Figure S1. Behavioral response to rotational and translational stimuli. Related to Figure 1.
Each stimulus has a spatial period of 45° and a temporal frequency (TF) of 4 Hz.
Error patches represent standard error of the mean.
Icons indicate the stimulus presented (outside) and the behavioral response measured (inside).
(**A**) 0.25 contrast rotational (**i**), front-to-back (FtB) (**ii**), and back-to-front (BtF) (**iii**) sine wave grating. 0.5 contrast counterphase grating (**iv**). A counterphase grating is equal to the sum of a 0.25 contrast leftward and rightward sine wave grating.
(**B**) Fly behavioral response to single 1s presentations of a stimulus. 10 responses to single presentations overlaid on top of the mean (black) (*top*). Heat map of fly turning response to 50 single presentations where color axis shows 99% of the data (*bottom*). First 10 examples are plotted in (*top*). (**i**) Fly turning in response to rotational rightward sine waves. (**ii**) Fly slowing in response to FtB translational sine waves.
(**C**) Average walking response of 15 flies as a function of TF in units of mm/s (**i**) and fold change where fly responses have been divided by each fly's average walking speed during a middle gray stimulus (**ii**). Average walking response of all flies as a function of TF in units of mm/s (**iii**) and fold change (**iv**). n=18
(**D**) Absolute value of the turning response when presented with a FtB (**i**) and BtF (**ii**) translational sine wave with and without a middle gray stripe occluding 45° centered on the expanding/contracting pole at the front of the stimulus. Baseline absolute turning marked with dashed gray line. Average walking response as a function of TF to a FtB (**iii**) and BtF (**iv**) translational sine wave with and without a middle gray stripe occluding the expanding pole of the stimulus. Occluding the expanding pole attenuated responses to FtB stimuli but did not strongly affect BtF stimuli. FtB, n=9; BtF, n=14.
(**E**) Turning (**i**) and slowing (**iii**) response over time when presented with rotational sine waves with different TFs. Stimulus presented during shaded gray region. Average turning (**ii**) and slowing (**iv**) response to rotational sine waves as a function of TF. The turning response (**ii**) can be measured in fly path curvature (Buchner, 1976; Götz and Wenking, 1973), in units of °/mm, over the full second (*purple*). Since flies both turn and slow late in the stimulus, this metric incorporates both behaviors. To isolate turning, one may also average turning, measured in °/s, during the first 250 ms of stimulus (*orange*), and find a similar tuning curve. This is the metric for turning we use throughout. n=69.
(**F**) Mean fly slowing to FtB (green, n=18) and BtF (brown, n=14) sine wave gratings and counterphase gratings (purple, n=19) averaged over the first 250 ms of stimulus. The response to counterphase gratings is emphasized but still significantly less than the response to FtB and BtF sine waves above 2 Hz. n=18.
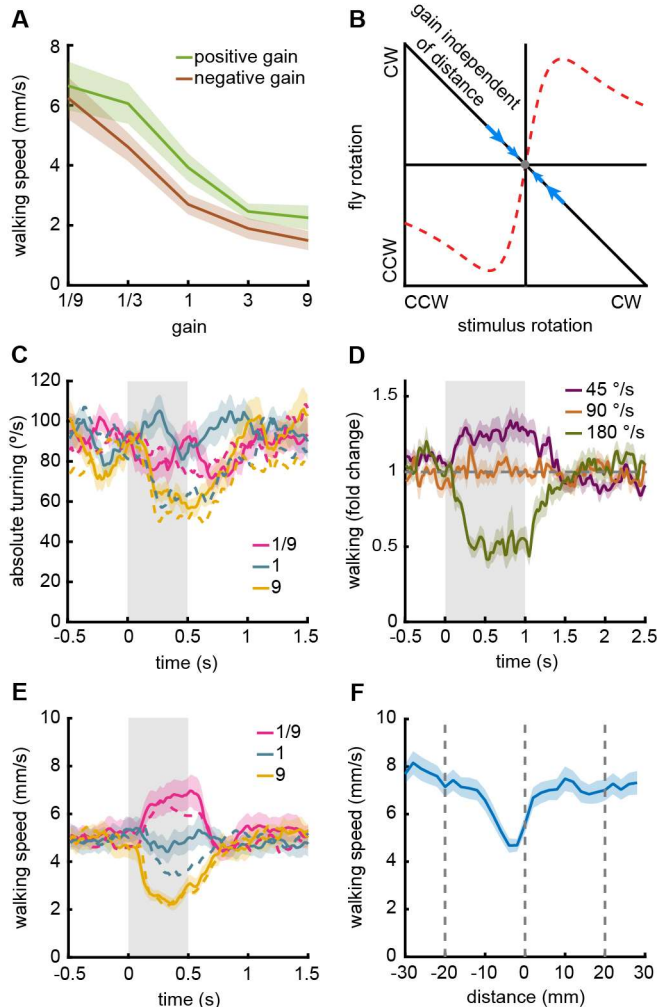
Figure S2. Behavioral response to translational sine waves in closed-loop. Related to Figure 2.

