therefore quite possible that photoselection gives rise to a population of radical-pair states with some degree of rotational order. Thus, even a completely randomly oriented array of cryptochrome molecules in a cell could allow the cell to act as a directional sensor (106).

Two further deductions follow from the photoselection concept. First, the response of a magnetoreceptor cell should be sensitive to the plane of polarization of the incoming light. From the

[16]Sunlight is naturally polarized as a result of scattering in the atmosphere. The degree of polarization can be up to 75% in a north–south-oriented band in the sky at dawn and dusk.

above argument, rays of horizontally and vertically polarized light hitting the retina are expected to produce different rotational distributions of radical pairs that would have different responses to the geomagnetic field. Experimental evidence for an interaction between polarized light and light-dependent magnetic-compass orientation in trained zebra finches has recently been reported (141). Second, even unpolarized light should show photoselection effects because the electric vector is always perpendicular to the direction of propagation. The fact that the electric vector of unpolarized light is not isotropically distributed in space means that when it strikes a cell in the retina, it produces an anisotropic distribution of radical pairs. Although photoselection effects are expected to be more pronounced for polarized light, it is possible, in principle, that randomly oriented cryptochromes excited by unpolarized light could provide the primary signal for a magnetic direction sensor (106).

Structural and Kinetic Aspects of Cryptochromes

An additional requirement for a radical-pair compass is that the singlet and triplet states have very similar energies, which means that the radicals must not be too close to one another. A loose analogy would be two side-by-side bar magnets that attract if they have opposite poles facing (↑↓, a singlet) but repel if one of them is flipped over (↑↑, a triplet). If the radicals are more than approximately 1.5 to 2.0 nm apart, the singlet and triplet states are expected have quite similar energies (38) (think of small bar magnets separated by more than 15 to 20 cm). It is probably important that the exchange and dipolar interactions of the two spins (38), which cause the singlet and triplet states to have different energies, are comparable to or preferably smaller than the interaction with the Earth's magnetic field for only then can the latter have a significant effect on the spin dynamics (**Figures 4** and **7**). However, the radicals should not be too far apart because then (*a*) their formation may not be fast enough to compete efficiently with other processes (e.g., fluorescence or intersystem crossing) and (*b*) their recombination (the k_S step in **Figure 8*b***) may be too slow to compete with spin relaxation. It can be argued (169) that this problem has been avoided in cryptochrome by consecutive electron transfers along the Trp triad. The distances involved in the three steps (i.e., FAD–Trp_A, Trp_B–Trp_A, and Trp_C–Trp_B) are all less than 0.6 nm (169), ensuring that the two electron spins are separated rapidly and efficiently to a distance of ∼2 nm where recombination can proceed on a microsecond timescale. This point is discussed in more detail in Reference 169. There is also the possibility that, at a separation of ∼2 nm, the (exchange and dipolar) contributions to the singlet and triplet energies partially cancel, allowing singlet-triplet interconversion to proceed more easily (38). Finally, there is the possibility of a further electron transfer from an electron donor to the Trp_C radical in RP1 to produce a radical pair with a larger separation and therefore smaller spin-spin interactions and larger magnetic-field effects. Possible electron donors could be a tyrosine residue (59) or a fourth tryptophan residue (28, 143) in the cryptochrome or a group in another protein bound to the cryptochrome.

BIOLOGICAL ASPECTS OF MAGNETIC SENSING

Signaling

The identity of the magnetic signaling state in a cryptochrome magnetoreceptor is presently unclear. At least in *Arabidopsis* cryptochromes, signaling appears to be triggered by a change in the conformation of the C terminus of the protein when the FAD is photoreduced to $FADH^{\bullet}$ (8, 70, 102, 153). If this occurs via electron transfer from the Trp triad, then it seems plausible that the signaling state could arise in a two-step process. First, RP1 is converted to the longer-lived

RP2 state (**Figure 8**). Second, RP2 is then further stabilized against reverse electron transfer by reduction of the tryptophan radical by an extrinsic electron donor. In this way, the magnetic-field effect on RP1, whose ~1-μs lifetime probably makes it too short-lived to be the signaling state, could be transferred via RP2 to a much longer-lived state of the protein that could trigger signal transduction.

But how could changes in the levels of a signaling state lead to changes in neuronal signals that can be processed within the brain? When receptor proteins are activated by the relevant physical and/or chemical stimuli, in this case a combination of light and the geomagnetic field, they usually undergo a conformational change. This change in the shape of a protein, in turn, activates the first step of a specific signaling cascade, which can include one, two, or many consecutive chemical reactions (61). Many signaling cascades involve G proteins (150).[17] In each of these steps, the signal is amplified, and overall amplification factors of more than a million can occur (61). In other words, biological systems can achieve very high levels of amplification and can thus reliably detect even very weak primary signals provided they are distinguishable from noise.

At the end of a signaling cascade, a conformational change in an ion-channel protein usually results in the opening or closing of the channel, which changes the rates at which ions move in and/or out of the cell and so alters the cell's membrane potential (90). This, in turn, modifies the rate and/or relative timing of action potentials[18] in the form of voltage spikes traveling along a neuron and/or the rate of release of neurotransmitters that affect neighboring neurons (61). Identification of the signaling state, the signaling cascade, and the ion channels involved in magnetic-compass sensing in birds would be a major step forward. At present, essentially nothing is known about the protein-protein interactions that might allow an avian cryptochrome to transduce directional magnetic information (37). So far, the only experimentally suggested interaction partner is a *Drosophila* homolog of the bacterial iron-sulfur cluster assembly protein, IscA (160).

A very different kind of signaling mechanism has been proposed. Stoneham et al. (185) suggested that if a radical-pair reaction produces a long-lived, charge-separated triplet state with a large electric dipole moment, then its electric field could influence the isomerization of the photoreceptor pigment retinal, and therefore modulate the visual signals transmitted from the photoreceptor cells to the brain. In this way, magnetic sensing would not need its own, separate, biochemical signaling pathway. At present, there is no experimental evidence for this idea, which may not be compatible with cryptochrome photochemistry. Nor is it clear that a cryptochrome could physically get close enough to a rhodopsin for the electric field effect to be significant. Furthermore, if the cryptochrome and retinal pathways were intertwined, the essential separation of standard vision and magnetoreception signals would be even more difficult than if there were strictly separate pathways (see the section titled Neuronal Processing and Perception—Separation of Light Intensity Changes and Magnetic Information).

Neuroanatomy

The brains of vertebrates such as birds are structured in a modular fashion (82, 84, 137, 161, 176), and most areas in the brain are dedicated to processing a specific kind of information.[19] There are visual areas, auditory areas, somatosensory areas, and so on. A purely visual area does not process

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[17] G proteins are guanine nucleotide–binding proteins that operate as molecular switches in many different signaling cascades.

[18] Action potentials can be thought of as the fundamental binary code on which most neural processing of information in the brain is based.

[19] There are also specific brain areas that integrate information from different senses and others that are involved, for example, in storing and retrieving memory.

auditory or somatosensory information and vice versa. Each area is characterized by its location and connectivity within the brain—factors that are consistent between individuals (82, 161, 176)—and by a suite of neurotransmitters and receptor proteins (84, 188). The field of neuroanatomy maps these characteristics.

