

Polarized Light Cues Underlie Compass Calibration in Migratory Songbirds

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Migratory songbirds use the geomagnetic field, stars, the Sun, and polarized light patterns to determine their migratory direction. To prevent navigational errors, it is necessary to calibrate all of these compass systems to a common reference. We show that migratory Savannah sparrows use polarized light cues from the region of sky near the horizon to recalibrate the magnetic compass at both sunrise and sunset. We suggest that skylight polarization patterns are used to derive an absolute (i.e., geographic) directional system that provides the primary calibration reference for all of the compasses of migratory songbirds.

Studies of migratory songbirds provide evidence that they use multiple compasses (1–5), but the integration of these multimodal directional cues is poorly understood. Although many species of songbirds have been shown to have an innate sense of migratory direction derived from magnetic (6–8) and/or celestial (9–11) cues, these compass systems are “recalibrated” when exposed to conflicting directional information. Such conflicts occur naturally when magnetic declination changes; this change is especially pronounced at high latitudes (12). When cue availability changes with time of day and/or weather conditions, avoiding navigational errors requires that information from all of the compasses be calibrated with respect to a common reference (13). Here, we show that the primary calibration reference is derived from horizon-polarized light cues at sunrise (SR) and sunset (SS).

Research on the integration of compass information by migratory songbirds has focused on experiments in which birds were given conflicting directional information from two or more cues to determine which of the cues is given greater saliency and whether the conflict results in recalibration of one or more of the compass systems. Such experiments have produced variable and sometimes contradictory findings. Most studies suggest that birds give precedence to celestial light cues and use these cues to recalibrate the magnetic compass before the onset of migration (6, 7, 14–16) but that the reverse is true once birds begin migration; that is, the magnetic compass takes precedence over and is used to calibrate celestial light cues (17–19). However, two studies demonstrated recalibration of the magnetic compass with respect to SS cues during migration (i.e., the cue hierarchy normally observed during the premigratory period) (20, 21). In a recent re-

view (13), we found that recalibration of the magnetic compass with respect to SS (or SR) cues occurred during both the premigratory and migratory periods when birds exposed to conflicting information were able to see celestial polarized light cues from the horizon sky. In studies carried out during migration that failed to show magnetic compass recalibration, birds were exposed to cue conflicts in orientation cages/funnels that blocked a view of the sky near the horizon (13). When deprived of polarized light cues from this region of sky, birds gave precedence to magnetic cues and secondarily calibrated other celestial cues (e.g., star patterns and/or overhead polarized light cues) with respect to the magnetic field (22–29).

To clarify the role of polarized light cues in calibration of the magnetic compass, we tested whether wild-caught Savannah sparrows, *Passerculus sandwichensis*, recalibrated their magnetic compass when exposed to conflicting magnetic and polarized light cues near the horizon at SR or SS. We captured juvenile and adult birds in the Yukon Delta National Wildlife Refuge, Alaska, during autumn 2005. The birds were held indoors under the natural photoperiod without access to natural visual cues (30). All orientation experiments were started at around SS and carried out indoors in the ambient magnetic field in the absence of celestial cues, thus requiring the birds to use their magnetic compass for orientation (30). The magnetic orientation of individual birds selected for experimental exposures is shown in Fig. 1B (see also table S1). They were given a single exposure for 60 min around SR or SS to a cue conflict between the ambient magnetic field and an artificial polarization pattern rotated by $\pm 90^\circ$ relative to the natural polarization pattern at that time of day (Fig. 1C). During exposure, the birds had a full view of the surroundings, including the horizon, through the polarization filters that produced the artificial pattern (30).

After exposure to the cue conflict, the magnetic compass orientation of the birds was again tested indoors. The distribution of bearings was indistinguishable from random (SR, mean bear-

ing or axis $\alpha = 138^\circ/318^\circ$, $r = 0.08$, $P = 0.82$, $N = 30$; SS, $\alpha = 309^\circ$, $r = 0.17$, $P = 0.59$, $N = 20$; Fig. 1D and table S1) and was significantly different from the birds' initial responses (Fig. 1B; nonparametric two-sample Watson U^2 test: SR, $U^2 = 0.33$, $P < 0.005$; SS, $U^2 = 0.42$, $P < 0.001$). The absence of significant clustering of bearings after cue-conflict exposure suggests that the birds did not orient in a consistent direction or axis relative to the magnetic field. Thus, the birds had not calibrated the magnetic compass in a fixed relationship (e.g., perpendicular or parallel) to the polarization pattern or to the Sun's position, which was visible to some birds during the cue-conflict exposure (Fig. 1, D and E, open symbols; table S1). However, when each bird's response after cue-conflict exposure (Fig. 1D) was plotted as a deviation from its initial response (Fig. 1B), the deviations were bimodally distributed along an axis perpendicular to their earlier response (SR, $\alpha = 85^\circ/265^\circ$, $r = 0.54$, $P < 0.001$, $N = 30$; SS, $\alpha = 94^\circ/274^\circ$, $r = 0.58$, $P < 0.001$, $N = 20$; Fig. 1E and table S1).