Each stimulus has a spatial period of 45° and a temporal frequency (TF) of 4 Hz.

Error patches represent standard error of the mean.

(**A**) Average walking speed during presentation of translational closed-loop sine waves with different gains. n=29.

(**B**) Simple feedforward model of fly turning behavior in closed-loop. Fly turning response (*red*) as a function of stimulus rotation, measured in open-loop. Natural stimulus gain (*black*) indicating how the visual world rotates when the fly rotates. The stable fixed point is indicated in gray; blue arrows indicate flow towards the fixed point. See **Methods** for a detailed analysis.

(**C**) Absolute turning during presentation of translational closed-loop sine waves with different gains as in **Fig. 2A**. Dotted lines indicate negative gain. n=29.

(**D**) Fly slowing response over time when presented with open-loop front-to-back (FtB) sinewaves at a velocity of 45, 90, and 180 °/s. The pre-stimulus interval was 5 s of 90 °/s FtB stimulation. When the stimulus switches to a lower stimulus velocity the fly speeds up and when the stimulus switches to a higher velocity the fly slows down. This is similar to the speeding up and slowing down observed in closed loop (**Fig. 2A**). n=20.

(**E**) Fly walking speed over time when presented in open-loop with the identical stimulus generated by a fly in closed-loop. Fly response is broadly similar whether it is in open- or closed-loop with the stimulus (**Fig. 2A**). n=29.