Functional neuroanatomy links neuroanatomy with function (83, 124). It uses the fact that the activation of certain genes called immediate early genes (83, 124), and consequently the levels of certain proteins (such as egr-1 and c-fos), in a given brain area correlates with the degree of neuronal activity in the previous 30–60 min[20] and can therefore be used to map which parts of the brain an animal primarily uses for a certain task (72, 83, 110, 113, 124, 136).[21] When genes are "read" (transcribed), messenger RNA (mRNA) is produced in the nucleus of the cell and translated into proteins in the cytoplasm. The expression levels of immediate early genes in different parts of the brain can therefore be quantified as mRNA by in situ hybridization,[22] or as proteins using antibodies (immunohistochemistry[23]).

The retinal ganglion cells in both eyes and a forebrain area named Cluster N in both halves of the brain have been shown, through the use of functional neuroanatomy, to be by far the most active parts of the nervous system when birds use magnetic-compass information in orientation behavior[24] (46, 66, 67, 113, 136, 139, 216). The activation of Cluster N requires light: Its activity disappears when light is prevented from reaching the eyes (67, 113, 136). A mapping of the connections in the bird's brain using neuronal tracing showed that Cluster N receives its input from the dorsal lateral geniculate nucleus (GLD) in the visual thalamus, which, in turn, receives its input from the retinal ganglion cells in the eyes (71). This pathway is known as the thalamofugal visual pathway (71). Because it ends in the so-called visual wulst, we can conclude that Cluster N is a small part of the visual wulst (71, 137). When Cluster N is destroyed, night-migratory songbirds can no longer use their magnetic compass, whereas their sun and star compasses still function normally (215). Thus, magnetic-compass information is processed in Cluster N.

The facts that Cluster N is active in both brain hemispheres and is part of the visual system are very strong evidence that the magnetic compass is light dependent, that birds perceive magnetic-compass information as a visual impression, and that the primary sensor must be located in both eyes (137, 138). An earlier claim (207) that the magnetic compass is located only in the bird's right eye has turned out to be false (41, 66, 67, 113). Functional neuroanatomy data alone cannot identify which cell types within the eyes contain the primary magnetic sensory molecules because all information leaving the eyes is transmitted through the retinal ganglion cells to the rest of the brain (36, 199), and the retinal ganglion cells (and a few amacrine cells) are the only cell types within the eye that generate action potentials (189) and express egr-1 and c-fos. Thus, there are no presently known molecular activity markers available to determine which of the other cell types in the eye are highly active during magnetic-compass orientation behavior.

[20]There are other immediate early genes that appear on different timescales.

[21]The challenge is to design a behavioral experiment in which the task of interest is isolated as far as possible from other behaviors. If a bird performs different tasks simultaneously, many parts of the brain will show high levels of activity, making it difficult to associate brain areas with specific behaviors.

[22]In situ hybridization uses a labeled complementary RNA (or DNA) strand to localize a specific RNA (or DNA) sequence.

[23]Immunohistochemistry refers to the process of detecting the presence of proteins in the cells of a tissue section by using fluorophore-labeled antibodies that bind specifically to the protein in question.

[24]The eye is generally considered to be a separate but integral part of the brain; it is sequestered from the brain early in development, keeping its connections with the brain intact through the optic nerve made up of ganglion cell axons. Furthermore, processing takes place in a large number of interneurons within the eye so that the information from ~100 million photoreceptor cells is compressed and sent through ~100 times fewer ganglion cells that communicate with the rest of the brain.

To sum up, because of the specifically dedicated, modular structure of the bird's brain, the functional neuroanatomical data from night-migratory songbirds provide very strong support for the existence of a light-dependent magnetoreception mechanism with the primary detector molecules located in the eyes. One consequence of this is that the magnetic field is a secondary stimulus modulating a primary light-dependent effect. This creates some additional challenges, which we consider next.

Neuronal Processing and Perception—Separation of Light-Intensity Changes and Magnetic Information

How photoreceptor-based magnetic information is processed within the cell and in the nervous system is not known at present. Virtually all natural sensory systems are based on detecting changes in physical and/or chemical parameters rather than absolute levels. In magnetic-compass sensing, this suggests that birds should compare or scan different directions using their magnetic-compass sensor looking for maxima and/or minima from which the direction of the magnetic field can be inferred. Some birds perform characteristic head scans typically covering 90° or 180° in the horizontal plane in order to sense the direction of the Earth's magnetic field (135). As mentioned above, in light-dependent magnetoreception, the magnetic-field effect is a secondary modulation of a primary light-detection mechanism. Consequently, being able to distinguish changes in light-intensity from magnetic-field effects will be a major challenge for the bird's nervous system.

Because the first step in light-dependent magnetoreception is light detection, not magnetoreception, a magnetic field is not expected to affect a photoreceptor-based magnetic sensory system in complete darkness. Furthermore, if we consider a single light-dependent magnetoreceptor in isolation, a change in the intensity of the light would have the same qualitative effect on it as a change in the magnetic stimulus (163). This is analogous to the situation in color vision, in which a single color receptor cannot determine whether an increased activation is due to a general increase of light intensity or a change in the wavelength distribution. Color vision is achieved by comparing the responses of two neighboring receptors that are sensitive to different parts of the visual spectrum (123).

In a similar fashion, the separation of light and magnetic-field effects can most elegantly be achieved by having two populations of identical receptor molecules in close proximity to each other, with different, ideally perpendicular, orientations. This arrangement could be achieved either within one cell or in neighboring cells, containing receptors oriented in different directions. Because of their close spatial proximity, the light input will be approximately the same, but the magnetic-field effects will be different. Comparison of the outputs of the two receptor populations could be achieved in the early stages of neuronal processing, and the resulting signal could then be processed in a specialized neuronal information channel dedicated to magnetic sensing, separate from image-formation processing. Here, it is particularly interesting to consider the double-cone photoreceptor cells consisting of two "grown-together" cones (**Figure 9**), which are abundant in bird retinas. Their function is currently a mystery, but they would be a particularly well-suited location for light-dependent magnetoreception and/or polarized light detection if the cryptochromes and/or opsins, respectively, were orthogonally oriented in the two cones (**Figure 9**). There are of course many other theoretically possible structural and neuronal processing arrangements within a bird's retina that could be used to separate magnetic and light-intensity changes, but the one outlined above seems to be the most straightforward (for more detail, see Reference 163).

The outer and/or inner segments of the photoreceptor cells (rods and/or cones) would seem to be the ideal cellular locations for cryptochromes involved in detecting magnetic-compass information because they contain oriented membranes to which cryptochromes could be attached and thereby aligned (182). The downside is that these cells are teeming with the visual pigments used for normal vision (rhodopsin in the rods and various opsins in the cones). Owing to the much higher abundance and light absorption cross section of the (rhod)opsins, they will dominate changes in the membrane potential of the photoreceptor cell and therefore the release of neurotransmitters. However, there are several ways out of this dilemma.

The rod photoreceptor cells are active under low-light conditions, whereas the cone cells are active at higher-light intensities. The low-light intensities available at night are below the threshold intensity needed to activate the cone opsins, and therefore the membrane potential of the cones is not affected by the opsins at night. Consequently, cryptochrome-based, light-dependent magnetic sensors could work during the night if they were located in the cones because any membrane potential changes the cryptochromes generated would not compete with opsin signals. In contrast, a cryptochrome-based magnetic compass located in cone cells would almost certainly not work during the day because it would have to compete with very strong membrane potential changes generated by the much more abundant opsins. One consequence of these considerations is that the magnetic compass of night-migratory songbirds would—if the primary sensors are located in photoreceptor cells—almost certainly be located in one of the cone photoreceptor cell types and would then work only during the night. This idea is supported by a brain activation study that showed that Cluster N—the processing center for magnetic-compass information (see above)—in diurnally and nocturnally migrating meadow pipits is highly active at night but not during the day (216).

