



Magnetic fields and childhood leukaemia – candidate mechanistic pathways

Report by Ian Jones of a Think Tank sponsored by Children with Cancer UK held at the Holiday Inn Regent's Park, London, 22–23 September 2014

Executive summary

Life on Earth has evolved in the presence of the Earth's magnetic field. Indeed, in recent decades it has become clear that a wide range of organisms can detect this magnetic field and use it to guide navigation. Likely magnetosensitive detectors in birds and other species have also been identified.

For billions of years, the Earth's magnetic field was the only one that organisms ever encountered. Since the development of electric power, however, multiple new sources of artificial electric and magnetic fields have appeared with the potential to affect magnetosensory systems or other aspects of animal biology.

Magnetic fields have been reported to have short- and long-term effects on health, and epidemiological studies point to an increased risk of certain cancers after exposure to even low field strengths^{1 2 3}. The International Agency for Research on Cancer (IARC) has classified so-called Extremely Low Frequency (ELF) magnetic fields (MFs) as a Class 2B 'possible carcinogen', whereas the evidence for ELF electric fields was classed as "inadequate to judge". Hence, the focus in this meeting was on ELF magnetic fields.

However, the mechanistic pathways by which magnetic fields may affect health risks are not clear.

A variety of EMF interaction mechanisms with biological system based on ELF-MF effects on ions have been discussed^{4 5 6}. Despite physical differences in and incompleteness of these mechanisms, all of them relate ELF effects with ion cyclotron resonance frequencies and their harmonics or subharmonics^{7 8}.

¹ Ahlbom A et al. A pooled analysis of magnetic fields and childhood leukaemia. *Br J Cancer*. 2000;83:692–8

² Kheifets L et al. Occupational electromagnetic fields and leukaemia and brain cancer: An update to two meta-analyses. *J Occup Environ Med*. 2008;50:677–88.

³ O'Carroll MJ, Henshaw DL. Aggregating epidemiological evidence: comparing two seminal EMF reviews. *Risk Analysis*. 2008;28:225–34.

⁴ Binh VN, Alipov YD, Belyaev IY. Effect of static magnetic field on E. coli cells and individual rotations of ion-protein complexes. *Bioelectromagnetics*. 2001;22(2):79–86.

⁵ Halgamuge MN, Abeyrathne CD. Behavior of charged particles in a biological cell exposed to AC-DC electromagnetic fields. *Environ Engineering Sci*. 2011;28(1):1–10

⁶ Foletti A et al. Bioelectromagnetic medicine: The role of resonance signaling. *Electromagn Biol Med*. 2013;32(4):484–99.

⁷ Belyaev IY, Alipov ED. Frequency-dependent effects of ELF magnetic field on chromatin conformation in Escherichia coli cells and human lymphocytes. *Biochim Biophys Acta*. 2001;1526(3):269–76.

⁸ Sarimov R et al. Exposure to ELF magnetic field tuned to Zn inhibits growth of cancer cells. *Bioelectromagnetics*. 2005;26(8):631–8.



At this meeting, two further possible biological detector systems for magnetic fields were discussed: **magnetic particles**, probably based on the biomineral **magnetite**; and the **radical pair mechanism**, through which magnetic fields may influence biochemical reactions by affecting the quantum properties of electrons in biomolecules.

Cryptochrome has emerged as a strong candidate protein molecule through which the radical pair mechanism might operate in the avian magnetic compass. Cryptochrome is also a component of the endogenous **molecular circadian clock**, providing a potential link to natural daily body rhythms – disruptions of which are known to increase the risk of cancer and other conditions.

A better understanding of the mechanisms of disease may suggest ways in which magnetic fields could increase the risk of cancer. In the most common cancer of children, **acute lymphoblastic leukaemia (ALL)**, a ‘window of vulnerability’ may exist before birth, when a specific population of hematopoietic stem/progenitor cells may be present. Initiating mutations in this population of cells may predispose to the later development of ALL, if another ‘second hit’ or hits occur.

Magnetic fields have been found to enhance the effects of genome-damaging chemicals, promoting **genome instability** – rapid accumulation of multiple mutations in cells.

They have also been found to alter **chromatin conformation**, potentially by introducing DNA double strand breaks. Effects are greatest at particular ‘frequency windows’, which show an interesting correlation with the frequencies to which, according to theoretical calculations, divalent ions should be sensitive.

Circadian rhythms are found in nearly all living organisms. They have a strong influence on physiology, and disruption of circadian rhythms has detrimental effects on health. Cyclical production of **melatonin** by the pineal gland may be particularly important. Magnetic fields could exert their effects by influencing melatonin production in the pineal gland, or potentially by altering calcium homeostasis or storage of iron in ferritin.

Melatonin is also produced by the **placenta**. It may have an important role in mitigating the effects of oxidative stress in the placenta, protecting the growing fetus.

There is some evidence that magnetic fields affect the melatonin system in animals and humans, the strongest evidence typically coming from natural exposures to varying fields.

As well as many simple organisms, several vertebrate species, including fish, birds, ruminants and dogs have been shown to **align to natural magnetic fields**, and some species have also been shown to detect magnetic field intensity, both evidence of magnetosensory detection. Artificial magnetic fields, for example from power lines, have been shown to disrupt magnetic alignment.

Magnetite-based magnetodetection systems have been identified in bacteria. They have been proposed to exist in the pigeon beak, but this has been questioned. Magnetite particles have also been visualised in cells of the trout olfactory system. There have been several reports of magnetite



particles in the human brain, but at least some of these may reflect contamination of laboratory materials with iron oxide particles.

The **radical pair mechanism** has been convincingly demonstrated in chemical systems and has been proposed as the potential basis for magnetic field detection in living organisms, for example in 'chemical magnetic compasses' to guide bird or insect migration or orientation.

The fundamental **quantum physics** of the radical pair mechanism places constraints on the type of magnetic field that could be detected. While the Earth's magnetic field is sufficiently strong to exert effects on radical pairs, current theoretical calculations suggest that smaller fields, such as those from artificial sources implicated in cancer, would be highly unlikely to have a significant impact on radical pairs. Such fields could, however, be amplified by the presence of magnetic particles, placing them in a regimen where they could affect chemical reactions⁹.

Electromagnetic 'noise' at radio wave frequencies has recently been shown to disrupt the innate magnetic compass of migratory robins. The mechanism of this disruption is unclear, and theoretical considerations again argue against a role for the radical pair mechanism.

Although some published evidence points to radical pair effects in **thermally driven enzyme systems**, key findings have not been replicated. Evidence is stronger for the radical pair mechanism in **light-driven systems**, such as those involving cryptochromes. Studies of cryptochromes in a model organism such as the fruit fly could provide important insight into the links between magnetic fields and biological responses.

A tripartite chemical has been developed as an optimised system for demonstrating the radical pair mechanism. It is sensitive to magnetic fields in the 50 μT range, supporting the idea that a chemical magnetosensory system is feasible in living organisms.

Background

Opening the meeting, **Professor Denis Henshaw** (Bristol) pointed out that magnetic fields have a long history on Earth – the Earth was created some 4.5 billion years ago, complete with its natural magnetic field. There is good evidence for very ancient biological sensitivity to natural magnetic fields, including the evolution of magnetotactic bacteria 2 billion years ago and the appearance of magnetosensitive proteins, cryptochromes, 2.5 billion years ago. An avian magnetic compass has been dated back to 90 million years ago. Hence, when human ancestors appeared some 2 - 3 million years ago, biological systems had grown up with, and evolved in the context of, magnetic fields.

