

Polarized light modulates light-dependent magnetic compass orientation in birds

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Magnetoreception of the light-dependent magnetic compass in birds is suggested to be mediated by a radical-pair mechanism taking place in the avian retina. Biophysical models on magnetic field effects on radical pairs generally assume that the light activating the magnetoreceptor molecules is nondirectional and unpolarized, and that light absorption is isotropic. However, natural skylight enters the avian retina unidirectionally, through the cornea and the lens, and is often partially polarized. In addition, cryptochromes, the putative magnetoreceptor molecules, absorb light anisotropically, i.e., they preferentially absorb light of a specific direction and polarization, implying that the light-dependent magnetic compass is intrinsically polarization sensitive. To test putative interactions between the avian magnetic compass and polarized light, we developed a spatial orientation assay and trained zebra finches to magnetic and/or overhead polarized light cues in a four-arm “plus” maze. The birds did not use overhead polarized light near the zenith for sky compass orientation. Instead, overhead polarized light modulated light-dependent magnetic compass orientation, i.e., how the birds perceive the magnetic field. Birds were well oriented when tested with the polarized light axis aligned parallel to the magnetic field. When the polarized light axis was aligned perpendicular to the magnetic field, the birds became disoriented. These findings are the first behavioral evidence to our knowledge for a direct interaction between polarized light and the light-dependent magnetic compass in an animal. They reveal a fundamentally new property of the radical pair-based magnetoreceptor with key implications for how birds and other animals perceive the Earth’s magnetic field.

magnetoreception | magnetic compass | orientation | skylight polarization | radical-pair process

A range of animals, including birds, use directional information from the Earth’s magnetic field for orientation and navigation (1–6). There is growing evidence that this ability is by far not restricted to migratory animals, but that it is likely an omnipresent capability of the majority of organisms, playing a fundamental role in the animals’ daily routines in all stages of life (7). Behavioral and physiological studies on taxonomically diverse animals suggest the presence of two fundamentally different, independent magnetoreception mechanisms that detect different parameters of the Earth’s magnetic field (1, 8–11): A light-dependent magnetic compass detects the axial alignment of the magnetic field, and an iron mineral-based mechanism provides positional magnetic map information.

Magnetoreception of the light-dependent magnetic compass is suggested to be mediated by light-induced, biochemical reactions taking place in specialized photoreceptors (8, 10–12). Upon light excitation, the photopigment molecules form magnetically sensitive radical-pair intermediates. The magnetic field then alters the ratio of the spin states of the radical pairs (i.e., singlet vs. triplet state) and, thereby, affects the photopigments’ response to light. Such magneto-sensitive photoreceptors arranged in an ordered array in the avian retina would show increased or decreased sensitivity to light, depending on their alignment to the magnetic field (8, 10, 12, 13). The animals would perceive the magnetic field as a magnetic modulation pattern centered on the magnetic field lines, either

superimposed on the visual field or mediated by a separate channel (12). Cryptochromes have been proposed as putative candidate receptor molecules (8, 14) and found to be expressed in the retinas of birds exhibiting magnetic orientation behavior (15–17). Biophysical models on magnetic field effects on radical pairs generally assume that light reaching the magnetoreceptor molecules is nondirectional and unpolarized, and that light absorption is isotropic, i.e., that the probability of excitation by light is equal for all receptor molecules (8, 10, 12). Likewise, behavioral experiments testing the mechanisms of magnetic compass orientation in migratory birds have typically been carried out under depolarized light (e.g., refs. 1 and 18–22). As recently pointed out by Lau et al. (13), traditional radical-pair models thus do not take into consideration that natural skylight always enters the eyes directionally through the cornea and lens and that the magnetoreceptor molecules absorb light anisotropically, i.e., they preferentially absorb light of a specific direction and polarization. The probability of photoexcitation and, thereby, formation of radical pairs will therefore differ between magnetoreceptors across the retina and result in a photoselected population of magnetoreceptors that the magnetic field can act upon (13, 23, 24). The ratio of singlet and triplet states and, thereby, the magnetic field effect, will in turn depend on the relative alignment of the radical pair to the magnetic field (8, 10). Such a photoselection effect has been shown to be strong enough to allow for a functional magnetic compass without major rotational restrictions of the receptor molecules (13).