Could diurnal birds have a light-dependent, cryptochrome-based magnetic compass that could work during the day? This is not completely inconceivable provided the cryptochromes were located in rod photoreceptor cells. It would, however, require that the cryptochrome signals would be detectable as a modulation of the level of neurotransmitter release found in the light-saturated rod cells during the day. Following the same line of argument as that used for cones, the rods are highly unlikely to harbor magnetoreceptive cryptochromes in night-migratory songbirds because the primary visual processes in the rods would almost certainly mask any cryptochrome signals from within those cells at night.

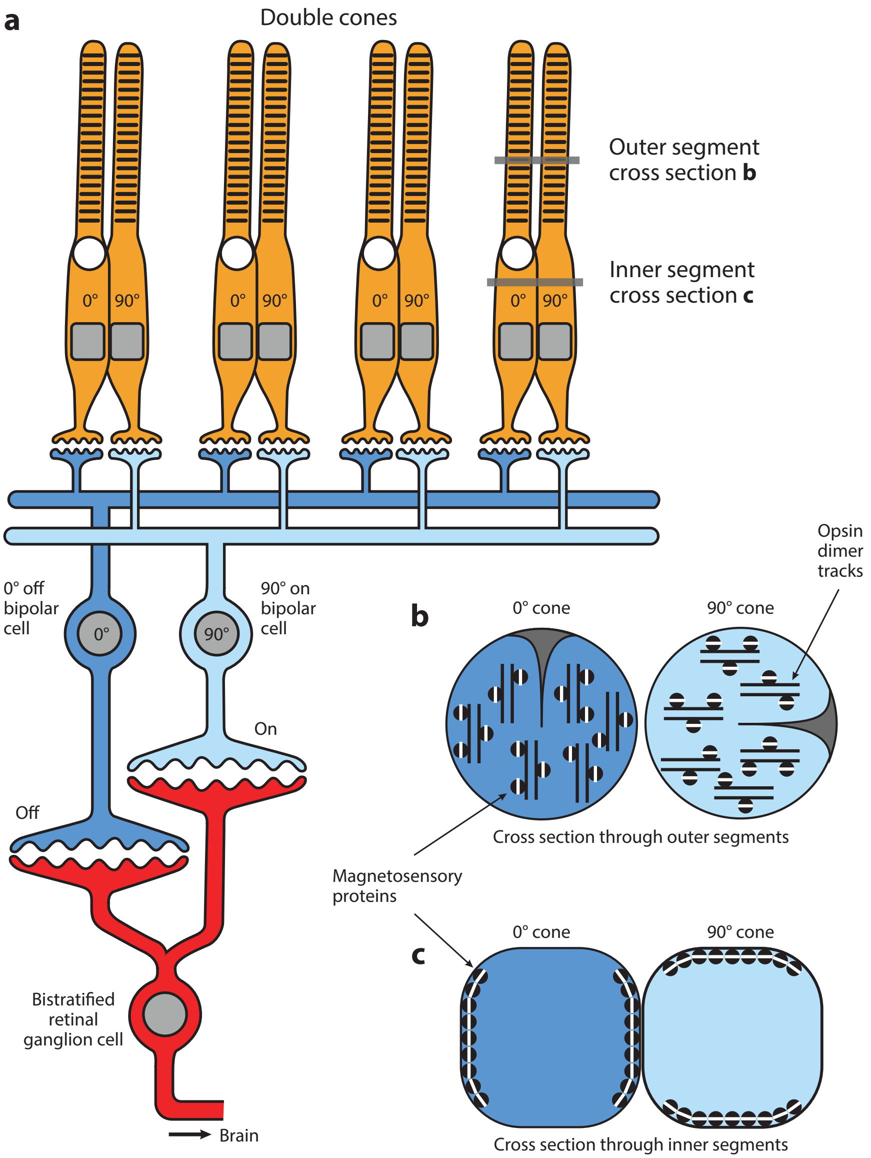
There is one other, somewhat less likely, hypothetical solution, which could bypass membrane potential competition between cryptochromes and opsins. The cryptochrome-signaling pathway could be enzymatic and activate, for example, a kinase, which in turn could produce a diffusible messenger such as nitric oxide, whose release to neighboring cells would be independent of the membrane potential. However, no such pathway is currently known in photoreceptor cells of any animal.

To sum up, it seems easier to imagine how a light-dependent magnetic compass could work in the retina during the night than during the day, and by far the strongest evidence supporting light-mediated, radical-pair–based magnetoreception comes from songbirds migrating exclusively during the night. But is there enough light available at night? We discuss this in the next section.

How Much Light Is Needed for Light-Dependent Magnetoreception?

The fact that many bird species migrate only at night appears, at first sight, to contradict the notion of light-dependent magnetoreception. However, the night sky is never totally dark—some light is always available; after all, birds need to see to be able to fly. Birds can see at night using the rod pathway, which relies on the photopigment rhodopsin. Rhodopsin requires only a few photons to detect light, and it is expected that cryptochromes could also work near this physical detection limit. Thus, for the radical-pair mechanism to work, it would, in principle, require only tens to a few thousands of photons in order to build up the signaling-state statistics needed to determine the axis of the magnetic-field lines (see **Figures 1** and **6**). Thus, night migration and light-dependent magnetoreception are not incompatible.

It is, however, clear that the weaker the magnetic-field effect, the more photons would be needed, and it is therefore worth taking a closer look at how many photons actually enter the eye of a migratory bird at night. It is known that birds can orient in free flight using their magnetic compass on moonless, dark starry nights with light intensities down to 3×10^{-4} lux (32). At this light intensity, only approximately 3 photons hit 1 μm^2 of pupil area per second. The area of the retina is approximately 10–30 times larger than the area of the pupil, and the diameter of the outer segment of a cone is approximately 2 μm in a night-migratory songbird (A. Meyer, unpublished data). Consequently, on very dark nights each cone outer segment receives no more



than approximately 1 photon s^{-1} (assuming no absorption of light between the cornea and the photoreceptor). Seen from the perspective of radical-pair–based magnetoreception, this is an extraordinarily small number. This estimate, however, assumes uniform illumination of the visual field, which is the case under overcast skies, but clearly not for a star-lit night. Photoreceptor outer segments "looking at" a star receive many more photons per second as the following argument suggests. Of the stars visible to the human eye, all but the faintest can be seen using the fovea, which contains only cones (189). The threshold for human cone vision is a little above that required to read a book by the light of a full moon (i.e., at least 10^3 photons s^{-1} per photoreceptor outer segment). Consequently, all stars visible in the fovea should illuminate at least one cone with at least 10^3 photons s^{-1}. Because the intensity of a bright star (class 0) is approximately 100 times that of the faintest stars visible to the human fovea (~class 5), a photoreceptor outer segment "looking at" a star should be exposed to approximately 10^3–10^5 photons s^{-1}.

The lack of point light sources providing a high photon flux may be one of several reasons why radar observations have shown that birds tend to be significantly more disoriented during periods of sustained overcast skies than under clear skies (39) and why migrating birds hardly ever perform level flight within clouds. Instead, they fly either above or below the clouds (T. Alerstam, personal communication) where either the stars are visible or dead reckoning on landmarks is possible. Furthermore, catastrophic mortalities of migratory birds have been reported during extended periods of thick overcast and fog when flight above or below the clouds is not possible (4, 145).

Even though the opsins in the cones do not absorb enough light to trigger their signaling cascade, they should still absorb incoming photons. If the cryptochromes would be located in the outer segments of the photoreceptors, they would have to compete directly with the opsins for the few incoming photons. Owing to the much higher abundance and absorption cross section of the opsins, this would mean that only 1 in 100 to 1 in 10,000 photons would be absorbed by the cryptochromes, which could be too few to build up the signaling-state statistics needed to determine the axis of the magnetic-field lines.

