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Walking *Drosophila* align with the e-vector of linearly polarized light through directed modulation of angular acceleration

Mariel M. Velez,

Department of neurobiology, Stanford University, Stanford, CA 94305, USA

Mathias F. Wernet,

Department of neurobiology, Stanford University, Stanford, CA 94305, USA

Damon A. Clark, and

Department of neurobiology, Stanford University, Stanford, CA 94305, USA

Thomas R. Clandinin

Department of neurobiology, Stanford University, Stanford, CA 94305, USA

Thomas R. Clandinin: trc@stanford.edu

Abstract

Understanding the mechanisms that link sensory stimuli to animal behavior is a central challenge in neuroscience. The quantitative description of behavioral responses to defined stimuli has led to a rich understanding of different behavioral strategies in many species. One important navigational cue perceived by many vertebrates and insects is the e-vector orientation of linearly polarized light. *Drosophila* manifests an innate orientation response to this cue ('polarotaxis'), aligning its body axis with the e-vector field. We have established a population-based behavioral paradigm for the genetic dissection of neural circuits guiding polarotaxis to both celestial as well as reflected polarized stimuli. However, the behavioral mechanisms by which flies align with a linearly polarized stimulus remain unknown. Here, we present a detailed quantitative description of *Drosophila* polarotaxis, systematically measuring behavioral parameters that are modulated by the stimulus. We show that angular acceleration is modulated during alignment, and this single parameter may be sufficient for alignment. Furthermore, using monocular deprivation, we show that each eye is necessary for modulating turns in the ipsilateral direction. This analysis lays the foundation for understanding how neural circuits guide these important visual behaviors.

Keywords

Polarization; Behavior; *Drosophila*; Monocular

Correspondence to: Thomas R. Clandinin, trc@stanford.edu.

Present Address: D. A. Clark, Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06511, USA

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Introduction

Animals that rely on vision must transform the richness of the visual world into specific behavioral changes that subserve ethological goals. Understanding quantitatively how a given visual stimulus alters animal behavior provides a lower bound on the computations that must be performed by the brain, and thus represents a critical first step in unraveling circuit function. For example, in the context of motion vision, understanding how behavioral responses vary as a function of stimulus contrast provided critical insights into the development of the underlying model, the Hassenstein–Reichardt Correlator (Hassenstein and Reichardt 1956; Yonehara and Roska 2013), a powerful computational framework whose circuit implementation remains an area of ongoing research (Maisak et al. 2013; Takemura et al. 2013). However, for most visual stimuli, no such quantitative framework exists. Here, we measure the responses of freely walking *Drosophila melanogaster* to linearly polarized light and describe how their movements are modulated as a function of electric vector (e-vector) orientation.

Polarized light provides a valuable orienting cue (Wehner 2001). Sunlight becomes polarized through two distinct mechanisms (Wehner 2001). First, linearly polarized skylight is created by differential scattering of sunlight in the atmosphere, creating a predictable pattern of polarization across the celestial hemisphere that is detected by a variety of animals (Marshall et al. 2007; Kamermans and Hawryshyn 2011; Wiltshko and Wiltshko 2012), including many insects (Von Frisch 1949; Vowles 1950; Brunner and Labhart 1987). For example, polarized light signals guide the long-range migrations of monarch butterflies (Reppert et al. 2004) and direct the navigation of honeybees and desert ants toward food (Rossel and Wehner 1986; Wehner 2003). Second, polarized light is also created by reflections off of certain surfaces, like water and leaves (Wehner 2001), creating stimuli that attract dragonflies and water bugs to apparent water surfaces for oviposition or feeding (Schwind 1983; Wildermuth 1998), while repelling desert locusts (Shashar et al. 2005). In flies, previous studies have shown that freely moving Tabanids are attracted by polarized reflections (Horvath et al. 2008), and quantitative studies have demonstrated that polarized light signals can guide the turning responses of flying *Drosophila* (Wolf et al. 1980; Weir and Dickinson 2012). We have previously reported the responses of freely walking flies to polarized light signals (Wernet et al. 2012); here, we present a more detailed quantitative description of these responses so as to define the behavioral mechanisms involved.

