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Animal behaviour

Stabilizing responses to sideslip disturbances in *Drosophila melanogaster* are modulated by the density of moving elements on the ground

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Stabilizing responses to sideslip disturbances are a critical part of the flight control system in flies. While strongly mediated by mechanoreception, much of the final response results from the wide-field motion detection system associated with vision. In order to be effective, these responses must match the disturbance they are aimed to correct. To do this, flies must estimate the velocity of the disturbance, although it is not known how they accomplish this task when presented with natural images or dot fields. The recent finding, that motion parallax in dot fields can modulate stabilizing responses only if perceived below the fly, raises the question of whether other image statistics are also processed differently between eye regions. One such parameter is the density of elements moving in translational optic flow. Depending on the habitat, there might be strong differences in the density of elements providing information about self-motion above and below the fly, which in turn could act as selective pressures tuning the visual system to process this parameter on a regional basis. By presenting laterally moving dot fields of different densities we found that, in *Drosophila melanogaster*, the amplitude of the stabilizing response is significantly affected by the number of elements in the field of view. Flies countersteer strongly within a relatively low and narrow range of element densities. But this effect is exclusive to the ventral region of the eye, and dorsal stimuli elicit an unaltered and stereotypical response regardless of the density of elements in the flow. This highlights local specialization of the eye and suggests the lower region may play a more critical role in translational flight stabilization.

1. Introduction

Most flies are visual animals with a big part of their heads devoted to the perception and processing of visual information for flight control [1]. Successful navigation requires them to correct for any deviations from their intended track, which in turn implies distinguishing between self-elicited motion and unintended motion caused by external disturbances. Optomotor responses counteract visual translational and rotational deviations [2] and are refined by the integration of multisensory input [3,4]. When pushed laterally by a gust of wind, for example, flies countersteer to return to their original route [5,6].

When evaluating a visual scene for course correction during translation, near objects are more informative than far ones because distant references are less reliable for determining the magnitude of deviations [7–9]. Since objects below the horizon are typically closer to a fly, there is potentially strong selective pressure to evaluate translational disturbances below the horizon in detail [10,11]. In fact, the ventral region of the eye is mostly insensitive to certain types of rotation [12], while highly responsive to translation [13]. This functional compartmentalization likely