Indeed, there is considerable evidence that humans, as well as many other animals, are sensitive to magnetic fields. For example, superimposed on the Earth's static magnetic field of around 50 μT in the UK, solar storms generate low intensity, $\sim 0.1 \mu\text{T}$, ultralow frequency magnetic field fluctuations that

⁹ Binhi VN. Magnetic nano-particles and risk of leukaemia. *Int J Radiat Biol.* 2008;84(7):569–79.



have a range of short-term effects on physiology and health (such as blood pressure changes, heart rate variability and melatonin disruption)¹⁰. Generally lasting for a few hours and occurring a few times a year, such storms seem to affect a susceptible 10–15% of the population.

It is only in the past 150 years, however, that these natural exposures have been supplemented by additional exposures linked to human activity, with the advent of electric power and its accompanying magnetic fields.

Average exposures in the home are low, typically around 0.05 μT . Close to some appliances exposures can exceed 10 μT but such exposures are usually short term in nature. Field levels under high voltage powerline cables are typically a few μT but can be as high as tens of μT .

Over many years, much epidemiological evidence has emerged to suggest that exposure to artificial magnetic fields is a risk factor for the development of some cancers. Field strengths of 0.3/0.4 μT , for example, are associated with an approximate doubling of the risk of childhood leukaemia¹¹. This evidence led the International Agency for Research on Cancer (IARC) to classify magnetic fields as a 'possible carcinogen'. Indeed, several review bodies have examined links, and there is a significant degree of agreement about increased risk for a range of exposures and for certain cancer types¹².

However, epidemiological studies provide little insight into possible mechanisms linking exposure to magnetic fields to the development of cancer. Various mechanisms can be envisaged. In the particular case of high voltage powerlines, a second mechanism has been proposed. The **corona ion hypothesis**, points out that electric fields can ionise the air and subsequently inhaled charged aerosols may be preferentially deposited in the lungs¹³. There is evidence this can occur, but its impact is unclear. Alternatively, clouds of corona ions emitted from powerlines could disrupt melatonin metabolism and circadian rhythms, by virtue of the disturbances they create in the Earth's natural DC electric field¹⁴.

In vitro studies have demonstrated a range of effects of magnetic fields on biological systems. Notably, high strength fields have been shown to promote production of potentially damaging reactive oxygen species in monocytes¹⁵, a possible route by which immune cells could become leukaemogenic. Indeed, in a meta-analysis of 41 studies, 36 provided evidence of increased reactive oxygen species production after exposure to magnetic fields¹⁶.

¹⁰ <http://www.electric-fields.bris.ac.uk/geomagneticfields.pdf>

¹¹ Zhao L et al. *Magnetic fields exposure and childhood leukemia risk: a meta-analysis based on 11,699 cases and 13,194 controls*. *Leuk Res*. 2014 Mar;38(3):269-74.

¹² O'Carroll MJ, Henshaw DL. *Aggregating disparate epidemiological evidence: comparing two seminal EMF reviews*. *Risk Anal*. 2008;28(1):225–34.

¹³ Fews AP et al. *Corona ions from powerlines and increased exposure to pollutant aerosols*. *Int J Radiat Biol*. 1999;75(12):1523–31.

¹⁴ Henshaw DL, Ward JP, Matthews JC. *Can disturbances in the atmospheric electric field created by powerline corona ions disrupt melatonin production in the pineal gland?* *J Pineal Res*. 2008;45(4):341–50.

¹⁵ Lupke M, Rollwitz J, Simkó M. *Cell activating capacity of 50 Hz magnetic fields to release reactive oxygen intermediates in human umbilical cord blood-derived monocytes and in Mono Mac 6 cells*. *Free Radic Res*. 2004;38(9):985–93.

¹⁶ Mattsson MO, Simkó M. *Grouping of Experimental Conditions as an Approach to Evaluate Effects of Extremely Low-Frequency Magnetic Fields on Oxidative Response in vitro Studies*. *Front Public Health*. 2014 Sep 2;2:132.



Since cancer is a genetic disease, ultimately magnetic fields must have some impact on DNA to have a carcinogenic effect. For many years, it was argued that magnetic fields, unlike ionising radiation, lacked the energy to damage DNA. However, with the discovery of the '**bystander effect**' in the field of ionising radiation – where cells not exposed to ionising radiation can show signs of DNA damage in the presence of exposed cells – it became clear that genome damage could be triggered indirectly. Indeed, after low-dose ionising radiation, DNA damage can appear not immediately but after multiple generations of cell division, a phenomenon known as **Genomic Instability**, or **GI**. The latter has recently been reported following magnetic field exposure to cells *in-vitro*.

One challenging question is whether, in the presence of a static 50 μT background field, small fluctuations are likely to have any impact. The evidence from both the natural world and from experimental studies is that they do. As well as the documented impact of geomagnetic storms, magnetic fields have been found to affect animal navigation. Many experimental studies have shown that magnetic fields can affect biological systems.

A further important question is the point at which interference occurs. Magnetosensitive organisms must have a **detector** that can sense changes in magnetic fields, but signalling pathways must link these to effects or systems responsible for the response to magnetic signal inputs. Artificial magnetic fields could in theory interfere with the primary detector or at points downstream of detection.

In terms of primary detectors, **magnetic particles** are one obvious possibility. Magnetic particles might physically rotate when subject to a magnetic field (or exert a force if tethered). For smaller particles, rather than the particle itself rotating, an applied magnetic field might result in rotation of the induced field. Of potential biological interest is **ferritin**, an iron storage protein containing small, super-paramagnetic particles that may have the potential to magnify magnetic fields locally¹⁷.

A second possible means of detection is the **radical pair mechanism**. In essence, by influencing electron spin states (see below), magnetic fields can affect the properties of pairs of molecules, and ultimately influence the kinetics of chemical reactions. This effect can be harnessed to create a 'chemical magnetic compass', and may underlie the magnetosensitive abilities of migratory birds. These abilities are thought to reflect the ability of bound cofactors and particular amino acid residues in cryptochromes to establish radical pairs sensitive to magnetic fields¹⁸.

Since cryptochromes are also components of the oscillating molecular networks that regulate **circadian rhythms**, they provide a tantalising link between detection of magnetic fields and disruption of circadian rhythms – a known risk factor for the development of cancer.

¹⁷ Binhi V. Do naturally occurring magnetic nanoparticles in the human body mediate increased risk of childhood leukaemia with EMF exposure? *Int J Radiat Biol.* 2008;84(7):569–79.

¹⁸ Ritz T, Adem S, Schulten K. A model for photoreceptor-based magnetoreception in birds. *Biophys J.* 2000;78(2):707–18.



The origins of childhood leukaemia

A better understanding of the mechanistic origins of childhood cancer could suggest ways in which magnetic fields might influence their development. **Professor Tariq Enver** (UCL) provided an update of what is known of the mechanisms underlying the most common cancer of children, acute lymphoblastic leukaemia (ALL).

A key question is why the disease specifically affects children. Professor Enver presented evidence suggesting that there is a 'window of vulnerability' when an initiating mutation, or 'first hit', occurs in a specific B-cell lineage present only before birth. By itself, this mutation does not trigger cancer, but by slightly altering the proliferative potential of cells, it increases the likelihood that they acquire further genetic changes ultimately leading to full-blown ALL.

