Non-preferred contrast responses in the *Drosophila* motion pathways reveal a receptive field structure that explains a common visual illusion

**Highlights**
- Whole-cell recordings of T4 and T5 responses to ON-OFF visual stimuli
- T4 and T5 have a similar receptive field but with opposite contrast selectivity
- Biophysical model fit to ON and OFF responses predicts directional selectivity
- The model explains cellular and behavioral responses to reverse-phi illusion

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**In brief**
Recording whole-cell responses from ON and OFF cells in the *Drosophila* motion pathway, Gruntman et al. show that these cells share a receptive field structure for their non-preferred contrast responses. This structure explains the perception of directional inversion to the reverse-phi illusion.
Non-preferred contrast responses in the Drosophila motion pathways reveal a receptive field structure that explains a common visual illusion

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SUMMARY

Diverse sensory systems, from audition to thermosensation, feature a separation of inputs into ON (increments) and OFF (decrements) signals. In the Drosophila visual system, separate ON and OFF pathways compute the direction of motion, yet anatomical and functional studies have identified some crosstalk between these channels. We used this well-studied circuit to ask whether the motion computation depends on ON-OFF pathway crosstalk. Using whole-cell electrophysiology, we recorded visual responses of T4 (ON) and T5 (OFF) cells, mapped their composite ON-OFF receptive fields, and found that they share a similar spatiotemporal structure. We fit a biophysical model to these receptive fields that accurately predicts directionally selective T4 and T5 responses to both ON and OFF moving stimuli. This model also provides a detailed mechanistic explanation for the directional preference inversion in response to the prominent reverse-phi illusion. Finally, we used the steering responses of tethered flying flies to validate the model’s predicted effects of varying stimulus parameters on the behavioral turning inversion.

INTRODUCTION

In both invertebrate and vertebrate visual systems, neuronal signals bifurcate into parallel pathways, within which many neurons preferentially encode luminance increments (ON) or luminance decrements (OFF) within 1 to 2 synaptic layers downstream of photoreceptors.1 The direction of local motion is computed separately within these pathways a few synapses downstream of the ON-OFF split.1–3 Splitting sensory signals into increments and decrements is common to different modalities4–6 and may enable more efficient stimulus encoding.7 However, in the mammalian retina, motion is also computed in ON-OFF cells,8 and the separate motion pathways in the fly show clear evidence of crosstalk.9,10 What is the benefit of mixing between pathways? An effective tool for studying this crosstalk in the motion pathway is the “reverse-phi” visual illusion,11–15 in which inverting the contrast of moving objects induces an illusory inversion in the detected motion direction.13 Similarities between the computations carried out in the fly and vertebrate visual systems suggest that understanding ON-OFF crosstalk in the Drosophila motion circuit could reveal fundamental aspects of visual processing and may uncover more general, conserved aspects of sensory processing.

Anatomical studies of the Drosophila medulla identified two major pathways,16,17 later determined to primarily encode ON and OFF signals.18–21 Each pathway contains motion computing circuits; the T4 neurons (ON) and T5 neurons (OFF) are the first cells in the visual system to show directionally selective responses.22 Connectomic reconstructions mapped the T4 and T5 inputs and showed most connections were between neurons within the same pathway. However, anatomical interactions between prominent cells of the ON and OFF pathways have been described,9,17,23 with likely functional contributions.24 For example, a key T4 input is primarily OFF-responding,25–27 and T5 neurons show responses to some ON stimuli.28,29 The classic experiments that established the analysis of motion computation in insects already showed inverted responses to ON-OFF combinations.12 Later studies found neuronal correlates for this behavioral inversion in neurons downstream of T4 and T5,11,30 and recent studies showed that T4 and T5 exhibit an inverted directional preference to reverse-phi stimuli.26,31 Localizing this inversion to the directionally selective neurons suggests that mixing ON and OFF signals is a central feature of computing motion, but it remains unclear why or precisely how T4 and T5 are susceptible to the reverse-phi illusion.

In previous works, we described how T4 and T5 neurons generate directionally selective responses to moving bright and dark objects, respectively.32,33 Using static stimuli of the preferred contrast (PC; bright for T4, dark for T5), we showed that both cell types share a similar spatiotemporal receptive field (RF). This was unexpected since their input neurons have quite different properties.15,34 We modeled this RF as generated by a fast excitatory conductance and a slower, spatially offset inhibitory conductance.30,33 In this study, we found that responses of T4 and T5 cells to moving bars of their non-preferred contrast (NC; dark for T4, bright for T5) were still directionally selective.

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Therefore, we sought to understand how NC inputs affect T4 and T5 responses. First, we characterized T4 and T5 responses to PC and NC moving bars and mapped their RFs using both bright and dark stimuli. This revealed that T4 and T5 share a largely similar NC spatial RF. Next, we proposed a unified, conductance-based model to capture the PC/NC RF and showed that this model can explain the generation of directionally selective responses to both PC and NC moving bars. Remarkably, this model also provides an explanatory mechanism for the reverse-phi illusion. Finally, we corroborate these model predictions with functional recordings and behavioral responses to reverse-phi motion and show that NC responses are essential for understanding the motion computation.

RESULTS

T4 and T5 directionally selective responses to NC moving bars exhibit distinct dynamics

We used targeted in vivo whole-cell electrophysiology to localize the RF center for individual T4 or T5 neurons, identify their primary motion axis, and record responses to a panel of RF-aligned stimuli (Figure 1A). Bright stimuli are the PC for T4 but NC for T5, while dark stimuli are T5’s PC and T4’s NC (Figure 1B). Moving bars are a compound stimulus, containing a leading edge of one contrast transition and a trailing edge with the opposite contrast transition. When T4 cells were presented with bright bars moving in the preferred and non-preferred directions (PD and ND, respectively), their responses were directionally selective (Figure 1C, top), as expected.22,32,33,35 When presented with fast moving (56 /sec) dark narrow bars, T4 were largely unresponsive to both directions (Figure 1C, bottom). However, when presented with 4 × wider dark bars moving at the same speed, T4 responses were large and directionally selective (Figure 1C, bottom right). T5 cells responded similarly to bright moving stimuli: narrow bars evoked small responses, while wide bars evoked larger, directionally selective responses (Figure 1C, bottom right). We presented NC bars of different widths and speeds (Figure S1) and found that fast, narrow NC bars did not evoke directionally selective responses while slow, wide NC bars did. Additionally, the difference between the peak PD and ND responses for these slow and wide NC moving bars is comparable in magnitude to this difference for the corresponding PC bars (Figures 1D and S1).

T4 (T5) cells are typically referred to as ON (OFF) cells as a shorthand to indicate that they are strongly selective for luminance increments (decrements). Since a moving dark bar has both a leading luminance-decrement edge and a trailing luminance-increment edge, the recorded T4 responses to a dark moving bar could simply be a delayed response to the trailing edge (Figure 1E). We compared T4 responses to PD motion of bright and dark bars (see also Figure S1) and noted 3 key differences: (1) in response to bright bar motion, T4 depolarization preceded hyperpolarization while in response to dark bar motion hyperpolarization preceded (Figure 1C, arrowhead); (2) decay of dark bar responses were slower than for the bright bar (compare slope of green and black traces, Figure 1E); and (3) the dark bar response peak was delayed compared to bright bar peak, even after temporally aligning to the appearance of the light-increment edge (compare light green trace to black trace, Figures 1E, and S1A). The same 3 differences in the response dynamics were seen when we compared responses to PD motion of dark (PC) and bright (NC) bars in T5 cells (Figures 1Eii and S1). The above differences show that T4 and T5 directionally selective responses to NC moving bars cannot be explained as only a PC response to the trailing edge and, therefore, may reveal contributions from additional mechanisms.

T4 and T5 neurons have a similar NC RF structure

To uncover the mechanism for generating directionally selective responses to NC stimuli in T4 and T5 cells, we mapped the “static” RFs of both cells. Since our moving bars stimuli are composed of discrete steps, we can decompose them into single-step duration bar flashes presented at each position along the movement trajectory (Figure 1A). The PC RF has the same overall structure previously reported:34 responses on the leading side of the RF are depolarizing, while responses on the trailing side feature rapid depolarization followed by sustained hyperpolarization (Figure 2A; T5 data from same recordings as Gruntman et al.35). The NC RF structure is distinct from the PC RF yet remarkably similar between T4 and T5 (Figure 2A). For both cell types, depolarizing responses induced by flashing an NC bar are toward the trailing side, while hyperpolarizing responses are shifted toward the center (defined by PC responses; Figures 2A and 2B). Interestingly, this NC hyperpolarizing component is reminiscent of crosstalk inhibition found in the mammalian retina, where opposing contrast leads to increased inhibition when excitatory inputs decrease and vice versa.36,37 Although this RF structure is most evident in responses to long-duration flashes of wide bars, it is also measured in responses to narrower bars (Figure 2C). Next, we highlight three features of NC flash responses that differentiate them from PC responses yet are common to T4 and T5.

