The Journal of Experimental Biology 210, 3171-3178
Published by The Company of Biologists 2007
doi:10.1242/jeb.007567

Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules

Sönke Johnsen[1,*], Erin Mattern[2] and Thorsten Ritz[2]
[1]*Biology Department, Duke University, Durham, NC 27708, USA and* [2]*Physics Department, University of California, Irvine, CA 92697, USA*
*Author for correspondence (e-mail: sjohnsen@duke.edu)

Accepted 9 July 2007

Summary

Dozens of experiments on magnetosensitive, migratory birds have shown that their magnetic orientation behavior depends on the spectrum of light under which they are tested. However, it is not certain whether this is due to a direct effect on the magnetoreceptive system and which photosensitive molecules may be involved. We examined 62 experiments of light-dependent magnetoreception in three crepuscular and nocturnal migrants (48 for the European robin *Erithacus rubecula*, ten for the silvereye *Zosterops lateralis*, and four on the garden warbler *Sylvia borin*). For each experiment, we calculated the relative quantum catches of seven of the eight known photosensitive molecules found in the eyes of passerine birds: a short- (SW), medium- (MW) and long-wavelength (LW) cone pigment, rhodopsin, melanopsin, and cryptochrome in its fully-oxidized and semiquinone state. The following five opponency processes were also calculated: LW–SW, LW–MW, MW–SW, LW–(MW+SW), and cryptochrome–semiquinone. While the results do not clearly show which receptor system may be responsible for magnetoreception, it suggests several candidates that may inhibit the process. The two significant inhibitors of magnetoreceptive behavior were overall irradiances (from 400 to 700 nm) higher than those found at sunset and high quantum catch by the LW receptor. The results were also consistent with the hypothesis that high quantum catch by the semiquinone form of cryptochrome inhibits magnetoreception. The opponency mechanism that best separated oriented from non-oriented behavior was LW–MW, where a difference above a certain level inhibited orientation. Certain regions of experimental spectral space have been over-sampled, while large regions have not been sampled at all, including: (1) from 440 to 500 nm at all irradiance levels, (2) for wavelengths longer than 570 nm from 10^{12} to 3×10^{12} photons s^{-1} cm^{-2} and (3) for wavelengths less than 560 nm from 10^{12} to 3×10^{12} photons s^{-1} cm^{-2} and below 5×10^{11} photons s^{-1} cm^{-2}. Experiments under these conditions are needed to draw further conclusions.

Key words: cryptochrome, magnetoreception, navigation, orientation, vision.

Introduction

Certain species possess a physiological magnetic compass that allows them to identify their correct migratory or homing direction. A wealth of behavioral experiments, primarily on amphibians and birds, has shown that the behavior of some of these magnetoreceptive species depends on the spectrum of the light under which the animals are tested (reviewed by Wiltschko and Wiltschko, 1995). Early tests in the geomagnetic field with European robins, Australian silvereyes, garden warblers and pigeons showed oriented responses under monochromatic short-wavelength light, but disorientation under long-wavelength light, with a transition between orientation and disorientation around 570 nm (Wiltschko et al., 1993; Wiltschko and Wiltschko, 1999). In recent years, the pattern of responses under different light conditions has become considerably more complex, with responses ranging from correct orientation, complete disorientation, and both axial and unimodal orientation towards an incorrect and generally fixed direction.

The biological significance of this light dependence is unclear, but may involve effects on the compass mechanism itself. One of the three major proposed mechanisms for magnetoreception in animals involves magnetic-field-dependent chemical reactions (reviewed by Johnsen and Lohmann, 2005; Mouritsen and Ritz, 2005; Wiltschko and Wiltschko, 2005). Various lines of evidence have suggested that at least one of the reactants is a photosensitive molecule found in the cephalic or pineal eye (Wiltschko et al., 2002; Ritz et al., 2004). In this model, the light-dependent behavior is due to differential absorption by photosensitive molecules that initiate the magnetosensitive chemical reactions (Ritz et al., 2000). It is also possible that the lights change the behavior of the animals by affecting their motivational state. Regardless of what explanation one favors, the fact remains that photosensitive molecules are linked in some way to magnetic orientation responses.

In this study, we calculate the relative quantum catches of seven of the eight known photosensitive molecules found in the eyes of passerine birds: a short-, medium- and long-wavelength cone pigment, rhodopsin, melanopsin, and

