Light-dependent magnetoreception in birds: analysis of the behaviour under red light after pre-exposure to red light

Wolfgang Wiltschko*, Andrea Möller, Marcus Gesson, Catrin Noll and Roswitha Wiltschko

*Fachbereich Biologie und Informatik, Zoologie, J. W. Goethe-Universität Frankfurt, Siesmayerstrasse 70, D-60054
Frankfurt am Main, Germany*

*Author for correspondence (e-mail: wiltschko@zoology.uni-frankfurt.de)

Summary

In previous experiments, migratory birds had been disoriented under 635 nm red light, apparently unable to use their magnetic compass. The present study with European robins, *Erithacus rubecula*, confirms these findings for red light at the levels of 6×10^{15} quanta s^{-1} m^{-2} and 43×10^{15} quanta s^{-1} m^{-2}, suggesting that the disorientation under red light was not caused by the test light being below the threshold for magnetoreception. However, pre-exposure to red light for 1 h immediately before the critical tests under red light of $6–7 \times 10^{15}$ quanta s^{-1} m^{-2} enabled robins to orient in their seasonally appropriate migratory direction in spring as well as in autumn. Pre-exposure to darkness, by contrast, failed to induce orientation under red light. Under green light of 7×10^{15} quanta s^{-1} m^{-2}, the birds were oriented in their migratory orientation after both types of pre-exposure. These findings suggest that the newly gained ability to orient under red light might be based on learning to interpret a novel pattern of activation of the magnetoreceptors and hence may represent a parallel to the previously described enlargement of the functional window to new magnetic intensities. Mechanisms involving two types of spectral mechanisms with different absorbance maxima and their possible interactions are discussed.

Key words: migratory orientation, magnetoreception, magnetic compass, wavelength dependency, photopigment, *Erithacus rubecula*.

Introduction

The use of a magnetic compass by European robins, *Erithacus rubecula*, was first demonstrated more than 30 years ago; meanwhile, many other animals are known to obtain directional information from the geomagnetic field (R. Wiltschko and Wiltschko, 1995). The biophysical mechanisms underlying magnetoreception are still largely unclear, however. The radical pair model proposed by Schulten and Windemuth (1986) and Ritz et al. (2000) assumes that the direction of the geomagnetic field is perceived in the eye with the help of specialised photopigments. They are raised to excited singlet states by photon absorption and generate radical pairs, which, by hyperfine interactions, may be converted into triplet pairs (see Ritz et al., 2000 for details). Since the triplet yield depends on the alignment of the respective macromolecules with the axis of the magnetic field lines, comparing the triplet yield in the various spatial directions on the hemispherically shaped retina may be the first step leading to processes obtaining directional information from the magnetic field.

Because the initial step of the proposed mechanism is photon absorption, magnetoreception would be light dependent. Results of behavioural experiments with homing pigeons and caged migratory birds support the model by indicating that magnetoreception indeed occurs in the eye (W. Wiltschko et al., 2002a) and that light from a certain wavelength range is required for magnetic compass orientation. Migratory birds were well oriented in their migratory direction in the presence of light from the blue and green part of the spectrum up to 565 nm, whereas they were disoriented under monochromatic 590 nm yellow or 635 nm red light (see W. Wiltschko and Wiltschko, 2002 for a review). These experiments used LEDs (light-emitting diodes) with a range of half bandwidths of ~30 nm to produce the test light; a recent study using even narrower bandwidths of ~10 nm produced by interference filters reported disorientation already at 568 nm (Muheim et al., 2002). The wavelength range of vision in birds extends up to ~680 nm (Maier, 1992); the range allowing magnetic compass orientation thus seemed to be markedly shorter, with the long-wavelength part of the visual spectrum not being able to initiate the processes leading to magnetoreception.

This seemed strange, because neurophysiological recordings from the nucleus of the basal optic root (nBOR) in pigeons had identified direction-selective cells that responded to magnetic stimuli under long-wavelength light. Some cells showed peak

responses at 580 nm and continued to respond at a fairly high level up to 674 nm (Semm and Demaine, 1986), i.e. at wavelengths that appeared to be beyond the birds' range for compass orientation. This discrepancy between electrophysiological and behavioural data on the wavelength dependency of magnetoreception in birds caused us to analyse further the behaviour under red light. If neurons responding under red light carried information about the magnetic field, one might expect that birds were, in principle, able to make use of this information.

An earlier study had shown that exposing robins to magnetic intensities outside the normal functional window of the magnetic compass enabled these birds to orient at the respective intensities (W. Wiltschko, 1978). By analogy to these experiments, we pre-exposed European robins to red light before testing them under the same red light, and when they were found to be oriented (Möller et al., 2001) we began to analyse this orientation by varying the pre-exposure conditions and comparing the response under red light to that under green light.

Materials and methods

The experiments described here were performed in Frankfurt a.M. during three migratory seasons, in pre-spring from 11 January to 13 February 1999 and 10 January to 9 February 2000 and in autumn from 2 to 27 October 2000.

Test birds

Our test birds were European robins (*Erithacus rubecula*, Turdidae), small passerines that migrate at night. All birds were young ones in their first year of life that had been mistnetted during autumn migration in September and early October 1998, 1999 and 2000 in the Botanical Garden near the Zoological Institute in Frankfurt a.M. (50°08′ N, 8°40′ E). They were housed indoors in individual cages under a photoperiod that simulated the natural one, gradually decreasing from 13 h:11 h L:D in early September until 8 h:16 h L:D was reached in December. The autumn tests in 2000 with 16 birds were carried out while the birds stayed under this photoperiodic regime.

For the spring experiments, we increased the photoperiod after New Year to 13 h:11 h L:D in order to induce premature spring migratory activity so that the tests could start in the first half of January. After the tests were completed, the robins remained in captivity until the last week of March when the natural photoperiod outside had reached 13 h:11 h L:D; then they were released.

