Bimodal Optomotor Response to Plaids in Blowflies: Mechanisms of Component Selectivity and Evidence for Pattern Selectivity

Aman B. Saleem,^{1,2*} Kit D. Longden,^{1*} Daniel A. Schwyn,¹ Holger G. Krapp,¹ and Simon R. Schultz¹

¹Department of Bioengineering, Imperial College London, London, SW7 2AZ, United Kingdom, and ²Institute of Ophthalmology, University College London, London, EC1V 9EL, United Kingdom

Many animals estimate their self-motion and the movement of external objects by exploiting panoramic patterns of visual motion. To probe how visual systems process compound motion patterns, superimposed visual gratings moving in different directions, plaid stimuli, have been successfully used in vertebrates. Surprisingly, nothing is known about how visually guided insects process plaids. Here, we explored in the blowfly how the well characterized yaw optomotor reflex and the activity of identified visual interneurons depend on plaid stimuli. We show that contrary to previous expectations, the yaw optomotor reflex shows a bimodal directional tuning for certain plaid stimuli. To understand the neural correlates of this behavior, we recorded the responses of a visual interneuron supporting the reflex, the H1 cell, which was also bimodally tuned to the plaid direction. Using a computational model, we identified the essential neural processing steps required to capture the observed response properties. These processing steps have functional parallels with mechanisms found in the primate visual system, despite different biophysical implementations. By characterizing other visual neurons supporting visually guided behaviors, we found responses that ranged from being bimodally tuned to the stimulus direction (component-selective), to responses that appear to be tuned to the direction of the global pattern (pattern-selective). Our results extend the current understanding of neural mechanisms of motion processing in insects, and indicate that the fly employs a wider range of behavioral responses to multiple motion cues than previously reported.

Introduction

Moving creates patterns of visual motion across our eyes that provide valuable information for controlling our balance, motor actions and gaze. Exploiting these patterns for behavioral control requires the nervous system to detect the direction of visual motion. The neurophysiological mechanisms of motion perception have been studied in a number of systems, including insects and primates, leading to an understanding of some common principles of local motion detection (Hassenstein and Reichardt, 1956; Adelson and Bergen, 1985). A fruitful approach has been to take a pair of drifting sinusoidal gratings and combine them additively to produce a plaid stimulus (Fig. 1*a*). In a breakthrough study for the identification of the neural correlates of perception (Newsome et al., 1989), the behavioral measure of plaid perception in primates correlated with the activity of a subset of neurons within visual cortical area V5/MT. Plaid stimuli have been used since to

make significant progress in accounting for the properties of cells involved in primate motion perception (Simoncelli and Heeger, 1998; Rust et al., 2006).

At the same time, our understanding of visual motion processing benefits from studies of model systems in which individually identifiable cells contribute to visually guided behavior (Hartline et al., 1956; Bialek et al., 1991; Gabbiani et al., 2002). The blowfly has well described flight and gaze stabilization reflexes, both of which are heavily supported by a set of optic flow-processing interneurons, the lobula plate tangential cells (LPTCs), that have been extensively studied (Hausen, 1993; Borst and Haag, 2002; Krapp and Wickelmaier, 2008). LPTCs integrate the outputs of multiple local, direction-selective elements, known as elementary motion detectors (EMDs), to form extended motion-sensitive receptive fields (Hassenstein and Reichardt, 1956; Egelhaaf and Borst, 1993; Krapp and Hengstenberg, 1996). The computations performed by EMDs are well understood mathematically, and the compound eye geometry places simplifying constraints on the spatial organization of their visual inputs (Petrovitz et al., 2000; Sanes and Zipursky, 2010).

We have investigated for the first time how a visually guided insect processes plaids. Previous studies on the integration of two-dimensional pattern motion in LPTCs have assumed that a cell's output is well described by the inner product between the stimulus direction and the cell's preferred direction (Eriksson, 1984; Reichardt and Schlögl, 1988; Borst et al., 1993), exactly as in the pattern cell model of mammalian visual motion studies (Eq. 1, Materials and Methods; Movshon et al., 1985). This assumption predicts that LPTCs and the behaviors that they control

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*A.B.S. and K.D.L. contributed equally to this study.

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Correspondence should be addressed to Kit D. Longden or Aman B. Saleem, Department of Bioengineering, Imperial College London, London, SW7 2AZ, UK. E-mail: kit@imperial.ac.uk or aman.saleem@ucl.ac.uk.