(**F**) Fly walking speed as a function of position in the narrowed virtual hallway when presented in open-loop with the identical stimulus generated by a fly in closed-loop. n=35. Fly response is broadly similar whether it is in open- or closed-loop with the stimulus (compare with **Fig. 2B**). n=35.
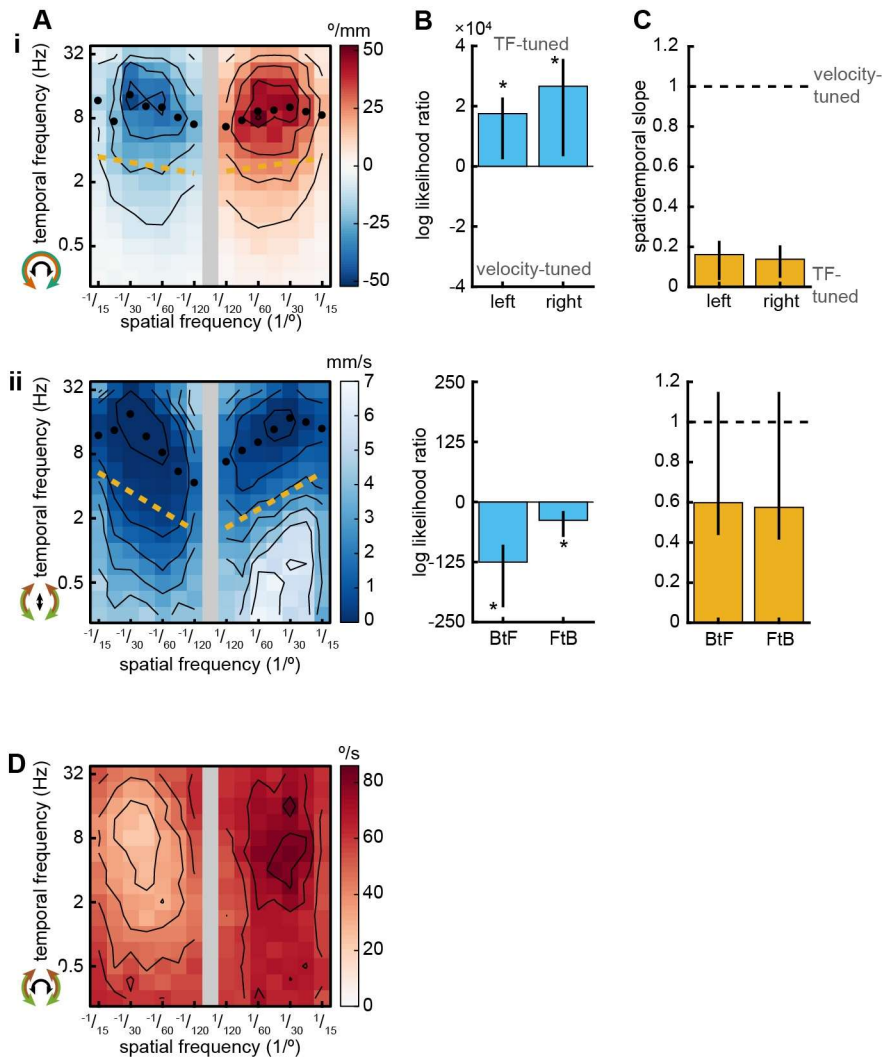
Figure S3. Turning and walking metrics over spatiotemporal frequency space. Related to Figure 3.

(**A**) Behavioral response measured over a suite of spatiotemporal frequencies. (**i**) Turning response measured in curvature (°/mm) when presented with rotational sine waves. Positive/negative spatial frequencies represent rightward/leftward sine waves. Black isoresponse lines are plotted every 0.2-fold change for walking speed. Black circles mark the TF of the maximal response for each wavelength. Gold line is the spatiotemporal slope (STS). n=467. (**ii**) Walking response measured in mm/s when presented with translational sine waves. Positive and negative spatial frequencies represent front-to-back (FtB) and back-to-front (BtF) sine waves respectively. FtB, n=124; BtF, n=104.

(**B**) The log likelihood ratio of TF-tuned to speed-tuned models (see **Methods**). Positive values indicate that a TF-tuned model is more likely while negative values indicate that a velocity-tuned model is more likely. Error bars are 95% confidence intervals and asterisks represent significant difference from 0 at α=0.05.

(**C**) STS of plots in (**A**), where a value of 0 corresponds to perfect TF-tuning and 1 corresponds to perfect speed-tuning (see **Methods**). Error bars are 95% confidence intervals.