These findings indicate that the birds had shifted their orientation relative to the magnetic field by $\pm 90^\circ$, corresponding to the rotation of the artificial polarization pattern relative to the natural pattern at the same time of day (i.e., SR or SS; Fig. 1E). The alternative hypothesis that calibration of the magnetic compass occurs only at SS or only at SR is excluded by the data (Fig. 1E, triangles outside circle) (30). Moreover, both juvenile and adult birds recalibrated their magnetic compass (adult birds were exposed and tested only at SR) (30) (table S1). Birds that recalibrated the magnetic compass at SR subsequently did so again at SS, and vice versa (30) (table S1).

A small sample of birds exposed to a rotated polarization axis that did not include the horizon exhibited orientation that was indistinguishable from their responses before exposure, indicating that the magnetic compass had not been recalibrated (fig. S1E and table S2) (30).

Our findings support the following conclusions: (i) The magnetic compass is recalibrated with respect to polarized light cues at both SR and SS; a conflict between magnetic and polarized light cues at either time of day resulted in recalibration of the magnetic compass. (ii) This recalibration occurs both before [as shown by previous investigators, reviewed in (13)] and during migration, and in both juvenile and adult birds. (iii) A view of the polarization patterns from the sky near the horizon is required for magnetic compass recalibration. Thus, the failure to observe magnetic compass recalibration in many studies carried out during migration is probably the result of exposure to the cue conflict in cages/funnels that obscured the natural horizon (13). In conjunction with earlier work showing that Sun and star compass calibrations are secondarily derived from magnetic and polarized light cues (23, 24, 31), these

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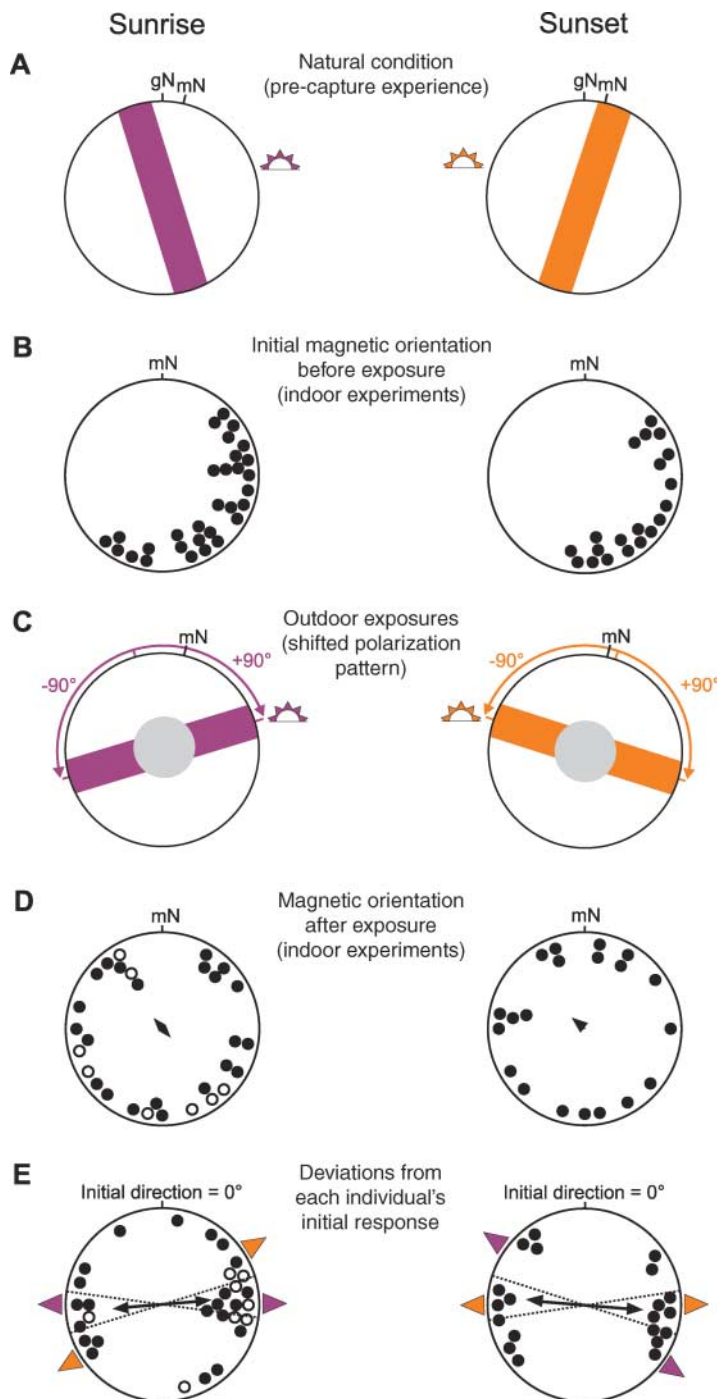