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Figure 1. Bimodal optomotor response to plaid stimulus. *a*, A plaid is formed from two half-contrast gratings, to form a full contrast pattern. Gray arrows indicate direction of motion of gratings, black arrow indicates direction of plaid motion. θ_p denotes the plaid separation angle. *b*, Diagram of apparatus. Positive and negative torque directions for the torque compensator (TC) are shown. The monitor is centered at azimuth angle, Φ , of 30°. *c*, Temporal frequency (F) of plaid stimulus, and example torque compensator trace. *d*, Responses to a single grating (gray), 150° plaid (black), and predicted pattern cell response (dashed). Mean $\pm$ SE shown ($N = 5$). Gray arrows indicate the angles at which the plaid components are moving horizontally, 105° and 255°.

should have a unimodal tuning to plaids (Fig. 1*d*). We show that, in fact, the blowfly optomotor reflex can have a bimodal tuning to plaids, as can the activity of a supporting LPTC, the H1 cell. To explain these results, we show it is necessary to model properties of EMDs whose functional consequences have not previously been fully appreciated (Zanker, 1990; Borst, 2004). We also show that the properties of other LPTCs involved in visually guided behavior range from bimodal responses to plaid components, to unimodal responses that appear to be selective to the direction of pattern motion.

Materials and Methods

Optomotor experiments

Male blowflies, *Calliphora vicina*, aged 2–4 weeks, were used. A mixture of beeswax and rosin was used to wax a small strip of cardboard to the fly's thorax, by which the fly was held in a torque compensator (Götz, 1964). The fly rested for ≥ 1 h with food and water before experiments.

Each plaid stimulus was generated by the superimposition of two half-contrast gratings moving in different directions (Fig. 1*a*). The directions of the underlying gratings were rotated to form a plaid stimulus moving with a specific plaid direction. The half-contrast gratings had a spatial wavelength of 11.5° and a temporal frequency of 2 cycles/s. The stimuli were displayed with a Michelson contrast of 60% in a circular aperture of diameter 60° centered at 30° azimuth (az.) 0° elevation (el.). The stimuli were displayed on an Iiyama 19 inch CRT monitor (Vision Master Pro 454 monitor) at 200 Hz, and generated using Psychophysics Toolbox (Brainard, 1997). Stimuli were displayed in a random order of orientations between 90° and 270°, in 10° steps, with 20 trials at every orientation. The stimulus was presented for 5 cycles in a trial. In a cycle, the

pattern moved in the positive direction for 1 s, then in the opposite direction for 1 s. An isoluminant screen was presented before and after trials for ≥ 2 min, while the fly rested and fed.

The yaw torque was measured using a torque compensator (MPI), sampled at 5 kHz with a NI USB-6251 data acquisition card (National Instruments Corp.). We calculated the mean response to each stimulus cycle by pooling responses to all cycles, across all trials. The response to the first stimulus cycle was excluded because the responses at the start of flight were highly variable. To determine the response amplitude and phase, we fit a 0.5 Hz sine wave to the mean cycle response using the Nelder-Mead algorithm (Nelder and Mead, 1965). To correct for interindividual variations in yaw torques generated, the directional tuning curves of each fly were normalized by the mean response to the half-contrast grating stimuli.

Electrophysiology

Female flies aged 4–11 d, were used. The animal preparation and extracellular recording equipment was as described in Longden and Krapp (2009). The H1, V1 and V2 cells were identified by their motion preferences throughout the visual field (Hausen, 1984; Krapp et al., 2001; Longden and Krapp, 2009). The temperature was 23.0–24.4°C.

Plaid stimuli were displayed in a circular aperture of diameter 24°, centered at -30° az. 0° el. for the H1 and V1 cells, and at 105° az. and 0° el. for the V2 cell. The Michelson contrast of plaids and gratings was 20% for the H1 cell, 30% for the V2 cell, and 30% (see Fig. 5) or 20% for the V1 cell. Stimuli were displayed as for the optomotor experiments, except where stated. Stimuli were randomly ordered, with directions of 0–360°, in steps of 15°, with 25

trials per direction. In a trial, an isoluminant screen was displayed for 250 ms before and after the stimulus, which was displayed for 500 ms. The response was calculated as the mean spike rate during the 500 ms stimulus. The spontaneous spike rate was calculated over the remaining 500 ms of every trial.

Pattern/component classification

The partial correlations and z-scores of the response to the component and pattern cell predictions were calculated as described by Smith et al. (2005). The pattern cell response, $r_{\text{pat}}(\theta)$, to the stimulus oriented in direction θ , was calculated as

$$r_{\text{pat}}(\theta) = (r_{\text{grat}}(\theta) - r_{\text{spont}}) \left[\frac{1}{2} \cos\left(\theta + \frac{\theta_p}{2}\right) + \frac{1}{2} \cos\left(\theta - \frac{\theta_p}{2}\right) \right] + r_{\text{spont}} \quad (1)$$

where $r_{\text{grat}}(\theta)$ is the response to the grating oriented in the same direction, r_{spont} is the spontaneous spike rate, and θ_p is the plaid separation angle. The component cell response, $r_{\text{comp}}(\theta)$, was calculated as

$$r_{\text{comp}}(\theta) = \frac{1}{2} \left[r_{\text{grat}}\left(\theta + \frac{\theta_p}{2}\right) + r_{\text{grat}}\left(\theta - \frac{\theta_p}{2}\right) \right] \quad (2)$$