Work in ants, bees, crickets and flies has highlighted the importance of a specialized subset of ommatidia in the dorsal rim of the retina, the DRA, in detecting polarized light (Labhart 1980; Wunderer and Smola 1982; Labhart 1986a; Zufall et al. 1989; Meyer and Domanico 1999). Ommatidia in the DRA are anatomically distinct from those in other parts of the eye and contain photo-receptors with untwisted rhabdomeres that preferentially absorb specific e-vector orientations (Labhart and Meyer 1999). In flies, each ommatidium in the DRA contains a pair of UV-sensitive photoreceptors (called R7 and R8) with orthogonally oriented rhabdomeric analyzers whose outputs can be compared to estimate the orientation of the e-vector, independent of light intensity (Hardie 1984; Fortini and Rubin 1991). In *Drosophila*, the DRA comprises a narrow band of ommatidia extending from equator to

equator across the dorsal-most edge of each eye (Wada 1974; Wernet et al. 2003). While the neural correlates of polarization vision in flies are unknown, electrophysiological recordings in crickets reveal that outputs from the DRA converge onto polarization-opponent interneurons, which integrate inputs from many analyzers, and are maximally sensitive to particular e-vector orientations relative to the body axis (Labhart 1988). Studies in locusts and crickets demonstrate that central complex neurons represent a higher order step in a computational hierarchy, comparing outputs from POL interneurons to become tuned to the orientation of individual e-vectors relative to the head of the animal, acting like an internal compass (Heinze and Homberg 2007; Sakura et al. 2008; Homberg et al. 2011). However, how polarization-sensitive neural circuits translate stimuli into behavioral responses remains relatively poorly understood.

Behavioral studies in several insect species, including Tabanid flies and backswimmers, have demonstrated that polarized light reflections can also be detected by the ventral half of the compound eye (Schwind 1983; Wildermuth 1998; Shashar et al. 2005). In *Drosophila*, polarized light perception can be mediated by photoreceptors outside the DRA and can be elicited at wavelengths that the inner photoreceptors of the DRA cannot detect (Wolf et al. 1980; Wernet et al. 2012). Thus, while the DRA plays a critical role in the detection of celestial polarized light, additional parts of the fruit fly retina also appear to be polarization-sensitive. recently, we have described morphological specializations in the ventral retina of *Drosophila*, providing a substrate for the behavioral responses to ventrally presented POL stimuli (Wernet et al. 2012). However, whether the DRA and the ‘ventral polarization area’ (VPA) converge on the same neural circuitry to guide behavior, or represent two distinct polarization pathways, remains unresolved. Moreover, there have been no recordings of polarization-sensitive interneurons that receive inputs from the ventral retina of any insect.

Polarotactic behavior is defined as an animal's innate preference for aligning its body with a “preferred” e-vector orientation. For example, walking crickets exhibit spontaneous, periodic changes in turning tendency when exposed to a rotating e-vector field, a response that remains even when the degree of polarization is low, and the aperture through which the e-vector is viewed is small (Labhart 1986b; Brunner and Labhart 1987). In a similar experimental paradigm, houseflies displayed a preferred e-vector that was most often perpendicular to its body axis and tended to avoid e-vectors parallel to it (von Philipsborn and Labhart 1990). given that these animals display spontaneous modulation in response to polarized light, it has been postulated that polarized light is an important cue for stabilizing the animal's course, in good agreement with results obtained for flying insects (Wolf et al. 1980; Weir and Dickinson 2012).

In our previous study (Wernet et al. 2012), we found that *Drosophila* orient to plane-polarized light and that this behavior is mediated by photoreceptors located in both the dorsal and ventral region of the eye. What are the behavioral strategies used by flies to achieve alignment with the e-vector? Computer-based tracking of large numbers of freely moving flies under a variety of stimulus conditions has provided a useful tool for identifying circuits involved in motion vision (Katsov and Clandinin 2008; Zhu et al. 2009; Silies et al. 2013). Using analogous experimental paradigms, combined with a model of walking fly

behavior, we define behavioral correlates of polarotactic behavior. In particular, we demonstrate that as flies align to e-vectors, they modulate their angular acceleration relative to the e-vector, thus accelerating towards alignment in a pendulum-like fashion. This same pattern of modulation is used when polarized light stimuli are detected by either the dorsal or ventral retina. Thus, the two classes of polarized light detectors in the retina appear to use the same behavioral mechanism to achieve alignment. Using monocular deprivation, we find that polarized light input to a single eye is sufficient to guide polarotactic behavior, but that this perturbation causes a systematic change in the preferred orientation. These studies reveal an unexpected dimension to the visual control of walking behavior, as polarized visual input to a single eye only modulates turning in one direction.

Materials and methods

Animals

All stocks were maintained on molasses, under 12:12 light/dark cycles, with circadian temperature changes between 18 and 25 °C, under 45–60 % humidity. 66 mated female flies were collected 1–3 days after eclosion and sorted onto fresh food. After 2 days, flies were tested within 3 h after the onset of light, or 4 h before the offset of light. All experiments were performed at 34 °C.

