

A Role for Melanopsin in Alpha Retinal Ganglion Cells and Contrast Detection

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SUMMARY

Distinct subclasses of retinal ganglion cells (RGCs) mediate **vision and nonimage-forming functions such as circadian photoentrainment**. This distinction stems from studies that ablated melanopsin-expressing intrinsically photosensitive RGCs (ipRGCs) and showed **deficits in nonimage-forming behaviors, but not image vision**. However, we show that the ON alpha RGC, a conventional RGC type, is intrinsically photosensitive in mammals. In addition to their classical response to fast changes in contrast through rod/cone signaling, **melanopsin expression allows ON alpha RGCs to signal prior light exposure and environmental luminance over long periods of time**. Consistent with the high contrast sensitivity of ON alpha RGCs, mice lacking either melanopsin or ON alpha RGCs have behavioral deficits in contrast sensitivity. These findings indicate a surprising role for melanopsin and ipRGCs in vision.

INTRODUCTION

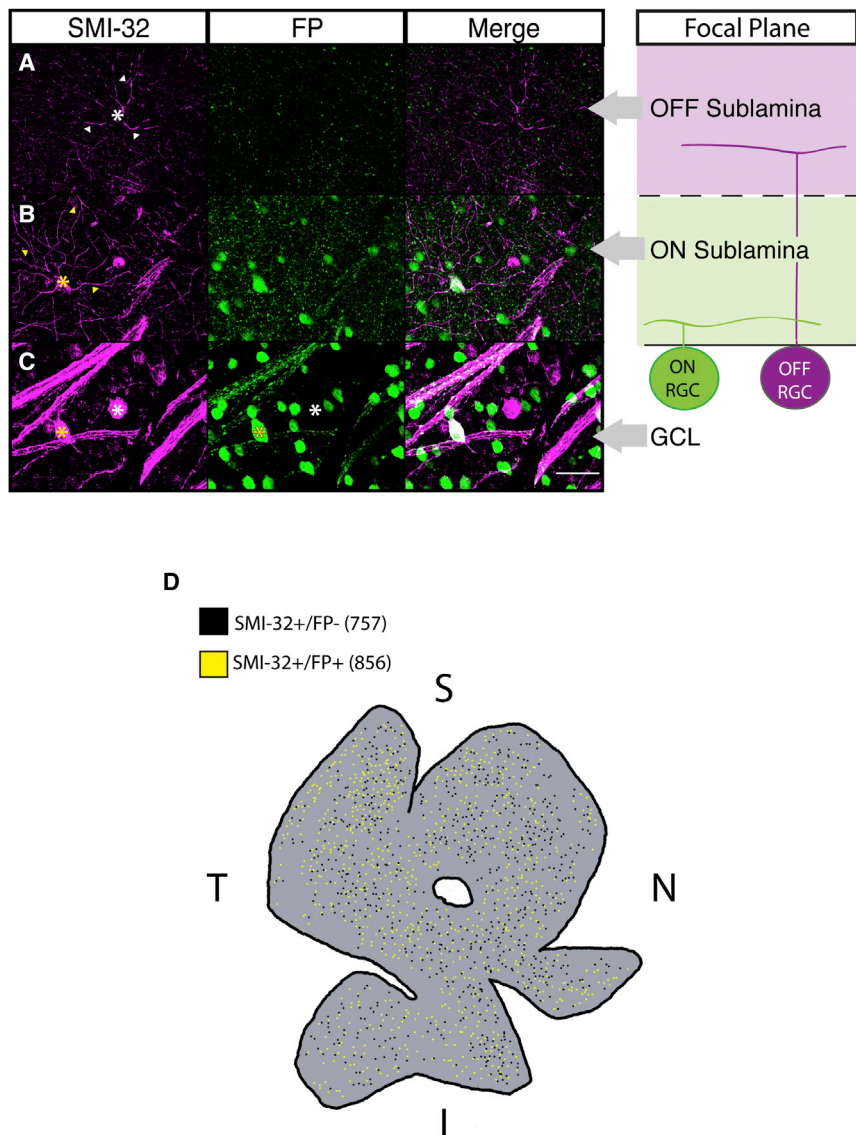
The visual system can be grossly divided into two systems: the image-forming visual system, which involves conscious perception of images, and **the nonimage-forming visual system, which includes functions that occur outside of conscious awareness such as circadian photoentrainment and the pupillary light reflex**. The image-forming visual system relies on rod and cone photoreceptors to detect light. These signals are then processed in the retina and relayed via conventional retinal ganglion cells (RGCs) to visual nuclei such as the dorsal lateral geniculate nucleus (dLGN) for further processing. The nonimage-forming visual system, however, relies almost exclusively on a distinct subset of RGCs that are intrinsically photosensitive RGCs (ipRGCs) and express melanopsin (Berson et al., 2002; Güler et al., 2008; Hattar et al., 2002). These photoreceptive neurons project to brain regions such as **the suprachiasmatic nucleus (SCN)**, which mediates circadian photoentrainment, and the olivary pretectal nucleus (OPN), which drives the pupillary light reflex (Hattar et al., 2006). Because genetic ablation of ipRGCs results in loss of nonimage-forming behaviors but retention of image-