The first feature is prominent in the RF center: NC responses exhibited a strong hyperpolarization aligned to stimulus onset (Figure 2A, first vertical line in each panel) and a depolarization following stimulus offset (Figure 2A, second vertical line in each panel). The offset of a dark (bright) flash is accompanied by a luminance increment (decrement), which is expected to evoke a depolarizing response in T4 (T5). However, we find that the onset of these stimuli also evoked substantial responses. In response to NC bar onset, T4 and T5 were hyperpolarized in the RF center but depolarized on the trailing side (see also Wienecke et al.28). The depolarization response rise times (Figure 2D) for PC stimuli in both T4 and T5 are position-independent and follow stimulus onset (t = 0). Conversely, rise start times for NC bar flash responses show a clear positional dependence: following stimulus offset on the RF leading side and center yet following stimulus onset on the trailing side (for T5 also on the leading side; Figure 2D). These results suggest a specific interaction between ON and OFF signals, with T4 receiving both depolarizing and hyperpolarizing inputs induced by luminance decrements, and T5 receiving both depolarizing and hyperpolarizing inputs induced by luminance increments.

The second common feature: hyperpolarization in response to NC flashes decayed faster than hyperpolarization in response to PC flashes (Figure 2E). PC responses can be modeled by a combination of a fast excitatory conductance and a slow inhibitory one.32,33 Consequently, the response decay time is likely
dominated by the persistent inhibitory conductance pulling the membrane potential down. Since the hyperpolarization present in the NC flash at the RF center preceded the depolarizing component, we deduce that this hyperpolarization wanes faster than the slower PC hyperpolarization. These results suggest there are (at least) 2 separate sources of inhibition affecting the RF structure.

The third common feature: stimulus-duration-dependent offset depolarization. In response to a short dark flash, T4 cells hyperpolarized during stimulus presentation and then returned.

Further details of NC moving bar responses provided in Figure S1.

Figure 1. T4 and T5 directionally selective responses to non-preferred contrast moving bars exhibit distinct dynamics
(A) Schematic of experimental setup and procedure. Whole cell recordings were targeted to GFP-labeled somata. The receptive field (RF) center and preferred direction (PD) were located for each cell. Further RF characterization was reduced to presenting stimuli along the PD-ND axis.
(B) Summary of stimulus conventions used throughout. PD, preferred direction; ND, non-preferred direction; PC, preferred contrast; NC, non-preferred contrast. By convention, in all figures, the PD is aligned to movement from left to right.
(Ci) Baseline subtracted T4 responses (n = 9 cells diagonally aligned; mean ± SEM) to bars of width 1 and 4 LEDs (2.25° and 9°) moving at 56°/s (40 ms per LED step) in the PD and ND with both PC (top) and NC (bottom).
(Cii) Same as Ci for T5 (n = 12 cells diagonally aligned).
(Di) Boxplots for difference in the peak response to motion in both directions of T4 by bar contrast, width, and speed (n = 14 cells). Filled boxes denote means significantly different from zero (p < 0.01).
(Dii) Same as Di for T5 (n = 17 cells).
(Ei) Baseline subtracted T4 responses to 4-LED-wide PD moving bars with PC and NC overlaid (same data as Ci). Light green trace shows PC response temporally shifted to align with trailing edge (bottom: schematics of stimulus luminance levels).
(Eii) Same as Ei for T5.
Further details of NC moving bar responses provided in Figure S1.
Figure 2. T4 and T5 neurons have a similar non-preferred contrast receptive field structure
(A) Baseline subtracted responses (mean ± SEM) of T4 (middle; n = 15 cells) and T5 (bottom; n = 17 cells) to 160 ms bright (green) and dark (black) 4-LED-wide bar flashes at different position along the PD-ND axis. Gray vertical lines indicate stimulus onset and offset. Top: schematic for stimulus position within the RF.
(B) Maximal response depolarization and hyperpolarization by position for the same dataset in (A). Large dots represent the mean max response at each position and small dots the individual cell response mean. Points above (below) the zero line represent maximal depolarization (hyperpolarization). Green line in the T4 NC plot and black line in T5 NC plot are the respective PC means.
(C) Same as (B), for mean maximal responses to 1- and 2-LED-wide bar flashes.
(D) Boxplots of rise start time by position for responses presented in B for T4 (top) and T5 (bottom). Stimulus onset: t = 0; stimulus offset: t = 160 ms (gray lines).
(E) Boxplots of the response decay times for trailing side responses presented in (B) (positions [0:2]) for T4 (top; n = 15 cells) and T5 (bottom; n = 12 cells).
(F) Baseline subtracted responses to non-preferred contrast 4-LED-wide bar flashes presented for 40 and 160 ms at a single position (−1; T4 n = 15 cells; T5 n = 12 cells; mean ± SEM).
(G) Mean response amplitude for the same NC stimuli as in (F) (averaged over positions [-2:0]) compared to a linear approximation. These non-linear responses are unlikely to arise from intrinsic mechanisms, as detailed in Figure S2.
Figure 3. A unified model architecture explains the generation of both preferred and non-preferred contrast directionally selective responses in T4 and T5

(A) Schematic of T4 and T5 4-conductance model highlighting the temporal aspects. The schematic depicts a T4 responding to a dark stimulus, with a pulse signal for the duration of the light decrement feeding into the E-I decrement pair and a delta signal marking the time of light increment feeding into the E-I increment pair. Colored temporal filters represent input and conductance time-series for this input.

(B) Mean measured responses to 4-LED-wide flashes of dark and bright bars at 10 different positions (gray) compared to predicted model responses (green and black); model was optimized to fit the averaged T4 responses (n = 5 cardioidly aligned cells). Bottom: Gaussian spatial filters for the 4 conductances in the model (μ ± σ; dashed line indicates extended filter scope).

(C) Mean measured responses to fast (40 ms step, left) and slow (160 ms step, right), dark (top) and bright (bottom) bars moving in the preferred and non-preferred directions overlaid with predicted responses from the same model cell with the same model parameters as in (B).

(D) Peak measured responses compared to peak predicted responses for moving dark (top) and bright (bottom) bar stimuli (same widths and speeds as Figure 1D). Marker size denotes speed (large: slower). Marker color denotes movement direction, as in (C).

(E) Comparing the measured and the predicted responses, same as (B) but for the averaged T5 cell (n = 5 cardioidly aligned cells). Note that the T5-optimized model has E_rs fed by a spatial filter on the leading side of the receptive field to generate the leading-side bright stimulus onset component.

(F) Same as (D) for the same averaged T5 cell as in (E), presenting both dark (top) and bright (bottom) moving bar responses.

(G) Example T5 moving bar responses to explain the generation of directional selectivity. The numbers on the right correspond to numbered stages in (A). Top row: mean measured responses to 4-LED-wide dark (left columns) and bright (right columns) bars moving in the PD and ND overlaid with predicted responses. Middle

(legend continued on next page)
to baseline. When the same bar was presented for longer, its disappearance evoked a strong depolarization (Figure 2F, top). The dependence on stimulus duration is not linear (Figure 2G; paired sample t-test p < 0.001) and does not appear to rely on an intrinsic mechanism (Figure S2). In T5 cells, a different stimulus-duration dependence is seen at the offset of a bright flash (Figure 2F, bottom) since they exhibit a weak sublinearity (Figure 2G; paired sample t-test p < 0.01).

A unified model architecture explains the generation of both PC and NC directionally selective responses in T4 and T5

Our previous work showed that a model based solely on the PC flash responses of T4 and T5 cells can predict their responses to PC moving stimuli. Here, we evaluate whether a model of the PC-NC composite RF can explain the generation of directionally selective responses to both dark and bright moving stimuli. We established a model that allowed us to capture responses to both the onset and offset of luminance increments and decrements. In our model, we focus on the effects of NC and PC stimuli on the conductance inputs into T4 and T5 neurons and have deliberately avoided modeling the complete ON and OFF pathways.

We expanded our previous modeling framework with three major modifications: (1) inputs that represent stimulus offset, (2) preferred and non-preferred inputs, and (3) two additional conductances corresponding to NC inputs (Figure 3A). We designed the model to simulate either T4 or T5 neurons but used T4 responses to a dark bar flash (depicted in Figure 3A, right side) to provide intuition for these components. To enable differential responses to stimulus onset and offset, we represented flashes as an input pulse with the duration of the flash. We accounted for the instantaneous luminance change that occurs at stimulus offset with a delta input. The duration of the preceding flash scales the amplitude of this delta input to account for the history-dependence of the NC offset response (Figure 2F,G), which is later filtered (τO parameter; see STAR Methods).

