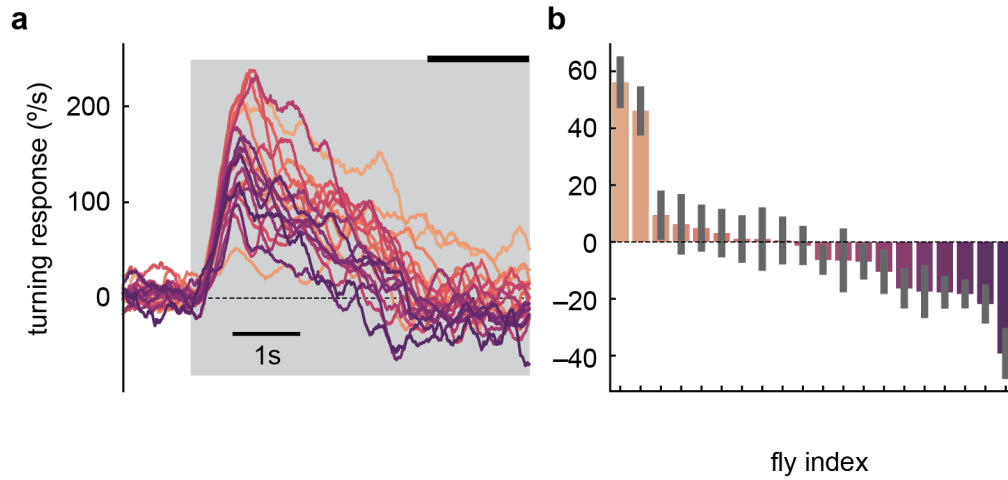
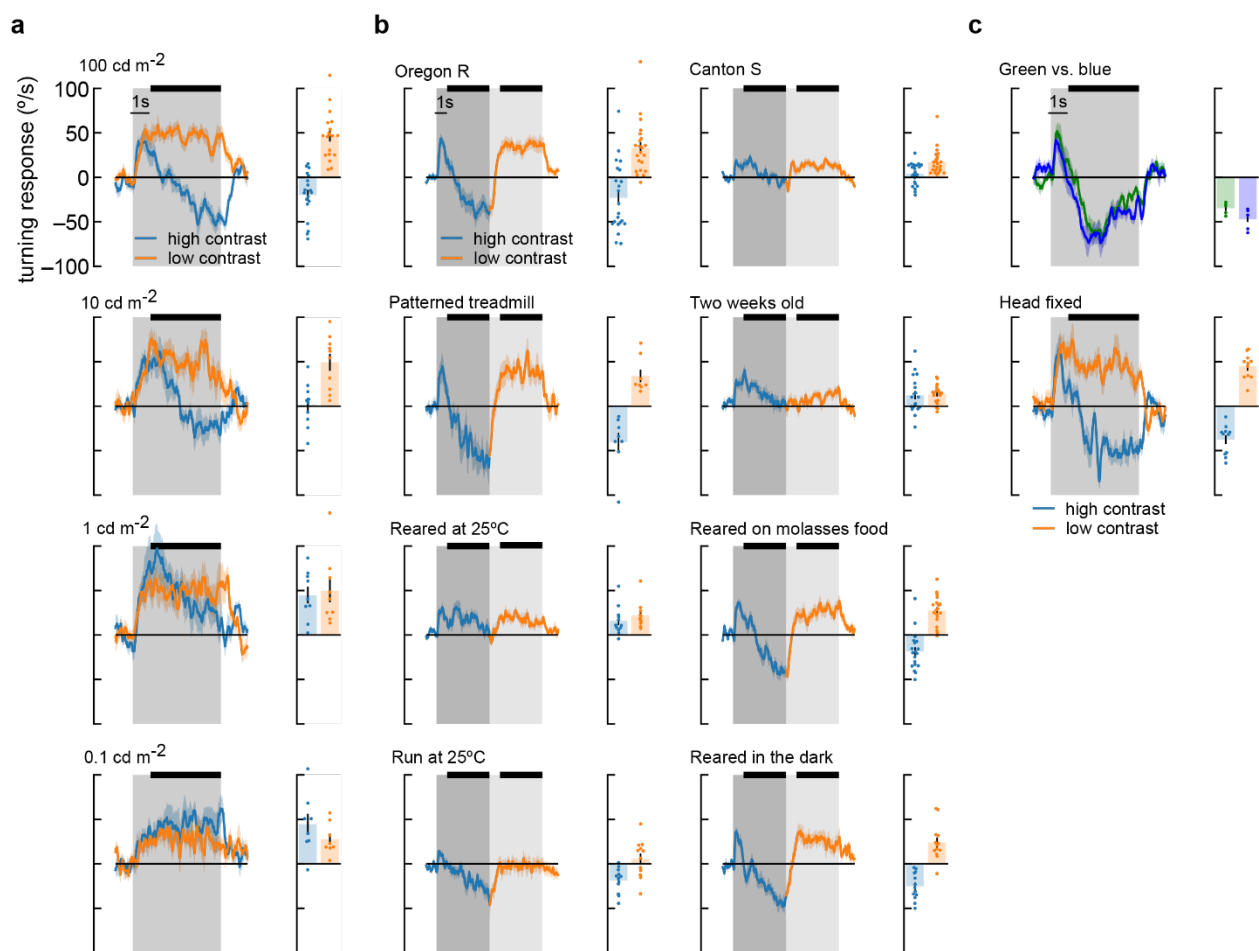


## Supplementary Figures



### Supplementary Figure S1. Individual *D. melanogaster* flies in TRC lab experiments show anti-directional turning.

- Mean time traces of individual fly responses to the high contrast stimulus, averaged over trials. The flies are those in **Fig. 1d**.
- Long-timescale responses of individual flies, averaged over the last 1.5 s of the 5-second stimulus in panel (a) (indicated by thick black line). Mean and SEM shown are over the trials presented to that fly.

