Article

Multilevel visual motion opponency in *Drosophila*

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Supplementary Table 1. List of all experimental genotypes

Figure	Short Name	Genotype
Figure 1b-c	T4/T5 > ChR2	norpA[7]; UAS-ChR2-H134R; R42F06-Gal4
	VS > ChR2	w+; UAS-ChR2-H134R; R24E09-Gal4
Figure 1d,h	VS > ArcLight	w+; UAS-ArcLight/ +; R24E09-Gal4/ +
Figure 1e,i	LPi3-4 > ArcLight	w+; UAS-ArcLight/ +; R20D01-Gal4/ +
Figure 1f,j	LPi4-3 > ArcLight	w+; UAS-ArcLight/ +; R38G02-Gal4/ +
Figure 1g,k	T4c > ArcLight	w+/ w-; VT15785-AD/ UAS-ArcLight; VT50384-DBD/ +
Figure 2a-c	VS > ArcLight (control)	w+/ w-; UAS-ArcLight/ +; R24E09-Gal4/ lexAop-TNT
	VS > ArcLight (T4/T5c block)	w+/ w-; UAS-ArcLight/ +; R24E09-Gal4/ VT50384-lexA, lexAop-TNT
Figure 2d-f	LPi3-4 > ArcLight (control)	w+/ w-; UAS-ArcLight/ +; R20D01-Gal4/ lexAop-TNT
	LPi3-4 > ArcLight (T4/T5c block)	w+/ w-; UAS-ArcLight/ +; R20D01-Gal4/ VT50384-lexA, lexAop-TNT
Figure 2g-i	LPi4-3 > ArcLight (control)	w+/ w-; UAS-ArcLight/ +; R38G02-Gal4/ lexAop-TNT
	LPi4-3 > ArcLight (T4/T5c block)	w+/ w-; UAS-ArcLight/ +; R38G02-Gal4/ VT50384-lexA, lexAop-TNT
Figure 3i-j	wildtype	Canton S
Figure 4a	VS > GluClα-GFP	w-; VT58487-AD/ UAS-GluClα-GFP; R35G07-DBD/ UAS-tdTomato
Figure 4b	LPi3-4 > GluClα-GFP	w-; VT61221-AD/ UAS-GluClα-GFP; VT45575-DBD/ UAS-tdTomato
Figure 4c	LPi4-3 > GluClα-GFP	w-; R38G02-AD/ UAS-GluClα-GFP; R24A07-DBD/ UAS-tdTomato
Figure 4d	T4/T5c > GluClα-GFP	w-; UAS-GluClα-GFP/+; VT50384-Gal4/ UAS-tdTomato
Figure 4e-g	LPi4-3 > ArcLight (RNAi control)	w+; UAS-ArcLight/ UAS-GluRIA-RNAi; R38G02-Gal4/ +
	LPi4-3 > ArcLight (GluClα RNAi)	w+; UAS-ArcLight/ UAS-GluClα-RNAi; R38G02-Gal4/ +
Figure 5a-b	TNT control	w+/ w-; +; lexAop-TNT/ +
	T4/T5c block	w+/ w-; +; VT50384-lexA, lexAop-TNT/ +
Figure 5c-d	LPi4-3 > GC6f (control)	w+/ w-; UAS-GCaMP6f/ +; R38G02-Gal4/ lexAop-TNT
	LPi4-3 > GC6f (T4/T5c block)	w+/ w-; UAS-GCaMP6f/ +; R38G02-Gal4/ VT50384-lexA, lexAop-TNT
Figure 5e-f	T4/T5c > GC6m (control)	w+/ w-; UAS-TNT/ +; VT50384-lexA, lexAop-GCaMP6m/ +
	T4/T5c > GC6m (LPi4-3 block)	w+/ w-; UAS-TNT/ R38G02-AD; VT50384-lexA, lexAop-GCaMP6m/ R24A07-DBD
Figure 6a-e	VS > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R24E09-Gal4
	LPi3-4 > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R20D01-Gal4
	LPi4-3 > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R38G02-Gal4
	T4/T5c > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ VT50384-Gal4
Figure 6g-h	T4/T5c > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ VT50384-Gal4
	T4/T5 > GC6f (control)	w+/ w-; UAS-GCaMP6f/ +; R42F06-Gal4/ +
	T4/T5 > GC6f (T4/T5c block)	w+/ w-; UAS-GCaMP6f/ +; R42F06-Gal4/ VT50384-lexA, lexAop-TNT
Figure 7c-h	wildtype	Canton S
Ext. Fig. 1	VS > ArcLight	w+; UAS-ArcLight/ +; R24E09-Gal4/ +
Ext. Fig. 2	VS > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R24E09-Gal4
	LPi3-4 > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R20D01-Gal4
	LPi4-3 > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R38G02-Gal4
	T4c > GC6f	w+; VT15785-AD/ UAS-GCaMP6f; VT50384-DBD/ UAS-GCaMP6f
Ext. Fig. 3c	wildtype	Canton S
Ext. Fig. 4a	LPi3-4 > nAChRα7-GFP	w-; VT61221-AD/ UAS-tdTomato; VT45575-DBD/ UAS-nAChRα7-GFP
Ext. Fig. 4b	LPi4-3 > nAChRα7-GFP	w-; R38G02-AD/ UAS-tdTomato; R24A07-DBD/ UAS-nAChRα7-GFP
Ext. Fig. 5a,b	LPi4-3 > GC6f	w+/ w-; UAS-GCaMP6f/ +; UAS-GCaMP6f/ R38G02-Gal4
Ext. Fig. 5c,d	T4/T5c > GC6f	w+; UAS-GCaMP6f/+; VT50384-Gal4/+
Ext. Fig. 5e,f	T4c > GC6f	w+; VT15785-AD/ UAS-GCaMP6f; VT50384-DBD/ UAS-GCaMP6f
Ext. Fig. 5g,h	T4c > ArcLight	w+/ w-; VT15785-AD/ UAS-ArcLight; VT50384-DBD/ +
Ext. Fig. 6	T4c > GC6f	w+; VT15785-AD/ UAS-GCaMP6f; VT50384-DBD/ UAS-GCaMP6f
Ext. Fig. 8a-c	wildtype	Canton S

