Published in final edited form as:

Curr Biol. 2012 January 10; 22(1): 21–27. doi:10.1016/j.cub.2011.11.026.

Flying *Drosophila* Orient to Sky Polarization

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Summary

Insects maintain a constant bearing across a wide range of spatial scales. Monarch butterflies and locusts traverse continents [1, 2], foraging bees and ants travel hundreds of meters to return to their nest [1, 3, 4], whereas many other insects fly straight for only a few centimeters before changing direction. Despite this variation in spatial scale, the brain region thought to underlie long-distance navigation is remarkably conserved [5, 6], suggesting that the use of celestial cues for navigation is a general and perhaps ancient behavioral capability of insects. Laboratory studies of *Drosophila* have identified a local search mode in which short straight segments are interspersed with rapid turns [7, 8]. Such flight modes, however, are inconsistent with measures of gene flow between geographically-separated populations [9-11], and individual *Drosophila* have been observed to travel 10 km across desert terrain in a single night [9, 12, 13] – a feat that would be impossible without prolonged periods of straight flight. To directly examine orientation behavior under outdoor conditions, we built a portable flight arena in which a fly viewed the natural sky through a liquid crystal device that could experimentally rotate the angle of polarization. Our findings indicate that flying *Drosophila* actively orient using the sky's natural polarization pattern.

Results

To observe flight orientation of *Drosophila* under a natural sky, we tethered wild type flies within a portable magnetic arena [7] with a clear ceiling equipped with a digital video camera for automatically tracking flight heading (Figure 1A). During the hour before and after sunset, we recorded the headings of flies relative to arena coordinates for 12 minutes (Figure 1B). To test whether flies oriented using celestial cues rather than some unaccounted for feature of the arena itself, we rotated the arena by 90° every 3 minutes. When the sky light reaching them was not altered by optical filters, some flies compensate for rotations of the arena, thereby maintaining a consistent heading in world coordinates (Figure 1C, Supplemental Movie 1). To quantify the flies' response to the rotation of the arena, we computed the circular mean of each animal's relative change in heading after each of the 3

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rotations. The population circular mean of these individual averages was significantly shifted in the direction opposite to the arena rotation, as expected for an animal that corrected for the angular disturbance by maintaining a real world heading. In order to determine which features of the sky flies used to accomplish this compensation, we covered the arena with a circularly polarizing filter, which eliminates the natural linear polarization pattern. In this condition, flies' headings did not shift significantly with respect to the arena upon rotation. This manipulation was motivated by a small number of studies in *Drosophila melanogaster* [14-16] and other dipteran species [17-21] indicating that flies possess a neural pathway specialized for the detection of linearly polarized light. One caveat associated with use of a circular polarizer is that it decreases the total light intensity reaching the fly and severely attenuates ultraviolet frequencies (Supplemental Figure 1). We tested if these effects could explain the flies' lack of orientation under a circular polarizer by covering the arena with two control filters: a blue bandpass filter that severely restricted the range of wavelengths reaching the fly (even more so than the circular polarizer), and a neutral density (gray) filter that diminished total light intensity by roughly the same factor as the circular polarizer. Under these conditions, flies compensated for the rotations in a manner similar to flies under unfiltered sky light, although not quite as effectively (Figure 1D). Not surprisingly, when we conducted the same experiment indoors with the arena covered by an opaque black cloth, flies were completely unable to compensate for the physical rotation of the arena.

We examined the flies' behavior for the entire duration of the experiment by computing fictive trajectories for each fly assuming an arbitrary constant forward flight speed of 0.5 m $\sec^{-1}$ and integrating the headings in world coordinates (Figure 1E). Inspection of these calculated trajectories indicates that flies under the circular polarizer followed more circuitous routes, tending to end the experiment at a shorter calculated distance from the fictive 'release point'. We quantified this effect by computing the total distance traveled under our constant flight speed assumption (Figure 1F). Flies with access to polarized sky light ended the trial significantly 'farther' from where they started than flies covered by the circular polarizer. The fictive distances covered by flies navigating under the blue bandpass filter and neutral density filter were indistinguishable from the unfiltered condition. The fictive distances traveled by flies in the dark serve as baseline measurements for the performance expected in our arena in the complete absence of visual cues. To evaluate individual fly performance, we calculated the mean heading during 24, 30-second segments for each fly. We used the Rayleigh test for uniformity [22] at the $p<.05$ level to determine if an individual managed to hold a straight course for the duration of the experiment. 12 out of 21, 13 out of 19, and 7 out of 12 flies showed stable courses in the no filter, blue filter, and gray filter conditions, respectively. Only 4 out of 21 flies under the circular polarizer and 2 out of 18 flies in the dark showed significant directional preferences under the same analysis.