forming vision via conventional RGCs, the logical assumption has been that RGCs driving nonimage- and image-forming behaviors are distinct (Güler et al., 2008). However, here we show that a well-described RGC type, the ON alpha (A-type) cell, characterized by large soma and dendritic field sizes (Boycott and Wässle, 1974), expresses melanopsin, is intrinsically photosensitive, and **encodes irradiance over long periods**. We also demonstrate that in ground squirrels **the ON alpha-like RGCs, which have a transient rod/cone-driven response, are also intrinsically photosensitive**. Moreover, mice lacking melanopsin (*Opn4*^{-/-}), or with ON alpha RGCs ablated (*Opn4*^{Cre/+}; *Brn3b*^{zDTA/+}; Chen et al., 2011), are defective in contrast detection, consistent with the demonstrated high contrast sensitivity of alpha RGCs (Enroth-Cugell and Robson, 1966; Shapley and Victor, 1978; Zaghloul et al., 2003). These findings reveal a functional role for melanopsin signaling in contrast detection in the presence of fully functional rod/cone signaling pathways.

RESULTS

ON Alpha RGCs Are Intrinsically Photosensitive

We found that a subset of fluorescently labeled ipRGCs (*Opn4*^{Cre/+}; *Brainbow-1.0* mice; Ecker et al., 2010; Livet et al., 2007) with large somas ($\geq 20 \mu\text{m}$) are immunopositive for the nonphosphorylated form of the neurofilament heavy chain protein (SMI-32; Figure 1C), which **strongly labels alpha RGCs, in addition to weakly labeling other conventional RGC subtypes** (Coombs et al., 2006; Lin et al., 2004). This is surprising because alpha cells project to image-forming brain regions and have high contrast sensitivity, which is essential for image formation. **Alpha cells are divided into two populations that arborize in either the ON or OFF sublamina of the inner plexiform layer (IPL)**. These morphological divisions correlate with physiological responses to light increments (ON-center RGCs) or decrements (OFF-center RGCs) respectively (Famiglietti and Kolb, 1976; Kuffler, 1953). We found that only SMI-32-positive RGCs with dendrites stratifying in the ON sublamina expressed the melanopsin reporter (Figures 1B and 1C). Conversely, **SMI-32-positive cells that stratified in the OFF sublamina were consistently negative for the fluorescent reporter** (Figures 1A and 1C). However, we observed no colocalization when we immunostained for melanopsin and SMI-32, even with the most sensitive melanopsin antibody (Ecker et al., 2010; Provencio et al., 2002; Figure S1



SMI-32-positive RGCs expressing the melanopsin reporter share all of these properties. This supports the hypothesis that **ON alpha RGCs are the same cell population as those previously designated M4 ipRGCs.**

Though it has been shown that M4 ipRGCs are “alpha-like” (Ecker et al., 2010; Estevez et al., 2012), it remains unclear whether all ON alpha RGCs are intrinsically photosensitive. To directly test this, we performed whole-cell recordings in wild-type (WT) retinas from putative alpha RGCs randomly selected based on soma size ($\geq 20 \mu$ m; Pang et al., 2003). We encountered both ON-

available online), confirming previous reports (Coombs et al., 2006; Lin et al., 2004). **These surprising results show that ON, but not OFF, alpha cells colabel with a melanopsin reporter.**

Nearly half of SMI-32-labeled cells (n = 1,715/3,646 cells from N = 3 mice) were positive for the melanopsin fluorescent reporter in whole-mount retinas from *Opn4^{Cre/+};Brainbow-1.0* mice. The fluorescent protein-positive and -negative populations were distributed evenly throughout the retina (Figure 1D). Previously, five different subtypes of ipRGCs were identified by labeling with the *Opn4^{Cre}* reporter system on the basis of their morphological properties (Ecker et al., 2010). One distinctive subtype, **M4**, has been described as having “alpha-like morphology” and **has the largest soma size of all ipRGC subtypes, dendrites that stratify in the ON sublamina**, and does not stain with the most sensitive antibody against melanopsin unless amplification techniques are used (Figure S1; Coombs et al., 2006; Ecker et al., 2010; Estevez et al., 2012; Lin et al., 2004). As described above,

and OFF-center alpha RGCs, which responded with a fast depolarization or hyperpolarization, respectively, to a 30 s bright, full-field 480 nm light stimulus (Figures 2B and 2H). After application of a cocktail of pharmacological agents to block synaptic transmission of rod and cone signals, **ON (31/31; Figure 2C), but not OFF (0/8; Figure 2I), alpha RGCs responded intrinsically to light**, showing that ON alpha RGCs are intrinsically photosensitive. We then filled each recorded cell with Neurobiotin and immunostained it for SMI-32. All recorded ON alpha RGCs were **intrinsically photosensitive** (n = 31; Figures 2A–2C), had **large dendritic field diameter** ($397.7 \pm 51.77 \mu$ m, n = 8), **large soma diameter** ($31.47 \pm 3.73 \mu$ m, n = 8), and were **positive for SMI-32** (Figure 2A; Coombs et al., 2006; Lin et al., 2004). Importantly, **ON RGCs that were not intrinsically photosensitive were SMI-32 negative (3/3) and had morphologies dissimilar to alpha RGCs.** OFF alpha RGCs, in contrast, were all SMI-32 positive but **none were intrinsically photosensitive** (n = 8; Figure 2G).