To implement both dark and bright stimuli, we split the input such that the bright and dark input streams determine which conductance pair (increment/decrement) will receive which input type (pulse/delta). Since the stimulus cannot be both dark and bright at the same position at the same time, we simply integrate the two parallel input streams and their resulting conductances. The left side in the schematic (Figure 3A, faded) conveys bright inputs and is inactive during a dark bar stimulus.

The expanded model requires 4 conductances: an excitatory-inhibitory (E-I) pair responding to increments (Einc and Iinc) and another responding to decrements (Edec and Idec). Each pair receives the same type of input depending on which stimulus was presented at that position. For the dark bar example, the decrement-responding E-I pair receives the pulse input, and the increment-responding pair receives the delta input. Each conductance has its own temporal filter (Figure 3A) and its own spatial filter (not shown).

We fit and simulated responses from a full model as well as a model where we further minimized the total number of parameters (see STAR Methods). We optimized model parameters for T4 and T5 separately by using an iterative least-squares procedure that minimized the difference between our simulation results and the measured dark and bright responses. We present results from the reduced version in the primary figures, but detailed results from both models are in Figure S3. Since the reduced model requires fewer parameters and could be fit using only responses to non-moving stimuli, it is the more useful version.

As expected, the T4-optimized model’s responses were well-matched to the measured responses to both bright and dark flashes at all positions along the RF (Figure 3B). Reassuringly, despite having two additional conductances, the optimization procedure converged on spatial filters for Einc and Iinc that were similar to our prior (PC-only) T4 model: Einc in the RF center and Iinc toward the trailing side (depicted by horizontal lines below traces, Figure 3B). In the T4-optimized model, Edec appears to play a minor role, fit to a spatial filter on the far trailing side (Figure 3B) and with minimal amplitude. The Idec conductance is toward the leading side and contributes the hyperpolarizing current in response to a dark bar’s onset. We next used the same model parameters from the bar-flash optimization to simulate moving bar responses and compared them to our recordings (Figures 3C, 3D, and S3E–S3H). The model captures both the dynamics and the magnitude of the moving bar responses and predicts directionally selective responses to both dark and bright moving bars (illustrated for T5 in Figure 3G).

The T5-optimized model was fit using the identical architecture and accurately reproduces T5 responses to both bright and dark flashing bars (Figure 3E). Since T5 prefer luminance decrements, this model has Edec in the RF center and Idec toward the trailing side (Figure 3E, bottom). This asymmetric configuration generates a directional preference for moving dark bars.3 The spatial filter for Iinc is toward the leading side and generates the hyperpolarizing current in response to a bright bar onset. Einc is further toward the leading side and captures the weak depolarization in leading positions’ responses to bright flashing bar onset (Figure 2A, left-most positions). This T5-optimized model accurately predicted the magnitude of the directionally selective responses to both bright and dark moving bars (Figures 3F, 3G, top row, and S3E–S3H).

We use T5 responses to slow moving bars to illustrate a mechanism by which these four conductances contribute to directional selectivity. The model responses (Figure 3G, top row) closely match the recorded data for dark (PC) and bright (NC) bars moving in both directions. For dark moving bars, the simulated response is dominated by 2 conductances: Edec and Idec (Figure 3G, middle row, 2 left columns). For bright moving bars (Figure 3G, middle row, 2 right columns), all 4 conductances contribute, with Iinc playing a prominent role. During ND motion for both dark (Figure 3G, 1st column) and bright (Figure 3G, 3rd column) bars, the total excitatory and inhibitory conductances largely overlap (Figure 3G, bottom row), reducing changes in
membrane potential. In response to PD motion (Figure 3G, dark, 2nd column; bright, 4th column), the excitation and inhibition maxima are well-separated in time, reducing suppression. For dark stimuli, the excitation peak precedes inhibition, while for bright stimuli the excitatory conductance peaks in between the $I_{on}$ onset peak and the $I_{off}$ offset peak (similar to results of the T4-optimized model; Figures S3E and S3F). Note, the top 5% of optimization solutions always included parameter sets for simulations that feature the 2 inhibitory peak configurations (example in Figure S4). Taken together, these results demonstrate that a 4-conductance model optimized to reproduce the static RF can quantitatively account for PC and NC directional selectivity. To critically test the broader utility of our expanded model, we chose stimuli that include novel combinations of bright and dark components.

**4-conductance model explains illusory motion perception**

The mechanism we describe for generating directionally selective responses to NC moving bars should require a trailing stimulus edge, since it provides the offset signal that drives the excitatory responses to NC moving bars should require a trailing edge, featuring a prominent offset depolarization when the edge moves (Figure 3G), but for NC bar motion, they respond to the trailing edge delta input. To test this requirement, we recorded and simulated T5 responses to dark and bright moving edges (Figure 4A, see inset). The model, simulated with the same parameters as in Figure 3, predicts the magnitude, dynamics, and directional selectivity of the measured T5 responses to moving dark edges (Figure 4A, left). Both the recordings and the model predictions show similar responses to a bright moving edge, featuring a prominent offset depolarization when the edge disappears (Figure 4A, right). Importantly, these offset responses do not differentiate between the two directions. These results confirm the hypothesis that directional selectivity to NC stimuli requires a trailing luminance-change boundary and show that moving PC, but not NC, edges drive directionally selective responses.

Can this model predict T4 and T5 responses to the specific ON-OFF combination that give rise to the reverse-phi illusion? In standard “phi” motion, discrete sequential edge displacements are perceived as smooth motion in the direction of displacement. In the reverse-phi motion illusion (documented in many animals11,13,15,38), displacement is combined with contrast inversion (dark turns bright and vice versa), resulting in motion perceived in the direction opposite to the displacement. A minimal version of reverse-phi illusion motion can be evoked by the sequential presentation of 2 adjacent bars with opposite contrasts.11,26,39 We presented an NC bar followed by an adjacent PC bar in multiple positions along each cell’s RF. On the leading side of T4’s RF, we measured bar pair responses consistent with the cell’s typical directional preference, despite the presence of the dark bar (Figure 4B). However, on the trailing side, the dark-bright bar pair evoked responses with an inverted directional preference (see positions [-1,3] and [1,5]; peak responses summarized in Figures 4C, 4D, and S5A). We measured T5 responses to bright-dark bar pairs and found a similar structure (Figures 4D and S5B). Responses to leading-side stimuli had the same directional preference as to moving PC bars, but responses showed an inverted directional preference for pairs that included trailing-side positions (Figure 4D). This directional preference inversion was largest for wider bars presented for longer durations (Figures 4D, S5A, and S5B). We used the T4- and T5-optimized models (same parameters as in Figure 3) to simulate responses to these NC-PC bar pairs and found they accurately predicted the inversion in directionality preference in both cells, including the specific relationship between the inversion and RF positions (Figure 4E).

Furthermore, we found good agreement between the model’s predictions and T5 responses to a variant of this bar-pair stimulus (Figure S5C).

We next used a simple example to provide intuition for how the RF structure accounts for the reverse-phi inversion. An NC bar (Figure 4F) in the RF center evokes a strong inhibitory response ($I_{on}$). When followed by a PC bar on the trailing side, another strong inhibitory response is evoked ($I_{off}$). Thus, while the bars “move” in the PD, the response is strongly inhibited (Figure 4F, top). Conversely, when the NC bar first appears on the trailing side, it evokes an excitatory response ($E_{on}$) activated at stimulus offset. A subsequent PC bar in the RF center evokes a strong depolarizing response (same $E_{off}$ conductance) due to the stimulus onset. Therefore, although the bars “move” in the ND, the net response is strongly excitatory (Figure 4F, bottom).

