Magnetoreception in birds: different physical processes for two types of directional responses

Roswitha Wiltschko,[1] Katrin Stapput,[1] Thorsten Ritz,[2] Peter Thalau,[1] and Wolfgang Wiltschko[1]

[1]Fachbereich Biowissenschaften, J. W. Goethe-Universität Frankfurt, Siesmayerstrasse 70, D-60054 Frankfurt a.M, Germany
[2]Department of Physics and Astronomy, University of California, Irvine, California 92697-4575

Migratory orientation in birds involves an inclination compass based on radical-pair processes. Under certain light regimes, however, "fixed-direction" responses are observed that do not undergo the seasonal change between spring and autumn typical for migratory orientation. To identify the underlying transduction mechanisms, we analyzed a fixed-direction response under a combination of 502 nm turquoise and 590 nm yellow light, with migratory orientation under 565 nm green light serving as the control. High-frequency fields, diagnostic for a radical-pair mechanism, disrupted migratory orientation without affecting fixed-direction responses. Local anaesthesia of the upper beak where magnetite is found in birds, in contrast, disrupted the fixed-direction response without affecting migratory orientation. The two types of responses are thus based on different physical principles, with the compass response based on a radical pair mechanism and the fixed-direction responses probably originating in magnetite-based receptors in the upper beak. Directional input from these receptors seems to affect the behavior only when the regular inclination compass does not work properly. Evolutionary considerations suggest that magnetite-based receptors may represent an ancient mechanism that, in birds, has been replaced by the modern inclination compass based on radical-pair processes now used for directional orientation. [DOI: 10.2976/1.2714294]

CORRESPONDENCE
Roswitha Wiltschko: wiltschko@bio.uni-frankfurt.de

Many animals use information from the geomagnetic field for orientation and navigation (R. Wiltschko and Wiltschko, 1995; W. Wiltschko and Wiltschko, 2005). For the reception mechanisms providing this information, two models are discussed. The "radical-pair model" proposes magnetoreception by light-induced processes in specialized photopigments (Schulten, 1982; Ritz et al., 2000). The geomagnetic field can affect electron-transfer reactions of photopigments with radical-pair intermediates, and the model suggests that this sensitivity provides the basis for the ability of birds to obtain directional information from the geomagnetic field. Magnetoreception would take place in the eye; the effect of magnetic fields would modulate photoreceptor signaling and could manifest itself by forming direction-dependent activation patterns (Ritz et al., 2000; Wang et al., 2006).

The alternative model assumes magnetoreception based on magnetite, a special form of iron oxide that is rather widespread among animals (e.g., Yorke, 1979; Kirschvink and Gould, 1981; Kirschvink et al., 1985; Davila et al., 2003). In birds, remanence measurements indicated single domain magnetite particles in the heads of various species (Walcott et al., 1979; Presti and Pettigrew, 1980; Edwards et al., 1992), with concentrations in the ethmoid region (Beason and Brennan, 1986). Histological studies also showed iron-rich particles in the ethmoid region of passerine birds and pigeons (Beason and Nichols, 1984; Williams and Wild, 2001). Crystallographic methods identified clusters of smaller superparamagnetic particles within specific structures in the skin of

the upper beak of pigeons (Hanzlik *et al.*, 2000; Winklhofer *et al.*, 2001), with subsequent histological studies revealing that they are associated with a series of small iron-based platelets all embedded within sensory nerve terminals of the ophthalmic nerve (Fleissner *et al.*, 2003).

So far, only in the case of the magnetic compass of birds, has the underlying mechanism been identified. Using high-frequency fields in the megahertz range as a diagnostic tool in experiments with migratory birds, the avian inclination compass was identified as based on a radical-pair mechanism (Ritz *et al.*, 2004; Thalau *et al.*, 2006; R. Wiltschko *et al.*, 2005). However, under certain light regimes, an odd type of directional response has been observed (W. Wiltschko *et al.*, 2000, 2003, 2004a; R. Wiltschko *et al.*, 2005): birds headed in "fixed" directions that were different from the normal migratory direction and did not show the seasonal change between spring and autumn. These headings could be shifted by altering magnetic north, i.e., the birds obtained directional information from the magnetic field, but did not use it for seasonally appropriate orientation.