Table 1. *Spectra and sources examined in this study*

Peak ± half maximum range (nm)	Irradiance ($\times 10^{11}$ quanta s^{-1} cm^{-2})	Orientation	Subject
White incandescent light	3.9	Disorientation	Robin[1]
White incandescent light	39	Disorientation	Robin[1]
568±10	2.9	Disorientation	Robin[1]
568±10	14	Disorientation	Robin[1]
568±10	29	Disorientation	Robin[1]
617±11	32	Disorientation	Robin[1]
590±25	8.7	Disorientation	Robin[2]
590±16	7	Disorientation	Robin[3]
590±16	43	Disorientation	Robin[3]
635±20	6.5	Disorientation	Robin[4]
635±20	43	Disorientation	Robin[4]
645±21	6.5	Disorientation	Robin[4]
635±20	43	Disorientation	Robin[5]
565±15	26	Disorientation	Robin[6]
565±13 and 645±20	12	Disorientation	Robin[7]
424±27	42	Disorientation	Robin[7]
424±27	57	Disorientation	Robin[7]
424±27	30	Disorientation	Robin[7]
565±27	36	Disorientation	Robin[7]
565±27	72.5	Disorientation	Robin[7]
633±20	8.7	Disorientation	Silvereye[8]
590±18	8.7	Disorientation	Warbler[9]
630±26	8.7	Disorientation	Warbler[9]
565±18	43	Fixed axial	Robin[3]
565±15	38	Fixed axial	Robin[6]
565±15	51	Fixed axial	Robin[6]
617±11	3.2	Fixed unimodal	Robin[1]
502±16	54	Fixed unimodal	Robin[10]
424±25 and 590±16	14	Fixed unimodal	Robin[11]
565±15 and 590±16	14	Fixed unimodal	Robin[11]
502±18 and 580±17	12	Fixed unimodal	Robin[7]
645±21	3.2	Fixed unimodal	Robin[7]
424±25 and 645±21	14	Fixed unimodal	Robin[7]
565±15 and 590±16	5.4	Fixed unimodal	Robin[7]
502±18 and 590±17	12	Fixed unimodal	Robin[7]
565±23	44	Fixed unimodal	Silvereye[12]
565±15	43	Fixed unimodal	Silvereye[13]
561±9	2.9	Orientation	Robin[1]
443±39	8.7	Orientation	Robin[2]
565±25	8.7	Orientation	Robin[2]
424±21	7	Orientation	Robin[3]
510±18	7	Orientation	Robin[3]
565±18	7	Orientation	Robin[3]
White incandescent light	56.4	Orientation	Robin[4]
565±15	6.5	Orientation	Robin[4]
565±20	7	Orientation	Robin[11]
502±16	8	Orientation	Robin[10]
565±15	6	Orientation	Robin[6]
565±13	6	Orientation	Robin[7]
424±27	6	Orientation	Robin[7]
502±18	6	Orientation	Robin[7]
565±13	5.4	Orientation	Robin[7]
565±27	7.7	Orientation	Robin[7]
White incandescent light	8.7	Orientation	Silvereye[8]
571±17	8.7	Orientation	Silvereye[8]
443±39	8.7	Orientation	Silvereye[8]
565±23	6.5	Orientation	Silvereye[12]
565±23	22	Orientation	Silvereye[12]
424±25	7	Orientation	Silvereye[13]
565±15	7	Orientation	Silvereye[13]
565±15	8.7	Orientation	Warbler[9]
White incandescent light	8.7	Orientation	Warbler[9]

[1](Muheim et al., 2002); [2](Wiltschko and Wiltschko, 1999); [3](Wiltschko and Wiltschko, 2001); [4](Wiltschko et al., 2004a); [5](Wiltschko and Wiltschko, 2002); [6](Wiltschko and Wiltschko, 2005); [7](Staput, 2006); [8](Wiltschko et al., 1993); [9](Rappl et al., 2000); [10](Wiltschko et al., 2005b); [11](Wiltschko et al., 2004b); [12](Wiltschko et al., 2000); [13](Wiltschko et al., 2003).

cryptochrome in its fully-oxidized and semiquinone form. Certain birds also possess a UV-sensitive cone, but because the vast majority of tests were performed under visible light, we will not consider this receptor. While quantum catches of photoreceptors represent a better measure of the photoreceptor signal output than irradiances, they should only be considered a step towards a full quantitative description that also includes the biochemical output and amplification mechanisms. In view of this, we have limited our study to experiments in which birds were exposed to light conditions in the magnetic testing arena without having been exposed to these light conditions previously. Birds were chosen because 62 published experiments have been performed on them, more than for any other animal group. Of these, 48 experiments were performed on the European robin *Erithacus rubecula* L., ten on the silvereye *Zosterops lateralis* Latham 1802, and four on the garden warbler *Sylvia borin* Boddaert 1783. All three species are crepuscular and nocturnal migrants. In addition, because the observed magnetoreception behavior is so strongly wavelength dependent, we also modeled five possible opponency mechanisms, where the quantum catch of one molecule is subtracted from the quantum catch of another.

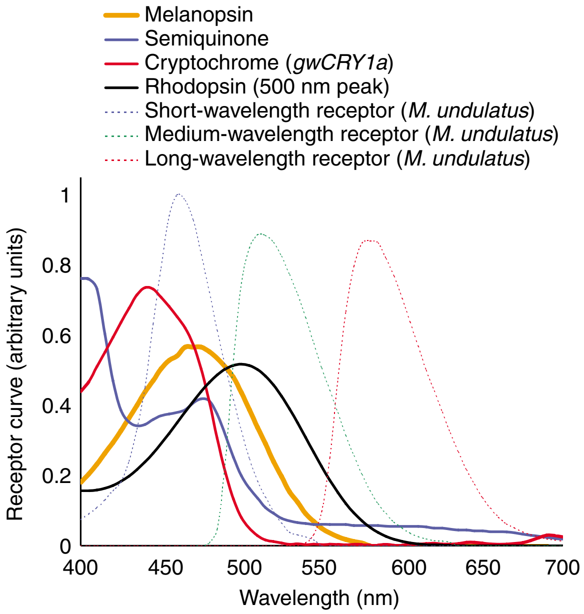

Fig. 1. Receptor curves used in study. For clarity, all are normalized so that the integral under each curve is identical.

Materials and methods

The spectra under which experimental birds were illuminated were taken from the sources listed in Table 1. Light-emitting diode (LED) spectra were generally modeled as Gaussian curves using the peak wavelength and the width of the spectrum in nm across the points where the irradiance was 50% of the peak radiance (i.e. full-width-half-maximum). White light and other, more complex spectra were digitized (GetData Graph Digitizer; S. Federov). All spectra were digitized or calculated at 5 nm resolution. After determining the shapes of the spectra, the absolute values of the data points were adjusted so that the integrated quantal irradiances (from 400–700 nm) matched those given in the references.

