

## RESEARCH ARTICLE

Edge detection depends on achromatic channel in *Drosophila melanogaster*

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## SUMMARY

Edges represent important information in object recognition, and thus edge detection is crucial for animal survival. Various types of edges result from visual contrast, such as luminance contrast and color contrast. So far, the molecular and neural mechanisms underlying edge detection and the relationship between different edge information-processing pathways have been largely undemonstrated. In the present study, using a color light-emitting-diode-based Buridan's paradigm, we demonstrated that a blue/green demarcation is able to generate edge-orientation behavior in the adult fly. There is a blue/green intensity ratio, the so-called point of equal luminance, at which wild-type flies did not show obvious orientation behavior towards edges. This suggests that orientation behavior towards edges is dependent on luminance contrast in *Drosophila*. The results of mutants *ninaE*<sup>17</sup> and *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> demonstrated that achromatic R1–R6 photoreceptor cells, but not chromatic R7/R8 photoreceptor cells, were necessary for orientation behavior towards edges. Moreover, ectopic expression of rhodopsin 4 (Rh4), Rh5 or Rh6 could efficiently restore the edge-orientation defect in the *ninaE*<sup>17</sup> mutant. Altogether, our results show that R1–R6 photoreceptor cells are both necessary and sufficient for orientation behavior towards edges in *Drosophila*.

Key words: Buridan's paradigm, edge detection, equiluminance, photoreceptor cell, rhodopsin mutant, *Drosophila melanogaster*.

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## INTRODUCTION

Resulting from the contrast between neighboring areas, edges are one of the most important visual cues for object segmentation and recognition (Marr and Hildreth, 1980; Biederman, 1987; Laws et al., 2003). Invertebrates to vertebrates, such as flies, honeybees, beetles, goldfish and budgerigars, showed a strong tendency to orient toward an edge (Varju, 1976; Lehrer et al., 1990; Osorio et al., 1990; Bhagavatula et al., 2009; Kim and Jung, 2010). The potential cues to define an edge include brightness, color, texture and relative motion (Mullen, 1985; Livingstone and Hubel, 1987; Regan et al., 1992; De Weerd et al., 1994; Greene and Brown, 1995; Reppas et al., 1997; Leventhal et al., 1998).

In insects, it has been shown that honeybees can detect boundaries that originate in brightness contrast. Behavioral analysis in honeybees suggested that edge detection is a color-independent process, which originates in the green-sensitive photoreceptor (Lehrer et al., 1990). Further experiments showed that honeybees could also use texture or the relative motion of different textures to define a boundary (Srinivasan et al., 1990; Lehrer and Srinivasan, 1993), and the combination of cues that define an edge could not enhance edge detection (Lehrer and Srinivasan, 1993). The honeybee's visual system processes motion/edge information in one channel and color information in another. In *Drosophila* it has been reported that flies tend to walk or fly toward simple objects such as a vertical black bar on white background (Reichardt and Wenking, 1969; Horn and Wehner, 1975; Reichardt and Poggio, 1976; Bülthoff et al., 1982; Neuser et al., 2008). Flies could also use vertical edges to control flight speed and judge the distance to objects (Frye and Dickinson, 2007).

In each *Drosophila* compound eye, there are about 800 ommatidia, each containing eight photoreceptor cells (R1–R8; Clandinin and Zipursky, 2002). All the ommatidia contain the major photoreceptor cells R1–R6, which express blue and UV-sensitive rhodopsin 1 (Rh1), and photoreceptor cells R7/R8, which express different combinations of rhodopsins, such as Rh3/Rh5 or Rh4/Rh6. Approximately 70% of the ommatidia are named the 'yellow' type, and they express UV-sensitive Rh4 in photoreceptor cell R7, and green-sensitive Rh6 in photoreceptor cell R8. The other 30% of the ommatidia, named the 'pale' type, express UV-sensitive Rh3 in photoreceptor R7, and blue-sensitive Rh5 in photoreceptor cell R8 (Cook and Desplan, 2001). Axons of R1–R6 photoreceptor cells project to the lamina, the first neuropile in the optic lobe, while axons of photoreceptor cells R7 and R8 project directly to the medulla, the second neuropile in the optic lobe (Hardie, 1985). The variety of rhodopsin in the retina and complex neural circuitry in the optic lobe provide the possible physiological basis for color vision in *Drosophila* (Gao et al., 2008; Morante and Desplan, 2008).

The R1–R6 photoreceptor channel has been shown to participate in phototaxis, tropotaxis and motion detection (Heisenberg and Buchner, 1977; Rister et al., 2007; Yamaguchi et al., 2008). However, the R7/R8 photoreceptor channel is thought to be crucial for color vision and the detection of e-vector orientation but not motion detection (Heisenberg and Wolf, 1984; Hardie, 1985; Yamaguchi et al., 2008). Previous experiments showed that R1–R6 were necessary for fixation and optomotor behavior in both flying and walking flies, and R7 played a secondary role (Heisenberg and Buchner, 1977; Morton and Cosens, 1978; Mimura, 1982; Coombe, 1984; Strauss et al., 2001). Lamina pathways L1, L2 and L3, which

are downstream of R1–R6, are also suggested to be necessary in orientation behavior (Rister et al., 2007). All these experiments showed that the R1–R6 channel is important in orientation behavior. However, there has been no definitive data about the function of R7/R8 channel in orientation behavior towards edges, making it necessary to selectively switch off the whole color channel to fully separate orientation behavior towards edges from color vision. In the present study, using a new color light-emitting diode (LED)-based Buridan's paradigm in which edges were produced by adjustable colors, we investigated the relationship between these photoreceptor channels and edge detection in the fly. We found that motion channel (R1–R6) is necessary and sufficient for edge detection, and that the color channel (R7/R8) is dispensable for edge detection.

## MATERIALS AND METHODS

### Animals

The flies used in all experiments were raised on a standard medium of corn meal and molasses (Guo et al., 1996) in a 12h:12h light:dark cycle at 25°C and 60% humidity. Three- to five-day-old flies were tested at least 24h after their wings had been shortened to one-third of their normal length under CO<sub>2</sub> anesthesia. The following flies were used in the experiments: *wild-type Canton S* (WTCS), *wild-type Berlin* (WTB), *ninaE*<sup>17</sup> (Hanai et al., 2008), *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> (Yamaguchi et al., 2008), *ninaE*<sup>17</sup>, *P[Rhl>3]* and *w*; *ninaE*<sup>17</sup> *e[s]*, *P[Rhl>4]* (Feiler et al., 1992), *yw*; *ninaE*<sup>17</sup>, *P[Rhl>5, y<sup>+</sup>]* (Chou et al., 1996), and *yw*; *ninaE*<sup>17</sup>, *P[Rhl>6, y<sup>+</sup>]* (Salcedo et al., 1999), *ninaE*<sup>17</sup> *e[s]*, *P[Rhl>4]*, *ninaE*<sup>17</sup>, *P[Rhl>5, y<sup>+</sup>]* and *ninaE*<sup>17</sup>, *P[Rhl>6, y<sup>+</sup>]*.

### Behavior assay

We used Buridan's paradigm (Bülthoff et al., 1982) with modifications. During the experiment, a wing-cut fly was allowed to walk for 3 min on a circular platform, 86 mm in diameter, surrounded by a water-filled moat to prevent its escape (Fig. 1A). The moat was surrounded by a panoramic LED display, 290 mm in diameter and 345 mm in height. The LED display was a cylinder of evenly distributed 128 (row) × 32 (column) LED units and was computer-controlled with LED Studio software (Shenzhen Sinorad Medical Electronics, Shenzhen, China). The refresh rate of the LED panels was 400 Hz. A camera (WV-BP330, Panasonic System Networks, Suzhou, China) directly above the arena was connected to a computer to record the fly's walking track at a rate of 12 frames s<sup>-1</sup>, and position coordinates of the fly in each frame were calculated using Limelight software (Coulbourn Instruments, Whitehall, PA, USA). Male and female flies were used alternately.