Test conditions

All tests took place in wooden houses in the garden of the Zoological Institute in the local geomagnetic field (46 000 nT, 66° inclination). The test lights were monochromatic red and green lights produced by LEDs. In 1999, we used the same red LEDs as in previous studies (e.g. Wiltschko et al., 1993; W. Wiltschko and Wiltschko, 1995), with a peak wavelength of

635 nm, with $\lambda/2$ at 617 nm and 657 nm. In 2000, another type of LED with a peak wavelength of 645 nm and $\lambda/2$ at 625 nm and 666 nm was used. Tests in 1999 under both types of red light had shown that the birds' behaviour did not differ in any way (see R and R_2 in Table 1, upper section). The green LEDs were the same as those used in previous experiments (e.g. W. Wiltschko and Wiltschko, 1995, 1999), with a peak wavelength of 565 nm and $\lambda/2$ at 553 nm and 583 nm. The intensity of the red and green test light was adjusted to be at an equal quantal flux of $6–7\times10^{15}$ quanta s^{-1} m^{-2} (test conditions R, G), an intensity where birds in earlier tests had shown excellent orientation under green and blue light (see W. Wiltschko and Wiltschko, 2002). This corresponded to 2.0 mW m^{-2} and 1.8 mW m^{-2} for red light and 2.4 mW m^{-2} and 2.1 mW m^{-2} for green light (in 1999 and 2000, respectively). In pre-spring 1999, the birds were also tested under bright red light with a higher intensity of 43×10^{15} quanta s^{-1} m^{-2} (condition RX; 13.0 mW m^{-2}). Control tests (C) took place under 'white' light produced by an incandescent light bulb with an intensity of approximately 24 mW m^{-2}.

For the tests in the above-mentioned C, R, RX and G conditions, the birds were caught in their housing cages shortly before the 'white' room light went off and were put immediately into the test cages. Birds that were to be tested after pre-exposure to red light were moved for the period of these tests into a smaller room that was lit during daytime by fluorescent lamps. Roughly 1 h before this white light went off, a red light with a peak wavelength of 635 nm and an intensity of 3.1 lux (~15 mW m^{-2}), produced by brilliant red LEDs, was added to the white room light; after the white light went off, the birds were exposed to the red light alone for approximately 1 h before their tests under red (conditions RpeR and RpeRX) or green (condition RpeG) light began. During spring 2000, we also tested birds after pre-exposure to total darkness for 1 h. These birds stayed in their housing cages after the room lights went off; approximately 1 h later, they were caught in darkness, put into the test cages and tested under red or green light (conditions DpeR and DpeG, respectively).

Test apparatus and performance

Orientation behaviour was recorded in funnel cages (Emlen and Emlen, 1966) lined with typewriter correction paper (BIC, Germany; formerly Tipp-Ex), where the birds were tested one at a time (see W. Wiltschko and Wiltschko, 1995, 2001). Each funnel cage was placed in an aluminium cylinder, the top of which consisted of the plastic disk carrying the LEDs. The light passed through at least two sets of diffusers before it reached the bird. The light intensity in the test cages was measured as irradiance using Optometer P9710-1 (Gigahertz-Optik, Puchheim, Germany) with the radiometric probe 'Visible' RW-3703-2, a silicium photoelement for the wavelength range 400–800 nm.

Recording in conditions C, R, RX and G began in the evening at about the time when the light went off in the housing cages; those in conditions with pre-exposure to red light or

Table 1. *Orientation behaviour of European robins under red light*

Test condition	Peak λ (nm)	Intensity (mW m^{-2})	Light condition before testing	N	Grand mean vector α_N	r_N	Significant difference to control
Effect of pre-exposure to red light on the orientation behavior under red light							
C	'White'	24.4	'White' light	12	5°	0.96***	
R	635	2.1	'White' light	11	46°	0.34^{NS}	***
RX	635	13.0	'White' light	11	37°	0.34^{NS}	***
RpeR	635	2.1	635 nm red	11	25°	0.81***	NS
RpeRX	635	13.0	635 nm red	12	32°	0.62**	**
RpeG	565	2.4	635 nm red	12	357°	0.56*	**
R$_2$	645	2.1	'White' light	11	154°	0.33^{NS}	**
Comparing the aftereffect of exposure to red light with that of exposure to darkness							
C	'White'	24.4	'White' light	12	15°	0.81***	
R	645	1.8	'White' light	12	278°	0.30^{NS}	**
G	565	2.1	'White' light	11	6°	0.94***	NS
RpeR	645	1.8	635 nm Red	11	358°	0.79***	NS
RpeG	565	2.1	635 nm Red	11	357°	0.49^{NS}	NS
DpeR	645	1.8	Total darkness	12	322°	0.24^{NS}	*
DpeG	565	2.1	Total darkness	11	17°	0.88***	NS
Seasonal change in heading between spring and autumn migration							
C	'White'	24.4	'White' light	16	201°	0.64***	
G	565	2.1	'White' light	16	200°	0.68***	NS
RepR	645	1.8	635 nm red	15	181°	0.62**	NS

Peak λ, peak wavelength of LED spectrum; N, number of birds tested; α_N, direction of the grand mean vector; r_N, length of the grand mean vector; asterisks at r_N indicate significant directional preference by the Rayleigh test; the asterisks in the Significance column indicate significance of the difference to the control tests under 'white' light. Significance levels: *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant ($P>0.05$).

darkness began ~1 h later (see above). The tests lasted ~75 min. When active, the birds left scratch marks on the coating of the inclined walls that documented the distribution of their activity. The birds were tested three times in each condition involving red or green light; in the control condition, they were tested up to five times.

Data analysis

After removal from the cage, the coated paper was divided into 24 sectors, and the scratch marks in each sector were counted. Recordings with a total of <35 scratches were excluded from the analysis because of too little activity (see W. Wiltschko and Wiltschko, 1995 for details).

From the distribution of the activity within the cage, the heading and the concentration of the respective test were calculated. From the headings of a bird under each condition, we calculated the mean vector of that bird, with the direction α_b and the length r_b. The α_b of the 12 or 16 birds tested were comprised in the grand mean vector for each condition, with the direction α_N and the length r_N. The grand mean vectors were tested by the Rayleigh test for significant directional preferences. The orientation in the various conditions was tested by the non-parametric Mardia Watson Wheeler test for differences in distribution (Batschelet, 1981) and by the Mann–Whitney *U*-test applied to the differences of the birds'

mean headings from the grand mean to test for differences in variance.