The most common initial genetic change in ALL is a chromosomal rearrangement that fuses two genes involved in haematopoietic stem cell survival and differentiation. This **TEL–AML1 fusion** is seen in around one third to one half of ALL patients.

Analysis of Guthrie spots (blood spots collected at birth) suggests that the TEL–AML1 fusion typically occurs before birth. This is backed up by a study of identical twins, one of whom developed ALL. Genetic analysis suggested that both twins had immune cells with a TEL–AML1 fusion, but only one had subsequently acquired additional mutations driving the development of ALL. Hence it is likely that a 'pre-leukaemic cell' had arisen *in utero* and colonised both twins while they were sharing a blood supply in the womb¹⁹.

Professor Enver has generated a model of this pre-leukaemic cell in mice, and shown that it has a distinct set of properties that could explain its propensity to generate ALL. In particular, it shows altered responses to immune signalling molecules such as TGF- β and enhanced proliferation in response to infection²⁰. Although its growth advantage is only small, this provides additional opportunities for other mutations to arise that further enhance proliferation.

According to traditional models, cancer cells develop by sequential accumulation of growth-promoting mutations. Work in ALL (and other cancers) has revealed this picture to be too simplistic. In reality, from a common ancestor, populations of cells arise each carrying different combinations of mutations – generating a 'family tree' of related cancer clones²¹. These clones vary in their 'fitness', and the most 'fit' tend to dominate. When a new mutation adds to a cell's fitness, that clone will expand at the expense of others.

Hence, a patient with ALL will typically have multiple cancer clones with slightly different sets of genetic aberrations. This has important implications for therapy, which may wipe out dominant clones but leave space into which previously minor clones can expand, leading to relapse²². Notably,

¹⁹Ford AM et al. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. Proc Natl Acad Sci USA. 1998;95(8):4584-8.

²⁰Ford AM et al. The TEL-AML1 leukemia fusion gene dysregulates the TGF-beta pathway in early B lineage progenitor cells. J Clin Invest. 2009;119(4):826-36.

²¹Anderson K et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469(7330):356-61.

²²Lutz C et al. Quiescent leukaemic cells account for minimal residual disease in childhood lymphoblastic leukaemia. Leukemia. 2013;27(5):1204-7.



chemotherapy typically selects for relatively quiescent clones, which are less affected by treatments that generally target cell division. These quiescent cells are hard to kill and can act as a source of new actively proliferating cancer cells.

In recent work, Professor Enver has been attempting to identify and characterise the *in utero* B-cell lineage that may generate ALL. He has isolated early B-cell progenitor cells expressing marker proteins on their surface characteristic of both lymphoid and myeloid lineages, consistent with the mixed potential of ALL cells.

To produce sufficient material to work with, he is now attempting to create B-cell precursors from induced pluripotent stem cells – adult cells that have been reprogrammed into a state that can generate all the different cell types of the body. Through new ‘genome-editing’ techniques, he is introducing artificial TEL–AML1 fusion genes into such cells. As well as providing an opportunity to explore the biology of TEL–AML1-containing B-cell precursors, they will also enable the effects of additional mutations to be studied, to identify critical second and further hits on the road to ALL.

Genomic instability

An important trait associated with cancer is genome instability – rapid accumulation of DNA damage such as DNA breaks or other mutations. **Professor Jukka Juutilainen** (University of Eastern Finland, Kuopio) discussed how magnetic fields might affect genome instability.

The concept of genomic instability emerged from radiation biology. While ionising radiation can damage DNA directly, it also has delayed effects, with damage becoming apparent only after several rounds of cell division.

Since then, other classes of agent have been found to have similar effects, including chemicals. The dioxin-like compound TCDD, for example, has no immediate apparent impact on DNA, but by eight days distinctive signs of chromosomal disintegration, such as micronuclei, appear.

To assess whether magnetic fields contribute to genome instability, Professor Juutilainen exposed mouse cells to a 50 Hz 100 μ T field before adding menadione, an agent known to induce DNA damage. Pre-exposure to magnetic fields led to increased levels of cell cycle defects²³. Similar results were seen with a human cell line, with clear evidence of DNA damage such as micronuclei formation and increased rates of DNA repair up to 15 days after exposure²⁴.

The mechanisms of genomic instability remain unclear, but one possible mediating factor could be higher levels of reactive oxygen species. Levels of reactive oxygen species were increased by pre-

²³ Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. *Int J Radiat Biol.* 2008;84(9):742–51.

²⁴ Luukkonen J et al. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. *PLoS One.* 2011;6(3):e18021.



exposure to magnetic fields, and there was also some evidence of abnormalities in mitochondrial function²⁵.

However, in new studies looking over longer time periods, Professor Juutilainen found that the antioxidant N-acetylcysteine did not suppress micronuclei formation. Hence the increase in reactive oxygen species may be a secondary effect rather than causal of DNA damage.

In other new work, Professor Juutilainen has also been exploring the possibility that the radical pair mechanism might be involved in genomic instability, by examining the potential involvement of cryptochrome. If it is important, the impact of magnetic fields is likely to be affected by blue light (the wavelength that cryptochrome is most sensitive to). In fact, magnetic field effects were observed even in the absence of blue light and, curiously, blue light actually seemed to suppress some of the effects of magnetic field pre-exposure.

Effects of magnetic fields on DNA

Continuing the theme of genomic instability, **Professor Igor Belyaev** (Cancer Research Institute, Bratislava, Slovakia) summarised studies exploring the impact of magnetic fields on DNA, and how genomic changes in a pre-leukaemic cell line could promote the development of ALL.

The effects of magnetic fields, Professor Belyaev pointed out, depend on both physical and biological factors. Key physical factors include the frequency and intensity of the applied field. Important biological factors include the nature of the cells studied, their genetic background and cell density. Chemical factors such as the presence of free radicals, radical scavengers, antioxidants and ion levels also have to be taken into account. Hence there is likely to be considerable individual variability in responses to magnetic fields.

To assess the impact of magnetic fields on DNA, Professor Belyaev has made use of an assay that provides a measure of DNA compaction. The '**anomalous viscosity time dependence**' (AVTD) assay exploits the fact that high molecular weight DNA-protein complexes such as nucleoids radially migrate in a rotary viscometer under increased shear stress. The migration of large DNA complexes towards a rotating rotor is determined by several parameters of chromatin conformation such as nucleoid rigidity, hydrodynamic radius, molecular weight of nucleoids and compactness²⁶. Any treatments that affect compaction, such as relaxation of loops by double-strand DNA breaks, will alter migration and hence AVTD measurements.

²⁵ Luukkonen J et al. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. *Mutat Res Fundam Mol Mech Mutagen*. 2014;760:33-41.

²⁶ Belyaev IY et al. Effects of ethidium bromide on DNA loop organisation in human lymphocytes measured by anomalous viscosity time dependence and single cell gel electrophoresis. *Biochim Biophys Acta*. 1999;1428(2-3):348-56.



Using this approach, Professor Belyaev showed that externally applied magnetic fields affected DNA conformation, but only at particular 'frequency windows'²⁷. At fixed frequency, magnetic field effect size was dependent on the amplitude of the applied field²⁸. As well as human lymphocytes, this effect was also seen in bacterial cells, with periodic 'intensity windows' apparent in which applied fields had greatest effect. The effects were significantly greater for collinear than for perpendicular AC/DC fields.