These considerations speak against a location of magnetoreceptive cryptochromes in the outer segment membrane disks of the photoreceptor cells. If the cryptochromes were located in the inner segments of the photoreceptor cells, or in any retinal cell type other than the photoreceptor cells, they would be located in front of the opsins in the light pathway, would get a chance to absorb

Figure 9

Hypothetical illustration suggesting how the avian double cones could be responsible for light-dependent magnetoreception. (*a*) Double cones consist of two closely attached cone cells. (*b*) It is possible that the opsins in the outer segments of the photoreceptor cells are arranged in parallel rows of dimers at night (but not during the day) like those recently found in mouse rod cells under low-light conditions (64). If the cryptochromes were attached to such dimer tracks, they could be highly oriented within the disk membranes and therefore respond in unison to the direction of the magnetic field. If these tracks were oriented at 90° to each other in the two halves of any given double cone, this could form the basis for an opponent processing pathway similar to the ones known for color vision in vertebrates and polarization vision in insects (12, 123). For instance, a 0° "off" bipolar cell could receive input from a number of 0° members of the double cones, whereas a 90° "on" bipolar cell could receive input from a matching number of 90° members of the same double cones. These bipolar cells could project onto a bistratified compass ganglion cell, which would then send the information to the rest of the brain for further processing. There are many other processing designs, which could for instance involve two "on" bipolar cell types and one inhibitory amacrine cell type, which could lead to the same opponent processing function. (*c*) As an alternative to a location between the outer segment disks, the cryptochromes could also be associated with the highly directed inner segment membranes. This would have the advantage that the cryptochromes would not have to compete with opsins for the incoming photons.

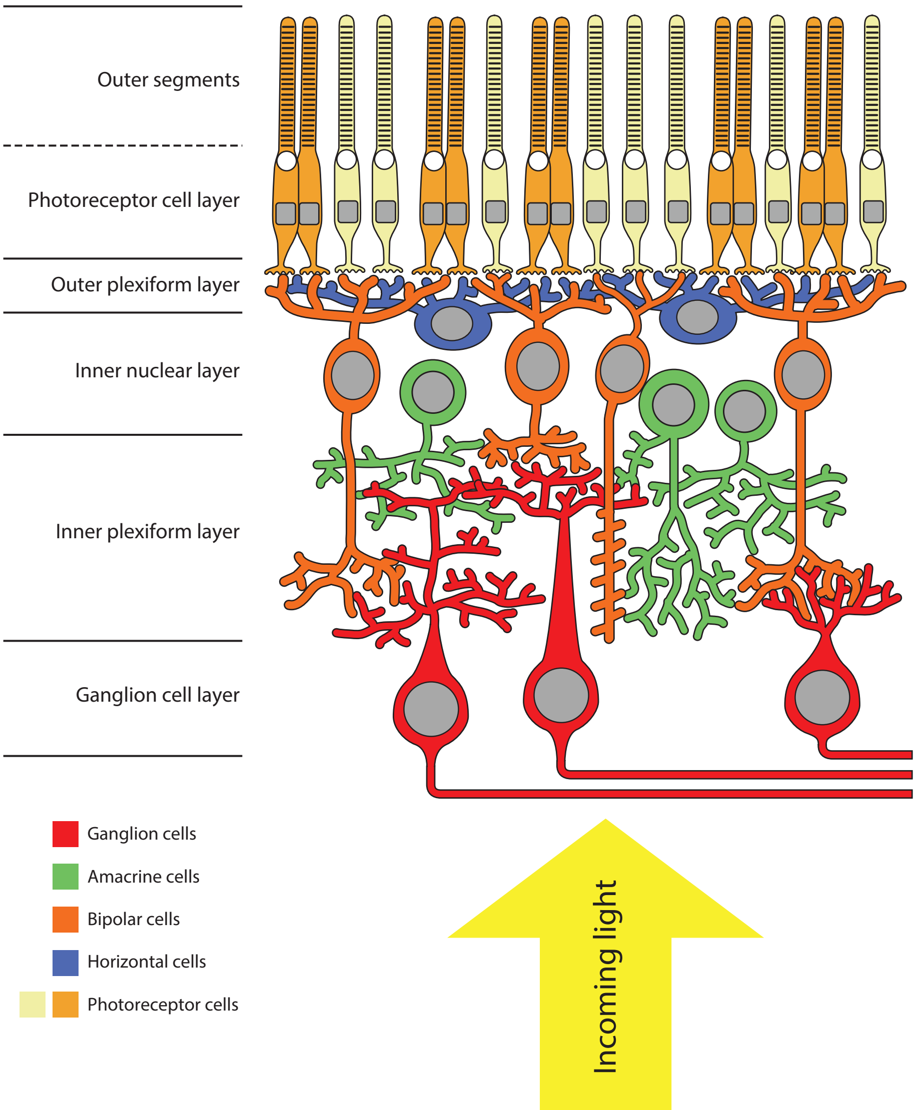


Figure 10

Schematic cross section through the retina showing the locations of the various cell types relative to the direction of the incoming light. Light enters the retina from the ganglion cell side so that it has to pass through the ganglion cells, amacrine cells, bipolar cells, and horizontal cells as well as the inner segments of the photoreceptor cells before it reaches the outer segments of the photoreceptor cells, which contain all the (rhod)opsins.

the photons first, and so would not have to compete with the opsins for the few photons that reach the retina at night (see **Figure 10**). The melanopsin pathway (63) used, for example, to control pupil dilation in many vertebrates is one example of a pathway in which non-image-forming photoreceptor molecules have been placed in front of the outer segments (in this case in ganglion cells). The potential problem with locating the cryptochromes in the nonphotoreceptor cell types of the retina—ganglion cells, amacrine cells, horizontal cells, and bipolar cells—is that there are no obvious, stable, aligned structures within these cell types to which the cryptochromes could attach. Therefore, at this moment the highly parallel, cylindrical, inner segments of the cones may provide the best compromise between the need to immobilize and align the cryptochromes (182) and the need to avoid competition with the opsins for the incoming photons (see **Figure 10**).

No matter where in the retina the sensors are located, the primary separation of light-intensity and magnetic-field effects almost certainly takes place within the retina. This preprocessed information must be transmitted to the brain for further processing: As described above, that the thalamofugal visual pathway including Cluster N is involved is almost certain. However, to be used for navigation, the magnetic-compass information has to be integrated with navigation-relevant information derived from a variety of other senses and stimuli (134, 137). Exactly where in the brain this is done is not known at present, but a number of suggestions based on current knowledge of the avian brain have recently been presented (137).

Visual Modulation Patterns

There have been attempts to construct visual modulation patterns (i.e., pictorial representations of how a bird might perceive the information derived from a radical-pair compass sensor) (74, 106, 162, 182). Such patterns are useful when explaining light-dependent magnetoreception and are also a convenient way of summarizing the effect of the geomagnetic field on model radical pairs at different locations in the retina, but they should not be taken too literally.

How information from the primary magnetoreceptor cells is processed is completely unknown, so any attempt to model what the bird actually perceives is necessarily naïve and potentially misleading. Consequently, any attempt to project such patterns onto the walls of a behavioral testing chamber in order to measure the behavioral or physiological responses of migratory birds would almost certainly teach us nothing about light-dependent magnetoreception.