(**D**) Absolute turning response measured when presented with translational sine waves. Positive/negative spatial frequencies represent FtB/BtF sine waves. FtB, n=124; BtF, n=104.
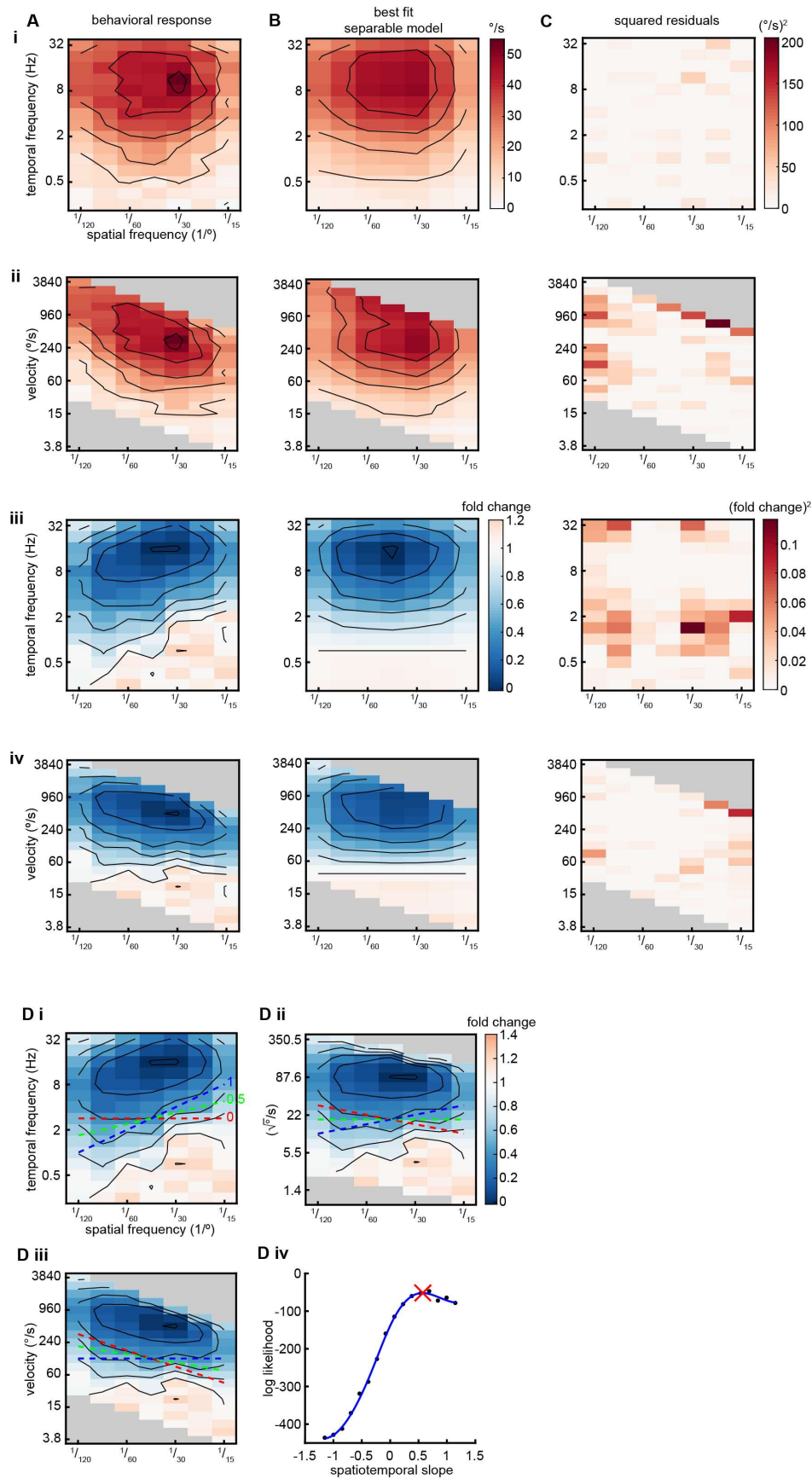
Figure S4. Computing the error between data and temporal-frequency-tuned or velocity-tuned models. Related to Figures 3, 4, 5, and 6.

Black isoresponse lines are plotted every 10 °/s for turning (**i, ii**) and every 0.2 fold change for walking (**iii, iv**).

(**A**) Behavioral data. (**i, ii**) Turning response to rotational sine waves plotted against temporal frequency (**i**) and velocity (**ii**). n=467. (**iii, iv**) Slowing response to FtB sine waves plotted against temporal frequency (**iii**) and velocity (**iv**). n=124.

(**B**) A separable model of the data $f(x, y)$ represents the function as the outer product of two functions, so that $f(x, y) = g(x)h(y)$.

(**C**) Squared error between data and the model. The log likelihood ratio for the two models was computed from the sum of the squared residuals for each model type.

(**D**) Finding the maximum likelihood spatiotemporal slope (STS). STS=0 corresponds to perfect temporal-frequency-tuning and STS=1 corresponds to perfect speed-tuning. Dotted lines show the slope when STS=0 (**red**), STS=0.5 (**green**), and STS=1 (**blue**). n=124.

(**D i**) Slowing response to FtB sine waves plotted with the y-axis in units of temporal frequency (STS=0).