### Visual stimulus

Each LED unit on the display included three diodes that separately emitted red, green and blue light. The absolute irradiance of the blue and green LEDs in all the visual stimulation was measured with the Spectroradiometer USB2000+ (Ocean Optics, Dunedin, FL, USA). The peak of the blue LED's spectrum curve was at 466 nm, while the peak of the green LED was at 519 nm (Fig. 1B). We used the red/green/blue (RGB) value to describe the brightness and chromatic properties of a visual stimulus. In the RGB color model, brightness ranging from completely dark to fully bright was divided into 256 grades, with 0 as the darkest and 255 as the brightest. RGB[0,0,255] (absolute irradiance value:  $2.9483 \times 10^{13}$  quanta cm<sup>-2</sup> s<sup>-1</sup>) represents the brightest blue with the red and green diodes off; RGB[0,255,0] (absolute irradiance value:  $3.9557 \times 10^{13}$  quanta cm<sup>-2</sup> s<sup>-1</sup>) represents the brightest green with the

red and blue diodes off (Fig. 1B). In some cases, we simply use B[m] and G[n] to represent blue color of RGB[0,0,m] and green color of RGB[0,n,0] for convenience.

### Date analysis

A circle composed of color dots, as seen in Fig. 1C, is used to represent the horizontal view of the visual stimulus on the LED cylinder. Different angles are marked around the circle (Fig. 1C). The orientation of a fly towards a certain angular position ( $\theta$  in Fig. 1C) on the LED cylinder is indicated by the projection of a vector formed by two consecutive position points (red dot and green dot in Fig. 1C) of the fly on the platform (Fig. 1C). The error angle  $\alpha$  represents the smallest angle between a fly's orientation and an edge. The range of error angle is -102 to 102 deg (Strauss and Pichler, 1998). The probability distribution of a group of flies' orientation over 3 min is schematically shown in a polar map (Fig. 1D). The fly's orientation towards an edge is quantified by the performance index of orientation behavior towards edges (PIEO). When two edges are present at 0 and 180 deg on the circle by setting 0–180 deg of the circle as one color and 180–360 deg of the circle as the other color, the circle is divided into four equal sections of 45–135, 135–225, 225–315 and 315–0–45 deg. The 135–225 and 315–0–45 deg sections correspond to an edge, the other two sections to the absence of an edge. The probabilities of a fly's orientation in sections with or without an edge are defined as  $P_{\text{edge}}$  and  $P_{\text{edgeless}}$ . The PIEO is then defined as  $(P_{\text{edge}} - P_{\text{edgeless}}) / (P_{\text{edge}} + P_{\text{edgeless}})$  (Fig. 1E), and the performance index of color preference (PICP) is used to measure the fly's preference for different colors. The probabilities of the fly's orientation in 0–180 and 180–360 deg sections are defined as  $P_{0-180}$  and  $P_{180-360}$ . PICP is then defined as  $(P_{0-180} - P_{180-360}) / (P_{0-180} + P_{180-360})$  (Fig. 1F). The mean walking speed is defined as total walking distance divided by its time. Turning angle is the change of a fly's orientation between two frames.

### Statistical analysis

The Lilliefors test was used to estimate whether data followed a normal distribution. We used the *t*-test to determine the significance of differences among groups. The *t*-test was also used to compare the PIEO (PICP) value of a group to zero, which is the theoretical random value.

## RESULTS

### Wild-type flies orient towards blue/green edges

We adopted a revised Buridan's paradigm, in which two edges could form between the blue half and green half of an arena; hence edges are constituted of both color contrast and luminance contrast. Luminance of the two colors was adjusted to the highest level in the RGB mode (see Materials and methods). Individual WTB or WTCS flies with clipped wings were allowed to walk freely for 180 s on the platform surrounded by the LED arena. If the arena was made of a homogenous mixture of blue and green (RGB[0,255,255]), the flies showed even distribution of orientation (Fig. 2A,B for WTB; Fig. 2I,J for WTCS) and the direction preference for edge represented by PIEO was not significantly different from zero (theoretical value in random distribution, Fig. 2E for WTB; Fig. 2M for WTCS). In contrast, in the presence of a blue/green edge, i.e. one half of the arena was blue and the other half green, the flies showed a higher probability of walking towards the blue/green edges (Fig. 2C,D for WTB; Fig. 2K,L for WTCS). PIEOs were significantly higher than zero (Fig. 2E for WTB; Fig. 2M for WTCS). The error angle distributions of WTB and WTCS in the presence of a homogenous mixture of blue and green are flat,

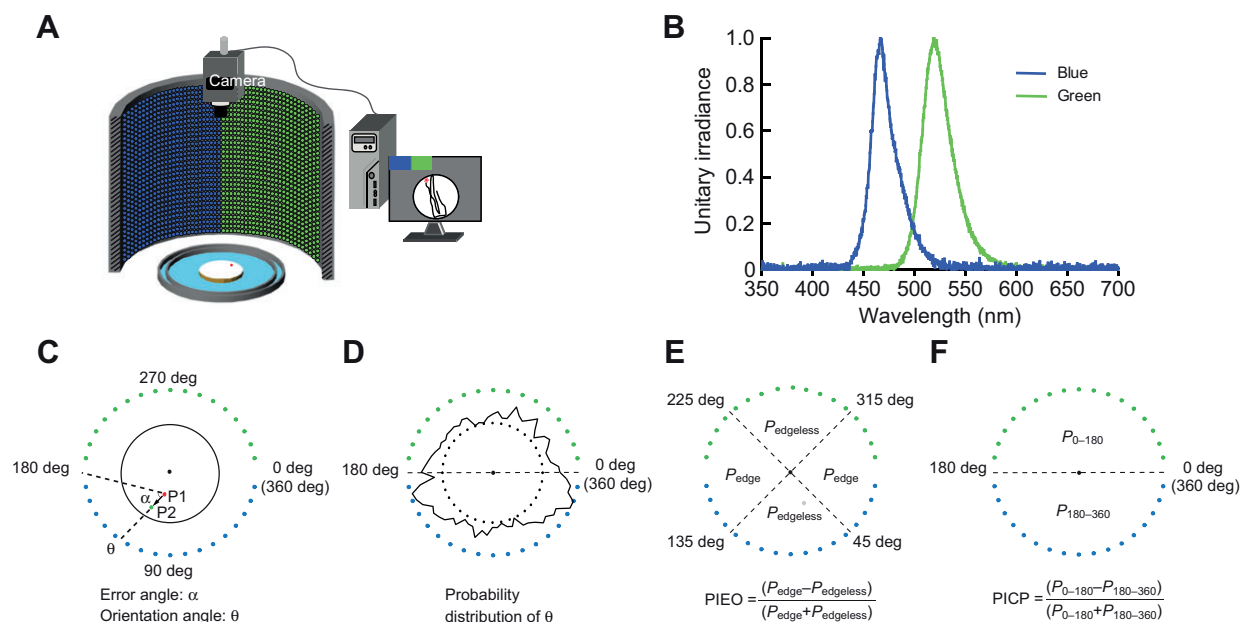


Fig. 1. Modified Buridan's paradigm for examining orientation behavior towards edges in *Drosophila*. (A) Schematic representation of the experimental apparatus. Only half of the LED is displayed. (B) Unitary irradiance photons of blue and green light used in the experiments. (C) The horizontal view of the visual stimulus on the LED cylinder represented by a color dotted circle. The angular positions of the boundaries of blue/green are arbitrarily set to be 0 and 180 deg. P1 (in red) and P2 (in green) are the positions of a fly on the platform (inner black circle) in two consecutive frames recorded by the camera. The angular position ( $\theta$ ) corresponds to the projected point of a vector (P1 to P2) onto the circle, indicating the orientation of the fly. The angle  $\alpha$  represents the smallest error angle between a fly's orientation and an edge. (D) A polar map showing the average probability distribution of a group of flies' orientation. The probability distribution is represented by a continuous black line. (E) Definition of the performance index of orientation behavior towards edges (PIEO).  $P_{\text{edge}}$  was the probability of the fly's orientation in sections with edges (135–225 and 315–0–45 deg).  $P_{\text{edgeless}}$  was the probability of the fly's orientation in sections without edges (45–135 and 135–225 deg). (F) Definition of performance index of color preference (PICP).  $P_{0-180}$  was the probability of the fly's orientation in a section with one color (0–180 deg).  $P_{180-360}$  was the probability of the fly's orientation in a section with another colour (180–360 deg).