Although these results suggested that flies can use polarization cues from the sky to stabilize heading, we desired a more direct test to determine whether flies will reorient when only the pattern of polarization, and no other celestial feature, changes. For these experiments we used an optoelectronic polarization switcher (Figure 2A), which rotates the plane of polarization of transmitted light by 90° when in the active, switched state. In the passive, un-switched state, the polarization of the transmitted light is not altered. In either mode, other parameters of the transmitted light such as intensity, color, and degree of polarization are unchanged by transmission through this device. To a human, who is unable to detect the polarization angle of light, the device appears as a clear glass window in both the switched and un-switched states. We first tested flies outdoors with a diffuser to block clouds or other visual features in the natural sky, but with a polarizer above the switcher to polarize the transmitted light (Figure 2B). Because there is 2-fold symmetry of such artificially polarized light, we treated the headings in this experiment as axial in subsequent analyses ($p=2$ in Supplemental Experimental Procedures equations 1 and 4). Flies exhibited course adjustments when we switched the polarization, compared to control flies for which the polarization was unswitched, as indicated by several different analyses (Figure 2, Supplementary Movie 2). First, the average autocorrelation of the time series data from all flies shows marked periodicity at the switch frequency of 0.5 cycles min^{-1} (Figure 2E). This periodicity was absent in control experiments in which the polarization was either not switched, or the polarizer was placed above the diffusing paper, ensuring that only unpolarized light reached the fly. The individual shown in Figure 2C and 2D reliably altered course in response to switching the polarization, leading to a large oscillation amplitude in its autocorrelation at the switch frequency. Other flies contributing to the average in Figure 2F showed weaker responses, resulting in a smaller average oscillation amplitude. Possible reasons for this variation across individuals are discussed below.

The influence of the rotation of the polarization angle is also manifest by a change in the angular speed averaged over all flies: immediately following the 90° rotation, the flies' angular speed increased (Figure 2F), indicating a turning response. By contrast, the averaged response of the flies in both control conditions showed no significant change in angular speed. Note that in these experiments, we would not expect to observe the same change in mean heading that we measured in the first experiment, because for a fly, interpreting the instantaneous shift of polarization by 90° as a clockwise or a counterclockwise rotation are equally valid. We also calculated the mean heading during 10, 6-second segments within each trial and compared these samples between trials for which the polarization was switched or unswitched. Using the Watson test for equal means [22], at the $p<.05$ level, 6 out of 13 flies showed differences between the trial types when the polarization was switched, as opposed to only 1 out of 14 when the polarization was not switched and 1 out of 13 when the diffuser was below the polarizer so that the incident light was unpolarized.

In the experiments described above, the presence of the diffuser served to even out gradients across the natural sky, providing a homogeneous field of light, which passed through a linear polarizer before reaching the fly. This result indicates that flies can orient using artificially polarized natural light, but it does not directly demonstrate the ability to orient using sun light that is naturally polarized by the atmosphere. In order to test flies' ability to react to a change in the orientation of naturally polarized sky light, we repeated the experiments using the optoelectronic polarization switcher, but without the diffuser and polarizer. We performed one set of control experiments in which we placed a diffuser over the arena to remove polarization cues and another in which we simply did not switch the rotator on and off. Most flies responded to the 90° rotations of the polarization angle of natural sky light with course adjustments in a manner similar to that under artificially polarized sky light (Figure 3, Supplementary Movie 3). Flies made no such adjustments when either the polarization was not switched or when the light was not polarized because of the diffuser. We observed the 2-minute periodicity in the autocorrelograms characteristic of behavioral dependence on trial type. (Note that here we did not treat the angles as axial, because in this case other cues, principally spectral and intensity gradients, were present in the sky light to disambiguate angles separated by 180°.) The individual fly in Figure 3B and 3C showed a strong response to switching the polarization and maintained a very consistent course, resulting in a larger autocorrelation of its heading compared to the population average. Flies increased their turning rate in response to switched polarization (Figure 3E and 3F), but not in the control conditions. Performing the same statistical tests as above, we found that at the $p<.05$ level, 11 out of 16 flies showed differences between the trial types when the polarization was switched, as opposed to only 3 out of 12 when the polarization was not switched and 2 out of 11 when the light was unpolarized (the diffuser was above the fly). This result was surprising, given the plethora of other cues present in sky light that the flies could potentially use to navigate, suggesting that polarization vision is an important component of the course control system in flies under a natural sky.