(**D ii**) Same data re-plotted with the y-axis with units of square root degrees per second (STS=0.5). n=124.

(**D iii**) Same data re-plotted with the y-axis in units of degrees per second (STS=1).

(**D iv**) Log likelihood of a separable model fit as a function of STS. The maximum likelihood value is marked by the red X. Blue curve is a cubic spline fit.
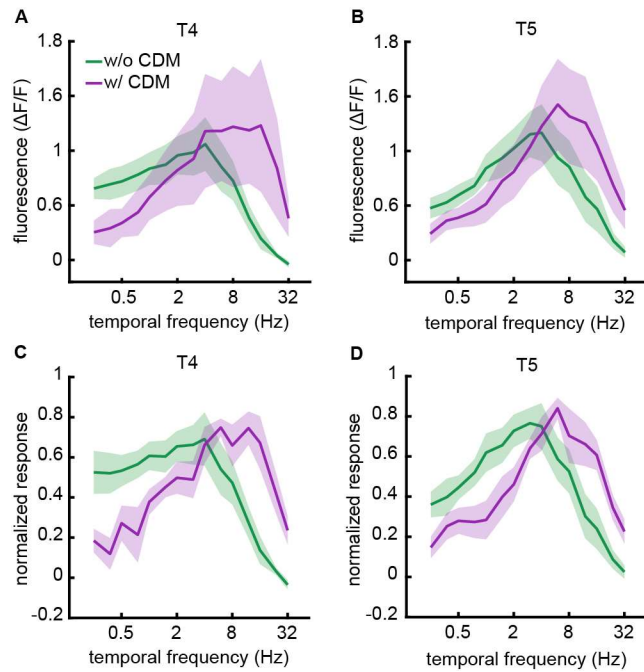
Figure S5. Calcium imaging of temporal frequency tuning response curve for T4 and T5. Related to Figure 5.

(**AB**) Average response of T4 (**A**) and T5 (**B**) as a function of temporal frequency (TF) to a sine wave with spatial wavelength 45°. Error patches represent standard error of the mean. With (*green*) and without (*purple*) chlordimeform (CDM). T4, n=6; T4+CDM, n=6; T5, n=7; T5+CDM, n=7.