The partial correlation of the response to the pattern prediction, Ω_{pat} , was calculated from the correlation of the neuron's response to the pattern cell and component cell predictions, ρ_{pat} and ρ_{comp} , respectively:

$$\Omega_{\text{pat}} = \frac{(\rho_{\text{pat}} - \rho_{\text{comp}}\rho_{\text{pc}})}{\sqrt{(1 - \rho_{\text{comp}}^2)(1 - \rho_{\text{pc}}^2)}} \quad (3)$$

where ρ_{pc} is the correlation between the pattern and component response (Movshon et al., 1985). The partial correlation of the response to the component prediction, Ω_{comp} , was similarly calculated. The z -score was then calculated using the Fisher r -to- Z transform to generate significance values,

$$Z_p = \frac{\frac{1}{2} \log\left(\frac{1 + \Omega_{pat}}{1 - \Omega_{pat}}\right)}{\sqrt{df}}, \quad (4)$$

where df is the degrees of freedom and is equal to the number of points in the tuning curve minus 3 (Movshon et al., 2003; Smith et al., 2005). The significance value, p , of Z_c exceeding Z_p by 1.28 is $p = 0.1$.

Computational model of the H1 cell

We modeled the processing of directional motion stimuli by the H1 cell as a cascade of processing stages. Our “H1 cascade model” has four stages.

(1) *EMD subunit directional tuning function.* The EMD subunits’ directional tuning functions were modeled as a circular Gaussian, von Mises function. The response of the tuning function c_n of the n th EMD subunit, in direction θ , was calculated as:

$$c_n(\theta) = \frac{1}{\sum_j \exp[\kappa \cos(\theta_j - \theta_n)]} \exp[\kappa \cos(\theta_j - \theta_n)], \quad (5)$$

where θ_n was the preferred direction of the subunit, j indexed the stimulus directions, and κ was a free parameter for the width of tuning of all the EMD subunits.

(2) *EMD subunit output function.* The response of the n th EMD subunit, v_n , was calculated as a static sigmoid nonlinearity of c_n :

$$v_n = \frac{1}{1 + \exp[\lambda(c_n - \epsilon)]}. \quad (6)$$

where λ and ϵ were free parameters.

(3) *EMD subunit weights.* The outputs of the EMD subunits were integrated with weights, w , to generate an input current, u , to the H1 cell:

$$u = \sum_n w_n v_n \quad (7)$$

where n indexes the EMD subunit directions.

(4) *H1 output function.* The predicted output firing rate of the H1 neuron, $\tilde{r}$, was calculated as a static exponential nonlinearity,

$$\tilde{r} = \omega \exp(\eta u), \quad (8)$$

where ω and η were free parameters.

To identify how the non-cosine direction tuning function and the nonlinearities of the model contributed to capturing features of the data, we first created a “simplified H1 cascade model,” in which we replaced the direction tuning function by a cosine function and the two nonlinear stages with linear functions. Specifically, the EMD subunit output function was the identity, ($v_n = c_n$), and the H1 output function was the identity, ($\tilde{r} = u$). We then added non-cosine direction tuning and the nonlinearities one-by-one and compared the performance of the model, with and without each component.

Analysis of prediction accuracy

We used two measures to estimate how well any of the models predicted the neuronal response. The first was the root mean squared error between the predicted and measured response,

$$RMS = (\langle (r - \tilde{r})^2 \rangle_{stim}^{trials})^{1/2}, \quad (9)$$

where r is the measured response of the cell, $\tilde{r}$ is the predicted response, and angular brackets denote averaging over stimuli (*stim*) and trials (*tri*-

als). As the measured response is inherently noisy, we also calculated the fraction of explainable variance β as (Machens et al., 2004):

$$\beta = \frac{\sigma_r^2 - \sigma_e^2}{\sigma_r^2 - \sigma_n^2}, \quad (10)$$

where σ_r^2 is the variance in the response power,

$$\sigma_r^2 = \langle \langle r^2 \rangle_{stim} \rangle_{trials}, \quad (11)$$

σ_e^2 is the square of the RMS error, and σ_n^2 is the residual noise power, calculated as:

$$\sigma_n^2 = \frac{n}{n-1} [\langle \langle r^2 \rangle_{stim} \rangle_{trials} - \langle \langle r \rangle_{trials}^2 \rangle_{stim}]. \quad (12)$$

The mean and SDs of the model performances were calculated by fitting the models to the data multiple times, and calculating the mean and SDs of the model performances. The number of times the model was fit, N_{fits} , was 150 for the H1 cell models, and 175 for the V2 cell models.

Model fitting

The electrophysiology data from each cell type was pooled. There were 5 plaid separation angles, each presented in 24 directions, resulting in 120 different stimuli. Each time the model was fit, 100 stimuli were randomly chosen, and all the responses to these stimuli were allocated to the training set. The responses to the remaining 20 stimuli formed the test set. Thus the model was fit to 83% of all the data for each cell type, and tested on the remaining 17%. For the H1 cells, there were 150 trials for each stimulus, and for the V2 cells there were 175 trials per stimulus.