Results

Table 1 gives the grand mean vectors numerically for all test conditions and indicates differences to the control tests under 'white' light, where the birds always showed significant orientation in their seasonally appropriate migratory direction. Tables A1–A3 list the mean vectors of the individual birds.

Effect of pre-exposure to red light on the orientation response under red light

The tests in pre-spring 1999 focussed on the question of whether orientation under red light could be induced either by increasing the intensity of the red test light sixfold or by pre-exposure to red light.

The mean headings of the test birds and the grand mean vectors are given in Fig. 1. Under 635 nm red light of both light levels tested, the birds were disoriented. After pre-exposure to red light, however, robins were significantly oriented in the migratory direction under red light of both light levels – pre-exposure to long wavelengths enables robins to obtain directional information under these long wavelengths (see Möller et al., 2001). The orientation under red light was

Fig. 1. Orientation behaviour of European robins under red light in spring 1999. The test conditions are indicated within the circular diagrams: C, control tests under 'white' light; R, red light with a low intensity of 7×10^{15} quanta s^{-1} m^{-2}; RX, red light with a higher intensity of 43×10^{15} quanta s^{-1} m^{-2}; RpeR, tests under low-intensity red light after the test birds had been pre-exposed to red light; RpeRX, corresponding tests under higher intensity red light after pre-exposure to red light. The triangles at the periphery of the circles mark the mean headings of individual birds; the arrow represents the grand mean vector, with its length proportional to the radius of the circle=1. The two inner circles are the 5% (broken) and 1% significance borders of the Rayleigh test.
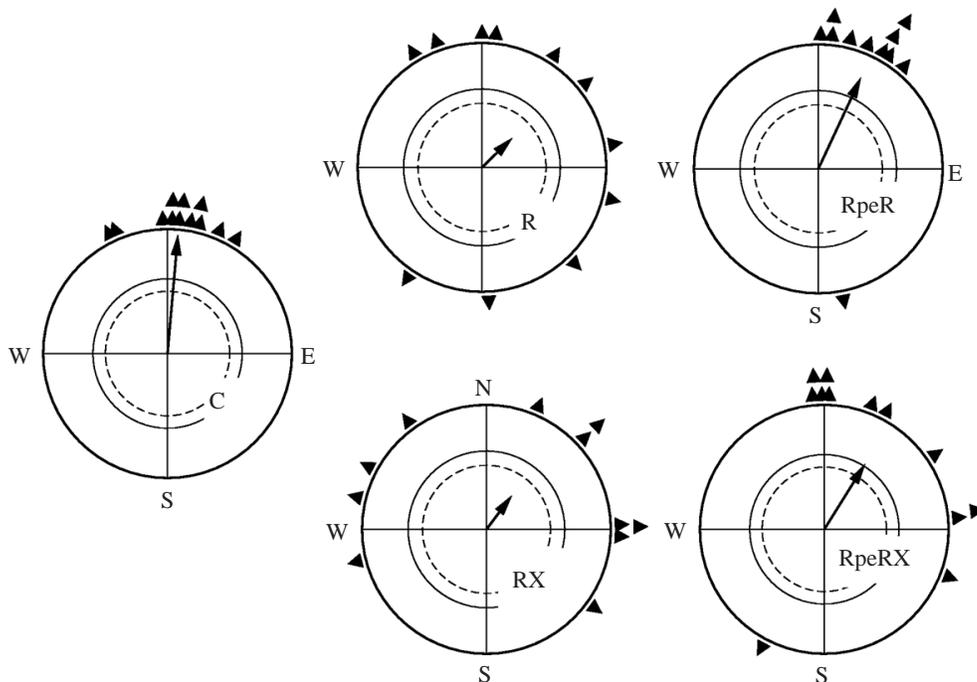
indistinguishable from the behaviour under control conditions; under bright red light, the birds showed a significant increase in scatter compared with the control, but their directional preference was still significant.

Additionally, birds that had been pre-exposed to red light were tested under green light (see Table 1, upper section, RpeG); these data are included in Fig. 2 as open symbols.
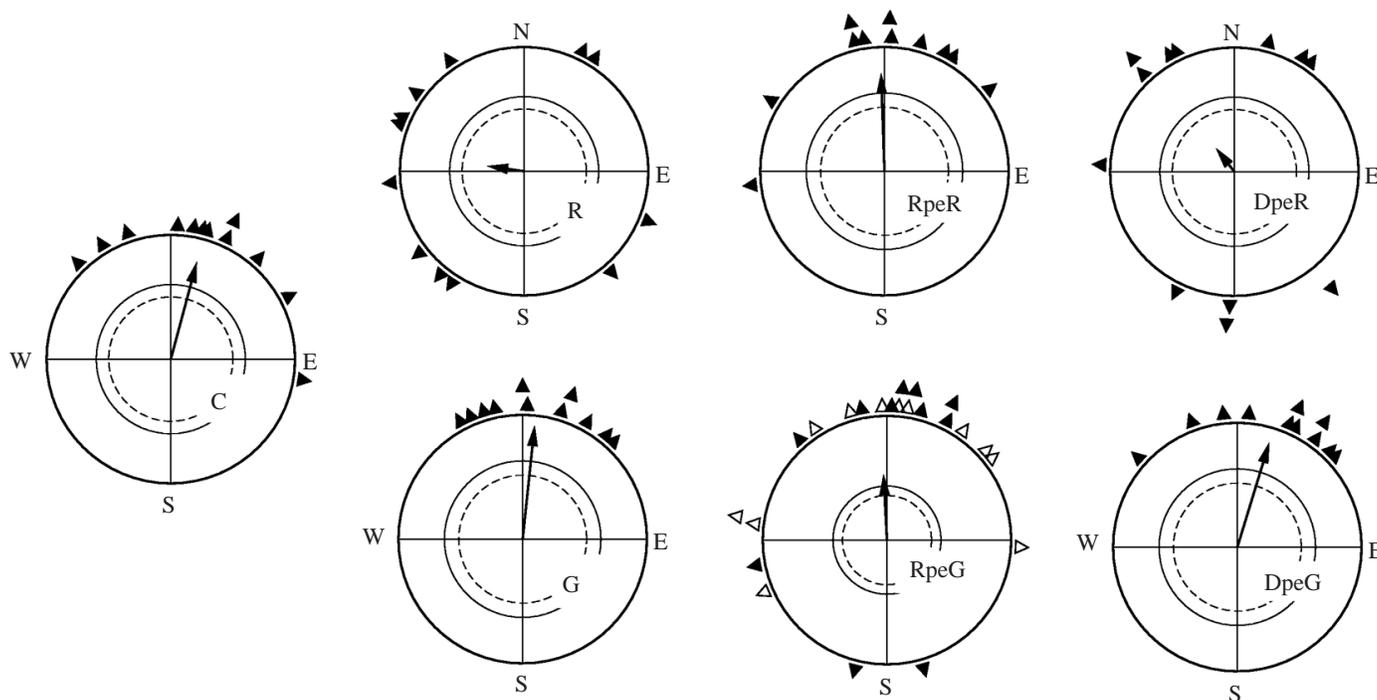