Supplementary Table 2. Statistical analysis

Figure	Comparison	Statistical test	p-value	
Figure 1l	Correlation: LPi3-4 – LPi4-3	Wald test	1.1407×10^{-8}	***
Figure 2c	MOI: VS (ctrl) – VS (T4/T5c block)	Welch's t-test	8.1344×10^{-4}	***
	LDir: VS (ctrl) – VS (T4/T5c block)	Welch's t-test	5.3057×10^{-4}	***
Figure 2f	MOI: LPi3-4 (ctrl) – LPi3-4 (T4/T5c block)	Welch's t-test	1.2751×10^{-3}	**
	LDir: LPi3-4 (ctrl) – LPi3-4 (T4/T5c block)	Welch's t-test	3.1044×10^{-3}	**
Figure 2i	MOI: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Welch's t-test	2.8558×10^{-5}	***
	LDir: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Welch's t-test	6.5523×10^{-3}	**
Figure 3j	PD local – ND local	Wilcoxon signed-rank test	0.32587	ns
Figure 4g	MOI: LPi4-3 (ctrl) – LPi4-3 (GluClα-RNAi)	Welch's t-test	9.5774×10^{-5}	***
	LDir: LPi4-3 (ctrl) – LPi4-3 (GluClα-RNAi)	Welch's t-test	0.01865	*
Figure 5b	MSI: VS (ctrl) – VS (T4/T5c block)	Student's t-test	1.0934×10^{-5}	***
Figure 5d	PD: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Welch's t-test	0.40606	ns
	ND: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Welch's t-test	9.0649×10^{-3}	**
	TM: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Welch's t-test	4.9512×10^{-4}	***
	MSI: LPi4-3 (ctrl) – LPi4-3 (T4/T5c block)	Student's t-test	7.7754×10^{-5}	***
Figure 5f	PD: T4/T5c (ctrl) – T4/T5c (LPi4-3 block)	Welch's t-test	0.82811	ns
	ND: T4/T5c (ctrl) – T4/T5c (LPi4-3 block)	Welch's t-test	0.44474	ns
	TM: T4/T5c (ctrl) – T4/T5c (LPi4-3 block)	Welch's t-test	1.1206×10^{-3}	**
	MSI: T4/T5c (ctrl) – T4/T5c (LPi4-3 block)	Student's t-test	2.5241×10^{-5}	***
Figure 6d	RF area: VS – LPi3-4	Mann-Whitney U-test, Holm-corrected	2.3722×10^{-16}	***
	RF area: VS – LPi4-3	Mann-Whitney U-test, Holm-corrected	3.3756×10^{-16}	***
	RF area: LPi3-4 – T4/T5c	Mann-Whitney U-test, Holm-corrected	2.0986×10^{-65}	***
	RF area: LPi4-3 – T4/T5c	Mann-Whitney U-test, Holm-corrected	1.5924×10^{-61}	***
Figure 6e	tau: VS – LPi3-4	Mann-Whitney U-test, Holm-corrected	3.3009×10^{-12}	***
	tau: VS – LPi4-3	Mann-Whitney U-test, Holm-corrected	6.4809×10^{-13}	***
	tau: LPi3-4 – T4/T5c	Mann-Whitney U-test, Holm-corrected	1.1806×10^{-31}	***
	tau: LPi4-3 – T4/T5c	Mann-Whitney U-test, Holm-corrected	7.7733×10^{-18}	***
Figure 6h	T4/T5c	Rayleigh z-test	0.02834	*
	T4/T5d	Rayleigh z-test	0.02287	*
	T4/T5d (T4/T5c block)	Rayleigh z-test	0.66186	ns