This raised the question about the physical mechanism underlying the "fixed-direction" responses. Information from an iron-based receptor seemed a possibility. Hence we decided to analyze the physical principles the fixed-direction responses in birds are based on, testing, on the one hand, for radical-pair processes and, on the other hand, for an involvement of the known iron-based receptors in their manifestation. The results are compared with corresponding findings on compass responses.

RESULTS
Our test birds were European robins, *Erithacus rubecula* (Turdidae), a night migrating species. Their orientation under low 565 nm green light served as an example of compass responses (W. Wiltschko *et al.*, 2001), with the birds preferring their seasonally appropriate migratory direction, showing southerly headings in autumn and northerly headings in spring (see Fig. 1, upper diagrams). Their orientation under a combination of 502 nm turquoise light and 590 nm yellow light, where the birds showed headings slightly south of east in both seasons, served as an example for a fixed-direction response (Fig. 1, lower diagrams).

To test for a radical-pair mechanism, the birds were subjected to a broadband oscillating field with frequencies from 0.1 to 10 MHz added to the geomagnetic field. Under green light, this had caused disorientation (Ritz *et al.*, 2004). Under turquoise-and-yellow light, in contrast, this high-frequency field did not have a disrupting effect (Fig. 1, center diagrams): the birds continued to head in easterly directions such as in the geomagnetic field alone (see Table I). This shows that the compass response under green light is based on a radical-pair mechanism, whereas the fixed-direction response under turquoise-and-yellow light is not.

To test for a possible involvement of magnetite-based re-

ceptors, we made use of the fact that magnetite had been found in the skin of the upper beak of pigeons (Fleissner *et al.*, 2003). Assuming a similar arrangement of magnetite crystals in passerines, we anesthetized the potential iron-based receptors by gently rubbing a cotton bud soaked with the local anaesthetic Xylocain along the edges of the upper beak. This treatment with the anaesthetic had no affect on the compass orientation under green light. Under turquoise-and-yellow light, however, it caused disorientation (Fig. 1, right diagrams), with the distribution of headings significantly different from that of the same birds when untreated (see Table I). This effect indicates that the orientation observed under turquoise-and-yellow light is most likely based on input originating from the iron-based receptors in the skin of the upper beak (Fleissner *et al.*, 2003).

DISCUSSION
Our findings document the different nature of the two types of directional responses observed in passerine birds: The compass responses are based on a radical-pair mechanism alone, without involving the iron-based receptors in the beak, as indicated by the lack of a detectable effect of the Xylocain treatment on the orientation under green light. The fixed-direction responses, on the other hand, do not involve radical-pair processes, but appear to be controlled by the iron-based receptors, as they break down when these receptors are temporarily deactivated by the local anaesthetic.

Information provided by magnetite-based receptors
The finding that the iron-based receptors provide directional information is rather surprising, because so far, the existing evidence seemed to suggest that these receptors provide information on magnetic intensity only. Electrophysiological recordings from the ophthalmic nerve, a branch of the trigeminal nerve that innervates the beak and ethmoid region, and from the trigeminal ganglion of a passerine species revealed units responding to changes in magnetic intensity. Units of the trigeminal system were activated by changes in intensity also when the direction of the magnetic field was held constant, with some units responding to changes as small as 200 nT (Semm and Beason, 1990). In conditioning tests using a local anomaly with large intensity changes as stimulus, the pigeons' ability to discriminate between two alternatives broke down when the ophthalmic nerve was sectioned (Mora *et al.*, 2004), indicating that this nerve transported information on the stimulus.