The data from our two experiments collected using the polarization rotator indicate that while some flies unambiguously altered their heading in response to the rotation of the polarization angle, there is a large variability in the response across flies. Whereas some flies exhibited a robust reaction, others showed no obvious response to the experimental change in polarization angle. Such behavioral variation might arise from a number of factors. Although we took efforts to perform experiments under comparable atmospheric conditions by restricting our studies to within a two hour time window each day, the intensity of light reaching the flies, the degree of polarization of that light, chromatic gradients, and other aspects undoubtedly varied from trial to trial. Thus, the actual experimental conditions in each experiment were different, and this is an inherent consequence of using a natural stimulus such as sky light. Second, unlike with studies of long distance migrants such as monarch butterflies or locusts, we have no guarantee that our subjects were actually motivated to fly straight, and some individuals may have been operating in a local search mode in which they ignored celestial cues. Third, the genetic diversity within our lab stock, descended from 200 wild-caught females, may have contributed to the differences among flies. Finally, it is worth noting that because of the physical restriction of our flight arena, the area of sky visible to the flies was rather small, extending over roughly ~35% of the dorsal rim area of the compound eye - the region thought to mediate polarization vision in insects [23, 24] – and less than 20% of their entire visual world [25]. Given these experimental constraints, together with the statistical significance of the response in population averages and in roughly 60% of all individual flies, we are confident that our results demonstrate that *Drosophila* can navigate using sky light polarization.

Discussion

Collectively, our results indicate that *Drosophila* possess the optic and neural machinery to navigate, if in a rudimentary fashion, using the pattern of sky light polarization. They can hold a straighter course when provided with a natural polarization pattern than they can when this signal is scrambled by a circular polarizer (Figure 1). When an artificial pattern of linear polarization (but naturalistic in terms of color and intensity) was shifted instantaneously by 90°, flies changed course accordingly (Figure 2). When the unaltered polarization pattern of sky light was shifted by 90° without changing its other features, flies also responded with course adjustments (Figure 3).

Central place foragers such as bees and desert ants have been the subject of intensive investigation into the role of a celestial compass in insect navigation. Among other topics, the important concepts of time compensation [3, 4], path integration [26, 27], and multisensory integration [3, 28] have been examined in detail in these organisms. A small specialized region of the eye called the dorsal rim area is thought to be critical for these behaviors in many species [23, 24], although the evidence in flies is somewhat contradictory. Flies possess a dorsal rim area which has been implicated in polarization responses [18], but prior experiments using a tethered flight arena suggest that the rest of the eye may play a role in responses to polarized light [16]. Our results do not bear directly on this discrepancy, because our sky stimulus was visible to ommatidia both within and outside the dorsal rim area. Within the dorsal rim area, photoreceptors R7 and R8, which have been proposed to underlie polarization vision, both express an opsin with a peak sensitivity in the ultraviolet. Thus, our observation of polarization dependent responses to wavelengths longer than 400 nm provides further indirect evidence for the role of other photoreceptors besides R7/8 within the dorsal rim. We cannot, however, rule out their involvement because it is possible that they exhibit some small, but functional sensitivity to the wavelengths used in our experiments. The possible existence of alternate, spectrally-distinct pathways for detecting polarized light may have contributed to the variability we measured in experiments in which UV light was attenuated by filters.