Figure 7g	Model with inhibition: local – global	paired Student's t-test	1.3108×10^{-169}	***
	Model w/o inhibition: local – global	paired Student's t-test	0.53761	ns
	Experiment: local – global	Wilcoxon signed-rank test	0.01738	*
Figure 7h	Model with inhibition: PD/ND sum – local	paired Student's t-test	9.5638×10^{-252}	***
	Model w/o inhibition: PD/ND sum – local	paired Student's t-test	6.6408×10^{-175}	***
	Experiment: PD/ND sum – local	Wilcoxon signed-rank test	0.00228	**
Figure 7l	Correlation: Diff. gain – diff. conduct. (PD)	Wald test	1.3917×10^{-3}	**
	Correlation: Diff. gain – diff. conduct. (ND)	Wald test	3.6991×10^{-3}	**
Ext. Fig. 1c	Correlation: VS (ephys) – VS (ArcLight)	Wald test	6.2240×10^{-7}	***
Ext. Fig. 1d	MOI: VS (ephys) – VS (ArcLight)	Student's t-test	0.22568	ns
	LDir: VS (ephys) – VS (ArcLight)	Student's t-test	0.58362	ns
Ext. Fig. 2k	MOI: VS (ArcLight) – VS (GCaMP)	Student's t-test	1.3514×10^{-7}	***
	MOI: LPi3-4 (ArcLight) – LPi3-4 (GCaMP)	Student's t-test	2.9343×10^{-6}	***
	MOI: LPi4-3 (ArcLight) – LPi4-3 (GCaMP)	Student's t-test	7.6028×10^{-7}	***
	MOI: T4c (ArcLight) – T4c (GCaMP)	Student's t-test	0.90496	ns
Ext. Fig. 2l	LDir: VS (ArcLight) – VS (GCaMP)	Student's t-test	2.5110×10^{-4}	***
	LDir: LPi3-4 (ArcLight) – LPi3-4 (GCaMP)	Student's t-test	2.9359×10^{-6}	***
	LDir: LPi4-3 (ArcLight) – LPi4-3 (GCaMP)	Student's t-test	1.7654×10^{-7}	***
	LDir: T4c (ArcLight) – T4c (GCaMP)	Student's t-test	0.13371	ns
Ext. Fig. 2m	PD: VS (ArcLight) – VS (GCaMP)	Student's t-test	0.50524	ns
	PD: LPi3-4 (ArcLight) – LPi3-4 (GCaMP)	Student's t-test	0.79030	ns
	PD: LPi4-3 (ArcLight) – LPi4-3 (GCaMP)	Student's t-test	0.18735	ns
	PD: T4c (ArcLight) – T4c (GCaMP)	Student's t-test	0.28244	ns
Ext. Fig. 3c	PD local – ND local	Wilcoxon signed-rank test	0.32587	ns
	PD global – ND global	Wilcoxon signed-rank test	0.64166	ns
Ext. Fig. 6d	PD (90°) sine wave: dend – axon	paired Student's t-test	0.06152	ns
	ND (270°) sine wave: dend – axon	paired Student's t-test	3.2141×10^{-3}	**
Ext. Fig. 6e	MOI sine wave: dend – axon	paired Student's t-test	4.0932×10^{-3}	**
	LDir sine wave: dend – axon	paired Student's t-test	0.01250	*
	PrefDir sine wave: dend – axon	paired Student's t-test	0.05741	ns
Ext. Fig. 6h	PD (90°) dot motion: dend – axon	paired Student's t-test	0.82709	ns
	ND (270°) dot motion: dend – axon	paired Student's t-test	2.7845×10^{-3}	**
Ext. Fig. 6i	MOI dot motion: dend – axon	paired Student's t-test	0.45529	ns

