Remaining Rod Activity Mediates Visual Behavior in Adult Rpe65−/− mice.

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PURPOSE. C57Bl/6, Cpf1l−/− (cone photoreceptors function loss 1; pure rod function), Gnat1a−/− (rod α-transducin; pure cone function), and Rpe65−/−;Rho−/− double-knockout mice were studied to distinguish the respective contributions of the different photoreceptor (PR) systems that enable light perception and mediate a visual reflex in adult Rpe65−/− mice, with a simple behavioral procedure.

METHODS. Visual function was estimated using a rotating automated optomotor drum covered with vertical black-and-white stripes at spatial frequencies of 0.025 to 0.5 cycles per degree (cyc/deg) in both photopic and scotopic conditions. Mouse strains with different luminances were tested to evaluate the contribution and the light-intensity threshold of each PR system.

RESULTS. Stripe rotation elicited head movements in the wild-type (WT) animals in photopic and scotopic conditions, depending on the spatial frequency, whereas the Cpf1l−/− mice show a reduced activity in the photopic condition and the Gnat1a−/− mice an almost absent response in the scotopic condition. A robust visual response was obtained with Rpe65−/− knockout mice at 0.075 and 0.1 cyc/deg in the photopic condition. The residual rod function in the Rpe65−/− animals was demonstrated by testing Rpe65−/−;Rho−/− mice that present no response in photopic conditions.

CONCLUSIONS. The optomotor test is a simple method of estimating the visual function and evaluating the respective contributions of rod and cone systems. This test was used to demonstrate that in Rpe65−/− mice, devoid of functional cones and of detectable 11-cis-retinal protein, the rods mimic cone function in part, by mediating vision in photopic conditions. The optomotor test allows easy recording of the head movement component of this reflex; this procedure has been shown to be possible simply by controlling the direction of the drum rotation without having to use occluders or suturing eyelids.

Among various methods that can attest to visual function, the optokinetic reflex reflects the activity of subcortical areas in mice, and an independent testing of the two eyes is possible simply by controlling the direction of the drum rotation without having to use occluders or suturing eyelids. The optomotor test allows easy recording of the head movement component of this reflex; this procedure has been shown to be

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efficient in estimating visual acuity in commonly used wild-type mice (C57/BL/6J, 129/SvPas, and CD1) and visually deficient mice: C3HeB/FeJ (Rd1) and Rbo−/−;Cnga3−/−.19,21–24

Because C57/B6 mice showed significant variability between males and females,22 we decided to use only male animals. In addition, since changes in intensities reflect the transition from rod to cone vision,25 we tested the visual acuity at different luminances in wild-type and mutant mice lacking rods, cones, or both, to evaluate whether rods allow the formation of a light-sensitive response and are sufficient to mediate visual function in Rpe65−/− mice. In the present study, we show that the rod photoreceptor system mediates OR in Rpe65−/− mice.

METHODS

Animals

The animals were handled in accordance with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research and approved protocols of the local institutional committee (Service Vétérinaire du Canton de Vaud, Lausanne, Switzerland). The mice were bred and housed in communal cages (five to six animals per cage), maintained in controlled temperature (22°C) and light (12-hour light–dark cycle), with free access to food and water. The behavioral study included wild-type C57/B6 mice (provided by Charles River, Lyon, France), Gnat1a−/− mice (provided by Janis Lem, Tufts University, Boston, MA), Cpb1−/− mice (provided by Bo Chang, The Jackson Laboratory, Bar Harbor, ME), Rpe65−/− mice (provided by T. Michael Redmond, National Eye Institute, Bethesda, MD), and Rpe65−/−;Rbo−/− mice (provided by Christian Grimm, Laboratory of Retinal Cell Biology, University of Zurich, Zürich, Switzerland; n = 5 males per group). Ages of the tested animals ranged from 4 to 8 weeks.

Behavioral Tests

The experimental setup consisted of a central platform (10-cm diameter) surrounded by a vertical drum (66-cm diameter) rotating at a velocity of nine rotations per minute. The wall of the drum was covered with white and black vertical stripes at various spatial frequencies, or with a uniform white and then black setting without stripes, to evaluate the baseline anxiety and the OR threshold of each strain tested (Fig. 1).