Through studies of migratory insects such as monarch butterflies and locusts, the neural circuitry that underlies polarization vision and its influence on motor behavior has begun to be elucidated. Researchers have traced the polarization vision pathway from the eye, to the central brain, to neurons arborizing in the thoracic ganglion [29-34]. This electrophysiological evidence suggests that the central complex, a series of unpaired neuropils of the central brain, plays a key role in processing polarized light. The ubiquity of this brain region along with the relevance of polarization vision to the life history of a variety of species suggests that orientation responses using polarized light may represent a rather ancient component of insect behavior [5, 6]. At first glance, the fruit fly, which is neither a central place forager nor known as a seasonal migrant, seems a strange choice of species in which to study polarization vision. Because long distance directed flights, either for migration or homing, have not been directly observed in flies, one cannot rely on innate motivation to navigate to a specific location when designing experiments. Nonetheless, a fly (or any insect for that matter) that finds itself in a resource-poor area, without observable attractive cues, faces a critical challenge. Maintaining a straight path ensures that it does not waste limited resources repeatedly traversing the same ground. Indeed, evidence suggests that several species of fruit flies, including *Drosophila melanogaster*, could fly over 10 kilometers across a desert without access to food or water [12, 13]. Given the energy resources of even a well-fed fly [35-37], this feat would only be possible by maintaining a straight heading. Because the sun is often obscured by clouds, masked by local features, or below the horizon, an alternative source of compass information – such as that available from sky light polarization - would be extremely useful for animals attempting to maintain a heading relative to global coordinates. An intrinsic compass preference would not be necessary, simply the ability to choose a heading and maintain it. Our experiments were designed to mimic this situation, and we observed that flies did indeed use sky light polarization to help maintain a steady course. The fruit fly, too often thought of without reference to its evolutionary history, thus displays another of the almost implausibly complex behaviors found in the insect world. The wealth of behavioral, physiological, and genetic tools available in *Drosophila* make it an ideal system in which to examine the open questions surrounding this behavior. Our observation of flies using celestial polarization to hold a course is a step in this direction.

Experimental Procedures

Portable magnetic arena

To examine fly behavior under a natural sky we modified the magnetic tether arena developed by Bender et al. [7]. An axially-symmetric magnetic field held the fly in place, but it was free to rotate in the yaw direction (Figure 1A). A 25.4 mm tall by 12.7 mm diameter cylindrical magnet was fixed in the center of a 152.4 mm diameter 6.4 mm thick disk of glass by another 12.7 mm diameter, 21.2 mm tall cylindrical magnet. Below, a V-shaped aperture held the pin in place above a 25.4 mm outer diameter, 12.7 mm inner diameter, 25.4 mm tall ring magnet. The walls and floor of the arena were matte gray, except for white plastic covering the ends of both magnets closest to the fly. No dark glossy surfaces, which can act as polarizers, were visible to the fly (see chapter 34 in [38]). When in the arena, a fly could view the sky through a ring-shaped window (measured from vertical: outer diameter = 58.5°, inner diameter = 30.6°), encompassing the view angles of approximately 17% of the fly's ommatidia [25]. In experiments using optical filters, we placed the filter directly above this window. We recorded videos [39] of the fly from below through the hole in the ring magnet, at either 290 or 130 fps. An infrared LED provided illumination through the same hole. Wavelengths emitted by this LED were such that it was not visible to the fly. The fly's heading was later calculated by custom machine vision analysis routines written in Python. The entire arena could be manually rotated on a bearing at its base, which was equipped with a spirit level to ensure a consistent upright orientation.

We placed each fly in the arena and filmed its heading for 12 minutes. Every 3 minutes we rotated the arena 90°. Although we attempted to make the interior of the arena radially symmetrical, this rotation controlled for any subtle intrinsic features of the arena that the flies could orient to independent of the exterior sky as well as radial inhomogeneities of the magnetic field. Each experiment was conducted in one of five conditions. In the first condition, there was no filter and only the glass window separated the fly from the sky. In the second condition, we placed a circular polarizing filter (Left Handed PFC Circular Polarizer, Aflash Photonics, Hollywood Park, TX) above the window, thereby effectively eliminating the linear polarization information from the sky. This filter also blocked wavelengths shorter than 400 nm and attenuated over half the intensity transmitted in the rest of the spectrum. In the third condition, we used a blue bandpass filter (Roscolux #74: Night Blue, Rosco Laboratories, Stamford, CT) that was more restrictive both in wavelengths and total intensity transmitted. In the fourth condition, we used a grey neutral density filter (Roscolux #398: Neutral Grey, Rosco Laboratories, Stamford, CT) to block the same amount of total intensity as the polarizing filter, but without restricting the wavelengths. Spectra of sky light transmitted through these filters are shown in Supplemental Figure S1. In the fifth condition, we tested flies indoors in total darkness, by covering the arena with a dark cloth.

