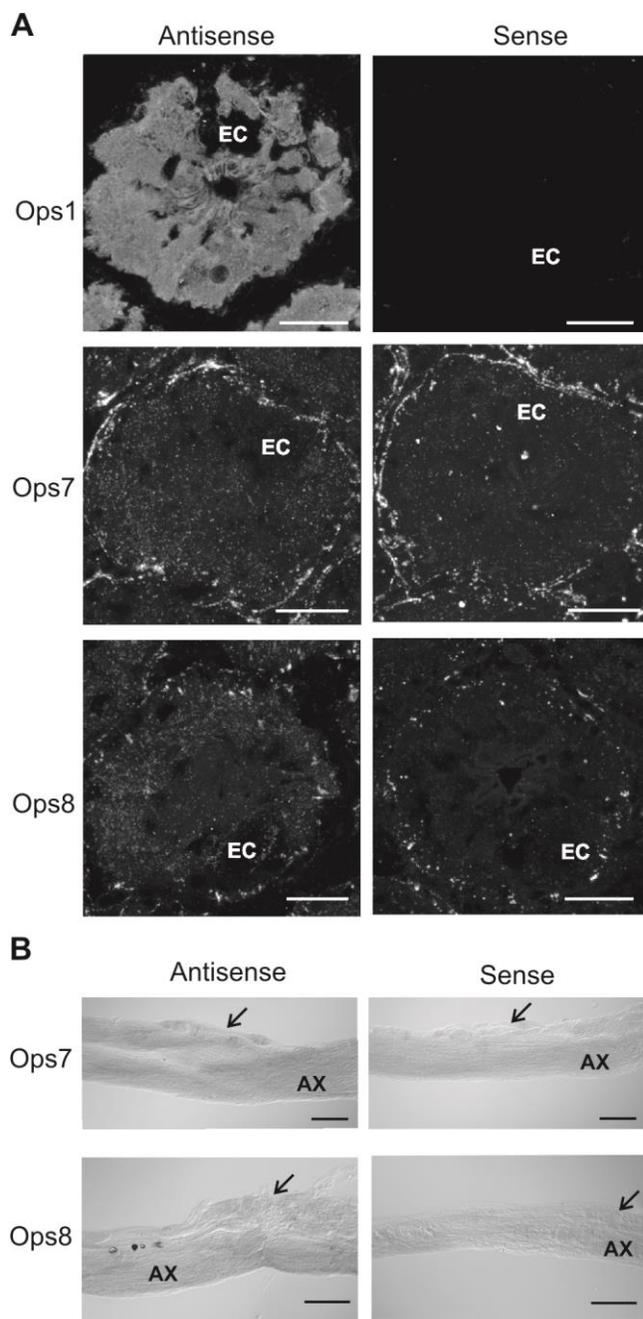


Supplemental Figure 1. LpOps6-ir is not detected in rhabdoms of LE reticular cells or small or giant photoreceptors in VE. A. Maximum projections of cross sections of LE (7-11 μm stacks) incubated with anti- $G_q\alpha$ (1:1000 dilution) to show rhabdoms and anti-LpOps6 (1:25 dilution) or anti-LpOps6 (1:25 dilution) that had been preincubated with antigen (Ops6 -Abs). Scale, 50 μm. B. Maximum projections of small ventral photoreceptors (10-14 μm stacks) obtained from whole mounts of ventral optic nerves incubated with anti- $G_q\alpha$ (1:500) to show rhabdoms and anti-LpOps6 (1:25) or anti-LpOps6 that had been preincubated with antigen (Ops6-Abs). Upper panel: a small photoreceptor with an internal rhabdom. Lower panel: a small photoreceptor with an external rhabdom. Scale, 10 μm. C. Maximum projections (8-11 μm stacks) of giant ventral photoreceptors immunostained as described in B. Scale, 25 μm. Each set of images was collected during a single confocal setting using identical settings.



