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Fast and slow light-induced changes in murine outer retina OCT: Complimentary high spatial resolution functional biomarkers

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Abstract

Fast (seconds) and slow (minutes to hours) optical coherence tomography (OCT) responses to light stimulation have been developed to probe outer retinal function with higher spatial resolution than the classical full-field electroretinogram (ERG). However, the relationships of functional information revealed by OCT and ERG are largely unexplored. In this study, we directly compared the fast and slow OCT responses with the ERG. Fast responses [i.e., the optoretinogram (ORG)] are dominated by reflectance changes in the outer segment (OS) and the inner segment ellipsoid zone (ISez). The ORG OS response has faster kinetics and a higher light sensitivity than the ISez response, and both differ significantly with ERG parameters. Sildenafil-inhibition of phototransduction reduced the ORG light sensitivity, suggesting a complete phototransduction pathway is needed for ORG responses. Slower OCT responses were dominated by light-induced changes in the external limiting membrane to retinal pigment epithelium (ELM-RPE) thickness and photoreceptor-tip hyporeflective band (HB) magnitudes, with the biggest changes occurring after prolonged light stimulation. Mice with high (129S6/ev) vs. low (C57BL/6J) ATP synthesis efficiency show similar fast ORG but dissimilar slow OCT responses. We propose that the ORG reflects passive physiology, such as water movement from photoreceptors, in response to the photocurrent response (measurable by ERG), whereas the slow OCT responses measure mitochondria-driven physiology in the outer retina, such as dark-provoked water removal from the subretinal space.
Significance Statement:

This study revealed distinct properties of fast and slow light-induced responses recorded with optical coherence tomography, which are mediated by direct photoreceptor activity and metabolic regulation in outer retina, respectively. A combination of these non-invasive imaging tools provides high spatial resolution measurements for a more comprehensive view of retina function than previously available, that is complementary to electrical signal recorded with electroretinogram.
Introduction

Light triggers a photochemical transduction cascade in photoreceptors that generates an electrical signal that is eventually transmitted to brain to elicit a visual percept. These electrical signals can be non-invasively recorded from the eye as an electroretinogram (ERG), which has long served as the sole means for non-invasive probing of retinal function. For the ERG responses recorded from the dark-adapted eye, the ERG a-wave is mainly linked to photocurrent changes from rod photoreceptors, and the ERG b-wave from activity of the second order neurons. The ERG c-wave, on the other hand, is produced by RPE and Muller cells as an indirect response to photoreceptor activity. However, the full-field ERG reports only on the whole retinal response so that local variations in dysfunction and focal treatment responses are often not detectable. In addition, ERG is known to only incompletely interrogate the retinal physiology. For example, it is hard to extract indices of mitochondria activity, a central aspect of retinal function, from the monovalent ion changes on a sub-second time scale measured by the ERG.

To address this problem, high spatial resolution measurement of optical changes following light stimulation using optical coherence tomography (OCT) in retinal tissue has been suggested. So far, both fast (seconds) and slow (minutes to hours) OCT changes show promise as retinal function biomarkers. Fast OCT responses have been designated as the optoretinogram (ORG), which can be revealed both as reflectance changes and as subtle (submicron) thickness alterations. In addition, a faster response peaked within 10 milliseconds has also been reported. A prominent ORG response is the increase of outer retinal reflectivity of the inner segment ellipsoid zone...
(ISez) and the outer segment (OS) bands upon light stimulation\(^5,7,15\). These fast responses are linked to light-induced rhodopsin isomerization and passive, osmotic alteration in water content as a consequence of phototransduction steps\(^5\). However, exactly how these osmotic changes are produced and the direction of water movement after light stimulation are still under debate\(^5,16\). In addition, potential contributions from mitochondria function have been proposed to underlie ORG responses, but this hypothesis has not been fully tested\(^17,18\). Furthermore, although the fast OCT responses have been reported in human patients\(^19\), it requires specialized custom-built OCT systems that often not available in most eye clinics.

Slow responses have been measured by diffusion MRI and OCT in rat and mouse outer retina after long room light or dark adaptation\(^4,20-22\). For example, robust light-dark changes in mice retina include larger (4-6 \(\mu\)m) changes in outer retina thickness (from ELM to RPE), and large changes in hyporeflective band (HB) intensity between the photoreceptor tip and RPE layers\(^4,22-24\). The physiology of the ELM-RPE thickness change is well understood (for more details see the Discussion) and closely linked to mitochondria-induced pH changes in the subretinal space\(^25,26\). A recent study found that the light-dark HB signal intensity changes to be correlated with those of the ELM-RPE thickness but in a pH-insensitive manner, suggesting a link to mitochondria activity as well\(^24\). Intriguingly, the light-dark ELM-RPE thickness change measured with OCT measured can be used to detect oxidative stress in outer retina\(^23,25,26\).