Outdoor switching arena

When partially polarized light passes through a polarizing filter the intensity transmitted depends on the orientation of the filter. Because sky light is partially polarized, this resulted in changes in the global intensity pattern when we rotated the arena with the circular polarizer. We aligned the filter with its transmission axis approximately 45° to the main celestial polarization direction to alleviate this problem, but some intensity change was inevitable. We designed a second portable arena to ensure complete isolation of the effect of celestial polarization (Figure 2A). As in the first experiment, we used a magnetically tethered fly enclosed in an arena. In this arena, however, the window above the fly was an optoelectronic liquid crystal polarization rotator (Crystal Vision, Borlänge, Sweden). This device either leaves the transmitted light unchanged or it can rotate the plane of polarization by 90° (we call this mode “switched” in order to avoid confusion with a physical rotation). Changing modes does not alter the wavelength, intensity, or degree of polarization of the transmitted light. Supplemental Figure S2 demonstrates the operation of this device by displaying transmission spectra of skylight passing through it in both states when between two linear polarizers. There is some deviation from perfect 90° rotation of the polarization angle for wavelengths different from 500 nm. For experiments with the optoelectronic rotator, we used the same size magnets as the first arena, but in a slightly different configuration. The two top magnets were in contact and both were above the window. The fly tether directly contacted the window, with no bearing. We found that the magnetic field was sufficient to keep it centered in place. The resulting outer diameter of the visible window was the same as before (58.3° outward from vertical), but the inner diameter was smaller: 24.6°, viewable by approximately 19% of the fly's ommatidia [25]. The interior of the arena was painted entirely white, and its interior diameter was 50mm. The fly was illuminated by 4 infrared LEDs below an infrared pass filter painted white on top.

In the second set of experiments we covered the window of this arena with a sheet of diffusing paper that eliminated the linear polarization pattern of the transmitted light (Supplemental Figure S3). In the first condition, we placed a linear polarizing filter below the diffuser, such that light reaching the fly was artificially polarized and its polarization angle could be rotated by the polarization rotator (Figure 2B). We switched the rotator every 60 seconds for twelve minutes. In the first control condition, the filter configuration was the same, but we did not switch the polarization rotator. In the second control condition, we placed the diffuser below the polarizer, such that unpolarized light reached the fly, to control for effects of switching the rotator state.

In the third set of experiments, we used only the natural polarization pattern in sky light. The first control was again with no filter, but without switching the rotator. The second control was to cover the arena with the diffusing filter, eliminating polarization in the arena, and controlling for effects of switching (Figure 3A).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Marie P. Suver for contributing to a set of preliminary experiments and useful conversations throughout the project. This work was supported by a National Science Foundation FIBR award 0623527 (M.H.D.) and a National Institutes of Health training grant 5-T32-MH019138 (P. T. W.).

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Highlights

- A liquid crystal polarization rotator was used to study *Drosophila* flight outdoors.
- Flies maintain heading in the face of external disturbances using celestial cues.
- Rotating the angle of naturally polarized light causes compensatory turns by flies.

