Supporting Information

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SI Materials and Methods

Animals. WT mice were C57BL/6J strain (The Jackson Laboratory, Bar Harbor, ME). Melanopsin null ($Opn4^{-/-}$) mice were originally created by Panda et al. (1). The mice were considered to be P0 on their day of birth. Melanopsin null mice were genotyped by PCR analysis of tail-snip–derived DNA (2) and phenotyped by melanopsin antibody (UF006, gift from Ignacio Provencio, University of Virginia, Charlottesville, VA) staining of retina. Dams were given ad libitum access to food and water. Litters were housed on a 12:12-h light–dark schedule. Institutional animal care and use committees at the University of California at San Francisco and Washington University approved all animal procedures, and all of the experiments met the guidelines of the NIH, PHS Policy, and the Society for Neuroscience Policy on the Use of Animals in Neuroscience Research.

Phototaxis Assay. Dams and their pups were dark adapted for at least 1 h before the tests. An individual pup was removed from the cage and placed in the test chamber that was warmed from beneath by a heating pad. Transfer was done in either dim red light or in darkness using night vision goggles (B.E. Meyers). The ends of the test chamber, which was 3.8 cm in diameter and 12.5 cm long, were covered with transparent glass slides. Pups could easily turn back and forth in the test chamber. If stimulation lights were shone from both ends of the chamber simultaneously, pups would reverse direction 5–10 times during a 5-min test period. The activity of each pup was monitored with an infrared camera (Watek-902H3). Analog images were converted to digital format using a Canopus converter (ADVC-110) and then recorded onto the hard drive of a computer (Apple) using BTV Pro (version 5.4.1) for later analysis. For most experiments, each pup's behavior was analyzed for 5 min in complete darkness and for 5 min in the presence of a bright light directed into one end of the cylindrical chamber. For littermate control experiments (Fig. 5), 2-min periods of movement in light and in darkness were recorded. In all experiments, the light was directed into the pup's face at the light onset. The sequence of darkness followed by light was reversed in some experiments with no apparent effect on phototaxis. Immediately after the testing period the pup was returned to the cage with its mother. Each pup was tested only once per day except when circadian variations were examined. All tests were carried out on freely moving, unanesthetized pups from postnatal ages P6 to P9. The cylindrical chamber was wiped down with an alcohol pad between tests. Neonatal rat pups tested in the chamber exhibited negative phototaxis very comparable to mice. Phototaxis assays were performed between 12 noon and 7 PM, during subjective daytime for the pups. To assess whether negative phototaxic behavior depended on whether pups were tested during the day or night we compared the latency to first turn on pups tested during the day (12 noon to 2 PM) and night (11 PM to 1 AM) over 2 d. Figure S4, plotting the average latencies to first turn, shows comparable values for day-tested versus night-tested WT pups. These results accord with findings from a recent study showing that ipRGC light sensitivity was minimally changed across a day/night cycle (3).

Light Stimuli. A bright blue LED (Jameco 183222, 468 nm λmax, 0.2 mW/cm^2) provided photic stimulation. The LED was positioned 4.5 cm from the end of the cylindrical chamber. Unattenuated light produced 4.7×10^{14} photons/s/cm^2 on the closed eyelids of the pups. Assuming that blue light was attenuated 2 log units by the eyelids (4), this unattenuated light produced 4.7×10^{12} photons/s/cm^2 at the cornea.

Analysis of Movies. Digital movies of phototaxis behavior were viewed offline using Quicktime (Apple). Most experiments were manually scored by at least two observers. Individual movies were scrambled so the observers were blind as to the mouse strain and, a majority of the time, as to whether the movie was taken from a dark or light epoch. Over each 5-min epoch we measured the cumulative time each pup faced in its original placed position. This measured time included the period before pups turned around and any time when the pup turned back around. We also measured the latency before a pup turned 180° from its original position. For littermate control experiments, the total distance traversed by each pup during the last 1 min of darkness and the first min of light was measured using a custom centroid tracking routine implemented in Matlab (Mathworks).

