Long timescale anti-directional rotation in *Drosophila* optomotor behavior

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Abstract

Locomotor movements cause visual images to be displaced across the eye, a retinal slip that is counteracted by stabilizing reflexes in many animals. In insects, optomotor turning causes the animal to turn in the direction of rotating visual stimuli, thereby reducing retinal slip and stabilizing trajectories through the world. This behavior has formed the basis for extensive dissections of motion vision. Here, we report that under certain stimulus conditions, two *Drosophila* species, including the widely studied *D. melanogaster*, can suppress and even reverse the optomotor turning response over several seconds. Such 'anti-directional turning' is most strongly evoked by long-lasting, high-contrast, slow-moving visual stimuli that are distinct from those that promote syn-directional optomotor turning. Anti-directional turning, like the syn-directional optomotor response, requires the local motion detecting neurons T4 and T5. A subset of lobula plate tangential cells, CH cells, show involvement in these responses. Imaging from a variety of direction-selective cells in the lobula plate shows no evidence of dynamics that match the behavior, suggesting that the observed inversion in turning direction emerges downstream of the lobula plate. Further, anti-directional turning declines with age and exposure to light. These results show that *Drosophila* optomotor turning behaviors contain rich, stimulus-dependent dynamics that are inconsistent with simple reflexive stabilization responses.

Intro

Visual navigation requires active mechanisms to stabilize trajectories through the world. Insects exhibit an optomotor turning response, a behavior in which they rotate their bodies in the direction of visual patterns that rotate about them [1-3]. This behavior is analogous to optomotor

turning responses in fish [4] and the optokinetic response in mammals [5]. In insects, this response is thought to be a course-stabilization mechanism that minimizes retinal slip, allowing animals to maintain their trajectory in the face of external or unexpected rotational forces [2,6]. For instance, if an insect attempts to walk in a straight line, it may slip and turn to the right. From the point of view of the insect, this turn is observed as optic flow rotating to the left. By responding to this leftward optic flow with a leftward turn, the insect can recover its original trajectory.

In fruit flies, the optomotor response relies on well-characterized circuitry [7]. Photoreceptor signals are split into parallel ON and OFF pathways in the lamina and medulla [8-11], which are not direction-selective. These signals provide input to T4 and T5 cells, which compute direction-selective responses along four directions at every point in the fly visual field [12-16]. The outputs of T4 and T5 cells are then summed across visual space by lobula plate tangential cells (LPTCs) [12,17-20]. Different LPTCs provide distinct signals about the overall pattern of motion surrounding the fly, and have been linked to head and body movements [21-23].

Interestingly, there have been several reports of flies turning in the direction opposite to what is predicted by the optomotor turning response. In some cases, these counter-intuitive behaviors were observed using periodic stimuli with spatial wavelengths smaller than the receptive field of individual ommatidia, and thus can be accounted for by aliasing [3,24,25]. Work in a tethered flight simulator showed that when a moving pattern is presented in front of the fly, the animal turned in the direction of the stimulus motion [26], as expected [27]. However, if the moving pattern was presented behind the fly, it attempted to turn in the direction opposite to stimulus motion [26]. In a different experimental preparation, rotational patterns were presented on a dome around freely-walking flies [28]. Under these conditions, flies generally turned in the direction of motion of the stimulus, but these rotations were often punctuated by brief, large-magnitude saccades in the opposite direction. Similarly, experiments using flight simulators have reported spikes in the torque in the direction opposite the stimulus rotation [29].

Here we show that rotational stimuli can elicit strong, consistent anti-directional turning behavior in two drosophilid species, *D. melanogaster* and *D. yakuba*. We report that flies respond to high contrast, high luminance rotational motion stimuli by first turning in the direction of stimulus motion, and then reversing their trajectory after approximately one second, depending on the species. In *Drosophila melanogaster*, we characterize the dynamics of this behavior and the stimuli that drive it, showing that it is distinct from prior observations of anti-directional turning. The behavior depends critically on adaptation to back-to-front motion. We use the genetic tools available in *Drosophila melanogaster* to show that this behavior relies on the motion detecting neurons T4 and T5. Silencing HS and CH, two widefield neurons downstream of T4 and T5, resulted in small changes in this complex turning behavior. However, the visually evoked responses of these direction-selective neurons could not account for the anti-directional behavior. Thus, the observed reversal must be mediated by downstream circuitry. Overall, these results show that circuits in the fly generate behaviors that oppose the direction of wide-field visual motion, showing that *Drosophila* turning responses are more complex than a simple stabilizing reflex.

Results

Anti-directional turning responses to high contrast stimuli

Optomotor turning responses are central to gaze stabilization, so we sought to examine this response across different conditions. Many studies have investigated this behavior using stimuli with low contrast, low light intensity, or both [2,3,30-33], at a variety of different speeds. However, natural scenes can have relatively high contrast and luminance, conditions have been poorly explored in the laboratory. In this experiment, we presented flies with rotational stimuli using high contrast and relatively high luminance.

We tethered individual female *D. melanogaster* above a freely rotating ball to characterize the optomotor response [3,34] (**Fig. 1a**). As expected, low contrast, slow-moving sinusoidal gratings caused flies to turn in the same direction as the moving gratings via the classical optomotor turning response (**Fig. 1b**) [1,3,8,25,26,30,31,35-42]. However, when we changed the stimulus to high contrast sinusoidal gratings (nominal 100% Weber contrast), flies turned in the stimulus direction for approximately 1 second, but then reversed course, and turned in the direction opposite to the stimulus motion for the duration of the stimulus presentation. Because this turning response is in the opposite direction of stimulus and the syn-directional optomotor turning response, we refer to it as anti-directional turning.

We swept a range of contrasts and compared the fly turning in the first 500 milliseconds to the turning after one second (**Fig. 1c**). As contrast increased, the flies turned faster during the first half second of stimulus presentation, reaching a plateau at around 0.5 contrast, consistent with previous results [3,35,36,43-45]. Fly behavior after the first second of stimulation was more complex. As contrast increased from 0 to 0.25, flies turned in the same direction as the stimulus, with faster turning as the contrast increased. When the contrast was greater than 0.25, turning decreased, lowering to no net sustained turning at around 0.8 contrast. Above a contrast of 0.8, flies began to turn in the direction opposite the stimulus.

These initial experiments took place in the lab of author DAC. To confirm that these unexpected responses did not reflect some idiosyncrasy of one specific behavioral apparatus or environment, we repeated these experiments in a second lab, that of author TRC. Under similar conditions, using the same strain of *Drosophila melanogaster*, we reproduced the rapid deceleration after an initial, transient syn-directional response (**Fig. 1d**), with some individual flies exhibiting significant anti-directional turning (**Supp. Fig. S1**). This demonstrates that the key features of this behavioral response are stable across experimental systems and laboratories, though the magnitude of anti-directional turning behavior in *D. melanogaster* is sensitive to some unknown experimental parameter differences between the laboratories.

Individual strains of *D. melanogaster*, and other drosophilid species, display significant variation in their locomotor patterns during walking [46]. Indeed, when we tested a Canton-S *D. melanogaster* strain, we observed milder but significant anti-directional turning at long timescales (**Supp. Fig. S2b**). We reasoned that a strong test of the generality of anti-directional turning would be to examine turning behavior in another species, and selected *D. yakuba*. Strikingly, *D. yakuba* also displayed anti-directional turning behavior under similar conditions (**Fig. 1e**). Thus, this behavior is not an idiosyncratic feature of a single laboratory strain.

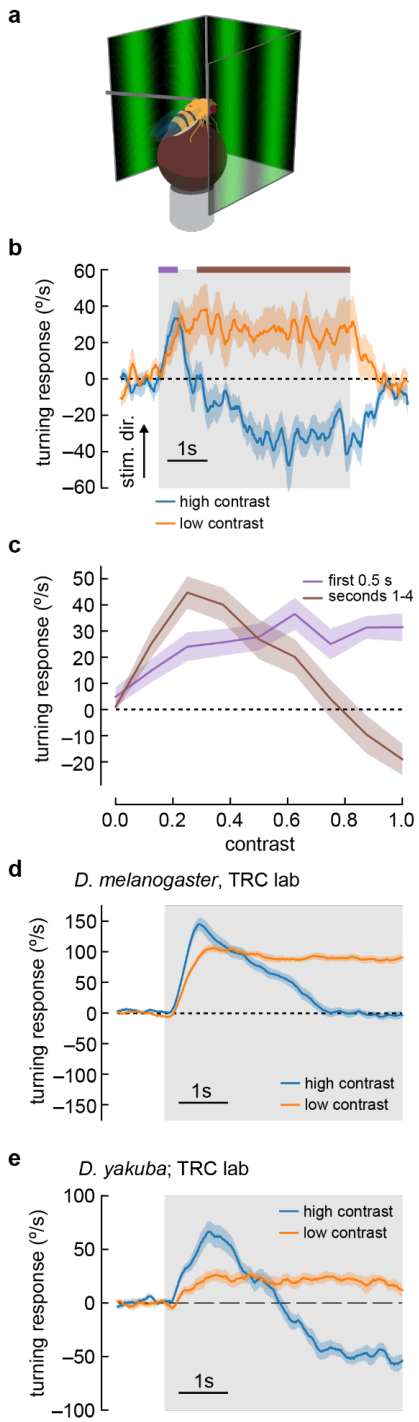

Figure 1. Flies turn opposite to the stimulus direction in high contrast conditions

a) We measured fly turning behavior as they walked on an air-suspended ball. Stimuli were presented over 270 degrees around the fly.

b) We presented drifting sinusoidal gratings for 5 seconds (shaded region) with either high contrast (c = 1.0) or low contrast (c = 0.25). When high contrast sinusoidal gratings were presented, flies initially turned in the same direction as the stimulus, then started turning

in the opposite direction after ~1 second of stimulation. Under low contrast conditions, flies turned continuously in the same direction as the stimulus. In these experiments, the sine waves had a wavelength of 60º and a temporal frequency of 1 Hz. Shaded patches represent ±1 SEM. N= 10 flies.

c) We swept contrast between 0 and 1 and measured the mean turning response during the first 0.5 seconds (purple, purple bar in **b**) and during the last 4 seconds of the stimulus (brown, brown line in **b**). The response in the first 0.5 seconds increased with increasing contrast, while the response in the last four seconds increased from c = 0 to c = 0.25, and then decreased with increasing contrast, until flies turned in the direction opposite the stimulus direction at the highest contrasts. N = 20 flies.

d) We repeated the presentation of drifting sinusoidal gratings, this time in the lab of author TRC, using a similar behavioral apparatus. Stimulus parameters were as described in (b). In these experiments, the population average shows that flies proceeded to zero net turning at high contrasts, but some individual flies exhibited anti-directional turning responses. N = 20 flies.

e) We repeated the experiments with *D. yakuba*, also in the lab of TRC, and observed that this species exhibited a robust anti-directional turning response to high contrast gratings and a classical syn-directional turning response to low contrast gratings. N = 11 flies.