while the distributions in the presence of a blue/green edge show a peak near 0 deg, but the peak is smaller in *WTCS* (Fig. 2F for *WTB*; Fig. 2N for *WTCS*). Yet the distributions of turning frequency of *WTB* in the presence of a homogenous mixture of blue and green and in the presence of a blue/green edge are similar (Fig. 2G), while the turning frequency distribution of *WTCS* is less steep in the presence of a blue/green edge as in a homogenous mixture of blue and green (Fig. 2O). The mean walking speed of *WTB* is higher in the presence of a blue/green edge than in a homogeneous mixture of blue and green (Fig. 2H), while the mean walking speed of *WTCS* is decreased (Fig. 2P). Such edge-orientation ability was further confirmed by changing the number of edges. As shown in Fig. 2R,S more edges in the arena led to preferences for corresponding directions. We gradually changed the luminance from maximum to zero in the LED cylinder to form a visual stimulus with only one obvious edge. It is interesting that when only one edge was present, the flies showed preferences not only for the edge, but also for the direction opposing the edge (Fig. 2Q). This so-called anti-fixation behavior has been reported in previous experiments with Buridan's paradigm (Reichardt and Poggio, 1976). In addition to orientation behavior towards edges, we also noticed that flies demonstrated a preference for the blue area over the green area, as indicated by PICP in Fig. 2E,M.

Taken together, these results show that a blue/green demarcation is able to generate edge-orientation behavior in the adult fly.

#### Flies' orientation behavior towards edges is caused by luminance contrast, but not color contrast

As the blue/green edge constituted a difference not only in color but also in brightness, we wondered whether both or only one of these factors contributes to the fly's orientation behavior towards

edges. So we tried to separate the contribution of brightness contrast and color contrast in our visual stimulus by adjusting the color or luminance that creates the edges. We first examined the orientation behavior of wild-type flies in the presence of blue/green edges with various blue/green intensity ratios, which was achieved by linearly changing the luminance of the blue light and fixing the luminance of the green light at G[255] (Fig. 3A). We found that there was a blue/green intensity ratio, the so-called point of equal luminance (POE), at which *WTB* did not show significant orientation behavior towards edges. The POE corresponded to a blue/green ratio of B[226]/G[255] (Fig. 3B). Furthermore, we noticed that *WTB* flies showed a preference for the blue area not only at the POE but also at other blue color intensities, such as B[220], B[232] and B[255] (Fig. 3C). To test the equiluminant stimuli at a range of mean luminances, we fixed the luminance of the green light at lower intensities of G[232] and G[197] and linearly changed the luminance of the blue light. We also found corresponding POE (Fig. 3D–I). However, when we fixed the luminance of the blue light at intensities of B[197] and linearly changed the luminance of the green light, the POE was not as clear as in previous cases (Fig. 3J,K). It is worth noting that according to the unitary irradiance spectrum, the blue/green irradiance ratio at the POE in most cases is about 0.4 (Fig. 3B,E).

Because at POE the blue/green contrast still existed, the loss of orientation behavior towards edges suggests that orientation behavior towards edges is independent of color contrast but dependent on luminance contrast. We then tested whether flies could use only luminance contrast-based edge to control their orientation. So we tried two types of luminance contrast-based edges: G[255]/G[197] in green and B[255]/B[197] in blue. In both cases, *WTB* flies showed

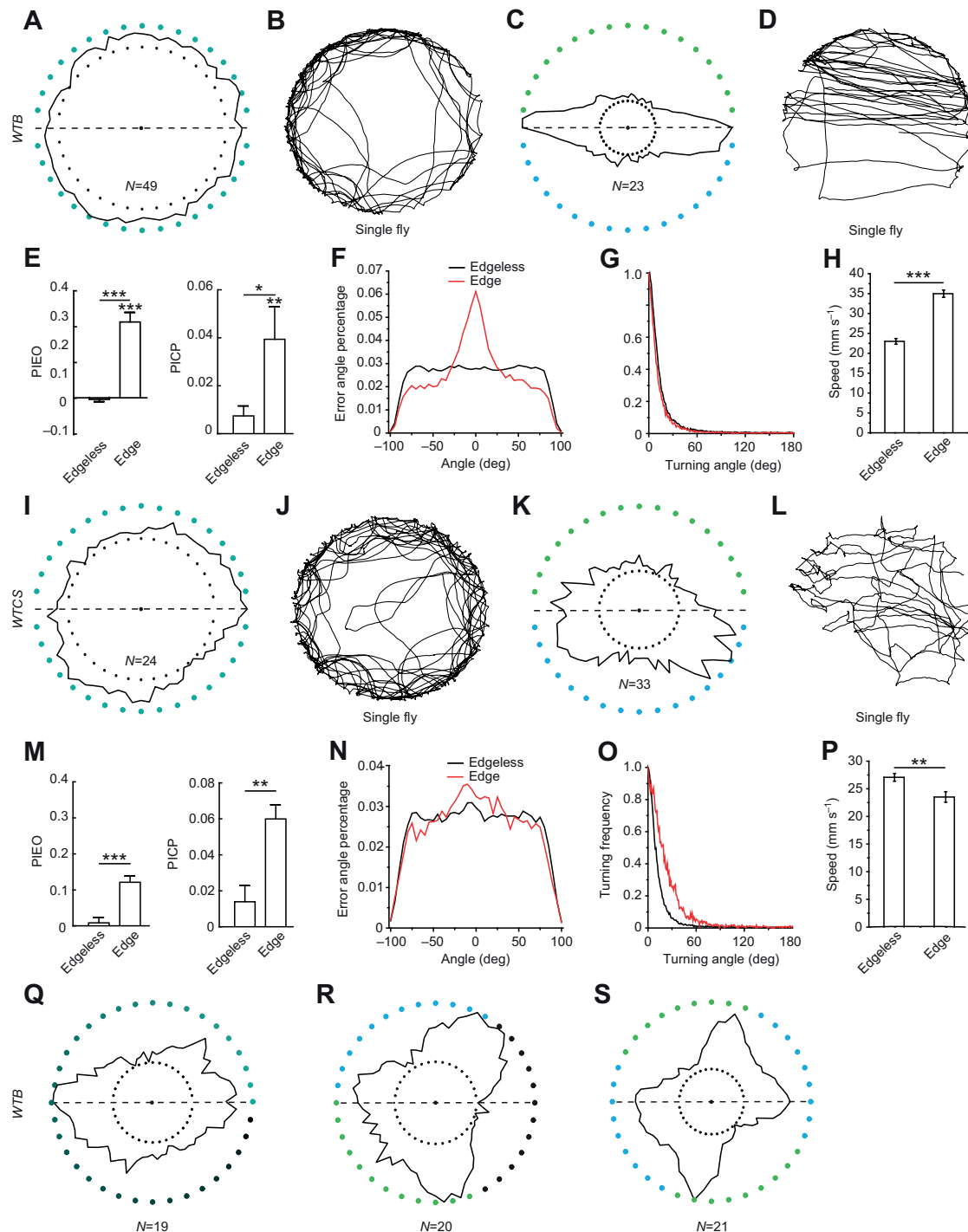


Fig. 2. Wild-type flies were able to orient towards blue/green edges. (A–H) *WTb* showed significant orientation preference in a visual panorama with edges constituted of half green (RGB[0,255,0]) and half blue (RGB[0,0,255]), but not in a homogenous visual panorama consisting of a mixture of blue and green (RGB[0,255,255]). (A) Polar map of *WTb* flies ( $N=49$ ) in a homogenous mixture of blue and green (RGB[0,255,255]) visual panorama. (B) Walking trajectory of a representative single fly in (A). (C) Polar map of *WTb* flies ( $N=23$ ) in a half green (RGB[0,255,0]) and half blue (RGB[0,0,255]) visual panorama. (D) A representative walking trajectory of the fly in C. (E) PIEO and PICP of *WTb* in (C) are significantly higher than in (A). (F) Error angle percentage distribution of *WTb* in A and C. (G) Turning angle distribution of *WTb* in (A) and (C). (H) Mean walking speeds of *WTb* in C are significantly higher than in A. (I–P) *WTCS* also showed a significant orientation preference towards blue/green edges. (I) Polar map of *WTCS* flies ( $N=24$ ) in a homogenous mixture of blue and green (RGB[0,255,255]) visual panorama. (J) Walking trajectory of a representative single fly in I. (K) Polar map of *WTCS* flies ( $N=33$ ) in a half green (RGB[0,255,0]) and half blue (RGB[0,0,255]) visual panorama. (L) Walking trajectory of a representative single fly in K. (M) PIEO and PICP of *WTb* in (I) are significantly higher than in K. (N) Error angle percentage distribution of *WTb* in I and K. (O) Turning frequency distribution of *WTb* in (I) and (K). (P) Mean walking speeds of *WTb* in I are significantly higher than in K. (Q–S) *WTb* flies showed orientation behavior towards edges in the presence of various numbers of edges. (Q) Preferred edge direction of flies in the presence of only one edge. Note that the flies also walked in the opposite direction to the edge. Flies showed three or four preferred edge directions in the presence of three (R) or four edges (S). Error bars are s.e.m.; for comparison between bars: \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ ; for comparison between bar and zero (theoretical value of random distribution): \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .



obvious edge-orientation behavior (Fig. 3M). The PIEO and PICP values of *WTB* in the blue luminance contrast were not significantly different from the results in the green luminance contrast, as shown in Fig. 3N. Finally we wanted to know whether the color contrast contributes to orientation behavior towards edges in the presence of luminance contrast, so we tried to compare the orientation

behavior towards edges under the same luminance contrast with and without color contrast. We found that the POE of blue/green ratio is B[226]/G[255] (see Fig. 3B), which means that flies' perception for B[226] and G[255] is equal luminance. Then we tried two types of edges: B[226]/B[255] and G[255]/B[255]. Since B[226] and G[255] are equal luminance, two types of edges of

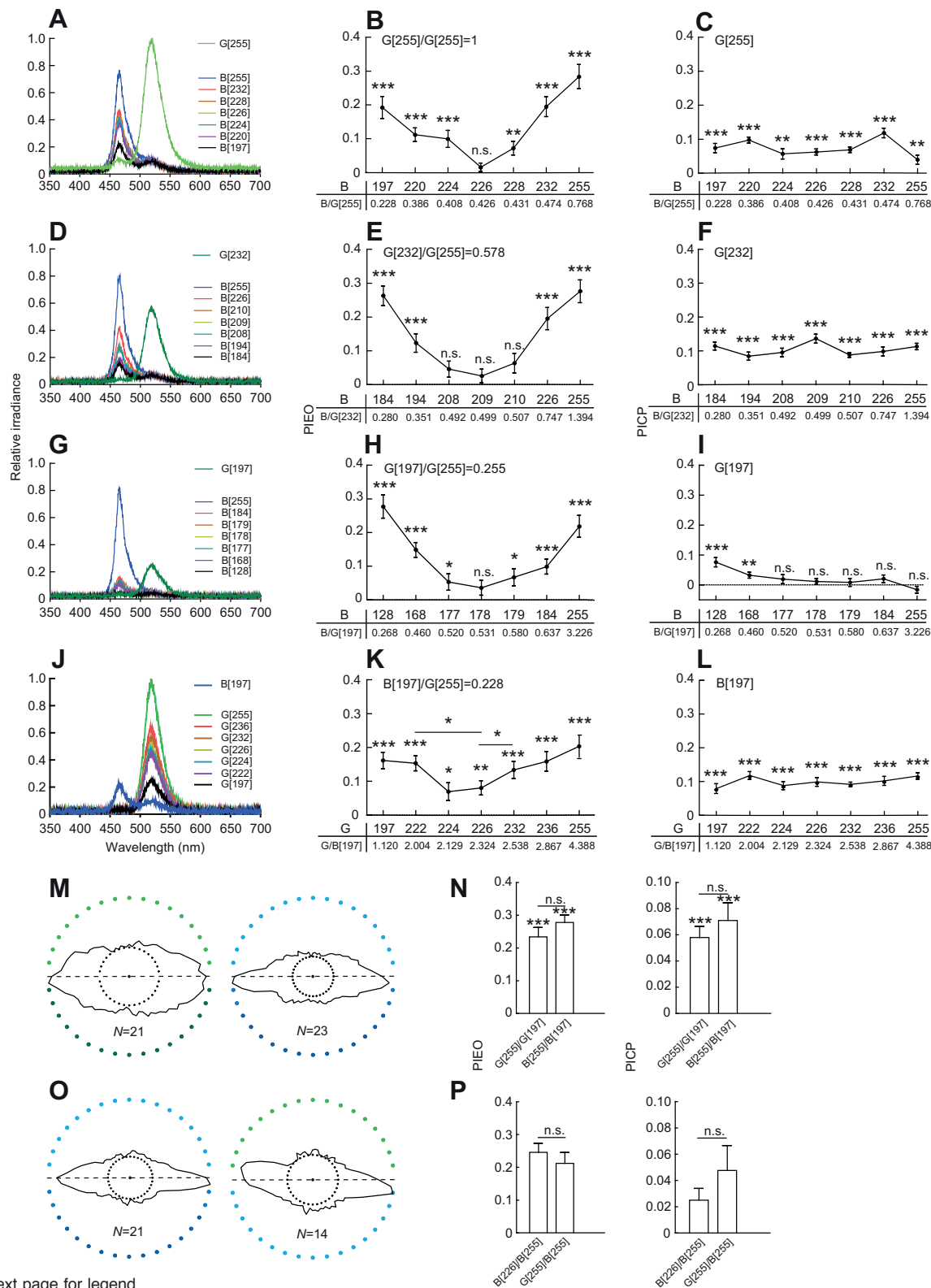


Fig. 3. See next page for legend.

Fig. 3. Orientation behavior towards edges is caused by luminance contrast but not color contrast. (A) Relative irradiance photons of blue and green light of different intensities used in (B,C) experiments. (B) PIEO of *WTB* in presence of various luminance contrasts, which were achieved by maintaining the luminance of the green light constantly at G[255] while changing the luminance of the blue light from B[197] to B[255]. Note that orientation behavior towards edges was significant at all blue light intensities except for B[226], which was at the point of equal luminance (POE) for *WTB*. All the relative luminance is shown below the RGB value (from left to right,  $N=23, 16, 21, 38, 20, 26$  and  $23$ ). (C) PICP of *WTB* in presence of a series of luminance contrasts. *WTB* flies showed a preference for the blue area over the green area at all blue light intensities. (D) Relative irradiance photons of blue and green light of different intensities used in (E,F) experiments. (E) PIEO of *WTB* in presence of various luminance contrasts, which were achieved by maintaining the luminance of the green light constantly at G[232] while changing the luminance of the blue light from B[184] to B[255]. Orientation behavior towards edges was significant at all blue light intensities except for B[208], B[209] and B[210] (from left to right,  $N=16, 20, 19, 29, 11, 20$  and  $20$ ). (F) PICP of *WTB* in presence of a series of luminance contrasts. *WTB* flies showed a preference for the blue area over the green area at all blue light intensities. (G) Relative irradiance photons of blue and green light of different intensities used in (H,I) experiments. (H) PIEO of *WTB* in presence of various luminance contrasts, which were achieved by maintaining the luminance of the green light constantly at G[197] while changing the luminance of the blue light from B[128] to B[255]. Note that orientation behavior towards edges was significant at all blue light intensities except for B[178] (from left to right,  $N=17, 24, 18, 20, 19, 18$  and  $19$ ). (I) PICP of *WTB* in presence of a series of luminance contrasts. *WTB* flies showed a preference for the blue area over the green area at B[128] and B[168]. (J) Relative irradiance photons of blue and green light of different intensities used in (K,L) experiments. (K) PIEO of *WTB* in presence of various luminance contrasts, which were achieved by maintaining the luminance of the blue light constantly at B[197] while changing the luminance of the green light from G[197] to G[255]. Orientation behavior towards edges was significant at all blue light intensities, but G[224] was the closest to the POE (from left to right,  $N=20, 17, 19, 19, 18, 14$  and  $20$ ). (L) PICP of *WTB* in presence of a series of luminance contrasts. *WTB* flies showed a preference for the blue area over the green area at all green light intensities. (M,N) *WTB* were able to orient toward edges produced only by luminance contrast. (M) Polar maps of *WTB* in presence of green luminance contrast-based edges G[255]/G[197] and in presence of blue luminance contrast-based edges B[255]/B[197]. (N) PIEO and PICP of *WTB* in presence of green luminance contrast-based edges ( $N=21$ ) and blue luminance contrast-based edges ( $N=23$ ). (O,P) Color contrast did not enhance the detection of luminance contrast-based edges. (O) Polar maps of *WTB* in presence of blue luminance contrast-based edges B[255]/B[226] and in green–blue contrast-based edges B[255]/G[255]. According to POE in (B), B[226] and G[255] are of equal luminance to *WTB* flies. (P) PIEO and PICP of *WTB* in presence of blue luminance contrast-based edges ( $N=21$ ) and green–blue contrast-based edges ( $N=14$ ). Error bars are s.e.m.; for comparison between bars: n.s., not significant; \* $P<0.05$ ; for comparison between bar and zero (theoretical value of random distribution): n.s., not significant; \*\* $P<0.05$ , \*\*\* $P<0.01$ , \*\*\*\* $P<0.001$ .