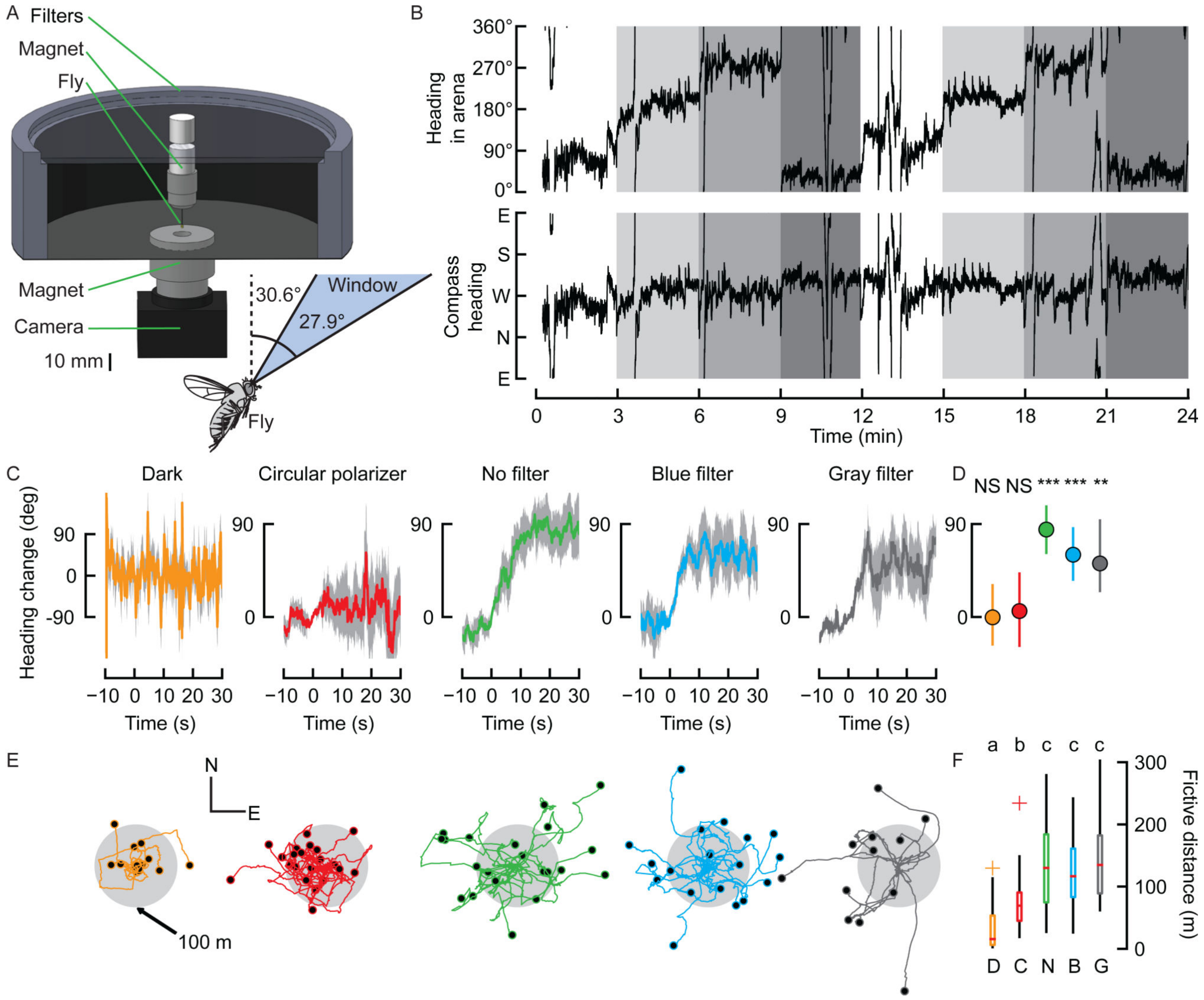

Figure 1.

Flies correct for external rotations by using polarized sky light (A) The rotating arena used in experiment 1. The fly is glued to a steel pin and suspended between two magnets. It can view the sky through a glass window (spanning the region 30.6° to 58.5° elevation from vertical, inset), above which various filters were placed. A camera below the fly records its azimuthal orientation. The entire arena was rotated about its vertical axis by 90° every 3 minutes. (B) An example trace showing 24 minutes of flight orientation in arena coordinates (above) and outside world coordinates (below). Changes in background grayscale level indicate when the arena was rotated. Only the first 12 minutes were used in subsequent analyses, in order to increase rate of data collection. (C) Circular mean (colored line) and circular variance (gray patch) of change in heading with respect to arena after a rotation at time t=0. A change of 90° would indicate perfect compensation for external rotation. For each fly, a single response was calculated by averaging its responses to all three rotations during the experiment. The mean and variance of these single fly responses are displayed. In this and subsequent panels, different experimental conditions and sample sizes (N, the total number of individual flies tested), from left to right, were as follows: orange: complete darkness (experiment conducted indoors) N = 18; red: arena covered with circular polarizer, N = 21; green: only glass window between the fly and the sky, N = 21; blue: blue bandpass filter above glass window, N = 19; gray: neutral density filter above glass window, N = 12. See also Figure S1. (D) Circular mean of change in heading between 10 and 30 seconds after rotation of arena. Bars indicate 95% confidence intervals as computed by bootstrap method in [22]. Asterisks indicate with what confidence mean is different from zero (*** p<.001, ** p<.01, NS p>.05). 95% confidence intervals include 90° for no filter and gray filter conditions, 99% confidence interval includes 90° for blue filter condition. (E) Fictive trajectories assuming constant forward flight speed of 0.5 m s^{-1} in world coordinates. Gray background circles indicate radius of 100 m. Black circles indicate position at the end of the experiment for each fly. (F) Fictive distance traveled at the end of 12 minute experiment (distance from the origin of the black dots in E). A fly orienting perfectly in one direction would ‘travel’ 360 m. Median indicated by horizontal red line, box extends from lower to upper quartile values. Vertical black lines extend to most extreme data point within 150% of the interquartile range. Outliers, defined as any points outside the range of the black lines, are shown as crosses. Lowercase letters above the plot indicate different groups at the p<.05 level as computed by the Bonferroni-corrected one-tailed Mann-Whitney U test.