Conditions for anti-directional turning behaviors

While anti-directional turning behaviors have been reported before, other groups have presented similar stimuli without observing anti-directional behavior [2,3,30-33]. We wondered what aspects of our experimental setup could lead to these behavioral differences. In our experiments, anti-directional turning was strongly linked to display brightness (**Supp. Fig. S2a**). When the mean brightness of the screens was reduced from 100 cd/m^2 to 1 cd/m^2, we saw no anti-directional turning in 5 second trials (though average optomotor behavior did decrease over the course of the stimulus presentation). When we further reduced the mean brightness to 0.1 cd/m^2, flies persisted in their optomotor behavior throughout the stimulus presentation. We note that in these low luminance experiments, low levels of ambient light in the nominally dark experimental rig could also reduce the effective contrast of the stimulus.

We tested a variety of other factors that might affect anti-directional turning. Anti-directional turning occurred when experiments were run both at hot temperatures and at room temperature (**Supp. Fig. S2b**). We also observed anti-directional behavior when flies were reared in the dark and on different media. We also tested several other experiment conditions (**Supp. Fig. S2c**). Flies responded with anti-directional turning to high contrast stimuli presented at both blue and green wavelengths. We glued fly heads to their thorax to ensure stimuli could not be affected by head movements [21,22], but found no difference between head-fixed and head-free flies. We did find a few factors that modulated anti-directional turning behavior. In particular, rearing *D. melanogaster* at 25°C instead of 20°C or testing flies that were two weeks old instead of 12-60 hours old both reduced overall turning behavior and eliminated anti-directional turning. In these cases, optomotor turning still decreased over the course of the 5 second, high contrast trials, but

did not reverse. As details of rearing temperature and the age at which behavior tests are run often vary across labs, it is likely that these factors, as well as stimulus brightness, account for the differences between our observations and the previous literature.

Distinct spatiotemporal tuning of the anti-directional behavioral response

To further characterize the anti-directional response, we swept the spatial and temporal frequency of the sinusoidal grating stimulus. Using only Weber contrasts of 1, we compared the early response (first quarter second, **Fig. 2a**) to the late response (after one second, **Fig. 2b**). *Drosophila melanogaster* always turned in the optomotor direction during the early stimulus response. In this early response, flies turned most vigorously to stimuli with short spatial frequencies (~20º wavelength) and fast temporal frequencies (~8 Hz), in agreement with earlier studies [26,37,39]. However, during the longer-timescale response to high-contrast stimuli, flies only turned in the optomotor direction at very high temporal frequencies (> ~16 Hz) and at very low temporal frequencies (<0.5 Hz). At intermediate temporal frequencies, flies showed a sustained anti-directional response. The maximal anti-directional response was achieved at 1 Hz and 45º wavelength, distinct from the conditions for peak classical turning responses. Interestingly, the stimuli that elicit the strongest anti-directional response appear similar to those that maximally activate T4 and T5 neurons when those neurons are measured in head-fixed flies [12,37,39,47-49].

Anti-directional turning results from adaptation effects

We were intrigued by the switch from syn-directional to anti-directional turning behavior. To investigate the dynamics of these changes, we presented a rotating sinusoidal stimulus at contrast 1 for five seconds, and then changed the contrast to 0.25 (**Fig. 2c**). After the switch to low contrast, the flies quickly reverted classical, syn-directional optomotor behavior, demonstrating that no long-term switch in directional turning occurs during high contrast stimulus presentation. This effect did not depend on the periodic nature of these stimuli: a rotating stimulus consisting of 5º-wide vertical bars with randomly-chosen, binary contrasts [38] yielded similar behavioral responses (**Fig. 2d**).

To further isolate the causes of this switch in behavior, we developed a stimulus to adapt the fly to different stimuli before presenting high-contrast rotational sinusoidal gratings to elicit the anti-directional turning response. This adapting stimulus consisted of five seconds of high contrast 'translational' stimuli, which was then followed by a rotational stimulus (**Fig. 2e**). The translational stimuli consisted of both left and right hemifields moving either front-to-back or back-to-front across the fly's two eyes [39]. These stimuli resulted in no net turning by the flies [39,42]. Adapting the fly with front-to-back stimuli did not have a strong effect on the subsequent response to rotational stimuli. However, adapting with back-to-front stimuli generated responses that no longer showed an initial syn-directional turning response, but instead exhibited anti-directional turning immediately after the rotational stimulus began. This result indicates that the anti-directional turning results from slow-timescale changes that depend on strong back-to-front motion stimulation.

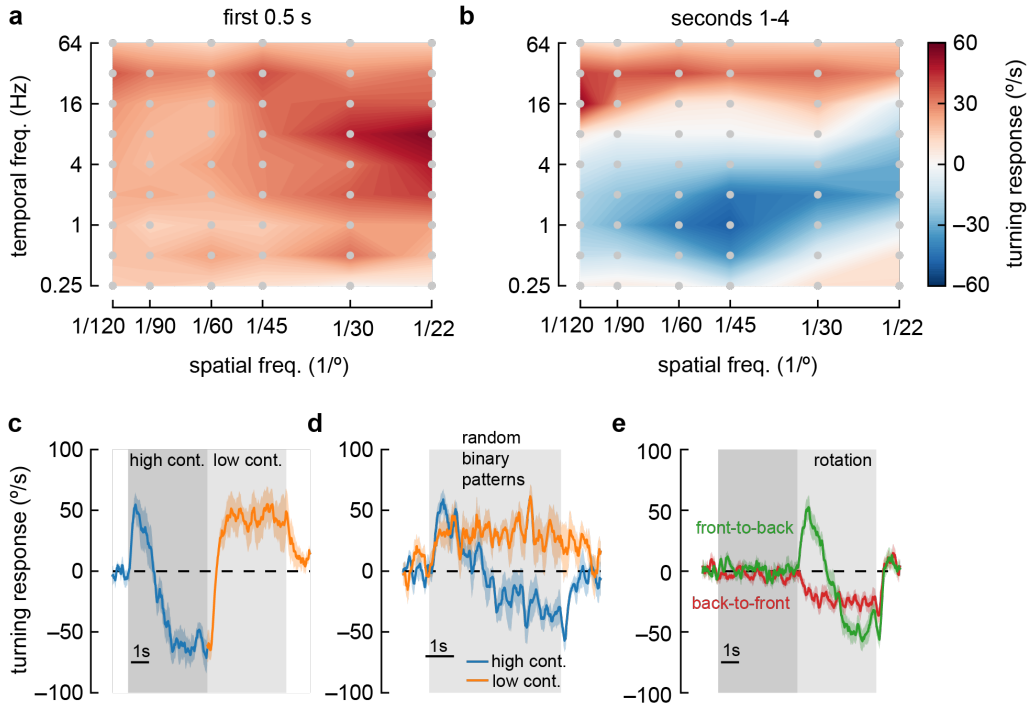

Figure 2. Anti-directional turning behavior has distinct tuning and is driven by adaptation.

a) Heatmap of fly turning velocity during the first 0.5 seconds of sinusoidal grating stimulation under high contrast conditions and variable temporal and spatial frequencies. The flies turned in the direction of the stimulus across all conditions and responded most to 8 Hz, 22-degree stimuli. N = 16,21,17,21,7, and 22 flies for spatial frequencies 1/120, 1/90, 1/60, 1/45, 1/30 and 1/22 degrees respectively.

b) Heatmap as in (**a**), measured during the last four seconds of stimulation. Flies turned in the same direction as the stimulus at high and low temporal frequencies, but in the opposite direction of the stimulus at intermediate temporal frequencies, with a maximal anti-directional response at wavelengths between 30º and 60º.

c) Switching stimulus contrast from high to low after 5 seconds caused flies to revert to syn-directional behavior after the anti-directional response. N = 7 flies.

d) Presenting rotating random binary patterns (5-degree vertical strips rotating at 150 degrees/second) induced anti-directional turning similar to that elicited by rotating sine wave gratings. N = 7 flies.

e) We presented flies with five seconds of "translational" stimuli (dark shaded region), with high contrast sinusoidal gratings moving either front-to-back or back-to-front, bilaterally, for five seconds. After that, we presented high contrast rotational sinusoidal grating stimuli (60º wavelength, 1 Hz). Front-to-back stimulation did not affect the subsequent response to rotational stimuli, but back-to-front stimuli caused flies to turn immediately in the opposite direction of the stimulus. N = 18 flies.

Anti-directional turning is elicited when stimuli are presented in front of the fly

A previous report of anti-directional turning behavior in flying tethered flies showed that flies turn in the opposite direction to stimuli that are presented behind their midline [26]. To test whether our results were caused by this effect, we split our stimulus into three regions: 90 degrees in front of the fly, 45 degrees in front of the midline on either side of the fly, and 45 degrees behind the midline on either side of the fly (**Fig. 3a**). We found that flies displayed anti-directional turning when presented with stimuli only in the front region or only just in front of the midline (**Fig. 3bc**). They did not display anti-directional turning when moving stimuli were presented behind the midline (**Fig. 3bc**). This suggests a different mechanism from the behaviors that depend on posterior spatial location to elicit reverse-turning [26].

Anti-directional responses do not depend on saccades

Anti-directional saccades have been reported in walking and flying flies [28,29]. In walking flies [28], flies largely turned in syn-directionally, but these turns were sometimes interrupted by brief, high-amplitude saccades in the opposite direction, against the stimulus direction. If such saccades were frequent or high amplitude, the net effect could shift the average turning we measured, creating apparent anti-directional turning. To investigate this possibility, we plotted the turning response on a per-trial basis (**Fig. 3d**). We then discarded information about the magnitude of the turns and considered only the direction of the turning at each point in time (**Fig. 3e**). Strikingly, in many trials, flies continued to turn opposite to the stimulus for several seconds, a behavior unlike brief saccades. We then calculated a turning index for each response timepoint (sampled at 60 Hz). This turning index represented the fraction of trials where the fly turned in the direction of the stimulus at each timepoint minus the fraction of trials where the fly turned in the opposite direction (**Fig. 3f**). Since this turning index does not include the magnitude of turning, it is strongly affected by sustained low-amplitude turns and discounts any brief high-amplitude saccades. When presented with high contrast stimuli, flies maintained a negative turning index, indicating that sustained turns, and not high velocity saccades, underlie this anti-directional turning behavior. As such, it appears distinct from the reports of anti-directional saccades.