B[226]/B[255] and G[255]/B[255] should present a similar luminance contrast, but their color contrasts are different (Fig. 3O). We found that there was no significant difference between the orientation behavior towards edges under these two conditions (Fig. 3P), indicating that color does not enhance orientation at perceptually approximate luminance contrasts. These results suggest that flies could use only luminance contrast-based edges but not color contrast-based edges to guide their walking orientation.

#### R1–R6 but not R7/R8 contributes to orientation behavior towards edges

As orientation behavior towards edges is luminance dependent and color independent, we wanted to establish the underlying

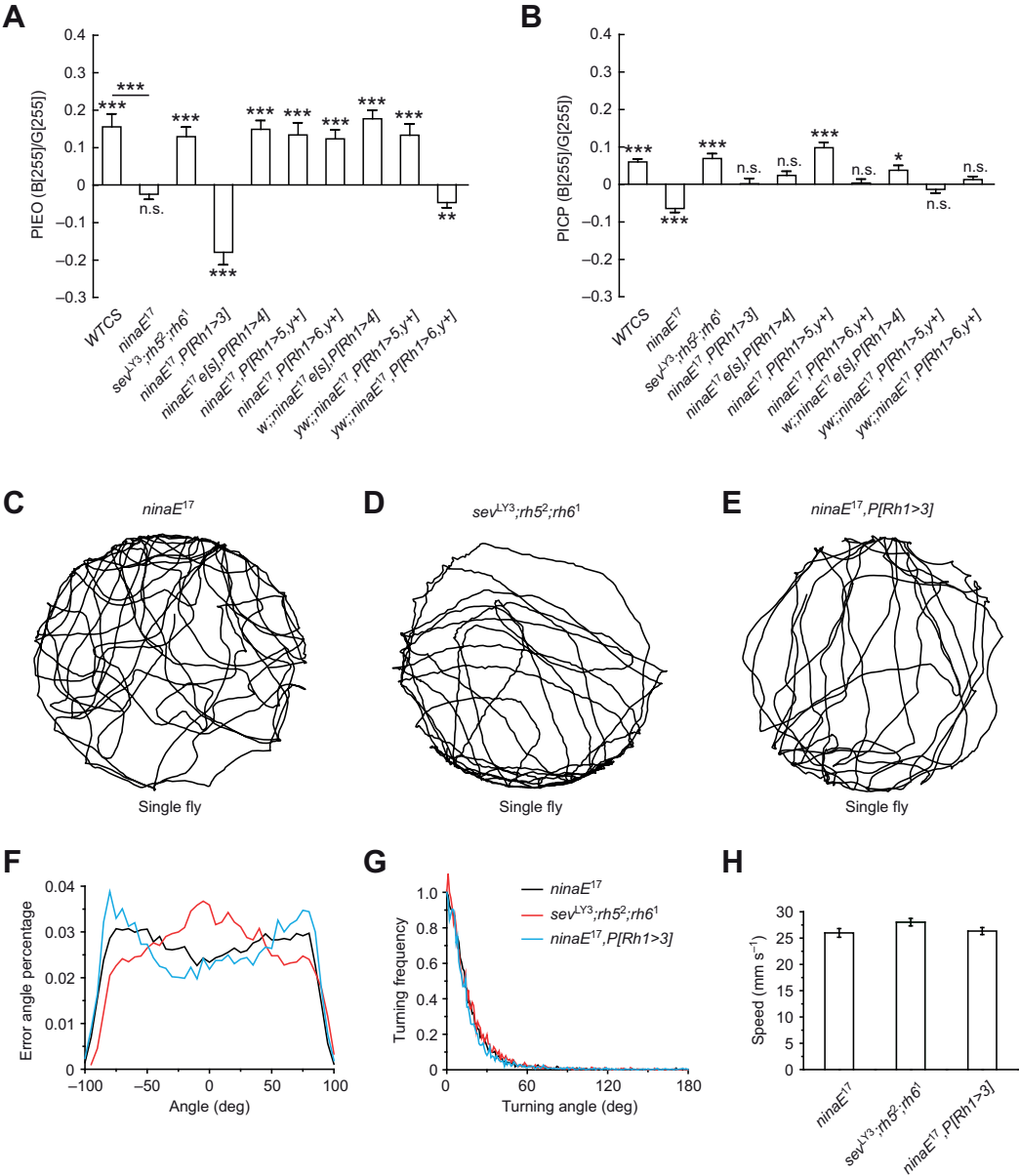
physiological mechanism. For this, we used an Rh1 mutant, *ninaE*<sup>17</sup>, in which the monochromatic R1–R6 photoreceptor cells are dysfunctional, but the chromatic R7/R8 photoreceptor cells are normal. Thus this mutant is normal in color sensing but abnormal in luminance sensation and motion detection (Yamaguchi et al., 2008). Another mutant line we used was *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup>, in which the chromatic photoreceptor cells R7 are absent and the working rhodopsins of Rh5 and Rh6 in photoreceptor cells R8 are abnormal. Thus this mutant is normal in luminance sensation but abnormal in color sensation. As shown in Fig. 4A,C, *ninaE*<sup>17</sup> appeared to be defective in orientation behavior towards edges while *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> was not significantly different from the wild-type control in orientation behavior towards edges (Fig. 4A,C,D). These results demonstrate that R1–R6 photoreceptor cells, but not R7/R8, are necessary for orientation behavior towards edges. Besides, both *WTCS* and *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> showed a preference for the blue area, and yet *ninaE*<sup>17</sup> showed a preference for the green area.

To establish whether R1–R6 photoreceptor cells are sufficient for orientation behavior towards edges, we ectopically expressed different rhodopsins in the R1–R6 photoreceptor cells in the Rh1 mutant background flies. We found that over-expression of Rh4, Rh5 or Rh6 could efficiently restore the edge-orientation defect in the *ninaE*<sup>17</sup> mutant, but expression of Rh3 failed to restore the edge-orientation defect (Fig. 4A,E). Expression of Rh3 actually resulted in an edge-avoiding effect, which was an unexpected result (Fig. 4A). An over-expression of Rh5 could also restore the color preference in the *ninaE*<sup>17</sup> mutant to the *WTCS* level. The peak of error angle distribution of *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> in the presence of blue/green edges was near 0 deg, while that of the distributions of *ninaE*<sup>17</sup> and *ninaE*<sup>17</sup>; *P[Rh1>3]* was near 100 and –100 deg (Fig. 4J). Yet the distributions of turning frequency of *ninaE*<sup>17</sup>, *sev*<sup>LY3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> and *ninaE*<sup>17</sup>; *P[Rh1>3]* in the presence of blue/green edges were similar (Fig. 4K). The mean walking speed of these three lines were also not significantly different (Fig. 4L). Ectopic expression of Rh4 or Rh5 in R1–R6 photoreceptor cells could fully restore the defect of *w*; *ninaE*<sup>17</sup> to the *WTCS* level, but expression of Rh6 failed to restore the edge-orientation defect in *yw*; *ninaE*<sup>17</sup>. Such ectopic expression of Rh4 or Rh5 switched the preference of *w*; *ninaE*<sup>17</sup> from green to blue, but the expression of Rh6 resulted in loss of color preference between blue and green (Fig. 4B). Taken together, these results show that R1–R6 photoreceptor cells are both sufficient and necessary for orientation behavior towards edges in *Drosophila*.