Notably, the effect of an applied field was inhibited by chelators of divalent ions such as EDTA. Divalent ions, such as zinc, calcium and magnesium, play fundamental roles in multiple enzymatic processes. Theoretical calculations suggest they should be sensitive to particular frequencies of magnetic field, and there is striking concordance between these theoretical frequencies and the experimentally observed frequency windows.

Further studies indicated that the effects of external fields on bacteria depend on growth phase and cell density, hinting at the potential importance of cell-cell interactions. Furthermore, effects were markedly reduced by the free radical scavenger glycerol. Different responses were also seen in different strains of bacteria, and in different types of mammalian cell, highlighting the high degree of heterogeneity in responses to magnetic fields.

Turning to ALL, Professor Belyaev has analysed umbilical cord blood samples to assess the incidence of pre-leukaemic gene fusions, including TEL-AML1. In 500 samples from Slovakia, TEL-AML1 fusions were found at low levels in around 2% of samples²⁹. Professor Belyaev suggested that the presence of pre-leukaemic gene fusions may affect DNA stability, leading to additional DNA breaks and 'second hits' promoting the development of ALL³⁰. Indeed, haematopoietic stem cells harbouring pre-leukaemic gene fusions may have reduced DNA repair capacity. However, there is also evidence that defective regulation of the RAG system, involved in the shuffling of immune-related genes, plays an important role in generating secondary events in ALL³¹.

Melatonin and circadian rhythms

The spectrum of non-ionising magnetic fields and electromagnetic radiation spans wavelengths from 100 nm to several thousand kilometres. Humans have evolved to detect just a small section of this spectrum, visible light at wavelengths of 390–700 nm. **Professor Richard Stevens** (University of Connecticut, USA) discussed the important impact visible light has on our biology.

²⁷ Belyaev IY, Alipov ED. Frequency-dependent effects of ELF magnetic field on chromatin conformation in *Escherichia coli* cells and human lymphocytes. *Biochim Biophys Acta*. 2001;1526(3):269–76.

²⁸ Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. *Bioelectromagnetics*. 2011 Oct;32(7):570-9

²⁹ Škorvaga M et al. Incidence of common preleukemic gene fusions in umbilical cord blood in Slovak population. *PLoS One*. 2014;9(3):e91116.

³⁰ Somsedikova A et al. Constitutive 53BP1/γH2AX foci are increased in cells of ALL patients dependent on BCR-ABL and TEL-AML1 preleukemic gene fusions. *Neoplasma*. 2014;61(5):617–25.

³¹ Papaemmanuil E et al. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. *Nat Genet*. 2014;46(2):116–25.



Until around 130 years ago, most life on Earth experienced regular approximately 12-hour cycles of light and dark. Following the invention of electric lighting, this picture has changed completely, with people now typically exposed not only to more light at night but also less light during the day, particularly those working indoors. This is likely to have profound impact on our biology and health.

Earth's natural 24-hour cycles are reflected in innate endogenous circadian rhythms seen in almost all living organisms. In the absence of external inputs, circadian rhythms cycle naturally at around 24 hours. In practice, they are entrained by external light signals, synchronising organisms to natural day–night cycles and allowing for anticipation of key transitions such as dawn.

Circadian rhythms influence a wide variety of physiological processes. As well as obvious sleep–wake cycles, circadian patterns are seen in core body temperature, physical activity, appetite, metabolism and hormone production. Circadian rhythm disruption has been implicated in various cancers and other conditions, including obesity, diabetes and mood disorders.

In mammals, a key role is played by the body's 'master clock', the **suprachiasmatic nucleus**, which regulates the activity of multiple peripheral clocks. Indeed, each cell contains an endogenous clock, a complex oscillating gene network generating rhythmic 24-hour outputs³². Notably, up to 10% of human genes show circadian patterns in gene expression, including many key cell cycle genes and others of potential importance to cell proliferation and cancer.

One of the most intensively studied circadian rhythms is the nightly production of **melatonin** by the pineal gland. The detrimental effects of circadian rhythm disruption may be mediated by changes in production of melatonin, a molecule with a multitude of functions in the body.

In the 1980s, it was discovered that intense light suppresses night-time melatonin production in humans³³. Later, it was shown that blue light is far more effective than red light in suppressing melatonin production³⁴, and that people vary significantly in their sensitivity to melatonin suppression³⁵, possibly because of their genetic inheritance³⁶. Notably, the wavelengths most effective at suppressing melatonin production correspond almost exactly with the peak intensity wavelengths of natural daylight.

In humans, circadian rhythms are entrained by light signals detected by a specific photoreceptor cells in the eye, **retinal ganglion cells**, which signal to the suprachiasmatic nucleus. The light-detecting molecule in these cells is **melanopsin**, an evolutionarily ancient opsin molecule.

³² Takahashi JS et al. [The genetics of mammalian circadian order and disorder: implications for physiology and disease](#). *Nat Rev Genet.* 2008;9(10):764–75.

³³ Lewy AJ et al. [Light suppresses melatonin secretion in humans](#). *Science.* 1980;210(4475):1267–9.

³⁴ Brainard GC et al. [Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor](#). *J Neurosci.* 2001;21(16):6405–12.

³⁵ McIntyre IM et al. [Melatonin supersensitivity to dim light in seasonal affective disorder](#). *Lancet.* 1990;335(8687):488.

³⁶ Chellappa SL et al. [Human melatonin and alerting response to blue-enriched light depend on a polymorphism in the clock gene PER3](#). *J Clin Endocrinol Metab.* 2012;97(3):E433–7.



Outside the visible light spectrum, the detectors of non-ionising low frequency magnetic fields or high frequency electromagnetic radiation are less clear, and it is less obvious how detection might trigger changes that harm health. Professor Stevens drew attention to two possible mechanisms – mobilisation of iron from **ferritin** and disruption of **calcium homeostasis** – both of which have the potential to lead to increased oxidative stress.

Iron is essential to life and a key component of many important enzymes and biomolecules, not least haemoglobin. But it also has potential to cause harm, particularly by generating oxidative stress. Iron is stored by a special protein, ferritin, which sequesters free iron to avoid damage and releases iron when required. It forms large multiprotein complexes each capable of holding more than 2000 iron atoms.

Calcium has a particularly important role as a signalling molecule, in a multitude of physiological processes. Interference in its function by magnetic fields could therefore potentially have major impact.

Melatonin and pregnancy

As well as in the pineal gland within the brain, melatonin is also produced in a wide range of peripheral tissues, as discussed by **Professor Cathy Vaillancourt** (Institut National de la Recherche Scientifique (INRS)-Institut Armand Frappier, Université du Québec, Canada). This local production does not contribute significantly to circulating melatonin and is not related to melatonin's circadian role. Local concentrations can be remarkably high, particularly in organelles such as mitochondria.

Melatonin has a wide range of functions, but may be particularly important as a free radical scavenger, contributing to protection against oxidative stress. It is an evolutionarily ancient, well-conserved molecule, with powerful anti-oxidant properties. Notably, its scavenging activities generate new products that themselves have anti-oxidant properties, creating a cascade whereby each melatonin molecule can ultimately quench up to ten free radicals.

Melatonin has been implicated in multiple other physiological processes, in a wide range of organ systems. There is also some evidence that it may offer protection against cancer, potentially through a wide range of mechanisms.