The animals were tested with spatial frequencies increasing from 0.025 to 0.5 cycles per degree (cyc/deg): 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, and 0.5 cyc/deg. Behavior was recorded for 4 minutes in each test session with a camcorder that enabled infrared recording in scotopic conditions (CDR-HC90E; Sony, Tokyo, Japan). During each session, the drum rotated alternately clockwise and counterclockwise, with the rotation direction inverted at mid session. The drum was lighted by a white LED, placed above the cylinder. Neutral-density filters were superimposed to obtain light intensities ranging from 0.01 to 1750 lux, measured on the platform center, with the following parameters: 0.01, 0.15, 0.6, 3.2, 18.5, 150, and 1750 lux.

Procedure

In the first step, the animals were trained by handling the day before the task, to reduce baseline anxiety and to adapt the mice to the setup. Two successive sessions with background settings were run without recording, and the animals were returned to their communal cages in the animal facility. The tests were first performed in photopic conditions with a light intensity of 650 lux, measured with an illuminance meter (T-10; Konica-Minolta, Osaka, Japan). The mouse was placed on the platform, allowed to move freely, and adapted during 1 minute to the setup before the test session. Then, the sessions were repeated with each spatial frequency. For scotopic conditions, the mice were dark-adapted overnight, and tests were performed under dim illumination (<0.01 lux). The procedure was the same as for photopic conditions. Each animal was videotaped, and head movements were then scored manually. Clockwise movement drives tracking through the left eye, whereas counterclockwise motion activates the right eye.37 A movement was counted when the animal followed the drum rotation at the same angular speed and in the same direction. We considered a one-movement event to be when the head axis (frontal-occipital) was clearly rotated by minimum 15°. We used the same quantification method to evaluate the background. All movements slower or faster than drum speed were not taken into account. We checked first the response obtained with the left and then the right eye and observed no significant differences between eyes. In consequence, the ORs from the clockwise and counterclockwise stimuli were then pooled.

In the Rpe65−/− mice, the first tested eye (left) did not respond as well as the second (right) eye, thus precluding pooling of the results. This finding could be explained by the fact that individuals with low vision need a longer time to adapt. To abolish these disparities, we performed test sessions of 6 minutes in the mice, changing the direction of the drum every 2 minutes, and we collected data during the last 4 minutes only. In this condition, we observed no significant differences between eyes.

Tissue Processing and Immunolabeling

For immunohistochemical analyses, Rpe65−/− and Rpe65−/−;Rbo−/− mice were killed at 4 weeks of age after optomotor recordings. The eyes were cataractized for orientation indication, enucleated, fixed in 4% paraformaldehyde, rinsed in PBS, cryoprotected in 30% sucrose overnight, embedded in albumin from hen egg whites (Fluka, Buchs, Switzerland), and cut into 14-μm cryostat sections. Immunohistochemistry was performed as described previously.26 Briefly, the first antibody was incubated overnight at 4°C in PBS containing 0.2% TritonX-100 and 10% normal horse serum. Sections were then washed and incubated with the secondary antibody at 37°C for 1 hour. The following primary antibodies were used: goat polyclonal blue-sensitive opsin (N20; 1/1000) and rabbit polyclonal GNAT2 (1:100; both from Santa Cruz Biochemicals, Santa Cruz, CA). For visualization, fluorescence-labeled secondary antibodies (AlexaFluor 488 and AlexaFluor 633; Molecular Probes, Eugene, OR) were used. Sections were counter-
stained with DAPI (Molecular Probes) and mounted under coverslips (Mowiol 4-88 Reagent; VWR International AG, Lucerne, Switzerland).

**Data Analysis and Statistics**

We measured the average between the results found in both white and black settings (background) for each mouse strain, and the mean value was carried over in the statistical analysis to determine whether the number of head movements (at spatial frequency settings) was significant compared with the background. The data were analyzed by one-way, repeated-measures ANOVA (Prism 5.0 Software; GraphPad Software Inc., San Diego, CA). The Turkey and Dunnett tests were used for post hoc analysis. A level of $P < 0.05$ (two-tailed) was considered significant.