Experimental setup

An unpolarized light source (see below) illuminated a filter set consisting of a polarizer and a diffuser ('polarizer/diffuser pair'), which was rotated by a computer-controlled motor [software: 'NMC Simple Sequencer' (Jeffrey Kerr)]. Within a large temperature-controlled (Peltier device) chamber, 66 flies were contained in a small arena formed by a heavily sanded, plexiglass ring ($\varnothing = 7.5$ cm, height = 2.5 cm) between two plates of UV-transparent plexiglass. The distance from polarizer/diffuser pair was 3.5 cm for flies walking on the 'ceiling' of the arena and 6 cm for those on the floor. An infrared light source (an 880-nm LED array), invisible to the animals, illuminated the flies from below; IR reflections were captured by an infrared-sensitive video camera, filtered so as to eliminate UV and visible light. Custom tracking software extracted the position and orientation of each animal within a population at 30 Hz (Katsov and Clandinin 2008).

Stimulus

The light of an EXFO X-cite *exacte* DC light source passed one of three bandpass filter combinations. UV: Schott UV1 (365 ± 10 nm) + Thorlabs FGB37S, BLUE: Newport (20BPF10-460 (460 ± 10 nm) + FGL435S), or GREEN: Newport (20BPF10-510 (510 ± 10 nm) + FGL435S). All stimuli were calibrated with an Ocean Optics USB 2000 spectrophotometer. Polarizer (HN42HE, Polaroid) and diffuser (two sheets of tracing paper: 'Transparent paper', Max Bringmann KG, Germany) were illuminated through a 35-mm Zeiss collimating adapter. The light stimulus was either linearly polarized or unpolarized depending on which side of the polarizer/diffuser pair faced the flies, while keeping stimulus intensity constant across all experiments. This polarizer/diffuser ("pol/diff") pairing could be rotated by a computer-controlled motor. The stimulus aperture was limited to 5 cm using a

black plastic sheet with circular opening. Only flies walking directly under this aperture were tracked. The intensity of the stimulus was 1.7×10^{12} photons/cm²/s.

Each experiment was divided into epochs during which the diffuser/polarizer pair was rotated 45° at 18°/s for 2.5 s, was then stopped for 5 s, and then rotated again, eventually making 10 full turns (Supplemental Fig. S1b). In typical experiments, populations of female flies were exposed to static POL stimuli for 10 min. From these populations, we acquired approximately 5,000 fly trajectories, each providing positional information about single flies. Histograms of the average orientation of each fly trajectory during each of the four possible orientations of the filter, taken during the 5-s epochs when the filter was not turning, revealed that fly heading varied systematically with the orientation of the polarizing filter (Supplemental Fig. S1c).

Our experimental apparatus allowed flies to freely walk on the top and bottom surface of the arena; thus, flies could be oriented either right side up, with the dorsal part of the eye facing the stimulus, or upside down, with the ventral eye facing the stimulus. Using our system, we specifically monitored flies walking on either surface of the chamber, in separate experiments. Since flies exhibited strong phototactic responses toward the UV light source, and thus often aggregated at the top of the chamber, we modified our apparatus to track flies oriented with their dorsal eye facing the stimulus by including a diffuse, unpolarized white light at the bottom of the chamber.

Metrics

The ‘alignment metric’, A , quantification of the behavioral response is extracted in several steps. (1) All fly angular headings during the stopped epochs for a given experiment are binned in 2° increments from 0° to 360° and transformed into a probability distribution. (2) This probability distribution was fitted to $B \cos(2\theta + \phi) + b$ (where B is the modulation amplitude, θ is the fly heading angle, ϕ the phase shift of the cosine function, and b is the offset). (3) A modulation index $A = \text{abs}(\frac{B}{b} \cos(\phi))$ was then computed. Thus, if the phase shift ϕ is zero, then $A = \text{abs}(\frac{B}{b})$. However, if the distribution is shifted away from alignment, then A decreases. Statistical significance between experiments was determined using a Student's t test. In all experiments, “***” designates a p value of <0.01 and “*” designates a p value of <0.05 . Only fly trajectories obtained while the POL stimulus was static were analyzed.

Modeling of fly behavior

Fly behavior was modeled as a non-linear dynamical system with additive noise. A fly's trajectory then becomes a trajectory through a plane of (θ, ν) , where θ is the angular position and ν is the angular velocity. A mean acceleration and a noise term are applied at each point in time, and depend only on the trajectory's current position in (θ, ν) . We first obtained the empirical mean and standard deviation of acceleration at each point in (θ, ν) for top and bottom flies in both the normal and unpolarized ‘inverted’ cases. We modeled the angular accelerations $a(\theta, \nu)$ as:

$$\alpha(\theta, \nu) = f(\theta) + g(\nu) + \eta$$

where $f(\theta)$ is a modulation found from the normal filter position data, $g(\nu)$ is a basal deceleration (friction-like term) found from the inverted filter data, and η is an uncorrelated noise term, approximated as Gaussian with zero mean and standard deviation equal to that measured across all trajectories. The theta- and velocity-dependent functions were found by averaging over all trajectories and serve to smooth the acceleration field.