Supplemental Figure 2. Transcripts encoding LpOps7 and 8 are not detected in LE retinal cells or eccentric cells or in VE photoreceptors. A. Cross sections of daytime LEs were incubated in parallel with sense and antisense probes for LpOps1-2, 7 and 8. Maximum projections (7-11 μm stacks) of optical images are shown. Each pair of images was collected during the same confocal session using identical settings. The antisense probe for LpOps1-2 labeled retinal cells but not eccentric cell bodies (EC). No label was observed over photoreceptors with the sense probe for LpOps1-2. Antisense probes for LpOps7 and 8 did not label retinal cells or eccentric cell bodies. Antisense and sense probes both labeled glia surrounding ommatidia; therefore this label is considered non-specific. Scale, 50 μm . B. Whole mounts of ventral eye nerves were incubated with sense and antisense probes for LpOps7 and 8. No label was detected in photoreceptor cells bodies with either probe. Arrows, clusters of photoreceptor cell bodies; Ax, axon. Scale, 200 μm .

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LpPerOps1 MASSTELGSVDLNMSLGETPEFOADKDTYLVDSTFPTSTHKA VGIYLVIVGILGTFGNCIIITMFIRFRLLTPNLLLI 80
LpPerOps2 --TSNEFGSVNLNKSKNETFEISIGDVFLAMDAYFPPSTHIVVGIYLVIVGILGTIGNCAIITMFIRFRALTPPSLLLI 78

LpPerOps1 LAVSDLGHIIFGFPPSASSSFANRWLFNEGCCQYARMGFLFGSAHIGV LALLGLDRYLITCRIDFRKLT-----YK 154
LpPerOps2 NLAASDLGICL-----RWLFBYICCOLYAFTSFLFGSAHIGV LALLGLDRYMMTCKIDL SKWVHSIFYSRYR 145

LpPerOps1 RYCOMICAVVWYAFWVSMPLIGW-----RYGPE SITTCIDWRHNDGSYKSFILVYFVLGFLVPELLIAI 222
LpPerOps2 RYLCIICCV AYAFWAMMPLGWGRFLVIVLLSEFNRYGP LSIATCIDWRHND SKYKSFILAYFVLGFLVPELLIAI 225

LpPerOps1 CYFNHARQLSVKFWAPSLRSA CDQWANERNVTMMCLVIVITFVVSWSFPYAHVCLWTVFKPPESTVFSVLTLPPLFAKAS 302
LpPerOps2 CYYKARCOLNLKPFPLHLHSTTCQDQWANERNVTLVCLIVVIAFLVAWTPYATLCLWTVFRPEFTVPPYFTLMPPLFAKAS 305

LpPerOps1 TVFNPIIYYLTNPRLRMGIIATITCSCELEGEIIP-----VSSNPEATPEHESI 352
LpPerOps2 AVFNPIIYYVANPRLRMGIRALKCSDDFNGMBENNPEALSEIROVPPNPEALLEIHOE- 365

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Supplemental Figure 3. LpPerOps1 and 2 may be products of a recent gene duplication.

Clustal W alignment of the amino acid sequences of LpPerOps1, predicted from cDNA sequence cloned from VE and CNS and LpPerOps2 predicted from an assembly of the *Limulus* genome (<http://ftp.ncbi.nlm.nih.gov/genbank/genomes/Eukaryotes/invertebrates/>; scaffold 1009, position 135709-135885; 150733-150855; 151959-152129; 152328-152408; 155132-155407; 156180-156494). The sequences are 57% identical and 67% similar. LpPerOps2 transcripts were not detected in ME, LE or VE cDNA.

Table S1. Primers used in this study

Oligo name	5' to 3'
LpOps6-F1	ATGACTGGCTGGAATCCCTCAC
LpOps6-R1	TCAGGGAGATGATTCCGGA
LpOps7-F1	TCTACTACCATGGTTGAGTGGAATC
LpOps7-R1	GCTTCTGTCGTCGAATTGTTTTTCTC
LpOps8-F1	ATGTTAGACATTATCTCCTCCTTCTCCC
LpOps8-R1	TAAAGTAGAAATCTTAAAAGGCAGTTG GA
LpPerOps1-F1	CCGCTCAGACCCTTCCTGTT
LpPerOps1-F2	TCGCCATCTGCTATTTCAAC
LpPerOps1-F3	GTCTAGTGATTGTTATCACTTTCGTG
LpPerOps1-F4	CTCTTCGGTTTCCCATTCTCCGC
LpPerOps1-F5	GCCTCCTTCTACAGTTCCTTCCG
LpPerOps1-F6	GGTGACAGCAGCGGCAGAT
LpPerOps1-R1	TGTGTTTCGGGTGTGGC
LpPerOps1-R2	GTTGAAATAGCAGATGGCGA
LpPerOps1-R3	CACGAAAGTGATAACAATCACTAGAC
LpPerOps1-R4	GCGGAGAATGGGAAACCGAAGAG
LpPerOps1-R5	CATTAATGAGCCACCTAAATACATGCTC
LpPerOps2-F4	TTTTCGAGCTTTAACCACGCCAACAAG
LpPerOps2-R6	AATAAGGGGGAACAGTAAATGGAGGTCGG