(**CD**) Same as in (**AB**) but where each fly's response has been divided by its maximum response across all TFs.
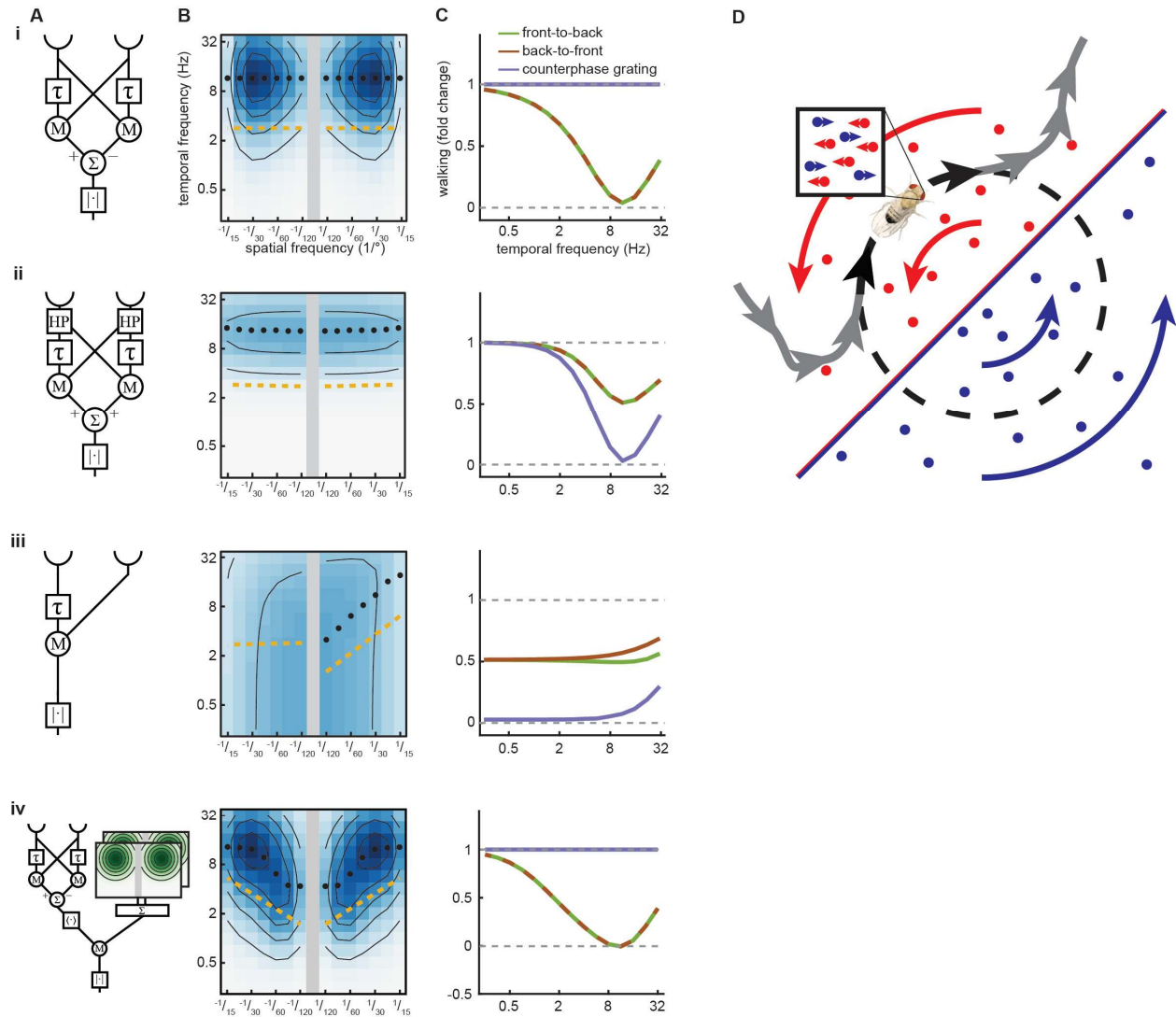
Figure S6. Alternative models of speed tuning. Related to Figure 6 and Table S5.

Model plots are scaled so that their maximum response to any of the stimuli was 0.

(**A**) Schematic of the models. τ indicates a first-order lowpass filter. HP indicates a first-order highpass filter. M denotes a multiplication of the two inputs. Σ indicates summation of the inputs. |·| indicates taking the absolute value of the signal. ⟨·⟩ indicates averaging over space and time.

(**B**) Mean model response to a variety of spatiotemporal frequencies. Positive/negative spatial frequencies indicate front-to-back/back-to-front sine waves. Black isoresponse lines are plotted every 0.2-fold change for walking speed. Black circles mark the TF of the maximal response for each wavelength. Gold line is the spatiotemporal slope (STS).

(**C**) Mean model response to FtB sine wave, BtF sine wave, and counterphase gratings at a spatial wavelength of 45°. Dashed lines simply indicate when responses to FtB and BtF are equivalent.

(**i**) The Hassenstein-Reichardt correlator (HRC) (Borst and Egelhaaf, 1989; Hassenstein and Reichardt, 1956).

(**ii**) Sum of two halves of an HRC (Higgins, 2004).

(**iii**) A single half correlator (Zanker et al., 1999).

(**iv**) Multiplication of an HRC with a non-direction-selective speed tuned cell. Multiplication with the HRC reduces the model response to counterphase gratings.

(**D**) Diagram is of a fly walking through a star field (for illustrative purposes). As the fly walks along an arc of a circle, objects between the fly and the center of that circle move front-to-back (red dots), while objects farther away than the center of the circle move from back-to-front (blue dots). Inset: motion percept from the view of the fly.

Table S1. List of fly genotypes. Related to all figures.