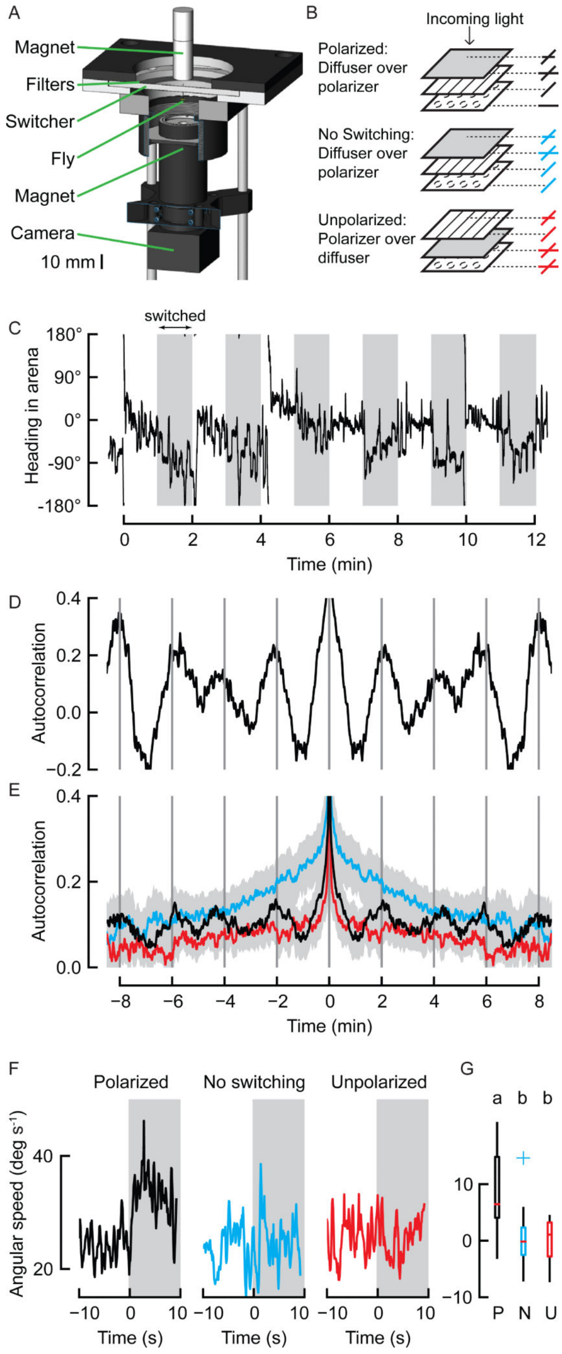

Figure 2.

Flies Turn in Response to Changing the Angle of Artificially Polarized Light. (A) Polarization switching arena. As in Experiment 1, the fly is suspended between two magnets and free to rotate about its yaw axis while being filmed from below. The glass window has been replaced by a polarization switcher, which can rotate the polarization angle of transmitted light by 90° depending on the voltage applied across it. In both rotating and unrotating states, it does not change other properties (intensity, color, or degree of polarization) of the light. See also Figure S2. Exterior angle of transparent window is roughly the same as in Experiment 1, 58.4°, interior angle is 24.6°. (B) Schematic of the three experimental conditions. Colored bars on right indicate the polarization state of the light at each level. (C) An example trace showing fly heading for 12 minutes in the Polarized condition (panel B, top), during which the polarization was unaltered for six minutes (white background) and rotated by 90° for six minutes (gray background). 0° corresponds to flying parallel to polarization axis. (D) Autocorrelation plot of headings from C. Time axis is the same as panel E. Vertical gray lines depict the lag corresponding to the switching cycle during our experiments. (E-G) Average responses for all flies. Black: trials in which the polarization was switched, sample size N=13 flies; blue: polarization was not switched, N=14; red: polarization switcher active, but diffuser below polarizer, eliminating polarization, N=13. (E) Mean autocorrelations plotted as lines, standard error of the mean in gray. (F) Mean of the flies' angular speeds after polarization was switched at time t=0. A single average response was determined for each fly by averaging its responses to all 12 switches during the experiment. The mean of these single fly responses are plotted here. Gray background indicates time after switch. (G) Average changes in angular speed. The fly's mean angular speed for 10 seconds before each switch was subtracted from the fly's mean angular speed for 10 seconds after that switch. The mean of these differences for each fly are shown in the boxplots. Boxplots were constructed as in Figure 1F. Lowercase letters above the plot indicate different groups at the $p<.01$ level as computed by the Bonferroni-corrected one-tailed Mann-Whitney U test.