**Behaviorally measured perception of reverse-phi stimuli corroborates model predictions**

Since T4 and T5 cells are the major—if not exclusive—source of motion information in the fly’s visual system,40 we hypothesized that reverse-phi stimuli inducing stronger directional preference reversal in T4 and T5 should also evoke stronger reversal in the fly’s behavioral response. Tethered flying flies turn in the direction of a rotating grating pattern, a reaction known as the synchronous optomotor response.41 However, in response to a reverse-phi stimulus (Figure 5A), the flies turn against the pattern’s rotation.41 The RF mapping (Figure 2B,C) showed that longer durations of flashes and wider NC bars evoked stronger responses, while the minimal reverse-phi responses (Figures 4, S5A, and S5B) showed that longer flashes and wider bars, and reduced overlap between bar-pairs, evoked a stronger reversal of the directional preference. To test this conjecture, we presented flies with both standard and reverse-phi gratings comprised of bars with 1 of 4 widths, moving with either step sizes corresponding to the width of the bars in the grating or with single-pixel steps (Figure 5A, schematic examples). The bar-width steps were used to mimic the bar-pair stimuli used during our electrophysiology experiments (Figure 4). When presented with gratings composed of 1-LED-wide bars moving every 40 ms (~47/s), flying flies exhibited a robust behavioral response (Figure 5B, left-most panel, black). However, a reverse-phi stimulus with the same bar width and step duration failed to evoke any response (Figure 5B, red). Increasing the bar width (and the corresponding “bar width” step size) increased the response to standard motion modestly but had a dramatic effect on the response to reverse-phi motion (Figure 5B). Comparing the responses to reverse-phi gratings of different widths reveals a significant increase with increasing width (Figure 5C). To test the prediction that overlap between bar presentations in sequential frames will reduce reverse-phi responses, we compared gratings moving with bar-width steps (no
Figure 4. 4-conductance model explains illusory motion percept
(A) Mean baseline subtracted measured responses (n = 3; individual cells in lighter shades) of T5 cells to dark and bright edges moving in the PD and ND, together with predicted moving edge responses from the same averaged T5-optimized model (with same parameters) as in Figure 3. Inset shows the space-time plot for a local moving edge stimulus.
(B) Baseline subtracted T4 responses (mean ± SEM) to dark-bright bar pairs, a minimal reverse-phi stimulus, presented along the PD-ND axis (n = 7, 9, 8, and 9 cells for panels from left to right). Schematics above (below) traces depict positions for PD (ND) stimuli with respect to the center of the receptive field (extended central column). Faint gray and green rectangles demarcate presentation time (160 ms) of flashed bars.
(C) Boxplot summary of measured response maxima for the time series in (B).
(D) Boxplot summary of measured response maxima differences between PD and ND minimal reverse-phi bar-pair flashes for T4 and T5 cells (dots: mean individual cell responses). Bolded labels: T4 responses for the position pairs presented in (B) and (C).
(E) Predicted response maxima differences between PD and ND bar-pair flashes for the T4 and T5 models (with same parameters) as in Figure 3.
(F) Schematic explaining the inversion in directional preference in response to a minimal reverse-phi stimulus. Spatial filters depicted above stimulus sketches, with bold lines representing the primary active conductance.
T4 and T5 responses to additional reverse-phi bar-pair stimuli are presented in Figures S5A–S5C.
overlap) to the same gratings moving with 1-LED steps (maximal overlap). The no overlap stimuli resulted in significantly increased reverse-phi responses, from no net response to 1-LED step grating to the strongest recorded response for each width (except width 1; Figure 5 C). Model simulations of T5 responses to 1-LED steps or bar-width steps (Figures S5 D and S5E) confirm that non-overlapping bars near the RF center generate a stronger response in the ND (stronger E_{dec} activation) and weaker response in the PD (stronger I_{inc} and I_{dec} activation), in agreement with stronger reverse-phi inversion for the bar-width steps (Figure S5 E, position \(9^\circ\)). Filled boxplots are significantly different from zero. Asterisk below brackets indicate significant difference (2-sided Student’s t-test; *p < 0.001; NS, not significant). Model predictions of T5 responses to both bar-width and single-LED step reverse-phi stimuli are presented in Figures S5 D and S5E.

Next, we tested the response to gratings composed of bars of the same width moving with different step durations (focusing on 40 and 160 ms steps). Increasing step duration (slower speed) resulted in reduced turning responses to standard motion but increased responses to the reverse-phi motion (Figure 5C), in agreement with our predictions. Since changing step size/duration changes rotational speed, and since changing rotational speed affects the optomotor response,\(^{42,43}\) we compare 2-LED-wide bars moving with single-LED steps every 20 ms to bar-width steps every 40 ms (both move at ~94°/s). For standard motion gratings, these stimuli yielded responses of similar magnitude. However, for reverse-phi motion, the change dramatically flipped the response from weak and syn-directional to strong and anti-directional. These behavioral results corroborate our predictions: wider, slower bars drive stronger responses from NC conductances (T4: E_{dec} and I_{dec}; T5: E_{inc} and I_{inc}), which are necessary for inverting the T4 and T5 directional preference. Consequently, slower, wider-bar gratings without overlap evoke a strong illusory motion percept and, therefore, a stronger behavioral inversion.

**DISCUSSION**

In this study, we used whole-cell recordings from the directionally selective ON-preferring T4 cells and the OFF-preferring T5 cells and showed that they can report the direction of motion of wider NC moving bars (Figure 1). We mapped their composite ON-OFF RFs and revealed a structure that is common to both T4 and T5 (Figure 2), suggesting that both cell types receive direct inputs with non-canonical properties (T4 receive OFF signals; T5 receive ON signals). We proposed a unified model architecture to capture this composite RF and showed that this model accurately predicts directionally selective responses to moving bars of either contrast (Figure 3). The model also predicted the inversion in T4’s and T5’s PD in response to minimal reverse-phi motion and the inversion’s dependence on the RF position (Figure 4). Finally, we measured behavioral turning of tethered
flying flies to reverse-phi motion and found strong evidence that the stimuli that evoked stronger NC responses in T4 and T5 also evoked stronger behavioral-turning inversion (Figure 5).

The model presented here (Figure 3) extends our prior model of T4 and T5.32,33 Beyond the specific ON-OFF interactions of the reverse-phi model, does this updated model offer any advantages? We simulated responses to grating patterns using both the model introduced here and our previous T5 model (Gruntman et al.;33 Figure 6). We find that the current model responds to all tested gratings, while the prior model can only respond to movement from dark bars. More importantly, the new model responds with greater directional selectivity to all 3 grating configurations (Figure 6), suggesting that in order to understand responses to this most classical stimulus, it is essential to account for these ON-OFF interactions.

Connecting our data-driven model to motion pathway circuitry

One important goal of systems neuroscience is to link functional measurements and algorithmic models with neural circuit mechanisms. The Drosophila motion-detection circuit, with a fully reconstructed connectome, genetic access, and neuronal functional studies, is an exciting system for making these connections. Nevertheless, it is not straightforward to map specific model conductances to inputs from specific upstream neurons. While both T4 and T5 neurons receive multiple PC-encoding excitatory inputs9,23 and functional studies have shown these neuron types to have different temporal and spatial filters,20,25,27,29,44 we were able to model the PC depolarization in the RF center with a single excitatory conductance. Similarly, we could model a single PC inhibitory conductance, while connectomics studies have revealed at least 3 potential inhibitory inputs on the trailing side of the T4 dendrite.9 It appears that T4 and T5 responses may mask additional complexity not yet uncovered or their upstream inputs could interact to produce simpler downstream effects. Although assigning model conductances to individual cell types may be too simplistic, it is important to combine our modeling approach with the powerful circuit-constrained models pioneered by other groups29,44–47 and attempt to reconcile the above discrepancies. One such future step would be to replace our generic excitatory and inhibitory inputs with specific conductances based on the known characteristics of the upstream neurons that provide inputs to T4 and T5.

What are the sources of the NC-mediated hyperpolarization?

The T4-optimized model places this conductance just to the leading side of the RF center. M9 depolarizes to dark stimuli,25,27 is glutamatergic34 (likely inhibitory48), and could contribute to this conductance. However, the synapses from this cell type are found at the leading edge of T4’s dendrite9 where we measure minimal NC-mediated hyperpolarization (Figure 2). Furthermore, although T5 cells show a similar NC hyperpolarization, no source of NC inhibition has been described among the major T5 inputs (but see Ramos-Traslosheros29 for ON responses in T5 inputs). These discrepancies suggest that the circuit function of M9 remains unresolved. Regardless of its source, the NC inhibitory conductances in our model (Idec in T4 and Ibn in T5) may reflect the functional role of crossover inhibition. Although we are unable to measure excitatory and inhibitory currents directly, it appears that, like in the mammalian retina, this inhibitory input is largest when the excitatory input is smallest.36 These properties have been shown to linearize responses in neurons with rectified inputs and are postulated to reduce the net conductance change, thus stabilizing responses to other inputs.37
Toward a unified model architecture

Prior to the discovery of ON-OFF rectification by medulla neurons,18–20 nearly all models of fly motion detection, including the famous Hassenstein-Reichardt Elementary Motion Detector (HREMD), used non-rectified inputs signaling both luminance increments and decrements.49,50 These classic models could reproduce the reverse-phi illusion, yet their reliance on non-rectified inputs renders them less relevant for explaining the inverted selectivity to reverse-phi motion.

Although our model was not explicitly constructed to model reverse-phi responses, it is still instructive to compare it with models that were. Most of these models employ a 3-armed architecture, corresponding to input from 3 spatial locations, which was (to our knowledge) first suggested by Mo and Koch,51 based on the classic Torre and Poggio52 passive neuron model. To explain reverse-phi responses in cortical neurons, Mo and Koch modified the original model by adding a second inhibitory “veto” mechanism responding to inputs of the opposite contrast polarity. A more recent model for reverse-phi responses in the fly used a different architecture combining two full HREMDs: one for ON and the other for OFF.31 However, since individual T4 and T5 cells already exhibit the reverse-phi directional preference inversion and since one-half HREMD is roughly equivalent to a single cell, this phenomenological model is also of less interest to our discussion.