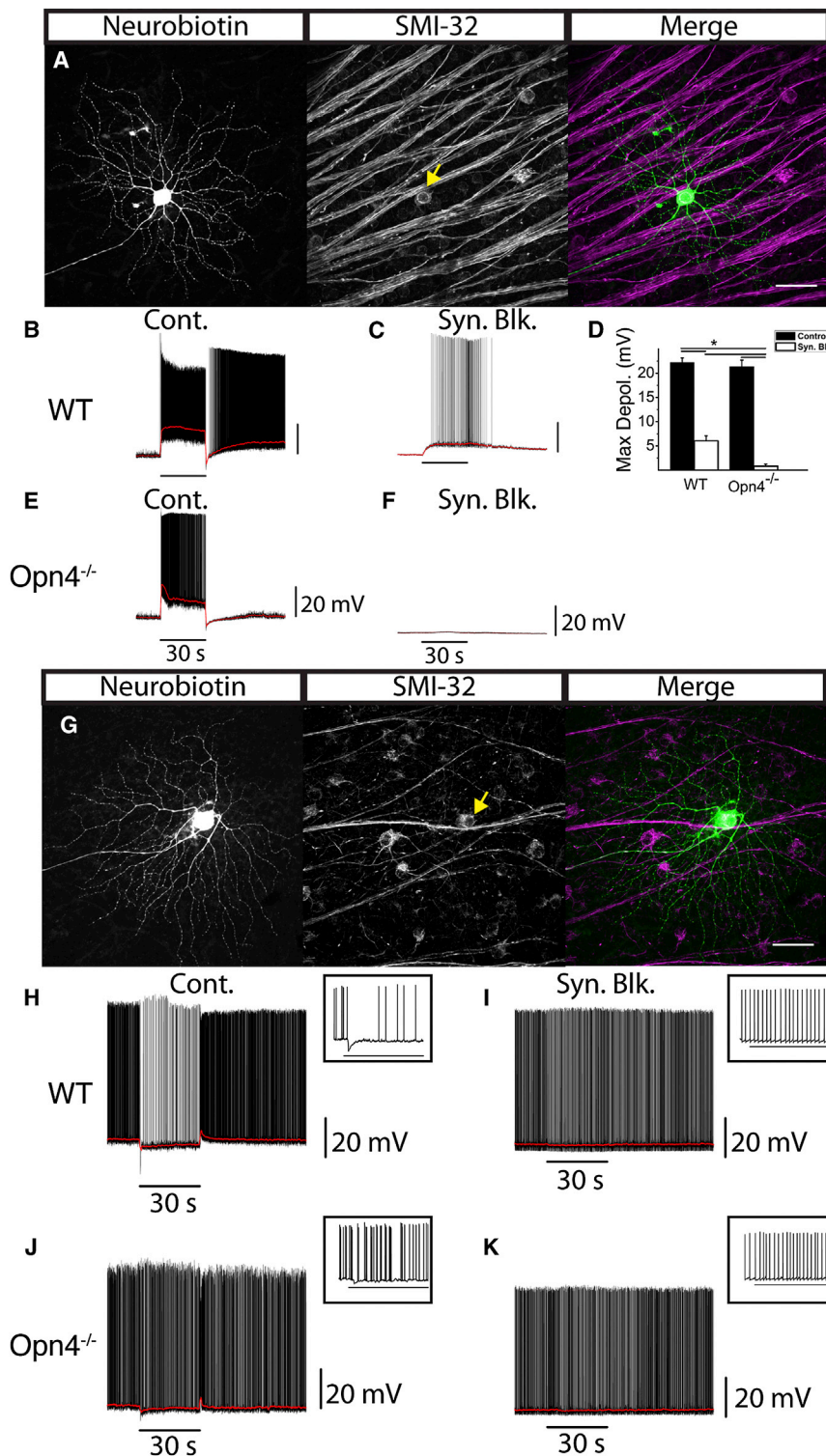


Figure 2. ON, but Not OFF, Alpha Cells Are Intrinsically Photosensitive

Cells with the largest soma sizes were randomly targeted for whole-cell recordings. (A) Cell recorded in (B) filled with Neurobiotin (left) and coimmunostained for SMI-32 (middle). Merged image (right) shows colocalization of Neurobiotin (green) and SMI-32 (magenta). (B) Whole-cell current-clamp recording of light response from ON alpha RGC in WT retina. (C) Cell from (B) recorded in the presence of a cocktail of synaptic blockers. (D) Mean \pm SEM maximum depolarization in ON alpha (SMI-32+) RGCs from WT ($n = 8$) and *Opn4*^{-/-} ($n = 5$) mice under control conditions (black bars) and then in the presence of synaptic blockers (white bars). (E) Whole-cell current-clamp recording from ON alpha RGC in *Opn4*^{-/-} retina. (F) Cell from (E) recorded in the presence of synaptic blockers. (G) Cell recorded from (H) filled with Neurobiotin (left) and coimmunostained for SMI-32 (middle). Merged image (right) shows colocalization of Neurobiotin (green) and SMI-32 (magenta). (H) Whole-cell current-clamp recording from OFF alpha RGC in a WT mouse. (I) Cell from (H) recorded in the presence of a cocktail of synaptic blockers. (J) Whole-cell current-clamp recording from OFF alpha RGC in *Opn4*^{-/-} retina. (K) Cell from (J) recorded in the presence of synaptic blockers. Scale bars in (A) and (G), 50 μ m. (B–E and H–K) All cells were stimulated with 30 s, full-field, 480 nm stimulus. Horizontal bars indicate 30 s light stimulus. (H–K) Inset shows first ~ 3.5 s of response following light onset. * $p < 0.01$. RGC, retinal ganglion cell.