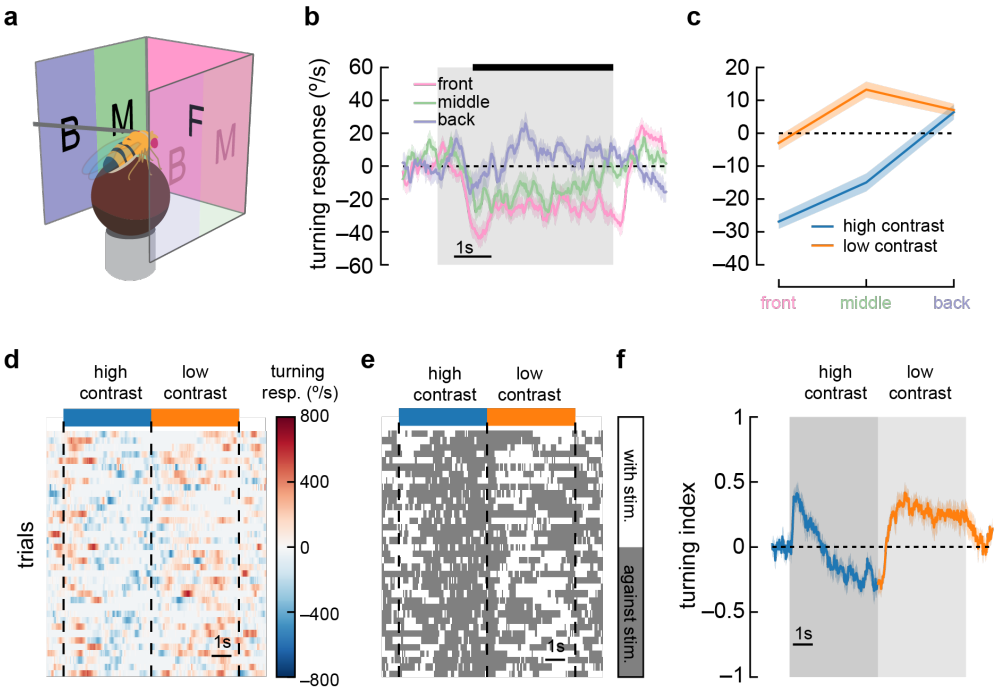

Figure 3. Anti-directional turning is driven by stimuli in the forward-facing visual field and is not driven by saccades.

- **a)** We divided our panoramic display into three sections — the front 90º, the 45º behind the fly on either side, and a middle 45º.
- **b)** High contrast sinusoidal gratings were presented on each of these three display sections, with the remaining sections blank. Flies turned syn-directionally when stimuli were presented behind the fly, and turned anti-directionally when stimuli were presented in front of the fly. Shaded patches represent ±1 SEM. N = 55 flies.
- **c)** Average turning in the last 4 seconds of the stimulus (black bar in **b**), in low contrast and high contrast conditions. Shaded patches in the time trace plots represent ±1 SEM. N = 55 flies.
- **d)** A single fly responds to many trials of sinusoidal grating stimuli at high contrast (blue bar) and low contrast (orange bar). We show a heatmap of the fly's responses over time (horizontal axis) and across trials (vertical axis).
- **e)** We can ignore the magnitude of the turning and instead only quantify whether the fly was turning in the same direction as the stimulus (white area) or in the opposite direction (dark gray area). This shows sustained anti-directional turning, not brief saccades.
- **f)** Averaging the direction (but not magnitude) of turning across trials and across flies yields a turning index for each point in time. Shaded patches in the time trace plots represent ±1 SEM. N = 7 flies.

Anti-directional turning requires elementary motion detectors

What neurons are involved in this anti-directional turning behavior? Previous work demonstrated that T4 and T5 are required for directional neural responses [18], as well as for optomotor turning [12,40,50], for walking speed regulation [39], and for responses to visual looming stimuli [51]. We

silenced the neurons T4 and T5 using shibire^{ts} [52] and measured responses to sinusoidal stimuli that switched from high to low contrast (**Fig. 4a**). Flies in which T4 and T5 had been silenced displayed only minimal responses to motion stimuli, with anti-directional turning suppressed along with classical syn-directional turning. Thus, we conclude that, like optomotor turning behaviors, this anti-directional behavior depends critically on signals from T4 and T5.

Anti-directional turning requires the CH lobula plate tangential cell

Since the switch from optomotor to anti-directional behavior seems to be dependent on the direction of motion adaptation (**Fig. 2e**), we reasoned that neurons involved in this behavior were likely to be downstream from T4 and T5. Relatively little is known about circuitry that connects the neurons T4 and T5 to optomotor turning behavior. However, Horizontal System (HS) cells are well-studied postsynaptic partners of T4 and T5 [9,20]. These lobula plate tangential cells integrate information from front-to-back and back-to-front selective T4 and T5 cells across the fly's visual field [17]. HS cells have been implicated in visually-evoked head turns [21] and body rotations in flight [22] and in maintenance of direction during walking [53]. When we silenced HS neurons, we found small deficits in syn-directional turning behavior, consistent with prior results, but no deficits in anti-directional turning (**Fig. 4b**), indicating that HS cells synaptic output is not required specifically for anti-directional turning behavior.

Next, we turned to the CH lobula plate tangential cells. These cells are GABAergic and are both pre-synaptic and post-synaptic in the lobula plate [54]. In blowflies, these neurons play an inhibitory role in an interconnected LPTC circuit that shapes behavior [55]. When we silenced CH neurons, we found a small increase in syn-directional turning and a decrease in anti-directional turning (**Fig. 4c**). Overall, silencing this neuron type caused the flies to turn more in the direction of motion. This result suggests that CH activity contributes to the anti-directional turning response. However, since adapting to back-to-front translational stimuli significantly affected the dynamics of anti-directional turning, it seems likely that other neurons beyond HS and CH are involved, since these two neurons both respond selectively to front-to-back motion [20,56].

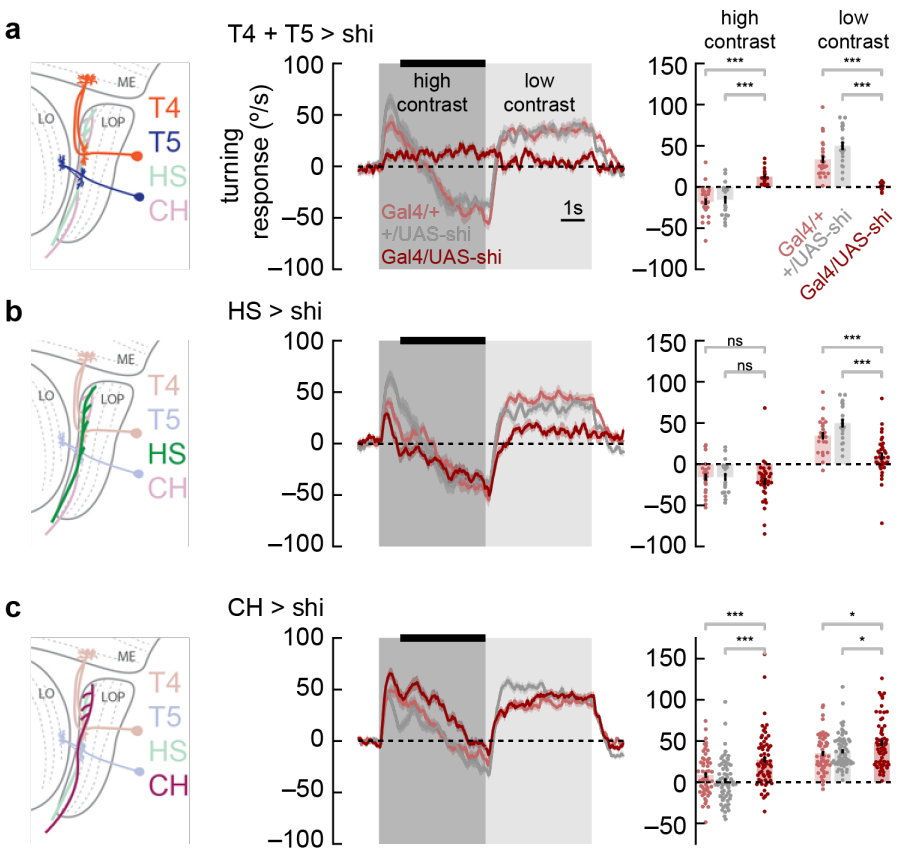

Figure 4. Syn-directional and anti-directional turning share common circuitry

a) We silenced T4 and T5 neurons by expressing shibire^{ts} selectively in those neurons. We measured turning behavior during a contrast-switching stimulus (as in **Fig. 2c**). Results from flies with T4 and T5 silenced shown in dark red, while controls are in light red and gray. Average fly behavior during the last four seconds of the first contrast (black bar on left) shown as bars on the right, with individual fly behavior shown as dots. Note that the data labeled "low contrast" are from experiments in which the low-contrast stimulus was shown before the high contrast stimulus. Shaded patches in the time trace plots represent ±1 SEM, as do vertical lines on bar plots. *** indicates experimental results are significantly different from results, $P < 0.001$ via a two-sample Student t-test. * indicates $P < 0.05$. N = 17, 24, 19 flies with genotypes T4T5/Shibire^{ts}, T4T5/+, +/Shibire^{ts}.

b) Results from HS silencing as in **a**. Silencing HS reduced syn-directional turning behavior ($P < 0.001$) but did not have a strong effect on anti-directional turning. N = 34, 21, 19 flies with genotypes HS/Shibire^{ts}, HS/+, +/Shibire^{ts}.

c) Results from CH silencing as in **a**. CH silencing reduced the degree of anti-directional turning ($P < 0.001$). N = 63, 57, 70 flies with genotypes CH/Shibire^{ts}, CH/+, +/Shibire^{ts}.