Relative quantum catches for the short- (SW), medium- (MW) and long-wavelength (LW) photoreceptors found in the budgerigar *Melopsittacus undulatus* were calculated from the integral of the spectra weighted by the receptor curves:

$$Q_\mathrm{i} = f_\mathrm{i} \int_{400}^{700} S(\lambda) V_\mathrm{i}(\lambda) \mathrm{d}\lambda \,, \qquad (1)$$

where $S(\lambda)$ is the given irradiance spectrum and $V_\mathrm{i}(\lambda)$ and f_i are the receptor curves and relative abundance of the short-, medium- and long-wavelength receptors in the bird's retina (SW:MW:LW=1:2:2) (Goldsmith and Butler, 2003). The receptor curves included the effects of oil droplets in front of the photoreceptors and were all normalized to have the same integral (Fig. 1). Unfortunately, the receptor curves for *Erithacus rubecula* are not known. However, the peak wavelengths of the visual pigments of passerine birds are well conserved (J. Partridge, personal communication) and small differences in the receptor curves are unlikely to affect the general conclusions of this study.

Quantum catches were also calculated for cryptochrome in its fully oxidized and semiquinone state, shown in Fig. 1. The absorption spectrum of cryptochrome (*gwCRY1a* in its fully oxidized state) has been measured for the cryptochrome found in another passerine bird (the garden warbler *Sylvia borin*) (H. Mouritsen, personal communication), but no spectral information exists yet for bird cryptochrome in its semiquinone state, which is likely anionic rather than the neutral semiquinone found in plants. We use the recently measured semiquinone absorption spectrum from the fruit fly *Drosophila melanogaster* (Berndt et al., 2007). The relative values of the fully oxidized cryptochrome to semiquinone absorption could not be calculated because their relative concentrations are variable. While this makes the exact values of the semiquinone and cryptochrome data points in Figs 4 and 6 impossible to calculate, it does preserve their topological relationships (i.e. grouped data points in Fig. 6 remain grouped for any relative concentrations of the two molecules). Finally, we assumed that the concentration of these two molecules was not high enough to broaden the absorption curve relative to the shape of the absorbance curve (due to saturation at wavelengths close to the peak, as occurs in very long photoreceptors).

Finally, quantum catches were calculated for a typical 500 nm peak rhodopsin and 470 nm peak melanopsin (Brainard et al., 2001). In both cases, the absorbance curves were used, which approximate the shapes of the absorption curves for all but high concentrations of the molecules.

Two processes were considered to potentially affect magnetoreception behavior. One was relative quantum catch by a given light-absorptive molecule (as calculated in Eqn 1). The other was an opponency process between two sets of molecules or receptors. We modeled the opponency (O) processes in the following fashion:

$$O_\mathrm{ij} = \frac{Q_\mathrm{i} - Q_\mathrm{j}}{Q_\mathrm{i} + Q_\mathrm{j}} \,, \qquad (2)$$

where Q_i and Q_j are the quantum catches of two different molecules (e.g. *gwCRY1a* and semiquinone). Thus, if no photons are captured by receptor j, the opponency value is 1. If no photons are captured by receptor i, the opponency value is –1. This was then plotted against $Q_\mathrm{i}+Q_\mathrm{j}$, the total relative catch of the two receptors or set of molecules. Given the inability to directly compare semiquinone and cryptochrome with the cone pigments (due to the lack of information on the concentration of the former two), the two groups were treated separately. For similar reasons, no opponency calculations were performed for melanopsin or rhodopsin. The following five opponency processes were considered in addition to total relative quantum catch: LW–SW, LW–MW, MW–SW, LW–(MW+SW) and cryptochrome–semiquinone.

All data were grouped by the four types of magnetoreceptive behavior: significant and biologically useful orientation (i.e. correct migratory direction), no significant orientation, fixed unimodal orientation and fixed axial orientation. While the first two behaviors are self-explanatory, the latter two require further definition. Fixed unimodal orientation is oriented behavior that is not useful in the migratory context. In general, this consists of orientation that is significantly different from the trained direction or the correct direction of migration. The direction of orientation is also unaffected by season, which generally changes the correct orientation of these migrating birds. Fixed axial orientation is simply fixed unimodal orientation, except that two opposite directions are chosen rather than just one (i.e. orientation to both north and south by different birds in the experiment).

Results

Spectral characteristics versus magnetoreception behavior

The two primary differences between the spectra under which oriented and disoriented behavior occurred are: (1) total quantal irradiance for oriented behavior was generally less than that for disoriented behavior (though significant overlap occurred), and (2) with the exception of two of the white light conditions, spectra under which oriented behavior occurred did not extend above ~600 nm (Fig. 2A,B, Fig. 3). For single LED conditions, spectra under which fixed unimodal orientation occurred were not obviously distinguishable from those resulting in disorientation (Fig. 2C, Fig. 3). For double LED conditions, however, spectra under which fixed unimodal orientation occurred were similar in intensity to those resulting in orientation but always had one LED in the long wavelength range (Fig. 2D). Due to small sample size (N=3), little can be said about the spectra under which fixed axial orientation occurred (Fig. 2E, Fig. 3). All three were relatively bright, with a peak wavelength of 565 nm, but similar spectra also resulted in both fixed unimodal and disoriented behavior.