22.2 ± 0.9 mV $n = 8$, one-way ANOVA, $p < 0.01$; Figure 2D). Furthermore, their membrane potential remained elevated throughout the duration of the recording, even minutes after light offset, with only one out of eight cells decaying to 37% of maximum within the recording period. These properties implicate melanopsin-phototransduction in driving these intrinsic responses.

To test whether the intrinsic light responses of ON alpha RGCs is melanopsin dependent, we recorded from ON alpha RGCs in the retinas of *Opn4*^{-/-} mice. Under control conditions, ON alpha RGCs in *Opn4*^{-/-} mice responded to light (maximum depolarization = 21.3 ± 1.4 mV, $n = 5$) similarly to ON alpha cells in WT mice (maximum depolarization = 22.2 ± 0.9 mV, $n = 8$, one-way ANOVA,

The onset of light responses of ON alpha cells were significantly slower (change in 10%–90% RT = 18.9 ± 2.9 s, $n = 8$, t test, $p < 0.01$; Figure 2C) and smaller in the presence of synaptic blockers (maximum depolarization = 6.0 ± 1.1 mV, $n = 8$) compared to control conditions (maximum depolarization =

$p > 0.05$; Figures 2D and 2E). However, in contrast to the intrinsic response of WT mice (6.0 ± 1.1 mV, $n = 8$; Figure 2C), the light response of ON alpha RGCs was completely abolished in *Opn4*^{-/-} mice upon application of synaptic blockers (0.8 ± 0.4 mV, $n = 5$, one-way ANOVA, $p < 0.01$; Figures 2D and 2F),

