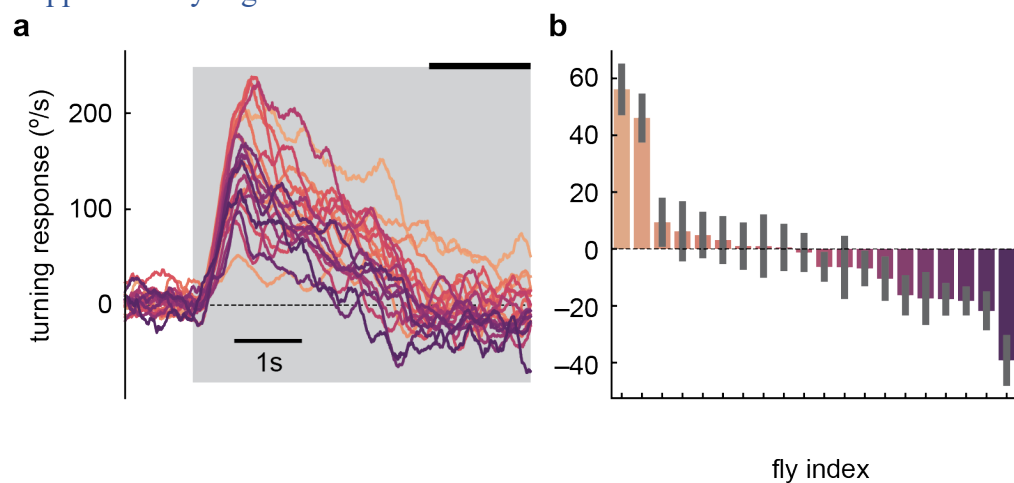


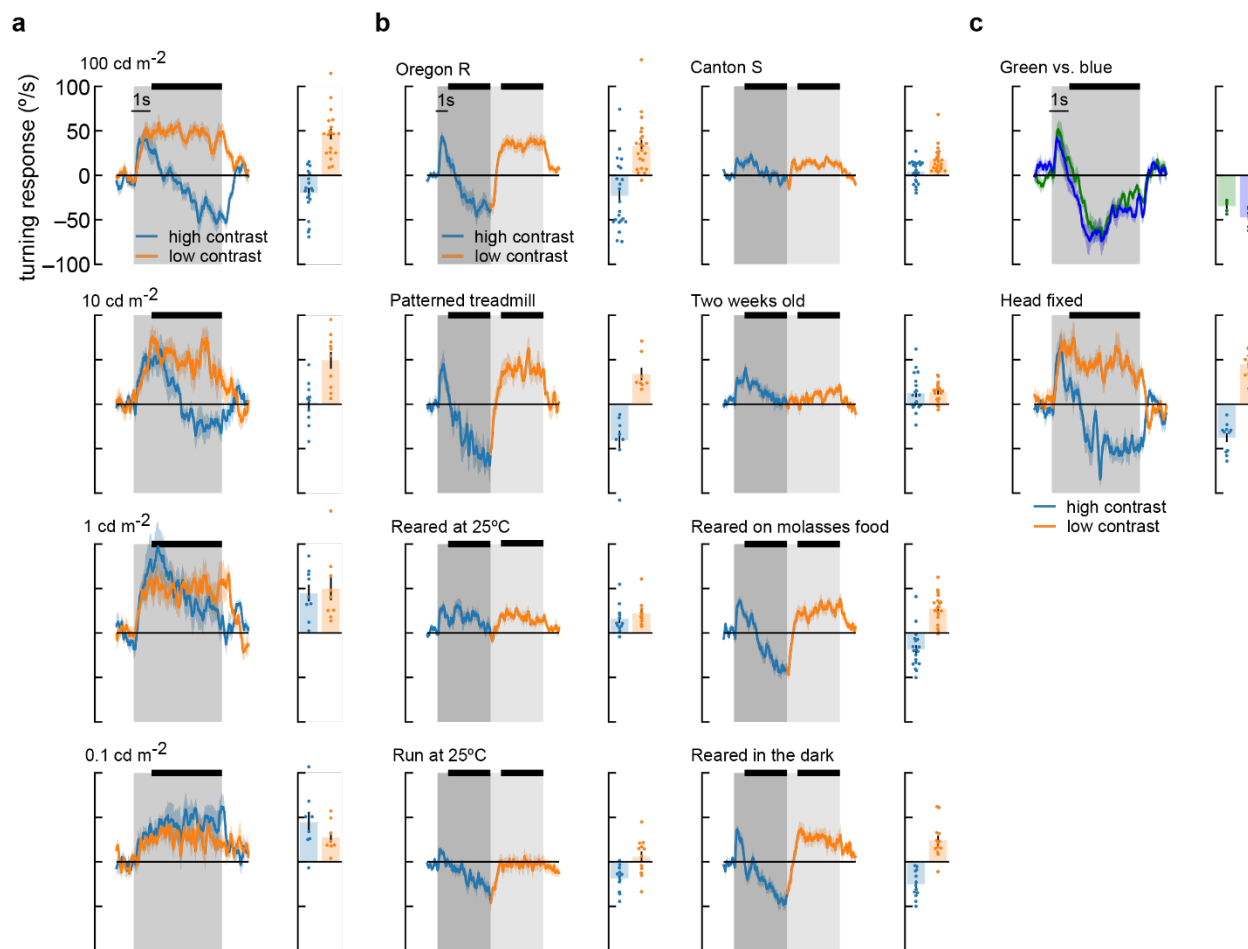
591 **Supplementary Figures**



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

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

600



601

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

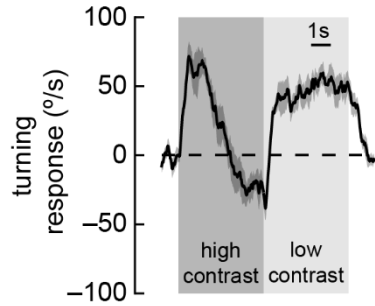
604 a) Fly turning behavior at different mean screen brightness. We swept brightness from 100
 605 cd/m² to 0.1 cd/m² and measured turning responses to high and low contrast stimuli. Flies
 606 performed the most anti-directional behavior in response to high brightness stimuli. At 1
 607 cd/m², flies never turned in the opposite direction of the stimulus, and at 0.1 cd/m², flies
 608 turned continuously in the same direction as the stimulus, even in high contrast
 609 conditions. We also measured average turning during the last four seconds of stimulation
 610 (black bar above time traces). Average fly behavior shown as bars on the right, with
 611 individual fly behavior shown as dots. Shaded patches in the time trace plots represent ± 1
 612 SEM, as do vertical lines on bar plots. N = 19, 10, 9, 8 flies, top to bottom.

613 b) Our wildtype flies were Oregon-R strain⁷² raised at 20 degrees. They were grown on
 614 glucose-based food media with 12-hour light-dark cycles. Experiments were run at high
 615 temperature, 12-60 hours after eclosion. We used uniform, red balls to avoid visual
 616 feedback from walking. The response of these wildtype flies to a contrast-switching