Statistical Analysis. Dark/light differences in each age group were tested for significance using unpaired t tests (Prism4, GraphPad Software). Comparable significance levels were achieved when median rather than mean values were used. Because some of the same mice were tested on different days we used the Wilcoxon signed-rank test for cluster-correlated data (5) to investigate whether a lack of independence of observations in the different age groups might have skewed our conclusions. For this purpose we derived the Larocque statistic, $\overline{W_n}$. Using our original data, we calculated $\overline{W_n}$ (data) for each of 1,000 randomly assigned clusterings of mice. The median ($\overline{W_n}^*$) of the 1,000 computed values of $\overline{W_n}$ (data) was 2.85 for dark/light latency differences and 3.34 for dark/light duration differences. To compute the range of $\overline{W_n}$ that would be expected if there were truly no differences between the light and dark settings, we randomly shuffled the sign of the light–dark differences (i.e., randomly permuted the light–dark labels for the observations for each mouse at each age point) for each of the above randomly assigned clusters. The median value of $\overline{W_n}$ (data) was computed for 1,000 permutations of the sign differences for each cluster. In none of the simulations of clustered groups did $\overline{W_n}^*$(random) exceed 2.85 for latency differences or 3.34 for duration differences. On this basis we estimate the light–dark differences were significant ($P < 0.001$). Similar nested simulations were run on the data from the $Opn4^{-/-}$ mice. For the latency data, $\overline{W_n}^*$ (random) exceeded the magnitude of $\overline{W_n}$ (data) in 637 of the 1,000 simulations. For the duration data, $\overline{W_n}^*$ (random) exceeded the magnitude of $\overline{W_n}$ (data) in 240 of the 1,000 simulations. On this basis there is no reason to reject the null hypotheses that the means of the latencies and durations for the $Opn4^{-/-}$ mice in light were indistinguishable from those measured in the dark.

Multielectrode Array Extracellular Recording from Retina. Multielectrode array (MEA-60, MCS) recordings of light-evoked ganglion cell spiking were acquired and analyzed as described previously (6, 7). Briefly, the MEA chambers consisted of an array of 60 planar electrodes, each 10 μm in diameter, in eight rows and spaced 100 μm apart for a total array size of 700 μm^2. Acquired voltage signals were bandpass filtered at 0.1 Hz to 3 kHz and sampled at 20 kHz (MC_Rack, version 2.0; MultiChannel Systems). Offline, action potential waveforms from high-pass-filtered data (100 Hz lower cutoff) were detected by threshold crossing and then clustered based on the first two principal components, as described previously (7). Cluster contours in principal components space were either manually selected or derived from a k-means algorithm (OfflineSorter, version 1.3; Plexon). The algorithm eliminated outlier waveforms at a threshold of 1.3 times the mean distance from the calculated cluster center. Obvious auto-

matic sorting errors were corrected for each cluster manually. Time stamps for each action potential of each sorted RGC were used to generate peristimulus time histograms and peristimulus spike rasters. Light stimuli were presented from a monitor (Dell Ultrascan P780; 100 Hz vertical refresh) imaged onto the retinal surface at an approximate intensity of 0.35 W/cm^2.

1. Panda S, et al. (2003) Melanopsin is required for non-image forming photic responses in blind mice. *Science* 301:525–527.
2. Zhu Y, et al. (2007) Melanopsin-dependent persistence and photopotentiation of murine pupillary light responses. *Invest Ophthalmol Vis Sci* 48:1268–1275.
3. Weng S, Wong KY, Berson DM (2009) Circadian modulation of melanopsin-driven light response in rat ganglion-cell photoreceptors. *J Biol Rhythms* 24:391–402.
4. Crawford ML, Marc RE (1976) Light transmission of cat and monkey eyelids. *Vision Res* 16:323–324.
5. Larocque D (2005) The Wilcoxon signed-rank test for cluster correlated data. *Statistical Modeling and Analysis for Complex Data Problems*, eds Duchesne P, Remillard B (Springer, New York), pp 309–323.
6. Tian N, Copenhagen DR (2003) Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron* 39:85–96.
7. Rentería RC, et al. (2006) Intrinsic ON responses of the retinal OFF pathway are suppressed by the ON pathway. *J Neurosci* 26:11857–11869.