When $g(\nu)$ acts to bring trajectories back towards 0 velocity and $f(\theta)$ acts to modulate the acceleration sinusoidally, this equation looks very much like the equation for a damped pendulum, so that the system will tend towards stable fixed points at (0, 0) and (180, 0). To see exactly what steady state is predicted when the noise term is included, we calculated the model's transition probability from each (θ, ν) to every other (θ', ν') in the subsequent time point, given η . Flies were returned to 0 velocity and random orientation if they left the phase space (when speeds became too high). To include jumping and colliding flies, each point in the phase space also had a small constant probability of returning to 0 velocity and random orientation. This led to an effective trajectory lifetime of 4 s. The eigenvector of the full transition matrix with eigenvalue 1 is the steady state distribution of flies predicted by the model. From that eigenvector, we calculated the steady-state distribution in θ , as shown in Fig. 3.

This model makes some simplifying assumptions. First, fly turns, even when random, occur on the time scale of hundreds of milliseconds; we impose a noise term that changes every data frame (33 ms). A more realistic noise term would have longer time correlations. Second, the model assumes that flies will leave the tracking program if they turn too fast. Those flies are “reinjecting” into the system with random orientation and zero velocity, a reasonable construct that does not bias the steady-state heading. Last, we assume that each fly at each point in time has a constant probability of being lost (through jumping or collision). To keep the number of flies constant, they are “reinjecting” with random orientation and 0 velocity. With these assumptions, we show that a simple phase plane analysis with an additive noise term is sufficient to explain the accumulation and distribution of flies in alignment with the polarization field.

Results

Characterization of *Drosophila* polarotactic responses

To examine how populations of flies respond to polarized light, we developed a high-throughput behavioral assay designed to simultaneously capture the trajectories of many freely moving flies (Supplemental Fig. S1a; Wernet et al. 2012). A number of important controls were performed to demonstrate that these orienting responses reflected the orientation of the e-vector field, rather than subtle differences in intensity produced by the polarizing filter (Wernet et al. 2012). Among these, the Pol/Diff combination was oriented within the light path in such the polarization filter was proximal to the flies (the “Pol” condition, Fig. 1a), or away from the flies (the “inverted” condition; Fig. 1e). In the former

case, the filter presented polarized light of uniform intensity to the animals; in the latter case, diffused, and therefore unpolarized light of uniform intensity was presented as a control (as previously reported; Wernet et al. 2012). Fly headings were preferentially oriented along the axis of the e-vector field when the filter was oriented such that polarized light was presented to the fly (Fig. 1b), and were uniformly distributed under the inverted condition (Fig. 1f).

To capture fly responses with a single metric, the A value, we first transformed these histograms into probability distributions and then fit them with a cosine function with an amplitude and a phase (Fig. 1c, g). We then computed the normalized probability modulation, multiplied this value by the cosine of the phase, and took the absolute value of the result as the A value (see “Materials and methods”). This metric thus captures both the strength of the behavioral response as well as its alignment with the e-field. Using this system, we observed that, as expected from previous work in other insects, flies consistently orient to linearly polarized light presented to the dorsal eye (Fig. 1d); a response that is eliminated under the “inverted” condition (Fig. 1h).

Previous work demonstrated that *Drosophila* exhibits polarotactic responses when polarized light is presented to their ventral eye (Wolf et al. 1980). Consistent with this, flies tracked only at the top of the chamber, with their ventral eye pointed towards the stimulus, robustly oriented to the e-vector orientation of polarized light (Wernet et al. 2012). Again, consistent with the qualitative observations of the histograms of fly heading, we observed robust alignment of wild-type flies when the diffuser–polarizer pair was in the POL configuration, and no alignment when both eyes were painted with black paint (Fig. 1i). Furthermore, the ventral polarotactic response was abolished in the unpolarized ‘inverted’ stimulus condition, or when using a quarter wave plate that transforms linear polarization into circular polarization, which is perceived by animals as unpolarized (Labhart 1986b; Wernet et al. 2012). To eliminate the possibility that upside-down flies were orienting to a polarized reflection from the bottom surface (using their dorsal eyes to detect the reflection), we performed a variety of controls aimed at eliminating reflections. These included (1) tilting the bottom surface so that any possible reflection was aimed away from the flies (30° or 45° , see Supplemental Information), (2) placing an antireflective plate at the bottom that greatly reduced UV reflections, and (3) placing mesh at the bottom surface so there was no bottom surface to allow cohesive reflections. In all of these experiments upside-down flies robustly oriented to the stimulus (Supplemental Fig. S2). Thus, polarotactic behavior in *Drosophila* can be mediated by either the dorsal retina or the ventral retina, independently, as previously reported (Wernet et al. 2012).