Natural skylight is also always to some degree polarized, with the exception of totally overcast or foggy conditions (25–27). The degree of polarization and the alignment of the polarization axis vary across the sky with respect to the position of the sun and depend on weather conditions (25–27). Magnetoreceptor molecules in different

Significance

Birds have a light-dependent magnetic compass that is suggested to be mediated by light-induced, biochemical reactions in specialized magnetoreceptor molecules in the avian retina. Natural skylight reaching these receptors is always directional and to some degree polarized, which has largely been neglected in biophysical models and behavioral experiments on the magnetic compass sense. Training zebra finches in a spatial orientation assay, we show that overhead polarized light modulates radical pair-based magnetic compass orientation. The magnetic compass is only operational when overhead polarized light is aligned parallel, but not perpendicular, to the magnetic field. These findings reveal fundamentally new properties of the light-dependent magnetoreceptor that significantly advance our understanding of how birds, and animals in general, perceive the Earth’s magnetic field.

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parts of the avian retina will therefore receive light of different degrees and angles of polarization, depending on their location in the retina. Cryptochromes, like all photopigments, are linearly dichroic and, thereby, intrinsically polarization sensitive. The isoalloxazine ring of the light-responsive chromophore in cryptochromes, the flavin adenine dinucleotide, preferentially absorbs light polarized parallel to the ring formation (13, 28). Consequently, the population of cryptochrome receptors with their transition dipoles aligned parallel to the e-vector of light will be preferentially excited, i.e., polarization selected (13, 23, 24). Recent models suggest that partially or fully polarized light enhances the photoselection effects on the radical-pair magnetoreceptors described above (13). Assuming that the lens, ocular media, or retinal tissue in the avian eye do not significantly depolarize the incoming light before reaching the magnetoreceptor molecules, the light-dependent magnetic compass is therefore expected to be based on a photo- and polarization-selected population of magnetoreceptors whose signaling state depends on the relative alignment of the receptor molecules with polarized light and the magnetic field (13, 23, 24). Magnetic compass orientation in birds and other animals is, therefore, expected to be influenced by polarized

light aligned at different angles to the magnetic field. Alternatively, or additionally, a cryptochrome-based reception system could be involved in polarized light reception and mediate polarized light information (29, 30). Sensitivity to polarized light is well understood in invertebrates (31), but little is known about how vertebrates, including birds, sense polarized light (30, 31). There are no obvious anatomical structures in the avian retina specialized for polarized light reception, and no viable theory exists on how birds, and most other vertebrates, can perceive polarized light (32). The functional and physiological prerequisites for a radical pair-based magnetic compass could in principle also apply for a polarized light receptor, which suggests that polarized light and magnetic compass reception could be based on a similar, or possibly the same, receptor mechanism in at least some responses to these two types of stimuli.

To investigate putative interactions between polarized light and the magnetic compass, we developed a spatial orientation assay and trained zebra finches (*Taeniopygia guttata*) to locate a hidden food reward in a four-arm plus maze by using magnetic compass and/or overhead linearly polarized light cues (Fig. 1A and Methods).