Other behavioral experiments aimed at testing for an involvement of iron-based receptors in avian navigation subjected migratory birds and homing pigeons to a brief, strong magnetic pulse that was designed to alter the magnetization of magnetite. It was predicted that strong pulses such as the ones used should change the output of magnetite-based receptors dramatically, even if it was not yet possible to specify
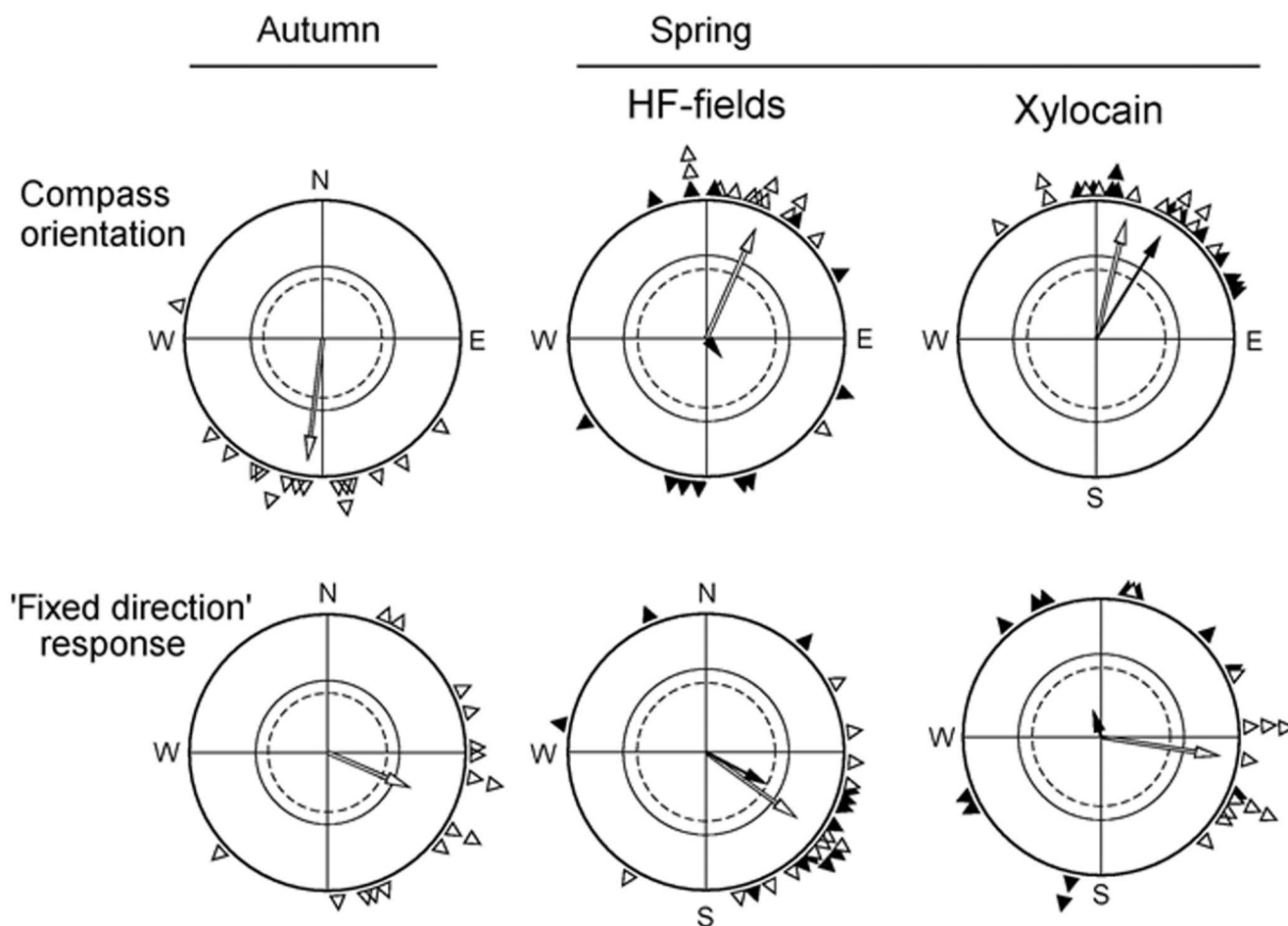
Figure 1. Orientation of European robins, *Erithacus rubecula*, in the local geomagnetic field; effects of a broadband high-frequency field and of Xylocain, a local anaesthetic, applied to the skin of the upper beak. Upper diagrams: compass orientation in the migratory direction under 565 nm green light; lower diagrams: fixed-direction responses under a combination of 502 nm turquoise and 590 nm yellow light. The symbols at the periphery of the circle mark the mean headings of the test birds based on three recordings each. Open symbols: when untreated, solid symbols, with high-frequency fields added or with the local anaesthetic applied, respectively. The arrows represent the corresponding mean vectors, and the two inner circles are the 5% (dotted) and the 1% significance border of the Rayleigh test (Batschelet, 1981).