Early direction-selective cells do not adapt to the stimulus

The anti-directional turning response is preceded by an initial syn-directional response. This change in behavior must be the result of changes in neural activity, but this change could happen

329 at any point along the neural pathway between photoreceptors and motor neurons. In order to
330 constrain possible mechanisms for generating the anti-directional turning behaviors, we used
331 calcium imaging to interrogate the activity of direction selective neurons during high and low
332 contrast stimulation (**Fig. 5a**). However, as calcium imaging experiments using two photon
333 microscopy require additional spectral filtering of the projector, we first confirmed that these
334 spectral differences did not alter anti-directional turning responses. To do this, we re-measuring
335 the anti-directional turning behavior using optical filtering matched to the conditions needed for
336 imaging. Using this spectrally distinct illuminant, we observed both syn-directional and anti-
337 directional turning behaviors, following the previously observed dynamics (**Supp. Fig. S3**).

338 As T4 and T5 neurons play a critical role in both the syn- and anti-directional turning responses,
339 we first measured the calcium activity of these neurons as they responded to sine wave gratings
340 at a range of contrasts in their preferred and null directions. The T4 and T5 neurons responded to
341 sine wave gratings in their preferred direction by increasing their calcium activity for the full
342 duration of the stimulus presentation, reaching a plateau after approximately 1 second (**Fig. 5bc**,
343 middle). As we increased the contrast of the preferred direction stimuli, we found that both T4
344 and T5 cells had increased calcium activity throughout the contrast range (**Fig. 5bc**, right),
345 consistent with prior measurements [12]. Thus, the responses of T4 and T5 cells do not capture the
346 transition from syn-directional to anti-directional turning behavior.

347 Next we examined two LPTCs downstream of T4 and T5 cells. Calcium activity in HS cells
348 followed similar trends to T4 and T5. Calcium signals increased at the start of preferred direction
349 stimuli presentation and stayed high until the end of the presentation (**Fig. 5d**, middle).
350 Increasing contrast caused stronger calcium responses with a mild saturation effect at high
351 contrast (**Fig. 5d**, right), consistent with prior voltage measurements [20]. These results indicate
352 that the changes in the time course of optomotor behavior at high contrast are not related to
353 changes in HS activity. Finally, we measured calcium activity in CH cells. CH cells responded to
354 visual stimuli more quickly than HS cells (**Fig. 5e**, middle), and showed decreased calcium
355 signals in response to null direction stimuli (**Fig. 5e**, right). However, they also showed sustained
356 responses to high contrast stimuli, as in T4, T5, and HS. These measurements suggest that the
357 switch from syn- to anti-directional turning behavior is driven by cells downstream of or parallel
358 to T4, T5, HS, and CH.

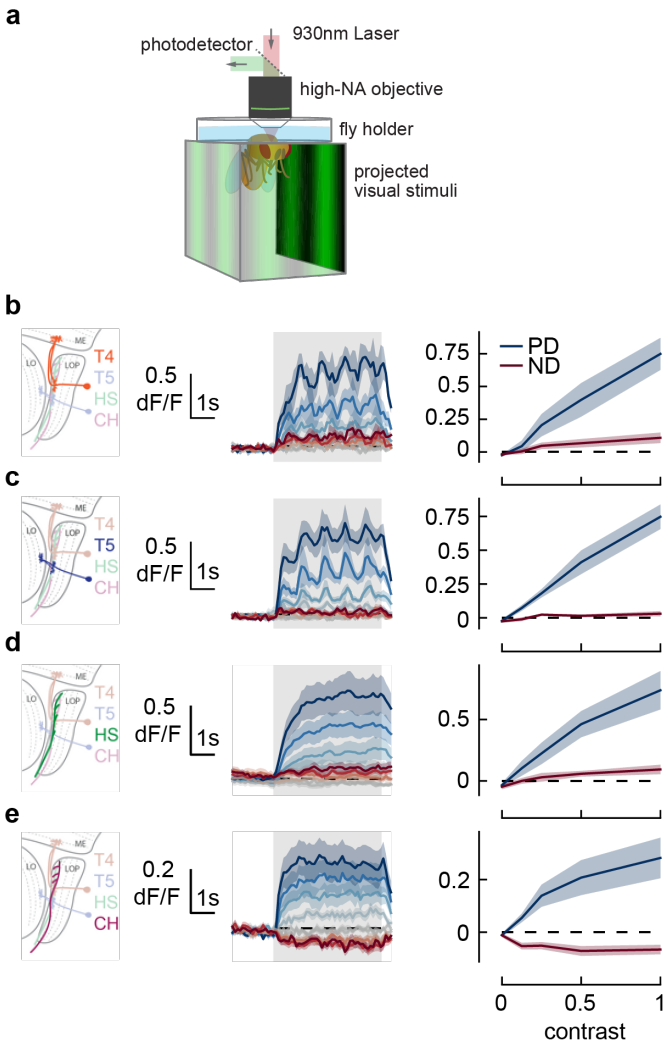

Figure 5. Responses in early direction-selective cells do not show a reduction or reversal of response on the timescale of the behavior.

a) We used two-photon microscopy to measure calcium activity in lobula plate neurons while presenting sinusoidal gratings at a range of contrasts.

b) T4 cells, marked in orange (*left*), responded to drifting sinusoidal gratings with increased calcium activity (*middle*). Darker colors indicate higher contrast, preferred direction in blue, null direction in red. When integrated across the stimulus presentation (*right*), calcium activity increased with stimulus contrast. N = 8 flies.

c-e) As in **b)** measuring calcium activity in T5, HS, and CH cells. N = 8, 10, 15 flies.

Adult plasticity in anti-directional turning behavior

In behaving flies, the strength of anti-directional turning was dependent both on rearing temperature, which alters the rate of growth, and on age (**Supp. Fig. S2**). This raises the possibility that syn- and anti-directional turning responses might be plastic during the early adult stages of development. To probe this possibility, we presented 1 Hz, high-contrast, rotating

sinusoidal grating at various stages during early adulthood (**Fig. 6**). Strikingly, as flies aged from 0.5 to 4 days post eclosion (dpe), the initial syn-directional turning became less transient and more sustained, indicative of a weaker anti-directional turning drive. We then wondered whether this plasticity was intrinsically programmed, or dependent on visual input. To disambiguate these possibilities, we reared flies in darkness to 2 or 4 dpe and measured their turning responses (**Fig. 6**, *gray*). Dark-reared flies exhibited a stronger deceleration away from syn-directional turning, similar to that found in more juvenile flies, arguing that visual input may sculpt the balance of syn- and anti-directional turning. Finally, we examined whether optomotor response plasticity could be detected in *D. yakuba*. However, in this species, anti-directional responses were stable across the first four days of adulthood, arguing that the role of visual experience in shaping these responses is itself evolutionarily tuned in drosophilids (**Supp. Fig. 4**).

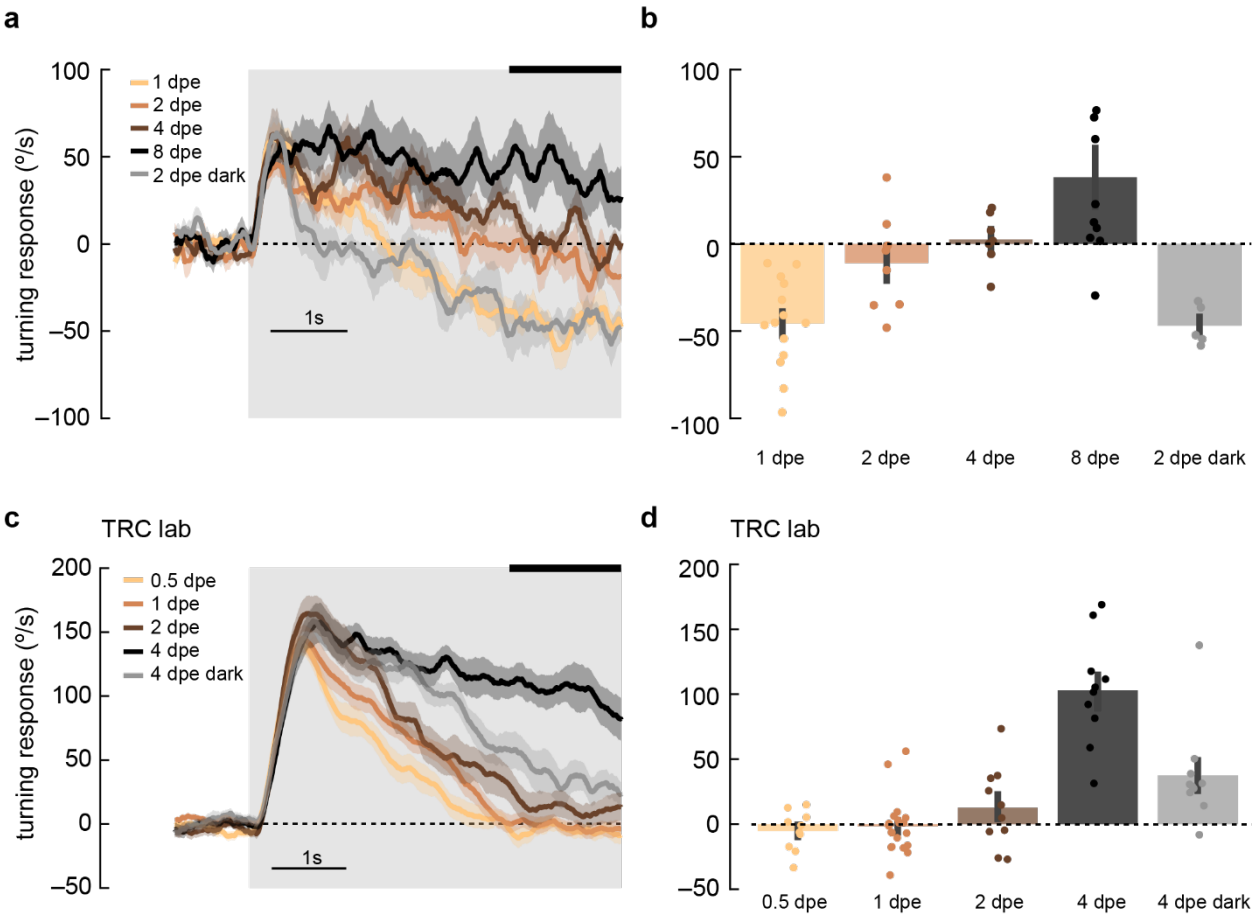

Figure 6. Maturation of optomotor response in early adulthood

a) Adult flies at various ages post eclosion were presented with 5-second, high-contrast, rotating sinusoidal gratings as in **Fig 2b**. As the flies aged from 1 day post eclosion (dpe) to 2, 4, and 8 dpe, the initial anti-directional turning response transitioned into syn-directional turning. Dark-rearing flies at 2 dpe reduced this maturation effect. Shaded patches represent ±1 SEM. N = 5-14 flies.

b) The last 1.5 seconds of the mean turning velocity of each fly was averaged, and the population response was plotted.

c) As in (a) but in the TRC lab, using 0.5, 1, 2, and 4 dpe, with dark rearing for 4 dpe. With maturation, the syn-directional turning became less transient. N = 9-15 flies.

d) As in (b) but for data in (c).