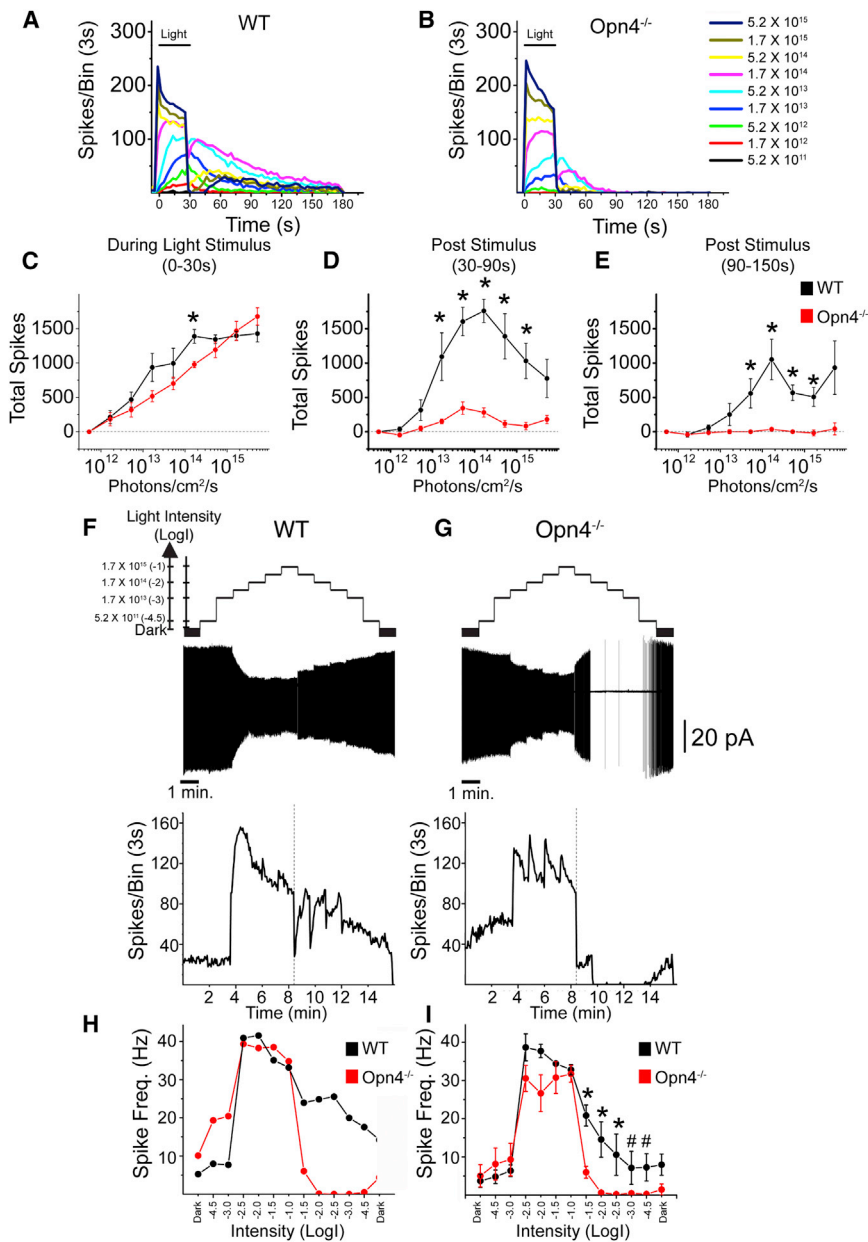


Figure 3. ON Alpha RGCs Signal Long-Term Lighting History and Irradiance

ON alpha RGCs of WT and *Opn4*^{-/-} mice were recorded extracellularly in loose-patch or cell-attached configuration. (A and B) Responses (spikes/3 s bin) of WT (A) and *Opn4*^{-/-} (B) ON alpha RGCs to increasing intensities of 480 nm light for 30 s (horizontal black bar) at various intensities. (C) Mean ± SEM total spikes recorded during 30 s light stimulus for ON alpha RGCs in WT (black, n = 4) and *Opn4*^{-/-} (red, n = 5) retinas. (D) Mean ± SEM total spikes of ON alpha RGCs recorded 0–60 s after light offset in WT (black) and *Opn4*^{-/-} (red) retinas. (E) Mean ± SEM total spikes of ON alpha RGCs recorded 60–120 s after light offset in WT (black) and *Opn4*^{-/-} (red) retinas. (F and G) ON alpha RGCs were exposed to 1 min steps of increasing and then decreasing irradiance from 5.2 × 10¹¹ to 1.7 × 10¹⁵ photons/cm²/s. Top: representative examples of WT (F) and *Opn4*^{-/-} (G) ON alpha cells exposed to this light stimulus. Bottom: spikes/3 s bin of same WT (F) or *Opn4*^{-/-} (G) cells on the same timescale. Dotted line indicates point at which light intensities began to decrease. (H) Spike frequency for single WT (black) and *Opn4*^{-/-} (red) cells shown in (F) and (G) over 1 min at each light intensity (x axis = Log(intensity)). (I) Mean ± SEM spike frequency (Hz) for WT (black, n = 4) and *Opn4*^{-/-} (red, n = 4) ON alpha cells over 1 min at each light intensity (x axis = Log(intensity)). Horizontal scale bar in (F) and (G) represents 1 min of recording time and vertical scale bar (F) and (G) represents 20 pA. *p < 0.05, #p = 0.057 (Mann-Whitney U test).

showing that the intrinsic photosensitivity of ON alpha RGCs is melanopsin dependent. The synaptic response of OFF alpha RGCs in *Opn4*^{-/-} mice (n = 3) was similar to WT (Figures 2H and 2J), and no intrinsic response was detected in these cells (Figure 2K). Each recorded ON and OFF cell in *Opn4*^{-/-} mice was SMI-32 positive, indicating that we were sampling the same alpha RGC population in *Opn4*^{-/-} retinas as in WT.

The ON alpha cells of the mouse show sustained rod/cone-driven responses to light, which differ from the transient rod/cone-driven response of the classically identified cat ON alpha RGCs (Cleland and Levick, 1974). Therefore, we examined whether the ON alpha-like RGCs of the diurnal ground squirrel (*Ictidomys tridecemlineatus*), which has a functional melanopsin gene (Figure S2E; Provencio et al., 2000), are intrinsically photo-

sensitive. We randomly selected RGCs with the largest somas and confirmed ON alpha-like morphology, positive staining for SMI-32, and stratification in layer S4/S5 of the IPL by dye filling (Figures S2A–S2C and S2F; Linberg et al., 1996). ON alpha-like RGCs in squirrel responded to light with a transient inward current at light onset (Cleland et al., 1973; Jakiela and Enroth-Cugell, 1976; Figure S2D). Upon application of synaptic

blockers, we found that ON alpha-like RGCs had a sluggish and sustained inward current resembling a melanopsin-mediated light response (Figure S2E; n = 4), which persisted throughout the duration of the 30 s light stimulus and recovered slowly after stimulus offset (Figure S2E; n = 4). This indicates that the intrinsic photosensitivity of ON alpha-like RGCs exists in a diurnal mammal with a cone-dominated retina and may thus be found across a variety of species.