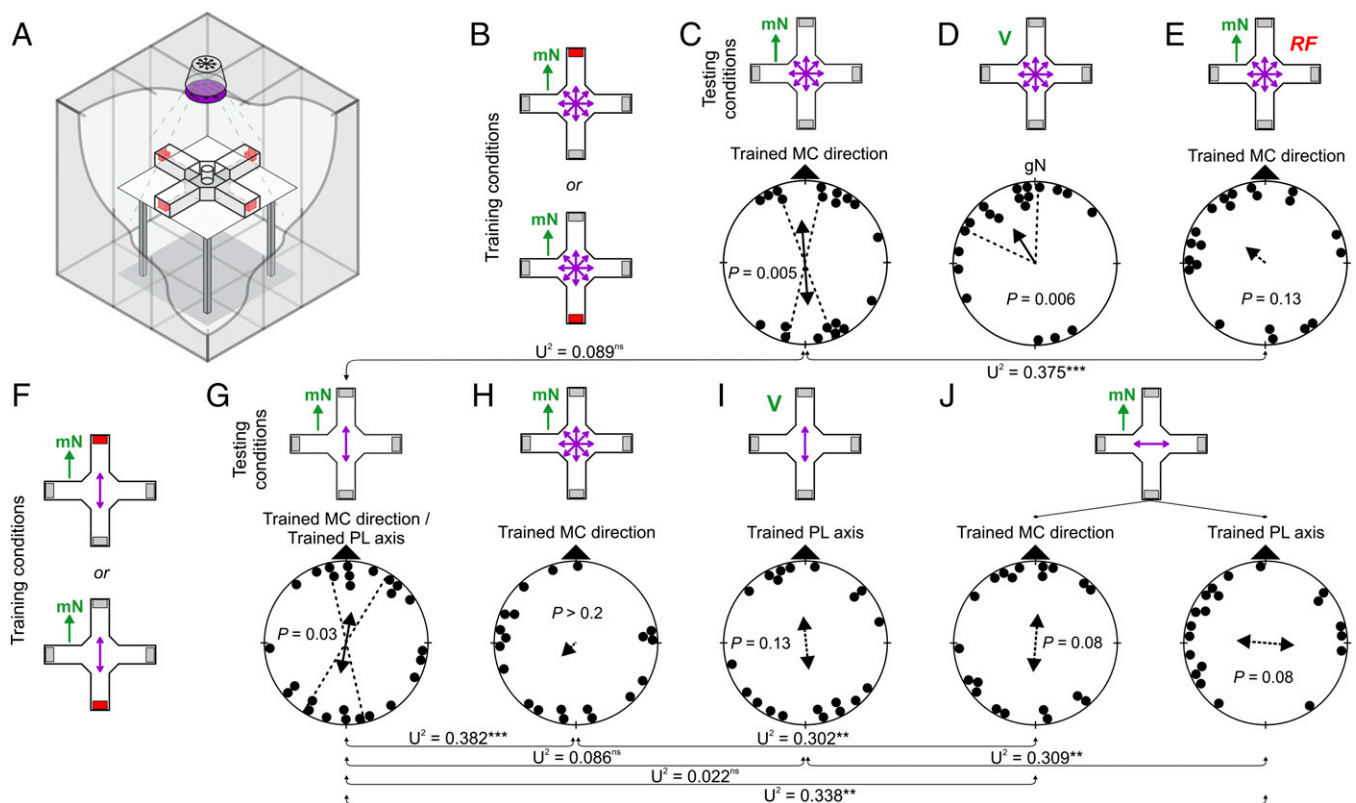


Fig. 1. (A) Illustration of the experimental setup. The plus maze is centered on a wooden table inside the magnetic coil (Merritt design). At the end of each of the four arms, the food reward (during training trials only) is hidden in one of the four red trays. The cylinder in the center of the maze shows the release device from where the birds are remotely released to explore the maze. The unpolarized light source is centered above the maze. Linear polarizers and/or depolarizers can be inserted in a filter holder just below the light source. (B–E) Orientation of zebra finches trained to relocate a food reward in the spatial orientation assay using magnetic compass cues. (B) Individual birds were trained to mN (Top) or mS (Bottom) under unpolarized light. Orientation of birds tested under the training condition (C), in a vertical magnetic field (V) without any directional magnetic information (D), and in the presence of a 1.4 MHz RF field at 80 nT (RF) (E). (F–J) Orientation of zebra finches trained to relocate a food reward using magnetic compass cues in the presence of overhead polarized light aligned parallel to the magnetic field. (F) Individual birds were trained to either mN or mS, with the e-vector of overhead polarized light (violet double-headed arrow) aligned parallel to the magnetic field. Orientation of birds tested in the presence of both cues (training condition) (G), in the presence of magnetic compass cues only (H), in a vertical magnetic field with access to polarized light only (I), and with magnetic compass and polarized light cues aligned perpendicular to each other (J). Maze illustrations: Green arrows with mN symbolize training or testing in the presence of an Earth-strength magnetic field. The gray boxes at the end of the maze indicate the food trays, with the red tray containing the reward (training only). The four violet double arrows in the center of the maze indicate unpolarized light. Circular diagrams: Each dot represents the orientation of one bird; the arrows give the mean orientation of the group of birds; solid arrows with two dotted lines indicate significantly orientated groups ($P < 0.05$, Rayleigh test) with 95% CI; dashed arrows indicate nonsignificant distributions; P values of the Rayleigh test are given inside each circular diagram. MC, magnetic compass; PL, polarized light. Watson U^2 test statistics are given for comparisons between experimental groups: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, $P \geq 0.05$. For detailed statistics, see Tables S1–S3.