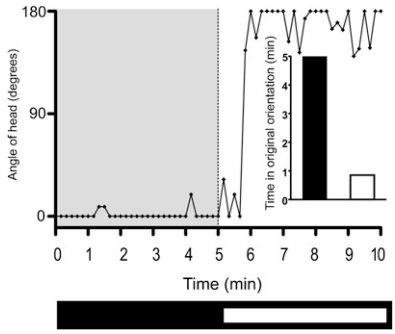


Fig. S1. Example of a pup's movement within the test chamber. The pup was placed into the chamber and left for 5 min in complete darkness. The LED was turned on at 5 min. The angle of the head was measured using the skewness tool in ImageJ (NIH). Head angle every 10 s is plotted as the absolute angular deviation from 0° or 180° once the pup turned around. 0° is taken as the original orientation. Inset plots the total time the pup faced in its original position in darkness (black bar) and toward the light once the LED was turned on (white bar).

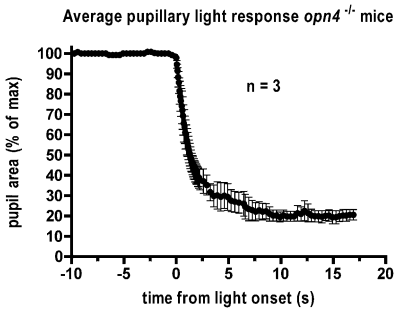


Fig. S2. Pupillary responses are intact in *Opn4*$^{-/-}$ mice. Pupil responses were measured using the procedure described previously (1). Briefly the consensual light response in one eye was recorded before and after stimulation of the opposite eye with 480 nm light from an LED. Infrared images of the eye were captured at 15 frames/s. Pupil diameter was measured in every fifth frame using ImageJ (NIH). Results from three older, genotypically verified *Opn4*$^{-/-}$ mice (P370, P297, and P521) are plotted (mean ± SEM). These data confirm earlier studies demonstrating light-induced pupil constrictions in *Opn4*$^{-/-}$ mice and indicate visual signaling originating in rods and cones.

1. Johnson J, et al. (2007) Vesicular glutamate transporter 1 is required for photoreceptor synaptic signaling but not for intrinsic visual functions. *J Neurosci* 27:7245–7255.

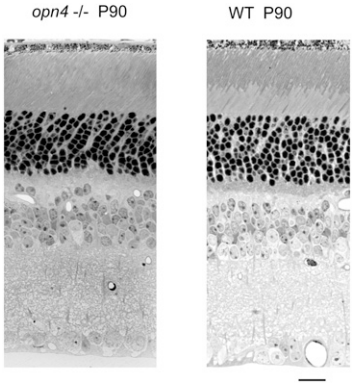


Fig. S3. Comparison of retinas from WT and *Opn4*$^{-/-}$ mice. For comparative purposes retinas from P15, P30 and P90 wild type and opn4$^{-/-}$ mice were examined. The two images in Figure S3 compare retinas from P90 aged mice. Mice were killed with carbon dioxide inhalation and immediately perfused intracardially with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde. The eyes were removed, bisected along the vertical meridian, postfixed in osmium tetroxide, and embedded in an Epon-Araldite mixture. Sections of the entire retina were cut at 1 μm thickness and stained with toluidine blue, as described elsewhere (1). No discernible difference was noted in overall morphology of WT and age-matched O*pn4*$^{-/-}$ retinas.

1. LaVail MM, Battelle BA (1975) Influence of eye pigmentation and light deprivation on inherited retinal dystrophy in the rat. *Exp Eye Res* 21:167–192.