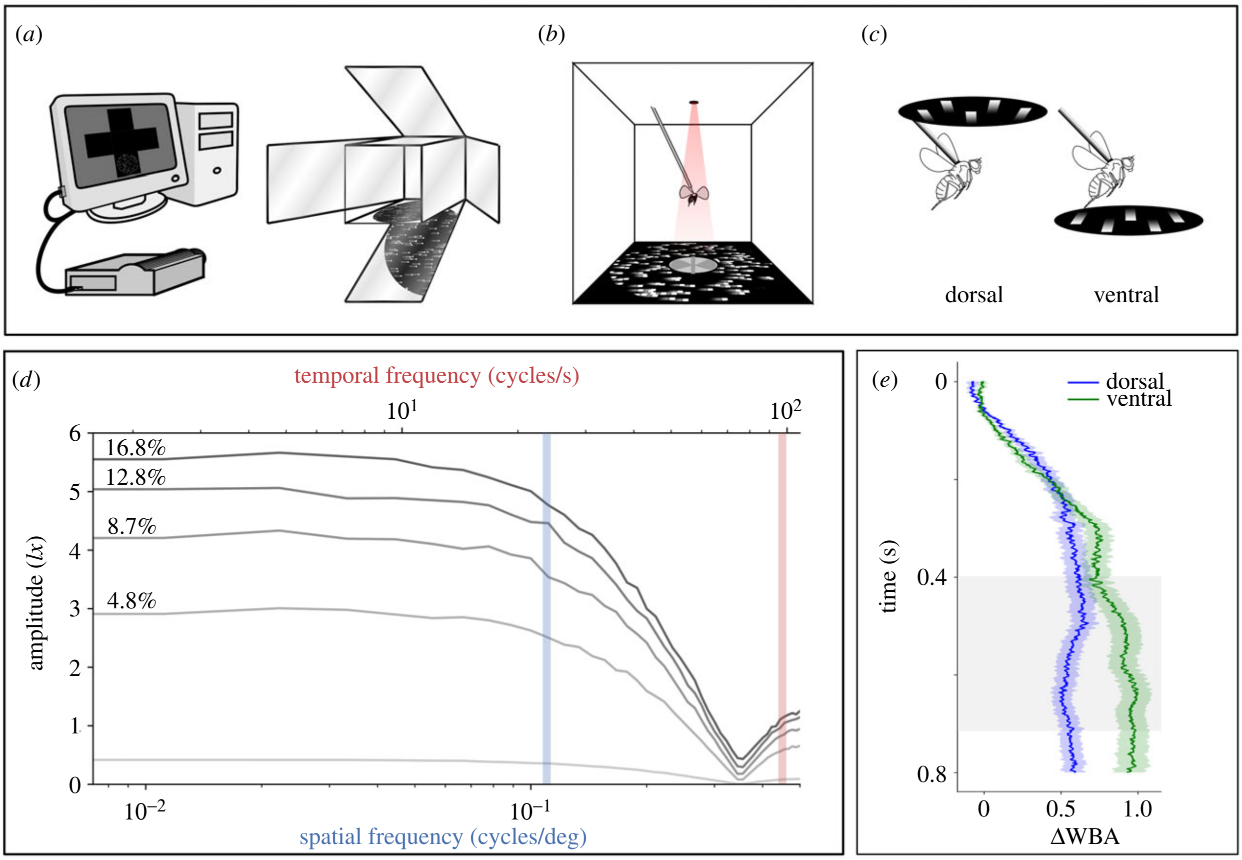

Figure 1. (*a*) Projection arena used to present stimuli to specific regions of the visual field on flies. (*b*) Tethered flies held under an IR light at the centre of the arena cast a shadow over a sensor below. Changes in wing-beat amplitude (ΔWBA) alter the size of the shadow of each wing and are registered by the sensor as voltages. Differences in amplitude between both wings represent steering attempts. (*c*) Responses to regional stimuli were elicited by exposing the flies to laterally moving dot patterns projected on the upper or lower faces of the projection arena. (*d*) Spatial and temporal frequencies in the stimuli. Coloured regions represent limits of perception for spatial (blue), and temporal (red) frequencies in flies. (*e*) Mean steering response (solid lines) and standard error of the mean (s.e.m.) (colour shading) of 50 flies, elicited by a moving dotfield with 4.6% element density, presented to the dorsal and ventral region of the eye at $t = 0$. The shading represents the interval over which responses were compared between treatments.

reduces redundancy and enhances accuracy in the perception of self-motion during flight (e.g. in different insects: [14–16])

Optomotor responses are further affected by image parameters such as light intensity, contrast and spatial frequency composition [17,18]. Within certain ranges, different scenes are accommodated by changes in photoreceptor behaviour [19], or visual processing [20], allowing insects to navigate in a broader set of conditions, but past these responses saturate [21]. The finding that ventral motion parallax mediates steering responses, but dorsal motion parallax does not [22,23], raises the question of whether flies evaluate other image statistics differently between regions of the eye. In a similar fashion to parallax, the density of moving elements in natural scenes may vary predictably by region, conveying information such as spatial structure and contour distribution in the surroundings [7], in addition to the magnitude of perturbations. To determine the regional effect of element density in the optic flow, we measured the corrective steering responses of *Drosophila melanogaster* to sideslipping dot fields, with a different number of elements, across the dorsal and ventral regions of the eye.

2. Methods

(a) Subjects

We measured steering responses from 50 female *Drosophila melanogaster* 3–5 days after eclosion. The flies came from our laboratory colony where they were fed with Instant *Drosophila* Medium (Formula 4–24®, Carolina Biological Supply) and kept under a 12 L : 12 D cycle at 21°C. We tethered cold-immobilized flies by glueing them to a tungsten rod by the mesonotum. They then recovered for at least 30 min in the dark while holding a piece of paper to avoid triggering the tarsal reflex [24]. Upon recovery, we placed each fly in the centre of a projection arena and removed the paper to elicit flight. We tested each fly only once to prevent experience-biased responses.