We fit the model to the response to plaid stimuli and not the grating stimuli, as the grating and the plaid stimuli had the same Michelson contrast and mean luminance values, and therefore different root mean square contrast (RMS contrast, the SD of the pixel luminance). For plaid separation angles between 30° and 150°, the calculated RMS contrast of our stimuli is nearly constant (30°: 4.8%, 60°: 5.0%, 90°: 5.0%, 120°: 5.0%, 150°: 5.2%), but for the grating it is much larger (7.1%). EMDs compare the luminance of small patches of the visual stimulus, corresponding to the acceptance angles of the ommatidia, so the grating is likely to be an effectively higher contrast stimulus than the plaids. To capture the responses to the full grating, the model would need to include contrast gain control mechanisms (Harris et al., 2000; Rust et al., 2006).

A nested fitting procedure was used to fit the possible models in five steps: (1) the free parameters κ , λ , and ϵ were fixed. (2) v_n was calculated for all possible stimuli. (3) Assuming $r = u$, the optimal weights w_n for the these free parameters were calculated by ridge regression, using a regularization parameter of 10^{-2} (Hastie et al., 2001). (4) The parameters of the H1 output nonlinearity, ω and η were fit using linear regression. (5) The overall training error of this fit, $Error_{train}$, was calculated as the mean squared error:

$$Error_{train} = \sqrt{\sum_{k_{train}} (r - \tilde{r})^2}, \quad (13)$$

where k_{train} indexes the training data, r is the response of the H1 cell, and $\tilde{r}$ the predicted response of the model. We iterated through steps 1–5 to find the free parameters κ , λ , and ϵ that minimize $Error_{train}$ using the `fminsearch` function of MATLAB (The MathWorks Inc.).

To estimate the goodness of fit, the test error, $Error_{test}$, was calculated over the test data, indexed by k_{test} :

$$Error_{test} = \sqrt{\sum_{k_{test}} (r - \tilde{r})^2}. \quad (14)$$

The simplified H1 cascade models were optimized on a reduced set of free parameters. We avoided overfitting when adding the non-cosine direction tuning and nonlinearities to the simplified cascade model by assessing the model performance using separate testing and training data, which penalizes overfitting. We also judged the success of the model by its ability to capture features of the data, in addition to the overall fit.

Figure 2. Component selectivity of the H1 cell to plaids. **a**, Diagram of electrophysiology setup. **b**, Example H1 cell response to a 60° plaid. Black bar indicates stimulus period. **c**, Mean H1 responses to plaids. Mean $\pm$ SE shown ($N = 6$). **d**, Comparison between the H1 response to the 150° plaid, and pattern and component cell predictions. Black asterisks indicate significant differences between peak H1 responses, and pattern cell predictions ($p < 0.005$, paired t test, $N = 6$). Gray asterisks indicate significant differences between H1 responses and component cell predictions ($p < 0.05$, paired t test, $N = 6$). **e**, Normalized correlation coefficients between H1 responses and pattern cell predictions, Z_p , and component cell predictions, Z_c , for 120° plaids (blue circles) and 150° plaids (red triangles). Symbols with error bars indicate the mean $\pm$ SE. Gray lines denote statistically significant ($p = 0.10$) bias to pattern or component cell correlation (Smith et al., 2005).

Results

Optomotor response to plaid stimuli

We measured the yaw torque responses of tethered flies in flight to motion stimuli moving back and forth along different orientations (Fig. 1*b*). The orientation tuning to a single grating was unimodal, peaking at 180°, and consistent with the expected optomotor response to single gratings (Hausen and Wehrhahn, 1983) (Fig. 1*c,d*). The orientation tuning to the plaid, however, had a bimodal dependence on the stimulus, with peaks at 140° and 210° (Fig. 1*d*). The bimodal tuning was not well described by the tuning predicted by the inner product between the stimulus direction and the preferred direction of the fly's response to the single grating (Fig. 1*d*). To understand how this behavior could be generated, we recorded the responses of a neuron involved in the yaw optomotor reflex.

Component motion selectivity of H1 cell: neural correlate of yaw optomotor response

The H1 cell is part of a network of LPTCs sensitive to horizontal optic flow that mediates the optomotor yaw response (Hausen and Wehrhahn, 1989, 1990; Haag and Borst, 2001). Its mean spike rate is correlated to the yaw torque generated in response to moving gratings (Eckert, 1980). The tuning of the H1 cell to the direction of a 150° plaid was bimodal, consistent with the bimodal optomotor yaw response to a 150° plaid (Fig. 2). The results were not well described by the unimodal tuning predicted by the pattern cell model, with significant differences to the H1 response peaks at 120° and 225° (Fig. 2*d*; $p < 0.002$, paired t test, $N = 6$, with Bonferroni correction, $N_{\text{tests}} = 24$).