Discussion

In this study, we found we could elicit robust turning in the opposite direction of high contrast motion stimuli (**Fig. 1**). This behavior is qualitatively different from other turning behaviors reported in the literature (**Figs. 2** and **3**), but shares elements with the circuitry necessary for optomotor behavior (**Fig. 4**). However, the switch from syn-directional turning behavior to anti-directional turning behavior is not a reflection of changes in the activity of known direction-selective neuron types in the early visual system (**Fig. 5**). Moreover, this anti-directional turning behavior exhibits a degree of experience-dependent plasticity (**Fig. 6**).

Anti-directional turning is distinct from other against-stimuli behaviors

The anti-directional turning behavior we have characterized is distinct from previous reports of flies turning in the direction opposite to the stimulus motion. First, some opposite-direction turning behaviors can be explained by stimulus aliasing [3]. Aliasing cannot explain our results because the stimulus that maximally activates anti-directional behavior has a spatial frequency of 1/60 cycles per degree, well below the Nyquist frequency of the fly eye (~1/10 cycles per degree) [3,24] and below reports of higher acuity vision in flies [57]. Aliasing would also not explain the dependence on stimulus contrast.

Second, our observations also cannot be explained by stimuli to the rear of the fly driving it in the opposite direction [26], since we observe anti-directional turning even when stimuli are only presented in only the 90 degrees in front of the fly (**Fig. 3**).

Third, it is also distinct from previous reports of reverse body saccades [28] since it manifests in persistent turns in the opposite direction of the stimulus and can be measured even when the magnitude of the turns is discarded (**Fig. 3**).

Fourth, the behavior observed here also appears to be distinct from previously-observed stimulus-density dependent behavioral reversals [58]. Those previously reported behaviors showed immediate reversals, but it took ~1 second for flies in our paradigm to switch between optomotor and anti-directional behaviors.

Anti-directional turning is unlikely to be due to adaptation to contrast alone

In mammalian retina, the direction preference of cells can switch because of upstream circuit adaptation [59,60]. However, we do not believe the anti-directional turning we observe has similar causes. In the mammalian retina, direction switching occurs when non-direction-selective neurons adapt to high contrast stimuli, which distorts the downstream direction-selective computation. Since the adaptation in those experiments occurs in non-direction-selective neurons, it cannot be affected by the direction of the adapter stimulus. However, we see

differences in turning behavior depending on whether we adapt with front-to-back or back-to-front stimuli (**Fig. 2e**). This observation rules out a mechanism based solely on contrast, since the contrast content of front-to-back and back-to-front stimuli are identical.

The fly's visual system, however, adapts its gain to stimulus contrast [61,62]. Importantly, the phenomenology of the anti-directional turning also argues that the contrast adaptation is incomplete or heterogeneous among neurons, since contrast 1 and contrast 0.25 stimuli result in such different behaviors. Contrast adaptation reported in the fly is also faster than the 1-2 seconds preceding the shift to anti-directional turning in these experiments.

Anti-directional turning behavior may require specific experimental and rearing conditions

Despite these previous reports of anti-directional turning under certain conditions, other labs have measured sustained optomotor turning in response to high contrast stimuli [2,31,32,36]. Two major causes of this difference are likely display brightness and rearing conditions. Some experiments employ displays with mean luminances less than 5 cd/m^2 [31,33,36]. Our screens, with a mean luminance of 100 cd/m^2, are substantially brighter, but not especially bright when compared to natural scenes. In daytime natural scenes, foliage and the ground have average luminances of 200-500 cd/m^2 and the sky has an average luminance of around 4000 cd/m^2 [63]. We suspect that as researchers move to using displays that can more accurately depict natural scene luminances, anti-directional turning behaviors will be encountered more frequently.

Rearing conditions also significant influenced anti-directional turning behavior. Flies reared at 25°C showed less anti-directional behavior than those reared at 20°C. Temperature has known developmental effects on neural connectivity [64]. We also found differences based on fly age and fly strain. Notably, all three of these parameters vary significantly across the field, with prior studies varying rearing temperatures from 18 to 20 to 25ºC (see for instance [36,39,57,65,66]), ages from 1 day to 10 days (see for instance [26,30,42]), and strain between CantonS or OregonR (see for instance [8,33]). Thus, these factors likely account for the fact that this phenomenon has not previously been reported, even as rotating sinusoids have been widely used in behavioral experiments.

Tuning of anti-directional turning matches tuning of direction selective neurons

The study of anti-directional turning behavior may yield clues about the temporal tuning of fly motion detectors. Optomotor behavior is tuned to visual stimuli in the range of 8-22 Hz [26,37,39,67], while anti-directional behavior is tuned to stimuli in the range of 0.5-4 Hz (**Fig. 2**). Intriguingly, this slower tuning matches the tuning of T4, T5, and HS neurons, as measured via calcium imaging or electrophysiology [12,20,39,68]. Previous studies have suggested that the difference in tuning between behavior and imaging are due to octopamine that is released during behavior but not necessarily released during imaging [37,47,68]. In this work, we demonstrate a motion-related behavior tuned to low frequencies, comparable to those in neural measurements, during behavior that requires T4 and T5 neurons. Overall, this suggests that T4 and T5 are required for behaviors with very different temporal tuning, which in turn suggests that the temporal tuning of behavior is not determined solely by T4 and T5 tuning, but by other, parallel pathways as well.

Anti-directional turning reveals circuits that turn the fly counter to visual motion

Experiments that show a decrease of turning over time to high contrast stimuli (e.g., **Fig. 1d**) could plausibly be explained by some kind of gain reduction or adaptation over time. However, the existence of turning in the direction opposite the stimulus motion in *D. melanogaster* and in *D. yakuba* requires a different explanation. These experiments reveal that over long timescales, a circuit that opposes the syn-directional optomotor turning response can dominate the behavioral response. Thus, this circuit is not simply scaling the magnitude of turning responses, but rather must be implementing an antagonistic, subtractive operation. Measurements of free walking behavior have shown that the time constant of the autocorrelation of fly turning is around 100 ms [69,70]. Opposing syn- and anti-directional turning circuits could be used to balance and tune the strength of turning responses on short timescales, while the anti-directional turning is revealed on longer timescales. This sort of subtractive processing predominates in computing motion signals in the visual systems of insects [17] and mammals [71], and could also explain the existence of syn- and anti-directional turning behaviors.

In summary, we have presented evidence of a transition from syn-directional turning to no turning or to anti-directional turning when high contrast stimuli are presented to the fly. This persists across laboratory environments and across *Drosophila* species and shows plasticity with age. This behavior suggests that turning in response to rotational stimuli is not a simple reflex. Instead, the turning is likely driven by circuits with opposing influences on turning direction. These circuits appear to differentially adapt to the direction and contrast of the stimulus. This complexity makes the optomotor response a model for studying the interactions of circuits as they control the low-dimensional behaviors that change an animal's orientation.

Contributions

OM, MC, RT, MSC, NCBM, JS, and BAB collected data. OM, MC, TRC, and DAC wrote the paper.

Acknowledgements

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507 # Methods

508 ## Data availability

509 Raw behavioral and imaging data, along with code to run the analyses and create the plots in this
510 paper, are available on Dryad: https://doi.org/10.5061/dryad.stqjq2c77.

511 ## Fly strains

512 Strains used in these experiments are listed in the tables below:

513 Table 1: Parental stock genotypes

Name	Genotype	Source	Stock #
Wildtype	+; +; + (IsoD1)	[72]	N/A
T4T5-Gal4	+; +; R42F06-Gal4 (IsoD1 background)	BDSC	BDSC 41253
HS-Gal4	+; +; R27B03-Gal4 (IsoD1 bg)	[31]	BDSC 49211
CH-Gal4	w; +; R35A10-Gal4 (Janelia bg)	BDSC	BDSC 49897
UAS-Shibire^{ts}	+; +; UAS-Shibire^{ts} (IsoD1 bg)	[42]	N/A
Empty Gal4	w; +; pBDPGAL4.1Uw (Janelia bg)	BDSC	BDSC 68384
GCaMP6f	w; UAS-GCaMP6f; +	BDSC	BDSC 42747
jGCaMP7b	w; +; UAS-jGCaMP7b	BDSC	BDSC 79029
mtdTomato	w; +; UAS-mtdTomato	BDSC	BDSC 30124

514

515 Table 2: Genotypes of flies used in behavior experiments

Experimental	Gal4 Control	UAS Control	Background Control
T4T5-Gal4 x UAS-Shibire^{ts}: +; +; R42F06-Gal4/UAS-Shibire^{ts}	T4T5-Gal4 x IsoD1: +;+;R42F06-Gal4/+	IsoD1 x UAS-Shibire^{ts}: +; +; +/UAS-Shibire^{ts}	IsoD1: +; +; +
HS-Gal4 x UAS-Shibire^{ts}: +; +; R27B03-Gal4/UAS-Shibire^{ts}	HS-Gal4 x IsoD1: +; +; R27B03-Gal4/+	IsoD1 x UAS-Shibire^{ts}: +; +; +/UAS-Shibire^{ts}	IsoD1: +; +; +
CH-Gal4 x UAS-Shibire^{ts}: w/+; +; R35A10-Gal4/UAS-Shibire^{ts}	CH-Gal4 x IsoD1: w/+; +; R35A10-Gal4/+	Empty Gal4 x UAS-Shibire^{ts}: +/w; +; pBDPGAL4.1Uw /UAS-Shibire^{ts}	Empty Gal4 X IsoD1: +/w; +; +/ pBDPGAL4.1Uw

516

517 Genotypes of files used in imaging experiments: +; +; HS-Gal4/UAS-jGCaMP7b, +; UAS-
518 GC6f/+; T4T5-Gal4/UAS-mtdTomato, w/+; +; CH-Gal4/UAS-jGCaMP7b.