According to above results, the POE should be decided by the spectral sensitivity of photoreceptors R1–R6. To verify whether ectopic expression of other rhodopsins in R1–R6 might shift the POE, we examined the orientation behavior of all the rescue flies in the presence of blue/green edges with various blue/green intensity ratios (Fig. 5). We found that POE was drastically shifted with ectopic expression of rhodopsin in R1–R6, e.g. replacing Rh1 with Rh5 compared with WT flies (Fig. 3B), less blue light was required to balance the green intensity (Fig. 5G,H), giving a blue/green ratio of 0.169 compared with 0.426 for WT flies.

#### DISCUSSION

We have shown that edge-orientation behavior in *Drosophila* can be elicited by luminance contrast but not by color contrast. The monochromatic channel, outer photoreceptor cells R1–R6, contribute to edge detection, though the color channel (inner photoreceptor cells R7 and R8) does not. There are several lines of evidence for this conclusion. First, wild-type flies can orient towards achromatic edges but not to edges defined by equiluminant color contrast. Second, color contrast did not enhance orientation behavior



towards edges at a salient luminance contrast. Third, flies with mutated R1–R6 photoreceptor cells could not perform edge detection. Fourth, genetic removal of the function of photoreceptor cells R7 and R8 did not diminish the orientation index to edges. Finally, flies with the Rh1 in R1–R6 photoreceptor cells changed to Rh4, Rh5 or Rh6 could perform orientation behavior towards edges, whereas flies with the Rh1 changed to Rh3 showed edge aversion. Furthermore, such ectopic expression of different rhodopsins in outer photoreceptors altered the POE.

Equal absolute irradiance green and blue will be perceived by the human eye as brighter green (Rovamo et al., 1996). Similarly a wild-type fly may perceive blue and green as having a different brightness. In flies, we do not even know all the luminosity functions, so we could not certify whether two stimuli had exactly the same luminance contrast. However, according to the results of POE experiments in Fig. 3B, we speculate that the stimuli used in Fig. 3O (B[226]/B[255] and G[255]/B[255]) should present a similar luminance contrast.

At the point of POE (Fig. 3B), the relative photon catch between Rh4 and Rh6 receptors for the blue field is about 0.051, while for

the green field it is close to zero, which means that there is a significant color contrast for R7 and R8. Moreover, previous research found a type of medulla neuron contacting the R8 photoreceptors in different ommatidia, which could be the potential neural substructure for comparing the light signal between Rh5 and Rh6 (Morante and Desplan, 2008). These two points suggest that there may well be effective color contrast between the blue and green stimuli at the point of POE.

Expressing Rh3 in R1–R6 photoreceptor cells did not restore the edge-orientation defect in Rh1 mutant background flies, but gave rise to an inverted edge preference. This indicated that replacing Rh1 with Rh3 could not support the orientation behavior towards edges in flies, but such flies may be able to detect edges. Previous electrophysiological experiments reported that flies with Rh1 changed to Rh3 in R1–R6 photoreceptor cells showed similar but slight slower response to UV flash than wild-type flies (Feiler et al., 1992; Liu et al., 2008), which also suggests that the substitution of Rh1 with Rh3 may cause other unpredicted effects. Expression of Rh6 in R1–R6 did not rescue the orientation behavior towards edges in *yw;ninaE17* but rescued the orientation behavior towards

Fig. 4. R1–R6 cells but not R7/R8 contributed to orientation behavior towards edges. (A) PIEO of *ninaE17* ( $N=49$ ) was not significantly different from zero, but it was significantly different from *WTCS* ( $N=19$ ). PIEO of *sevLY3;rh52;rh61* ( $N=21$ ) was not significantly different from *WTCS*. Ectopic expression of Rh4 ( $N=19$ ) or Rh5 ( $N=16$ ) or Rh6 ( $N=19$ ) in R1–R6 photoreceptor cells could fully restore the defect of *ninaE17* to the *WTCS* level, but expression of Rh3 ( $N=20$ ) failed to restore the edge-orientation defect in *ninaE17*. Ectopic expression of Rh4 ( $N=21$ ) or Rh5 ( $N=20$ ) in R1–R6 photoreceptor cells could fully restore the defect of *w;ninaE17* to the *WTCS* level, but expression of Rh6 ( $N=20$ ) failed to restore the edge-orientation defect in *w;ninaE17*. (B) PICP of *ninaE17*, *sevLY3;rh52;rh61* and all the rescue flies. Error bars are s.e.m.; \*\*\* $P<0.001$ ; a,  $P<0.001$ , b,  $P<0.01$ , c,  $P<0.05$ , n.s., not significant. (C–E) A representative single fly walking trajectory of *ninaE17*, *sevLY3;rh52;rh61* and *ninaE17;P[Rh1>3]*. (J) Error angle percentage distribution of *ninaE17*, *sevLY3;rh52;rh61* and *ninaE17;P[Rh1>3]*. (K) Turning angle distribution of *ninaE17*, *sevLY3;rh52;rh61* and *ninaE17;P[Rh1>3]*. (L) Mean walking speed of *ninaE17*, *sevLY3;rh52;rh61* and *ninaE17;P[Rh1>3]*.