One of melatonin's most important roles may be in maintaining **placenta function** and protecting the health of the fetus *in utero*. The first 1000 days of life, including time spent in the womb, are critical to both immediate and long-term health, and placental function is vital for much of this period.

An important barrier between maternal and fetal blood circulation in the placenta is formed by the **villous trophoblast**. Turnover of cells in the villous trophoblast is carefully regulated, but can be affected by oxidative stress, leading to enhanced cell turnover and compromised placental function. This increases the risk of intrauterine growth restriction, pre-eclampsia and pre-term birth.



Given its anti-oxidant properties, Professor Vaillancourt suggested that melatonin might have a role in preventing oxidative damage in the placenta. Indeed, circulating levels of melatonin are significantly higher during pregnancy, peaking at birth³⁷. Professor Vaillancourt found that the enzymes synthesising melatonin were active in placental tissue³⁸. In addition to significance for disease, serum melatonin levels were markedly reduced in both pre-eclampsia and fetal growth restriction. Furthermore, the activity of melatonin-producing enzymes and melatonin production itself were both lower in women with pre-eclampsia³⁹.

To explore further, Professor Vaillancourt established an *in vitro* model based on cultured villous trophoblast cells. A hypoxic insult to these cells triggered a damaging response, including enhanced release of reactive oxygen species associated with increased apoptosis, and raised levels of inflammatory mediators. These responses were substantially mitigated when melatonin was added to the culture, arguing for an important protective role⁴⁰. Clinical trials of melatonin supplementation have now begun in Australia in an attempt to reduce the risk of pre-eclampsia and growth restriction, both associated with increased oxidative stress in placenta, mother and foetus.

Professor Vaillancourt also pointed out that there is growing evidence that maternal melatonin can influence fetal circadian rhythms, by influencing the fetal suprachiasmatic nucleus directly or indirectly through its effects on other maternal rhythms.

Disruption of melatonin release by magnetic fields

With evidence growing of the harmful impact of disruption of night-time melatonin production and natural circadian rhythms, the system is one on which magnetic fields could act to increase the risk of cancer. **Professor Denis Henshaw** (Bristol) reviewed the evidence that electric or magnetic fields influence the melatonin system in animals and humans.

Interestingly, back in the 1970s, it was discovered that shielding subjects from the Earth's natural electric field affected circadian rhythms, while exposure to artificial electric fields lengthened the circadian day⁴¹.

In 2005, Henshaw and Reiter reviewed studies examining the impact of magnetic fields on pineal cell function and melatonin production in animals and humans⁴². In animals, results were mixed, with effects typically seen following exposure to non-sinusoidal or non-smoothly varying fields and in animals such as cows in natural environments.

³⁷ Kivelä A. Serum melatonin during human pregnancy. *Acta Endocrinol (Copenh)*. 1991;124(3):233–7.

³⁸ Lanoix D et al. Human placental trophoblasts synthesize melatonin and express its receptors. *J Pineal Res*. 2008;45(1):50–60.

³⁹ Lanoix D, Guérin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. *J Pineal Res*. 2012;53(4):417–25.

⁴⁰ Lanoix D, Lacasse AA, Reiter RJ, Vaillancourt C. Melatonin: the watchdog of villous trophoblast homeostasis against hypoxia/reoxygenation-induced oxidative stress and apoptosis. *Mol Cell Endocrinol*. 2013;381(1-2):35–45.

⁴¹ Wever R. The effects of electric fields on circadian rhythmicity in men. *Life Sci Space Res*. 1970;8:177–87.

⁴² Henshaw DL, Reiter RJ. Do magnetic fields cause increased risk of childhood leukemia via melatonin disruption? *Bioelectromagnetics*. 2005;Suppl 7:S86–97.



In humans, laboratory studies typically provided little evidence that magnetic fields affected the melatonin system. However, effects were seen more often in studies of natural populations experiencing chronic exposure, even at very low field strengths (down to $0.2\mu\text{T}$).

In 2012 Touitou and Selmaoui provided an updated review examining the effects of magnetic and geomagnetic fields on melatonin⁴³. They reviewed 33 studies, which again showed conflicting results. For 11 papers, a clear effect was seen; for the remaining 22 papers, Touitou and Selmaoui concluded that no effect was apparent.

Touitou and Selmaoui concluded that, on the balance of evidence, magnetic fields were unlikely to be having an effect on the melatonin system. Their conclusion was heavily influenced by results of their own study on French power industry workers, some of whom were chronically exposed to magnetic fields⁴⁴. No differences in melatonin metabolism were seen in exposed individuals compared to their unexposed colleagues, leading the authors to reject the 'melatonin hypothesis'.

However, pointed out Professor Henshaw, several of the 'negative' papers did in fact identify effects, while two other papers were outside the remit of the review. Furthermore, the average number of participants in 'positive' studies was around 150, but was just 42 in 'negative' studies. Resolving power calculations suggest that studies of the size used in these so-called negative studies would not be sufficient to identify an effect on the scale that might be expected.

Overall, he suggested, the updated evidence presented by Touitou and Selmaoui was inconsistent with there being no effect, and reinforced the conclusion that chronic exposure to magnetic fields in 'natural' settings can have a significant impact on the melatonin system.

Magnetic alignment in vertebrates

Magnetosensitivity has now been demonstrated in a very wide range of organisms. **Professor Hynek Burda** (University of Duisburg-Essen, Germany) described what is known about the alignment in the Earth's magnetic field in one particular group, vertebrates.

The first convincing demonstration of magnetosensitivity came from work on Ansell's mole rat⁴⁵. These underground rodents build extensive networks of burrows that can stretch for several kilometres. Strikingly, they are able to build new tunnels in absolutely straight lines. Laboratory studies of nest building revealed that the animals have a preference for the south-east quarters of circular arenas, which can be altered by external magnetic fields, suggesting that the animals can detect naturally occurring magnetic fields.

⁴³ Touitou Y, Selmaoui B. *The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system*. *Dialogues Clin Neurosci*. 2012;14(4):381–99.

⁴⁴ Touitou Y et al. *Magnetic fields and the melatonin hypothesis: a study of workers chronically exposed to 50-Hz magnetic fields*. *Am J Physiol Regul Integr Comp Physiol*. 2003;284(6):R1529–35.

⁴⁵ Burda H et al. *Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae)*. *Experientia*. 1990;46(5):528–30.



Since then, other rodents, as well as unrelated mammals such as bats, have been shown to orientate themselves according to the magnetic compass. For larger animals, it becomes more challenging to identify magnetosensitivity. Indirect evidence has come from large-scale migrations (and their failure, such as whale strandings). Anecdotal evidence, such as long-distance cat homing, also hints at magnetosensitivity capacities.

A convincing demonstration of magnetic alignment must take into account other factors that might influence an animal's alignment, such as the position of the sun, air or water currents, or the presence of other attractors. To this end, Professor Burda took the novel approach of analysing the orientation of cows on Google Earth images. This revealed a striking preference for north–south alignment – some 70% of animals were thus aligned⁴⁶. Further (unpublished) evidence of magnetic alignment in large animals has come from studies of deer beds in snow.

One study has published contradictory evidence, though Professor Burda pointed to significant flaws in such studies (including mis-identification of white rocks and hay bales as cows...). Independent confirmation has been obtained, though with effects depending on herd density⁴⁷.

Interestingly, Google Earth images also provided a way to examine the possible impact of power lines. Animal orientation close to such lines was essentially random, and reduced alignment was seen at distances up to 100 m, suggesting that their magnetic fields were interfering with animals' detection of the Earth's magnetic field⁴⁸.