Fig. 1. Magnetic orientation of Savannah sparrows exposed to polarization pattern shifted $\pm 90^\circ$ at sunrise (SR; left) and sunset (SS; right). (A and C) 360° view of sky under natural and experimental conditions (gN, geographic north; mN, magnetic north). Purple and orange bars indicate mean position of band of maximum polarization (BMP) at SR and SS, respectively; gray zones indicate areas of sky not visible during exposure. (A) Natural relationship between SR/SS celestial cues and geomagnetic field. (C) Alignment of $\pm 90^\circ$ shifted polarization axis during exposure to cue conflict. (B and D) Magnetic orientation of birds tested indoors, plotted relative to mN = 0° . Open symbols denote birds for which the disk of the Sun was visible during exposure. Arrowheads show mean bearing or axis; length (measure of concentration) is drawn relative to the radius of the circle = 1. (B) Orientation of birds selected for exposure; (D) magnetic orientation after exposure. (E) Deviations from each individual's initial response before exposure [initial direction (B) of each individual set to 0°]. Arrows show mean bearing or axis; dashed lines give 95% confidence intervals (38); triangles outside circles give predicted responses for a $\pm 90^\circ$ shift in BMP relative to the natural SR (purple) or SS (orange) position. See (30) and table S1.

findings suggest that horizon polarization patterns at SR and SS provide the primary calibration reference for all the compass systems of migratory songbirds.

At SR and SS, the band of maximum polarization (BMP) passes directly through the zenith (32, 33) and, along with the e-vector (electrical vector) of polarized light, is aligned vertically on the horizon (30) (fig. S2, A and B). In contrast to Sun position, therefore, the intersections of the BMP with the horizon at SR and SS are independent of topography (i.e., horizon height). In addition, because the BMP and e-vector are vertically aligned only at SR and SS, their use as a calibration reference would not require a time compensation mechanism (34). Averaging the intersections of the BMP with the horizon during a successive SR and SS would enable migratory birds to derive an absolute reference system that is “fixed” with respect to the north-south meridian at any location on Earth and thus independent of latitude and time of year (30, 34) (fig. S2C). Periodic updating of the relationship between the polarization patterns at SR and SS (i.e., their angular “split” on either side of the meridian) would make it possible to use either the SR or SS pattern to estimate the reference direction and calibrate other compass systems (30) (fig. S2, D and E).

Changes in latitude and time of year produce opposite shifts in the alignments of the BMP at SR and SS (fig. S3). Consequently, use of the polarization pattern at either SR or SS alone as an independent calibration reference (i.e., without averaging) can result in a gradually curving migratory route that may under some conditions be adaptive (30) (fig. S3). However, such routes depend on the timing of migration and would therefore be altered by delays such as those caused by extended periods of inclement weather.

In species like our Savannah sparrows that use both SR- and SS-polarized light patterns (Fig. 1), failure to integrate the information from these two times of day would produce an unpredictable “zig-zagging” migratory path depending on whether the clear skies necessary to see the polarization pattern occurred most recently at SR or at SS (30). Thus, not only does averaging of SR- and SS-polarized light cues provide a calibration reference that is unaffected by changes in latitude and time of year, but failure to do so would decrease the accuracy and increase the distance of migration. In species that use both SR- and SS-polarized light cues to calibrate other compass systems, therefore, both curving migratory routes and abrupt changes in migratory direction associated with major topographic features (such as oceans and mountain ranges) are likely to involve secondary adaptations rather than properties of the underlying calibration reference system (12, 30, 35–37).

References and Notes

1. G. Kramer, in *Ornithologie als biologische Wissenschaft*, E. Mayr, E. Schütz, Eds. (Winter-Universitätsverlag, Heidelberg, 1949), pp. 269–283.