Normalized quantum catch versus magnetoreception behavior

With the exception of melanopsin and rhodopsin, the quantum catches for conditions under which fixed axial orientation occurred were dramatically different from the quantum catches for the other behaviors (Fig. 4). However, due to the small sample size for these spectra and the fact that that the conditions were not chosen independently by the researchers, they were excluded from statistical analysis. The differences in quantum catches for the remaining three behaviors showed a significant effect of catch for the long wavelength cone (P<0.05, Kruskal–Wallis), due to the lower catch for the conditions under which oriented behavior occurred. Two-tailed *t*-tests comparing oriented behavior *versus* all other behaviors again were significant only for the quantum catch of the long wavelength cone (P<0.0025). Again, because the lighting conditions were not chosen independently and randomly by researchers, any statistical results should be treated with caution.

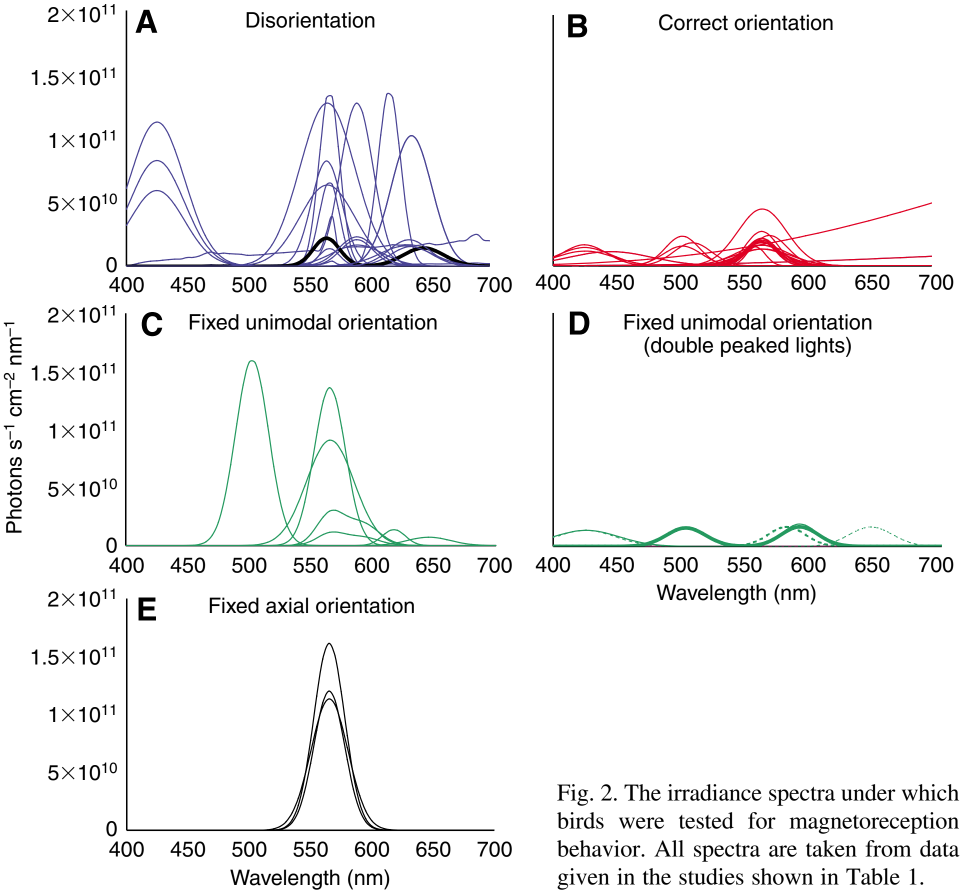

Fig. 2. The irradiance spectra under which birds were tested for magnetoreception behavior. All spectra are taken from data given in the studies shown in Table 1.

Opponency mechanisms

LW–MW

With few exceptions, conditions resulting in oriented behavior were tightly clustered around (0.24, 6×10^{11}) (Fig. 5A). However, LW–MW for four oriented conditions was approximately –1. Fixed unimodal behavior occurred

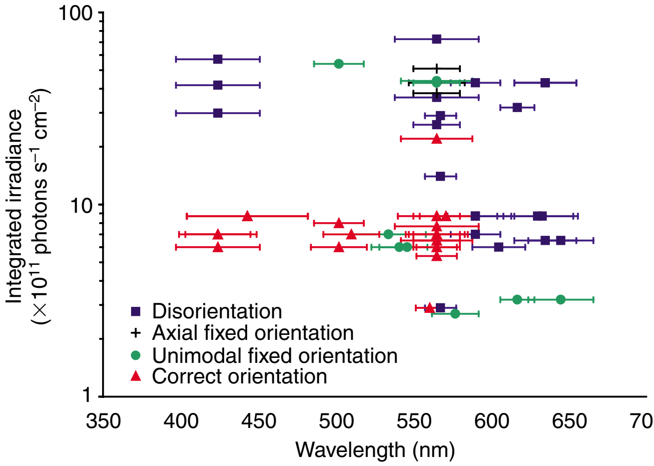

Fig. 3. Irradiance spectra of the single-LED conditions under which birds were tested. Values denote central wavelength; error bars denote the range over which intensity is at least half of that at the peak.

under conditions generally having a higher LW–MW, but with a similar total catch. Disoriented behavior occurred under conditions similar to fixed unimodal, but generally with a higher total catch, as did fixed axial orientation. The results for *E. rubecula* generally overlapped with those of the other two bird species (*Zosterops lateralis* and *S. borin*) in this and all the following cases. Another common factor to all opponency mechanisms was that oriented behavior only occurred when total catch was less than that at sunset.