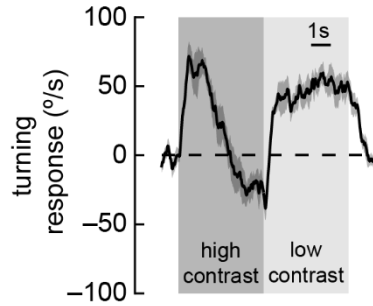


**Supplementary Figure S2. Flies perform anti-directional turning under a wide range of stimulus and growing conditions.**

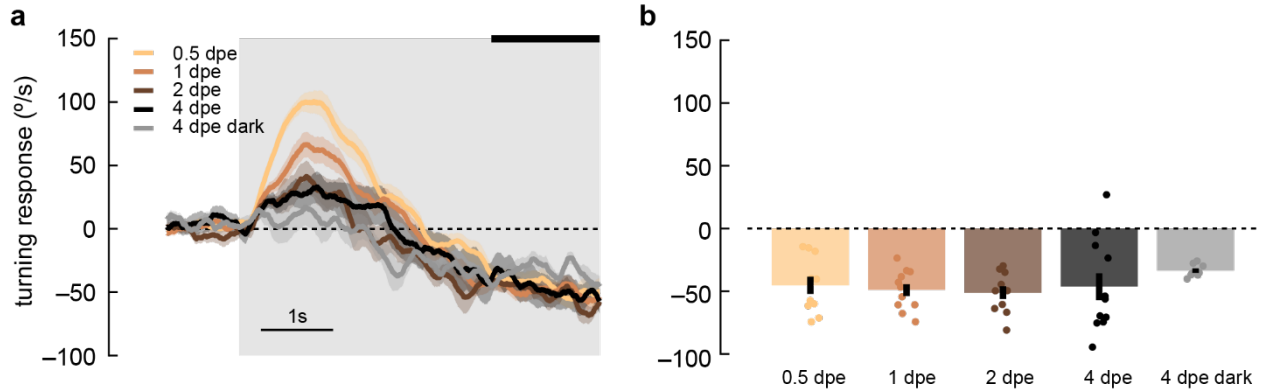
- a)** Fly turning behavior at different mean screen brightness. We swept brightness from 100  $\text{cd}/\text{m}^2$  to 0.1  $\text{cd}/\text{m}^2$  and measured turning responses to high and low contrast stimuli. Flies performed the most anti-directional behavior in response to high brightness stimuli. At 1  $\text{cd}/\text{m}^2$ , flies never turned in the opposite direction of the stimulus, and at 0.1  $\text{cd}/\text{m}^2$ , flies turned continuously in the same direction as the stimulus, even in high contrast conditions. We also measured average turning during the last four seconds of stimulation (black bar above time traces). Average fly behavior shown as bars on the right, with individual fly behavior shown as dots. Shaded patches in the time trace plots represent  $\pm 1$  SEM, as do vertical lines on bar plots.  $N = 19, 10, 9, 8$  flies, top to bottom.
- b)** Our wildtype flies were Oregon-R strain (Gohl *et al.*, 2011) raised at 20 degrees. They were grown on glucose-based food media with 12-hour light-dark cycles. Experiments were run at high temperature, 12-60 hours after eclosion. We used uniform, red balls to avoid visual feedback from walking. The response of these wildtype flies to a contrast-switching stimulus (as in **Fig. 2c**) is shown in the upper left corner. We also tested different variations of all these parameters. Canton-S flies turned less overall, and showed less anti-directional turning, but still turned in the opposite direction after 5 seconds of

high contrast stimuli. We tested flies walking on highly-visible silver balls with black dots and saw behavior similar to wildtype. Two-week-old flies showed reduced turning and much reduced anti-directional behavior. Flies raised at 25 degrees Celsius had behavior similar to two-week-old flies. When we performed experiments at 25 degrees, we saw much less optomotor turning, but anti-directional turning persisted. Rearing on molasses-based media or in the dark did not have strong effects on behavior. N = 22, 8, 12, 12, 24, 19, 19, 13 flies top to bottom, left to right.

- c) Other changes to the experimental setup did not cause large differences in behavior. We compared responses to high contrast stimuli presented with green light (peak wavelength: 525nm) and blue light (peak wavelength: 450), and did not see large differences in behavior. Head-fixed flies (middle) showed similar behavior to head-free flies (**a**, *top*). N = 5 and 11 flies, top to bottom.



**Supplementary Figure S3. Anti-directional turning behavior occurs when using the optical filters also employed in the two-photon imaging experiments.** High and low contrast sinusoidal stimuli were presented as in Figure 2c, but using the bandpass filters also used in our two-photon microscope stimulus presentation. N = 30 flies.



**Supplementary Figure S4. *D. yakuba* lacks plasticity of anti-directional responses in adulthood that is observed *D. melanogaster*.**

- a) Adult *yakuba* flies at various ages post eclosion were presented with 5-second, high-contrast, rotating sinusoidal gratings as in Fig. 6. Data was acquired in the TRC lab. Anti-directional responses stayed consistent from 0.5 days post eclosion (dpe) to 1, 2, and 4 dpe, although the initial optomotor response became smaller as the flies aged. Shaded patches represent  $\pm 1$  SEM. N = 7-11 flies.
- b) The last 1.5 seconds of the mean turning velocity of each fly was averaged, and the population response was plotted.