Melanopsin Influences ON Alpha RGC Signaling

To determine whether the intrinsic, melanopsin-based photoresponse contributes physiologically to ON alpha RGC signaling, we compared responses of ON alpha RGCs of WT and *Opn4*^{-/-} mice to 30 s stimuli of increasing irradiance (Figures 3A and 3B;

Figure S3). In WT and *Opn4*^{-/-} mice, ON alpha RGCs (n = 5 each) responded to increasing intensities of light with increasing spike frequencies during the 30 s light stimulus, reaching maximum average firing levels of >40 Hz (Figures 3A and 3C; Figure S3). However, a major difference between WT and *Opn4*^{-/-} animals was observed after cessation of the light stimulus. Four out of five ON alpha RGCs in WT mice showed continued elevation of spike frequencies after stimulus offset (Figure 3A; Figure S3) and were utilized for subsequent analyses. We found that the total number of spikes in the first 60 s after stimulus offset in WT ON alpha RGCs was intensity dependent (Figures 3A and 3D), reaching maximum spike output at 1.7×10^{14} photons/cm²/s (Figure 3D). In contrast, cells in *Opn4*^{-/-} mice showed markedly reduced levels of firing during this same period, though cells still showed slight increases in spike output between 5.2×10^{12} and 5.2×10^{13} photons/cm²/s (Figures 3B and 3D). A dramatic difference between ON alpha cell responses in WT and *Opn4*^{-/-} retinas was evident when we quantified the total number of spikes between 60 and 120 s after stimulus offset. As stimulus intensity increased, ON alpha RGCs continued to show steadily increasing poststimulus firing levels through 1.7×10^{14} photons/cm²/s in WT mice (Figure 3E), while cells in *Opn4*^{-/-} mice showed no appreciable increases (Figure 3E). The fact that ON alpha cells signal irradiance even after the light stimulus has ceased enables ON alpha RGCs to keep a record of lighting history.

As melanopsin is implicated in signaling ambient light intensity, we next recorded spike output from ON alpha RGCs in WT and *Opn4*^{-/-} retinas as the intensity of a 480 nm background light was steadily increased and then decreased at 1 min intervals with no intervening dark (see Supplemental Experimental Procedures; Figures 3F and 3G; Barlow and Levick, 1969). We found that ON alpha RGCs in WT (n = 4) and *Opn4*^{-/-} (n = 4) mice responded to the onset of illumination increases and were able to maintain elevated firing levels throughout the light stimulation (Figures 3F and 3G). As the light intensity was stepped down, ON alpha RGCs in WT mice responded to decreases in luminance with a fast initial offset of spiking followed by a robust rebound in spiking that was maintained throughout the remainder of the light pulse (Figures 3F, 3H, and 3I). However, cells in *Opn4*^{-/-} mice demonstrated complete cessation of firing by the second light decrement step (Figures 3G–3I). This repression was maintained throughout steps of decreasing intensity, consistent with the characteristics of an ON center RGC. Thus, while rods and cones mediate the response to sustained increases in illumination, melanopsin-mediated phototransduction enables ON alpha RGCs to signal ambient environmental irradiance regardless of whether the intensity has been increased or decreased.

Melanopsin Is Required for Normal Sensitivity

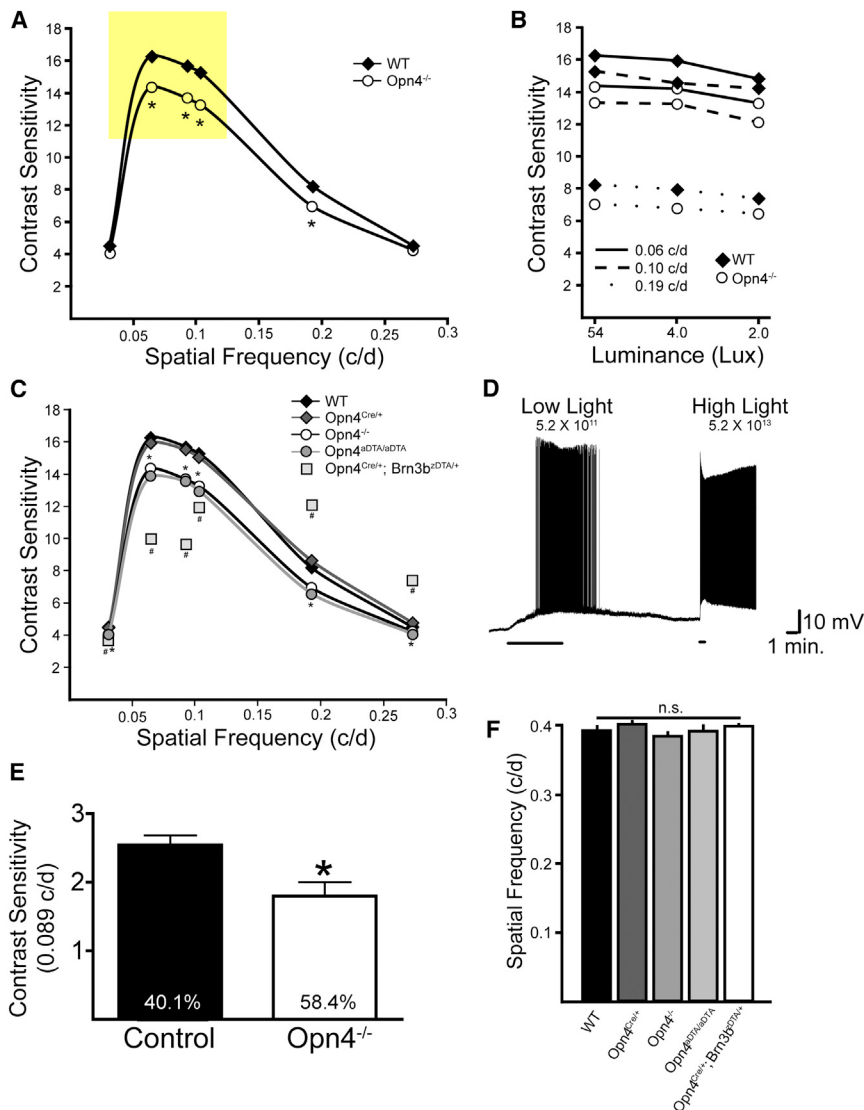
The contribution of melanopsin to the responses of ON alpha RGCs, which have high contrast sensitivity (Enroth-Cugell and Robson, 1966; Shapley and Victor, 1978; Zaghloul et al., 2003), raises the possibility that *Opn4*^{-/-} animals could have deficits in image-forming functions. We used optokinetic tracking (OKT; Douglas et al., 2005; Prusky et al., 2004) as an assay to measure contrast sensitivity and spatial frequency thresholds