(b) Visual stimuli

We back-projected onto a 200 mm Perspex cube [9] to display moving dot fields in a 90° diameter disk, directly above or below the fly [23] (figure 1*a–c*). We used dark-adapted flies in a dark room to ensure they perceived only light from the stimulus. Each experiment consisted of 10 open-loop, randomly ordered trials of white square-shaped dots of equal size, moving laterally on a black background. These were interspersed by closed-loop segments of bar-fixation, using a white bar on a black background, which generates robust flight and standardizes the behavioural state at the beginning of each trial [25,26]. Dot fields assumed one of five levels of density of moving elements (0.4–16.8%), moving coherently left or right, at a constant angular speed (216°/s) for 0.8 s, resulting in a transit time of 0.41 s for individual elements moving along the disk's equator. Densities of moving elements were measured as the mean relative fraction of the stimulus surface covered by dots. Individual dots subtended 2.86° on the visual field of the fly, their placement was random and overlapping

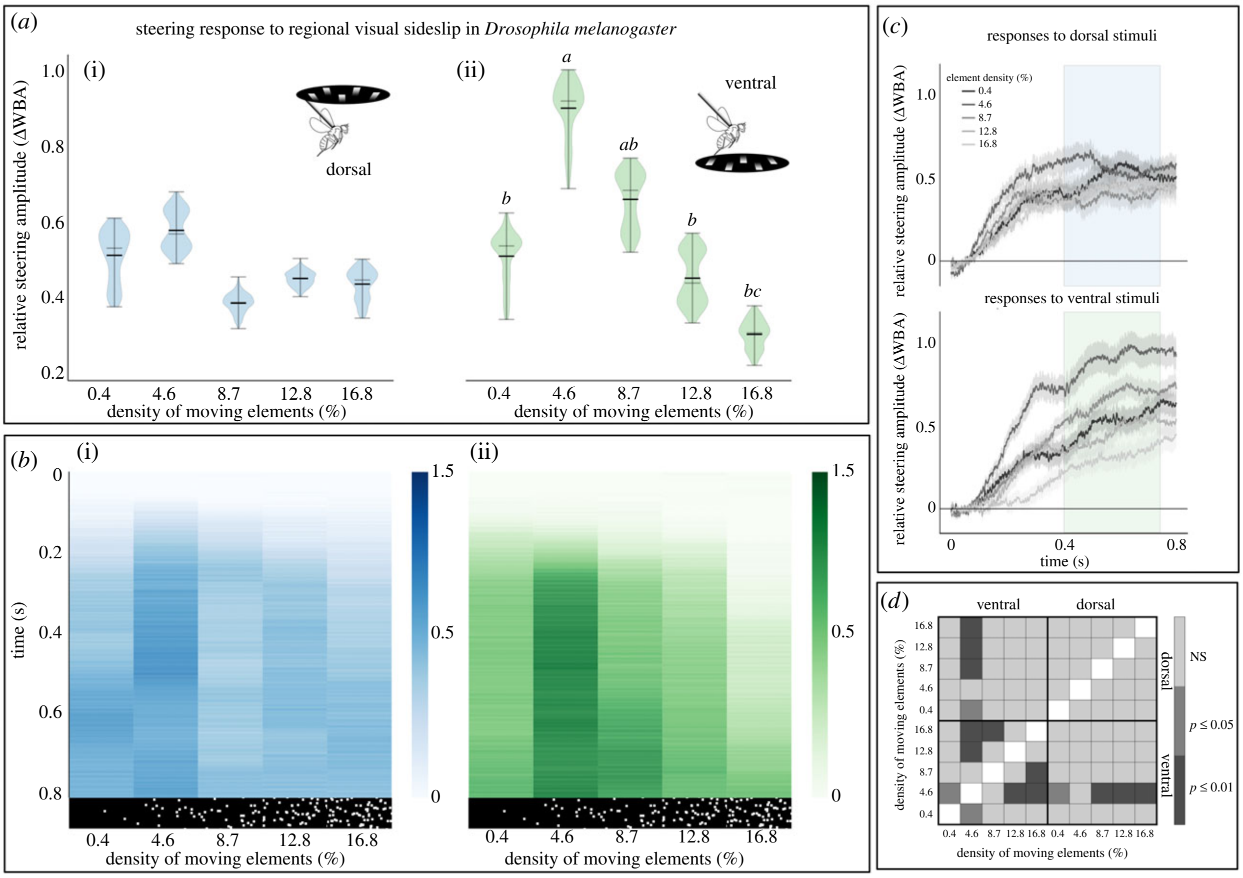

Figure 2. Relative steering responses (*Δ*WBA) to sideslip disturbances in coherently moving dot fields as a function of visual element density in dorsal (blue) and ventral (green) visual regions (FOV = 90°, *n* = 50). (*a*) Means (black) steering responses to dorsal (i) and ventral (ii) sideslip disturbances across five levels of element density. Violin plots represent the distribution of data within interquartile ranges, along with the median (grey). Mean responses labelled with different letters are significantly different when compared within the ventral region of the eye (Bonferroni post hoc analysis, $p \leq 0.05$). (*b*) Heatmaps of mean temporal series at each level of element density in the dorsal (i) and ventral (ii) regions of the eye. Dark tones represent relatively stronger responses (*Δ*WBA). (*c*) Mean time series of steering responses across levels of element density. Envelopes represent s.e.m. and coloured rectangles the time window within which responses were analysed. (*d*) Post hoc pairwise *t*-test for multiple comparisons among both regions of the eye and all levels of element density. Bonferroni-adjusted probabilities are represented as shades of grey according to their value. Not significant differences (NS) are represented in light grey.

