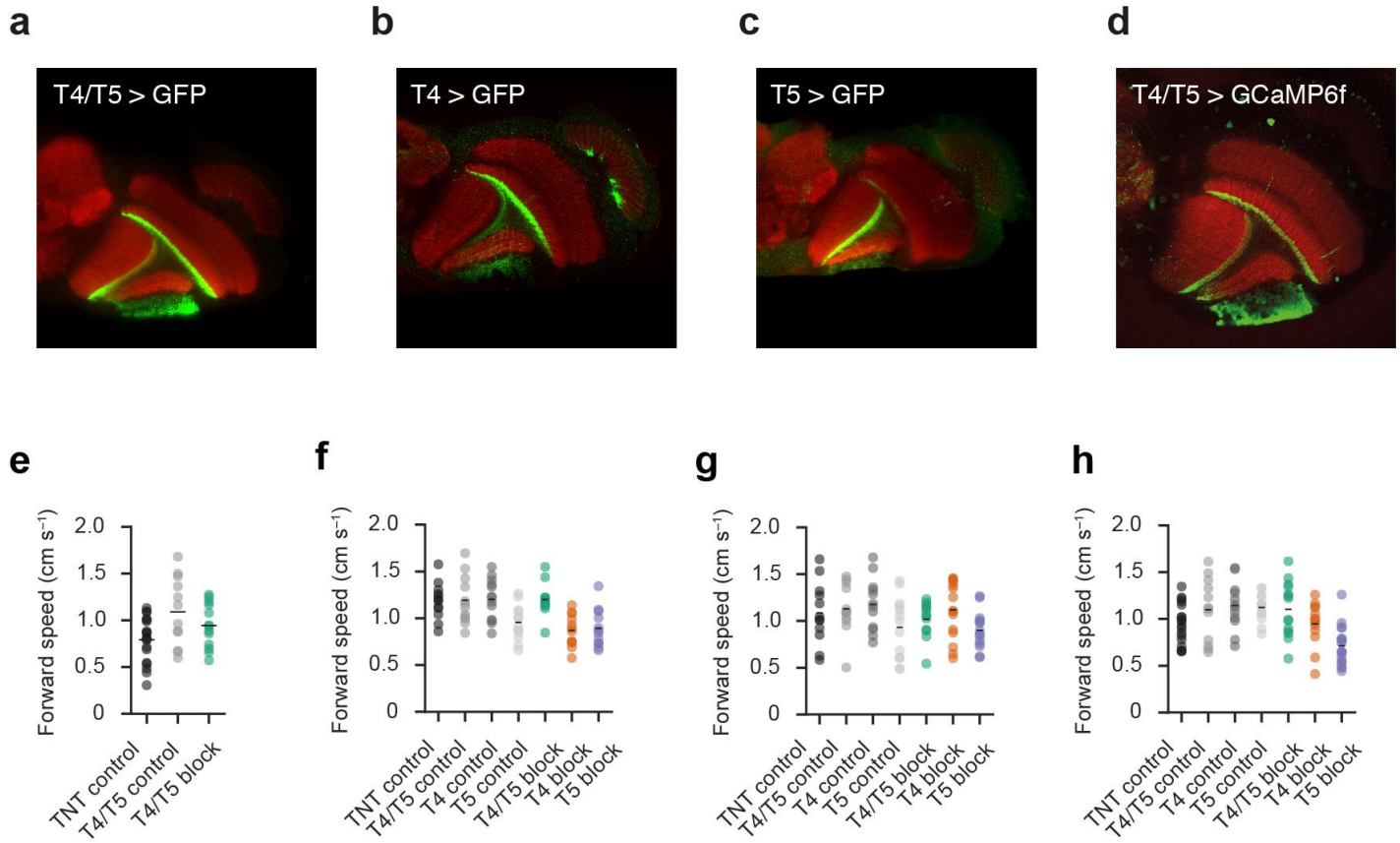