Sections were analyzed on a microscope equipped for epifluorescence (model BX60; Olympus Suisse SA, Aigle, Switzerland) and coupled to software (Cell-P; Soft Imaging System, Olympus, Hamburg, Germany). To quantify the number of cells positive for GNA12 and S-opsin, we counted the number of outer segments that stained positive throughout the retina of the most transverse section containing the optic nerve. Histologic counting of cone markers was analyzed by one-way ANOVA (Prism 5.0; GraphPad Software, Inc.) to determine the statistical significance between Rpe65<sup>−/−</sup> and Rpe65<sup>−/−</sup>;Rbo<sup>−/−</sup> mice.

**RESULTS**

**Background Noise**

We first investigated whether head movements can be elicited without the presence of a contrasting decor, resulting in a background threshold that allows discrimination between spontaneous activity (background noise) and visual acuity of mice induced by striped patterns. In photopic conditions, the C57/Bl6, Cpf1<sup>−/−</sup>, and Rpe65<sup>−/−</sup> mice showed similar background noise. In contrast, activities of the Gnat1a<sup>−/−</sup> (1.2 ± 0.2 mov/min) and Rpe65<sup>−/−</sup>;Rbo<sup>−/−</sup> (1.3 ± 0.27 mov/min) mice were significantly higher than those in the C57/Bl6 (P < 0.01) animals, indicating that spontaneous activity was increased in these strains (Fig. 2). Under scotopic conditions, we showed similar results. The background thresholds of the

**OR in Scotopic Conditions**

To estimate the visual acuity in scotopic conditions (<0.1 lux), we tested stripes with spatial frequencies ranging from 0.025 to 0.5 cyc/deg (Fig. 3, Table 1). As previously demonstrated,<sup>22–24</sup> the C57/Bl6 mice showed clear head movements in scotopic conditions between 0.025 and 0.2 cyc/deg. ORs in the C57/Bl6 mice reached maximum at 0.1 cyc/deg (17.3 ± 1.78 mov/min), and the visual reflex was limited to 0.3 cyc/deg (P < 0.05). Although the number of head movements per minute was reduced in the Cpf1<sup>−/−</sup> mice, the response range was comparable, being at its maximum at 0.1 cyc/deg (11.3 ± 0.83 mov/min), but the responses were significantly lower than those observed in the C57/Bl6 animals at 0.075 and 0.1 cyc/deg (P < 0.001). When spatial frequencies were higher than 0.2 cyc/deg, responses were equivalent to background values (P > 0.05). In contrast, no OR was scored in the Gnat1a<sup>−/−</sup> mice, which show cone function only. The values were similar to those obtained with background settings (P > 0.05), with the maximum number of head movements never exceeding 1.6 ± 0.10 mov/min at 0.1 cyc/deg. These results indicate that rods are essential in mediating ORs in scotopic conditions.

**Optomotor Response in Photopic Conditions**

A similar approach was undertaken in photopic conditions (650 lux), to reveal the respective contributions of rods and cones to the OR (Fig. 4, Table 2). The C57/Bl6 mice showed head movements between 0.025 and 0.3 cyc/deg, with the maximum response at 0.1 cyc/deg (12.5 ± 0.68 mov/min). At 0.4 and 0.5 cyc/deg, the OR decreased and became identical with that observed at background settings (P > 0.05). The Gnat1a<sup>−/−</sup> mice behaved similarly to the C57/Bl6 control animals when they were placed in photopic conditions, with a
maximum response observed at 0.2 cyc/deg (19 ± 0.84 mov/min). There was no major strain-related difference in the ORs compared with the C57/Bl6 control animals between 0.025 and 0.2 cyc/deg (P > 0.05), except at 0.3 cyc/deg (P < 0.01), which induced a more pronounced activity in the Gnat1a−/− mutants. With frequencies higher than 0.3 cyc/deg, no response was detected, and the number of head movements was not significant in regard to the baseline threshold. Compared with the C57/Bl6 and Gnat1a−/− mice, the Cpf1−/− cone-deficient mice were significantly less sensitive and less stimulated in photopic conditions between 0.025 and 0.5 cyc/deg (P < 0.001). However, the Cpf1−/− mice showed an OR significantly higher than background values (P < 0.01), and their OR was limited to 0.2 cyc/deg with a maximum OR obtained for 0.05 cyc/deg (5.4 ± 0.49 mov/min). These results suggest that cones are essential to properly induce OR with bright-light stimuli.