TIME-DEPENDENT MAGNETIC FIELDS

Effects of Time-Dependent Magnetic Fields

Arguably the most convincing evidence in favor of the radical-pair mechanism of magnetoreception comes from reports that migratory birds can be prevented from using their magnetic compass by being subjected to weak time-dependent magnetic fields (42, 92, 164, 165, 187, 203). Laboratory studies of small organic radicals have established that the effect of a static magnetic field on a radical-pair reaction can be modified by an additional, time-dependent magnetic field (20, 51, 168), and that this can be used as a diagnostic test for the operation of the radical-pair mechanism (68). These effects, which are entirely consistent with radical-pair theory, generally go by the name of reaction yield detected magnetic resonance (RYDMR).

The principal requirement for a RYDMR effect is that the time-dependent field must have a frequency component that matches one of the frequencies with which the radical pair oscillates between its singlet and triplet states in the static field (see **Figure 4**). This resonance effect may be likened to making a violin string vibrate using a tuning fork that emits sound at, or very close to, the natural frequency of the string. If the time-dependent field is in resonance with one or more of the natural frequencies of the radical pair, it can change the extent and timing of singlet-triplet interconversion and hence the yield of the reaction product (C in **Figure 6*a***).

Effects at the Larmor Frequency

To see how this would work, consider **Figure 11**, which shows histograms of the singlet-triplet interconversion frequencies for a few model radical pairs in a 50-μT static field, with hyperfine

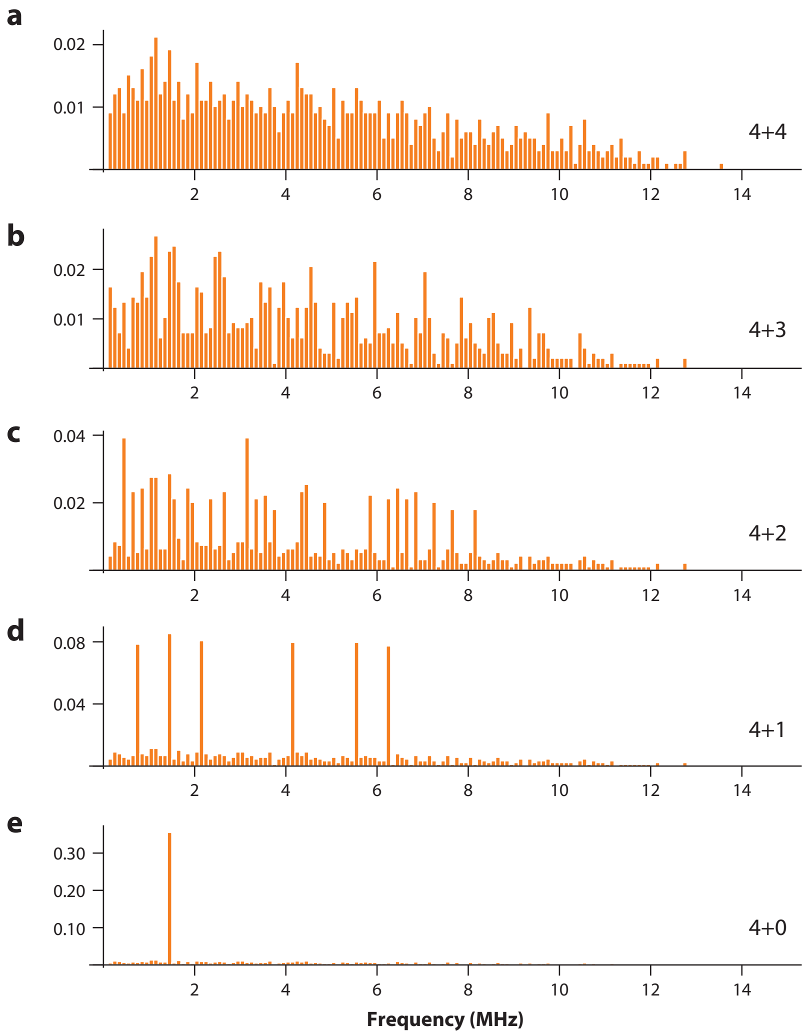


Figure 11

Singlet-triplet interconversion frequencies in model radical pairs. Histograms of the frequencies at which a time-dependent magnetic field could alter the response of a radical pair to a 50-μT static magnetic field. One radical has four ^1H hyperfine interactions; the other has (*a*) 4, (*b*) 3, (*c*) 2, (*d*) 1, and (*e*) 0. The principal values of the anisotropic hyperfine interactions were chosen randomly in the range of −250 to +250 μT. The widths of the histogram bins are 0.1 MHz. The five probability distributions have different vertical scales. In the case of panel *e*, the peak in the distribution at the Larmor frequency (1.4 MHz) is ∼30 times stronger than at any other single frequency. Note that the calculations shown here were performed for one orientation of the radical pair in the 50-μT magnetic field. When the distributions are recalculated for different orientations, the only feature that does not change is the strong one at 1.4 MHz in panel *e*.

interactions chosen randomly in the range of −250 to +250 μT. Each panel shows the fraction of the interconversion frequencies that fall into bins of width 0.1 MHz between 0.1 and 15 MHz. When both radicals have four hyperfine-coupled nuclear spins (**Figure 11*a***), the radical pair has a broad spread of interconversion frequencies. Because the heights of the histogram bars add up to 1, the bars all have low amplitude (<0.02 in this case). **Figure 11*b–e*** shows what happens when the number, N, of hyperfine interactions in the second radical is reduced from four to zero, keeping the number in the first radical fixed at four. When $N = 2$ (**Figure 11*c***), some frequencies start to stand out above the broad background. When $N = 1$ (**Figure 11*d***), just a handful of frequencies dominate the distribution, and when $N = 0$ (**Figure 11*e***) (i.e., when all of the hyperfine interactions have been removed from the second radical), there is just one strong component superimposed on a broad, weak background. This unique component occurs at the Larmor frequency (~1.4 MHz for a 50-μT field) regardless of what the hyperfine interactions are in the first radical. However, this is true only if the exchange and dipolar interactions in the radical pair are small compared to 50 μT, which in practice would require the radicals to be 50% further apart than $FAD^{\bullet -}$ and $TrpH^{\bullet +}$ in cryptochromes (38).

Assuming that the effect of a time-dependent magnetic field on a radical pair is proportional to the height of the histogram bar at the appropriate frequency, three cases can be identified from calculations such as those in **Figure 11**: (*a*) When both radicals have several hyperfine interactions, the radical pair is likely to be affected by time-dependent fields at a variety of different frequencies (determined by the hyperfine interactions in both radicals) with no particular frequencies being much more sensitive than any others (**Figure 11*a*,*b***); (*b*) when one of the radicals has only one or two hyperfine interactions, there should be significantly stronger responses at a few frequencies (determined by the hyperfine interactions in the simpler radical; **Figure 11*c*,*d***); and (*c*) only when one radical is devoid of hyperfine interactions will there be a strong resonance at the Larmor frequency (**Figure 11*e***) and then only if the interaction of the two electron spins is very small.

The origin of this behavior is clear: In case *c*, every radical pair has one radical with a magnetically isolated electron spin that interacts only with the geomagnetic field and so drives singlet-triplet interconversion at the Larmor frequency. In case *b*, the electron in the second radical contributes a small number of frequencies determined partly by the geomagnetic-field strength but mostly (because they are stronger) by the hyperfine interactions. In case *a*, both radicals are dominated by their hyperfine interactions, and no particular frequencies stand out. In general, the more hyperfine interactions there are in the radicals, the wider the spread of interconversion frequencies and the smaller the amplitude in each of the histogram bins.