in WT (N = 8) and *Opn4*^{-/-} (N = 8) animals (Figures 4A and 4B). Consistent with a role for melanopsin in alpha cell signaling, *Opn4*^{-/-} mice showed deficits in contrast sensitivity when compared to WT mice (Figure 4A). These were observed at the peak of the contrast sensitivity curve (0.064 c/d; one-way ANOVA, $p < 0.001$), as well as spatial frequencies of 0.092 c/d (one-way ANOVA; $p < 0.001$), 0.103 c/d (one-way ANOVA; $p < 0.001$), and 0.192 c/d (one-way ANOVA; $p < 0.01$; Figure 4A). Reduced contrast sensitivity in *Opn4*^{-/-} animals was also observed at various spatial frequencies as screen luminance was decreased from 54 lux to 2 lux ($p < 0.05$; Figure 4B). Consistent with the behavioral contrast sensitivity deficits at low light intensities, we were able to record melanopsin-mediated responses at similar, low light intensities in ON alpha RGCs in the isolated, dark-adapted retina (5.2×10^{11} photons/cm²/s; n = 6/6 cells; Figure 4D). We also measured contrast sensitivity in control (n = 8) and *Opn4*^{-/-} (n = 5) mice in a visual cortex-dependent assay: the visual water task (VWT; Prusky and Douglas, 2004). At 0.089 c/d, *Opn4*^{-/-} mice showed a significant reduction in contrast sensitivity compared to control mice (Figure 4E; t test; $p < 0.01$), consistent with the OKT results. The reduction in visual function in *Opn4*^{-/-} mice was specific to contrast sensitivity because spatial frequency thresholds in WT and *Opn4*^{-/-} mice did not differ (one-way ANOVA, $p > 0.05$; Figure 4F; Güler et al., 2008). Importantly, these findings also demonstrate that RGCs (such as ON alpha RGCs or other ipRGCs) that do not project to the accessory optic system, which is necessary for OKT, are nonetheless capable of modulating the optokinetic reflex (Figures 4A–4C).

To delineate the role of ipRGC subtypes in contrast sensitivity, we examined whether the ablation of M1 ipRGCs caused further deficits in contrast sensitivity using the *Opn4*^{aDTA/aDTA} mice, which have normal ON alpha RGC distribution but lack M1 ipRGCs (Figure S4). *Opn4*^{aDTA/aDTA} mice showed normal spatial frequency thresholds (one-way ANOVA, $p > 0.05$) and no deficits in contrast sensitivity beyond those observed in *Opn4*^{-/-} animals (Figures 4C and 4F). It is therefore unlikely that the contrast sensitivity deficits observed in *Opn4*^{-/-} animals are due to changes in retinal dopamine release since M1 ipRGCs are the only subtype of ipRGC that form a plexus with the dopaminergic amacrine cells (Vugler et al., 2007).