in detail the nature of the expected effect. Pulse treatment was indeed found to cause marked deflections in the headings of birds (W. Wiltschko *et al.*, 1994, 1998; Beason *et al.*, 1995, 1997), with pulses applied in different directions deflecting the headings to different sides (Beason *et al.*, 1995, 1997). However, the pulse affected only adult, experienced birds, whereas young, inexperienced migrants remained unaffected (Munro *et al.*, 1997). This suggested an effect on an experience-based mechanism and pointed to the "navigational map" used by birds to indicate position. This interpretation is in agreement with the electrophysiological data (Beason and Semm, 1987; Semm and Beason, 1990); changes in magnetic intensity could signal birds whether they are, e.g., north or south of their goal. At the same time, three observations indicate that the pulse did not affect compass orientation: (1) young birds after being subjected to the pulse continued to head in the migratory direction (Munro *et al.*, 1997), (2) the pulse effect could be suppressed by blocking the ophthalmic nerve, with the birds heading in the migratory direction (Beason and Semm, 1996), and (3) recent data document an intact inclination compass after pulsing (W. Wiltschko *et al.*, 2006). These findings argue against an involvement of the magnetite-based receptors in the avian magnetic compass.

Together, the findings mentioned above seemed to suggest that iron-based receptors innervated by the trigeminal system provide information on magnetic intensity as a component of the navigational "map." Our present findings show that these receptors are also the origin of the fixed directions, thus suggesting an additional function that becomes evident only under specific conditions that interfere with the inclination compass based on radical pairs.

Table I. Orientation behavior of European robins in the geomagnetic field: the effect of a broadband high-frequency field of $0.1-10$ MHz and of the local anaesthetic Xylocain applied to the upper bill on compass responses in the migratory direction and on fixed-direction responses

Season	Treatment	Controls without treatment				Experimentals				Difference[a]	
		N	r_b	α_N	r_N	N	r_b	a_N	r_N	Δ	Sign
Compass orientation response under 565 nm green light											
Autumn		16	0.79	190°	0.73***						
Spring	HF field[b]	12	0.93	16°	0.96***	12	0.49	(142°)	0.18[n.s.]	(+126°)	***
Spring	Xylocain	12	0.91	14°	0.88***	12	0.71	32°	0.88***	+18°	n.s.
Fixed-direction response toward east–southeast under bichromatic 502 nm turquoise and 590 nm yellow light											
Autumn		16	0.53	113°	0.64***						
Spring	HF field	12	0.73	126°	0.80***	12	0.89	118°	0.50*	−8°	n.s
Spring	Xylocain	12	0.86	99°	0.86***	12	0.49	(343°)	0.21[n.s.]	(−116°)	**

Note: N, number of birds tested.

r_b, median-vector length of the individual birds (based on three recordings each).

α_N, r_N, grand mean vector based on the mean headings of the 16 or 12 individual birds, with asterisks at r_N indicating significance by the Rayleigh test (Batschelet, 1981) and nonsignificant directions given in parentheses.

[a]The last two columns compare experimental and control birds, with differences involving nonsignificant directions given in parentheses and the last line indicating significance of the difference by the Mardia Watson Wheeler test (Batschelet, 1981).

[b]Data from Ritz et al. (2004) included for comparison.

The phenomenon of fixed-direction responses

The existence of fixed-direction responses is an odd phenomenon. It is unclear why birds appear to be unable to use the directional input from the magnetite-based receptors to locate their migratory direction. This input does not seem to provide a proper compass; instead, it causes birds to head into fixed directions whose significance is unclear. The easterly preference observed here under turquoise-and-yellow light cannot be related to any meaningful task or context. Under light regimes involving intense monochromatic or other bichromatic lights, different fixed directions have been observed: Australian silvereyes, *Zosterops l. lateralis*, preferred a west–northwesterly fixed direction under 565 nm green light about seven times as bright as the one used here (W. Wiltschko et al., 2000, 2003); European robins preferred northerly fixed direction under bright monochromatic turquoise light of similar intensity (R. Wiltschko et al., 2005). Fixed-direction responses were also observed under bichromatic lights of different color combinations, but with a similar quantal flux as the turquoise-and-yellow light used in this study: robins headed northward under a combination of green-and-yellow light, whereas they preferred southerly fixed directions under 424 nm blue-and-yellow light (W. Wiltschko et al., 2004a). In none of these cases, do we know what the respective fixed direction might mean. Fixed-direction responses only occur under unnatural light regimes designed to explore the limits of the magnetoreception system — hence a biological significance of these responses is unlikely; they seem to reflect the limitations of the perception system in critical conditions where different types of receptors are no longer in balance (see R. Wiltschko et al., 2007).