MW–SW

Because most of the test conditions contained little short-wavelength light, MW–SW was generally close to 1 (Fig. 5B). Although the positions of the data points are different, roughly similar groupings occurred as with LW–MW, with the following two major exceptions: (1) oriented conditions occurred in three groups, though with similar total catches, (2) MW–SW for five disoriented conditions was lower than those for most of the oriented conditions.

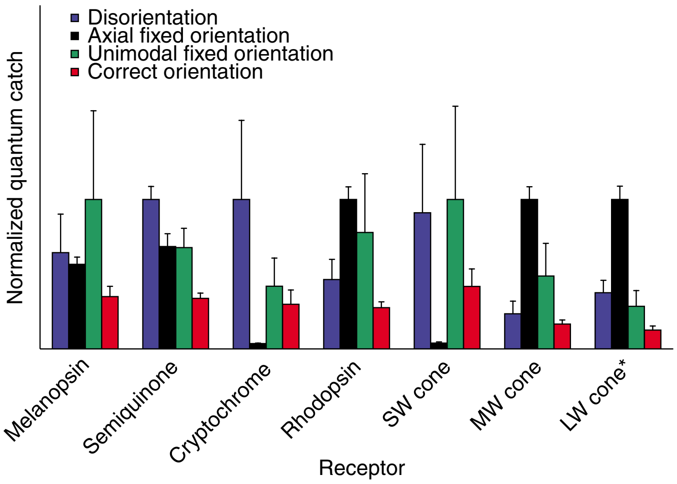

Fig. 4. Quantum catch of the various receptors grouped by magnetoreception behavior. Since one cannot compare the catches of the different receptors for reasons described in the text (with the exception of the cone pigments), the catches are normalized by their maximum value for clarity. Values are means ± s.e.m. *$P<0.05$.

LW–SW

As in MW–SW, LW–SW was generally close to 1 (Fig. 5C). Oriented behavior was generally found when LW–SW was either 1 or –1 and total relative catch by the two receptors was less than 6×10^{11}. Disorientation also occurred when LW–SW was 1 or –1, but generally at a higher total catch. In four cases, fixed unimodal orientation occurred at intermediate LW–SW, but otherwise was found at values close to 1.

LW–(MW+SW)

The results were nearly identical to those for LW–MW, most likely because the catch in the SW receptor was relatively small (Fig. 5D).

gwCry1a–semiquinone

All the lighting conditions fell into two well-separated clusters (Fig. 6). Conditions resulting in oriented behavior were either tightly clustered around (–0.9, 10^{11}) or loosely clustered around (0, 10^{12}). Disoriented behavior was generally found at higher total catches within each cluster. Fixed behavior occurred under conditions that could not be well separated from the rest.

Discussion

Intensity

Both the irradiance and quantum catch data show that the brightness of the ambient light correlated strongly with behavior. Generally, birds were disoriented under higher intensity lights and oriented in the correct migratory direction under lights with irradiances below that of sunset. The most pronounced difference in quantum catches between disoriented and correctly oriented behavior occur for the semiquinone form of cryptochrome and for the LW receptor, the two receptors that absorb most strongly at long wavelengths. The irradiances used during the most experimental conditions are low, corresponding to sunset and twilight conditions. Although no firm data exist, the fact that the experimental conditions were above the photopic threshold in humans (Wyszecki and Stiles, 1982) suggests that color vision was also still active in the birds.

The lack of orientation at higher irradiances could reflect a change in motivation. For example, brighter light could stimulate a diurnal behavior or inhibit migration. Circadian behaviors, such as migration, are often influenced by environmental light levels (reviewed by Johnsen and Lohmann, 2005; Cashmore et al., 1999). Mouritsen identified a forebrain area, cluster N, that was active during magnetic orientation experiments with night-migrants in low light intensities, but not during daylight. He suggested that processing of magnetic stimuli might occur in this brain area (Mouritsen et al., 2005), providing an additional reason for a transition in behavior at higher light intensities. However, neither the quantum catches of cryptochrome nor of melanopsin are good predictors of behavior. Nevertheless, the motivation could also be affected by the summed light absorption of several receptors.