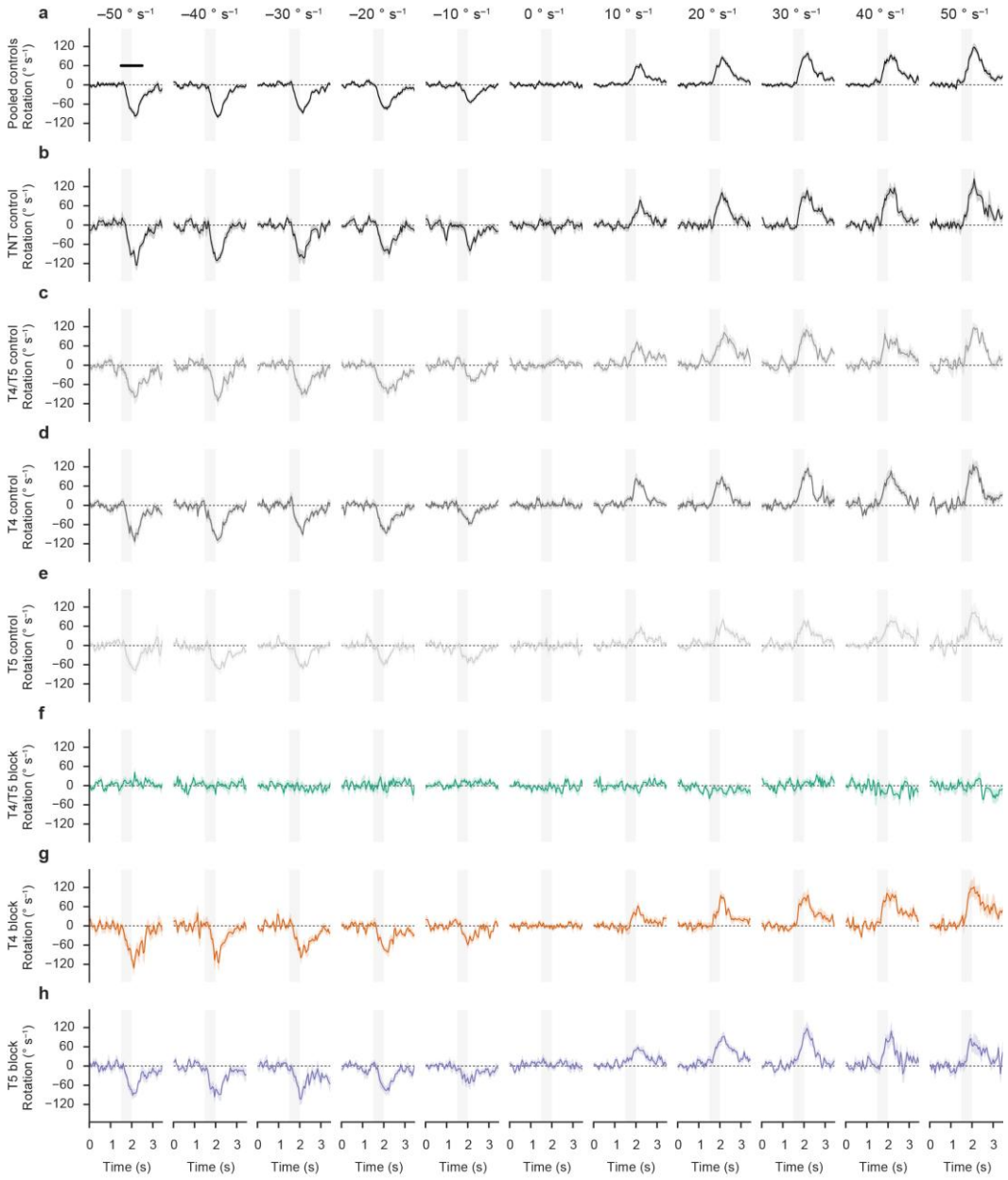