	LDir dot motion: dend – axon	paired Student's t-test	0.14394	ns
	PrefDir dot motion: dend – axon	paired Student's t-test	0.25177	ns
Ext. Fig. 6l	PD (90°) edges: dend – axon	Student's t-test	0.37722	ns
	ND (270°) edges: dend – axon	Student's t-test	0.59320	ns
Ext. Fig. 6m	MOI edges: dend – axon	Student's t-test	0.45927	ns
	LDir edges: dend – axon	Student's t-test	0.05213	ns
	PrefDir edges: dend – axon	Student's t-test	0.01100	*
Ext. Fig. 8c	Model with inhibition: PD/ND sum – global	paired Student's t-test	1.0	ns
	Model w/o inhibition: PD/ND sum – global	paired Student's t-test	1.0	ns
	Experiment: PD/ND sum – global	Wilcoxon signed-rank test	0.46300	ns

Supplementary Table 3. Sources of experimental animals, reagents and software

Resource	Source	Identifier
Antibodies		
anti-brp (nc82) mouse monoclonal antibody	DSHB	RRID: AB_2314866
anti-GFP chicken polyclonal antibody	Rockland	Cat# 600-901-215S
anti-DsRed rabbit polyclonal antibody	Takara Bio	RRID: AB_10013483
donkey anti-chicken-Alexa-488	Jackson Immuno Research	Cat# 703-545-155
goat anti-rabbit-Alexa-568	Invitogen	Cat# A-11011
goat anti-mouse-Alexa-647	Invitrogen	Cat# A32728
Experimental animals		
w-; +; R20D01-Gal4 (LPi3-4 cells)	BDSC	RRID: BDSC_48889
w-; VT61221-AD; VT45575-DBD (LPi3-4 cells)	BDSC	RRID: BDSC_86823
w-; +; R24E09-Gal4 (VS/HS cells)	BDSC	RRID: BDSC_49083
w-; VT58487-AD; R35G07-DBD (VS/HS cells)	BDSC	RRID: BDSC_86824
w-; +; R38G02-Gal4 (LPi4-3 cells)	BDSC	RRID: BDSC_50016
w-; R38G02-AD; R24A07-DBD (LPi4-3 cells)	BDSC	RRID: BDSC_76004
w-; +; R42F06-Gal4 (T4/T5 cells)	BDSC	RRID: BDSC_41253
w-; VT15785-AD; VT50384-DBD (T4c cells)	Tirian et al., bioRxiv, 2017	N/A
w-; +; VT50384-Gal4/Tm6b (T4/T5c cells)	Tirian et al., bioRxiv, 2017	N/A
w-; +; VT50384-lexA/Tm6b (T4/T5c cells)	Tirian et al., bioRxiv, 2017	N/A
w-; 20xUAS-IVS-GCaMP6f; +	BDSC	RRID: BDSC_42747
w-; +; 20xUAS-IVS-GCaMP6f	BDSC	RRID: BDSC_52869
w-; +; 13xlexAop-IVS-GCaMP6m	BDSC	RRID: BDSC_44277
w-; UAS-ArcLight; +	BDSC	RRID: BDSC_51057
w-; +; UAS-ArcLight	BDSC	RRID: BDSC_51056
w-; UAS-TNT; +	BDSC	RRID: BDSC_28838
w-; +; 13xlexAop-IVS-TNT:HA	Karuppudurai et al., Neuron, 2014	N/A
w+; UAS-GluClα-RNAi; +	BDSC	RRID: BDSC_53356
w+; UAS-GluRIA-RNAi; +	BDSC	RRID: BDSC_40844
w-; UAS-GluClα-GFP; +	BDSC	RRID: BDSC_92151
w-; UAS-Rdl-GFP; +	BDSC	RRID: BDSC_92150
w-; UAS-nAChR-Dα7-GFP; +	Fendl et al., eLife, 2020	N/A
w-; +; UAS-tdTomato	BDSC	RRID: BDSC_32221
norpA[7]; +; +	BDSC	RRID: BDSC_5685
w-; UAS-ChR2-H134R-mCherry; +	Mauss et al., JNeurosci, 2014	N/A
Software and algorithms		
MATLAB	MathWorks	mathworks.com
Python 2.7.15 and 3.8.8	Python Software Foundation	python.org
ScanImage 3.8	Vidrio Technologies	scanimage.vidriotechnologies.com
ImageJ/Fiji	National Institutes of Health	imagej.nih.gov/ij/
neuPrint	Janelia Research Campus	neuprint.janelia.org
Deposited data		
Data and code for this paper	G-Node GIN	doi: 10.12751/g-node.7v3pe6