The fixed-direction responses listed above have not yet been analyzed in view of the underlying reception mechanism except for the behavior under bright monochromatic turquoise light, which was found not to involve radical-pair processes (R. Wiltschko et al., 2005). It appears most likely, however, that the other fixed directions, too, originate in the iron-based receptors in the upper beak. The diversity of fixed directions under different light regimes — preferences in northerly, southerly, easterly, and westerly directions have been observed (W. Wiltschko et al., 2000, 2003, 2004a; R. Wiltschko et al., 2005)—indicates that they are controlled by the nature of the ambient light rather than by, e.g., the orientation of magnetite particles. In amphibians, a "fixed-axis" response along the magnetic north–south-axis has also been reported, which in part of the sample seemed to be related to the alignment of single domain magnetite particles in the head of newts (Phillips et al., 2002). The iron-containing structures in the upper beak of birds, in contrast, do not contain single domains, but superparamagnetic crystals of magnetite as well as iron-based platelets in different orientations (Fleissner et al., 2003), which may provide directional information, but do not seem suitable to cause specific alignments.

The phenomenon of the fixed directions raises another question. Fixed-direction responses occur only in some of the test conditions in which the regular inclination compass appears disrupted; in other conditions in the geomagnetic field, we observed disorientation rather than fixed directions. This was the case when, e.g., (1) birds are subjected to oscillating fields (Fig. 1, upper central diagram; Ritz et al., 2004; R. Wiltschko et al., 2005; Thalau et al., 2006), (2) under red light without pre-exposure (W. Wiltschko et al., 2004b), or

(3) when the right eye was covered (W. Wiltschko *et al.*, 2002). These conditions interfere with the radical-pair mechanism, but the magnetite-based system should remain operational, providing directional information. Neverthe-less, the input from these receptors does not appear to be uti-lized in these situations. This and the observation that the specific manifestation of the fixed directions depends on the light regime implies rather complex interactions between the radical-pair mechanism and the visual system on the one side and the iron-based receptors on the other side.

At this point, our considerations are handicapped by our limited knowledge on the processes mediating the magnetic directional information. The photopigment cryptochrome has been suggested as a promising candidate for the receptor molecule, because it could form the crucial magnetosensitive radical pair (Ritz *et al.*, 2000). Cryptochromes have been found in the retina of chicken (Bailey *et al.*, 2002), robins (Möller *et al.*, 2004), and another passerine species (Mouritsen *et al.*, 2004). Since the reception of magnetic compass information in birds takes place in the eyes (W. Wiltschko *et al.*, 2002), it seems likely that cryptochrome indeed plays a crucial role in this process. However, whether and how the cryptochrome-mediated input might interact with that of the normal visual system is not yet known (see R. Wiltschko *et al.*, 2007). Likewise, it is not known whether the fully oxidized or the semiquinone form of cryptochrome is the initial state for forming the magnetosensitive radical pair. The interaction with the iron-based mechanism in the upper beak must be assumed to take place at higher centers in the brain where the various information converge; they, too, are not yet understood and require further analysis. The fixed-axis response reported in amphibians has not yet been analyzed in detail. It is unclear whether it represents a paral-lel to the fixed-direction responses in birds.