Further support for magnetosensitivity has come from recent work on wild boars, sleeping horses and foxes hunting mice – which are dramatically more successful in their pounces in particular orientations⁴⁹.

A further intriguing example has come from studies of 'Christmas carp' in the Czech Republic⁵⁰. A popular seasonal treat, the carp are stored in large barrels, and an analysis of more than 18 000 fish revealed a preferential north–south alignment within barrels.

Professor Burda has shown that magnetic fields affect the orientation of defecating and urinating dogs, as well as the flight trajectories of landing ducks^{42, 43}. These studies – which won Professor Burda an Ig Nobel Award in 2014 – have the advantage that they are examining a natural, outdoor behaviour. Curiously, on some days, dogs were found to exhibit no preferential alignment. These days turned out to be marked by solar flares interfering with the Earth's magnetic field. Professor Burda suggested that

⁴⁶ Begall S et al. [Magnetic alignment in grazing and resting cattle and deer](#). *Proc Natl Acad Sci USA*. 2008;105(36):13451–5.

⁴⁷ Slaby P, Tomanova K, Vacha M. [Cattle on pastures do align along the North-South axis, but the alignment depends on herd density](#). *J Comp Physiol A*. 2013;199(8):695–701.

⁴⁸ Burda H et al. [Extremely low-frequency electromagnetic fields disrupt magnetic alignment of ruminants](#). *Proc Natl Acad Sci USA*. 2009;106(14):5708–13.

⁴⁹ Červený J et al. [Directional preference may enhance hunting accuracy in foraging foxes](#). *Biol Lett*. 2011;7(3):355–7.

⁵⁰ Hart V et al. [Magnetic alignment in carps: evidence from the Czech christmas fish market](#). *PLoS One*. 2012;7(12):e51100.



the ability to detect magnetic fields might work in conjunction with a cognitive map of the environment to support animals' orientation and navigation.

Mechanisms of magnetoreception

How animals detect magnetic fields is critical to understanding the basis of magnetosensitivity.

Professor Michael Winklhofer (Ludwig-Maximilians-University, Munich, Germany) reviewed what is currently known about detection mechanisms in bacteria, animals and humans.

The two most likely mechanisms for detecting weak natural fields (<1 mT), he suggested, would be a magnetic biomineral such as magnetite (a form of iron oxide) and the radical pair mechanism.

Concentrating on the former, Professor Winklhofer pointed out that magnetite is the most strongly magnetic of the different forms of iron oxide. This is particularly the case for 'single domain' particles (smaller than around 100 nm); interactions between domains in multidomain particles tend to reduce their field strength.

On the other hand, at very small sizes (less than 20 nm), magnetite particles become 'superparamagnetic': a particle's magnetic field can switch between a range of possible orientations, driven by the energy of thermal fluctuations. The stability of a particle's magnetic properties is thus strongly dependent on temperature.

In terms of biological context, magnetosensitive bacteria containing magnetite particles were discovered in the mid-1970s⁵¹. In animals, magnetic extracts were obtained from homogenised tissue in the 1980s⁵², and possible magnetoreceptor cells in the olfactory organ of trout were first described in the late 1990s⁵³. A striking magnetite-containing 'dendrite' was identified in the pigeon beak in 2003⁵⁴.

However, the existence of this structure was called into question in 2011, when it was reported that magnetite particles in the pigeon beak were present in macrophages⁵⁵. In fact, white blood cells are highly effective magnetic particle scavengers. Since macrophages do not make magnetite particles, their origin remains uncertain. Although they could be externally introduced via infection, they may also be a breakdown product from a magnetoreceptor cell.

⁵¹ Blakemore R. *Magnetotactic bacteria*. *Science*. 1975;190(4212):377–9.

⁵² Walker MM, Kirschvink JL, Chang SB, Dizon AE. *A Candidate Magnetic Sense Organ in the Yellowfin Tuna, Thunnus albacares*. *Science*. 1984;224(4650):751–3.

⁵³ Walker MM et al. *Structure and function of the vertebrate magnetic sense*. *Nature*. 1997;390(6658):371–6.

⁵⁴ Fleissner G et al. *Ultrastructural analysis of a putative magnetoreceptor in the beak of homing pigeons*. *J Comp Neurol*. 2003;458(4):350–60.

⁵⁵ Treiber CD et al. *Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons*. *Nature*. 2012;484(7394):367–70.



Recently, more detailed images have been produced of candidate magnetoreceptor cells in the trout olfactory organ⁵⁶.

A magnetosensitive structure within a cell could rotate in response to an externally applied magnetic field or, if tethered, generate a force. One possible mechanism by which this force could be transduced is via tethering to an ion channel. Theoretical models suggest that this mechanism would be capable of modulating ion channel function⁵⁷. Although a single superparamagnetic particle would not align with an external field, clusters of particles would be capable of detecting a field and theoretically able to influence ion channel opening (albeit rather inefficiently).

Whether humans have magnetosensitive structures is uncertain. In 1992, magnetite particles were detected in the human brain, particularly the meninges, and were suggested to have a possible role in Alzheimer's disease⁵⁸. However, although other studies have also obtained evidence for magnetite in brain samples, disposable laboratory materials have been found to be frequently contaminated with ferromagnetic particles⁵⁹. Despite the replications, all studies have been carried out on tissue extracts, and there is no evidence yet of cellular context.

Interestingly, recent work has pointed to possible ferritin dysfunction in Alzheimer's disease, with a significant enrichment of reduced forms of iron, rather than the more usual ferrihydrite⁶⁰. However, it is unclear whether this is a cause or consequence of disease.

Professor Winklhofer also discussed one other potential action of magnetic particles. Because of their magnetic properties, they may have a field amplification effect. This could in turn lead to an effect on biological processes via the radical pair mechanism. Indeed, the radical pair mechanism has been used to visualise magnetic fields around nanoparticles⁶¹.

From epidemiology to mechanism

Despite the epidemiological evidence linking both ELF magnetic and radio frequency electromagnetic fields to cancer, there is a lack of plausible causative mechanisms. **Dr Patricia Bounds** (IT'IS Foundation, Zurich, Switzerland) described the **Arimmora project**⁶², funded through the EU Framework Programme 7, which aims to bridge this gap.

⁵⁶ Eder SH et al. *Magnetic characterization of isolated candidate vertebrate magnetoreceptor cells*. Proc Natl Acad Sci USA. 2012;109(30):12022-7.

⁵⁷ Winklhofer M, Kirschvink JL. *A quantitative assessment of torque-transducer models for magnetoreception*. J R Soc Interface. 2010;7 Suppl 2:S273-89.

⁵⁸ Kirschvink JL, Kobayashi-Kirschvink A, Woodford BJ. *Magnetite biomineralization in the human brain*. Proc Natl Acad Sci USA. 1992;89(16):7683-7.

⁵⁹ Kobayashi AK, Kirschvink JL, Nesson MH. *Ferromagnetism and EMFs*. Nature. 1995;374(6518):123.

⁶⁰ Quintana C, Cowley JM, Marhic C. *Electron nanodiffraction and high-resolution electron microscopy studies of the structure and composition of physiological and pathological ferritin*. J Struct Biol. 2004;147(2):166-78.

⁶¹ Lee H, Yang N, Cohen AE. *Mapping nanomagnetic fields using a radical pair reaction*. Nano Lett. 2011;11(12):5367-72.