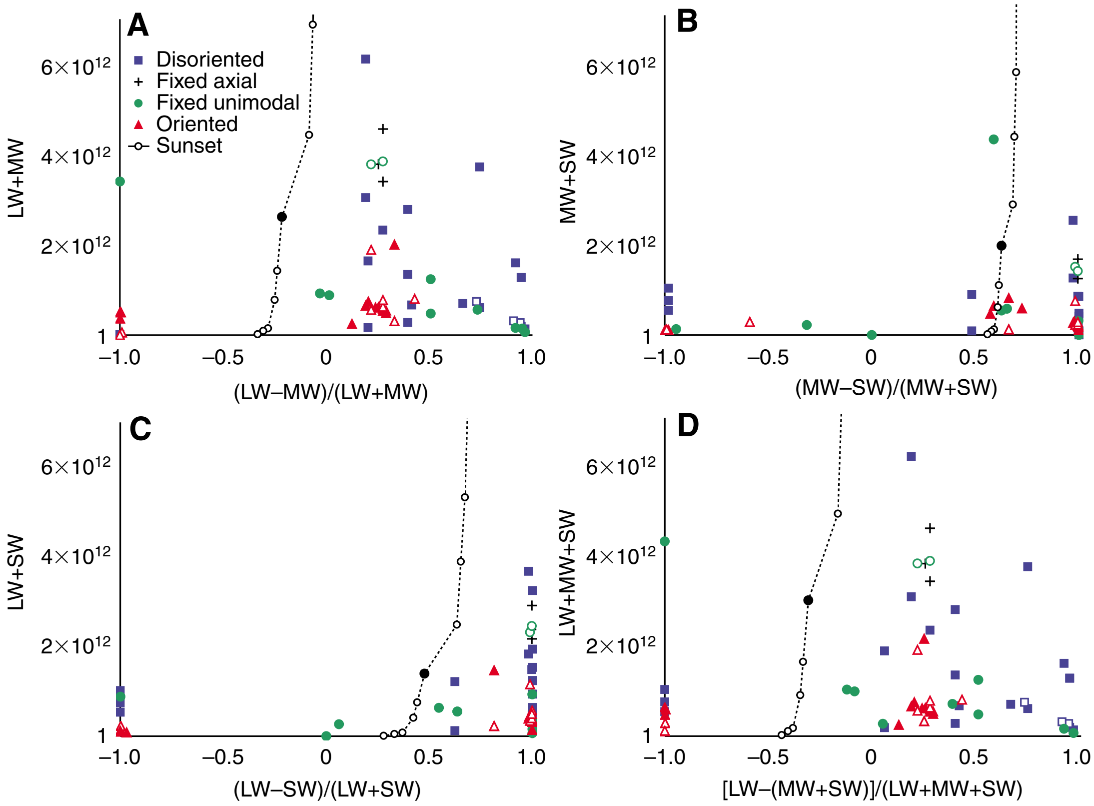

Fig. 5. Four possible cone opponency mechanisms plotted against total quantum catch for the receptors involved. (A) LW–MW, (B) MW–SW, (C) LW–SW, (D) LW–(MW+SW). The broken lines show the irradiance spectra during sunset. The black circles denote the conditions when the sun is at the horizon; open circles, solar elevations that are not sunset. The other points are for conditions separated by approximately 1° of solar elevation. Filled symbols, European robin; open symbols, other birds (see Table 1).

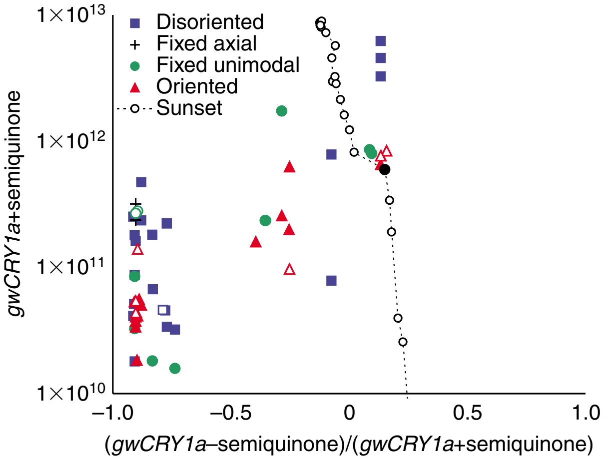

Fig. 6. A possible opponency mechanism involving *gwCry1a* and semiquinone. See Fig. 5 for further details.

Spectrum

In addition to low irradiance, the best predictor of orientation behavior is the difference in intensity of long and shorter-wavelength light, best shown as an opponency between the LW and MW receptor. If LW–MW is large, disorientation generally occurs. Orientation usually occurs when the quantum catch by the LW receptors is about 50% greater than the catch by the MW receptors, though it rarely occurs when there is no long-wavelength light at all (Fig. 5A). The lack of data between –1 and 0 for LW–MW cone opponency makes it impossible to assess whether the observed clustering around 0.24 and –1 is a real effect or an artifact of the biased choice of experimental conditions.

Cryptochrome and semiquinone

Aside from higher irradiances, the most striking inhibitor of correct orientation behavior is the presence of long-wavelength light, which in this study is best absorbed by the LW receptor and the semiquinone form of cryptochrome. Although statistics are problematic in a set of conditions that were not chosen randomly, low quantum catch by the semiquinone form of cryptochrome is a weak predictor of orientation behavior ($P<0.05$ in a one-tailed *t*-test between oriented behavior and all disoriented behaviors; Fig. 4). Cryptochromes are short-wavelength sensitive photoreceptors found in many organisms including bacteria, plants and animals. Their active chromophore is flavin, which can exist in three redox states: fully oxidized, semiquinone and fully reduced (see Fig. 7). Cryptochrome activation involves absorption of light while in the fully oxidized state, leading to formation of a flavosemiquinone state (Banerjee and Batschauer, 2005). Semiquinone has recently been shown to be the signaling state of cryptochromes 1 and 2 (Bouly et al., 2007; Banerjee et al., 2007) and can be converted to an inactive form by light absorption. Fully oxidized cryptochrome is recovered from the semiquinone or the fully reduced state in the presence of oxidization agents without light. Cryptochromes are the only known photopigments in birds that change oxidization states through radical pair reactions, a pre-requisite for magnetically sensitive chemical reactions. Effects of magnetic fields on light responses mediated by cryptochrome have in fact been found in plants (Ahmad et al., 2006) and could in principle change the rate of two reaction steps, as indicated in Fig. 7: the transition from activated FAD* to the semiquinone, which involves a triad of flavin–tryptophan radical pairs (Solov'yov et al., 2007), and the re-oxidation step from fully reduced to fully oxidized cryptochrome. The relative concentration of cryptochrome states (and their products) is thus initially set by the ambient light conditions and intrinsic reaction rates. Magnetic field effects on magnetically sensitive reaction rates would shift the concentrations from this operating point, resulting in a change of cryptochrome activation and, hence, light signaling. This signal modulation could provide the basis for magnetic sensing (Ritz et al., 2000; Wang et al., 2006).