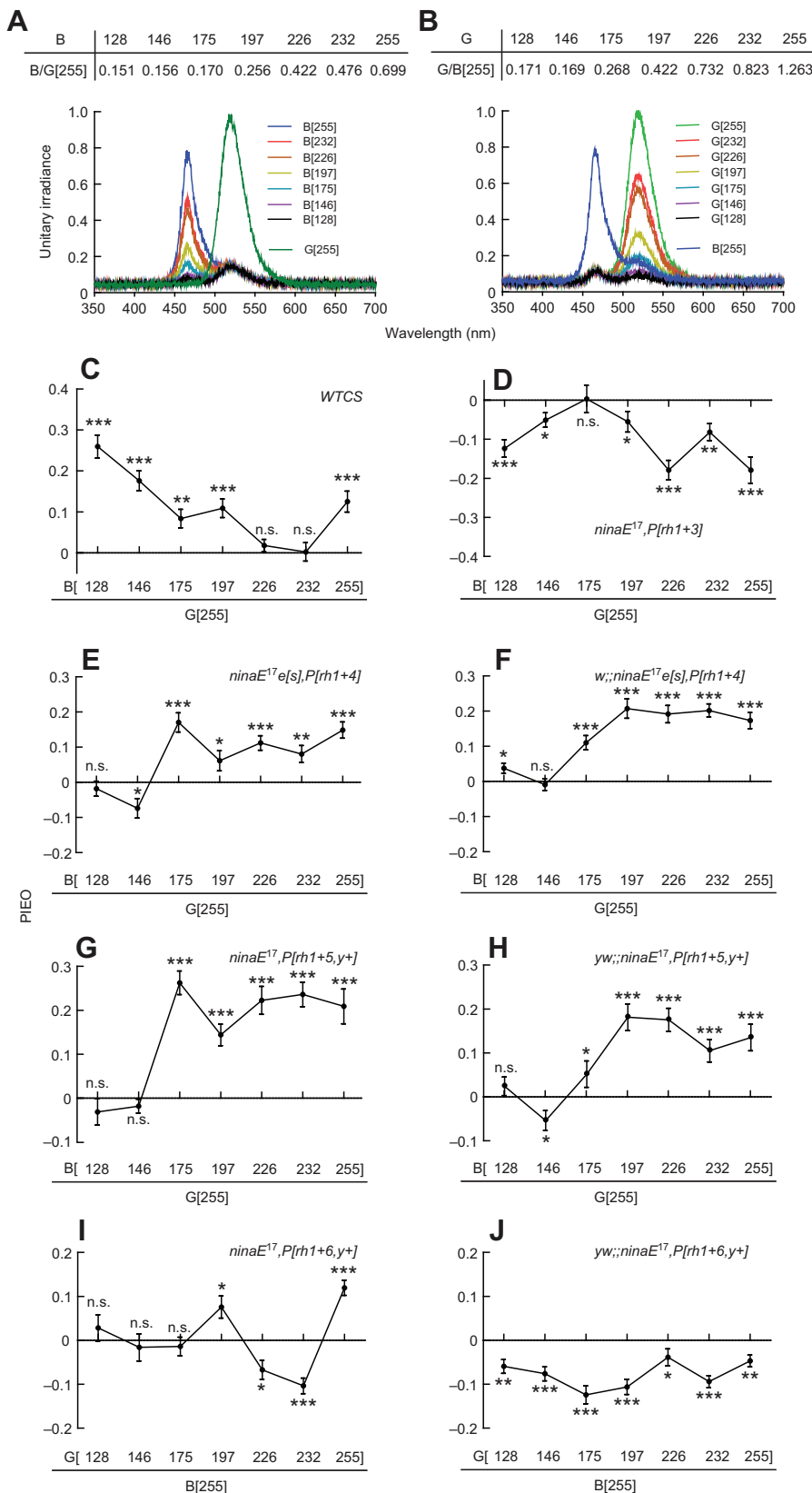


Fig. 5. POE shifted in ectopic expression of rhodopsin. (A) Relative luminance and relative irradiance photons of blue and green light of different intensities used in (C–H). (B) Relative luminance and relative irradiance photons of blue and green light of different intensities used in (I, J). (C–H) PIEO of WTCS (from left to right,  $N=24, 24, 24, 24, 20, 22$  and 17), *ninaE<sup>17</sup>,P[Rh1>3]* (from left to right,  $N=21, 21, 20, 22, 21, 20$  and 20), *ninaE<sup>17</sup>e[s],P[Rh1>4]* (from left to right,  $N=16, 9, 16, 24, 16, 16$  and 16), *w;;ninaE<sup>17</sup>e[s],P[Rh1>4]* (from left to right,  $N=24, 24, 24, 19, 21, 20$  and 19), *ninaE<sup>17</sup>,P[Rh1>5,y<sup>+</sup>]* (from left to right,  $N=10, 18, 22, 19, 21, 24, 24$ ), *yw;;ninaE<sup>17</sup>,P[Rh1>5,y<sup>+</sup>]* (from left to right,  $N=20, 22, 18, 19, 20, 24$  and 18) in the presence of various luminance contrasts, which were achieved by maintaining the luminance of the green light constantly at G[255] while changing the luminance of the blue light from B[128] to B[255]. (I, J) PIEO of *ninaE<sup>17</sup>,P[Rh1>6,y<sup>+</sup>]* (from left to right,  $N=24, 24, 24, 23, 15, 15$  and 20) and *yw;;ninaE<sup>17</sup>,P[Rh1>6,y<sup>+</sup>]* (from left to right,  $N=24, 24, 24, 24, 19, 19$  and 20) in the presence of various luminance contrasts, which were achieved by maintaining the luminance of the blue light constantly at B[255] while changing the luminance of the green light from G[128] to G[255].

edges in *ninaE<sup>17</sup>*. This suggests the difference in eye color may also cause other unpredicted effects.

Previous research showed that ocelli may function in phototaxis and edge fixation for walking blowflies (Wehrhahn, 1984).

Behavioral results in fruit fly showed that ocelli are not necessary in phototaxis behavior but may function in modulating compound eye sensitivity (Hu and Stark, 1980). In our experiments, flies with mutation in R1–R6 but with normally functioning ocelli showed no



obvious orientation behavior toward edges. This result indicates that ocelli do not participate in orientation behavior toward edges, which is consistent with previous reports.

Both a previous publication (Strauss et al., 2001) and our work have shown that orientation of *ninaE*<sup>17</sup> mutant is abnormal, although in the study of Strauss et al., *ninaE*<sup>17</sup> showed residual orientation in Buridan's paradigm. The difference between our results and previous results may mainly be caused by the obviously different experimental conditions. In our experiments, the visual stimulus was two edges formed between the blue half and green half of the LED arena. Luminance of the two colors was adjusted to the highest level (RGB[0,255,0]/RGB[0,0,255]) in the RGB mode. *ninaE*<sup>17</sup> was tested under the visual condition with both color contrast and luminance contrast. In our experimental conditions with color stimulation, *ninaE*<sup>17</sup> did not show edge orientation behavior but showed weak preference for the green area. In addition, genetic background is clearly important for this behavior, given that *WTB* showed an obviously higher orientation score than that of *WTCS* in Fig. 2.

There are two main causative factors in color preference – chromatic difference and luminance difference. Wild-type flies showed a color preference for the blue area at POE with relative high mean luminance, which could be caused by chromatic difference (Fig. 3C,F). However, flies did not show color preference at POE with low mean luminance (Fig. 3I). Flies also showed a similar level of color preference for the blue area at other blue/green brightness ratios as at POE (Fig. 3C,F); this could be caused by a chromatic difference in the blue and green area or residual luminance contrast, which could not be excluded in our experiments. In our experiments, *ninaE*<sup>17</sup> showed a preference for the green area and *sev*<sup>L<sup>Y</sup>3</sup>; *rh5*<sup>2</sup>; *rh6*<sup>1</sup> preferred the blue area (Fig. 4B). This is consistent with a previous study by Yamaguchi et al. (Yamaguchi et al., 2010).

Our work implies that edge detection is a color-blind process in the fruit fly, although the achromatic channel in *Drosophila* is blue and UV sensitive (Kirschfeld and Franceschini, 1977; Britt et al., 1993). Previous research in honeybees implied that edge detection is also a color-independent process (Lehrer et al., 1990; Srinivasan, 2011). The reason for achromatic processing of edges in these two species may have originated in the natural environment, in which achromatic processing of edges is sufficient for the insect to survive.

In goldfish, the edge-triggering response is an intrinsic factor of optomotor response, implying that edge detection has an internal relation with motion detection (Kim and Jung, 2010). In our experiments, orientation behavior towards edges originates from the monochromatic channel (outer photoreceptor cells R1–R6), which is also the motion channel. This also indicates that orientation behavior towards edges is related to the optomotor response.

Further research about the neural circuitry underlying edge detection in *Drosophila* could benefit from knowledge about the fly's visual system and powerful genetic tools in *Drosophila*, which will contribute to our understanding of how edge detection and parallel processing of visual information are executed in other animals.

#### LIST OF ABBREVIATIONS

LED	light-emitting diode
PICP	performance index of color preference
PIEO	performance index of orientation behavior towards edges
POE	point of equal luminance
RGB	red, green, blue
Rh1	rhodopsin 1
Rh3	rhodopsin 3
Rh4	rhodopsin 4
Rh5	rhodopsin 5
Rh6	rhodopsin 6