Supplementary Figure 1

Auxiliary data for Gal4 lines used throughout the study.

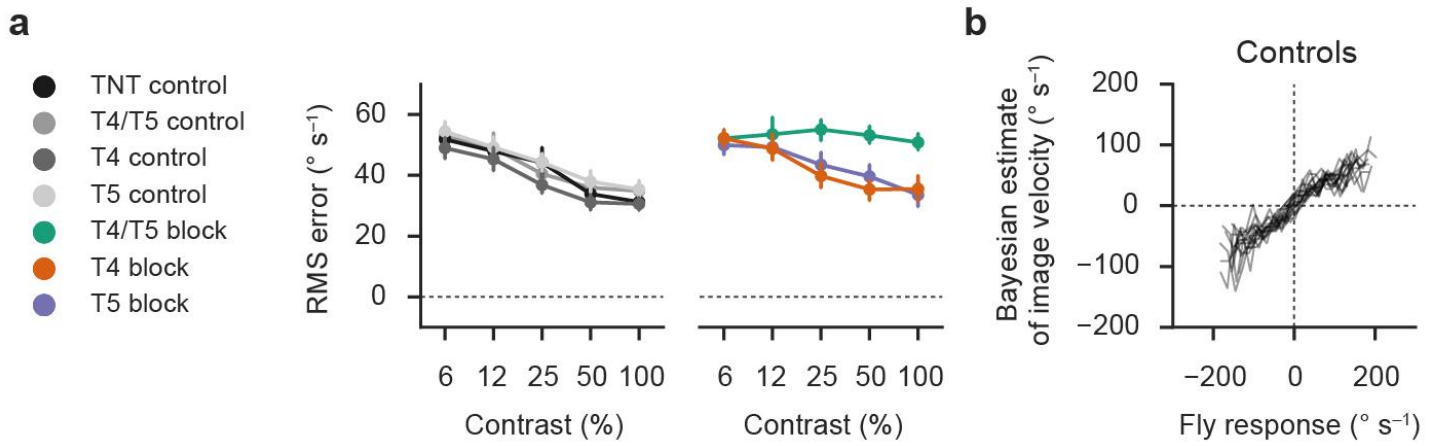
(**a-d**) UAS-mCD8GFP or UAS-GCaMP6f were driven by Gal4 driver lines used throughout the text and visualized using confocal images of the optic lobe. (**a**) GFP expression of splitGal4 line labeling T4 and T5. (**b**) GFP expression of Gal4 line labeling T4. (**c**) GFP expression of Gal4 line labeling T5. (**d**) GCaMP6f expression of combined Gal4 line labeling T4 and T5. See Online Methods for Gal4 line names and details of the immunohistochemistry procedures. (**e-h**) Locomotor integrity for each behavioral experiment was quantified as the mean forward velocity across conditions, with values close to control level indicating a general ability to respond to visual stimuli. (**e**) Walking speeds for closed-loop experiments (**Fig. 1**). (**f**) Walking speeds for open-loop experiments (**Fig. 2**). (**g**) Walking speeds for opposing edge experiments (**Fig. 4**). (**h**) Walking speeds for glider experiments (**Fig. 8**). Dots represent individual flies. Black bars mark the group mean for each genotype.



Supplementary Figure 2

Walking traces for open-loop velocity estimation experiment.

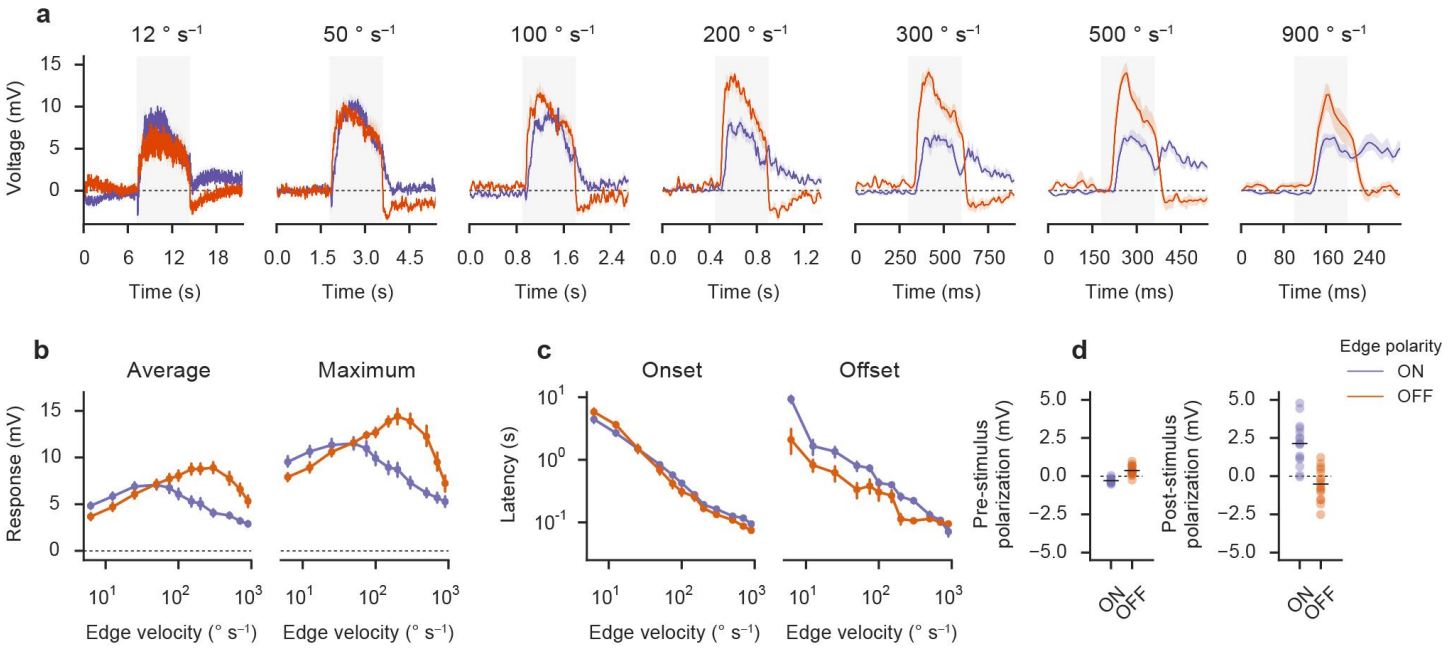
Binned response traces for all genotypes used throughout the stochastic open loop velocity estimation experiment (**Fig. 2**). In order to generate velocity-specific traces, stimulus velocities were sorted into bins spanning 5° s^{-1} centered about the value indicated above each column. The corresponding traces were then averaged for each fly. Shaded areas indicate the bootstrapped 68% confidence interval across flies (*N* as in main figure; **Fig. 2**). Nota bene, traces were not low-pass filtered and the sampling base for each fly decreases with distance from zero velocity due to the stimulus distribution. The black line in the top leftmost panel indicates the period over which we averaged in order to generate responses for main experiment (**Fig. 2**). See Online Methods for details. (**a**) Responses for pooled controls as in main experiment (**Fig. 2b**). (**b-h**) Responses for individual genotypes.