Fig. 2. Orientation behaviour of robins pre-exposed to red light and to total darkness tested under red and green light in spring 2000. C, control tests under 'white' light. Upper row: tests under low-intensity red light; R, normal tests; RpeR, after pre-exposure to red light; DpeR, after pre-exposure to total darkness. Lower row: tests under low-intensity green light; G, normal tests; RpeG, after pre-exposure to red light, with open symbols indicating data from 1999; DpeG, after pre-exposure to total darkness. Symbols as in Fig. 1.
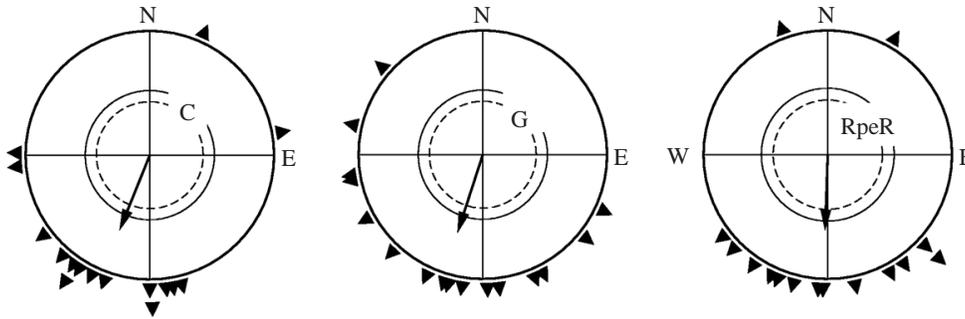
Fig. 3. Orientation behaviour of robins in autumn. C, control experiments under 'white' light; G, normal tests under green light; RpeR, birds pre-exposed to red light tested under red light. Symbols as in Fig. 1.

Comparing pre-exposure to red light with pre-exposure to total darkness

The tests in pre-spring 2000 focussed on the processes by which pre-exposure to red light induced the orientation under red light. Did pre-exposure to red light stimulate the receptors underlying the orientation under red light or not? If not, pre-exposure to red light should be equivalent to total darkness for these receptors that, as a result, might become more sensitive, enabling them to extract enough information from the short-wavelength end of the red LED spectrum to indicate directions. To check this possibility, we compared the effect of pre-exposure to red light with that of pre-exposure to total darkness. Additionally, we tested the birds under 565 nm green light to see whether the two types of pre-exposure also affected the behaviour at other wavelengths.

The results are given in Fig. 2. Under red light, the birds were once more disoriented, while under green light they were excellently oriented in the northerly migratory direction. Pre-exposure to red light led to oriented behaviour under red light as before, whereas a similar exposure to total darkness failed to induce an oriented response, with the behaviour not different from that under normal red light ($P>0.05$, Mardia Watson Wheeler test).

The birds were also tested under green light after both types of pre-exposure. Pre-exposure to darkness did not affect the orientation under green light, while pre-exposure to red light did not alter the general nature of the response but appeared to increase the scatter (see Table 1). The pooled data of 1999 and 2000 are significantly oriented (23 birds: $\alpha_N=357°$, $r_N=0.53$, $P<0.01$, Rayleigh test) but also show significantly more variance than the joint control sample ($P<0.01$, Mann–Whitney test).

Seasonal change in headings between spring and autumn

Responses to certain light regimes in Australian silvereyes (*Zosterops l. lateralis*) and European robins had turned out to be fixed responses, not changing between autumn and spring (W. Wiltschko et al., 2000, 2003, 2004). To check whether the induced orientation under red light showed the normal seasonal reversal, we tested birds in autumn, with tests under white and green light serving as controls.

The results are given in Fig. 3. The robins tested under red light after pre-exposure to red light preferred the seasonally appropriate southerly directions, with their behaviour not

different from that under white or green light ($P>0.05$, Mardia Watson Wheeler test).

Discussion

Earlier studies (e.g. W. Wiltschko and Wiltschko, 1995, 1999, 2001) seemed to suggest that the ability of robins to derive directional information from the magnetic field is generally restricted to the presence of light from the blue–green part of the spectrum. However, birds that had been previously exposed to red light are able to orient in migratory direction under light of longer wavelengths (Möller et al., 2001). This clearly shows that under red light with a peak wavelength of 635 nm or 645 nm, birds can, in principle, detect magnetic directions. Thus, the first finding indicating light-dependent magnetoreception in birds (W. Wiltschko et al., 1993) is no longer generally true (see also Muheim et al., 2002). Meanwhile, however, several other findings documenting a dependency of magnetic orientation on the ambient light regime have been described (e.g. W. Wiltschko et al., 2000, 2003; W. Wiltschko and Wiltschko, 2001) so that this mechanism of magnetoreception appears well established. The question arises, however, of how birds detect their migratory direction under long-wavelength light – does pre-exposure to red light affect the light-dependent magnetoreception system, initiating an ability to derive magnetic compass information from wavelengths beyond 580 nm, or is an entirely different mechanism involved?