Flies modulate rotational acceleration in response to linearly polarized light

Our previous studies had defined polarotaxis in terms of an alignment metric, but we did not examine how the turning responses of flies were altered by the stimulus. We next examined how polarized light alters fly movements to produce a bias in heading by measuring the rotational parameters that control turning, specifically angular velocity v and angular acceleration α . To determine how flies modulate these parameters as they align their body axis with the incident e-vector, we plotted the mean of these parameters as a function of fly

angular heading relative to the e-vector for both dorsally and ventrally facing flies (Fig. 2a–f). In all of these analyses, flies oriented at $\theta = 0^\circ$ were aligned with the e-vector, and flies oriented at $\theta = 90^\circ$ were orthogonal to the e-vector. We also followed the convention of denoting clockwise and counterclockwise turns with positive and negative velocities (with associated accelerations), respectively. Both populations were also examined under the (unpolarized) “Inverted” Pol/Diff condition to control for any modulations that were not associated with alignment behavior.

We initially hypothesized that flies would align with the e-vector by reducing their turning speed as they approach the preferred orientation, ceasing to turn once their bodies came into alignment. However, neither dorsally nor ventrally presented polarized light altered the mean angular velocity of the population across all values of θ (Fig. 2a–c). In particular, the average angular velocity of flies turning in either direction was approximately $75^\circ/\text{s}$ for flies walking on either the upper or lower surfaces of the arena, regardless of orientation relative to the e-vector (Fig. 2b, c). Thus, flies do not, on average, reduce their rate of turning when they are aligning with e-vector field.

By contrast, for both dorsally and ventrally stimulated flies, angular acceleration captured a profound modulation of behavior as a function of angular heading. In particular, flies adjusted their acceleration so that they were accelerating into alignment ($\theta = 0^\circ$); this occurred regardless of whether their angular velocity was taking them towards or away from alignment (Fig. 2d–f). This modulation reached a maximum value (either positive or negative) when flies were oriented at 45° relative to the e-vector. Consistent with this modulation being a specific response to the presentation of polarized light, the average angular acceleration of the population was constant as a function of theta in the absence of the polarization signal (when the filter was in the inverted configuration). These modulations were easily fitted by the function $a = a_0 \sin 2\theta$, where a denotes the acceleration, and θ denotes the angle relative to the e-field. In this analysis, the only difference between dorsally and ventrally stimulated flies was the slightly larger amplitudes necessary to describe ventral behavior. Thus, flies strongly modulate a as a function of angular position relative to the e-vector, a modulation that was qualitatively similar (but quantitatively different) for both ventral and dorsal POL behavior.

Polarization-sensitive photoreceptors produce strongest signals when their untwisted rhabdomeric microvilli are aligned with the preferred e-vector, and weak signals when orthogonal to it (Hardie 1984; Labhart et al. 1984). We reasoned that circuitry underlying polarotactic behavior must transform this input into an output signal that is sinusoidal and in phase with the $a = a_0 \sin 2\theta$ function that describes angular acceleration as a function of theta. As has previously been proposed, the simplest transformations of this signal that could generate this sinusoid would either be to take the temporal derivative of a photoreceptor signal with a single preferred e-vector orientation or by taking a spatial derivative of the pooled signals from an array of polarization-sensitive photoreceptors with different preferred e-vector orientations. A critical distinction between these two models lies in whether the strength of the modulation is affected by the angular velocity of the fly. In particular, if a temporal derivative from a single detector is used, the slope of the sinusoid

will be increased as flies turn more rapidly, sweeping the detector more rapidly across the e-vector field, and will be reduced when flies are turning more slowly. Conversely, a spatial derivative integrating over many polarization-sensitive photoreceptors should be unaffected by the speed of rotation of the array of detectors. To distinguish between these two possibilities, we measured the amplitude of α in flies turning at different angular velocities (Fig. 2e–f inset). For both dorsally and ventrally facing flies, we found that α modulation was only weakly affected by the angular velocity of the fly. Thus, these results are inconsistent with those predicted by a simple model based on a single temporal derivative.