Results and Discussion

Zebra Finches Use a Radical Pair-Based Magnetic Compass for Spatial Orientation. Zebra finches trained to relocate a food reward in the spatial orientation assay using directional magnetic compass information learned the task rapidly, in only three to four training trials (Fig. 1*B* and Table S1) (33). Birds trained to find the reward at either magnetic north (mN) or magnetic south (mS) were significantly oriented along the trained magnetic compass axis when subsequently tested in the presence of a magnetic field aligned in one of four topographic directions [$P < 0.05$, confidence interval (CI) test; Fig. 1*C*; see *SI Results and Discussion* for discussion of axial orientation]. When tested in a vertical magnetic field, the birds oriented toward topographic northwest (Fig. 1*D*) thus likely resorted to a fixed topographic response in the absence of trained directional cues. To determine which magnetoreception mechanism was involved in this learned response, we tested the zebra finches in the presence of a weak radio-frequency (RF) electromagnetic field. The use of low-intensity, oscillating RF fields at 0.1–10 MHz (intensity $< 1 \mu\text{T}$) is the most powerful tool to test for an involvement of a radical-pair mechanism in the primary magnetoreception process (19, 34). RF fields have been shown to influence the interconversion between the singlet and triplet excited states of the radical pairs and, thereby, alter or eliminate the effects produced by the Earth's magnetic field (19, 34). Consistent with predictions from the radical-pair theory, zebra finches tested in the presence of such RF fields at the Larmor frequency (1.4 MHz; intensity 80 nT) were disoriented (Fig. 1*E*). The failure to exhibit consistent orientation to the magnetic field in the presence of the RF field agrees with previous studies (19, 20, 35, 36) and provides the critical evidence that a radical-pair mechanism is involved in the trained magnetic orientation response shown by the zebra finches in the spatial orientation assay.

Interaction Between Magnetic Compass and Polarized Light Cues. To investigate putative interactions between polarized light and magnetic compass reception, we trained zebra finches to magnetic compass cues in the presence of overhead polarized light aligned parallel to the horizontal component of the magnetic field (Fig. 1*F* and Tables S2 and S3). Birds tested under the training condition were significantly oriented along the trained magnetic compass/polarized light axis ($P < 0.05$, CI test; Fig. 1*G*), comparable to the responses of birds trained and tested to magnetic compass cues under unpolarized light (Fig. 1*C*). In contrast, birds trained in the presence of both cues were not significantly oriented when tested under either cue presented alone during the probe trial (Fig. 1*H* and *I*). The orientation of the birds tested for magnetic compass orientation under unpolarized light (Fig. 1*H*) was significantly different from the control group (Fig. 1*G*), suggesting that the birds were not able to orient with the magnetic compass anymore. Birds tested in the presence of the overhead polarized light axis in a vertical magnetic field (Fig. 1*I*) showed a tendency to orient

along the trained polarized light axis, similar to the control group (Fig. 1*G*). Interestingly, when tested with the two cues aligned perpendicularly to each other, the birds tended to orient along the trained magnetic compass axis, but perpendicular to the polarized light axis (Fig. 1*J*). The difficulty of the birds trained to magnetic compass cues in the presence of overhead polarized light aligned parallel to the magnetic field to orient with access to only one of the two cues was surprising. It suggests one of the following explanations: (i) magnetic compass and overhead polarized light cues are two independent compass cues that have to be recalled together when learned together, (ii) changes in the polarization state of the overhead light alter the visual perception of the maze to such a degree that the birds become confused or create secondary cues that the birds could use for orientation, or (iii) overhead polarized light directly interacts with the magnetic compass and modulates how the birds perceive the magnetic field.

No Evidence for Overhead Polarized Light as Independent Compass Cue.

If the birds learned the spatial orientation task using two independent directional cues that both had to be available to solve the task (explanation 1), we would expect both the magnetic field and overhead polarized light to be independent compass cues. The avian magnetic compass is clearly an independent compass and functional without polarized light. Our zebra finches were able to learn to orient to magnetic compass cues in the maze under unpolarized light (Fig. 1*C*; see also Fig. 3*B* and *F* below). This finding is further supported by numerous experiments testing magnetic compass orientation in birds under fully depolarized light (e.g., refs. 1 and 18–22). It is unclear, however, whether birds use overhead polarized light as an independent compass cue. There is convincing evidence that birds use directional information from the skylight polarization pattern for orientation and compass calibration (reviewed by refs. 30 and 37). It has been suggested that polarized light cues from different parts of the sky have different functions (30, 38, 39). We have shown that migratory songbirds calibrate their magnetic compass exclusively with information from the vertically aligned e-vector near the horizon at sunrise and sunset, and not with polarized light information from the zenith (40, 41). Sunrise/sunset polarized light cues from the lower half of the sky are suggested to be used as compass cues to determine the departure direction (42, 43). It is not known, however, whether birds use overhead polarized light information near the zenith for sky compass orientation and whether they can perceive polarized light at times of the day other than sunrise and sunset (25, 26, 30).