ON Alpha RGC Deletion Causes Severe Deficits in Contrast Sensitivity

To determine the contribution of the intrinsically photosensitive ON alpha RGCs themselves to contrast sensitivity, we utilized animals in which ON alpha RGCs are ablated (*Opn4*^{Cre/+}; *Bmn3b*^{zDTA/+}; Chen et al., 2011). We found that SMI-32-positive cells were reduced by approximately 50% in these animals and those remaining were predominantly OFF stratified (t test, $p < 0.05$; Figure S4 and data not shown). These animals also lack other non-M1 ipRGC subtypes as well as the M1 ipRGCs that project to the OPN (Chen et al., 2011). Importantly, the *Opn4*^{Cre/+}; *Bmn3b*^{zDTA/+} animals, which exhibit normal circadian photoentrainment, contain 200 remaining M1 ipRGCs that arborize with dopaminergic amacrine cells and still contain melanopsin (Chen et al., 2011). *Opn4*^{Cre/+}; *Bmn3b*^{zDTA/+} animals showed severe contrast sensitivity deficits beyond those



observed in *Opn4*^{-/-} animals at the contrast sensitivity curve peak (0.064, 0.092, and 0.103 c/d; Figure 4C), indicating that **non-M1 ipRGCs contribute to contrast sensitivity**. As published previously, *Opn4*^{Cre/+}; *Brn3b*^{zDTA/+} animals show normal spatial frequency thresholds (one-way ANOVA, p > 0.05; Figure 4F; Chen et al., 2011), demonstrating the specificity of the non-M1 ipRGC contribution to contrast sensitivity. In addition, the number of choline acetyl transferase-positive amacrine cells in both the ganglion cell and inner nuclear layers was similar between *Opn4*^{Cre/+}; *Brn3b*^{zDTA/+} animals and control animals (Figure S4), indicating that these deficits are not due to defects in the direction selective circuitry. Interestingly, at higher spatial frequencies, *Opn4*^{Cre/+}; *Brn3b*^{zDTA/+} animals had enhanced contrast sensitivity (Figure 4C). The spatial frequencies where contrast sensitivity deficits were observed in *Opn4*^{Cre/+}; *Brn3b*^{zDTA/+} mice match the spatial frequencies over which

ON alpha RGCs respond to a sine wave grating stimulus in the isolated retina (Estevez et al., 2012), **making it likely that the contrast sensitivity deficits in these animals arise as a result of ablation of the intrinsically photosensitive ON alpha RGCs**.

DISCUSSION

ON alpha RGCs are intrinsically photosensitive and **melanopsin signaling influences the physiological properties of these conventional RGCs**. *Opn4*^{-/-} mice show defects in contrast sensitivity in both a **subcortical optokinetic tracking task** and a **visual cortex-dependent task**, providing a behavioral role for melanopsin in image-forming vision, even in the context of functional rods and cones. **This decrease in contrast sensitivity in *Opn4*^{-/-} mice does not occur through M1 ipRGCs** because ablation of M1 ipRGCs results in no further contrast sensitivity deficits beyond

those seen in melanopsin knockout animals. ON alpha RGCs were demonstrated to have high contrast sensitivity in vitro in other rodent models (Zaghoul et al., 2003), which suggests that melanopsin enhances behavioral contrast sensitivity at least in part through the ON alpha RGCs. Our data indicate that robust melanopsin responses can be driven in ON alpha RGCs at lower light intensities than previously appreciated, consistent with previous reports of melanopsin responses in ipRGCs at dim light (Wong, 2012) and consistent with a behavioral deficit in contrast sensitivity in mice lacking melanopsin at intensities of 2 lux (Figure 4B).

The severe contrast sensitivity deficits observed in mice where all non-M1 ipRGCs are ablated identify an important behavioral function for non-M1 ipRGCs and implicate this small population of ipRGCs in setting the thresholds for contrast detection. These results are consistent with recently identified non-M1 ipRGC connections to the dLGN in the mouse and primate (Dacey et al., 2005; Ecker et al., 2010; Estevez et al., 2012), as well as widespread melanopsin-derived light responses in 40% of light-sensitive neurons in the dLGN of mice (Brown et al., 2010). Collectively, these results show that melanopsin expression in non-M1 cells is not simply an evolutionary remnant but plays a crucial role in setting the threshold of contrast detection.

EXPERIMENTAL PROCEDURES

Animals

All animals were handled in accordance with guidelines of the Animal Care and Use Committees of Johns Hopkins University, the University of Minnesota, the National Eye Institute, or Cornell University. Specific details regarding strains used in each experiment as well as other methods can be found in the Supplemental Experimental Procedures.

Electrophysiology

All electrophysiological analyses were performed as described previously (Schmidt and Kofuji, 2011; and see the Supplemental Experimental Procedures). Light stimulation was full-field band-pass filtered 480 nm light of 1.66×10^{16} photons/cm²/s for mouse retina recordings and 1.94×10^{16} photons/cm²/s for ground squirrel retina recordings.

Immunohistochemistry

Immunohistochemical studies were performed as described previously (Li and DeVries, 2004; Schmidt and Kofuji, 2009).

Statistical Analyses

Statistical analyses were performed using Origin 7.5 (MicroCal) or GraphPad Prism. Statistical comparison of means was performed using a Student's t test or one-way ANOVA with Tukey's post hoc test or a Mann-Whitney U test and significance was concluded when $p < 0.05$. Data are presented as mean \pm SEM.

Behavioral Analyses

Optokinetic tracking experiments were performed as described previously (Prusky et al., 2004; OptoMotry, CerebralMechanics). The visual water task was performed essentially as described previously (Prusky et al., 2000, 2004) but with printed instead of electronic grating stimuli.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2014.03.022>.

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