Recent connectomics, transcriptomics, and functional studies9,25,34,44 have provided strong evidence for the proposal that T4 and T5 neurons receive inputs at approximately 3 spatial locations, and a 3-arm model with inhibitory inputs on both sides of an excitatory central arm was developed based on these findings.45 This model was configured to match major T4 inputs in their contrast responses, neurotransmitters, and connectivity along the dendritic axis. The modeled inhibitory inputs on the axon-proximal side of the dendrite respond to bright stimuli, while modeled inhibitory inputs on the axon-distal side respond to dark stimuli. This was used to propose a mechanism for PD enhancement in the PC motion computation. Although no claims about reverse-phi responses were made in the paper,45 we expect that with appropriate parameters this model should also produce directionally inverted responses to reverse-phi motion stimuli.

To explain reverse-phi responses in T4 and T5 cells, Salazar-Gatzimas et al.26 also proposed a 3-arm model that included NC inputs. A subsequent paper from the same group by Zavatone-Veth et al.16 used the same architecture to develop a more generalizable 3-arm model which they used to explain T4 function, including the reverse-phi inversion. This model also used inhibitory inputs of opposing contrasts flanking an excitatory center. The Zavatone-Veth model captures the higher-level phenomenology of the motion computation with a minimal model, while the objective of our model was to capture the lower-level RF structure with a biophysical model. Encouragingly, despite these disparate objectives the resulting models have several common features. Although the positions of the input conductances in our model were a result of an optimization procedure, both models have converged onto a similar 3-arm inhibition-excitation-inhibition architecture. Our model includes a fourth conductance (NC excitation) that was needed to account for the leading side NC responses of T5 (Figure 2A, left), but this conductance does not appear to play a central role in shaping the response dynamics to moving bars or reverse-phi stimuli (in part because we presented moving stimuli closer to the center). Both models also share 2 different input types. In the Zavatone-Veth model, the inhibitory arms respond for the duration of the stimulus (equivalent to pulse), while the excitatory arm responds to the appearance of the appropriate contrast change (equivalent to delta). In our model, we use pulse inputs to represent the duration of a stimulus and delta inputs to represent the instantaneous change in contrast at the disappearance of an opposite contrast stimulus, with both inhibitory and excitatory conductances receiving these two input types.

However, an important difference between the models is that our model is subject to the strong constraints imposed by reproducing the high temporal and spatial resolution whole-cell electrophysiological measurements. In contrast to the models that are tuned to produce higher-level response properties, our model is only trained to reproduce the static RF of T4 and T5 neurons. Therefore, we demonstrated that the spatiotemporal structure of the static RF is sufficient to explain T4 and T5 responses to all classes of tested, more complex, dynamic stimuli.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.09.072.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.G., S.R., and M.B.R.; methodology, E.G., P.R., and S.R.; software, E.G., P.R., and S.R.; formal analysis, E.G., P.R., and S.R.; investigation, E.G.; writing – original draft, E.G., P.R., S.R., and M.B.R.; writing – review & editing, E.G., S.R., and M.B.R.; visualization, E.G.; supervision, S.R. and M.R.; funding acquisition, S.R. and M.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Experimental models: Organisms/strains | | |
| *D. melanogaster*. T4: VT015785-p65ADZp (attP40); R42F06-ZpGdbd (attP2) | | |
| *D. melanogaster*. T5: VT055812-AD (attP40); R47H05-DBD(attP2) | | |
| *D. melanogaster*. GFP: pJFRC28-10XUAS-IVS-GFP-p10 | | |
| *D. melanogaster*. Empty Split: w^{1118}, pBPP65ADZp (attP40);pBPZpGAL4DBD (attP2) | | |
| *D. melanogaster*. Kir2.1: w (+DL);+(DL); pJFRC49-10XUAS-IVS-eGFP- Kir2.1(attP2) | | |

Software and algorithms

| MATLAB 2018b | MathWorks | https://www.mathworks.com/ |
| μManager V1.4 |  | https://micro-manager.org/ |
| Deposited data | figShare | figShare: https://doi.org/10.25378/janelia.c.5629663.v1 |
| Additional analysis code | gitHub | gitHub: https://github.com/gruyal/MatlabFunctions |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Michael B. Reiser (reiserm@janelia.hhmi.org).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Whole cell recording data, analysis code, and modeling optimization results have been deposited at Figshare (https://doi.org/10.25378/janelia.c.5629663).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Electrophysiology experiments were performed on 1-2 day old female *Drosophila melanogaster*; behavioral experiments were performed on 2-5 day old females. Flies were reared under 16:8 light:dark cycle at 24°C to mimic a long summer day and increase the duration of the pre-dusk period of higher activity. For T5 targeted recordings we used flies with the following genotype: pJFRC28-10XUAS-IVS-GFP-p10 in attP2 crossed to stable split-GAL4 SS25175 (w; VT055812-AD(attP40); R47H05-DBD(attP2)). For T4 we used pJFRC28-10XUAS-IVS-GFP-p10 in attP2 crossed to stable split-GAL4 SS02344 (VT015785-p65ADZp (attP40); R42F06-ZpGdbd (attP2)). Both genotypes were generously provided by Aljoscha Nern in Gerry Rubin’s lab. For behavioral experiments we used w^{1118}, pBPp65ADZp (attP40);pBPZpGAL4DBD (attP2) X w (+ DL);+(DL); pJFRC49-10XUAS-IVS-eGFP- Kir2.1(attP2), empty split GAL4 crossed to Kir2.1 flies. This line is commonly used as a control line in our lab, since the flies are relatively vigorous fliers.

METHOD DETAILS

Electrophysiology

T5 recording are from cells that were included in our previous paper (n = 17 cells), and were performed as published. T4 recordings are from a newly acquired dataset (n = 15 cells). The experimental methods are similar to our prior manuscript and will be briefly...
summarized below. Flies were anesthetized on ice and transferred to a chilled vacuum holder where they were mounted, with the head tilted down, to a customized platform machined from PEEK using UV-cured glue (Loctite 3972). To reduce brain motion, the proboscis was fixed to the head with a small amount of the same glue. The posterior part of the cuticle was removed using syringe needles and fine forceps. The perineural sheath was peeled using fine forceps and, if needed, further removed with a suction pipette under the microscope. To further reduce brain motion, muscle 16 was removed from between the antennae.

The brain was continuously perfused with an extracellular saline containing (in mM): 103 NaCl, 3 KCl, 1.5 CaCl2 2H2O, 4 MgCl2 6H2O, 1 NaH2PO4 H2O, 26 NaHCO3, 5 N-Tris (hydroxymethyl) methyl-2-aminoethane-sulfonic acid, 10 Glucose, and 10 Trehalose. Osmolarity was adjusted to 275 mOsm, and saline was bubbled with 95% O2 / 5% CO2 during the experiment to reach a final pH of 7.3. Pressure-polished patch-clamp electrodes were pulled for a resistance of 9.5-10.5 MΩ and filled with an intracellular saline containing (in mM): 140 KAsp, 10 HEPES, 0.1 CaCl2, 4 MgATP, 0.5 NaGTP, and 5 Glutathione. Pressure-polished patch-clamp electrodes were pulled for a resistance of 9.5-10.5 MΩ and filled with an intracellular saline containing (in mM): 140 KAsp, 10 HEPES, 0.1 CaCl2, 4 MgATP, 0.5 NaGTP, and 5 Glutathione. Pressure-polished patch-clamp electrodes were pulled for a resistance of 9.5-10.5 MΩ and filled with an intracellular saline containing (in mM): 140 KAsp, 10 HEPES, 0.1 CaCl2, 4 MgATP, 0.5 NaGTP, and 5 Glutathione. Pressure-polished patch-clamp electrodes were pulled for a resistance of 9.5-10.5 MΩ and filled with an intracellular saline containing (in mM): 140 KAsp, 10 HEPES, 0.1 CaCl2, 4 MgATP, 0.5 NaGTP, and 5 Glutathione.

The mounted, dissected flies were positioned on a rigid platform mounted on an air table. Recordings were obtained from labeled cell bodies under visual control using a Sutter SOM microscope with a 60X water-immersion objective. To visualize the GFP labeled cells, a monochrome, IR-sensitive CCD camera (ThorLabs 1500M-GE) was mounted to the microscope, an 850 nm LED provided oblique illumination (ThorLabs M850F2), and a 460 nm LED provided GFP excitation (Sutter TLED source). Images were acquired using Micro-Manager, to allow for automatic contrast adjustment.