To test whether birds can use an overhead polarized light axis as an independent orientation cue, we trained zebra finches to find a food reward in the two trays along the e-vector axis of overhead polarized light (Fig. 2*A* and Table S4). Despite repeated training, however, the birds did not consistently orient with respect of the trained polarized light axis (Fig. 2*B*). They seemed to be unable to

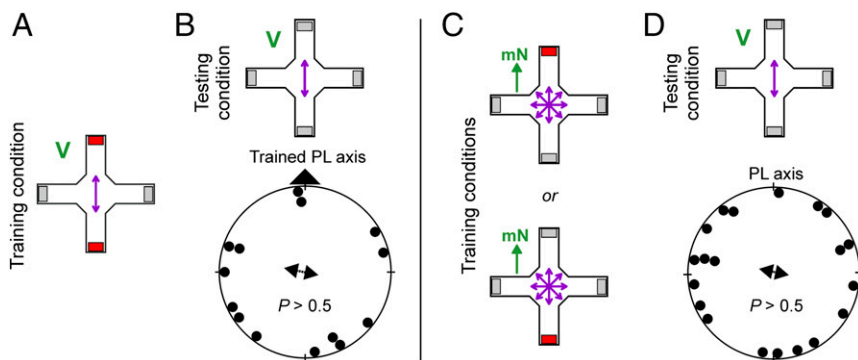


Fig. 2. Orientation of zebra finches trained to relocate a food reward using overhead polarized light (A and B) or magnetic compass cues (C and D). (A) Individual birds were trained in a vertical magnetic field to 100% overhead polarized light. Both maze arms along the e-vector axis contained rewards. (B) Orientation of birds tested under the training condition. (C) Individual birds were trained to mN (Top) or mS (Bottom) under unpolarized light. (D) Orientation of birds tested in a vertical magnetic field in the presence of polarized light. For explanations, see Fig. 1; for detailed statistics, see Tables S4 and S5.

use the e-vector alignment for orientation. It is therefore unlikely that polarized light cues near the zenith provide independent directional information for compass orientation. The skylight polarization compass and calibration reference sense(s) thus appear to respond uniquely to e-vectors of light from the region of sky at intermediate elevations and/or to vertical e-vectors near the horizon. The disorientation of the zebra finches trained to magnetic compass cues in the presence of polarized light aligned parallel to the magnetic field and tested under either one of the two cues separately (Fig. 1 *H* and *I*) can therefore not be explained by two independent compass cues that have to be recalled together when learned together (explanation 1).

No Innate Orientation Response to Polarized Light or Change in Visual Perception of Maze by Polarized Light. If the overhead polarized light axis does not provide compass information, could it change the visual environment in the maze or introduce light artifacts instead (explanation 2)? The weak alignment along the polarization axis shown by the birds trained to magnetic compass and polarized light cues and tested under polarized light cues in a vertical magnetic field (Fig. 1*I*) may be explained by an innate alignment response along the polarization axis or by light artifacts produced by differential reflection of the polarized light on the maze. However, the zebra finches did not show any innate preference for a specific polarization axis, as demonstrated by the random orientation of birds trained to magnetic compass cues (Fig. 2*C* and [Table S5](#)) and then tested in the presence of overhead polarized light in a vertical magnetic field (Fig. 2*D*). The total lack of orientation suggests that the birds did not use any light intensity artifacts to orient in the

maze, or that the polarized light generated a response pattern that the birds mistook for that generated by the magnetic field. The finding that the birds tested with the two cues aligned perpendicular to each other (Fig. 1*J*) tended to orient perpendicular to the polarized light axis further argues against an experimental artifact. The weak alignment of the birds along the polarization axis in Fig. 1*I* could instead be due to a calibration or transfer of information during training between the magnetic compass and polarized light cues in the lower part of the surroundings close to the maze arms.

There is to date no evidence that birds have true polarization vision (44, 45), i.e., that they can differentiate the angle of the e-vector of polarized light independently. We were unable to train zebra finches to polarized light stimuli presented on modified liquid crystal display screens in a two-choice conditioning experiment (45). Also, in control experiments where we trained zebra finches to color cues in the maze, we found no significant differences between birds tested under different alignments of overhead polarized light (Fig. S1 and [Tables S6](#) and [S7](#)). Thus, it is unlikely that the changes in the polarization state of the overhead light altered the birds' visual perception of the maze to such a degree that the birds became disoriented or created secondary cues that they could use for orientation (explanation 2).