The FAD–Trp radical pair is like case *a* because there are more than 10 nuclei in both radicals with significant hyperfine interactions (108). As such, one would not expect a much more sensitive response to a field oscillating at or close to the Larmor frequency than at, say, half or double that frequency. However, this is precisely what Ritz et al. (165) reported: a 15-nT radiofrequency field at 1.315 MHz (the Larmor frequency in Frankfurt am Main, Germany) was sufficient to prevent European robins from using their magnetic compass. By contrast, when the frequency was either 0.65 MHz or 2.63 MHz, the intensity of the time-dependent magnetic field had to be as large as 470–480 nT before the birds became disoriented. Magnetic disorientation caused by electromagnetic fields at the Larmor frequency has also been reported for garden warblers and cockroaches (92, 194).

The interpretation put on the finding of Ritz et al. (165) was that one of the radicals must be devoid of hyperfine interactions (i.e., case *c*). But then the problem is to know what this new radical could be and how the separation of the two radicals could be large enough to ensure that their mutual spin interaction is negligible. Superoxide, $O_2^{\bullet -}$, a radical form of dioxygen, has been suggested as a possible alternative to the tryptophan radical (119, 165). $O_2^{\bullet -}$ itself is free from hyperfine interactions (^{16}O has no spin; **Table 1**), and, other things being equal, an FAD-$O_2^{\bullet -}$ radical pair is expected to be much more sensitive to the geomagnetic field than is the FAD–Trp radical pair (108). Although attractive in biological terms [O_2 efficiently oxidizes reduced flavins and can, in principle, form FAD-$O_2^{\bullet -}$ radical pairs in the process (142, 158)], $O_2^{\bullet -}$ is expected to have exceedingly fast spin relaxation (86, 87). Unlike most organic radicals, which have much lower symmetry, the electron spin in $O_2^{\bullet-}$ couples strongly to the molecular axis[25] and so relaxes almost as fast as the molecule rotates [probably in nanoseconds (86, 87)]. It seems inconceivable that $O_2^{\bullet-}$ could bind strongly enough to cryptochrome (or any other immobile object) to prevent this from happening. We therefore cannot imagine that $O_2^{\bullet-}$ could form part of a magnetic-compass sensor.

Coming up with a biologically plausible radical (or metal ion) that satisfies the dual conditions of negligible hyperfine interactions and slow spin relaxation has not been possible. Apart from superoxide, essentially every biological radical one can think of has one or more hydrogen and/or nitrogen atoms in the neighborhood of the molecular orbital that contains the unpaired electron. In summary, one would not expect to see a resonance at the Larmor frequency from any radical pair that could reasonably arise in or from cryptochrome. Likewise, the claim by Ritz et al. (164) that a radiofrequency field oriented at a 24° or 48° angle to the geomagnetic field disrupted the birds' magnetic compass orientation, whereas a radiofrequency field aligned with the geomagnetic field did not, is only expected to be true when one radical has no hyperfine interactions and does not interact with its partner radical and is, thus, also incompatible with any realistic organic radical.

A very recent study (175), designed to replicate the experiments of Ritz et al. (165) under much more stringently controlled conditions (which involved testing birds double-blindly in a highly electromagnetically screened environment), failed to see specific effects on the birds' magnetic-compass orientation capabilities using fields oscillating at the Larmor frequency. These results seriously question whether the specific effects reported at the Larmor frequency are real. The new findings are more consistent with radical pairs such as the FAD–Trp pair in cryptochrome that have hyperfine interactions in both radicals.

The points made above also raise the question of why several other studies have reported disruption of the magnetic orientation of various animals exposed to Larmor-frequency fields (for instance, 92, 186, 194). In our opinion, the origin of these effects is unclear. None of these reports included measurements of the spectrum of the radiofrequency fields to which the animals were exposed. This raises concerns that the nominally single-frequency exposures were in fact contaminated by broadband components and/or significant sidebands that could have caused the reported effects. Accurate generation and measurement of single-frequency electromagnetic fields and broadband fields with well-defined bandwidths are challenging and require accurate signal generators and amplifiers and high-quality signal analyzers with appropriately designed and calibrated antennas. Unfortunately, such equipment is expensive. To allow reliable interpretation of future behavioral experiments, we consider it essential that both the magnetic and electric components of the time-dependent fields are carefully generated, measured, and reported (e.g., from ~10 kHz to 10 MHz or beyond).

It could be argued that such care in the design and execution of radiofrequency exposures is unnecessary if the aim is simply to determine whether a particular behavior originates in radical pairs or magnetite.[26] We do not share this view. Eventually, research on magnetoreception should strive for mechanistic explanations for the observed behaviors. Radiofrequency field effects on animal behavior can provide important clues to the origin, identities, and properties of the primary sensors but only if the experimental conditions are accurately known.

Furthermore, it is vital that all aspects of behavioral experiments are fully double-blinded to avoid the danger that researchers are unconsciously influenced by expectations of the outcomes.

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[25]A result of the strong spin-orbit coupling in $O_2^{\bullet-}$.

[26]A magnetite-based sensor in the interior of a cell would move far too slowly to track a radiofrequency field that changed direction more than a million times per second and had an intensity several orders of magnitude smaller than the geomagnetic field. There should therefore be no radiofrequency field effects on magnetite magnetoreceptors.

Not all predictions of the radical-pair model will be (or have been) equally reliable, simply because far too little is known about the radicals that might be involved. Double-blinded experimental procedures are the best guard against unintentional bias when testing model-based hypotheses.

Another puzzling aspect of the behavioral experiments is that the Larmor-frequency fields that appeared to disorient the robins are very weak indeed [~15 nT (165)]. How could a time-dependent field some 3,000 times smaller than 50 μT distort or corrupt the directional information coming from the Earth's magnetic field (91)? The only obvious explanation is that the spin coherence in the radical pair is extraordinarily long lived. We saw in the discussion of spin relaxation above that a time roughly equal to the Larmor period (~700 ns) is required for the Earth's field to have a significant impact on the spin dynamics of the radical pair. This argument can easily be extended to time-dependent fields. For a time-dependent field 3,000 times weaker than the Earth's field, one would have to wait at least 3,000 times longer (i.e., ~2 ms) before it could have a similar effect. Imagining how the spin relaxation could be that slow is extraordinarily difficult (89, 91).

Effects of Broadband Electromagnetic Noise

The disorientation of European robins by weak broadband electromagnetic fields (42, 175) is even more difficult to understand because the fields involved are so much weaker than those used in the Larmor-frequency experiments (165, 175). The birds' ability to orient in the geomagnetic field was found to be disrupted by electromagnetic noise (sometimes referred to as electrosmog) with a root-mean-square amplitude of 10 to 100 pT and a 40-min max-hold amplitude of 0.1 to 20 nT (measured with a 10-kHz bandwidth) in the frequency range of 2 kHz to 9 MHz (42, 175). Even though these fields contain components that oscillate at all possible singlet-triplet interconversion frequencies (e.g., **Figure 11*a***) so that there could be multiple additive effects, it is difficult to see how the spin relaxation could be slow enough to allow the electrosmog to corrupt the directional information coming from the much stronger geomagnetic field.

However, the effect of broadband noise may not be a direct magnetic interference with the spin dynamics of the radical-pair sensor: That the fluctuating magnetic field affects some other aspect of magnetic sensing or that it is the electric rather than the magnetic component of the electromagnetic field that is responsible cannot be excluded.