All recordings were obtained from the left side of the brain. Current clamp recordings were sampled at 20kHz and low-pass filtered at 10kHz using Axon multiClamp 700B amplifier (National Instrument PCle-7842R LX50 Multifunction RIO board) using custom LabView (2013 v.13.0.1f2; National Instruments) and MATLAB (Mathworks, Inc.) software. Shortly after breaking in, recordings were stabilized with a small injection of a hyperpolarizing current (0-3pA) setting the membrane potential to a range between ~60 to ~55mV (uncorrected for liquid junction potential). Occasionally, the injected current required adjustments, but these were done prior to the acquisition of the single bar flash data. To verify recording quality, current step injections were performed at the beginning of the experiment.

Current injection experiments

For the experiment presented in Figure S2 the current step injections described above were preceded with a hyperpolarizing current of different duration. This procedure was performed prior to the presentation of visual stimulation. The magnitude of the hyperpolarizing and the following depolarizing injection were adjusted manually to evoke a similar membrane voltage response between cells (~10 mV). Hyperpolarization current injections ranged between ~1 to ~2 pA, while depolarizing current injections varied between 0.5 and 2 pA.

Visual stimuli (electrophysiology)

The visual display for the electrophysiological experiments was the same setup used and described in our previous paper. Details are briefly summarized here. The display was constructed from an updated version of the LED panels previously described. The arena covered slightly more than one half of a cylinder (216° in azimuth and ~72° in elevation) of the fly’s visual field, with the diameter of each pixel subtending an angle of (at most) 2.25° on the fly eye. Green LEDs (emission peak: 565 nm) were used, bright and dark stimuli were presented on an intermediate intensity background of ~31 cd/m². The visual display for the behavioral experiments was constructed from an updated (faster, higher resolution) version of LED panel system (https://reisertlab.github.io/Modular-LED-Display/docs/G4-index.html). Each panel houses a 16 x 16 grid of ~570 nm LEDs, and are assembled into a ‘cylindrical’ 9 column, 4 row arena, that covers ~270° in azimuth and ~94° in elevation. Each pixel subtends a disc with a diameter of at most 1.875° on the eye of the fly.

Visual stimuli were generated using custom written MATLAB code that allowed rapid generation of stimuli based on individual cell responses. In contrast to the published stimulus control system, we have now implemented an FPGA-based panel display controller, using the same PCIe card (National Instrument PCle-7842R LX50 Multifunction RIO board) that also acquired the electrophysiology data. This new control system (implemented in LabView) streams pattern data directly from PC file storage, allowing for on-line stimulus generation.

To map the receptive field (RF) center of each recorded cell, three grids of flashing preferred contrast (dark for T5, bright for T4) squares were presented at increasing spatial resolution. Each flash stimulus was presented for 200ms. First, a 6 x 7 grid of non-overlapping 5 x 5 LEDs (~11° x 11°) preferred contrast squares was presented (Figure 1A). If a response was detected, a denser 3 x 3 grid with 50%-overlapping 5 x 5 LEDs (~11° x 11°) preferred and non-preferred contrast squares (to verify cell polarity) was presented at the estimated position of the RF center. If a recorded cell was consistently responsive to the first two mapping stimuli, a third protocol was presented to identify the RF center. A 5 x 5 grid of 3 x 3 LED squares (~7° x 7°) of the preferred contrast separated by 1 pixel-shifts was presented at the estimated center of the second grid stimulus. The location of the peak response to this stimulus was used as the RF center in subsequent experiments. Once the RF center was identified, a moving bar stimulus was presented in 8 directions with 80ms step duration (equivalent to ~28°/s). The bar was 9 pixels in height and 1, 2, or 4 pixels in width. When moving in the cardinal directions, the motion spanned 9 pixels. In the diagonal directions bar motion included more steps to cover the same distance (13 steps versus 9 steps). Once the preferred direction had been estimated, bright and dark bar flashes were presented on the relevant axis for widths 1, 2, and 4. To verify full coverage of RF, this stimulus was presented over an area larger than the original motion window (at least 13 positions; results in Figure 2). Following this procedure, cells were presented with additional stimuli using the same PD-ND axis and RF center as reference frames. All stimuli were presented in a pseudorandom order.
within stimulus blocks. All stimuli were presented 3 times, except for single bar flashes that were repeated 5 times. The inter-stimulus interval was 500ms for moving stimuli and 800ms for single bar flashes (to minimize the effect of ongoing inhibition on the responses to subsequent stimuli).

Other presented stimuli were:

1) **Moving bar.** After identifying the PD-ND axis, bright and dark moving bar stimuli were presented along this axis using either 40ms or 160ms steps (equivalent to 66 /sec or 14 /sec respectively). Bar height was the same as for the mapping stimuli (9 LEDs) and width was either 1, 2 or 4 LEDs (corresponding to 2.25\(^\circ\), 4.5\(^\circ\) or 9\(^\circ\)). Results are presented in Figures 1, 3, S3, and S4. The moving bar stimuli presented to T4 and T5 cells were not identical. T5 cells were presented with a bar moving into and out of a presentation “window.” Meaning, a 4-LED wide bar would first appear as a 1-LED bar and would only achieve its full width once the trailing edge crossed into the stimulus window. For T4 cells, the bar’s leading edge traversed the same distance but the stimuli appeared and disappeared as full width bars.

2) **Moving edges.** Moving edge stimuli were presented in the same stimulus windows as the moving bars (and spanned the same number of steps), and with the same two values of step durations. After the edge has passed through the entire stimulus window, it disappeared, and the entire window reverted to the background levels. Results in Figure 4A.

3) **Minimal Reverse Phi.** Bar pairs were presented such that the first bar was of the non-preferred contrast followed by a bar of the preferred contrast (bright-dark for T5; dark-bright for T4). For T5 cells, stimuli were presented in 2 different configurations. Either bars were of width 2 and delayed between the first and the second bar was adjusted to maintain a fixed speed (i.e., correcting the temporal delay to account for the spatial difference in positions), or bars were of width 4 and the second bar was presented directly after the first, regardless of positional difference. For T4 cells, only the second configuration was used since it elicited a stronger response. Results in Figures 4, S5A, and S5B. Responses presented in Figure S5A,B: stimuli with overlapping positions were 4-LED wide bars displayed with a 2-LED spatial overlap (bar center positions indicated along x-axis). Essentially, non-overlapping 4-LED wide bar pairs spanned 8 LEDs, while overlapping bar pairs spanned 6 LEDs, with the 2 middle LEDs inverting from non-preferred contrast to preferred contrast. In Figure SSC, the stimulus presented was a slight modification for the one described for T5, with the first bar persistently displayed until it disappeared together with the second.

**Behavioral experiments**

Flies were reared on standard cornmeal molasses medium on a 16:8 hours light:dark cycle and were tested 0-4 hours before their subjective night to increase activity levels. All experiments were conducted on female flies from a empty split crossed to Kir2.1 flies, a control line used in the lab. Flies were cold anesthetized and tethered to a tungsten wire with UV-cured glue. Flies were given at least 30 min to recover while holding a small piece of paper, to discourage tethered flight. For these experiments the flies were placed in a center of a different visual arena spanning 270\(^\circ\) in azimuth and ~120\(^\circ\) in elevation. The arena consisted of 192X64 LED array with the diameter of individual LED subtending ~1.875\(^\circ\). The stimulus control system was the same as for the electrophysiology. The fly’s wings were illuminated from above by an IR LED (ThorLabs M850F2) and their position was monitored by an optical wingbeat analyzer (JFI Electronics Laboratory, University of Chicago, Chicago, IL, USA). Data acquisition was performed with the same control system as for electrophysiology, but with a 1KHz sampling rate.

**Visual stimuli (behavior)**

Rotating grating (standard or reverse phi) stimuli were presented in 1.7 s open-loop trials interleaved with 3.6 s “stripe fixation” closed-loop trials, during which the fly actively controlled the position of a 30\(^\circ\) dark bar. These closed-loop trials were used to keep the fly flying and engaged in the task. Each open-loop stimulus was presented 4 times. Trials in which the fly stopped flying were excluded from the analysis. Stimuli presented in the open-loop condition were full field rotation gratings with a fixed 30\(^\circ\) cycle. To present different bar widths (1.875\(^\circ\), 3.75\(^\circ\), 5.625\(^\circ\), and 9.375\(^\circ\)) the duty cycle for each grating stimulus was changed. For example, since a 30\(^\circ\) cycle is comprised of 16 LEDs, to generate a 1.857\(^\circ\) bar, a single LED was turned dark and the rest (15 LEDs) were kept at background level. To match the electrophysiological stimuli, gratings were moved at 3 different step durations: 20, 40, and 160 ms (corresponding to each moving with 3 angular velocities: 30 /sec, 60 /sec, or 120 /sec) and 2 directions. Stimuli were presented either in the standard form, with a grating of bright over gray; or in the reverse phi form, with a bright (dark) over gray inverting to dark (bright) over gray with each motion step (see Figure 5A). Note that the effective angular size in the behavioral arena is different than our electrophysiological setup, therefore the bar widths used in the behavioral experiments subtend slightly different visual angles.