### OR in Rpe65−/− and Rpe65−/−;Rho−/− Mice

We measured ORs in Rpe65−/− mice at 8 weeks of age, when almost no more cones remained. Contrary to the C57/Bl6 and Cpf1−/− mice, the Rpe65−/− animals did not display a visual response under scotopic conditions. From 0.025 to 0.5 cyc/deg, we did not observe any significant difference in the Rpe65−/− responses compared with those at background settings or the Gnat1a−/− responses (Fig. 3, Table 1), indicating that Rpe65−/− mice are not responsive to dim stimuli in comparison to pure rod Cpf1−/− or C57/Bl6 animals. Similarly, no OR was detected in the Rpe65−/−;Rho−/− mice under scotopic conditions (Fig. 3, Table 1). Scores obtained between 0.025 and 0.5 cyc/deg were analogous and did not significantly differ from background data. This absence of OR is consistent with the lack of rod function in Rpe65−/−;Rho−/− mutants.

Under photopic conditions, the Rpe65−/− mouse showed a significant OR for 0.075, 0.1, and 0.2 cyc/deg (respectively, 9.9 ± 1.62, 9.9 ± 1.01, and 2.7 ± 0.50 mov/min; Fig. 4, Table 2). At 0.075 and 0.1 cyc/deg, no obvious difference in response was observed compared with those of the C57/Bl6 and Gnat1a−/− mice. At 0.2 cyc/deg, the OR decreased significantly compared with that of C57/Bl6 or Gnat1a−/− animals, but was still significant compared with the baseline level (P < 0.05). Nevertheless, when spatial frequencies were lower than 0.075 cyc/deg and greater than 0.2 cyc/deg, scores of the Rpe65−/− mice were analogous to those of the baseline threshold. These results indicate that, under photopic conditions, the OR of the Rpe65−/− mice was limited to a range of spatial frequencies between 0.075 and 0.2 cyc/deg.

To assess whether the photopic OR recorded in the Rpe65−/− mice was driven by rods, we investigated the ORs in 4- and 8-week-old double-knockout Rpe65−/−;Rho−/− mice. At 4 and 8 weeks after birth under photopic conditions, there were no differences in the ORs between the Rpe65−/−;Rho−/− age-matched animals and background values (Figs. 4, 5; Table 2). We next analyzed the expression of cone-specific markers in 4-week-old Rpe65−/− and Rpe65−/−;Rho−/− animals, an age at which the mouse retina can be considered mature, to verify that the absence of OR in the double-knockout was not due to a difference in the number of cones compared with those of the Rpe65−/− mice. We compared the retinal morphology and the expression of SWL-opsin in 8-week-old Rpe65−/− and Rpe65−/−;Rho−/− mice by immunostaining (Fig. 6) and did not observe significant differences in the expression pattern of SWL-opsin. In both strains, SWL-opsin expression was mislocalized to the axon and cone pedicle, indicating that there is no major difference in SWL-opsin expression and localization (Fig. 6). In addition, we quantified the number of cells positive for the cone opsin short wavelength (SWL-opsin) and the cone-specific transducin a-subunit (GNAT2), and it appeared that it was similar in both strains (Fig. 7). These results indicate that even if some cones were present in the Rpe65−/− and Rpe65−/−;Rho−/− animals, they did not mediate visual behavior.