519 ## Fly rearing (DAC lab)

520 Unless otherwise noted, flies were reared at 20 degrees Celsius in Panasonic MIR-154-PA
521 incubators (Panasonic/PHC, Tokyo, Japan). The flies were circadian entrained on 12-hour light-
522 dark cycles. Flies were raised on Archon Scientific glucose food (recipe D20102, Archon
523 Scientific, Durham, NC). We used CO_2 to anesthetize flies more than 12 hours before the
524 behavioral experiments.

Flies were tested for behavior in rigs built in the labs of DAC and TRC. Behavior shown in **Figs. 1d**, **1e**, **6c**, **6d**, **S1**, and **S4** was acquired in the lab of TRC, while the rest was obtained in the lab of DAC.

Fly rearing (TRC lab)

Flies were reared at 25°C, on molasses-based food, and circadian entrained on 12-hour light-dark cycles. Flies were collected within three hours of eclosion using brief CO2 anesthetization. *D. melanogaster* and *D. yakuba* were raised under identical conditions. Dark-reared flies were put in a dark chamber within 3 hours of eclosion. Flies tested at 0.5 days post eclosion were collected during the first two hours of the light cycle and were exposed to light until they were tested.

Stimulus generation and behavioral turning assays (DAC lab)

Stimuli were presented using DLP Lightcrafter (Texas Instruments, Dallas, TX) projectors [34]. Mirrors were used to bounce the projected light onto three screens made of back-projection material, surrounding the fly. The screens covered the front 270 degrees around the fly, and ~45 degrees in elevation above and below the fly. The projectors were set to monochrome mode (green unless otherwise noted), updating at 180 Hz. Stimulus video was generated through a custom MATLAB (Mathworks, Natick, MA) application using PsychToolbox [73]. Stimuli were mapped onto a virtual cylinder around the fly and the MATLAB application generated a viewpoint-corrected video signal.

Behavioral experiments were performed 12-60 hours after staging. For behavioral experiments, we selected female flies, and co-housed them with males after staging. Flies were cold-anesthetized and fixed to needles using UV-cured epoxy (Norland optical adhesive #63, Norland Products, Cranbury, NJ). Flies were then placed above air-suspended polypropylene balls. These balls were 6 mm in diameter and weighed ~120 mg. The balls were painted with two layers of marker coatings- a base silver layer and a red top layer. The motion of balls was detected by either a Parallax mouse sensor board (Parallax, Rocklin, CA) with an MCS-12086 sensor (Unity Opto Technology, Taipei, Taiwan), or a custom board with an ADNS 2080 sensor (Avago Technologies / Broadcom Inc, San Jose, TX). The data from these sensors were transferred to a custom MATLAB application via an Arduino Uno board.

Stimulus generation and behavioral turning assays (TRC lab)

Stimuli were presented using a DLP Lightcrafter (Texas Instruments, Dallas, TX) projector. Three coherent optic fibers were used to direct the projected light onto three screens made of back-projection material, surrounding the fly [8,38]. The screens covered the front 270 degrees around the fly, and ~45 degrees in elevation above and below the fly. The projectors were set to monochrome mode, updating at 120 Hz. Stimulus video was generated through Flystim (https://github.com/ClandininLab/flystim), a custom Python application developed in the Clandinin Lab [74]. Stimuli were mapped onto a virtual cylinder around the fly and Flystim generated a viewpoint-corrected video signal.

564 Behavioral experiments were performed 12-48 hours after eclosion, as described in the figures.
565 Flies were cold-anesthetized and fixed to needles using UV-cured adhesive (Bondic, Niagara
566 Falls, NY). Flies were then placed above air-suspended balls made with LAST-A-FOAM FR-
567 4615 polyurethane foam (General Plastics, Tacoma, WA). These balls were 9 mm in diameter
568 and weighed ~91.7 mg. The motion of balls was detected by a Flea3 FL3-U3-13Y3M camera
569 (Teledyne Flir, Wilsonville, OR) and Fictrac software [75].

570 Imaging procedures

571 Two photon imaging (**Fig. 5**) was performed as previously described [76]. Briefly, two-photon
572 images were acquired with a Scientifica microscope at between 6 and 13 Hz using a 930 nm
573 femtosecond laser (SpectraPhysics, Santa Clara, USA) using ScanImage [77]. Visual stimuli were
574 presented on three screens occupying 270º of azimuthal angle about the fly using projectors [34].
575 Optical filters on the projector and emission filters prevented the visual stimulus light from
576 leaking into the two-photon images.

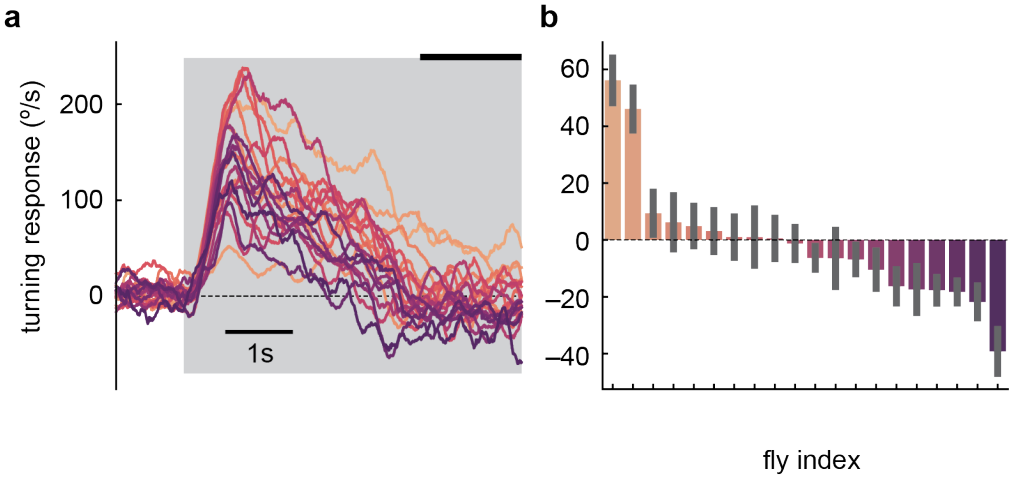
577 Regions of interest (ROIs) were extracted from image timeseries using a watershed algorithm.
578 Responsive ROIs were included in the analyses. For T4 and T5 neurons, each ROI was identified
579 as a T4-dominant or T5-dominant ROI by its response to light vs. dark edges, following prior
580 procedures [78]. For all neuron types, responses were averaged over ROIs and over trials of each
581 stimulus type to obtain a measurement for each fly; these fly measurements acted as the
582 independent measurements to compute means and standard error bars for the figure.

583 Statistical tests

584 Throughout the paper, each fly was considered an independent sample for statistical purposes.
585 Means and standard errors were computed over flies. For imaging experiments, regions of
586 interest from a specific neuron type were first averaged within each fly, creating a value for each
587 fly's response. These values were used to calculate means and standard errors over the tested
588 flies. In the silencing experiments, a 2-sample Student t-test was used to test for significant
589 differences between the experimental genotype and parental controls.

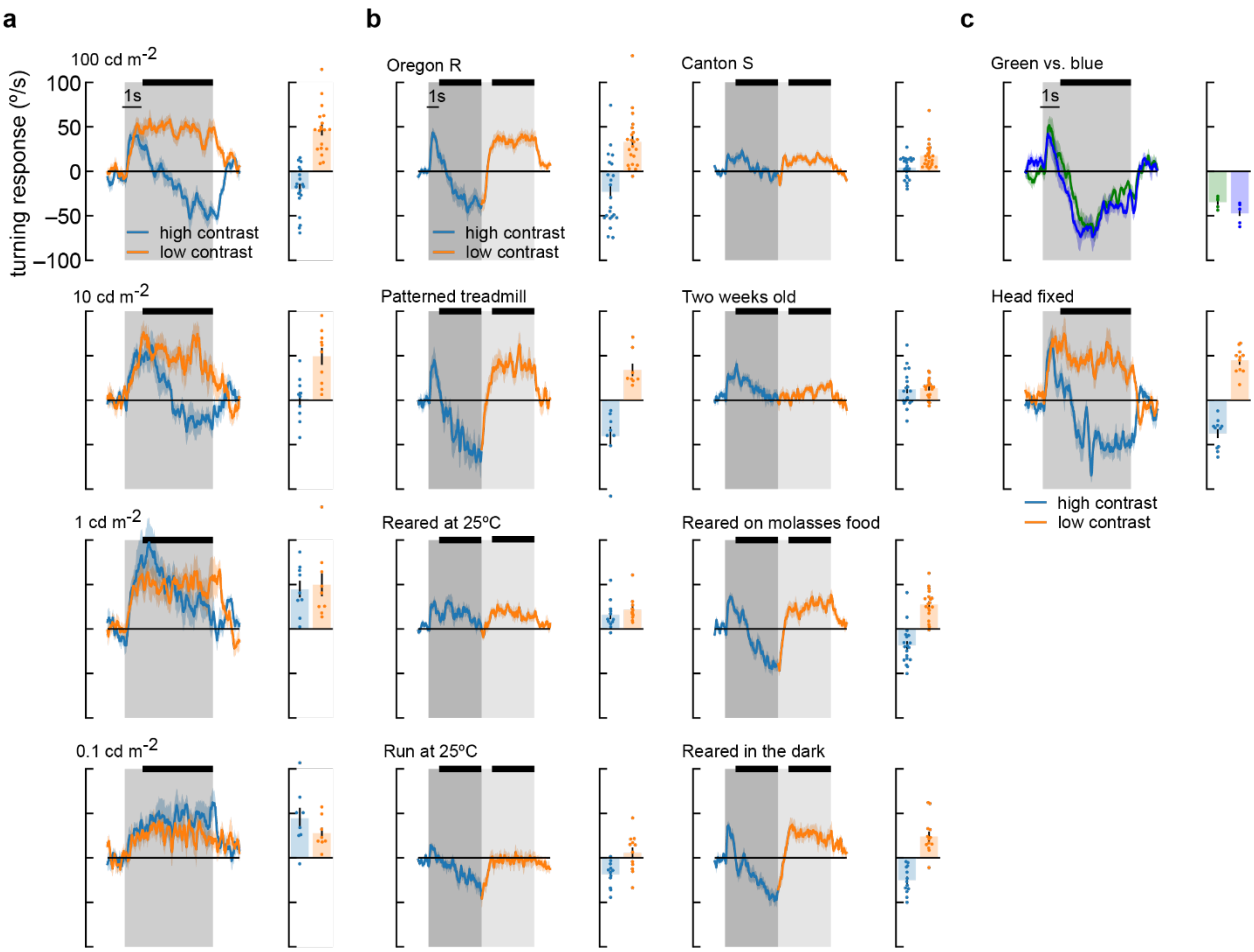
590

Supplementary Figures



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

a) Mean time traces of individual fly responses to the high contrast stimulus, averaged over trials. The flies are those in **Fig. 1d**.
b) 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.