Here, magnetite-based magnetoreception comes to mind. Crystals of magnetite have been found in numerous animals (see Kirschvink et al., 1985; R. Wiltschko and Wiltschko, 1995). In birds, they are located in the ethmoid region and in the upper beak (e.g. Beason and Brennan, 1986; Williams and Wild, 2001; Fleissner et al., 2003). However, behavioural data (Beason and Semm, 1996; Munro et al., 1997) as well as electrophysiological recordings (Semm and Beason, 1990) indicate that magnetite-based receptors do not provide birds with directions but with a different type of information: they appear to detect magnetic intensity used as a component of the navigational 'map'. Attributing the compass orientation observed in our experiments to magnetite would thus be at variance with these findings. Electrophysiological responses of neurons in the nBOR to changes in magnetic north in the presence of red light beyond 600 nm (Semm and Demaine,

1986), on the other hand, show that the light-dependent system is also active in the long-wavelength range. In view of this, our present findings strongly suggest that red light with a peak wavelength of 635 nm or 645 nm can, in principle, mediate the detection of magnetic directions.

Change in receptors or different receptors?

The observation that an increase in intensity to a sixfold level did not lead to orientation indicates that the reason for the disorientation normally observed under red light is not the light intensity being below threshold. The ability to orient under red light obviously depends on previous exposure to the same or similar wavelengths. The fact that exposure to total darkness failed to elicit oriented responses clearly shows that the birds' ability to extract information from the magnetic field under long-wavelength light is not based on the receptors becoming more sensitive when not stimulated. It is the pre-exposure to red light that affects the receptive system in some way that leads to the detection of magnetic direction under conditions where it is normally not possible.

One possibility is that red light changes the absorption spectrum of the receptor(s), causing a shift towards longer wavelengths by activating a second absorption peak in the long wavelength range, a phenomenon reported for certain pigments in plants. Photopigments with two absorption peaks have also been described in the parietal eye of lizards (Solessio and Engbretson, 1993) and are considered for salamanders by Phillips and Deutschlander (1997). The other possibility is that a second receptor with a peak at longer wavelengths is involved and provides the information for the newly gained orientation ability.

Electrophysiological recordings from the nBOR have indicated two types of neurons responding to changes in magnetic directions, one with a peak absorbance near 503 nm and the other with a peak absorbance near 582 nm (Semm and Demaine, 1986). This implies two different receptors as the origin of the information transmitted by these neurons. Also, the very abrupt transition from oriented behaviour under green light to disoriented behaviour under yellow light (Wiltschko and Wiltschko, 1999; Muheim et al., 2002), as well as the unexpected responses of birds to a combination of yellow and short-wavelength light (W. Wiltschko et al., 2004), can hardly be explained by one receptor alone. Hence, the assumption of a second type of receptor activated by longer wavelength light appears more likely.

Red light produces a different response pattern?

Why would birds be able to use information provided by this long-wavelength receptor only after they had experienced red light? The analysis of the avian magnetic compass revealed what appears to be a similar phenomenon with respect to magnetic intensities (W. Wiltschko, 1978): magnetic compass orientation was found to be narrowly tuned to the intensity of the ambient magnetic field, with an increase or decrease of only ~25% leading to disorientation; exposure to fields outside this range, however, enabled birds to orient under higher or lower intensities – obviously, birds could now interpret previously

unreadable magnetic fields. Interestingly, this newly gained ability seems to be limited to intensities that the birds had directly experienced: robins normally living at 46 000 nT and now exposed to 150 000 nT were able to orient at 46 000 nT and 150 000 nT but not at the intermediate intensity of 81 000 nT (W. Wiltschko, 1978).

The radical pair model of magnetoreception (Ritz et al., 2000) provides an explanation for this phenomenon: the processes mediating magnetoreception would result in specific patterns of activation across the retina, which are centrally symmetric to the axis of the magnetic field lines. Their size and pattern would vary with changing magnetic intensities. Hence, an abrupt increase or decrease in intensity would suddenly confront birds with a novel pattern, which might confuse them at first, resulting in disorientation. However, because the altered pattern would retain the central symmetry with respect to the axis of the field lines, the birds could learn to interpret the novel pattern and thus regain their ability to detect magnetic directions. Interpreting the induced ability to orient under red light as an analogous case would mean that red light alone causes a pattern of response on the retina that differs markedly from the one produced by white light or by light from the blue–green part of the spectrum. Yet this pattern, too, would necessarily be centrally symmetric to the axis of the field lines, and this might enable birds to learn to derive directional information from it.

The interpretation that the induced ability to orient under red light is caused by the birds becoming able to interpret a novel response pattern raises the question of why the pattern produced by red light alone should be initially unreadable. It implies that it must somehow differ from the patterns produced by the blue–green part of the spectrum or the combined pattern of both types of receptors under white light. This means that in the combined pattern, the part produced by blue–green light would dominate, as indicated by the birds' ability to orient at once when tested under monochromatic blue, turquoise and green light (e.g. W. Wiltschko et al., 1993; W. Wiltschko and Wiltschko, 1999, 2001). Red light would seem to produce the minor, complementary component of the joint pattern. Both patterns seem to act in a functionally synergistic way, providing birds with the same type of information. The abrupt change from orientation to disorientation around 570 nm (Wiltschko and Wiltschko, 1999; Muheim at al., 2002), on the other hand, appears to suggest an antagonistic interaction.

A spectral system as described for salamanders?

For amphibians, the other vertebrate group with a light-dependent magnetic compass, antagonistic interactions between two spectral components located either in the same or in two different receptors have been proposed (Phillips and Deutschlander, 1997; Deutschlander et al., 1999a). The available data suggest parallels, but also interesting differences, between the magnetic compass mechanisms of amphibians and birds.