Evolutionary considerations
The finding that aside from the radical-pair-based compass, magnetite-based receptors can also produce directional input in birds invites general speculations about the evolution of reception mechanisms for directional information from the geomagnetic field. Magnetite, a ferrimagnetic material of biogenous origin, was first discovered in "magnetotactic" bacteria (Blakemore, 1975). It is a product of the iron me-tabolism and has been reported from a wide variety of spe-cies from different phyla, among them all major groups of vertebrates (see Kirschvink *et al.*, 1985; Beason and Brennan, 1986; Walker *et al.*, 1997; Bassart *et al.*, 1999; Fleissner *et al.*, 2003). At the same time, the magnetic com-pass mechanisms of vertebrates are not uniform — two dis-tinctly different types have been described: the few species of fish and rodents analyzed so far have a "polarity compass" that detects the polarity of the magnetic field, whereas sala-manders, marine turtles, and birds have an "inclination com-pass" that detects the axial course of the field lines, obtaining

unimodal directional information by interpreting their incli-nation in space (R. Wiltschko and Wiltschko, 1995; W. Wiltschko and Wiltschko, 2005). This implies (at least) two different types of reception mechanisms.

The wide distribution of magnetite suggests that first magnetic receptors of the ancestral vertebrates may have been based on magnetite. Birds, however, (and possibly also salamanders and turtles) later developed a second type of re-ceptors based on radical-pair processes. By utilizing this type of processes, birds might have taken advantage of an already existing mechanism which, in other organisms, has different functions. Cryptochromes, a class of photopig-ments widespread among bacteria, plants, and animals (Sancar, 2003; Lin and Shalitin, 2003) where they act as blue-light receptors, have been suggested to form the magne-tosensitive radical pairs (Ritz *et al.*, 2004).

Cryptochrome photoreceptors were first identified in higher plants where they are ubiquitous and mediate a num-ber of blue-light-dependent developmental and growth re-sponses, such as hypocotyl elongation, anthocyanin accumu-lation, vegetative growth, floral initiation, and maintenance of circadian rhythms (Ahmad and Cashmore, 1993; Briggs and Olney, 2001; Ahmad, 2003; Sancar, 2003). Plant crypto-chromes have been shown to undergo a light-dependent electron-transfer reaction that leads to photoreduction of the flavin cofactor (Giovani *et al.*, 2003) and to the formation of long-lived radical pairs, a prerequisite for sensitivity to weak magnetic fields. An increase in the intensity of the ambient magnetic field about 12-fold was recently found to enhance growth inhibition in *Arabidopsis thaliana* (Magnoliatae, Brassicaceae) under blue light, when cryptochrome is the mediating pigment, but not under red light or in darkness; mutants lacking cryptochromes were likewise unaffected (Ahmad *et al.*, 2007). Other cryptochrome-dependent re-sponses, such as blue-light dependent anthocyanin accumu-lation and blue-light-dependent degradation of crypto-chrome 2 protein, were also enhanced at the higher magnetic intensity under blue light, indicating that plants are sensitive to the magnetic field in responses that are linked to cryptochrome-dependent signaling pathways (Ahmad *et al.*, 2007). In plants, the biological significance of the magneto-sensitivity of these responses is unclear; they may just be side effects of using cryptochromes to control light-dependent responses.

In animals, cryptochromes are involved, e.g., in the circa-dian system (Sancar, 2003). By modifying an aspect of the reaction that may have been without significance in other or-ganisms, birds may have developed the initial sensitivity to magnetic fields in the cause of evolution to a second, prob-ably more powerful mechanism to obtain directional infor-mation from the geomagnetic field. This mechanism re-placed the initial magnetite-based compass and provides the modern avian inclination compass.

Yet in birds, the magnetite-based mechanism did not degenerate, but appears to have undergone a change in function, now providing information on magnetic intensity for determining position as a component of their navigational "map" (Semm and Beason, 1990; Beason and Semm, 1996; Munro *et al.*, 1997). Its function of producing directional input still seems to persist to some extent, but it remains more or less dormant as long as the radical-pair mechanism provides compass information. Under certain light regimes, however, directional input from the magnetite-based receptors can affect directional behavior, but this input does not seem to provide proper compass information any longer, as it does not allow migrating species to locate their migratory direction.

These considerations are rather speculative and relate only to birds. In other vertebrate groups, magnetite-based mechanisms may continue to provide compass information. Indeed, the magnetic compass of rodents has been found not to involve radical-pair processes (Thalau *et al.*, 2006) and is probably based on magnetite (Marhold *et al.*, 1997; Wegner *et al.*, 2006). The physical principles underlying the compass mechanisms of other vertebrates, however, have not yet been identified and remain a challenge for future research.