A quantitative model of polarotactic behavior

To test whether this θ -dependent change in angular acceleration was sufficient to account for the observed polarotactic behavior, we constructed a Markov model of fly behavior and simulated the flies' response to polarized light (see “Materials and methods”). In this model, a fly's acceleration was found for every combination of orientation and rotational velocity, and this field of accelerations was used to predict where the fly would rotate next (see “Methods”). We modeled the fly's acceleration as a function of three additive components: the basal deceleration curves as a function of velocity (which describe fly movements independent of a POL signal), the modulation of acceleration as a function of orientation relative to the e-vector, and an additive noise term. The basal deceleration curves were drawn from flies in the inverted filter case, where there was no modulation (or heading bias) as a function of θ . rotational acceleration as a function of orientation was taken from the observed means. The noise was assumed to be Gaussian and uncorrelated in time, with standard deviation (SD) equal to the measured value at each point in phase space. With this model, we computed the transitions between each pair of points in phase space, where the transition probability is solely a function of current orientation and velocity (Fig. 3a–c) and is noise-dependent. When we computed the steady-state heading distribution predicted by this model, we found that the distribution of headings generated by the model was largely indistinguishable from that observed in the original data (Fig. 3d–f). As the only parameter in the model that was modulated as a function of orientation relative to the e-vector was angular acceleration, this simulation demonstrates that this single behavioral modulation is sufficient to account for polarotaxis.

Polarotactic behavior requires input from both eyes

We next tested whether flies can align to linearly polarized light using only monocular input, similar to what has been described for the detection of polarized light by desert ants (Wehner and Müller 1985). To do this we occluded either the right or left eye of all tested flies, using black paint, and examined the polarotactic responses of the respective population. Under these conditions, we observed that monocularly occluded flies aligned to the e-vector, although to a lesser degree than intact flies (Fig. 4a, b).

The A value measure of polarotactic behavior combines both the strength of a population's alignment and its alignment in the orientation of the e-vector. Thus, decreases in this metric can reflect either a diminution of the heading bias with respect the e-vector, a change that is reflected in the amplitude of the cosine fit to the orientation histogram, or a strong heading

bias that is systematically mis-aligned, a change that is reflected in the phase of the cosine fit. To determine why monocular flies exhibited decreased polarotactic responses, we first examined the phase (ϕ) of the cosine fit to the probability distribution of angular heading and plotted this on a unit circle (Fig. 4b–d). To our surprise, ventrally facing monocular flies exhibited a systematic ϕ shift that correlated with which eye was painted (-22° for left-eye-painted flies and $+26^\circ$ for right-eye-painted flies), with respect to the e-vector. This ϕ -shift contributes to the decreased A values seen for monocular flies (Fig. 4), since the A value is the projection of ϕ onto the x -axis and any shift away from 0° will cause the A value to shrink. Finally, dorsally facing flies also exhibited a significant ϕ -shift for right-eye-painted flies, but not for left-eye-painted flies (data not shown). As these changes were not systematic, we infer that the effects on monocular deprivation on dorsal POL were non-specific.

Polarized light stimuli only modulate turns toward the “open” eye

As monocular painting shifted the alignment axis for ventrally facing flies, we next examined whether the directed modulations of angular acceleration critical to polarotaxis were systematically altered by monocular deprivation. As expected, for control flies, the sinusoidal modulation of angular acceleration as a function of theta was indistinguishable, regardless of whether the flies were turning clockwise or counterclockwise (Fig. 5a). However, for monocularly occluded flies, this θ -dependent modulation of acceleration depended on the direction of turning relative to which eye was occluded. More specifically, ventrally facing flies sinusoidally modulated their acceleration as a function of angular heading when turning toward their open eye and did not demonstrate this modulation when turning toward their closed eye (Fig. 5b–d). Thus, each eye controls stimulus-dependent modulations in acceleration somewhat independently, altering only turns occurring in one direction, an observation that has direct implications on how the fly brain is organized with respect to polarized light stimuli.

Discussion

The visual system extracts different features from an animal's environment; further processing of this information leads to behavioral responses that can be quantified. The detailed description of insect visual behavior has led to powerful models for basic mechanisms guiding important animal behaviors, like the optomotor response (Hassenstein and Reichardt 1956). Navigating insects have long served as powerful models for the processing of multiple visual stimuli (Wehner 2003). The linear polarization of skylight, as well as polarized reflections, represents important cues that are also perceived by *Drosophila* (Wolf et al. 1980; Weir and Dickinson 2012; Wernet et al. 2012). However, the neural circuitry underlying this behavior as well as the quantitative description of precise behavioral strategies used by the animals to orient within an e-vector field have so far been missing. We have developed a high-throughput system for examining polarization-evoked behavior that allows us to probe different behavioral parameters in a robust and quantitative way in populations of freely moving *Drosophila*. Using this system, we have demonstrated

that free-walking *Drosophila* are sensitive to linearly polarized light presented both dorsally and ventrally, and tend to align their body axis parallel to the e-vector (Wernet et al. 2012).