was allowed. We measured spatial and temporal frequency composition as the average fast Fourier transform of linear strips of illuminance levels (along the direction of motion) in 1000 instances of the random dot pattern at different densities (figure 1*d*).

(c) Steering responses

Tethered flies were held at the centre of the arena where an infrared LED placed above cast a shadow of the flapping wings over a dual photodiode below (figure 1*b*). The difference in the wing-beat amplitude of the left and right wing generated during steering attempts [27] is captured by the dual sensor as a difference in voltage between both sides, due to the change in the size of the shadows of the wings [28,29]. Due to the bidimensional nature of the shadows, the wing-beat amplitude (*Δ*WBA) reported by the sensor includes roll and yaw attempts performed by the fly (figure 1*e*) [6].

(d) Data analysis

Steering responses were normalized based on the highest mean *Δ*WBA value exhibited by the flies across all treatments within the time window of interest. We used a one-way ANOVA to compare the effect of element density on the amplitude of normalized responses across visual fields (dorsal and ventral) at 0.4–0.7 s after the stimulus was projected (figure 1*d*). We evaluated significant differences by performing a post hoc pairwise *t*-test for multiple comparisons, adjusting the *p*-values using the Bonferroni one-step correction method.

3. Results

Flies steered with the stimulus direction regardless of the density of visual elements or the region of the eye perceiving motion. Although we found significant differences among responses to element density ($F = 6.27$, $p \ll 0.01$), they occurred regionally. The amplitude of the stabilizing responses to stimuli perceived by the upper eye was not affected by variations in the density of moving elements (figure 2*a*i, 2*d*). However, in the ventral eye, response amplitude was significantly affected by visual density, being stronger when 4.6 and 8.7% of the stimulus were covered by moving elements (figure 2*a*ii, 2*d*). Although at 4.6% of coverage by moving elements, responses seem to rise faster and peak at a higher amplitude for both eye regions (figure 2*b*), only in the lower eye this leads to a significant difference with responses elicited by dorsal stimuli (figure 2*d*). Further, response amplitude to dorsal stimuli was more stable over time across levels of element density (figure 2*c*) [30].