MATERIAL AND METHODS

Test birds

The test birds were mist netted as transmigrants in the botanical garden in Frankfurt a.M., Germany ($50°08'$N $8°40'$E) during the beginning of September. They were juveniles believed to be of Scandinavian origin. The birds were kept indoors in individual cages under white light from a fluorescent lamp in a photoperiod that simulated the natural one outside during the autumn tests and later. In the beginning of December, the photoperiod was decreased to L:D 8:16, and around 1 January, it was increased in two steps to L:D 13:11. This induced premature migratory restlessness so the spring tests could begin in the first week of January. During testing and thereafter, the photoperiod was maintained at L:D 13:11, and in the last week of March, when this photoperiod was reached outside, the birds were released at the site of capture.

Test conditions and experimental treatment

The test lights were produced by LEDs (light-emitting diodes). Green light had a peak wavelength of 565 nm (bandwidth of $553-583$ nm) and an intensity of 1.9 mW/m^2; under this light, robins had always shown excellent orientation in migratory direction (e.g., W. Wiltschko *et al.*, 2002, 2004b; Ritz *et al.*, 2004). The other test light consisted of a combination of 502 nm turquoise (bandwidth of $486-518$ nm, 2.4 mW/m^2) and 590 nm yellow (bandwidth of $572-609$ nm, 2.0 mW/m^2). Sets of 24 LEDs mounted in three circles on a plastic disk were suspended above the test cages (see below). The light passed through two sets of dif-

fusers before it reached the test bird. The intensity was measured as irradiance using Optometer P9710-1 (Gigahertz-Optik, Puchheim, Germany) with the radiometric probe "Visible" RW-3703-2, a silicon photoelement for the wavelength range of $400-800$ nm.

The broadband high-frequency field was produced by a coil antenna consisting of a single winding of coaxial cable with 2 cm of the screening removed. It was mounted on a horizontal wooden frame surrounding four test cages and was fed by oscillating currents from a high-frequency generator (for details, see Ritz *et al.*, 2004; data from that study are included here for comparison, see Table I and Fig. 1, upper central diagram), with the oscillating field vertical, i.e., at a 24° angle to the vector of the geomagnetic field.

To temporarily deactivate the magnetite-based receptors in the upper beak, a cotton bud soaked with the local anaesthetic Xylocain 2% (Astra Zeneca GmbH; active substance: Lidocainhydrochlorid 1 H$_2$O) was gently rubbed along the edges of the upper mandible of the birds with a cotton bud about 5 min before they were placed into the test cages.

Test performance

Testing took place in wooden huts in the garden of the Zoological Institute, where the local geomagnetic field was undisturbed with an intensity of 46 000 nT, and a +66° inclination. The directional tendencies of the birds were recorded in funnel cages lined with coated paper (typewriter correction paper BIC, Germany; formerly Tipp-Ex), where the birds were tested one at a time (see W. Wiltschko *et al.*, 2000, 2003, 2004a, 2004b; Ritz *et al.*, 2004; R. Wiltschko *et al.*, 2005). Each funnel cage was placed in a cylinder that isolated the cages against each other, with the top of the cylinder consisting of the plastic disk carrying the LEDs.

Recording the robins' orientation began in the evening at about the time when the light went off in the housing cages and lasted for 75 min. Tests under turquoise-and-yellow and under green light alone and in combination with the two treatments were performed in pseudorandom order. When active, the birds left scratch marks on the coating of the inclined walls of the cages, which documented the distribution of their activity. The birds were tested under the various test conditions until they had produced three recordings with sufficient activity ($\geq$35 scratches) in each.

Data analysis

After removal from the cage, the coated paper was divided into 24 sectors, and the scratch marks in each sector were counted. From the distribution of the scratches, the bird's heading in the respective recording was calculated. From the three headings of each bird in each condition, we calculated the mean vector of the bird for the respective condition, with direction α_b and length r_b. The mean headings α_b of the 12 or 16 test birds were comprised in the grand mean vector for each condition, with the direction α_N and the length r_N,

which were tested by the Rayleigh test for directional preferences (17). The effect of the treatments was determined by comparing the distributions of the mean headings with and without treatment with the nonparametric Mardia Watson Wheeler test (Batschelet, 1981). From the vector lengths r_b per bird, which represents the intraindividual variance, grand medians were calculated.

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