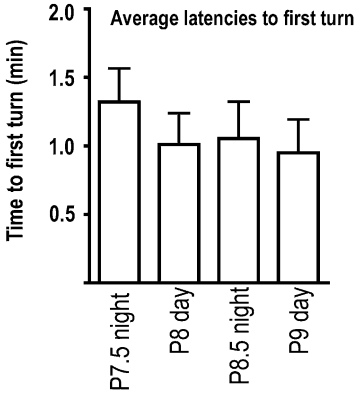
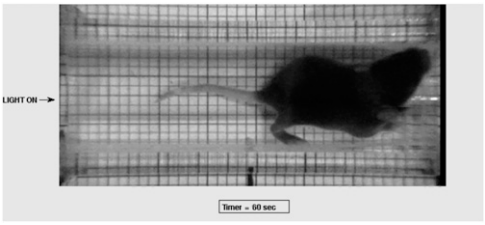
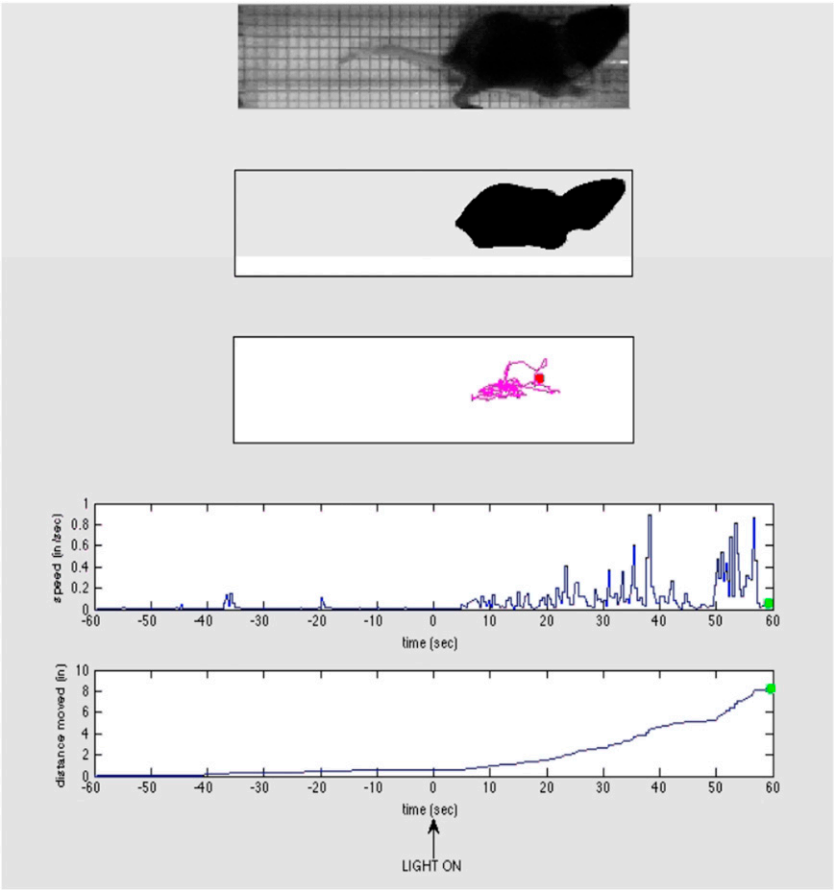


Fig. S4. Comparison of phototactic responses at night and during day. Negative phototaxis was sequentially measured during subjective day and night for two days. Twelve pups from two different litters of WT mice were tested four times over a 48-h period. Testing began on P7.5 during subjective daytime. Average latencies to first turn after light onset are plotted. Analysis of means (one-way ANOVA, Bonferroni multiple comparison test) failed to show any significant difference the mean latencies at the different testing times.



Movie S1. Infrared movie image of P8-aged pup in test chamber. Pup was in darkness for first 15 s of recording. Stimulus light was turned on at 0 s. Pup initially starting waving head back and forth (termed "pivoting") and then attempted to turn head and body away from light.

Movie S1



Movie S2. Quantification of pup's movement in test chamber. First (top) panel shows infrared image of P8-aged mouse in test chamber. The first 60 s (−60 to 0 s) shows the pup's position and movement in darkness. The stimulation light was turned on at 0 s. Second panel shows high-contrast image of the pup. Third panel plots x–y position of computed centroid of high-contrast image. Fourth panel plots computed velocity of the centroid. Last (bottom) panel shows the total x–y distance covered by the centroid.

Movie S2