Supplementary Figure 3

Bayesian analysis of open-loop behavioral data.

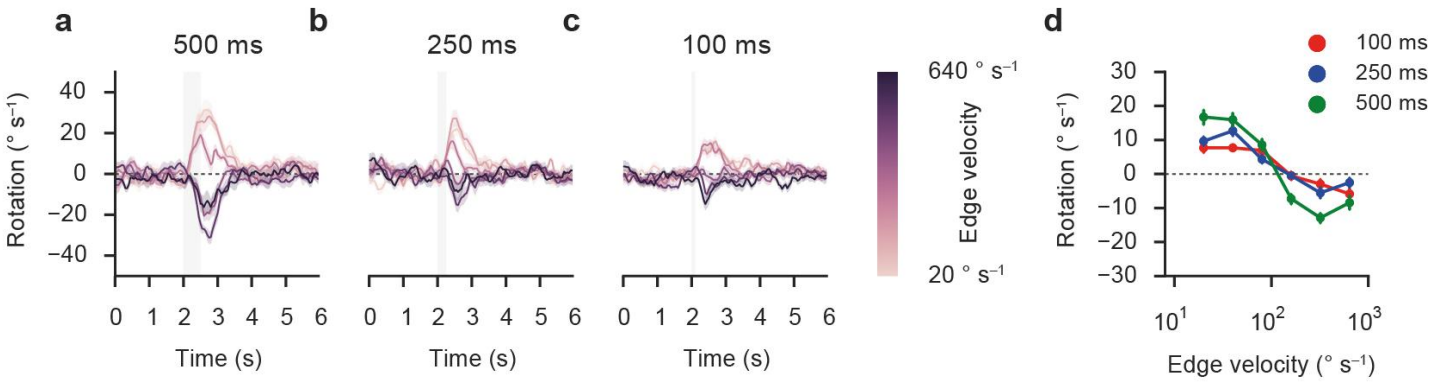
Using open-loop behavioral data (**Fig. 2**), we generated Bayesian decoders according to the procedure outlined in the Online Methods. For details about quantification and subject numbers, refer to main experiment (**Fig. 2**). (**a**) Mapping error across image contrast values, quantified as the root-mean-square error after application to the test data set. With higher contrasts, the quality of the estimate improves; this resembles results based on linear correlation. For T4/T5 block flies, the error stays flat. T4 or T5 block cannot be distinguished from wild-type behavior. (**b**) Visualization of resulting mapping functions, transforming fly responses into Bayesian estimates of input image velocity. Each line corresponds to a single fly. No significance tests were performed.