The detailed description of both walking and flying *Drosophila* solving different visual tasks has led to a dramatic increase in our knowledge about their behavioral strategies (Katsov and Clandinin 2008; Zhu et al. 2009; Clark et al. 2011; Straw et al. 2011; Tuthill et al. 2013; Silies et al. 2013). We have applied such a description to the polarotactic orientation response (Weir and Dickinson 2012; Wernet et al. 2012), focusing on the analysis of rotational velocity (v) and rotational acceleration (a). Our results show that walking fly populations align their body axis with the incident e-vector, a result consistent with *Drosophila* polarization vision studies performed with single flies in flight simulators (Wolf et al. 1980). Surprisingly, we find that v is not modulated by the linearly polarized stimulus. Instead, we find that the angular acceleration of animals aligning with the incident e-vector rapidly decreases away from $\theta = 0$, perhaps acting as a “braking” mechanism to prevent the flies from turning even further out of alignment. Interestingly, a returns to 0 as θ approaches 90° , suggesting that flies may modulate their acceleration in an unbiased way when they are completely orthogonal to the stimulus, since either direction will result in becoming more aligned with the e-vector than at $\theta = 90^\circ$. Hence, flies may use a strikingly simple mechanism to align with e-vectors by modulating a single turning parameter, their angular acceleration, relative to the e-vector, thus achieving biased headings by acting in a pendulum-like fashion. In this view, polarization cues bias the path of the animal, focusing the local search strategy around the dominant e-vector orientation in the presence of either celestial POL cues or polarized reflections.

We captured modulations of angular acceleration as a function of angular velocity and angular heading using a simple model. We successfully modeled polarotactic behavior using simple equations that reflected how a fly might transform a periodic signal mediated by neurons just downstream from the retina into modulations of angular acceleration. Using these methods we found a rather surprising result: a simple model that modulates acceleration based on orientation is sufficient to account for much of the data. The modulation function is proportional to the derivative of $\cos(2\theta)$, so that it could easily be computed as the spatial derivative of the polarization. This model appears distinct from the “scanning hypothesis”, postulated to describe orienting behavior in bees (Rossel and Wehner 1986), whereby the animal does not encode particular e-vectors, but instead orients to a celestial pattern in reference to a static internal representation. However, since the two experimental paradigms are very different, it remains to be seen whether bees would orient spontaneously to e-vectors and display similar results.

Using monocular deprivation, we show that, in principle, flies can align to an e-vector field using just one eye, results that are in agreement with previous experiments in desert ants (Wehner and Müller 1985). These authors described ‘intraocular transfer’ of POL information, which is particularly interesting since polarization-opponent interneurons innervated by the DRA of the ipsilateral eye in crickets (Labhart 1988) show prominent projections terminating in the optic lobes of the contralateral eye (Wehner 2003). Similar neurons have not yet been identified in *Drosophila* yet experiments in locusts have revealed

a POL vision pathway with similar neurons (Homberg et al. 2011). So far, little is known about the co-operation between the two eyes in coordinating the behavioral responses of flying or walking *Drosophila*, to different visual stimuli. However, we show that each eye controls stimulus-dependent modulations in acceleration at least partially independently, altering primarily turns occurring in one direction. Ongoing genetic screens for circuit elements involved in POL vision will reveal how the fly brain is organized to achieve such functional specialization.

Our studies provide the evidence that dorsal and ventral detectors can mediate strikingly similar polarotactic responses. These results demonstrate that flies use the same basic strategy to align with the polarization field, although different retinal receptors mediate the behavior and/or the activity of different polarization circuits for these two populations of flies. Further studies using genetic tools to determine the neural correlates of similarities and differences between these two behaviors are ongoing. In addition, we have defined a set of behavioral parameters that capture key aspects of the polarotactic response with the idea that changes in these metrics could be employed as behavioral signatures in a forward genetic screen. We anticipate that these methods will be useful to future studies that wish to identify the neural contributions to polarotactic behavior.

Supplementary Material

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Fig. 1.