**T4/T5 neuron model**

We modeled the membrane potential responses of T4 and T5 neurons with a single-compartment conductance-based neuron model\(^{32,33}\) whose dynamics are described by

\[
\tau_m \frac{d V}{dt} = - (V - V_L) - (E_{inc} + E_{dec})(V - V_E) - (I_{inc} + I_{dec})(V - V_I).
\]
The model has four conductances: One pair of excitatory (\(E_{\text{exc}}\)) and inhibitory (\(I_{\text{inc}}\)) conductances responding to luminance increments, and a second pair of conductances, \(E_{\text{dec}}\) and \(I_{\text{dec}}\), for luminance decrements. All the conductances are measured in units of leak conductance. The reversal potential for the leak, excitation, and inhibition are denoted by \(V_L\), \(V_E\), and \(V_I\), respectively.

We examined the model dynamics in the limit of small neuronal integration time \(\tau_m\).\(^{32,33}\) With this approximation, the dynamics of Equation 1 become

\[
\frac{V - V_L}{V_E - V_L} = \frac{E_{\text{tot}} - \alpha I_{\text{tot}}}{1 + E_{\text{tot}} + I_{\text{tot}}},
\]

where \(E_{\text{tot}} = E_{\text{exc}} + E_{\text{dec}}\) and \(I_{\text{tot}} = I_{\text{inc}} + I_{\text{dec}}\) are the total excitatory and inhibitory conductances respectively, and \(\alpha = \frac{V_L - V_E}{V_E - V_L}\).

Each of the four conductances is the sum of local contributions from receptive field locations along the PD-ND axis (relative to the location corresponding to the empirically measured center of the receptive field of a neuron, \(x = 0\)). Spatial locations \(x\) are discretized on a uniform grid with a spacing corresponding to the smallest width of flashed bars used in the experiment. Similarly, for the remaining conductances:

\[
E_{\text{dec}}(t) = \sum_j A_{E_{\text{dec}}} e^{\frac{(x-x_{E_{\text{dec}}})^2}{2\sigma_{E_{\text{dec}}}}^2} f_{E_{\text{dec}}}(t,x),
\]

\[
I_{\text{dec}}(t) = \sum_j A_{I_{\text{dec}}} e^{\frac{(x-x_{I_{\text{dec}}})^2}{2\sigma_{I_{\text{dec}}}}^2} f_{I_{\text{dec}}}(t,x).
\]

The time course of the local contribution to the conductances, \(f_j(x,t)\), is the output of two linear temporal filters in series, with time constants \(\tau_{\text{rise}}\) and \(\tau_{\text{decay}}\).

\[
\tau_{\text{decay}} \frac{d}{dt} f_j(x,t) = -f_j(x,t) + h_j(x,t)
\]

\[
\tau_{\text{rise}} \frac{d}{dt} h_j(x,t) = -h_j(x,t) + k_j(x,t),
\]

where \(k_j(x,t)\) is a function of the visual input that depends on the stimulus type (i.e., dark or bright).

**Input dynamics**

Here we describe the model of visual input processing upstream of the T4 and T5 model neurons. For bright stimuli, the input to the temporal filters for the light increment conductances is simply

\[
k_{E_{\text{exc}}}(x,t) = k_{E_{\text{exc}}}(x,t) = I_{\text{bright}}(x,t).
\]

The external input \(I_{\text{bright}}(x,t)\) assumes a value of 0 or 1 depending on whether a bright stimulus is absent or present at location \(x\) and time \(t\).

For light decrement conductances, the input is a filtered delta pulse centered at the time of bright stimulus offset \(t_{\text{off}}\):

\[
\frac{d}{dt} k_{E_{\text{exc}}}(x,t) = -k_{E_{\text{exc}}}(x,t) + C_{E_{\text{exc}}}(x) \delta(t - t_{\text{off}})
\]

\[
\frac{d}{dt} k_{I_{\text{dec}}}(x,t) = -k_{I_{\text{dec}}}(x,t) + C_{I_{\text{dec}}}(x) \delta(t - t_{\text{off}}),
\]

where \(\tau_{E_{\text{exc}}}\) and \(\tau_{I_{\text{dec}}}\) are the time constants of the filters. The amplitudes of the delta, \(C_{E_{\text{exc}}}(x)\) and \(C_{I_{\text{dec}}}(x)\), are functions of the bright stimulus duration at location \(x\) \((d_{\text{bright}}(x))\). The experimental data suggests that these are nonlinear functions. In the experiments,
however, we do not have a sufficiently dense sampling of possible stimulus durations to completely characterize this nonlinearity. Hence, we chose a simple rectified-linear function (ReLU):

\[
C_{E_{inc}}(x) = ReLU(m_{E_{inc}}d_{E_{inc}}(x) + b_{E_{inc}})
\]

(8)

\[
C_{I_{inc}}(x) = ReLU(m_{I_{inc}}d_{I_{inc}}(x) + b_{I_{inc}})
\]

(9)

with parameters \(m, b\).

For dark stimuli, light decrement conductances are directly driven by the presence of the dark stimulus \(E_{dark}(t, x)\), while light increment conductances receive a filtered delta pulse at the time of the dark stimulus offset \(t_{\text{off}}\):

\[
k_{E_{inc}}(t, x) = k_{I_{inc}}(t, x) = I_{dark}(t, x)
\]

(10)

\[
\frac{d}{dt}k_{E_{inc}}(t, x) = \frac{k_{E_{inc}}(t, x)}{\tau_{D_{E_{inc}}}} + C_{E_{inc}}(x)\delta(t - t_{\text{off}})
\]

(11)

\[
\frac{d}{dt}k_{I_{inc}}(t, x) = \frac{k_{I_{inc}}(t, x)}{\tau_{I_{inc}}} + C_{I_{inc}}(x)\delta(t - t_{\text{off}})
\]

Model optimization

We present two version of the model: a version we have named the ‘full model’ that contains independent parameters for each conductance and was optimized using both single bar flashes and moving bars (for reasons that will be discussed below), and a version named the ‘reduced model’ in which \(\tau_D\) is the same for all the conductances, \(\tau_{rise}\) is fixed at 1 for the excitatory conductances, and the model was optimized using only single bar flashes (see tables below). Early iterations of our optimization procedure have shown these were reasonable constraints that incurred only a modest price at the loss function (Compare full and reduced models in Figure S3). The full model is presented as an illustration of our modeling approach. First, we aimed to capture the receptive field structure qualitatively as well as we could, and then we systematically removed components until the reduced version we present was achieved. Since the reduced model could be optimized using solely responses to non-moving stimuli, we consider it more useful and focus on it in the main text.

In the reduced model (Figures 3 and S3E–S3H), parameters are randomly initialized within the bounds listed in the first table below and optimized to minimize the mean squared error (MSE) between simulated and measured voltage responses to PC and NC single bar flash stimuli of width [2,4] and duration [40ms, 160ms]. In the full model (Figure S3A–D), parameter bounds are given in second table below, and this model was optimized with PC and NC stimuli for both single bar flashes and moving bar stimuli of width [2,4], duration [40ms, 160 ms]. Moving bars were added to the optimization procedure in an attempt to increase the density of duration sampling for Equation 8. For a single position in the receptive field, bars of different widths moving at the same speed are equivalent to flashes of different durations (e.g., width 2 bar moving at 40 ms steps is equivalent to an 80 ms flash).

\[
\text{loss} = \sum_{\text{stim}} \sum_{\text{dur}} \sum_{\text{width}} \sum_{\text{contrast}} \text{MSE} \left( \text{Flash Response}_{\text{dur}, \text{width}, \text{contrast}} \right)
\]

(10)

where each stimulus condition was given equal weight in the cost function.

The full model (Figure S3A–D) parameter bounds are given in second table below, and this model was optimized with PC and NC stimuli for both single bar flashes and moving bar stimuli of width [2,4], duration [40ms, 160 ms]. Moving bars were added to the optimization procedure in an attempt to increase the density of duration sampling for Equation 8. For a single position in the receptive field, bars of different widths moving at the same speed are equivalent to flashes of different durations (e.g., width 2 bar moving at 40 ms steps is equivalent to an 80 ms flash).

\[
\text{loss} = \sum_{\text{stim}} \sum_{\text{dur}} \sum_{\text{width}} \sum_{\text{contrast}} \text{MSE} \left( \text{Response}_{\text{stim}, \text{dur}, \text{width}, \text{contrast}} \right)
\]

(11)

All simulated responses were delayed by 30 ms to match the measured transmission latencies from the omatidia to T4/T5 neurons.