Direct Interaction of Polarized Light with the Primary Magnetic Compass Receptor. If overhead polarized light does not provide directional information (explanation 1) and the behavior of the birds in Fig. 1 can be explained by neither an innate preference for a polarization axis nor by artifacts (explanation 2), could polarized light instead play a role in radical pair-based

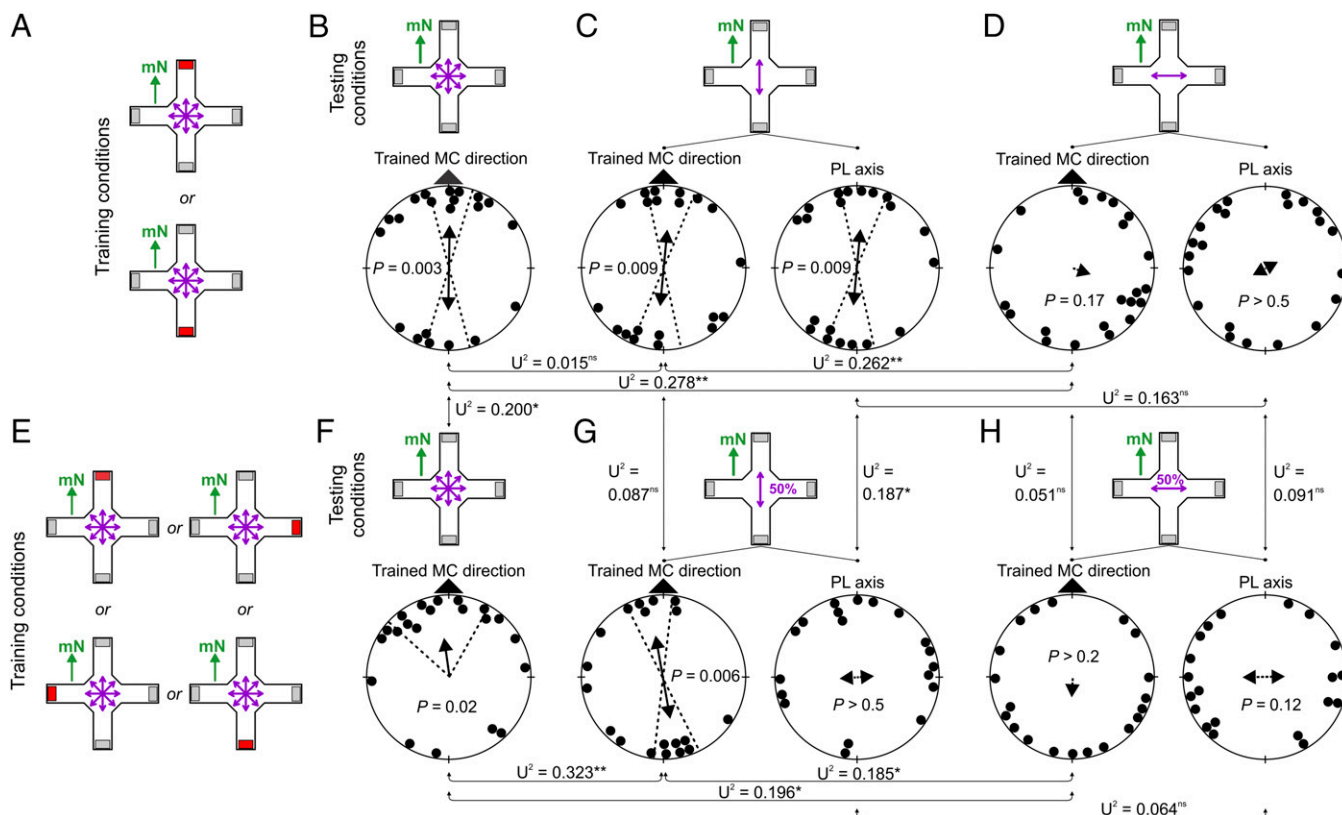


Fig. 3. Orientation of zebra finches trained to relocate a food reward by using magnetic compass cues. (A) Individual birds were trained to mN (Top) or mS (Bottom) under unpolarized light. Orientation of birds tested under the training condition (B), in the presence of 100% overhead polarized light aligned parallel to the magnetic field (C), and with 100% overhead polarized light aligned perpendicular to the magnetic field (D). (E) Individual birds were trained to mN, mS, mE, or mW under unpolarized light. Orientation of birds tested under the training condition (F), in the presence of overhead 50% polarized light aligned parallel to the magnetic field (G), and with 50% overhead polarized light aligned perpendicular to the magnetic field (H). For explanations see Fig. 1; for detailed statistics, see [Tables S8–S11](#).