THEORETICAL CONSIDERATIONS

Entanglement

The quantum nature of the radical-pair hypothesis has attracted the interest of theoreticians (7, 27, 56, 75, 101, 105, 154, 191, 218) and has been highlighted in several reviews (81, 104, 126, 219), popular science articles (6, 196), and a book (5) on the emerging field of quantum biology. One of the main reasons for all this attention is that the two electron spins in a singlet radical pair are entangled. Entanglement (a quantum phenomenon famously described by Einstein as "spooky action at a distance") in the context of a radical pair means that the behavior of one electron spin is affected by what happens to the other even though they may be well separated and have a negligible interaction energy. Entanglement is interesting in a variety of contexts. For example, entangled quantum bits, known as qubits, can allow certain computations to be performed much more efficiently than with classical bits in conventional processors. The problem is that entanglement is usually difficult to create and to preserve for long enough to do anything useful. So the notion that nature might have found a way to make an entangled state, maintain it for a microsecond or longer, and use it as the basis of a sensory mechanism is, to put it mildly, intriguing. It is, therefore, important to know whether entanglement actually conveys an advantage in terms of the performance of a radical pair as a compass sensor.

A simple way to approach this question is to compare the behavior of radical pairs that have been formed in a singlet state (see **Figures 4**, **6**, and **7**) with the behavior of those that have been formed in a triplet state (75). Up to now, we have considered only initial singlets because that is what happens in cryptochrome (69, 120, 179, 201). Imagine, for example, that the photoinduced electron transfers in cryptochrome are slow enough that the excited singlet state of the FAD, produced by light absorption, has time to switch to the excited triplet state, a process known as intersystem crossing (103). Electron transfer along the Trp triad would then generate a triplet radical pair because of the requirement to conserve spin. As far as we know, this does not happen in a cryptochrome, but if it did, it could be the basis of a compass sensor. In fact, reports of magnetic-field effects on initial triplet pairs (formed in organic radical reactions in liquids) are considerably more numerous than those on initial singlet pairs (184). The point is that the electron spins in a singlet radical pair are entangled—whereas those in a triplet pair need not be (75)—but magnetic-field effects can be seen in both cases. This simple argument shows that entanglement is not essential for a radical-pair compass.[27] As a consequence, most of the theoretical work on entanglement in this context, though intrinsically interesting, has little practical relevance to the mechanism of compass magnetoreception (27, 56, 101, 154, 190, 218).

Entanglement, therefore, seems to be something one gets "for free" in a cryptochrome—something that is not essential for the ability of the protein to act as a magnetic-compass sensor. Indeed, to spin chemists,[28] the very existence of spin entanglement is neither surprising nor particularly exciting. The two electron spins in a chemical bond in a molecule are essentially an entangled singlet so it is not terribly surprising that a radical pair formed by shifting one of those electrons rapidly to another part of the molecule should also be entangled. However, we cannot exclude the possibility that there is some completely unknown aspect of the radical-pair mechanism in which the entanglement plays an important or even crucial role in boosting the sensitivity or precision of the directional response to a weak magnetic field. New hypotheses of this sort would be welcome but, of course, would have to be (bio)chemically, as well as physically, plausible.

Compass Precision

Theoretical descriptions of radical-pair compasses have until very recently been unable to account for the high precision—better than 5°—with which birds are able to detect the direction of the Earth's magnetic field (3, 109). Using realistic models of [$FAD^{\bullet-}$ $TrpH^{\bullet+}$] radical pairs, Hiscock, Worster, and colleagues (74) have shown that when the spin coherence persists for longer than a few microseconds, the output of the sensor contains a sharp feature, referred to as a spike. The spike arises from avoided crossings of the quantum mechanical spin energy levels of the radicals and could deliver a heading precision sufficient to explain the navigational behavior of migratory birds in the wild. This study (*a*) affords new insights into radical-pair magnetoreception, (*b*) suggests ways in which the performance of the compass could have been optimized by evolution, (*c*) may provide the beginnings of an explanation for the magnetic disorientation of migratory birds exposed to anthropogenic electromagnetic noise, and (*d*) suggests that radical-pair magnetoreception may be more of a quantum biology phenomenon than previously realized (74).

[27]Even if not present initially, entanglement can, in principle, arise as a result of the coherent spin dynamics of the radical pair. This, too, is largely of academic interest.

[28]For more information on spin chemistry, see **http://spinportal.chem.ox.ac.uk/**.

Realistic Radicals

There has been relatively little work done on realistic models of the radical pairs that are known to be formed, or could feasibly be formed, in or from cryptochromes (25, 27, 31, 106, 108). Now that the basic principles of chemical direction sensing are well understood, it seems sensible to focus theoretical attention on cryptochromes rather than on abstract systems (7, 24, 29, 35, 75, 88, 100, 105, 107, 154, 157, 198, 212, 218) because they are the only candidate magnetoreceptor molecules we have at the moment and because theoretical predictions can be tested by experiment (albeit, sometimes, with considerable difficulty). By "realistic" here, we mean two things. First, for anything other than a qualitative treatment, the spin system needs to be realistically complex. Although treatments of very simple model systems undoubtedly have their place, they should not be expected to give a reliable picture of what happens in a biological context. Extrapolation, for example, from a model radical pair containing zero, one, or two nuclear spins to [$FAD^{\bullet-}$ $Trp^{\bullet+}$], which has more than 15 significant hyperfine interactions, is quite likely to be misleading (111, 121). Second, although calculations that either ignore spin relaxation or include it phenomenologically certainly have value (7, 29, 56, 105, 157, 190, 198, 213), they too can be deceptive. Considering the power of modern computers and the ingenuity of theoreticians, there is ample scope for detailed treatments of cryptochrome-derived radical pairs in which the electron transfer steps and the spin relaxation are incorporated by means of realistic magnetic interactions and realistic molecular dynamics (31, 89, 108, 118, 178, 179).

Finally, we would like to see a serious attempt to explain the quite remarkable finding that European robins are prevented from using their magnetic compass by broadband electromagnetic noise in the frequency range of ∼2 kHz to ∼9 MHz with root-mean-square amplitudes in the range 0.1 to 1.0 pT $Hz^{-1/2}$ (42, 175). We suspect that if the disorienting effect of these exceedingly weak fields can be shown to arise from a disruption in the spin dynamics (or even, unlikely as it may seem, to be a consequence of the entanglement) of a radical-pair sensor molecule, then in all likelihood, it will provide powerful insights into the detailed operation of the compass sensor.

CONCLUSIONS

Current evidence strongly suggests that night-migratory songbirds have a light-dependent magnetic-compass sensor located in their eyes and that the underlying mechanism relies on the quantum spin dynamics of photoinduced radical pairs probably generated in cryptochromes. As outlined in Appendix 1, night-migratory songbirds seem to have a separate magnetic-map sense based, at least in part, on input received through the ophthalmic branch of the trigeminal nerve.

By presenting and explaining the principles of the radical-pair mechanism, we have identified a number of critical areas where future research is needed to demonstrate whether, and to understand how, a radical-pair mechanism could enable migratory birds to sense the direction of the Earth's magnetic field. It is clear that truly multidisciplinary approaches involving quantum physics; chemistry; computer simulation; mathematical modeling; biochemistry; and molecular, neuro-, and behavioral biology will be needed to solve this important long-standing problem in biology.

APPENDIX 1: THE MAGNETITE HYPOTHESIS

There is another mechanism by which animals could, in principle, sense the Earth's magnetic field. Magnetite (Fe_3O_4), a crystalline form of iron oxide, can exist as ∼50-nm single-domain particles with permanent magnetic moments that are large enough that the particles can rotate into alignment with a 50-μT magnetic field (93–95, 180, 181, 197, 210). Smaller crystals (∼20 nm) are superparamagnetic, meaning that an external magnetic field can induce magnetic moments, which may cause adjacent particles to attract or repel (34, 94). In both cases, the movement of the crystals induced by the magnetic field could be detected, for example, by mechanoreceptors or by the opening of ion channels, and so form the basis of a magnetic intensity or direction sensor (85). Related iron-containing minerals with similar properties (e.g., maghemite, Fe_2O_3) have also been discussed (e.g., Reference 43).