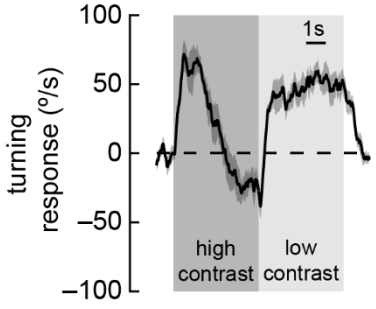
Supplementary Figure S2. Flies perform anti-directional turning under a wide range of stimulus and growing conditions.

a) Fly turning behavior at different mean screen brightness. We swept brightness from 100 cd/m^2 to 0.1 cd/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 cd/m^2, flies never turned in the opposite direction of the stimulus, and at 0.1 cd/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 ±1 SEM, as do vertical lines on bar plots. N = 19, 10, 9, 8 flies, top to bottom.

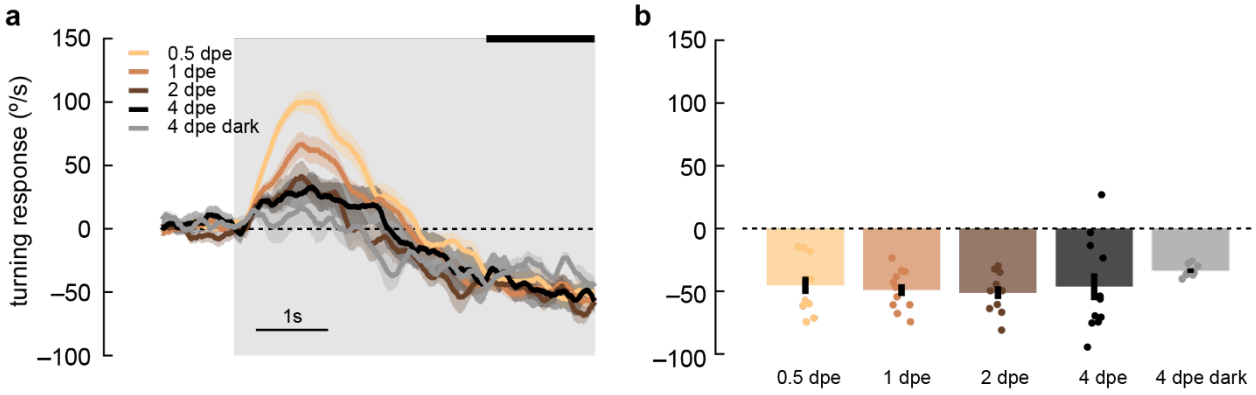
b) Our wildtype flies were Oregon-R strain [72] raised at 20 degrees. They were grown on glucose-based food media with 12-hour light-dark cycles. Experiments were run at high temperature, 12-60 hours after eclosion. We used uniform, red balls to avoid visual feedback from walking. The response of these 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 ±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.

Citations

1. Hassenstein, B., and Reichardt, W. (1956). Systemtheoretische Analyse der Zeit-, Reihenfolgen-und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers Chlorophanus. Zeits. Naturforsch. *11*, 513–524.

2. Götz, K., and Wenking, H. (1973). Visual control of locomotion in the walking fruitfly Drosophila. J. Comp. Physiol. A *85*, 235-266.

3. Buchner, E. (1976). Elementary movement detectors in an insect visual system. Biol. Cybern. *24*, 85-101.

4. Clark, D. (1981). Visual responses in developing zebrafish. Brachydanio rerio.

5. Koerner, F., and Schiller, P.H. (1972). The optokinetic response under open and closed loop conditions in the monkey. Exp. Brain Res. *14*, 318-330.

6. Götz, K.G. (1975). The optomotor equilibrium of theDrosophila navigation system. Journal of comparative physiology *99*, 187-210. 10.1007/BF00613835.

7. Yang, H.H., and Clandinin, T.R. (2018). Elementary motion detection in Drosophila: algorithms and mechanisms. Ann. Rev. Vis. Sci. *4*, 143-163.

8. Clark, D.A., Bursztyn, L., Horowitz, M.A., Schnitzer, M.J., and Clandinin, T.R. (2011). Defining the computational structure of the motion detector in Drosophila. Neuron *70*, 1165-1177.

9. Joesch, M., Schnell, B., Raghu, S., Reiff, D., and Borst, A. (2010). ON and OFF pathways in Drosophila motion vision. Nature *468*, 300-304.

10. Strother, J.A., Nern, A., and Reiser, M.B. (2014). Direct observation of ON and OFF pathways in the Drosophila visual system. Curr. Biol. *24*, 976-983.

11. Behnia, R., Clark, D.A., Carter, A.G., Clandinin, T.R., and Desplan, C. (2014). Processing properties of ON and OFF pathways for Drosophila motion detection. Nature *512*, 427-430.

12. Maisak, M.S., Haag, J., Ammer, G., Serbe, E., Meier, M., Leonhardt, A., Schilling, T., Bahl, A., Rubin, G.M., Nern, A., et al. (2013). A directional tuning map of Drosophila elementary motion detectors. Nature *500*, 212-216.

13. Takemura, S.-y., Bharioke, A., Lu, Z., Nern, A., Vitaladevuni, S., Rivlin, P.K., Katz, W.T., Olbris, D.J., Plaza, S.M., Winston, P., et al. (2013). A visual motion detection circuit suggested by Drosophila connectomics. Nature *500*, 175-181.

14. Shinomiya, K., Huang, G., Lu, Z., Parag, T., Xu, C.S., Aniceto, R., Ansari, N., Cheatham, N., Lauchie, S., Neace, E., et al. (2019). Comparisons between the ON-and OFF-edge motion pathways in the Drosophila brain. eLife *8*, e40025.

685 15. Bausenwein, B., Dittrich, A., and Fischbach, K.-F. (1992). The optic lobe of Drosophila
686 melanogaster. Cell Tissue Res. *267*, 17-28.

687 16. Henning, M., Ramos-Traslosheros, G., Gür, B., and Silies, M. (2022). Populations of
688 local direction–selective cells encode global motion patterns generated by self-motion.
689 Science advances *8*, eabi7112.

690 17. Mauss, A.S., Pankova, K., Arenz, A., Nern, A., Rubin, G.M., and Borst, A. (2015).
691 Neural circuit to integrate opposing motions in the visual field. Cell *162*, 351-362.

692 18. Schnell, B., Raghu, S.V., Nern, A., and Borst, A. (2012). Columnar cells necessary for
693 motion responses of wide-field visual interneurons in Drosophila. J. Comp. Physiol. A
694 *198*, 389-395.

695 19. Barnhart, E.L., Wang, I.E., Wei, H., Desplan, C., and Clandinin, T.R. (2018). Sequential
696 nonlinear filtering of local motion cues by global motion circuits. Neuron *100*, 229-243.
697 e223.

698 20. Joesch, M., Plett, J., Borst, A., and Reiff, D. (2008). Response properties of motion-
699 sensitive visual interneurons in the lobula plate of Drosophila melanogaster. Curr. Biol.
700 *18*, 368-374.

701 21. Kim, A.J., Fenk, L.M., Lyu, C., and Maimon, G. (2017). Quantitative predictions
702 orchestrate visual signaling in Drosophila. Cell *168*, 280-294. e212.

703 22. Haikala, V., Joesch, M., Borst, A., and Mauss, A.S. (2013). Optogenetic control of fly
704 optomotor responses. J. Neurosci. *33*, 13927-13934.

705 23. Krapp, H.G., and Hengstenberg, R. (1996). Estimation of self-motion by optic flow
706 processing in single visual interneurons. Nature *384*, 463-466.

707 24. Götz, K.G. (1970). Fractionation of *Drosophila* Populations According to Optomotor
708 Traits. Journal of Experimental Biology *52*, 419-436.

709 25. Götz, K. (1964). Optomotorische untersuchung des visuellen systems einiger
710 augenmutanten der fruchtfliege Drosophila. Biol. Cybern. *2*, 77-92.

711 26. Tammero, L., Frye, M., and Dickinson, M. (2004). Spatial organization of visuomotor
712 reflexes in Drosophila. J. Exp. Biol. *207*, 113-122.

713 27. Goetz, K.G. (1968). Flight control in Drosophila by visual perception of motion. Biol.
714 Cybern. *4*, 199-208.

715 28. Williamson, W.R., Peek, M.Y., Breads, P., Coop, B., and Card, G.M. (2018). Tools for
716 Rapid High-Resolution Behavioral Phenotyping of Automatically Isolated Drosophila.
717 Cell Rep. *25*, 1636-1649. e1635.

718 29. Wolf, R., and Heisenberg, M. (1990). Visual control of straight flight in Drosophila
719 melanogaster. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural,
720 and Behavioral Physiology *167*, 269-283.

721 30. Bahl, A., Ammer, G., Schilling, T., and Borst, A. (2013). Object tracking in motion-blind
722 flies. Nat. Neurosci. *16*, 730-738.

723 31. Seelig, J., Chiappe, M., Lott, G., Dutta, A., Osborne, J., Reiser, M., and Jayaraman, V.
724 (2010). Two-photon calcium imaging from head-fixed Drosophila during optomotor
725 walking behavior. Nat. Methods.

726 32. Bosch, D.S., van Swinderen, B., and Millard, S.S. (2015). Dscam2 affects visual
727 perception in Drosophila melanogaster. Frontiers in behavioral neuroscience *9*, 149.

728 33. Rister, J., Pauls, D., Schnell, B., Ting, C., Lee, C., Sinakevitch, I., Morante, J., Strausfeld,
729 N., Ito, K., and Heisenberg, M. (2007). Dissection of the peripheral motion channel in the
730 visual system of Drosophila melanogaster. Neuron *56*, 155-170.

731 34. Creamer, M.S., Mano, O., Tanaka, R., and Clark, D.A. (2019). A flexible geometry for
732 panoramic visual and optogenetic stimulation during behavior and physiology. J.
733 Neurosci. Methods *323*, 48-55.

734 35. Bahl, A., Serbe, E., Meier, M., Ammer, G., and Borst, A. (2015). Neural mechanisms for
735 Drosophila contrast vision. Neuron *88*, 1240-1252.