### Rod–Cone System Responses

To specify the ranges of rod and cone sensitivity and their contributions to visual acuity under various light intensities, we used a spatial frequency of 0.1 cyc/deg corresponding to...
the most efficient stimulus for most strains tested for both photopic and scotopic conditions (Fig. 8). As in the C57/Bl6 mice, the ORs were measured in dim light conditions (0.01 lux) as well as under intense illumination (1750 lux), with a maximum of head movements at 3.2 lux (8.1 m/min; 0.15 lux (1.1 m/min; 150 lux, the response decreased (11.5 ± 0.87 m/min)). Inversely, in Cpf1−/− mutant mice, rod photoreceptors were activated and contributed to visual response was detected by Schmucker et al.23 who estimated the highest sensitivity of pure cone function—the retina’s visual model, the mice responded significantly reduced compared with the C57/Bl6 and Wistar rats. Mice are nocturnal, rod-dominant with ERG recordings and an OR study of other mouse models, pure cone mice behaved similarly to the C57/Bl6 mice under photopic conditions but were unable to elicit head movements in scotopic conditions. A similar sensitivity to spatial frequency under photopic conditions was also described for these two mouse models.24 These data are in line with the results obtained with ERG recordings and an OR study of other mouse models of pure cone function—the Rho−/− mouse1,2,25 showing that the visual function of these animals is limited to the photopic range. The Gnat1−/− mice (pure rod model) are able to respond to the test in both photopic and scotopic conditions. However, ORs were significantly reduced compared with the C57/Bl6 and Gnat1−/− scores in photopic conditions indicating that cones are essential to respond properly to light stimuli and to provide daylight vision. This result also shows that rods can mediate some visual behavior in photopic conditions. Similarly, Schmucker et al.25 using an optomotor test, found that Cnga3−/− cone-deficient mice display visual acuity in photopic conditions. These results are in part in contrast to

### DISCUSSION

#### Visual Acuity in Wild-Type Mice and in Mice Lacking Rod (Gnat1−/−) or Cone (Cpf1−/−) Functions

The present findings indicate that the behavioral optomotor test is useful for estimating visual acuity and discriminating rod and cone contributions to vision. Previous studies and data have demonstrated that the optomotor test is a robust and efficient behavioral technique to assess vision in such species as zebrafish,25,26 chickens,27,28 and mice.19,21,22 Furthermore, the optomotor test provides an easy, noninvasive method of exploring the visual function in mice. In the current work, vision in the C57/Bl6 mice in both photopic and scotopic conditions is consistent with that in the study by Schmucker et al.23 who estimated the highest sensitivity at 0.3 c/deg. Similar to the results found by Umino et al.,24 the maximum number of responses was detected for spatial frequencies of the same range: from 0.025 to 0.2 c/deg (0.03–0.1 c/deg in Umino et al.,24 who used an automated system for speed and size of the rotating bands and for recording).

It is interesting to note that the scotopic OR was higher than the photopic OR. Mice are nocturnal, rod-dominant animals (97% of the photoreceptors),39 which could explain these variations in visual behavior in bright and dim illumination conditions. The Gnat1−/− pure cone mice behaved similarly to the C57/Bl6 mice under photopic conditions but were unable to elicit head movements in scotopic conditions. A similar sensitivity to spatial frequency under photopic conditions was also described for these two mouse models.24 These data are in line with the results obtained with ERG recordings and an OR study of other mouse models of pure cone function—the Rho−/− and Cngb1−/− mice,17,23,31 showing that the visual function of these animals is limited to the photopic range. The Cpf1−/− mice (pure rod model) are able to respond to the test in both photopic and scotopic conditions. However, ORs were significantly reduced compared with the C57/Bl6 and Gnat1−/− scores in photopic conditions indicating that cones are essential to respond properly to light stimuli and to provide daylight vision. This result also shows that rods can mediate some visual behavior in photopic conditions. Similarly, Schmucker et al.25 using an optomotor test, found that Cnga3−/− cone-deficient mice display visual acuity in photopic conditions. These results are in part in contrast to