In this study, we measured the following OCT biomarkers in the outer retina captured with a commercial OCT instrument: (1) light-induced changes in the mean intensity of the ISez and OS bands (i.e., the ORG response); (2) ELM-RPE thickness; and
(3) HB magnitude. We determined the kinetics and intensity-response relations of the light stimulation induced reflectance increases for both ISez and OS bands in outer retina. The light sensitivity and kinetics of these mouse ORG signals were compared to the outer retina ERG responses. In these same datasets, the ORG responses are compared with slow OCT biomarkers: ELM-RPE thickness and HB magnitude changes. We also asked how ATP synthesis efficacy \(^{25,27}\) and phototransduction modulated the ORG and OCT biomarkers. Our results support the notion that the fast ORG response is mediated by upstream events (e.g., the initial rod photoreceptor shape changes) and the passive light-induced osmotic water build up in the subretinal space are linked to phototransduction \(^5\). In other words, the ORG is a consequence of the ion charge movement-based transduction mechanism that is measured by the ERG \(^5,7,18\). On the other hand, the slow changes in outer retina thickness and HB intensity are consistent with downstream consequences of mitochondria-evoked shifts in water content in the dark compared to that in the light. Together, these different OCT modalities and biomarkers provide a complementary set of tools to non-invasively probe retinal function \textit{in-vivo} with high spatial resolution and sensitivity.

\section*{Results}

\textbf{Light-induced fast OCT intensity increases in outer retina bands:}

Most of study was performed with C57BL/6J (B6J) mice, a common mouse strain used to study the visual system. Figure 1 shows an example of OCT images captured from a dark-adapted eye (Fig. 1A left panel) and the same retina location 2 minutes after light exposure (Fig. 1A middle panel) using a commercial instrument (Bioptigen Envisu UHR2200) which constructs images based on logarithmic OCT intensity. OCT images
were exported in 8-bit (256 level) tiff format. Intensity of one grey level is referred as one a.u. throughout this study. A 2-minute light exposure was chosen as the ORG response metrics reached saturation at this time (see next section). Averaged intensity profiles of these two OCT images along retinal depth are shown on the right panel of Fig. 1A. Fig. 1B shows light-induced intensity difference of the two images in Fig. A with its intensity profile plotted in the right panel. It is clear that the largest differences in OCT intensity induced by light stimulation are in the outer retinal regions, i.e., ISez and OS. In this study, we focused on the outer retina regions for light-induced changes in the OCT image, as illustrated with a magnified view in Fig. 1C for OCT images and their intensity profile. Specifically, we measured light-induced OCT intensity changes for ISez and OS bands as indices ORG, and ELM-RPE thickness and magnitude of HB as indices of slow OCT responses.

**Kinetics of mouse ORG responses:**

To further characterize the ORG response, we then measured kinetics of the light-evoked signal intensity changes in the ORG for the ISez and OS bands to an 80-sec light stimulation step (Fig. 2A). The ISez responses appeared to show a slightly slower kinetics than the OS response. To quantitate the kinetics, the responses were fit with an exponential function (solid lines in Fig. 2A). The time constants for the average ON response of ISez and OS band reflectance increases are shown in Fig. 2B for both bright (4.0 log cd/m²) and dim (2.34 log cd/m²) light stimulations. There were significant differences observed (p<0.01) for the time constants of ISez and OS responses elicited by bright light stimulation. The time constants for ISez response elicited by dim light stimulation may have been slightly larger than those for OS responses, but this could not
be confirmed statistically. The amplitudes of ISez and OS responses for bright stimulation are shown in Fig. 2C. Although OS showed larger response than ISez, the difference is not statistically significant (p=0.08). Next, we asked if the signal intensity temporal trajectory changed in mice with higher (129/S6) or lower (B6J) ATP synthesis efficiency. Notably, light-induced reflectance changes in ISez and OS bands in S6 and B6J mice did not show significant differences in response amplitudes (p=0.78 for OS and p=0.80 for ISez, Fig. 2C). In contrast, S6 mice showed much smaller changes than B6J mice for slow OCT responses, i.e. long duration light adaptation induced reductions in ELM-RPE thickness and HB magnitude.