736 36. Strother, J.A., Wu, S.-T., Wong, A.M., Nern, A., Rogers, E.M., Le, J.Q., Rubin, G.M.,
737 and Reiser, M.B. (2017). The emergence of directional selectivity in the visual motion
738 pathway of Drosophila. Neuron *94*, 168-182. e110.

739 37. Strother, J.A., Wu, S.-T., Rogers, E.M., Eliason, J.L., Wong, A.M., Nern, A., and Reiser,
740 M.B. (2018). Behavioral state modulates the ON visual motion pathway of Drosophila.
741 Proc. Natl. Acad. Sci. USA *115*, E102-E111.

742 38. Clark, D.A., Fitzgerald, J.E., Ales, J.M., Gohl, D.M., Silies, M., Norcia, A.M., and
743 Clandinin, T.R. (2014). Flies and humans share a motion estimation strategy that exploits
744 natural scene statistics. Nat. Neurosci. *17*, 296-303.

745 39. Creamer, M.S., Mano, O., and Clark, D.A. (2018). Visual Control of Walking Speed in
746 Drosophila. Neuron *100*, 1460-1473.

747 40. Salazar-Gatzimas, E., Chen, J., Creamer, M.S., Mano, O., Mandel, H.B., Matulis, C.A.,
748 Pottackal, J., and Clark, D.A. (2016). Direct measurement of correlation responses in
749 Drosophila elementary motion detectors reveals fast timescale tuning. Neuron *92*, 227-
750 239.

751 41. Leonhardt, A., Ammer, G., Meier, M., Serbe, E., Bahl, A., and Borst, A. (2016).
752 Asymmetry of Drosophila ON and OFF motion detectors enhances real-world velocity
753 estimation. Nat. Neurosci. *19*, 706–715.

754 42. Silies, M., Gohl, D.M., Fisher, Y.E., Freifeld, L., Clark, D.A., and Clandinin, T.R.
755 (2013). Modular use of peripheral input channels tunes motion-detecting circuitry.
756 Neuron *79*, 111-127.

757 43. Heisenberg, M., and Buchner, E. (1977). The role of retinula cell types in visual behavior
758 ofDrosophila melanogaster. Journal of Comparative Physiology A: Neuroethology,
759 Sensory, Neural, and Behavioral Physiology *117*, 127-162.

760 44. McCann, G.D., and MacGinitie, G. (1965). Optomotor response studies of insect vision.
761 Proceedings of the Royal Society of London. Series B. Biological Sciences *163*, 369-401.

762 45. Duistermars, B., Chow, D., Condro, M., and Frye, M. (2007). The spatial, temporal and
763 contrast properties of expansion and rotation flight optomotor responses in Drosophila. J.
764 Exp. Biol. *210*, 3218.

765 46. York, R.A., Brezovec, L.E., Coughlan, J., Herbst, S., Krieger, A., Lee, S.-Y., Pratt, B.,
766 Smart, A.D., Song, E., and Suvorov, A. (2022). The evolutionary trajectory of
767 drosophilid walking. Curr. Biol.

768 47. Arenz, A., Drews, M.S., Richter, F.G., Ammer, G., and Borst, A. (2017). The temporal
769 tuning of the Drosophila motion detectors is determined by the dynamics of their input
770 elements. Curr. Biol. *27*, 929-944.

771 48. Leong, J.C.S., Esch, J.J., Poole, B., Ganguli, S., and Clandinin, T.R. (2016). Direction
772 selectivity in Drosophila emerges from preferred-direction enhancement and null-
773 direction suppression. J. Neurosci. *36*, 8078-8092.

774 49. Wienecke, C.F., Leong, J.C., and Clandinin, T.R. (2018). Linear Summation Underlies
775 Direction Selectivity in Drosophila. Neuron.

776 50. Salazar-Gatzimas, E., Agrochao, M., Fitzgerald, J.E., and Clark, D.A. (2018). The
777 Neuronal Basis of an Illusory Motion Percept Is Explained by Decorrelation of Parallel
778 Motion Pathways. Curr. Biol. *28*, 3748-3762.

779 51. Schilling, T., and Borst, A. (2015). Local motion detectors are required for the
780 computation of expansion flow-fields. Biology open, bio. 012690.

781 52. Kitamoto, T. (2001). Conditional modification of behavior in Drosophila by targeted
782 expression of a temperature-sensitive shibire allele in defined neurons. J. Neurobiol. *47*,
783 81-92.

784 53. Fujiwara, T., Brotas, M., and Chiappe, M.E. (2022). Walking strides direct rapid and
785 flexible recruitment of visual circuits for course control in Drosophila. Neuron.

786 54. Wei, H., Kyung, H.Y., Kim, P.J., and Desplan, C. (2020). The diversity of lobula plate
787 tangential cells (LPTCs) in the Drosophila motion vision system. J. Comp. Physiol. A
788 *206*, 139-148.

789 55. Borst, A., and Weber, F. (2011). Neural Action Fields for Optic Flow Based Navigation:
790 A Simulation Study of the Fly Lobula Plate Network. PLOS ONE *6*, e16303.
791 10.1371/journal.pone.0016303.

792 56. Eckert, H., and Dvorak, D.R. (1983). The centrifugal horizontal cells in the lobula plate
793 of the blowfly, Phaenicia sericata. J. Insect Physiol. *29*, 547-560.

794 57. Juusola, M., Dau, A., Song, Z., Solanki, N., Rien, D., Jaciuch, D., Dongre, S.A.,
795 Blanchard, F., de Polavieja, G.G., and Hardie, R.C. (2017). Microsaccadic sampling of
796 moving image information provides Drosophila hyperacute vision. Elife *6*, e26117.

797 58. Katsov, A., and Clandinin, T. (2008). Motion processing streams in Drosophila are
798 behaviorally specialized. Neuron *59*, 322-335.

799 59. Rivlin-Etzion, M., Wei, W., and Feller, M.B. (2012). Visual stimulation reverses the
800 directional preference of direction-selective retinal ganglion cells. Neuron *76*, 518-525.

801 60. Vlasits, A.L., Bos, R., Morrie, R.D., Fortuny, C., Flannery, J.G., Feller, M.B., and Rivlin-
802 Etzion, M. (2014). Visual stimulation switches the polarity of excitatory input to starburst
803 amacrine cells. Neuron *83*, 1172-1184.

804 61. Matulis, C.A., Chen, J., Gonzalez-Suarez, A., Behnia, R., and Clark, D.A. (2020).
805 Heterogeneous temporal contrast adaptation in Drosophila direction-selective circuits.
806 Curr. Biol. *30*, 222-236.

807 62. Drews, M.S., Leonhardt, A., Pirogova, N., Richter, F.G., Schuetzenberger, A., Braun, L.,
808 Serbe, E., and Borst, A. (2020). Dynamic Signal Compression for Robust Motion Vision
809 in Flies. Curr. Biol.

810 63. Frazor, R.A., and Geisler, W.S. (2006). Local luminance and contrast in natural images.
811 Vision Res. *46*, 1585-1598.

812 64. Kiral, F.R., Dutta, S.B., Linneweber, G.A., Hilgert, S., Poppa, C., Duch, C., von Kleist,
813 M., Hassan, B.A., and Hiesinger, P.R. (2021). Brain connectivity inversely scales with
814 developmental temperature in Drosophila. Cell Rep. *37*, 110145.

815 65. Mongeau, J.-M., and Frye, M.A. (2017). Drosophila spatiotemporally integrates visual
816 signals to control saccades. Curr. Biol. *27*, 2901-2914. e2902.

817 66. Ketkar, M.D., Sporar, K., Gür, B., Ramos-Traslosheros, G., Seifert, M., and Silies, M.
818 (2020). Luminance information is required for the accurate estimation of contrast in
819 rapidly changing visual contexts. Curr. Biol. *30*, 657-669. e654.

820 67. Tuthill, J.C., Nern, A., Holtz, S.L., Rubin, G.M., and Reiser, M.B. (2013). Contributions
821 of the 12 neuron classes in the fly lamina to motion vision. Neuron *79*, 128-140.

822 68. Chiappe, M.E., Seelig, J.D., Reiser, M.B., and Jayaraman, V. (2010). Walking modulates
823 speed sensitivity in Drosophila motion vision. Curr. Biol. *20*, 1470-1475.

824 69. DeAngelis, B.D., Zavatone-Veth, J.A., and Clark, D.A. (2019). The manifold structure of
825 limb coordination in walking Drosophila. eLife *8*, e46409.

826 70. Katsov, A.Y., Freifeld, L., Horowitz, M.A., Kuehn, S., and Clandinin, T.R. (2017).
827 Dynamic structure of locomotor behavior in walking fruit flies. eLife *6*, e26410.

828 71. Rust, N.C., Mante, V., Simoncelli, E.P., and Movshon, J.A. (2006). How MT cells
829 analyze the motion of visual patterns. Nat. Neurosci. *9*, 1421-1431.

830 72. Gohl, D.M., Silies, M.A., Gao, X.J., Bhalerao, S., Luongo, F.J., Lin, C.C., Potter, C.J.,
831 and Clandinin, T.R. (2011). A versatile in vivo system for directed dissection of gene
832 expression patterns. Nat. Methods *8*, 231-237.

833 73. Kleiner, M., Brainard, D., Pelli, D., Ingling, A., Murray, R., and Broussard, C. (2007).
834 What's new in Psychtoolbox-3. Perception *36*, 1.

835 74. Turner, M.H., Krieger, A., Pang, M.M., and Clandinin, T.R. (2022). Visual and motor
836 signatures of locomotion dynamically shape a population code for visual features in
837 Drosophila. bioRxiv.

838 75. Moore, R.J., Taylor, G.J., Paulk, A.C., Pearson, T., van Swinderen, B., and Srinivasan,
839 M.V. (2014). FicTrac: a visual method for tracking spherical motion and generating
840 fictive animal paths. J. Neurosci. Methods *225*, 106-119.

841 76. Tanaka, R., and Clark, D.A. (2022). Neural mechanisms to exploit positional geometry
842 for collision avoidance. Curr. Biol. *32*, 2357-2374.

843 77. Pologruto, T.A., Sabatini, B.L., and Svoboda, K. (2003). ScanImage: flexible software
844 for operating laser scanning microscopes. Biomed. Eng. Online *2*, 13.

845 78. Agrochao, M., Tanaka, R., Salazar-Gatzimas, E., and Clark, D.A. (2020). Mechanism for
846 analogous illusory motion perception in flies and humans. Proc. Natl. Acad. Sci. *117*,
847 23044-23053.

848