Supplemental material for "Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells"

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MATERIALS & METHODS

Animals

In the experiment shown in Fig. S1, *top trace,* the animals were *opn4*$^{Cre/+}$*;GFP* mice, and GFP-labelled ipRGCs were visualized using a 915-nm multiphoton laser. For the experiment described in Fig. S1, *middle trace*, we used *opn4*$^{Cre/Cre}$ mice in which both melanopsin promoters drive Cre recombinase; thus, the ipRGCs in this line lack functional melanopsin (Ecker *et al.*, 2010). Because this line has no GFP labelling, this experiment focused on M4 cells, and we identified them by targeting the largest somas in the ganglion cell layer for whole-cell recording. These cells were confirmed to be ipRGCs by their sustained extrinsic light responses recorded in the presence of normal Ames' medium, and by their M4-like intracellular dye fills. For the experiment shown in Fig. S1, *bottom trace*, the animals were *TRHR-GFP* mice in which GFP labels a subpopulation of ON-OFF directionally selective ganglion cells (Rivlin-Etzion *et al.*, 2011), and GFP-labelled cells were identified using a 915-nm multiphoton laser.

Chemicals and Solutions

The intracellular solution contained (in mM): 120 K-gluconate; 5 NaCl; 4 KCl; 10 HEPES; 2 EGTA; 4 Mg-ATP; 0.3 Na-GTP; 7 Tris-phosphocreatine; either ~0.1% Lucifer Yellow or ~0.001% Alexa Fluor568 hydrazide (Life Technologies, Grand Island, NY); and pH was adjusted to 7.3 with KOH. Two kinds of bathing solutions were used. In the experiment shown in Fig. S1, *top trace*, the Ringer contained (in mM): 120 NaCl; 3.1 KCl; 1.24 $MgCl_2$; 1 $CdCl_2$; 16 D-glucose; 22.6 $NaHCO_3$; and 0.5 L-glutamine. Elsewhere, the bathing solution was Ames' medium. L-AP4 (L-(+)-2-amino-4-phosphonobutyric acid), DNQX (6,7-dinitroquinoxaline-2,3-dione), D-AP5 (D-(-)-2-amino-5-phosphonopentanoic acid), UBP 310 ((S)-1-(2-amino-2-carboxyethyl)-3-(2-carboxy-thiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione), bicuculline

methochloride, CGP 52432 (3-[[(3,4-dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid), and TPMPA (1,2,5,6-tetrahydropyridin-4-yl)met-hylphosphinic acid) were purchased from Tocris (Minneapolis, MN). All other chemicals were purchased from Sigma (St. Louis, MO).

RESULTS

The low-amplitude hyperpolarizing photoresponses recorded from ipRGCs in the presence of 50 μM L-AP4, 40 μM DNQX and 25 μM D-AP5 (Fig. 3B) were unlikely caused by synaptic input resistant to these drugs, because they persisted in a Ringer containing a high concentration (1 mM) of the potent Ca^{2+} channel blocker Cd^{2+} (Figure S1, *top trace*), or in Ames' medium that included not only L-AP4, DNQX and D-AP5 but also 20 μM UBP 310 (kainate receptor antagonist) (Buldyrev *et al.*, 2012), 30 μM bicuculline ($GABA_A$ receptor antagonist), 5 μM CGP 52432 ($GABA_B$ receptor antagonist), 30 μM TPMPA ($GABA_C$ receptor antagonist), 10 μM strychnine (glycine receptor antagonist), and 100μM meclofenamic acid (gap junction blocker). These results seemed to suggest that the hyperpolarizing light responses were generated intrinsically by ipRGCs. However, such responses remained even in *opn4*$^{Cre/Cre}$ melanopsin-knockout mice (Fig. S1, *middle trace*) and since the ipRGCs in these animals lack GFP, they were not caused by photoactivation of this protein. These hyperpolarizing responses were also observed in *TRHR* cells, which are conventional, non-intrinsically photosensitive RGCs (Fig. S1, *bottom trace*).

CONCLUSION

These small hyperpolarizing light responses were probably neither melanopsin-based nor synaptically driven, but instead field potentials generated by the rod and/or cone photoreceptors.

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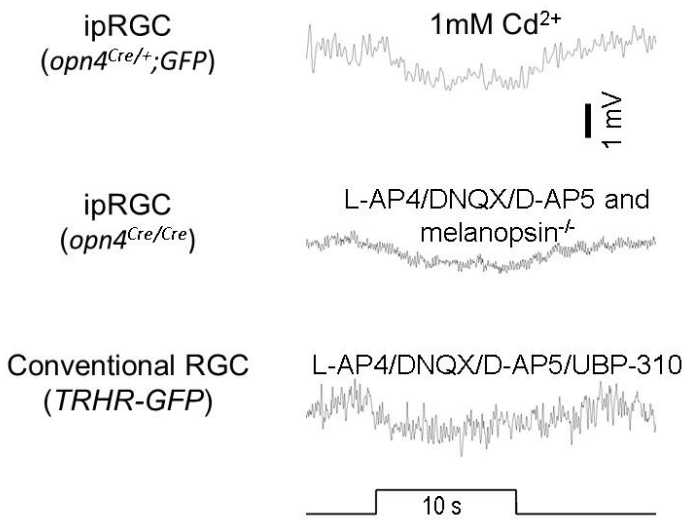

Figure S1. The low-amplitude hyperpolarizing photoresponses recorded from ipRGCs were neither melanopsin-mediated nor synaptically driven. *Top trace:* When synaptic release was blocked globally using 1mM Cd^{2+}, this M2 cell continued to hyperpolarize to a 9.5 log quanta cm^{-2} s^{-1} light step, which appeared to suggest that this was an intrinsic light response. *Middle trace:* However, even with melanopsin knocked out, the 9.5 log quanta cm^{-2} s^{-1} light still evoked hyperpolarizing responses from an M4 cell during synaptic block. *Bottom trace:* This light stimulus also evoked similar responses from a *TRHR-GFP* conventional ganglion cell in the presence of synaptic blockers.