In salamanders heading shoreward, the spectral range where monochromatic light produces the same responses as white

Table A1. *Tests in spring 1999*

Bird		C			R			RpeR			RX			RpeRX			RpeG			R₂	
	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b
98-1	4	10°	0.83	3	104°	0.38	2	31°	0.99	3	296°	0.74	3	1°	0.84	3	48°	0.88	3	45°	0.89
98-2	4	14°	0.82	3	177°	0.86	2	6°	0.99	3	89°	0.85	2	83°	0.71	3	344°	0.96	3	200°	0.46
98-3	5	358°	0.65	3	33°	0.34	3	29°	0.94	3	47°	0.87	3	2°	0.70	3	327°	0.64	3	19°	0.66
98-4	5	5°	0.90	3	136°	0.58	3	14°	1.00	3	47°	0.66	3	359°	0.95	3	35°	0.97	3	23°	0.94
98-5	5	337°	0.53	3	360°	0.34	3	30°	0.76	3	93°	0.95	3	355°	0.88	3	279°	0.90	3	186°	0.15
98-6	5	13°	0.85	3	51°	0.37	3	169°	0.97	3	324°	0.53	3	356°	0.78	3	358°	0.65	3	82°	0.78
98-7	5	334°	0.57	3	329°	0.65	3	40°	0.55	3	256°	0.48	3	28°	0.33	3	9°	0.60	3	255°	0.57
98-8	5	23°	0.75	3	340°	0.78	3	22°	0.61	3	284°	0.89	4	111°	0.33	3	277°	0.15	3	181°	0.33
98-9	5	2°	0.99	3	80°	0.95	3	32°	0.21	3	88°	0.13	4	21°	0.88	3	52°	0.41	3	155°	0.40
98-10	4	31°	0.57	3	6°	0.28	3	1°	0.87	3	23°	0.17	3	56°	0.95	3	5°	0.91	3	153°	0.98
98-11	5	2°	0.91	3	214°	0.72	3	6°	0.81	3	126°	0.47	3	85°	0.61	3	247°	0.33	3	198°	1.00
98-12	2	6°	0.92										1	208°	(1.00)	1	93°	(1.00)			
N=11 or 12		5,	0.83		46,	0.58		25,	0.87		37,	0.66		32,	0.78		357,	0.65		154,	0.66
		0.96***			0.34ᴺˢ			0.81***			0.34ᴺˢ			0.62**			0.56*			0.33ᴺˢ	

Test conditions: C, 'white' light, control; R, 635 nm red light (2.1 mW m⁻²); RpeR, red light after 1 h pre-exposure to red light; RX, 635 nm red light (13.0 mW m⁻²); RpeRX, bright red light after 1 h pre-exposure to red light; RpeG, 565 nm green light (2.4 mW m⁻²) after 1 h pre-exposure to red light; R₂, 645 nm red light (2.1 mW m⁻²).

Table A2. *Tests in spring 2000*

Bird		C			R			RpeR			DpeR			G			RpeG			DpeG	
	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b
99-1	3	14°	0.97	3	32°	0.88	3	3°	0.89	3	140°	0.47	3	348°	0.95	3	319°	0.97	3	35°	0.95
99-2	3	316°	0.98	3	232°	0.50	3	302°	0.73	3	182°	0.39	3	19°	0.96	3	194°	0.30	3	340°	0.35
99-3	3	341°	0.98	3	112°	0.99	3	347°	0.94	3	273°	0.24	3	29°	0.86	3	26°	0.68	3	46°	0.40
99-4	3	3°	0.39	3	213°	0.66	3	27°	0.14	3	206°	0.89	3	2°	0.66	3	259°	0.81	3	354°	0.66
99-5	3	41°	0.97	3	291°	0.43	3	31°	0.83	3	15°	0.51	3	337°	0.96	3	11°	0.96	3	26°	0.47
99-6	3	10°	0.99	3	26°	0.25	3	351°	0.79	3	183°	0.11	3	17°	0.98	3	349°	0.89	3	44°	0.50
99-7	3	25°	0.85	3	306°	0.57	3	347°	0.89	3	318°	0.29	3	332°	0.98	3	27°	0.66	3	5°	0.87
99-8	3	25°	0.98	3	139°	0.93	3	2°	0.85	3	331°	0.33	3	39°	0.70	3	2°	0.88	3	312°	0.63
99-9	3	329°	0.91	3	294°	0.51	3	53°	0.49	3	334°	0.55	3	360°	0.89	3	164°	0.47	3	24°	0.89
99-10	3	16°	1.00	3	265°	0.40	3	264°	0.65	3	35°	0.37	3	43°	0.38	3	15°	0.68	3	47°	0.54
99-11	3	62°	0.64	3	219°	0.61	3	16°	0.94	3	31°	0.44	3	343°	0.95	3	6°	0.87	3	23°	0.94
99-12	2	99°	0.33	3	326°	0.52				2	317°	0.94									
N=11 or 12		15,	0.97		278,	0.55		358,	0.83		322,	0.42		6,	0.95		357,	0.81		17,	0.63
		0.81***			0.30ᴺˢ			0.79***			0.24ᴺˢ			0.92***			0.49ᴺˢ			0.88***	

C, 'white' light, control; R, 645 nm red light (1.8 mW m⁻²); RpeR, red light after 1 h pre-exposure to red light; DpeR, red light after 1 h pre-exposure to darkness; G, 565 nm green light (2.1 mW m⁻²); RpeG, green light after 1 h pre-exposure to red light; DpeG, green light after 1 h pre-exposure to darkness.

Table A3. *Tests in autumn 2000*

Bird	C			G			RpeR		
	n	α_b	r_b	n	α_b	r_b	n	α_b	r_b
00-1	4	221°	0.66	3	224°	0.88	3	182°	0.13
00-2	4	214°	0.36	3	241°	0.68	3	202°	0.43
00-3	4	212°	0.59	3	259°	0.67	3	146°	0.49
00-4	4	265°	0.44	3	157°	0.54	3	341°	0.36
00-5	4	200°	0.93	3	153°	0.75	3	184°	0.36
00-6	1	215°	(1.00)	3	198°	0.73	3	205°	0.35
00-7	3	180°	0.36	3	189°	0.27	3	152°	0.96
00-8	4	164°	0.66	3	210°	0.83	3	133°	0.97
00-9	4	179°	0.48	3	195°	0.83	3	30°	0.17
00-10	2	80°	0.74	3	283°	0.63	3	225°	0.39
00-11	4	206°	0.37	3	115°	0.50	3	195°	0.73
00-12	4	271°	0.10	3	178°	0.73	3	232°	0.36
00-13	4	233°	0.52	3	212°	0.28	3	200°	0.78
00-14	4	166°	0.85	3	312°	0.43	3	133°	0.67
00-15	4	25°	0.21	3	173°	0.63	3	168°	0.96
00-16	4	170°	0.89	3	153°	0.99			
N=15 or 16		201, 0.64***	0.52		200, 0.68***	0.68		181, 0.62***	0.43

C, 'white' light, control; G, 565 nm green light (2.1 mW m^{-2}); RpeR, 645 nm red light (1.8 mW m^{-2}) after 1 h pre-exposure to red light.