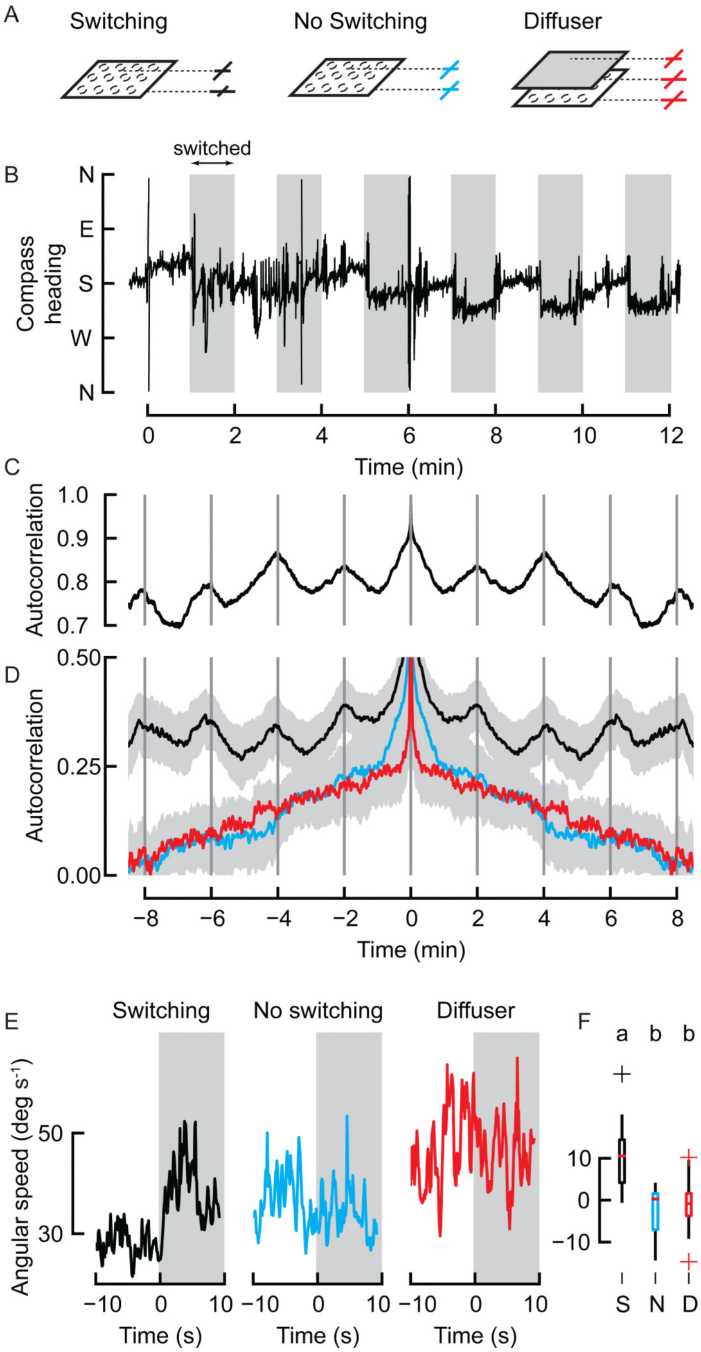

Figure 3.

Flies Turn in Response to Changing the Angle of Naturally Polarized Light (A) Schematic of experimental conditions. See also Figure S3. (B) An example trace showing fly heading for 12 minutes in the Switching condition (panel A, left), during which the polarization was unaltered for six minutes (white background) and rotated by 90° for six minutes (gray background). (C) Autocorrelation plot of headings from B. Time axis is the same as panel D. Vertical gray lines depict lag corresponding to the switching cycle during our experiments. (D-F) Average responses for all flies. Black: trials in which the polarization was switched, sample size N=16 flies; blue: polarization was not switched, N=12; red: polarization switcher active, but diffuser above arena, eliminating polarization, N=11. (D) Mean autocorrelations plotted as lines, standard error of the mean in gray. (E) Mean of the flies' angular speeds after polarization was switched at time t=0. A single average response was determined for each fly by averaging its responses to all 12 switches during the experiment. The mean of these single fly responses are plotted. Gray background indicates time after switch. (F) Average changes in angular speed. The fly's mean angular speed for 10 seconds before each switch was subtracted from the fly's mean angular speed for 10 seconds after that switch. The mean of these differences for each fly are shown in the boxplots. Boxplots were constructed as in Figure 1F. Lowercase letters above the plot indicate different groups at the p<.01 level as computed by the Bonferroni-corrected one-tailed Mann-Whitney U test.