Supplementary Figure 4

Physiological edge velocity tuning for fixed starting luminance.

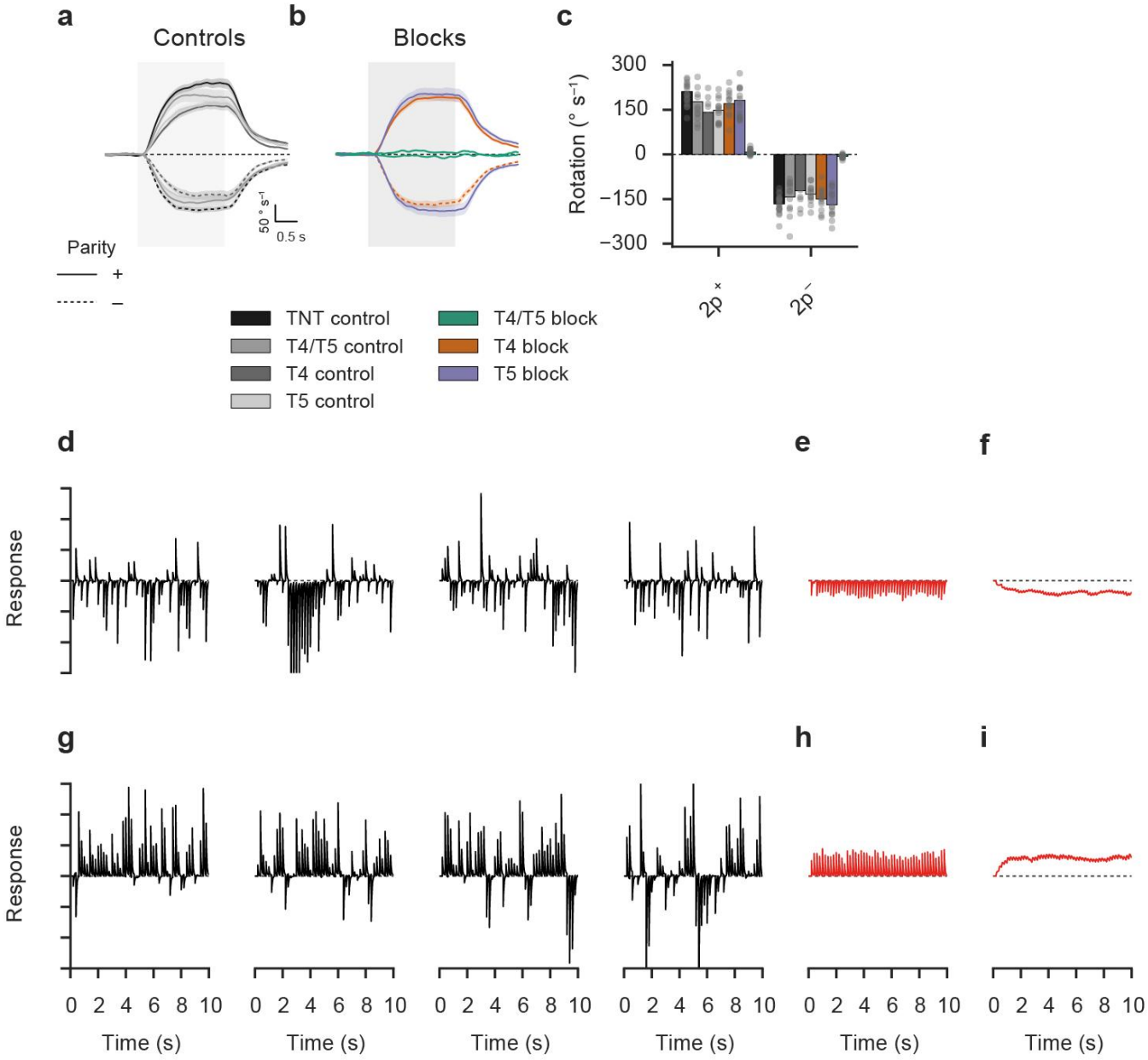
Lobula plate tangential cell responses to ON and OFF edges for equalized initial mean luminance (*N*=16 by pooling 12 vertical system/4 horizontal system cells). See legend of main experiment (**Fig. 3**) as well as Online Methods for details. (**a**) Response traces for edges moving at various velocities. Note that the timescale depends on edge velocity. (**b**) Quantification of velocity tuning. (**c**) Quantification of response dynamics (with latency being defined as the time to maximal response during stimulation for onset or time to minimal response after stimulation for offset). (**d**) Quantification of polarization before and after stimulus presentation. No significance tests were performed.