Many studies have documented particles of magnetite or other iron minerals in, for example, nematode worms, mollusks, insects, crustaceans, and a variety of vertebrates (96, 133, 204, 209). However, the mere existence of biogenic, iron-based, magnetic particles does not imply relevance to magnetoreception (133). Iron is required for the proper function of most organisms; iron homeostasis is therefore important, and iron mineral deposits may just be a way for an organism to store excess iron. Magnetic particles or structures can only be considered as possible magnetosensors if they are found at specific and consistent locations in the body, and if they are linked to the nervous system (132–134).

Chains of magnetite crystals—magnetosomes—are found in magnetotactic bacteria and lead to magnetically oriented swimming behavior (10, 18, 50). However, they are not part of an active sensory system and merely result in passive alignment of the bacterium in the Earth's magnetic field (133, 204). Nevertheless, magnetosomes do prove that living cells are capable of synthesizing magnetite particles with large enough magnetic moments that they can align with the geomagnetic field. Similar structures have repeatedly been suggested as the basis for avian magnetoreception (e.g., Reference 96), but so far magnetosomes have not been detected in the tissue of birds or any other vertebrate (133, 134, 204).

For a long time, iron-mineral structures—claimed to consist of magnetite spherules, maghemite platelets, and a vesicle—in the birds' upper beak were thought to function as magnetic sensors (43, 47). These structures were reported to be located in dendrites (sensory nerve endings) at three specific bilateral positions in the upper beak. However, detailed studies (192, 193) on more than 200 pigeons showed that they are almost certainly macrophages[29] rather than magnetosensitive neurons. That the structures reported by Treiber et al. (192, 193) included those previously interpreted as magnetic sensors (43) was subsequently independently confirmed (132). However, technical limitations of the Prussian blue staining method means that other iron-based magnetic sensors could have remained undetected (132).

Despite the controversy at the sensor level, a growing body of evidence suggests that the ophthalmic branch of the trigeminal nerve (V1), the only nonolfactory nerve entering the upper beak, is involved in magnetoreception. Convincing support for the relevance of V1 in specific tasks has come from studies in which the nerve has been ablated (see Reference 138 for a discussion of other methods of disabling V1). Several studies in which V1 had been surgically severed showed significant effects on birds' abilities to detect magnetic-field changes (128) or found a significant decrease in magnetically induced neural responses in trigemino-recipient hindbrain structures after either V1 ablation or removal of magnetic-field stimulation (72, 110). Thus, V1 does seem to convey magnetic information from the upper beak even though the primary sensors remain unknown.

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[29]Macrophages are immune cells characterized by their ability to engulf foreign particulate and colloidal material. Here, they are involved in iron homeostasis and contain ferritin clusters.

The magnetic information carried by V1 is unlikely to provide compass information because intact trigeminal nerves are neither necessary nor sufficient for magnetic-compass orientation (11, 215). In contrast, V1 most likely carries positional magnetic information to the brain. Migratory reed warblers correct for a virtual magnetic displacement (99) and can compensate for an actual 1,000-km displacement only if V1 remains intact (97). Furthermore, strong magnetic pulses, which would remagnetize an iron-containing sensor, lead to deflected orientation in adult migratory birds (77, 78).

In conclusion, birds appear to have a magnetic sense associated with V1. It seems to be involved in detecting magnetic-map information, not magnetic-compass information. The nature of the V1-associated magnetic sensors is unknown, but current evidence suggests that they are most likely to be iron-mineral–based.

APPENDIX 2: INTERACTIONS, FIELDS, FREQUENCIES, AND ENERGIES

The interaction of a spin with a magnetic field is known as a Zeeman interaction. The energy ΔE of the Zeeman interaction of an electron in a magnetic field of strength B is given by

$$\Delta E = h\nu_L = g\mu_B B, \quad 1.$$

where ν_L is the Larmor frequency, μ_B is the Bohr magneton (9.274×10^{-24} J T^{-1}), h is Planck's constant (6.626×10^{-34} J s), and g is the g-value of the electron. For a free electron, $g = 2.002319$, so that

$$\Delta E = 1.86 \times 10^{-23} B \text{ and } \nu_L = 2.80 \times 10^{10} B, \quad 2.$$

where ΔE is in joules, ν_L is in hertz (i.e., s^{-1}), and B is in teslas. When the Larmor frequency is expressed in kilohertz and the magnetic-field strength is in microteslas, the conversion factor is 28.0 kHz μT^{-1}. In a 50-μT field, $\Delta E = 9.28 \times 10^{-28}$ J, and $\nu_L = 1.40$ MHz.

The g-values of unpaired electrons in organic radicals differ a little from ∼2.0023, the exact value depending on the structure of the radical. For example, $CH_3^{\bullet}$ 2.0026, $CH_2^{\bullet}OH$ 2.0033, $CH_2^{\bullet}CHO$ 2.0045, $RC^{\bullet}O$ ∼2.0005, $R_2N^{\bullet}O$ ∼2.006, and $ROO^{\bullet}$ ∼2.015 (in which R is an alkyl group). These numbers are sufficiently close to 2.0023 that Equation 2 can still be used for the weak magnetic fields of concern here.

Nuclei have magnetic moments that are $\sim 10^3$ times smaller than the electron (because of their much larger mass). Nuclear Zeeman interactions in the Earth's field are therefore tiny and can be ignored here.

The energies of ^{1}H and ^{14}N hyperfine interactions in organic radicals are normally less than $\sim 4 \times 10^{-26}$ J or, using Equation 2, less than ∼2 mT or ∼60 MHz. Many hyperfine interactions are stronger than ∼50 μT. This does not stop the Earth's magnetic field affecting the radical-pair chemistry (see **Figure 2**).

The thermal energy associated with the random motions of molecules is $k_B T$, where k_B is Boltzmann's constant (1.381×10^{-23} J K^{-1}) and T is the temperature (in Kelvin). At 25°C (or 298 K), $k_B T \approx 4 \times 10^{-21}$ J. This is considerably larger than the energies mentioned above, meaning that the thermodynamic effect of the geomagnetic field on a radical-pair reaction is tiny. The fractional change in an equilibrium constant or the rate constant of a thermally activated chemical reaction caused by a 50-μT field at 298 K is at most $1 - \exp(-\Delta E/k_B T) \approx \Delta E/k_B T \approx 2 \times 10^{-7}$ (i.e., one part in 5 million). The Introduction and Spin Relaxation sections above explain why this is not a problem for the radical-pair mechanism.

The singlet (S) and three triplet states (T_{+1}, T_0, and T_{-1}) of a pair of electrons can be represented using arrows (↑ and ↓) to indicate the two allowed states of each spin, thus,[30]

$$S = (\uparrow_1\downarrow_2) - (\downarrow_1\uparrow_2)$$

$$T_{+1} = (\uparrow_1\uparrow_2); \quad T_0 = (\uparrow_1\downarrow_2) + (\downarrow_1\uparrow_2); \quad T_{-1} = (\downarrow_1\downarrow_2).$$

The subscripts 1 and 2 label the two electrons. The subscripts on the triplet states denote the size of the projection of the spin angular momentum vector onto an arbitrary axis, in units of $h/2\pi$.

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