However, due to the antagonistic effects of light absorption in fully oxidized cryptochrome *versus* semiquinone, changes in light conditions alone can also result in changes of cryptochrome activation, if they result in different absorption ratios of cryptochrome and semiquinone. Measurements of relative absorption strengths of cryptochromes indicate that the fully oxidized form absorbs light more strongly than the semiquinone form (Sancar, 2003), suggesting that weak absorption by semiquinone, as would occur under full spectrum light, does not interfere with magnetic sensing. However, relatively monochromatic light peaking at wavelengths above 570 nm would selectively be absorbed by semiquinone and would thus deactivate cryptochrome signaling (Bouly et al., 2007). Unfortunately the lack of knowledge about the exact nature of the bird cryptochrome photocycle, its reaction rates and the relative absorption strengths of cryptochromes, preclude more quantitative assessments at this point. Finally, it cannot be excluded that cryptochromes might be involved in photoreactions other than the discussed light signaling pathway, which could lead to formation of magnetosensitive radical pairs.

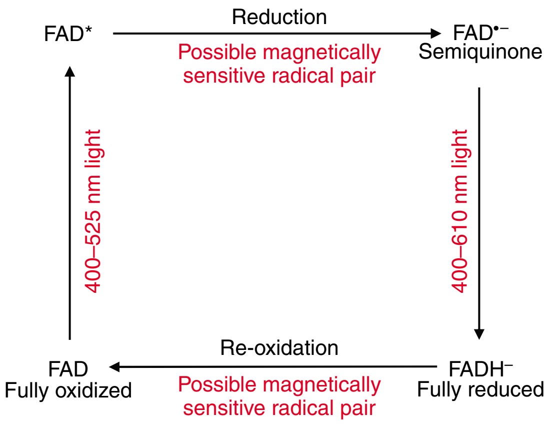

Fig. 7. Likely photocycle of crypotochrome in birds. Light can be absorbed either by the fully oxidized or semiquinone form of flavin, the active chromophore in cryptochrome. Magnetic field effects can, in principle, occur on the reduction from activated flavin (FAD*) to the semiquinone, or on the reoxidation from fully reduced FADH⁻ to fully oxidized FAD.

Fixed responses

Nearly all the bird studies involved tests during two seasons that had opposite 'correct' migration directions. While most resulted in either correct orientation during both seasons or no significant orientation in either season, about 22% resulted in significant orientation to an incorrect direction that was not affected by season. In three cases, the orientation was best described as axial. Because magnetic orientation, while incorrect, is nevertheless occurring, it is difficult to place these results in a consistent framework. Recently, however, it has been shown that anesthetization of the beak area containing iron-rich structures (Fleissner et al., 2003) results in disappearance of fixed responses but not normal magnetic compass orientation (Wiltschko et al., 2007), suggesting that fixed responses are mediated by magnetic field detection by iron-oxide structures in the beak. Indeed, it has been suggested that *E. rubecula* contains two magnetic systems, one for a compass and one for a navigational map. If true, why birds would use input from the iron-oxide beak system under certain light conditions and ignore this input under others is unknown. Fixed responses occurred mostly under lights of higher intensity or double peaks, but the conditions are not easily distinguishable from those that lead to orientation (Fig. 2). Phillips and co-workers (Phillips et al., 2001; Ritz et al., 2002) suggested that an antagonistic mechanism between a primary short-wavelength and a less sensitive long-wavelength receptor could explain fixed responses orthogonal to the expected migratory direction. Fixed responses have been observed in all kinds of directions relative to the expected migratory direction, however, making it doubtful that they can all be considered as one type of responses. Moreover, in red light, fixed responses have been observed for the lowest light intensities used; at higher intensities, birds were disoriented. These observations suggest that there may be a multitude of reasons for the occurrence of fixed responses and argue for a discussion of such responses on a case-by-case basis rather than treating them all as belonging to the same group in a statistical description.

Future experiments

While over 60 studies have been performed under many light conditions, large regions of the bird 'light space' remain untested. The three largest regions are: (1) from 440 to 500 nm at all irradiances, (2) for wavelengths longer than 570 nm from 10^{12} to 3×10^{12} photons s^{-1} cm^{-2}, and (3) for wavelengths shorter than 560 nm from 10^{12} to 3×10^{12} photons s^{-1} cm^{-2}, and below 5×10^{11} photons s^{-1} cm^{-2} (Fig. 3). The last is particularly important because it maps to the empty region between the two clusters of oriented behavior in the LW–MW and the cryptochrome–semiquinone opponency graphs (Fig. 5A, Fig. 6). Data from tests under these conditions could help determine whether the two clusters of conditions that lead to orientation behavior are simply the end points of one larger cluster, eliminating the need for some sort of bimodal mechanism.

We thank Dr Julian Partridge for providing information on the distribution of cone pigments in passerine birds. We thank Drs Eric Warrant, Kenneth Lohmann, and Roswitha and Wolfgang Wiltschko for helpful discussions and Dr Henrik Mouritsen for providing the cryptochrome spectrum. S.J. was supported in part by a grant from the National Science Foundation (IOB-0444674). T.R. was supported by a grant from the Human Frontier Science foundation, an Alfred P. Sloan Fellowship and a Cottrell Scholarship by the Research Cooperation.

References

Ahmad, M., Galland, P., Ritz, T., Wiltschko, R. and Wiltschko, W. (2006). Magnetic intensity affects cryptochrome-dependent responses in *Arabidopsis thaliana*. *Planta* **225**, 615-624.