We next compared the tuning of the H1 cell to the predictions of the “component cell” model, because this model is widely used to classify and understand the neural responses to plaids in vertebrate systems (Movshon et al., 2003). In the component cell model, each component of the plaid is processed independently, and the cell's response is the sum of these operations (Eq. 2, Materials and Methods) (Adelson and Movshon, 1982). The response to 150° plaids was better fit by the component cell model than the predicted unimodal tuning (Fig. 2*d*; $R^2 = 0.72$ vs 0.66), and the correlation was significantly higher for 4 of the 6 cells (Fig. 2*e*; $p < 0.10$; mean $Z_c - \text{mean } Z_p = 1.42$, $N = 6$). The component selectivity of the H1 cell responses to the 150° plaid

was present within the 50 ms after the stimulus onset for 5 of the six cells ($p < 0.10$; mean $Z_c - \text{mean } Z_p = 3.22$, $N = 6$).

Two observations indicated processing steps not captured by the component cell model. First, the peak responses to the 60° plaid were greater than for the 30° plaid (Fig. 2*c*; $p < 0.02$, paired t test, $N = 6$). Second, the directional tuning to the 150° plaid was narrower than predicted by the component cell model (Fig. 2*d*). This second factor limited the magnitude of the component selectivity correlation score, $Z_c - Z_p$, despite the qualitative good fit of the bimodal component predictions. Across all cells, $Z_c - Z_p$ was correlated for the 120° and 150° plaids (Pearson's correlation coefficient, $\rho = 0.69$). One of the H1 cells showed clear pattern selectivity for the 120° and 150° plaids (Fig. 2*e*), despite having bimodal responses to these plaids. This cell had a more variable response to the grating, with a mean SD of 21.8 Hz, compared with 14.0 Hz for the other cells, which skewed the pattern and component predictions for this cell.

Model of the H1 cell response

To understand the computational mechanisms underlying the bimodal directional tuning of LPTCs to plaids, we modeled the tuning of the H1 cell (Fig. 3). First we describe our H1 cascade model that includes both the EMD layer as well as the H1 cell. We then explain the contributions of the H1 cascade model components.

The initial layer of our H1 cascade model consists of 12 EMD subunits, corresponding to interactions between pairs of nearest and next-nearest ommatidia (Fig. 3*a*). In the light-adapted fly, the optomotor responses can be well explained by considering only these EMDs (Buchner, 1976, 1984). Individual EMD subunits were modeled using a set of directional tuning functions (Fig. 3*b*), whose output was processed by a saturating nonlinearity (Fig. 3*c*). The EMD subunit responses were integrated as a weighted sum (Fig. 3*d*), and an exponential nonlinearity converted this input into a spike rate (Fig. 3*e*). In total, the H1 cascade model has 5 parameters specifying the nonlinear functions and non-cosine direction tuning, and 12 parameters specifying the EMD subunit weights. The parameters were fit to a fraction of the data using a nested fitting procedure, and were validated on the remaining data.

The H1 cascade model was able to capture the essential features of the experimental data (Fig. 3*f*): the directional tuning of

Figure 3. H1 cascade model. *a–e*, H1 cascade model parameters, fitted values and equations. *a*, The 12 directions of EMD subunits (black arrows). *b*, EMD subunit directional tuning function. The fitted function (black) is shown with a cosine for comparison (gray). One parameter, κ , determines the directional tuning. *c*, EMD subunit output non-linearity. Two parameters, λ and ε , determine the sigmoid function. *d*, EMD subunit weight distribution. Excitatory (black) and inhibitory (gray) weights are shown. *e*, H1 output non-linearity. Two parameters, ω and η , determine the H1 output to the weighted EMD input. *f*, Mean responses of H1 cascade model to the test data (solid lines). Error bars show the variability (SE) of the H1 cell recordings, to demonstrate the accuracy of the H1 cascade model fit. *g*, Root mean squared errors between H1 cascade model responses and predictions of the pattern and component models. *h*, Fraction of explainable variance, β , accounted for by the models. *g, h*, Mean $\pm$ SE shown ($N_{\text{fits}} = 150$).

the cell obtained with the 150° plaid was bimodal, narrower than the component prediction, and the peak response to the 60° plaid was greater than that for the 30° plaid ($p < 0.01$, 2-sample t test; $N_{\text{fits}} = 150$). Across all plaid directions, the H1 cascade model fit the data more accurately than the pattern and component cell models (Fig. 3*g*; $p < 0.001$, 2-sample t test; $N_{\text{fits}} = 150$). We also used the fraction of explainable variance, β , to test the goodness of fit of the H1 cascade model. This measure takes into account the response variability across trials: a value of 1 identifies the best fit that can be expected from a model given this intrinsic variability. The H1 cascade model accounted for a significantly greater fraction of explainable variance than the pattern and component cell models (Fig. 3*h*; $p < 0.001$, 2-sample t test; $N_{\text{fits}} = 150$).