2. E. G. F. Sauer, *Z. Tierpsychol.* **14**, 20 (1957).
3. W. Wiltschko, R. Wiltschko, *Science* **176**, 62 (1972).
4. K. P. Able, *Nature* **299**, 550 (1982).
5. F. R. Moore, *Biol. Rev.* **62**, 65 (1987).
6. V. P. Bingman, *Behaviour* **87**, 43 (1983).
7. K. P. Able, M. A. Able, *Nature* **364**, 523 (1993).
8. P. Weindler, F. Böhme, V. Liepa, W. Wiltschko, *Behav. Ecol. Sociobiol.* **42**, 289 (1998).
9. S. T. Emlen, *Science* **170**, 1198 (1970).
10. W. Wiltschko, P. Daum, A. Fergenbauer-Kimmel, R. Wiltschko, *Ethology* **74**, 285 (1987).
11. P. Weindler, R. Wiltschko, W. Wiltschko, *Nature* **383**, 158 (1996).
12. T. Alerstam, G. A. Gudmundsson, M. Green, A. Hedenström, *Science* **291**, 300 (2001).
13. R. Muheim, F. R. Moore, J. B. Phillips, *J. Exp. Biol.* **209**, 2 (2006).
14. K. P. Able, M. A. Able, *Anim. Behav.* **39**, 905 (1990).
15. K. Prinz, W. Wiltschko, *Anim. Behav.* **44**, 539 (1992).
16. P. Weindler, V. Liepa, in *Proceedings of the 22nd International Ornithological Congress*, N. J. Adams, R. H. Slotow, Eds. (Birdlife South Africa, Johannesburg, 1999), pp. 979–987.
17. K. P. Able, *Trends Ecol. Evol.* **8**, 367 (1993).
18. S. Åkesson, *Anim. Behav.* **48**, 1379 (1994).
19. W. Wiltschko, P. Weindler, R. Wiltschko, *J. Avian Biol.* **29**, 606 (1998).
20. K. P. Able, M. A. Able, *Nature* **375**, 230 (1995).
21. W. W. Cochran, H. Mouritsen, M. Wikelski, *Science* **304**, 405 (2004).
22. W. Wiltschko, R. Wiltschko, *J. Comp. Physiol. A* **109**, 91 (1976).
23. R. C. Beason, *J. Ornithol.* **128**, 317 (1987).
24. V. P. Bingman, *Auk* **104**, 523 (1987).
25. V. P. Bingman, W. Wiltschko, *Ethology* **77**, 1 (1988).
26. W. Wiltschko, R. Wiltschko, U. Munro, H. Ford, *J. Comp. Physiol. A* **182**, 521 (1998).
27. R. Wiltschko, U. Munro, H. Ford, W. Wiltschko, *J. Avian Biol.* **30**, 56 (1999).
28. R. Sandberg, J. Bäckman, F. R. Moore, M. Löhms, *Anim. Behav.* **60**, 453 (2000).
29. R. Wiltschko, U. Munro, H. Ford, W. Wiltschko, *Anim. Behav.* **62**, 245 (2001).
30. See supporting material on Science Online.
31. J. B. Phillips, F. R. Moore, *Behav. Ecol. Sociobiol.* **31**, 189 (1992).
32. M. L. Brines, J. L. Gould, *J. Exp. Biol.* **96**, 69 (1982).
33. G. Horváth, D. Varjú, *Polarized Light in Animal Vision* (Springer, Berlin, 2004).
34. J. B. Phillips, J. A. Waldvogel, *J. Theor. Biol.* **131**, 55 (1988).
35. E. Gwinner, W. Wiltschko, *J. Comp. Physiol.* **125**, 267 (1978).
36. A. J. Helbig, P. Berthold, W. Wiltschko, *Ethology* **82**, 307 (1989).
37. T. Alerstam, in *Proceedings of the RIN05 Conference* (Royal Institute of Navigation, Reading University, Reading, UK, 2005), pp. 1–10.
38. E. Batschelet, *Circular Statistics in Biology* (Academic Press, London, 1981).
39. We thank the Swedish Polar Research Secretariat; Yukon Delta National Wildlife Refuge; B. J. McCaffery, M. Rearden, and the staff at the U.S. Fish and Wildlife Service for financial and logistic support and T. Alerstam for valuable comments on the manuscript. Permission to perform the experiments in Alaska was given by the U.S. Fish and Wildlife Service (permit 05-YDNWR-02). Supported by the Swedish Science Research Council (S.A.), a Swiss National Science Foundation postdoctoral fellowship (R.M.), and NSF grants IBN04-25712 and IBN02-16957 (J.B.P.).

Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S3

Tables S1 and S2

References

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Requirement for Coronin 1 in T Lymphocyte Trafficking and Cellular Homeostasis

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The evolutionarily conserved actin-related protein (Arp2/3) complex is a key component of actin filament networks that is dynamically regulated by nucleation-promoting and inhibitory factors. Although much is known about actin assembly, the physiologic functions of inhibitory proteins are unclear. We generated *coronin 1*^{-/-} mice and found that coronin 1 exerted an inhibitory effect on cellular steady-state F-actin formation via an Arp2/3-dependent mechanism. Whereas coronin 1 was required for chemokine-mediated migration, it was dispensable for T cell antigen receptor functions in T cells. Moreover, actin dynamics, through a mitochondrial pathway, was linked to lymphocyte homeostasis.

The integrity of the actin cytoskeletal network is critical for a diverse range of biological processes and is dynamically regulated by a cohort of actin-associated proteins. The Wiskott-Aldrich syndrome (WAS) and the suppressor of cyclic adenosine monophosphate (cAMP) receptor (SCAR) proteins promote actin nucleation and assembly via the Arp2/3 complex (1–3), whereas inhibitory proteins, which include coronin, tropomyosin, and caldesmon, oppose Arp2/3 function (4–6). The evolutionarily conserved coronin family of actin-binding proteins has been implicated in the regulation of multiple actin-mediated cellular functions, including cell migration, cytokinesis, and cell growth of *Dictyostelium discoideum* and *Saccharomyces cerevisiae* (7–12). Among the seven mammalian coronin family members,

coronin 1 (also known as corola, TACO, or p57) is preferentially expressed in cells of hematopoietic origin, where it is coexpressed with other more widely expressed coronin family members that include coronins 2, 3, and 7 (fig. S1A) (13). In mammals, coronin 1 colocalizes with F-actin surrounding phagocytic vesicles in neutrophils and macrophages and F-actin-rich membranes in activated T cells (14–16).

To investigate the physiological role of coronin 1, we generated *coronin 1*^{-/-} mice (fig. S1, B and C). No coronin 1 protein was detected in thymocytes, splenocytes, or bone marrow-derived cells isolated from *coronin 1*^{-/-} mice, and expression of coronins 2 and 3 was not altered (fig. S1D). Analysis of lymphoid tissues revealed normal segregation of T and B cells but a paucity of T cells in spleens and lymph nodes of *coronin 1*^{-/-} mice (Fig. 1A). Both CD4⁺ and CD8⁺ T cells were decreased in the blood, spleen, and lymph nodes (Fig. 1B). Naïve, but not memory/effector, splenic T cells were decreased, although both were reduced in the blood and lymph nodes of *coronin 1*^{-/-} mice. Thymic

cellularity and subpopulations were similar between *coronin 1*^{-/-} and *coronin 1*^{+/+} mice, although a small reduction in mature CD4⁺ and CD8⁺ (CD69⁺) *coronin 1*^{-/-} thymocytes was observed (Fig. 1C and fig. S1, I to J). An analysis of *coronin 1*^{-/-} mice bearing either major histocompatibility complex (MHC) class I restricted H-Y or class II restricted DO11.10 transgenic T cell antigen receptors (TCRs) revealed normal thymic development and decreased naïve T cells in lymph nodes (Fig. 1D).

The requirement for coronin in cell motility in *D. discoideum* prompted us to examine whether coronin 1 may play a role in thymic emigration and homing to secondary lymphoid organs. CD4⁺ *coronin 1*^{-/-} thymocytes demonstrated reduced spontaneous migration and transwell migration to CCL19, CXCL12, and CCL25 (Fig. 2A). Defects in chemotaxis were also observed in splenic CD4⁺ naïve and effector/memory *coronin 1*^{-/-} T cells (fig. S2B). *Coronin 1*^{-/-} T cells also demonstrated compromised migration in whole-organ thymic cultures and in vivo thymic egress (fig. S2, C and D). Lastly, adoptive transfer of differentially labeled *coronin 1*^{-/-} and *coronin 1*^{+/+} CD4⁺ thymocytes revealed ~60% decreased homing of *coronin 1*^{-/-} cells to lymph nodes (Fig. 3A). Thus, coronin 1 plays important functional roles in cell motility and chemokine-mediated homing of T lymphocytes to secondary lymphocyte organs.

Because the actin cytoskeleton is required for cellular polarization and lymphocyte migration, we analyzed the morphologic changes induced by CCL19. Whereas stimulated *coronin 1*^{+/+} T cells acquired a polarized phenotype with unipolar accumulation of talin beneath the cell membrane opposite of the uropod, *coronin 1*^{-/-} T cells failed to develop a uropod and formed multiple patch-like talin-rich

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