The optimization for both models was performed with the MATLAB function \texttt{fmincon()} and the default interior-point algorithm. In Figures 3, 4, 6, S3, and S5 we report the simulated responses from these optimization procedures. The parameters used in those simulations have been chosen among the top 1% optimization solutions based on the losses described above. Within these solutions, we selected the parameter sets resulting in the best match (smallest MSE) with moving bar stimuli not used in the loss functions for the reduced model.

2-conductance T5 model grating simulations

Model parameters for the simulation of Figure 6 were taken from our previous study\(^\text{23}\) (Example cell 2 in Figure 5D) and are available online at https://doi.org/10.25378/janelia.c.4771805.v1 (Figure 4 data and code modelFiles/309/result_cell_2). The parameters were used in the current 4-conductance model with the two increment conductances (\(E_{inc}\) and \(I_{inc}\) zeroed.
Analysis in this paper followed similar procedure as in our previous papers \cite{32,33} with certain necessary modification. All data analysis was performed in MATLAB using custom written code. Since the cells’ baseline was typically stable, we included only trials in which the mean pre-stimulus baseline did not differ from the overall pre-stimulus mean for that group of stimuli by more than 10 mV. We also verified that the pre-stimulus mean and overall mean for that trial did not differ by more than 15 mV (or 25 mV for slow moving bars, due to their strong responses). Responses were later aligned to the appearance of the bar stimulus and averaged (or the appearance of the bar in the central position in case of the 8-orientation moving bar).

**Determining PD**

After presenting the cell with 1- and 2-LED-wide bars moving in 8 different direction at 80 ms per LED step (speed that was optimal for determining directional selectivity for T4 cells \cite{32}), the preferred direction for the cell was determined by a visual estimate of the responses to determine the middle of the relatively wide range of large responding directions. The preferred direction was determined using only preferred contrast stimuli. Due to the structure of our display system (with LEDs organized in a rectangular grid), motion along diagonal directions included more steps when compared to motion along cardinal directions that span the same distance (13 rows of LEDs compared to 9). Accordingly, the responses are slightly different, and we therefore, separated the cells into groups of either cardinal-aligned PD-ND axis, or diagonally-align PD-ND axis (see Figure S1). When presenting average responses to moving stimuli, we present results from the diagonally-aligned T4 and T5 cells in Figure 1, and the cardinal-aligned cells in Figure 3. Model predictions presented in the main figures are always from T4- and T5-fits that were optimized to the cardinal-aligned cells. Figure S3 shows predications from models that were optimized to each one of the four groups independently.

**Single position flash response – depolarization**

Responses were defined as the 0.999 quantile (a robust estimate of the max) of the response during the ‘response window’ (defined below). If the response magnitude did not exceed 2.5 standard deviations of the pre-stimulus baseline (during a 200 ms window preceding the stimulus), the response was defined as zero. For 2- and 4-LED-wide bars the threshold was 2.7 and 2.9 standard deviations, since the responses were stronger. Due to the difference in dynamics for preferred and non-preferred contrast responses, the response window was defined as a function of both stimulus duration and stimulus contrast (200 ms for PC and 375 ms for NC + flash duration). Used in Figures 2B–2E. In Figure 2B, only positions that had 4 or more cells responding (with a missing response either being not recorded or not detected by the above criteria) have their averages displayed.

**Single position flash response – hyperpolarization**

same as above only the response window time was defined differently. For PC stimuli, the window was 800 ms + flash duration (since the hyperpolarization appears after the depolarization). For NC stimuli, the same window as the depolarization was used, since NC stimuli induced onset hyperpolarization. In addition, lower standard deviation thresholds were used (1.5, 1.7, and 1.9), due to lower magnitude of hyperpolarization. Used in Figures 2B–2E. In Figure 2B, only positions that had 4 or more cells responding (with a missing response either being not recorded or not detected by the above criteria) have their averages displayed.

**Rise start time**

Only presentations in which an average response was detected as depolarizing were used for this calculation. Start time was defined as the time from stimulus presentation (after correcting for arena delay) to 10% of the of the value of the maximal response for that position. Used in Figure 2D

**Decay time**

Same criteria was used as for rise time calculation. Decay time was defined as the temporal difference between reaching 80% and then 20% of the maximal response (after the response peak). Used in Figure 2E.

**Analysis (Behavior)**

All data analysis was performed in MATLAB using custom written code. Responses were quantified as left minus right wing-beat amplitude, a measure which has been shown to be proportional to yaw torque \cite{60}. In the beginning of each experiment, flies were presented with bi-directional rotational gratings to verify acceptable symmetry between left and right turns. Responses to both directions of the same stimulus condition were combined into an average after inverting the sign of responses to counterclockwise moving stimuli (standard and reverse phi). For each fly responses were normalized to the 0.95 quantile of the strongest single stimulus response. Mean responses were calculated by taking the average left minus right (turning) value within the 1-1.5 s response window. Used in Figure 5

**Statistics**

To determine whether mean responses in Figure 1D were (statistically) significantly different from zero, response groups were first tested for normality using the Lilliefors test, and then tested for a significant difference from zero using the one-sided Student’s t test. To test for differences between responses to long flashes and their equivalent linear sums in Figure 2G, a paired two-sided Student’s t test was performed. Finally, to test for the significance of behavioral responses in Figure 5C, we first test for normality using the Lilliefors test, and then performed a two-sided t test to determine whether responses are different from zero (corrected for multiple comparisons by controlling for the false discovery rate with q = 0.01). Paired two-sided t tests were used in all other pairwise comparisons in the figure. No statistical methods were used to pre-determine sample sizes, however our sample sizes are similar.
Data collection and analysis could not be performed blind to the conditions of the experiments.

**Data plotting conventions**

All boxplots presented were plotted with these conventions: box represents upper and lower quartile range, line represents median, whiskers were omitted, and in certain cases (that are clear from context) individual data points are overlaid on the box.

**Reduced model parameter bounds**

| Parameters | \( E_{\text{inc}} \) | \( I_{\text{inc}} \) | \( E_{\text{dec}} \) | \( I_{\text{dec}} \) | units |
|------------|-----------------|-----------------|-----------------|-----------------|-------|
| \( A \)    | \([0,10]\)      | \([0,10]\)      | \([0,10]\)      | \([0,10]\)      | unitless |
| \( \mu \)  | \([-7,7]\)      | \([-7,7]\)      | \([-7,7]\)      | \([-7,7]\)      | R.F. position |
| \( \sigma \)| \([0.1,5]\)     | \([0.1,5]\)     | \([0.1,5]\)     | \([0.1,5]\)     | R.F. position |
| \( \tau_{\text{rise}} \)| 1 | 1 | \([1,600]\) | \([1,600]\) | ms |
| \( \tau_{\text{decay}} \)| \([1,600]\) | \([1,600]\) | \(\tau_{\text{decay,Enc}}\) | \([1,600]\) | ms |
| \( M \)    | \([0.5]\)       | \([0.5]\)       | \([0.5]\)       | \([0.5]\)       | 1/ms |
| \( b \)    | \([-10,10]\)    | \([-10,10]\)    | \([-10,10]\)    | \([-10,10]\)    | unitless |
| \( \tau_D \)| \([1,600]\) | \(\tau_D,Enc\) | \(\tau_D,Enc\) | \(\tau_D,Enc\) | ms |

**Full model parameter bounds**

| parameters | \( E_{\text{inc}} \) | \( I_{\text{inc}} \) | \( E_{\text{dec}} \) | \( I_{\text{dec}} \) | units |
|------------|-----------------|-----------------|-----------------|-----------------|-------|
| \( A \)    | \([0,10]\)      | \([0,10]\)      | \([0,10]\)      | \([0,10]\)      | unitless |
| \( \mu \)  | \([-7,7]\)      | \([-7,7]\)      | \([-7,7]\)      | \([-7,7]\)      | R.F. position |
| \( \sigma \)| \([0.1,5]\) | \([0.1,5]\) | \([0.1,5]\) | \([0.1,5]\) | R.F. position |
| \( \tau_{\text{rise}} \)| \([1,600]\) | \([1,600]\) | \([1,600]\) | \([1,600]\) | ms |
| \( \tau_{\text{decay}} \)| \([1,600]\) | \([1,600]\) | \([1,600]\) | \([1,600]\) | ms |
| \( m \)    | \([0.5]\)       | \([0.5]\)       | \([0.5]\)       | \([0.5]\)       | 1/ms |
| \( b \)    | \([-10,10]\)    | \([-10,10]\)    | \([-10,10]\)    | \([-10,10]\)    | unitless |
| \( \tau_D \)| \([1,600]\) | \([1,600]\) | \([1,600]\) | \([1,600]\) | ms |