 617 stimulus (as in Fig. 2c) is shown in the upper left corner. We also tested different
 618 variations of all these parameters. Canton-S flies turned less overall, and showed less
 619 anti-directional turning, but still turned in the opposite direction after 5 seconds of high

620 contrast stimuli. We tested flies walking on highly-visible silver balls with black dots and
621 saw behavior similar to wildtype. Two-week-old flies showed reduced turning and much
622 reduced anti-directional behavior. Flies raised at 25 degrees Celsius had behavior similar
623 to two-week-old flies. When we performed experiments at 25 degrees, we saw much less
624 optomotor turning, but anti-directional turning persisted. Rearing on molasses-based
625 media or in the dark did not have strong effects on behavior. N = 22, 8, 12, 12, 24, 19, 19,
626 13 flies top to bottom, left to right.

627 **c)** Other changes to the experimental setup did not cause large differences in behavior. We
628 compared responses to high contrast stimuli presented with green light (peak wavelength:
629 525nm) and blue light (peak wavelength: 450), and did not see large differences in
630 behavior. Head-fixed flies (middle) showed similar behavior to head-free flies (**a**, *top*). N
631 = 5 and 11 flies, top to bottom.

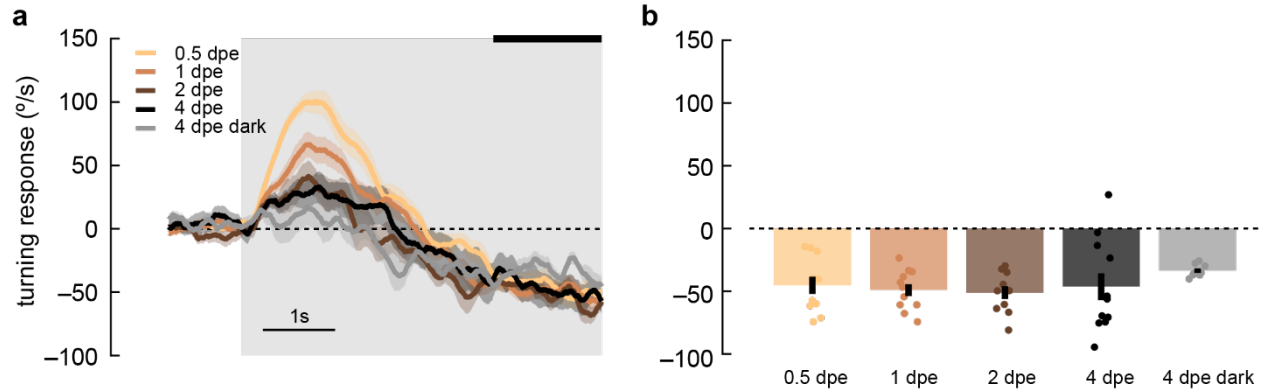
632



633

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

638



639

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

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

649