#### TABLE 2. Relative Responses in Wild-Type and Knockout Mice in Photopic Conditions

<table>
<thead>
<tr>
<th>Strain</th>
<th>0.025</th>
<th>0.05</th>
<th>0.075</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57/Bl6</td>
<td>10.0 ± 0.91***</td>
<td>10.0 ± 0.75***</td>
<td>10.0 ± 0.75***</td>
<td>12.5 ± 0.68***</td>
<td>11.8 ± 1.06***</td>
<td>4.8 ± 0.44***</td>
<td>2.0 ± 0.26</td>
<td>0.7 ± 0.11</td>
</tr>
<tr>
<td>Gnat1−/−</td>
<td>9.6 ± 0.59***</td>
<td>9.5 ± 0.78***</td>
<td>12.6 ± 0.77***</td>
<td>12.6 ± 0.85***</td>
<td>15.0 ± 0.84***</td>
<td>6.9 ± 0.32**</td>
<td>1.7 ± 0.05</td>
<td>1.7 ± 0.11</td>
</tr>
<tr>
<td>Cpf1−/−</td>
<td>2.9 ± 0.47*</td>
<td>5.4 ± 0.49*</td>
<td>4.5 ± 0.44*</td>
<td>5.1 ± 0.65*</td>
<td>4.1 ± 0.61*</td>
<td>0.8 ± 0.11</td>
<td>0.8 ± 0.10</td>
<td>0.8 ± 0.08</td>
</tr>
<tr>
<td>Rpe65−/−</td>
<td>0.9 ± 0.17</td>
<td>1.5 ± 0.38</td>
<td>9.9 ± 1.62***</td>
<td>9.9 ± 1.01***</td>
<td>2.7 ± 0.50*</td>
<td>1.0 ± 0.11</td>
<td>0.9 ± 0.10</td>
<td>1.0 ± 0.10</td>
</tr>
<tr>
<td>Rpe65−/−; Rho−/−</td>
<td>1.9 ± 0.19</td>
<td>1.9 ± 0.06</td>
<td>1.7 ± 0.15</td>
<td>1.9 ± 0.08</td>
<td>1.4 ± 0.08</td>
<td>1.85 ± 0.07</td>
<td>1.2 ± 0.08</td>
<td>1.2 ± 0.08</td>
</tr>
</tbody>
</table>

Number of head movements per minute (mean ± SEM; n = 10 per group) at different spatial frequencies was measured in wild-type (C57/Bl6) and mutant mice lacking functional rods, cones, or both (Gnat1−/−, Cpf1−/−, Rpe65−/−, and Rpe65−/−; Rho−/−). Strain background was used as the reference to determine the OR for each strain.

* Differences significantly from the values of the corresponding strain background. Strain-related differences were observed: **P < 0.01; ***P < 0.001, by one-way, repeated-measures ANOVA and Dunnett post hoc tests.
significantly reduced at 0.6 lux compared with the C57/Bl6 and in both photopic and scotopic conditions. However, ORs were stipulate that particularly high intensities (rod vision) cannot see above \( /H11002 \) level at 150 lux. Rpe65 old of the cone system is at 3.2 lux and reaches the maximum According to the results obtained, we stipulate that the thresh-

Contributions of Rods and Cones in Different Levels of Light

Our study also specifies the ranges of rod and cone sensitivity and their contributions to visual acuity under various light intensities. In vision-deficient mice, the activation thresholds of the two different photoreceptor systems were determined. According to the results obtained, we stipulate that the threshold of the cone system is at 3.2 lux and reaches the maximum level at 150 lux. Cpf1I\(^{-/-}\) mice were able to respond to the test in both photopic and scotopic conditions. However, ORs were significantly reduced at 0.6 lux compared with the C57/Bl6 and Gnat1a\(^{-/-}\) animals and declined significantly, with higher intensities showing that cones are essential to photopic vision, even though rods can mediate some visual behavior under this condition. Supporting these observations, the Cpf1I\(^{-/-}\) ERG recordings indicate that the photopic responses measured are due to rods that are not completely bleached.\(^{12}\) Thus, we stipulate that particularly high intensities (>1750 lux) are necessary to desensitize the system and to completely abolish rod responses. These results are in agreement with previous works evaluating the retinal activity and rod and cone contributions to OR.\(^{17,18,23,24}\) Combination of the different results obtained shows that rods mediate an appropriate visual function in mice at luminance levels of less than 18.5 lux (cf., Cpf1I\(^{-/-}\) results), defining the scotopic range, whereas cone contribution occurs at luminance levels above 3.2 lux (cf., Gnat1a\(^{-/-}\) results), specifying the photopic range. In consequence, a visual reflex can be activated from 0.01 to 1750 lux (at least) and cones and rods work together from 3.2 lux and higher until the rod system is bleached (>1750 lux).