Table S2. Accession numbers of opsins used to construct Figure 2

LpOps6	KM538950	<i>Limulus polyphemus</i>
LpOps7	KM538951	<i>Limulus polyphemus</i>
LpOps8	KM538952	<i>Limulus polyphemus</i>
Spider Rh1	AB251846	<i>Hasarius adansoni</i>
	BAG14334.1	<i>Plexippus Paykulli</i>
	HF549177	<i>Cupiennius salei</i>
LpOps1	L03781	<i>Limulus polyphemus</i>
LpOps2	L03782.1	<i>Limulus polyphemus</i>
Spider Rh2	AB251847	<i>Hasarius adansoni</i>
	BAG1433401	<i>Plexippus paykulli</i>
	HF549178	<i>Cupiennius salei</i>
Insect	O01668	<i>Drosophila melanogaster Rh6</i>
	AAA28733	<i>Drosophila melanogaster Rh1</i>
	P08099	<i>Drosophila melanogaster Rh2</i>
	NM00101163902	<i>Apis mellifera</i>
	NP001071293.1	<i>Apis mellifera</i>
Crustacean	S53494	<i>Procambarus clarkia</i>
	GQ221725	<i>Neogonodactylus oersterdii</i> isolate #1
	ABG37008.1	<i>Neogonodactylus oersterdii</i>
	ABG37009.1	<i>Neogonodactylus oersterdii</i>
	DQ852587	<i>Homarus gammarus</i> clone KC2162-c1
	DQ852581	<i>Holmesimysis costata</i> clone MP-Hcos-c1
	ABH00987	<i>Litopenaeus vannamei</i>
<i>Branchinella</i> RhC	AB293437	<i>Branchinella kugenumaensis</i>
<i>Branchinella</i> RhD	AB293438.1	<i>Branchinella kugenumaensis</i>
LpOps5	FJ791252	<i>Limulus polyphemus</i>

Crab opsin1	AB298794	<i>Branchinella kugenumaensis</i> RhB
	AB293431	<i>Tripos granarius</i> RhD
	AB293429.1	<i>Tripos granarius</i> RhB
	AB293432	<i>Tripos granarius</i> RhE
Crab opsin2	Q25158	<i>Hemigrapsus sanguineus</i>
	GO228847.1	<i>Uca vomeris</i>
	ADQ01810.1	<i>Uca pugilator</i>
	Q25157	<i>Hemigrapsus sanguineus</i>
	GQ228846.1	<i>Uca vomeris</i>
	ADQ01809.1	<i>Uca pugilator</i>
Triops RhA	AB293428.1	<i>Tripos granarius</i>
Spider Rh3	AB251848	<i>Hasarius adansoni</i>
	AB25185	<i>Plexippus paykulli</i>
	HF54917901	<i>Cupiennius salei</i>
<i>Limulus</i> UVOps1	AEL29244	<i>Limulus polyphemus</i>
<i>Daphnia</i> UV	EFX 81332.1	<i>Daphnia pulex</i>
Insect UV	P04950	<i>Drosophila melanogaster</i> Rh3
	P08255	<i>Drosophila melanogaster</i> Rh4
	ABW06837	<i>Tribolium castaneum</i>
	NP001011605.1	<i>Apis mellifera</i>
	AF414074	<i>Vanessa cardui</i>
Crustacean SWLS	AB293436	<i>Branchinella kugenumaensis</i> RhA
	AB293430	<i>Tripos granarius</i> RhC
	EFX75461.1	<i>Daphnia pulex</i> Blue
	ADQ01800.1	<i>Uca pugilator</i>
Insect SWLS	P91657	<i>Drosophila melanogaster</i> Rh5
	AAC13417.1	<i>Apis mellifera</i>
Mouse melanopsin	EU303118	<i>Mus Musculus</i>
Cephalopod opsin	AF000947	<i>Sepia officinalis</i>
	P24603	<i>Loligo forbesi</i>