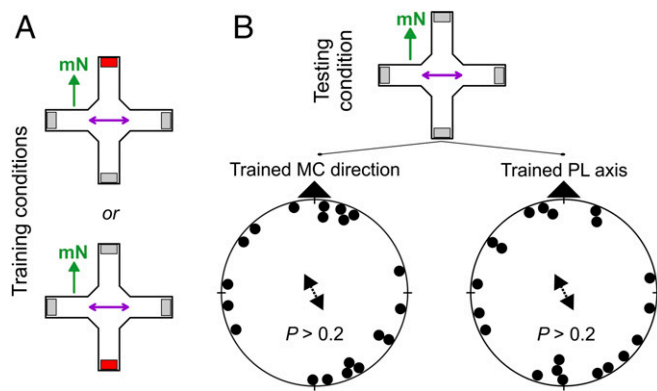


Fig. 4. Orientation of zebra finches trained to relocate a food reward by using magnetic compass cues in the presence of overhead polarized light aligned perpendicular to the magnetic field. (A) Individual birds were trained to mN (Top) or mS (Bottom) with overhead polarized light aligned perpendicular to the magnetic field. (B) Orientation of birds tested under the training condition. For explanations, see Fig. 1; for detailed statistics, see Table S12.

magnetoreception (explanation 3)? To test this hypothesis, we used zebra finches that had never been trained to polarized light. We trained these birds to magnetic compass cues under unpolarized light (Fig. 3A and Tables S8 and S9) and tested them in the presence of overhead polarized light aligned either parallel or perpendicular to the magnetic field (Fig. 3B–D). The behavior of the birds in the different testing conditions was strikingly different. The control group that was tested for magnetic compass cues under unpolarized light was oriented along the trained magnetic field axis (Fig. 3B; $P < 0.05$, CI test), comparable to the control group in Fig. 1C ($P > 0.2$, $U^2 = 0.07$, Watson U^2 test). Birds tested with polarized light aligned parallel to the magnetic field were likewise well oriented along the trained magnetic compass/polarized light axis (Fig. 3C; $P < 0.05$, CI test), suggesting that the parallel alignment was not affecting magnetic compass reception. However, the group of birds tested in the presence of perpendicularly aligned polarized light showed no significant pattern of orientation (Fig. 3D), indicating that the perception of the magnetic field had changed to such a degree that made magnetic compass information unreadable or uninterpretable.

Under natural conditions, the degree of polarized skylight is generally less than 80% (25–27). To test whether the observed effects of polarized light on the magnetic compass were present also at lower degrees of polarization, we repeated the experiments by using 50% instead of 100% polarized light. We trained birds under unpolarized light to relocate the food reward by using magnetic compass information at mN, mS, magnetic east (mE), or magnetic west (mW) (Fig. 3E and Tables S10 and S11). Birds subsequently tested under the training condition were significantly oriented toward the trained magnetic direction (Fig. 3F; $P < 0.05$, CI test; see *SI Text* for discussion of unimodal response). Birds tested in the presence of 50% polarized light aligned parallel to the magnetic field were significantly oriented along the trained magnetic compass axis (Fig. 3G; $P < 0.05$, CI test), comparable to the response observed under 100% polarized light (Fig. 3C). When tested with the axis of 50% polarized light aligned perpendicular to the magnetic field, the birds were not able to orient anymore (Fig. 3H), as has been observed under 100% polarized light (Fig. 3D).

Polarized Light-Sensitive Magnetic Compass. The differential magnetic compass response shown by zebra finches tested in the presence of polarized light aligned parallel (Fig. 3B and C) or perpendicular (Fig. 3F and G) to the magnetic field demonstrates that overhead polarized light affects light-dependent magnetic compass orientation and, thereby, changes the birds' perception of the magnetic field. The birds were equally well oriented under unpolarized as under fully or

partially polarized light with the e-vector axis aligned parallel to the horizontal component of the magnetic field (Figs. 1C and 3B, C, F, and G). However, birds tested with the two cues aligned perpendicularly to each other did not seem to be able to properly read the magnetic compass information (Figs. 1J and 3D and H). Thus, polarized light aligned perpendicular to the magnetic field appears to directly interfere with the primary magnetoreception of the light-dependent magnetic compass. If true, we would expect birds not to be able to learn to orient by using their magnetic compass when trained with overhead polarized light aligned perpendicular to the magnetic field. Indeed, zebra finches trained and tested for magnetic compass orientation in the presence of polarized light aligned perpendicular to the magnetic field were totally disoriented (Fig. 4 and Table S12).