Rod Activity and Visual Behavior in \( Rpe65^{+/--} \) Mice

The purpose of this work was to raise the question of rod-and cone-respective contributions to visual behavior in \( Rpe65^{+/--} \) mice. The OR was limited from 0.075 to 0.2 cyc/deg only under photopic conditions and the \( Rpe65^{+/--} \) mice started to respond significantly at 150 lux. The \( Rpe65^{+/--} \) mice had an OR only at high light intensity, suggesting that either cones respond to these stimuli, but with a lower sensitivity, or cone reaction is absent with a strong decrease of rod sensitivity. It is interesting to remember that children affected by the disease are photophilic. Indeed, both patients and \( Rpe65^{+/--} \) mice have a minute amount of chromophore\(^{30}\) that binds to rare opsin. In consequence, only a high flux of photons can stimulate photoreceptors. In accordance with our results, Aleman et al.\(^{55}\) indicate that \( Rpe65^{+/--} \) mice show a severe impairment of the transient pupillary light reflex (TPLR) compared with the \( Rpe65^{+/+} \) mice, but responses elicited with much higher-intensity stimuli in the \( Rpe65^{+/--} \) mice have properties similar to those evoked by lower intensities in control \( Rpe65^{+/+} \) mice. Because it has been shown that no cones are left at this age\(^{9}\) and the double-knockout \( Rpe65^{+/--}; Rho^{+/--} \) mice were not

![Figure 6](https://example.com/image6.png)

**Figure 6.** Localization of cone SWL-opsin. Retinal sections of 1-month-old mice were stained with antibodies against SWL-opsin in \( Rpe65^{+/--} \) and \( Rpe65^{+/--}; Rho^{+/--} \) retinas. Both strains showed that the S-opsin was mislocalized to the axons and cone pedicles.

![Figure 7](https://example.com/image7.png)

**Figure 7.** Cone marker quantification: quantification of the number of positive cells expressing GNAT2 and SWL-opsin in \( Rpe65^{+/--} \) and \( Rpe65^{+/--}; Rho^{+/--} \) mice at 4 weeks of age (mean ± SEM; \( n = 4 \) per group).

![Figure 8](https://example.com/image8.png)

**Figure 8.** Evaluation of rod and cone system contributions. Quantification of the ORs was measured at 0.1 cyc/deg as a function of illumination intensity (from 0.01–1750 lux) and is expressed in the number of head movements per minute in C57/Bl6, Gnat1a\(^{-/-}\), Cpf1I\(^{-/-}\), and \( Rpe65^{+/--} \) mice (mean ± SEM; \( n = 10 \) per group). Results represent the contribution of rod-cone systems. Statistical analysis: one-way, repeated-measures ANOVA and Dunnett post hoc tests.
able to respond in photopic conditions in the present study. 

**Optomotor Response in Rpe65−/− Mice**

Our study showed that cones and rods mediate ORs in response to light stimuli, but with different sensitivities and at different spatial frequencies. Using diverse mouse strains, it appears that, in wild-type mice, both photoreceptor systems (rods and cones) are essential to generate a visual reflex in scotopic and photopic conditions. In addition, this work provides evidence that the rod system is the source of a particular visual function in Rpe65−/− mice. Finally, the optomotor test is a simple behavioral method for estimating visual acuity in mice and is efficient to discriminate the relative contribution of different physiological structures.

**Acknowledgments**

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**References**