<i>WTB</i>	wild-type Berlin
<i>WTCS</i>	wild-type Canton S

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#### REFERENCES

- Bhagavatula, P., Claudianos, C., Ibbotson, M. and Srinivasan, M. (2009). Edge detection in landing budgerigars (*Melopsittacus undulatus*). *PLoS ONE* **4**, e7301.
- Biederman, I. (1987). Recognition-by-components: a theory of human image understanding. *Psychol. Rev.* **94**, 115–117.
- Britt, S. G., Feiler, R., Kirschfeld, K. and Zuker, C. S. (1993). Spectral tuning of rhodopsin and metarhodopsin *in vivo*. *Neuron* **11**, 29–39.
- Bülthoff, H., Götz, K. G. and Herre, M. (1982). Recurrent inversion of visual orientation in the walking fly, *Drosophila melanogaster*. *J. Comp. Physiol. A* **148**, 471–481.
- Chou, W. H., Hall, K. J., Wilson, D. B., Wideman, C. L., Townson, S. M., Chadwell, L. V. and Britt, S. G. (1996). Identification of a novel *Drosophila* opsin reveals specific patterning of the R7 and R8 photoreceptor cells. *Neuron* **17**, 1101–1115.
- Clandinin, T. R. and Zipursky, S. L. (2002). Making connections in the fly visual system. *Neuron* **35**, 827–841.
- Cook, T. and Desplan, C. (2001). Photoreceptor subtype specification: from flies to humans. *Semin. Cell Dev. Biol.* **12**, 509–518.
- Coombe, P. E. (1984). The role of retinula cell types in fixation behaviour of walking *Drosophila melanogaster*. *J. Comp. Physiol. A* **155**, 661–672.
- De Weerd, P., Sprague, J. M., Vandenbussche, E. and Orban, G. A. (1994). Two stages in visual texture segregation: a lesion study in the cat. *J. Neurosci.* **14**, 929–948.
- Feiler, R., Bjornson, R., Kirschfeld, K., Mismar, D., Rubin, G. M., Smith, D. P., Socolich, M. and Zuker, C. S. (1992). Ectopic expression of ultraviolet-rhodopsins in the blue photoreceptor cells of *Drosophila*: visual physiology and photochemistry of transgenic animals. *J. Neurosci.* **12**, 3862–3868.
- Frye, M. A. and Dickinson, M. H. (2007). Visual edge orientation shapes free-flight behavior in *Drosophila*. *Fly* **1**, 153–154.
- Gao, S., Takemura, S. Y., Ting, C. Y., Huang, S., Lu, Z., Luan, H., Rister, J., Thum, A. S., Yang, M., Hong, S. T. et al. (2008). The neural substrate of spectral preference in *Drosophila*. *Neuron* **60**, 328–342.
- Greene, H. H. and Brown, J. M. (1995). The effect of nearby luminance contrast polarity on color boundary localization. *Vision Res.* **35**, 2767–2771.
- Guo, A., Li, L., Xia, S. Z., Feng, C. H., Wolf, R. and Heisenberg, M. (1996). Conditioned visual flight orientation in *Drosophila*: dependence on age, practice, and diet. *Learn. Mem.* **3**, 49–59.
- Hanai, S., Hamasaka, Y. and Ishida, N. (2008). Circadian entrainment to red light in *Drosophila*: requirement of Rhodopsin 1 and Rhodopsin 6. *NeuroReport* **19**, 1441–1444.
- Hardie, R. (1985). Functional organization of the fly retina. *Prog. Sens. Physiol.* **5**, 2–79.
- Heisenberg, M. and Buchner, E. (1977). The role of retinula cell types in visual behavior of *Drosophila melanogaster*. *J. Comp. Physiol. A* **117**, 127–162.
- Heisenberg, M. and Wolf, R. (1984). *Vision in Drosophila*, pp. 1–250. Berlin: Springer.
- Horn, E. and Wehner, R. (1975). The mechanism of visual pattern fixation in the walking fly, *Drosophila melanogaster*. *J. Comp. Physiol. A* **101**, 39–56.
- Hu, K. G. and Stark, W. S. (1980). The roles of *Drosophila* ocelli and compound eyes in phototaxis. *J. Comp. Physiol. A* **135**, 85–95.
- Kim, S. H. and Jung, C. S. (2010). The role of the pattern edge in goldfish visual motion detection. *Korean J. Physiol. Pharmacol.* **14**, 413–417.
- Kirschfeld, K. and Franceschini, N. (1977). Evidence for a sensitising pigment in fly photoreceptors. *Nature* **269**, 386–390.
- Laws, K. R., Gale, T. M. and Leeson, V. C. (2003). The influence of surface and edge-based visual similarity on object recognition. *Brain Cogn.* **53**, 232–234.
- Lehrer, M. and Srinivasan, M. (1993). Object detection by honeybees: Why do they land on edges? *J. Comp. Physiol. A* **173**, 23–32.
- Lehrer, M., Srinivasan, M. and Zhang, S. (1990). Visual edge detection in the honeybee and its chromatic properties. *Proc. R. Soc. Lond. B* **238**, 321.
- Leventhal, A. G., Wang, Y., Schmolesky, M. T. and Zhou, Y. (1998). Neural correlates of boundary perception. *Vis. Neurosci.* **15**, 1107–1118.
- Liu, C. H., Satoh, A. K., Postma, M., Huang, J., Ready, D. F. and Hardie, R. C. (2008). Ca<sup>2+</sup>-dependent metarhodopsin inactivation mediated by calmodulin and NINAC myosin III. *Neuron* **59**, 778–789.
- Livingstone, M. S. and Hubel, D. H. (1987). Psychophysical evidence for separate channels for the perception of form, color, movement, and depth. *J. Neurosci.* **7**, 3416–3468.
- Marr, D. and Hildreth, E. (1980). Theory of edge detection. *Proc. R. Soc. Lond. B* **207**, 187–217.

- Mimura, K. (1982). Discrimination of some visual patterns in *Drosophila melanogaster*. *J. Comp. Physiol. A* **146**, 229-233.
- Morante, J. and Desplan, C. (2008). The color-vision circuit in the medulla of *Drosophila*. *Curr. Biol.* **18**, 553-565.
- Morton, P. D. and Cosens, D. (1978). Vision in *Drosophila*: evidence for the involvement of retinula cells 1-6 in the orientation behaviour of *Drosophila melanogaster*. *Physiol. Entomol.* **3**, 323-334.
- Mullen, K. T. (1985). The contrast sensitivity of human colour vision to red-green and blue-yellow chromatic gratings. *J. Physiol.* **359**, 381-400.
- Neuser, K., Triphan, T., Mronz, M., Poeck, B. and Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. *Nature* **453**, 1244-1247.
- Osorio, D., Srinivasan, M. V. and Pinter, R. B. (1990). What causes edge fixation in walking flies? *J. Exp. Biol.* **149**, 281-292.
- Regan, D., Giaschi, D., Sharpe, J. A. and Hong, X. H. (1992). Visual processing of motion-defined form: selective failure in patients with parietotemporal lesions. *J. Neurosci.* **12**, 2198-2210.
- Reichardt, W. and Poggio, T. (1976). Visual control of orientation behaviour in the fly. Part I. A quantitative analysis. *Q. Rev. Biophys.* **9**, 311-375, 428-438.
- Reichardt, W. and Wenking, H. (1969). Optical detection and fixation of objects by fixed flying flies. *Naturwissenschaften* **56**, 424-425.
- Reppas, J. B., Niyogi, S., Dale, A. M., Sereno, M. I. and Tootell, R. B. H. (1997). Representation of motion boundaries in retinotopic human visual cortical areas. *Nature* **388**, 175-179.
- Rister, J., Pauls, D., Schnell, B., Ting, C. Y., Lee, C. H., Sinakevitch, I., Morante, J., Strausfeld, N. J., Ito, K. and Heisenberg, M. (2007). Dissection of the peripheral motion channel in the visual system of *Drosophila melanogaster*. *Neuron* **56**, 155-170.
- Rovamo, J., Koljonen, T. and Näsänen, R. (1996). A new psychophysical method for determining the photopic spectral-luminosity function of the human eye. *Vision Res.* **36**, 2675-2680.
- Salcedo, E., Huber, A., Henrich, S., Chadwell, L. V., Chou, W. H., Paulsen, R. and Britt, S. G. (1999). Blue- and green-absorbing visual pigments of *Drosophila*: ectopic expression and physiological characterization of the R8 photoreceptor cell-specific Rh5 and Rh6 rhodopsins. *J. Neurosci.* **19**, 10716-10726.
- Srinivasan, M. V. (2011). Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics. *Physiol. Rev.* **91**, 413-460.
- Srinivasan, M. V., Lehrer, M. and Horridge, G. (1990). Visual figure-ground discrimination in the honeybee: the role of motion parallax at boundaries. *Proc. R. Soc. Lond. B Biol. Sci.* **238**, 331.
- Strauss, R. and Pichler, J. (1998). Persistence of orientation toward a temporarily invisible landmark in *Drosophila melanogaster*. *J. Comp. Physiol. A* **182**, 411-423.
- Strauss, R., Renner, M. and Götz, K. (2001). Task-specific association of photoreceptor systems and steering parameters in *Drosophila*. *J. Comp. Physiol. A* **187**, 617-632.
- Varju, D. (1976). Visual edge fixation and negative phototaxis in the mealworm beetle *Tenebrio molitor*. *Biol. Cybern.* **25**, 17-26.
- Wehrhahn, C. (1984). Ocellar vision and orientation in flies. *Proc. R. Soc. Lond. B* **222**, 409-411.
- Yamaguchi, S., Wolf, R., Desplan, C. and Heisenberg, M. (2008). Motion vision is independent of color in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **105**, 4910-4915.
- Yamaguchi, S., Desplan, C. and Heisenberg, M. (2010). Contribution of photoreceptor subtypes to spectral wavelength preference in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **107**, 5634-5639.