Magnetic compass orientation was affected in our zebra finches by overhead polarized light not only when polarization was maximal (100%), but also when only 50% of the light was polarized. Thus, the magnetic compass might be affected by overhead polarized light also under natural conditions. However, the degree of skylight polarization is $\geq 50\%$ only under optimal weather conditions and close to sunrise and sunset when the e-vector is aligned roughly along the geographic north–south axis (25–27). An effect would therefore only arise in geographic areas with magnetic declinations diverging significantly from 0° and/or at high latitudes close to summer and winter solstice, when the sun rises and sets close to geographic north/south. If magnetic compass orientation is impaired by perpendicularly aligned polarized light of $< 50\%$, it would affect animals primarily near solar noon, when the e-vector near the zenith is aligned along the geographic east–west axis. In theory, the interaction of polarized light with the magnetic compass could be a mechanism to weaken the magnetic modulation pattern and, thereby, minimize a potential interference with the visual system during times of day when vision is crucial for other tasks, like e.g., foraging or predator detection. Natural selection is likely to have influenced the design of the magnetic compass to reduce the confounding effects of the varying e-vector alignments of overhead polarization to a minimum, while using the advantages of the photo- and polarization selection effects (see below; ref. 13).

The effects of overhead polarized light on magnetic compass orientation demonstrated here reveal a fundamentally new property of the light-dependent, radical pair-based magnetic compass that has hitherto largely been neglected. Our findings provide convincing evidence that the primary magnetoreceptor is photo- and polarization selective, and thereby provide the foundation for a magnetic compass based on light-induced rotational order, as suggested by recent biophysical models (13, 24). This property relaxes the requirement for an intrinsic rotational order of the receptor molecules (as long as rotational motion is restricted) and opens for putative cryptochrome magnetoreceptors distributed in any, also nonrandomly oriented, cells in the avian retina (13). Similar effects are expected to occur also in other organisms orienting with a light-dependent magnetic compass based on radical-pair reactions. Our findings thereby add a new dimension to the understanding of how not only birds, but animals in general, perceive the Earth's magnetic field.

Methods

We trained female and male zebra finches, at least 6 mo of age, to use directional magnetic compass and/or polarized light cues to locate a hidden food reward in a visually symmetric four-arm (plus) maze (Fig. 1A). The maze was centered in a magnetic coil (Merritt design), thus the magnetic field could be directed toward any of the four maze arms [mN at geographic north (gN), geographic south (gS), geographic east (gE), or geographic west (gW)]. Individual birds were trained to find a food reward at the end of one of the maze arms under one of three training conditions (for example, see Fig. S2): (i) birds were trained to either mN or mS (Figs. 1B, 2C, and 3A) or to mN, mS, mE, or mW (Fig. 3E) under unpolarized light; (ii) birds were trained to either mN or mS in the presence of overhead polarized light aligned parallel (Fig. 1F) or perpendicular (Fig. 4A) to the horizontal component of the magnetic field; or (iii) birds were trained along an overhead polarized light axis, i.e., they were rewarded in the two trays on either side of the polarized

light axis, in a vertical magnetic field (Fig. 2A). Birds that successfully passed the training trials were tested in a probe trial without food reward. They were allowed to search the maze for 90 s, and their movement was tracked with a custom-made video tracking program that automatically counted the number of frames that the bird spent in each of the four arms. The orientation of an individual bird was calculated from the time (number of frames) spent in each of the four maze arms during the 90-s trial. The individual mean orientations for birds tested in the presence of a magnetic field were then recalculated relative to mN (0°, taking into consideration that different individuals were tested in one of four magnetic fields), and relative to the trained magnetic compass direction (correcting for whether a bird was trained to mN, mS, mE, or mW; Fig. S2). Similarly, the individual mean orientation of birds tested in the presence of polarized light was recalculated relative to the alignment of the polarized light axis during the probe trial, and relative to the trained polarized light axis (where applicable), whereby the north and east end of the polarization axis was set to 0°.

For each experimental condition, the mean orientation of the group of birds was calculated by using vector addition of the individual mean directions, disregarding the individual mean vector lengths. For all groups, we compared whether a unimodal or axial distribution best fitted the orientation data by calculating the mean vector length for the two distributions. To calculate the axial distributions, we doubled the individual mean angles (46). For the distribution that best described

the data, i.e., the one with the larger mean vector length, the Rayleigh test was performed to test for significance (46). We used the CI test to examine whether the groups of birds were oriented relative to the trained magnetic direction or polarized light axis, i.e., whether the trained direction was included in the 95% CI of the distributions of birds in significantly oriented groups (46). Watson U^2 tests were used to test for differences between experimental groups (46). See *SI Methods*, Fig. S3, and Table S13 for more details. All experiments were carried out in accordance with ethical permission from the Malmö-Lund Animal Ethics Committee, permits M 176-08, M 158-11 and M 423-12.

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