Supplementary Figure 5

Opposing edge responses for varying stimulus durations.

Presentation and quantification are analogous to main experiment (**Fig. 4**; see Online Methods and associated legend for details). Depicted flies were T4/T5 control flies. (**a-c)** Turning responses for edge pulses of 500 ms (*N*=12), 250 ms (*N*=12), and 100 ms (*N*=14) duration, respectively. (**d**) Quantification of turning responses.



Supplementary Figure 6

Extended data for higher-order motion experiments and simulations.

(**a-c**) T4 block flies and T5 block flies show 2-point glider responses at control level. (**a**) Control responses for 2-point gliders of positive or negative parity. (**b**) Block fly responses. (**c**) Summary of average turning tendency. Shaded area indicates stimulation period (see Online Methods and legend of main experiment for details; **Fig. 8**). (**d-i**) Time- and instantiation-resolved output of the asymmetric detector for converging 3-point gliders. Black traces are arbitrarily scaled detector responses for five random starting conditions of the pattern. (**d**) Single traces for positive parity. (**e**) Average time-resolved output for positive parity across 100 instantiations of the stimulus. (**f**) Low-pass filtered trace from **e** (first order with time constant of 500 ms followed by multiplicative scaling with a factor of four, approximating the behavioral response). (**g**) Single traces for negative parity. (**h**) Average time-resolved output for negative parity across 100 instantiations of the stimulus. (**i**) Low-pass filtered and scaled trace from **h** (procedure as in **f**).

Supplementary Table 1

Alias	Genotype	Experiments
T4/T5 block	w^+/w^-; UAS-TNT-E/Gal4-R59E08-AD; +/Gal4-R42F06	Figs. 1, 2, 4, 8, S1, S2, S3, S6
T4/T5 imaging	w^-; UAS-GCaMP6f; Gal4-VT25965/Gal4-VT37588	Fig. 3, S1
T4 block	w^+/w^-; UAS-TNT-E/+; +/Gal4-VT37588	Figs. 2, 4, 8, S1, S2, S3, S6
T5 block	w^+/w^-; UAS-TNT-E/+; +/Gal4-R42H07	Figs. 2, 4, 8, S1, S2, S3, S6
TNT control	w^+/w^-; UAS-TNT-E/+; +/+	Figs. 1, 2, 4, 8, S1, S2, S3, S6
T4/T5 control	w^+/w^-; +/Gal4-R59E08-AD; +/Gal4-R42F06	Figs. 1, 2, 4, 8, S1, S2, S3, S5, S6
T4 control	w^+/w^-; +/+; +/Gal4-VT37588	Figs. 2, 4, 8, S1, S2, S3, S6
T5 control	w^+/w^-; +/+; +/Gal4-R42H07	Figs. 2, 4, 8, S1, S2, S3, S6
Canton S	w^+; +/+; +/+	Figs. 3, 7, S4

Supplementary Table 2

12.5% contrast

Genotype		T4/T5 block (n=13)
UAS control	n	19
	t	9.27
	p	2.83e-10
Gal4 control	n	12
	t	11.2
	p	1.35e-9

25% contrast

Genotype		T4/T5 block (n=13)
UAS control	n	19
	t	12.2
	p	3.89e-13
Gal4 control	n	12
	t	16.4
	p	3.75e-14

50% contrast

Genotype		T4/T5 block (n=13)
UAS control	n	19
	t	14.4
	p	4.55e-13
Gal4 control	n	12
	t	13.7
	p	1.47e-12

100% contrast

Genotype		T4/T5 block (n=13)
UAS control	n	19
	t	12.9
	p	3.56e-12
Gal4 control	n	12
	t	13.9
	p	3.36e-12

Extended statistics for Fig. 1. For each contrast condition, we determined significance by comparing the block group to both control groups (UAS control and Gal4 control) using a two-tailed Student's *t* test. Blocks were declared significantly different if and only if both control groups were significantly different at a level of 0.05. For multiple comparisons, Bonferroni correction was applied. Red fields indicate significant differences after Bonferroni correction.The number indicated by n is the number of individual flies.