4. Discussion

Steering responses to translational flow in fruit flies are stronger when perceived below the equator of the eye [12,13]. In response to moving gratings, their amplitude depends on the temporal frequency of the stimulus [18,31]. However, accurate manoeuvring in the wild cannot rely only on this parameter due to the wide range of spatial frequencies and contrasts present in natural scenes. Several models attempt to account for such accuracy (e.g. [32,33]), but the actual mechanism remains unknown. Similar to natural scenery, randomly placed dots moving at a constant angular velocity do not provide the fly with a single temporal frequency to modulate their stabilizing responses (figure 1*d*), yet they respond to the faster motion by strengthening corrective manoeuvres [23].

Our results suggest that regardless of the actual algorithm used for estimating the magnitude of disturbances in dot fields, the density of moving elements can modulate motor responses, at least when disturbances are perceived on the ground. The strong responses elicited by the ventral region of the eye, within a narrow range of element densities in moving dot fields (figure 2*a*,*c*) imply an optimal level of visual elements beyond which a fly may estimate the magnitude of the disturbance less accurately. Interestingly, responses to sideslip presented above the fly suffered no such modulation (figure 2*a*,*c*), which could indicate subtle processing differences in dorsal and ventral regions of the eye.

Despite our finding that the density of elements in the dorsal stimuli has no significant effect on stabilizing responses, the time series for this region has a slightly higher amplitude at the same element density at which ventral stimulation produces its strongest response (figure 2*b*). This may imply flies process element density similarly in both regions of the eye, but its effect on the motor response is reduced in the dorsal region, keeping the system from saturation [21]. If this is the case, translational wide-field motion from ventral regions might be scrutinized for parameters such as texture, often irrelevant in dorsal sky views, making the ventral region susceptible to saturation when stimulus features may exceed the range of perception. Flies may further mediate responses by attending to different visual regions, when conditions imply some areas may contain more information [34,35].

Translational visual cues below the horizon are particularly relevant for controlling ground-speed and correcting changes in the position [13,36,37]. Blowflies, for example, encode motion parallax perceived in this region [22], and fruit flies do the same to modulate their steering responses during flight [23]. Strong sensory adaptation to variations in element density in the dorsal region of the eye could be an adaptive trait for flies traversing perturbed areas and edges of forests, where they would move frequently between structured vegetation and open spaces. Compared to visual elements above the fly that are highly variable, textures below might be more homogeneous and require enhanced sensitivity to small structural changes in order to be used for flight control. Moving from the understory to open areas with the sky as a background dramatically changes brightness and spatial distribution of contrasts above a fly. Exposure to these conditions could produce selective pressure for a regional gain-control mechanism tuned to these parameters [38]. The lower region, on the other hand, suffers a narrower range of variation in image statistics, such as spectral composition, partially because light perceived by this region is mostly reflected off the vegetation [39]. Such conditions could have led to enhanced sensitivity to image statistics conveying structural information, such as the density of moving elements, making the system more susceptible to saturation in this region than dorsally.

Fruit flies and their brachyceran relatives constitute the most ecologically diverse group of flies, many of which feed and reproduce on temporary resources and are forced to move through different habitats to find them [40]. Flight control plays such an important role in the survival of flies that we expect strong selective pressures to act on wide-field motion-detecting neurons [41–43]. If our finding represents an adaptive trait associated with high mobility, we will expect similar response patterns in flies with similar habits.

Finally, the levels of element density we tested likely do not saturate responses in free flight, in part because of multisensory integration. The mechanosensory input of halteres and antennae greatly increases the accuracy of the perception of positional and rotational disturbances [3,4], probably minimizing the effect of changes in element density. Nonetheless, our results provide additional information on how egomotion is perceived and treated regionally from the perspective of vision.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.vx0k6djq7 [30].

Authors' contributions. C.R. helped to develop the research question, carried out all of the laboratory work involved in the data collection, performed the data analysis and drafted the manuscript. J.T. helped to develop the research question, provided logistic support during the time the first author was collecting data and coordinated the study. J.T. also helped by reviewing the manuscript and helping shape the final document. Furthermore, J.T. provided the facilities and equipment necessary to run the experiments and collect the data. Both authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests

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