⁶² <http://arimmora-fp7.eu/>



Arimmora (Advanced Research on Interaction Mechanisms of Electromagnetic Exposures with Organisms for Risk Assessment) is an interdisciplinary programme spanning 10 centres in six countries, led from the IT'IS Foundation in Zurich, Switzerland. It is carrying out a range of research, spanning exposure studies on people, experimental investigation of magnetic and electromagnetic field exposure and modelling.

One of its core aims is to improve exposure assessment. It is following cohorts of children in Switzerland and Italy, using new instrumentation to measure daily exposure to magnetic and electromagnetic fields. This information will provide a better understanding of exposure patterns, helping to refine exposure models and risk assessment.

In vitro studies are focusing on the well-studied MAPK intracellular signalling pathway, which has been implicated in multiple cancers. The impact of magnetic fields is being examined in various cell lines, at high field strengths but also lower exposures (1 μ T and below). Other studies are examining epigenetic changes in haematopoietic stem cells.

In vivo studies are examining the impact of magnetic fields on immune cell function and other aspects of mice and rat physiology. A transgenic mouse has been developed expressing the TEL-AML1 fusion gene, which is being exposed to magnetic fields and followed up for two years to track the development of leukaemia.

A further strand of work focuses on computational modelling. Dosimetric modelling is being carried out on data collected on human, experimental animal and cellular exposure, and models are being developed to support comparisons between the different systems. Computational models are also being developed for different human postures.

Biophysical modelling studies aim to bridge the gap between dosimetric studies and subcellular effects. A literature review of existing studies has highlighted wide variation in experimental strategies, with particular issues surrounding the use of control or 'sham' exposures.

These studies are focusing on the radical pair mechanism and how this might impact on cellular processes such as cell cycle regulation, ion homeostasis and oxidative stress. Cryptochrome is a particular focus, including the possibility that electron transfer might generate radical pair effects in amino acid residues other than those conventionally implicated. The possible biological impact of other quantum effects is also being explored.



The radical pair mechanism

The radical pair mechanism has been proposed as a mechanism by which magnetic fields could affect biological processes, and hence form the basis of magnetodetection. **Professor Peter Hore** (Oxford) provided an overview of the mechanism and key factors influencing the potential of magnetic fields to affect radical pairs.

Radicals are characterised by the presence of unpaired electrons. Pairs of radicals can be formed during the breaking of chemical bonds, or by the transfer of electrons or hydrogen atoms. Electrons can be envisaged as spinning around their own axis, and when radicals are present in pairs, electrons can spin either in opposite directions (anti-parallel, the **singlet state**) or in the same direction (parallel, the **triplet state**). The '**entanglement**' of electrons, despite their physical separation, is one of the many curiosities of the quantum world – what Einstein called 'spooky action at a distance'.

Singlet and triplet states have similar energies and hence can rapidly interconvert. Importantly, the chemical reactivities of the singlet and triplet states may not be identical, so if external exposures such as magnetic fields influence interconversion, they can have an impact on the kinetics of chemical reactions and pathways in which the compounds generating the radical pairs are taking part.

Interconversion between singlet and triplet states is governed by '**hyperfine interactions**' –magnetic forces generated by the magnetic moment of the nucleus acting on electrons. Because of these hyperfine interactions, radical pairs show rapid oscillations between singlet and triplet forms, with a periodicity of a few tenths of a microsecond. An external magnetic field can generate its own pattern of interconversion, superimposed on the oscillations driven by hyperfine interactions.

Compared with the strength of a chemical bond, magnetic fields are extremely low energy and hence might not be expected to have any impact on the breakage or formation of bonds. However, they can have an effect by acting on a system when it is far from equilibrium, providing a small 'nudge' that can move the system from state to another.

It is well-established that the radical pair mechanism does exist in chemical systems. Professor Hore went on to describe the circumstances required for magnetic fields to have an impact on radical pairs. These include the need for hyperfine interactions in at least one radical, which is generally true for biological radicals. A larger effect would be seen if only one radical showed hyperfine interactions. Interactions between radicals must be small, and the lifetime of the radical pair must not be too short. But a crucial factor, suggested Professor Hore, is the **coherence lifetime** of the radical pair – the time during which singlet and triplet forms are able to interconvert coherently.

At best, magnetic fields have limited scope to affect chemical or biochemical reactions: under the most favourable circumstances, influencing radical pairs has been found to achieve a maximum of approximately a 25% change in yield of product. For weak magnetic fields, it is highly unlikely that



large magnetic field effects will occur. To date, magnetic field effects on radical pairs have not been seen below 40 μT , and theoretical calculations suggest that for, say, a 1 μT static field, coherence lifetimes would have to be far longer than is generally observed in order for magnetic field effects to be seen.

A 50 Hz oscillating field has similar or less potential to generate magnetic field effects on a chemical reaction, particularly if reaction steps are typically of the microsecond duration. However, it may be difficult to disentangle them from the background effects of the Earth's magnetic field. By contrast, radio frequency magnetic fields (1–100 MHz) have more potential to generate magnetic field effects. In particular, largest effects will be seen when the frequency matches that of the natural resonance frequency of the interconverting radical pair. Radio frequency magnetic fields would also be predicted to modify the effects of static fields.

One interesting example where radio frequency electromagnetic fields are having a biological impact is on the orientation abilities of **migratory robins**⁶³. Mouritsen and colleagues have shown that electromagnetic 'noise' on an urban university compass disrupts the ability of robins to orient themselves in their environment. The birds' innate compass could be restored by lining their living areas with aluminium sheets, as long as the sheets were grounded.

The screens are effective over a range from 2 kHz to 9 MHz, implying that the key frequencies 'jamming' the birds' compass lie in this range. Jamming was observed only on the university's urban campus and not outside the city. Notably, disruption was seen at exposure levels several orders of magnitude lower than WHO-recommended exposure guidelines for humans. In terms of mechanism, the intensities involved would seem to rule out a radical pair effect, as the required coherence time would need to be far greater than predictions suggest is possible.

The radical pair mechanism in proteins

Most of the key chemical reactions in biological systems are carried out by proteins, and **Dr Alex Jones** (Manchester) outlined some of the evidence that magnetic fields influence radical pairs in proteins.

Dr Jones reviewed two major areas of activity – in **thermally driven** and **light-driven** systems. For the former, although evidence of magnetic field effects has been published, replication of interesting initial findings has proven problematic.

⁶³ Engels S et al. Anthropogenic electromagnetic noise disrupts magnetic compass orientation in a migratory bird. *Nature*. 2014;509(7500):353–6.



Notably, magnetic field effects have been described in reactions catalysed by vitamin B₁₂-dependent enzymes. Radical pairs are generated after bond homolysis in the B₁₂ molecule during enzyme catalysis and were reported to be sensitive to magnetic fields. However, Dr Jones's group was unable to replicate these findings⁶⁴. The same radical pairs are generated by exposing B₁₂ to light in the absence of an enzyme, and the chemistry of these radical pairs *is* magnetically-sensitive. Magnetic field effects observed in B₁₂ photochemistry are enhanced by increasing the viscosity of the solvent, by making it more likely that radical pairs stay together, and by binding vitamin B₁₂ to an enzyme.