Supplementary Table 3

Correlation coefficient (Fig. 2d)

c = 6.25%

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-3.98	0.673	-0.862
	p	7.06e-4	0.508	0.398
Gal4 control	n	12	13	12
	t	-6.20	-1.95	-0.923
	p	3.89e-6	0.0631	0.368

c = 12.5%

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-9.15	0.968	-1.49
	p	4.05e-8	0.344	0.150
Gal4 control	n	12	13	12
	t	-14.7	-2.38	-1.57
	p	2.86e-12	0.0277	0.130

c = 25%

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-13.2	0.108	-0.545
	p	2.50e-11	0.915	0.591
Gal4 control	n	12	13	12
	t	-19.1	-2.53	0.0875
	p	7.56e-14	0.0198	0.931

c = 50%

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-31.5	-0.499	-2.02
	p	1.42e-17	0.624	0.0608
Gal4 control	n	12	13	12
	t	-28.2	-1.49	-0.832
	p	4.00e-18	0.156	0.415

c = 100%

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-24.0	-1.89	-2.25
	p	4.04e-13	0.0803	0.0362
Gal4 control	n	12	13	12
	t	-22.3	-2.17	-0.458
	p	6.25e-14	0.0495	0.652

Gain (Fig. 2e)

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-4.13	0.175	-1.34
	p	5.41e-4	0.863	0.193
Gal4 control	n	12	13	12
	t	-5.99	-1.81	-0.987
	p	1.15e-5	0.0853	0.336

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-8.66	0.0732	-1.58
	p	5.01e-7	0.942	0.129
Gal4 control	n	12	13	12
	t	-10.5	-1.55	-0.614
	p	9.04e-8	0.136	0.546

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-11.3	-0.161	-0.828
	p	4.18e-8	0.874	0.417
Gal4 control	n	12	13	12
	t	-14.4	-1.82	0.969
	p	2.00e-9	0.0810	0.344

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-19.3	-1.38	-2.35
	p	1.53e-10	0.185	0.0300
Gal4 control	n	12	13	12
	t	-17.3	-0.927	0.328
	p	7.31e-10	0.364	0.747

Genotype		T4/T5 block (n=11)	T4 block (n=12)	T5 block (n=12)
UAS control	n	12	12	12
	t	-16.0	-1.68	-2.00
	p	1.42e-9	0.110	0.0596
Gal4 control	n	12	13	12
	t	-19.2	-1.23	0.404
	p	4.77e-11	0.235	0.692

Extended statistics for Fig. 2. Test details were as in Supplementary Table 2. c denotes contrast. Red fields indicate significant differences after Bonferroni correction.The number indicated by n is the number of individual flies.

Supplementary Table 4

Feature		Mean (n=70)	Maximum (n=70)	Onset latency (n=70)	Offset latency (n=70)	Pre-stimulus polarization (n=70)	Post-stimulus polarization (n=70)
ON vs. OFF	t	-7.30	-5.50	5.18	5.63	-17.2	11.1
	p	3.76e-10	6.13e-7	2.13e-6	3.63e-7	1.12e-26	6.10e-17

Extended statistics for Fig. 3. We compared response features between ON and OFF edge presentation. Responses were always averaged across velocities and then tested using two-tailed Student's *t* tests at a significance level of 0.05. Red fields indicate significant differences. The number indicated by n is the number of individual cells pooled from vertical and horizontal system cells.

Supplementary Table 5

Difference from zero

v = 20 °/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	6.36	6.64	5.57	4.90
	p	5.34e-5	2.39e-5	1.67e-4	3.65e-4

v = 40 °/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	4.77	5.88	5.33	6.36
	p	5.77e-4	7.51e-5	2.40e-4	3.60e-5

v = 80 °/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	0.703	-0.765	-1.44	0.249
	p	0.497	0.459	0.178	0.808

v = 160 °/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	-4.57	-8.74	-5.78	-7.81
	p	8.02e-4	1.50e-6	1.23e-4	4.78e-6

v = 320 °/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	-5.67	-7.97	-5.44	-11.1
	p	1.45e-4	3.93e-6	2.04e-4	1.14e-7

v = 640°/s

Genotype		TNT control (n=12)	T4/T5 control (n=13)	T4 control (n=12)	T5 control (n=13)
versus 0	t	-2.50	-1.54	-1.15	-2.64
	p	0.0297	0.149	0.274	0.0216