Contributions of model components: mechanisms of component selectivity

To understand how the non-cosine direction tuning and nonlinearities of the H1 cascade model allowed us to capture features of the H1 cell responses to plaids, we started with a simplified H1 cascade model, that was just a weighted sum of cosine-tuned EMD subunit outputs. We then added the non-cosine direction tuning function and the two nonlinearities one-by-one, and compared the performance of the model in capturing features of the data, with and without each component (Fig. 4).

To generate the bimodal tuning, it was necessary to include an EMD subunit directional tuning function that was narrower than a cosine (Fig. 4*a*). Only then was the optimal tuning narrower than a cosine, in agreement with the narrow, bimodal tuning of the H1 cell to the 150° plaid (Fig. 4*a5*). With the non-cosine

directional tuning function, the H1 cascade model accounted for substantially more of the explainable variance (Fig. 4*a1*; β from 0.93 to 0.94; $p < 0.001$; 2-sample t test; $N_{\text{fits}} = 150$).

The addition of the sigmoidal nonlinearity applied to the output of each EMD subunit allowed the H1 cascade model to capture the greater peak response of the cell to a 60° plaid compared with a 30° plaid (Fig. 4*b2,3*). The nonlinearity suppressed the peak responses to each component grating, and effectively boosted the response to each component grating in the anti-preferred direction. It also increased the peak responses to the 120° and 150° plaids to a near perfect match with the cellular responses (Fig. 4*b4,5*), which improved the overall fit (Fig. 4*b1*; β from 0.94 to 0.96; $p < 0.001$; 2-sample t test).

Including an exponential H1 output nonlinearity resulted in a modest increase of the fit (Fig. 4*c1*; β from 0.964 to 0.975; $p < 0.01$; 2-sample t test). The nonlinearity amplified the difference in the peak responses to the 30° and 60° plaids (Fig. 4*c2,3*), and improved the fit of the H1 cascade model in the nonpreferred directions, where the cell is inhibited (Fig. 4*c4–5*). However, the H1 output nonlinearity reduced the fit of the 150° plaid, leading to an almost unchanged fit of the H1 cascade model overall (Fig. 4*c5*).

Motion selectivity of the V1 and V2 LPTCs: evidence for pattern selectivity

To understand whether bimodal directional tuning to plaids was a general property of LPTCs, we also recorded from two other spiking LPTCs, the V1 and V2 cells. The directional tuning of the V2 cell to plaids was qualitatively similar to that of the H1 cell (Fig. 5*a*), in particular with respect to the bimodal response to the

Figure 4. Contributions of H1 cascade model components. In every row, a component of the H1 cascade model is added to the simplified H1 cascade model, and the performance with and without the component is shown. **a1**, Fraction of explainable variance, β , accounted for by the simplified model when the directional tuning is a sinusoid (gray), or the nonlinear von Mises function (black). Mean $\pm$ SD across multiple fits ($N_{\text{fits}} = 150$). **a2**, Fit to the 30° plaid responses of the H1 cell (dashed) of the simplified model with the sinusoidal (gray), or von Mises (black) EMD subunit directional tuning. **a3–5**, As for **a2**, for 60°, 30°, 120°, and 150° plaids. **b**, As for **a**, but adding the sigmoid EMD subunit output nonlinearity (Sigmoid). **c**, As for **b**, but adding the exponential H1 output nonlinearity (Exponential).

Figure 5. Pattern selectivity of V1 cell and response of V2 cell. **a, b**, Responses of V2 (**a**) and V1 cells (**b**) to plaids. Gray arrows in **b** indicate secondary peaks in the response to the 150° plaid. Mean $\pm$ SE shown. V2, $N = 7$; V1, $N = 6$. **c**, Normalized correlation coefficients, as in Figure 2e, for the V2 cells (orange circles and purple triangles) and V1 cells (blue circles and green triangles).

150° plaid. However, the V2 response to the 150° plaid was narrower than the component cell prediction, such that the V2 responses could not be classified as pattern- or component-selective by their correlations with the pattern and component cell models (Fig. 5c).

A V2 cascade model, with a structure identical to the H1 cascade model, was able to fit the V2 cell data well (Fig. 6; $\beta = 0.98$ for V2, $N_{\text{fits}} = 175$). In particular, the V2 cascade model generated a peak output to the 60° plaid that was greater than for the 30° plaid, and a bimodal directional tuning to the 150° plaid (Fig. 6f). The V2 cascade model produced a narrower EMD subunit tuning function than the H1 cascade model (Fig. 6b). As the interommatidial angle is greater in the visual field where the V2 cell was stimulated, compared with the region used for the H1 cell (Petrowitz et al., 2000), the EMD subunit directional tuning function should indeed be narrower (Borst, 2004). Thus, identical computational mechanisms—in combination with the variations of the local spatial resolution of the compound eye—can explain the pattern motion response properties of both the H1 and V2 cells.

The tuning of the V1 cell was unimodal, and the peak preferred direction was not altered by the plaid separation angle (Fig.