Polarotactic responses of *Drosophila* populations. **a–d** Quantification of the polarotactic response. **a** Experimental “Pol” condition, in which the Pol/Diff filter combination is oriented such that the flies experience a linearly polarized UV stimulus. **b** Raw counts of fly angular headings during the stopped epochs plotted against theta (radians). Probability distributions (2° bins) are fitted by $B \times \cos(2 \times \theta + \phi) + b$. **d** Final $A = \text{abs}(B/b \times \cos(\phi))$, averaged across experiments. **e–h** Quantification of fly behavior in response to an “inverted” stimulus, in which the “Pol/Diff” filter combination is oriented such that the flies experience a diffused, unpolarized stimulus. Same data processing as above. note that the flies show no alignment response to the unpolarized stimulus. **i** Example of a fly with both eyes painted using custom black eye liner (see “Materials and methods”). **j** Alignment values (A) plotted for wild-type flies experiencing a linearly polarized (‘Pol’) stimulus (left), as well as flies unpolarised (inverted) stimulus (middle), and flies with both eyes painted, experiencing a ‘Pol’ stimulus. Painting of both eyes abolishes the polarotactic response, ruling out a sufficient role of ocelli in mediating this behavior

Fig. 2.

Behavioral dissection and modeling of fly polarotactic responses. **a–c** Angular velocity is not modulated by the linearly polarized stimulus. **a** Schematic illustration of angular velocity as a function of theta (θ). **b** Angular velocity as a function of theta for dorsal POL. **c** Angular velocity as a function of theta for ventral POL. In both cases, no detectable variation in the average angular velocity could be detected across all values of theta, and there were no differences between the presentation of a POL stimulus (magenta lines) and the presentation of a non-POL UV stimulus (black lines). **d–f** Modulation of angular acceleration by the linear polarized stimulus. **d** Schematic description of angular acceleration (A_r °/s/s). **e** A_r vs angular heading (θ , °) for flies facing a dorsal POL stimulus. Flies exhibit a decrease in A_r that minimizes at 45° away from alignment (from $\theta = 0^\circ$ to 90°) and an increase in A_r as flies

move towards alignment (from $\theta = 90^\circ$ to 180°). **f** A_r vs angular heading for flies experiencing a ventral POL stimulus. *Inset panels* in both **e** and **f** plots: the average magnitude of the A_r modulation at three different average angular velocities $s(v_r)$. **g** Alternative strategies involving temporal derivatives (*top*) or spatial derivatives (*bottom*) to deduce e-vector information from the sinusoidal modulation of photoreceptor signals. The result is a sinusoidal modulation of the fly's acceleration (*black curve*). *Right* model showing how the fly aligns to an e-vector field by decelerating out of alignment (*green T*) and accelerating into alignment (*green arrow*)

Fig. 3.

Behavioral dissection and modeling of fly polarotactic responses. **a, b** Acceleration fields for dorsally stimulated flies (**a**) and ventrally stimulated flies (**b**). *Top* measured acceleration as the joint function of orientation and rotational velocity (see *color panel* to left). *Middle* joint distribution modeled as simply the sum of the individual means shown in the traces, (**a**) and (**b**) (see “Materials and methods”). *Bottom* difference between the actual acceleration field and the “smoothed” acceleration field. There appear to be few systematic deviations, so that the sum is a reasonable model. The “smoothed” acceleration field, plus a noise term derived from the standard deviation at each point, was used to construct the transition matrix for the Markov model. **c** The phase space of a fly was modeled to consist of its orientation and velocity. An acceleration and noise at each point in this space govern trajectories through it. The predicted steady-state distribution of fly orientations matches the measured alignment of flies viewing a dorsally presented stimulus (**d**) and flies viewing a ventrally presented stimulus (**e**)

Fig. 4.

Monocular deprivation leads to systematic errors in the polarotactic response. **a** Alignment values of different fly populations (from *left to right*) the normal polarotactic alignment response of upside-down walking wild-type flies perceiving linearly polarized UV light with the ventral eye is lost upon painting both eyes with black eye liner, and systematically reduced when either one of the two eyes is painted over. Monocular deprivation of the left eye or right eyes leads to reductions of A that are statistically indistinguishable. **b–d** Detailed characterization of decreased polarotactic responses observed in monocular flies. **b** Unpainted flies: phase (ϕ) of the cosine fit to the probability distribution of angular heading plotted on a unit circle. **c, d** Ventrally facing monocular flies exhibited a systematic ϕ shift that correlated with which eye was painted, with respect to the e-vector. This ϕ -shift contributes to the decreased A values seen for monocular flies since the A value is the projection of ϕ onto the x -axis, and any shift away from 0° will cause the A value to shrink

Fig. 5.

The effect of monocular deprivation on sinusoidal modulation of A_r . **a–c** Angular accelerations (A_r) of different fly populations plotted over the orientation theta within the e-vector field. **a** In unpainted flies, A_r towards both eyes is modulated equally by theta. **b, c** When turning towards the occluded eye, A_r is no longer modulated by theta (*black trace*), whereas the modulation remains intact in the opposite direction (*red trace*). **d** Model summarizing the effect that both eyes have on the alignment of fly populations into alignment with an incident e-vector field