Appendix. Vectors of individual European robins under the various test conditions

In Tables A1–A3, n is the number of evaluable recordings and α_b and r_b are the direction and length of the mean vector, respectively. The summary line gives, under α_b, the grand mean vector and, under r_b, the median vector length of the respective test condition. In the few cases where a bird produced only one recording, the vector length 1.00 is given in parentheses and is not considered for the median. Significance levels compared with control: *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant ($P>0.05$).

light is considerably narrower than in birds, ending at ~450 nm (Phillips and Borland, 1992), in contrast to 565 nm in birds. The most important difference between the two groups, however, concerns the behaviour under long-wavelength light: from 500 nm onwards, the headings preferred by the salamanders shifted by ~90° counterclockwise with respect to those recorded under white light (Phillips and Borland, 1992; Deutschlander et al., 1999b). Salamanders that were kept under long wavelengths and had a chance to establish the shoreward direction under these light conditions preferred the true shoreward direction under red light but showed the reverse 90° shift when tested under white light. These observations seemed to imply that the directional information perceived under long-wavelength light differed from that under white or blue light. The authors speculate about two antagonistic spectral mechanisms indicating directions perpendicular to each other (see also Phillips and Deutschlander, 1997; Deutschlander et al., 1999a). At 475 nm, where both mechanisms would be equally stimulated, the salamanders were disoriented (Phillips and Borland, 1992). To reconcile this finding with the normal orientation observed under white light, where both mechanisms are likewise activated, Phillips and Deutschlander (1997) postulate that the short-wavelength part of the spectrum produces a stronger stimulus that dominates under full-spectrum light. Still, one would normally argue that a

mechanism producing what would seem false information would be selected against. In view of this, Phillips and colleagues (Philllips and Deutschander, 1997; Deutschlander et al., 1999a) propose that this second mechanism might be an intrinsic component of the magnetoreceptive system and discuss photopigments with two absorption peaks, as described in the pineal of lizards (Solessio and Engbretson, 1993), as possible receptors.

These findings clearly contrast with the disorientation normally observed in birds under yellow and red light at intensities of 6×10^{15} quanta s^{-1} m^{-2} or higher (W. Wiltschko et al., 1993; W. Wiltschko and Wiltschko, 1999; present study). Muheim et al. (2002), testing birds in autumn under red light of only ~3×10^{15} quanta s^{-1} m^{-2}, i.e. about half the intensity of that used in the present study, observed a westerly tendency that was different from the southerly migratory direction and which they interpreted as a shift in direction. Tests in spring, however, showed that this is misleading: the birds also headed west (271°, 0.52, $P<0.05$; W. Wiltschko and R. Wiltschko, unpublished), indicating that the response under dim red light is independent of the migratory direction; rather than a shift in compass direction, it appears to be a 'fixed direction' similar to the response observed, for example, in silvereyes under high-intensity green light (W. Wiltschko et al., 2000, 2003). This argues against a model such as the one proposed for

amphibians. The response of robins to a combination of yellow light with green or blue light (W. Wiltschko et al., 2004) likewise suggests that the interactions between the short-wavelength and the long-wavelength receptor in birds are far more complex than the model for amphibians suggests. The most important difference to salamanders, however, is the nature of the induced response under red light: birds show the same directional tendencies as under white light. The amphibian system of two mechanisms providing what would seem disagreeing information, perpendicular to each other, thus has no parallel in birds. Birds pre-exposed to red light oriented alike under red light and green light, with the induced orientation under red light showing the typical seasonal change, identifying the behaviour as true migratory orientation.

Another difference between amphibians and birds is the site of magnetoreception: in salamanders, magnetic directions are mediated by extraocular photoreceptors in the pineal (Deutschlander et al., 1999b), whereas magnetoreception in birds takes place in the eyes, in particular in the right eye (W. Wiltschko et al., 2002a). In view of this, marked differences in the type of receptors and in the way the receptors are connected with higher order units are not surprising.

A possible role of the minor component

Nevertheless, because birds can spontaneously orient under monochromatic short-wavelength light, but not under red light, we must also conclude that the long-wavelength mechanism provides the minor component of the combined pattern activated by white light. The biological function of this second component is not yet clear, in particular because both components appear to indicate the same directions.

The argument about a possible role of two spectral mechanisms in birds must consider that, while the information provided by both mechanisms is essentially the same, suggesting synergistic interactions, the sharp transition from orientation to disorientation around 570 nm indicates antagonistic interactions. To reconcile these seemingly contradictory findings, we can only speculate. For example, the type of second receptor might limit the area of the activation induced by the magnetic field in order to make the magnetic compass more precise – a possible analogue to lateral inhibition, as it is found to enhance the contrasts in the visual system. The size of a potential pattern of activation by magnetoreception in the eye is not known; the pictures given by Ritz et al. (2000) are purely arbitrary. Behavioural evidence from migrants that were repeatedly tested in cages indicate individual vectors based on 6–8 headings of ≥0.9 (data from Wiltschko et al., 1998, 2002b). Considering the circumstances of the tests, this implies a very high accuracy of the avian magnetic compass. Mechanisms improving the precision of magnetic compass readings thus do not appear unlikely, and the long-wavelength receptors would serve an important function in the magnetoreceptive system of birds.

References

Batschelet, E. (1981). *Circular Statistics in Biology*. London, New York: Academic Press.

Beason, R. C. and Brennan, W. J. (1986). Natural and induced magnetization in the bobolink, *Dolichonyx oryzivorus* (Aves: Icteridae). *J. Exp. Biol.* **125**, 49-56.

Beason, R. C. and Semm, P. (1996). Does the avian ophthalmic nerve carry magnetic navigational information? *J. Exp. Biol.* **199**, 1241-1244.

Deutschlander, M. E., Phillips, J. B. and Borland, S. C. (1999a). The case for light-dependent magnetic orientation in animals. *J. Exp. Biol.* **202**, 891-908.