5b). The responses to 120° and 150° plaids were better fit by the unimodal predicted responses than the component cell model (Fig. 5c), resulting in a significantly higher correlation with the pattern cell predictions than the component cell predictions, for 5 of 6 cells (Fig. 5c; $p < 0.01$; 120° plaid: mean $Z_p - \text{mean } Z_c = 3.0$, 150° plaid: mean $Z_p - \text{mean } Z_c = 2.9$, $N = 6$). The pattern selectivity of the V1 cell responses to the 150° plaid was present within the 50 ms after the stimulus onset for 3 of the six cells ($p < 0.10$), and for 4 of the 6 cells by 100 ms ($p < 0.10$; mean $Z_c - \text{mean } Z_p = 4.82$, $N = 6$). Thus, we found no clear evidence that the pattern selectivity of the V1 cell takes longer to develop than the component selectivity of the H1 cell, as it does in primate MT/V5 cells (Smith et al., 2005).

The tuning of the V1 cell to plaids differed from the pattern cell model predictions in two ways. There were secondary peak responses to the 120° and 150° plaids at plaid directions of 165° and 375° (Fig. 5b, gray arrows), and the response to the 60° plaid was greater than to the 30° plaid at the preferred direction (Fig. 5b; $p < 0.03$, paired t test, $N = 6$). The pattern selectivity of V1 was maintained at a lower contrast level of 20% (150° plaid: mean $Z_c - Z_p = -1.66$, $N = 5$).

Figure 6. V2 cascade model. *a–e*, V2 cascade model parameters, fitted values and equations. *a*, The 12 directions of EMD subunits (black arrows). *b*, EMD subunit directional tuning function. The fitted function (black) is shown with a cosine for comparison (gray). *c*, EMD subunit output nonlinearity. *d*, EMD subunit weight distribution. Excitatory (black) and inhibitory (gray) weights are shown. *e*, V2 output nonlinearity. *f*, Responses of V2 cascade model to the test data (solid lines). Error bars show the variability of the V2 cell recordings, to demonstrate the accuracy of the V2 cascade model fit. *g*, Root mean squared errors between V2 cascade model responses and predictions of the pattern and component models. *h*, Fraction of explainable variance, β , accounted for by the models. *g, h*, Mean $\pm$ SE shown ($N_{\text{fits}} = 175$).

Discussion

We have demonstrated that the blowfly's optomotor yaw response can be bimodally tuned to the direction of plaid stimuli, contrary to conclusions based on previous studies of two-dimensional motion processing in flies (Fig. 1*d*). The directional tuning to plaids of the H1 cell contributing to this reflex was likewise bimodal for the 120° and 150° plaids (Fig. 2*c*). By fitting a cascade model to the recorded activity of the H1 cell (Fig. 3), we identified the essential features of neural processing required to capture the response of these cells to two-dimensional pattern motion: (1) a non-cosine direction tuning of EMD subunits (Fig. 4*a*), and (2) a saturating nonlinearity at the output of the EMD subunits (Fig. 4*b*). The directional tunings of the other LPTCs investigated here, the V1 and V2 cells, range from between pattern- and component-selective responses, to pattern-selective responses (Fig. 5), indicating that a component-like tuning is not a general property of the population of LPTCs.

We assumed that the LPTCs we recorded from were entirely driven by EMD inputs. A number of LPTCs have properties consistent with cells that process higher-order motion, such as the figure-detecting (FD) cells. FD cells respond to the motion of small objects in their receptive fields and are thought to support object fixation behavior (Egelhaaf, 1985*a,b*; Hausen and Wehrhahn, 1990). Flies produce yaw optomotor responses when challenged with second order motion which are not fully accounted for by integrated EMD signals alone (Theobald et al., 2008). While it is possible that non-EMD-based visual processing contributes to the bimodal directional tuning of the yaw optomotor

reflex during plaid stimulation, EMD inputs provide the most parsimonious explanation of the inputs driving the H1 and V2 LPTCs (Quenzer and Zanker, 1991; Tuthill et al., 2011).

The behavioral responses to plaids have not been widely studied in invertebrate systems. Freely flying honeybees can learn to discriminate between the orientations of specific plaid patterns (Srinivasan et al., 1994). A flight situation that would generate plaid-like stimuli, for instance when the foreground and background appear to move in divergent directions, will likely generate additional visual motion cues, such as motion parallax or figure-ground discrimination, which the fly may use to guide its flight. For the behavioral responses driven by EMD inputs, our results and analysis show that neurons involved in the yaw response may allow the fly to preferentially follow one of the motion cues.

To our knowledge, the responses of motion-sensitive visual interneurons to plaids have not been investigated in detail in any invertebrate system. Eriksson (1984, 1989) recorded the responses of the H1 (Eriksson, 1984) and V1 (Eriksson, 1989) cells to two moving dots, when the direction of the second dot was varied. The responses of these cells were unchanged when the velocity of both dots along the preferred direction was held constant (Eriksson, 1984, 1989). These previous findings are consistent with our data for the V1 cell (Fig. 5*b*), but in apparent contradiction with our H1 cell data (Fig. 2). A possible explanation for the latter discrepancy between our results and the studies by Eriksson may be that the velocity of the second dot varied with

its motion direction. It is likely that changes in sensitivity to the direction of dot motion were affected by changes in the velocity.