Name in paper	Genotype	Origin
WT	+; +; +	Gohl et al., 2011
shibire	+/hs-hid; +; UAS-shibire[ts]	Kitamoto, 2001
GCaMP6f	+; UAS-GC6f; +	Chen et al., 2013
L1	+; GAL4-L1; +	Rister et al., 2007
L2	+; +; GAL4-L2	Rister et al., 2007
L3	+; +; GAL4-L3	Silies et al., 2013
T4 and T5	+; +; GAL4-R42F06	Maisak et al., 2013
	w; R59E08-AD; R42F06-DBD	Bahl et al., 2015

Table S2. Stimuli spatiotemporal frequencies. Related to all figures.

figure	spatial wavelength (°)	temporal frequency (Hz)
1DEFG, S1B	45	4
1HIJK, 6C, S1CDEiiEivF, S5	45	0.25, 0.375, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32
2A, S2AC	45	closed-loop
3D, 4BC, 6B, S3AD, S4ABCD	15, 22.5, 30, 45, 60, 90, 120	0.25, 0.375, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32
5CD	45	0.25, 1, 4, 16
5EF	22.5, 30, 45, 60, 90, 120	0.25, 0.375, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32
S1EiEiii	45	1, 4, 16
S2D	45	1, 2, 4

Table S3. Average fly walking speed. Related to Figure 4.

genotype	walking speed	standard deviation
WT	5.80	2.97
shibire/+	4.19	2.36
L1/+	5.93	1.96
L1/shibire	5.15	2.12
L2/+	4.09	2.14
L2/shibire	6.50	2.05
L3/+	5.75	2.86
L3/shibire	6.90	2.85
T4T5/+	3.92	2.12
T4T5/shibire	4.05	2.10

Table S4. Number of flies in each figure. Related to all figures.

figure	genotype	stimulus	n
1DEHI	WT	rotation	69
		counterphase	19
1FGJK	WT	FtB	18
		BtF	14
		counterphase	19
2A	WT	closed-loop	29
2B	WT	closed-loop	35
3 i,ii	WT	rotation	467
3 iii	WT	FtB	124
		BtF	104
4B	shibire/+	FtB	274
		BtF	247
4B i	L1/+	FtB	109
		BtF	115
4C i	L1>shibire	FtB	126
		BtF	146
4B ii	L2/+	FtB	83
		BtF	98
4C ii	L2>shibire	FtB	134
		BtF	126
4B iii	L3/+	FtB	121
		BtF	116
4C iii	L3>shibire	FtB	120
		BtF	131
4B iv	T4T5/+	FtB	95
		BtF	105
4C iv	T4T5>shibire	FtB	99
		BtF	96
5C	T4T5>GCaMP6f	rotation	7
5D	T4T5>GCaMP6f	rotation	8
5E i	T4T5>GCaMP6f	rotation	20
5F i	T4T5>GCaMP6f	rotation	22
5E ii	T4T5>GCaMP6f	rotation	13
5F ii	T4T5>GCaMP6f	rotation	12
S1CF	WT	FtB	18
S1D	WT	FtB	9
		BtF	14

S1E	WT	rotation	69
S2ACE	WT	closed-loop	29
S2D	WT	FtB	20
S2F	WT	closed-loop	35
S3A i	WT	rotation	467
3AiiD	WT	FtB	124
		BtF	104
S4AD	WT	rotation	467
		FtB	124
S5A	T4T5>GCaMP6f +CDM	rotation	6
			6
S5B	T4T5>GCaMP6f +CDM	rotation	7
			7

Table S5. Model Parameters. Related to Figures 6 and S6.

For explanation of form see **Methods**. $|\cdot|$ indicates taking the absolute value of the signal. $\langle \cdot \rangle$ indicates averaging over space and time. Numbers in parentheses were held constant at the value noted.

model	form	τ (ms)	φ (°)	α	β
Fig. 6D	<|R1+ R2|>	12.8	3.82	1.08	3.05
		51.8	10.87		1.66
Fig. S6 i	<|R|>	13.7	5.7	(1)	3.98
Fig. S6 ii	<|R|>	16	1.55	(-1)	4.2
Fig. S6 iii	<|R|>	3	3.2	(0)	0.98
Fig. S6 iv	<|R|>	10.4	3.2	(1)	1