Difference from control

v = 20 °/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	-2.66	-14.9	6.80
	p	0.0143	5.04e-13	1.32e-6
Gal4 control	n	13	12	13
	t	-2.08	-13.5	8.60
	p	0.0502	2.15e-11	1.27e-7

v = 40 °/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	-2.20	-13.1	8.85
	p	0.0399	4.12e-12	2.80e-8
Gal4 control	n	13	12	13
	t	-2.90	-12.4	9.65
	p	8.33e-3	1.26e-10	3.65e-8

v = 80 °/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	-0.324	-11.8	11.3
	p	0.749	1.07e-11	4.30e-10
Gal4 control	n	13	12	13
	t	0.921	-9.70	12.7
	p	0.367	6.14e-10	1.86e-9

v = 160 °/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	3.18	-6.82	12.3
	p	7.32e-3	4.96e-7	7.74e-12
Gal4 control	n	13	12	13
	t	6.02	-5.98	15.0
	p	1.29e-5	3.56e-6	9.84e-12

v = 320 °/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	3.99	-6.06	14.2
	p	9.22e-4	2.95e-6	1.16e-12
Gal4 control	n	13	12	13
	t	4.66	-6.45	19.0
	p	1.10e-4	1.24e-6	7.84e-15

v = 640°/s

Genotype		T4/T5 block (n=12)	T4 block (n=15)	T5 block (n=14)
UAS control	n	12	12	12
	t	2.25	-2.21	4.54
	p	0.0439	0.0368	1.89e-4
Gal4 control	n	13	12	13
	t	1.18	-4.80	5.05
	p	0.256	1.06e-4	3.73e-5

Extended statistics for Fig. 4. For each velocity condition, we determined significance by comparing control groups to zero or block groups to both corresponding control groups (UAS control and Gal4 control) using a two-tailed Student's t test. Blocks were declared significantly different if and only if both control groups were significantly different at a significance level of 0.05. v denotes velocity. For multiple comparisons, Bonferroni correction was applied. Red fields indicate significant differences after Bonferroni correction.The number indicated by n is the number of individual flies.

Supplementary Table 6

Stimulus		Random (n=16)	3p/conv/+ (n=16)	3p/conv/- (n=16)	3p/div/+ (n=16)	3p/div/- (n=16)
versus 0	t	-0.426	-2.33	18.4	-5.44	5.73
	p	0.676	0.0341	1.02e-11	6.89e-5	3.98e-5

Extended statistics for Fig. 7. We compared glider voltage responses to zero. Responses were tested using two-tailed Student's *t* tests at a significance level of 0.05. Red fields indicate significant differences.The number indicated by n is the number of individual cells pooled across cells from the horizontal and vertical systems.

Supplementary Table 7

Positive parity

2-point

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	-16.2	-2.41	-1.33
	p	2.17e-12	0.0228	0.194
Gal4 control	n	12	12	12
	t	-7.93	1.82	1.54
	p	5.91e-6	0.0814	0.136

3-point/conv.

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	7.82	16.7	-5.85
	p	2.73e-8	4.72e-16	1.85e-6
Gal4 control	n	12	12	12
	t	8.57	19.3	-5.49
	p	2.56e-7	2.39e-15	1.11e-5

3-point/div.

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	8.58	-3.34	10.8
	p	2.83e-8	2.32e-3	8.68e-12
Gal4 control	n	12	12	12
	t	5.36	-0.354	11.4
	p	1.85e-4	0.727	7.10e-10

Negative parity

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	21.3	1.29	-0.169
	p	1.43e-14	0.211	0.867
Gal4 control	n	12	12	12
	t	8.08	-1.79	-1.91
	p	5.28e-6	0.0869	0.0679

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	-6.44	-14.0	6.83
	p	3.12e-6	1.30e-13	2.01e-7
Gal4 control	n	12	12	12
	t	-12.4	-23.7	7.00
	p	4.45e-10	1.88e-15	1.00e-6

Genotype		T4/T5 block (n=14)	T4 block (n=13)	T5 block (n=17)
UAS control	n	18	18	18
	t	-9.25	4.52	-8.51
	p	8.57e-9	1.01e-4	1.36e-9
Gal4 control	n	12	12	12
	t	-6.82	0.991	-9.76
	p	2.12e-5	0.335	3.33e-10

Extended statistics for Fig. 8. Test details were as in Supplementary Table 2. Red fields indicate significant differences after Bonferroni correction.The number indicated by n is the number of individual flies.