The cascade model shows how the non-cosine direction tuning of EMD subunits generates a bimodal tuning to the plaids with large separation angles. A non-cosine directional tuning function is predicted by the EMD model (Varju, 1959; Götz, 1964; Zanker, 1990; Borst, 2004), but previous work indicated that it should only have an impact for much shorter spatial wavelengths than those used in our study (Zanker, 1990). For single grating stimuli of a spatial wavelength >4 times the inter-ommatidial angle ($\Delta\phi$) (Zanker, 1990), for both recordings and simulations of the H1 cell responses, the integration of multiple EMD outputs resulted in a directional tuning curve that was more similar to a sinusoid than the output of a modeled EMD. From this it was concluded that the non-cosine direction tuning of EMDs has little impact on the directional responses of LPTCs and the animal's optomotor behavior, with the possible exception of cases when wavelengths $<4 \Delta\phi$ are used. All previous studies have assumed, therefore, that the response to 2-dimensional patterns of visual motion is well approximated by the dot product between the direction of stimulus motion and the cell's preferred direction (Reichardt et al., 1988; Reichardt and Schlögl, 1988; van Hateren, 1990; Borst and Egelhaaf, 1993; Borst et al., 1993; Franz and Krapp, 2000; Weber et al., 2010). We have shown that the non-cosine direction tuning of EMD subunits can have a substantial impact on the LPTC and optomotor responses to visual stimuli that are more complex than single gratings, even when spatial wavelengths $>4 \Delta\phi$ are used.

Our data provides evidence that the V1 cell is pattern-selective. Unlike the H1 cell, the V1 cell receives its motion inputs from three other LPTCs, the VS1-3 cells, and not direct EMD inputs (Hausen, 1984; Krapp et al., 1998). The VS1 cell is sensitive to horizontal motion in addition to vertical motion (Krapp and Hengstenberg, 1996), which is the likely reason for the secondary peaks in the V1 cell response. We did not model the V1 cell response because the extra layer of cellular processing, compared with the H1 and V2 cells, requires a greater number of model parameters than we could fit with our data. In the future, intracellular recordings from VS cells will allow the mechanisms behind the V1 cell's unimodal tuning to plaid direction to be established, and confirm whether the cell is indeed selective for pattern motion.

Our cascade model of the H1 and V2 cells is directly comparable to a recent model of the responses to plaid stimuli of neurons in macaque monkey visual cortex area V5/MT (Rust et al., 2006). Both models share the same overall structure, the main difference being the use of a saturating nonlinearity in our model instead of directionally tuned and untuned normalization terms. Tuned inhibition is a well explored concept in striate cortex, which provides the early stage of visual processing in the MT model. Although tuned inhibition of the EMD subunits has not been described, lateral inhibition of some form has been suggested to operate in the early stages of visual processing in insects (Srinivasan et al., 1982; Zheng et al., 2006; Bolzon et al., 2009). By contrast, a saturating output nonlinearity has often been used in models of EMDs (Zanker, 1990; Franz and Krapp, 2000). In our H1 cascade model, the saturating output nonlinearity limits the maximum responses to motion in the preferred direction, achieving a comparable modulation of the cell's response as tuned inhibition achieves for the cascade model of V5/MT cells (Rust et al., 2006).

The responses of the LPTCs to plaid stimuli are qualitatively similar to those of neurons found in V5/MT, which may vary

along the continuum between pattern and component selectivity (Movshon et al., 1985). The calculated directional tuning curves of the EMD subunits are much broader than those of V1 (striate cortex) neurons. As a result, the challenge here is to explain how LPTCs generate a bimodal tuning to the plaid direction from broadly tuned EMD subunit responses, whereas for V5/MT it is to explain how a pattern cell-like response can be generated in some MT neurons from narrowly tuned V1 (striate cortex) neurons. In the model proposed by Rust et al. (2006), a broad distribution of excitatory and inhibitory weights generate a pattern motion selective response from narrowly tuned V1 (striate cortex) inputs. Rust et al. (2006) implemented tuned inhibition to ensure MT cell peak responses to plaid stimuli exceeded those to a single grating. In our H1 and V2 cascade models, the non-cosine directional tuning of the EMD subunits generated a bimodal tuning to the plaid direction, and the saturating EMD subunit output function ensured that the peak response to the 60° plaid exceeded that of the 30° plaid. Thus, our model shows how functionally comparable mechanisms may explain the MT and LPTC responses to plaid motion stimuli.

In summary, we have identified the processing steps necessary to explain an unexpected behavioral response to two-dimensional patterns of motion in the blowfly. The results also indicate that the fly may employ a range of visual processing to support behavioral responses to multiple motion cues. By taking advantage of the fly's well characterized visuomotor pathway, future recordings from identified cells will allow us to further specify the underlying physiological mechanisms.

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