Banerjee, R. and Batschauer, A. (2005). Plant blue-light receptors. *Planta* **220**, 498-502.

Banerjee, R., Schleicher, E., Meier, S., Viana, R. M., Pokorny, R., Ahmad, M., Bittl, R. and Batschauer, A. (2007). The signaling state of *Arabidopsis* cryptochrome 2 contains flavin semiquinone. *J. Biol. Chem.* **282**, 14916-14922.

Bouly, J. P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R. et al. (2007). Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J. Biol. Chem.* **282**, 9383-9391.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M. and Wolf, E. (2007). A novel photoreaction mechanism for the circadian blue-light photoreceptor *Drosophila* cryptochrome. *J. Biol. Chem.* **282**, 13011-13021.

Brainard, G. C., Hanifin, J. P., Greeson, J. M., Byrne, B., Glickman, G., Gerner, E. and Rollag, M. D. (2001). Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J. Neurosci.* **21**, 6405-6412.

Cashmore, A. R., Jarillo, J. A., Wu, Y. and Liu, D. (1999). Cryptochromes: blue light receptors for plants and animals. *Science* **284**, 760-765.

Fleissner, G., Holtkamp-Rötzler, E., Hanzlik, M., Winklhofer, M., Fleissner, G., Petersen, N. and Wiltschko, W. (2003). Ultrastructure analysis of a putative magnetoreceptor in the beak of homing pigeons. *J. Comp. Neurobiol.* **458**, 350-360.

Goldsmith, T. H. and Butler, B. K. (2003). Color vision of the budgerigar (*Melopsittacus undulatus*): hue matches, tetrachromacy, and intensity discrimination. *J. Comp. Physiol. A* **191**, 933-951.

Johnsen, S. and Lohmann, K. J. (2005). The physics and neurobiology of magnetoreception. *Nat. Rev. Neurosci.* **6**, 703-712.

Mouritsen, H. and Ritz, T. (2005). Magnetoreception and its use in bird navigation. *Curr. Opin. Neurobiol.* **15**, 406-414.

Mouritsen, H., Feenders, G., Liedvogel, M., Wada, K. and Jarvis, E. D. (2005). Night-vision brain area in migratory songbirds. *Proc. Natl. Acad. Sci. USA* **102**, 8339-8344.

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

Phillips, J. B., Deutschlander, M. E., Freake, M. J. and Borland, S. C. (2001). The role of extraocular photoreceptors in newt magnetic compass orientation: parallels between light-dependent magnetoreception and polarized light detection in vertebrates. *J. Exp. Biol.* **204**, 2543-2552.

Rappl, R., Wiltschko, R., Weindler, P., Berthold, P. and Wiltschko, W. (2000). Orientation behavior of garden warblers (*Sylvia borin*) under monochromatic light of various wavelengths. *Auk* **117**, 256-260.

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

Ritz, T., Dommer, D. H. and Phillips, J. B. (2002). Shedding light on vertebrate magnetoreception. *Neuron* **34**, 503-506.

Ritz, T., Thalau, P., Phillips, J. B., Wiltschko, R. and Wiltschko, W. (2004). Resonance effects indicate a radical-pair mechanism for avian magnetic compass. *Nature* **429**, 177-180.

Sancar, A. (2003). Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem. Rev.* **103**, 2203-2237.

Solov'yov, I. A., Chandler, D. and Schulten, K. (2007). Magnetic field effects in *Arabidopsis thaliana* cryptochrome-1. *Biophys. J.* **92**, 2711-2726.

Stapput, K. (2006). Verhaltensbiologische Untersuchungen zum Chemischen Magnetkompass bei Rotkehlchen (*Erithacus rubecula*). PhD thesis, J-W Goethe University, Frankfurt, Germany.

Wang, K., Mattern, E. and Ritz, T. (2006). On the use of magnets to disrupt the physiological compass of birds. *Phys. Biol.* **3**, 220-231.

Wiltschko, R., Ritz, T., Stapput, K., Thalau, P. and Wiltschko, W. (2005). Two different types of light-dependent responses to magnetic fields in birds. *Curr. Biol.* **15**, 1518-1523.

Wiltschko, R., Stapput, K., Bischof, H.-J. and Wiltschko, W. (2007). Light-dependent magnetoreception in birds: increasing intensity of monochromatic light changes the nature of the response. *Front. Zool.* **4**, doi: 10.1186/1742-9994-4-5.

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 behaviour 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. and Wiltschko, R. (2005). Magnetic orientation and magnetoreception in birds and other animals. *J. Comp. Physiol. A* **191**, 675-693.

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

Wiltschko, W., Wiltschko, R. and Munro, U. (2000). Light-dependent magnetoreception in birds: does directional information change with light intensity? *Naturwissenschaften* **87**, 36-40.

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

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 Biol. Sci.* **270**, 2133-2140.

Wiltschko, W., Möller, A., Gesson, M., Noll, C. and Wiltschko, R. (2004a). Light-dependent magnetoreception in birds: analysis of the behavior under red light after pre-exposure to red light. *J. Exp. Biol.* **207**, 1193-1202.

Wiltschko, W., Gesson, M., Stapput, K. and Wiltschko, R. (2004b). Light-dependent magnetoreception in birds: interaction of at least two different receptors. *Naturwissenschaften* **91**, 130-134.

Wyszecki, G. and Stiles, W. S. (1982). *Color Science: Concepts and Methods, Quantitative Data and Formulae.* New York: John Wiley.