However, when the B₁₂ radicals are generated thermally by the enzyme-catalysed reaction, all magnetic-sensitivity is removed. The enzyme stops the radical pair from recombining and therefore the reaction is no longer dependent on the radical pair spin-state (singlet or triplet) and is thus insensitive to applied magnetic fields. Similarly, magnetic field effects were reported for horseradish peroxidase, but again Dr Jones was unable to replicate the initial findings⁶⁵, which were an artefact of data analysis.

Finally, magnetic isotope effects were reported for enzymatic synthesis of ATP by creatine kinase. The production of ATP by this magnesium-dependent enzyme was reported to be affected by the particular isotope of magnesium used – an effect proposed to result from the distinct magnetic properties of one isotope. Reactions were suggested to generate radical pairs and to be sensitive to external magnetic fields. However, two independent groups, in Essex and the Republic of Ireland, were unable to reproduce these findings⁶⁶.

The jury is therefore out on the possibility of magnetic field effects in thermally driven enzyme systems, where the necessary conditions for magnetic-sensitivity might not be widespread. However, the evidence appears more robust for light-driven systems. Among the strongest evidence comes from work on the proposed 'chemical magnetic compass' of migrating birds, which can be disrupted by externally applied magnetic fields in a way consistent with interference with a radical pair mechanism⁶⁷.

Attention generally focuses on the **cryptochrome** family of proteins. An ancient family of proteins, cryptochromes take part in light-driven reactions in many species and are related to the DNA repair enzymes, photolyases. In mammals cryptochromes have been implicated in a wide range of processes and are a core component of the molecular circadian clock.

⁶⁴ Jones AR, Hay S, Woodward JR, Scrutton NS. [Magnetic field effect studies indicate reduced geminate recombination of the radical pair in substrate-bound adenosylcobalamin-dependent ethanolamine ammonia lyase.](#) J Am Chem Soc. 2007;129(50):15718–27.

⁶⁵ Jones AR, Scrutton NS, Woodward JR. [Magnetic field effects and radical pair mechanisms in enzymes: a reappraisal of the horseradish peroxidase system.](#) J Am Chem Soc. 2006;128(26):8408–9.

⁶⁶ Crotty D et al. [Reexamination of magnetic isotope and field effects on adenosine triphosphate production by creatine kinase.](#) Proc Natl Acad Sci USA. 2012;109(5):1437–42.

⁶⁷ Ritz T et al. [Resonance effects indicate a radical-pair mechanism for avian magnetic compass.](#) Nature. 2004;429(6988):177–80.



Cryptochromes typically bind a cofactor, **flavin adenine dinucleotide (FAD)**, which participates in a well-characterised electron transport chain that also involves key amino acid residues. Radical pairs are created during this electron transfer and therefore are potentially sensitive to magnetic fields. Evidence has accumulated that cryptochromes are involved in magnetoreception, although the intracellular signal transduction pathways involved remain elusive⁶⁸.

In fruit fly larvae, cryptochrome has been found to mediate the effect of light on seizure duration. Notably, this effect is enhanced by exposure to magnetic fields⁶⁹. The fact that this magnetic field effect is removed using typical anti-epileptic drugs is strongly indicative that the impact of fields is on the central nervous system. Other studies have shown that cryptochrome alters neuronal firing rate in the presence of blue light⁷⁰. Hence, this neuronal effect might represent a way in which magnetically-sensitive chemistry in a key biological molecule might result in an organism response.

Structural studies of cryptochrome could shed more light on its function and mechanisms potentially sensitive to magnetic fields. However, recent work has raised questions about the nature of the binding of FAD, critical to electron transport and radical pair formation, to mammalian cryptochrome⁷¹.

Optimised systems for detecting low-field effects

As **Dr Christiane Timmel** (Oxford) pointed out, it has been known for several decades that magnetic fields can influence chemical reactions via the radical pair mechanism. Indeed, low-field effects have been demonstrated in several systems. For example, when benzophenone is photoexcited in a lipid micelle, it can abstract a hydrogen atom from components of the micelle, creating a pair of radicals that are kept together by being trapped in a micelle 'microcontainer'. In other cases, such as pyrene and dimethylaniline mixtures, photoexcitation triggers electron transfer between compounds, generating radicals that are held together by electrostatic attraction.

Magnetic field effects have also been seen in biological systems, notably the electron transfer chain of the photosynthetic reaction centre of bacteria and plants. After light excitation, rapid energy transfer is funnelled to a special pair of chlorophyll molecules, which transfer electrons to cofactor molecules. Normally, electron transfer is so rapid that magnetic field effects cannot be observed. But if the system is chemically modified to stall electron transfer, radical pairs can be generated and magnetic field effects detected.

⁶⁸ Dodson CA, Hore PJ, Wallace MI. A radical sense of direction: signalling and mechanism in cryptochrome magnetoreception. *Trends Biochem Sci.* 2013;38(9):435–46.

⁶⁹ Marley R et al. Cryptochrome-dependent magnetic field effect on seizure response in *Drosophila* larvae. *Sci Rep.* 2014;4:5799.

⁷⁰ Fogle KJ, Parson KG, Dahm NA, Holmes TC. CRYPTOCHROME is a blue-light sensor that regulates neuronal firing rate. *Science.* 2011;331(6023):1409–13.

⁷¹ Xing W et al. SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. *Nature.* 2013;496(7443):64–8.



However, these effects are seen only with magnetic fields in the millitesla range – orders of magnitude larger than the Earth's magnetic field. Dr Timmel has sought to identify the most sensitive chemical system that might show responses to smaller fields.

Dr Timmel exhaustively scanned the literature to identify chemical systems that best met the criteria for magnetic field effects summarised by Professor Hore. The most favourable system identified was a three-moiety compound comprising a carotenoid, a porphyrin and a fullerene⁷². After photoexcitation of the porphyrin, an electron is transferred from the carotenoid to the fullerene, creating a relatively long lived radical pair.

Working at cryogenic temperatures (100K), Dr Timmel used laser flashes to generate radical pairs in this molecule, quantifying their creation by monitoring changes in absorbance in the presence or absence of magnetic fields. These studies revealed magnetic field effects, even at low field strengths (50 μ T). Given how favourable the properties of this chemical system are, Dr Timmel suggested it was just about the optimal system for detecting magnetic field effects on a radical pair.

In further work, Dr Timmel has been developing a new diagnostic test for radical pairs⁷³. The test is based on the use of a static magnetic field to interfere with singlet–triplet interconversion. An oscillating magnetic field is then applied, which at resonant frequencies, typically in the radio frequency range, can reverse the effect of the static field. If changes in reaction products are observed at the appropriate frequency, it is likely that a radical pair is present.

In addition, Dr Timmel's group has also been working on more sensitive methods of detection of magnetic field effects, based on changes in flavin fluorescence induced by photoexcitation⁷⁴. This approach, based on a newly developed spectrometer, is able to discern even small changes in fluorescence, and is significantly more sensitive than traditional absorbance-based methods.

⁷² Maeda K et al. [Chemical compass model of avian magnetoreception](#). *Nature*. 2008;453(7193):387–90.

⁷³ Henbest KB et al. [Radio frequency magnetic field effects on a radical recombination reaction: a diagnostic test for the radical pair mechanism](#). *J Am Chem Soc*. 2004;126(26):8102–3.

⁷⁴ Neil SR et al. [Broadband cavity-enhanced detection of magnetic field effects in chemical models of a cryptochrome magnetoreceptor](#). *J Phys Chem B*. 2014;118(15):4177–84.