Deutschlander, M. E., Phillips, J. B. and Borland, S. C. (1999b). Extraocular photoreceptors mediate the light-dependent magnetic compass of an amphibian. *Nature* **400**, 324-325.

Emlen, S. T. and Emlen, J. T. (1966). A technique for recording migratory orientation in captive birds. *Auk* **83**, 361-367.

Fleissner, G., Holtkamp-Rötzler, E., Hanzlik, M., Winklhofer, M., Fleissner, G., Petersen, N. and Wiltschko, W. (2003). Ultrastructural analysis of a putative magnetoreceptor in the beak of pigeons. *J. Comp. Neurol.* **448**, 350-360.

Kirschvink, J. L., Jones, D. S. and MacFadden, J. B., ed. (1985). *Magnetite Biomineralization and Magnetoreception in Organisms*. New York: Plenum Press.

Maier, E. J. (1992). Spectral sensitivities including the ultraviolet of the passeriform bird *Leiothrix lutea*. *J. Comp. Physiol. A* **170**, 709-714.

Möller, A., Gesson, M., Noll, C., Wiltschko, R. and Wiltschko, W. (2001). Light-dependent magnetoreception in migratory birds: previous exposure to red light alters the response to red light. In *Orientation and Navigation – Birds, Humans and other Animals*, pp. 61-66. Oxford: Royal Institute of Navigation.

Muheim, R., Bäckman, J. and Åkesson, S. (2002). Magnetic compass orientation in European robins is dependent on both wavelength and intensity of light. *J. Exp. Biol.* **205**, 3845-3856.

Munro, U., Munro, A. J., Phillips, J. B. and Wiltschko, W. (1997). Effect of wavelength of light and pulse magnetisation on different magnetoreception systems in a migratory bird. *Austr. J. Zool.* **45**, 189-198.

Phillips, J. B. and Borland, C. (1992). Behavioral evidence for use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* **359**, 142-144.

Phillips, J. B. and Deutschlander, M. E. (1997). Magnetoreception in terrestrial vertebrates: implication for possible mechanisms of EMF interaction with biological systems. In *The Melatonin Hypothesis: Electric Power and the Risk of Breast Cancer* (ed. R. G. Stevens, B. W. Wilson and L. E. Andrews), pp. 111-172. Columbus, Ohio: Batelle Press.

Ritz, T., Adem, S. and Schulten, K. (2000). A model for vision-based magnetoreception in birds. *Biophys. J.* **78**, 707-718.

Schulten, K. and Windemuth, A. (1986). Model for a physiological magnetic compass. In *Biophysical Effects of Steady Magnetic Fields* (ed. G. Maret, N. Boccara and J. Kiepenheuer), pp. 99-106. Berlin, Heidelberg, New York: Springer-Verlag.

Semm, P. and Beason, R. C. (1990). Responses to small magnetic variations by the trigeminal system of the bobolink. *Brain Res. Bull.* **25**, 735-740.

Semm, P. and Demaine, C. (1986). Neurophysiological properties of magnetic cells in the pigeon's visual system. *J. Comp. Physiol. A* **159**, 619-625.

Solessio, E. and Engbretson, G. (1993). Antagonistic chromatic mechanisms in photoreceptors of the parietal eye of lizards. *Nature* **364**, 442-445.

Williams, M. N. and Wild, J. M. (2001). Trigeminally innervated iron-containing structures in the beak of homing pigeons. *Brain Res.* **889**, 243-246.

Wiltschko, R. and Wiltschko, W. (1995). *Magnetic Orientation in Animals*. Berlin, Heidelberg, New York: Springer-Verlag.

Wiltschko, W. (1978). *Further analysis of the magnetic compass of migratory birds*. In *Animal Migration, Navigation and Homing* (ed. K. Schmidt-Koenig and W. T. Keeton), pp. 302-310. Berlin, Heidelberg, New York: Springer-Verlag.

Wiltschko, W. and Wiltschko, R. (1995). Migratory orientation of European robins is affected by the wavelength of light as well as by a magnetic pulse. *J. Comp. Physiol. A* **177**, 363-369.

Wiltschko, W. and Wiltschko, R. (1999). The effect of yellow and blue light on magnetic compass orientation in European robins, *Erithacus rubecula*. *J. Comp. Physiol. A* **184**, 295-299.

Wiltschko, W. and Wiltschko, R. (2001). Light-dependent magnetoreception in birds: the behavior of European robins, *Erithacus rubecula*, under monochromatic light of various wavelengths and intensities. *J. Exp. Biol.* **204**, 3295-3302.

Wiltschko, W. and Wiltschko, R. (2002). Magnetic compass orientation in birds and its physiological basis. *Naturwissenschaften* **89**, 445-452.

Wiltschko, W., Munro, U., Ford, H. and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525-527.

Wiltschko, W., Munro, U., Ford, H. and Wiltschko, R. (1998). Effect of a magnetic pulse on the orientation of silvereyes, *Zosterops l. lateralis*, during spring migration. *J. Exp. Biol.* **201**, 3257-3261.

Wiltschko, W., Wiltschko, R. and Munro, U. (2000). Light-dependent magnetoreception in birds: the effect of intensity of 565-nm green light. *Naturwissenschaften* **87**, 366-369.

Wiltschko, W., Traudt, J., Güntürkün, O., Prior, H. and Wiltschko, R. (2002a). Lateralization of magnetic compass orientation in a migratory bird. *Nature* **419**, 467-470.

Wiltschko, W., Munro, U., Wiltschko, W. and Kirschvink, J. L. (2002b). Magnetite-based magnetoreception in birds: the effect of a biasing field and a pulse on migratory behavior. *J. Exp. Biol.* **205**, 3031-3037.

Wiltschko, W., Munro, U., Ford, H. and Wiltschko, R. (2003). Magnetic orientation in birds: non-compass responses under monochromatic light of increased intensity. *Proc. R. Soc. Lond. B* **270**, 2133-2140.

Wiltschko, W., Gesson, M., Stapput, K. and Wiltschko, R. (2004). Light-dependent magnetoreception in birds: interaction of at least two different receptors. *Naturwissenschaften* doi: 10.1007/s0014-003-0500-x