The effects of light intensity on recovery kinetics are shown in Fig. 2D. After 80 seconds of bright light stimulation, both ISez and OS ORG signals persisted for several minutes. Ten minutes after bright light stimulation, their intensity had decayed ~30% on average. On the other hand, after a dim light stimulation, ORG signals for both ISez and OS returned to baseline within 2 minutes.

**Fast and slow light-induced changes of ELM-RPE thickness:**

The 80 seconds of light stimulation also induced a significant small extension of the outer retina (measured from ELM to RPE layer) thickness as shown in Fig. 3A for C57BL/6J mice. The ELM-RPE region expanded about 0.7 um (Fig. 3B), and similar magnitude of the response was also observed on S6 mice. The recovery time course of ELM-RPE thickness after an 80-sec light stimulation also depended on the stimulus light intensity. ELM-RPE thicknesses were persistently extended over 10 minutes after termination of a bright (4.0 log cd/m²) light stimulation. On the other hand, for dim (2.34
log cd/m$^2$) light stimulation, ELM-RPE thickness returned to baseline level within 5 minutes after termination of the light stimulation (Fig. 3C).

Using phase sensitive measurement, the light induced extension of photoreceptor OS length has been reliably detected$^{6,14,19}$. This change in OS length can be revealed on conventional OCT images$^5$. We also measured OS length changes in response to 80-sec light stimulation, and the results are shown in supplemental Fig. S1. However, the magnitude of OS length change is small, and none of light induced OS length changes reached statistical significance. Consequently, we only focused on ELM-RPE thickness changes in this study.

**Light-induced changes on the photoreceptor tip-HB magnitude:**

The HB magnitude, as illustrated in Fig. 1C, was increased from baseline after light stimulation. Fig. 4A showed HB magnitudes for each mouse eye before (baseline) and after an 80 sec stimulation with bright light (4.0 log cd/m$^2$). This increase of the HB magnitude was statistically significant. As S6 mice showed no light-induced the HB responses$^{24}$, no comparison with B6J mice was made. Recovery time course of the HB also depends on the stimulation light intensity. A fast recovery was observed for dim light stimulation, whereas the HB magnitude persisted for several minutes after termination of bright light stimulation (Fig. 4B).

**Effect of light-adaptation on OCT responses:**

Fig. 5 shows the effect of prolonged light adaption (>5 hr to room light) on the mouse OCT responses. Compared with a fully dark-adapted (over-night) mice, ORG responses were significantly diminished from mice adapted to room light (Fig. 5A). With an 80-sec bright light stimulation, the ISez response for dark-adapted mice averaged
10.13 ± 1.35 a.u. (mean ± SEM) but only 3.67 ± 1.35 a.u. for light-adapted animals. OS responses showed similar differences averaging 10.71 ± 1.12 a.u. for dark-adapted mice and 1.35 ± 0.88 a.u. of light-adapted mice. Similarly, light-adaptation also significantly diminished changes induced by 80-sec bright light stimulation on ELM-RPE thickness (Fig. 5B, left panel) and HB magnitude (Fig. 5B, right panel).

There is a significant difference (p<0.001) of ELM-RPE thickness at baseline for dark-adapted (51.86 ± 0.44 µm) and light-adapted (56.85 ± 0.67 µm) mice (Fig. 5C). The 80-sec of light stimulation only slightly increased ELM-RPE thickness for dark-adapted mice whereas no changes were observed for light-adapted mice. Consequently, there is still large significant difference of ELM-RPE thickness after light stimulation for these two groups of mice (p<0.01). The difference in ELM-RPE thickness for these two groups of mice is still significant even after 15 min of continued light stimulation (data not shown).

Mice light-adapted to room light had a larger HB magnitude (15.92 ± 1.41 a.u.) than those dark-adapted overnight (2.74 ± 0.57 a.u., p<0.001; Fig. 5D baseline). A short pulse of light stimulation elicited significant increases in the HB magnitude for dark-adapted mice (4.89 ± 0.99 a.u., p<0.05) but had no effect on light-adapted mice (15.04 ± 1.14 a.u., p=0.30).

**ORG vs. ERG:**

The dark-adapted ERG a-wave is mainly mediated by the electrical responses of rod photoreceptors, whereas the b-wave has a large contribution from bipolar cells in the inner retina, and the c-wave reflects voltage changes in both RPE and Muller cells in response to ionic changes in outer retina \(^1\). To compare the ORG and ERG light
sensitivity curves, we determined the intensity-response relation of the OCT ISez and OS bands and compared the ERG a- and c-wave responses. Fig. 6A showed examples of ERG transients to a step of light stimulation. The ERG a-wave is the first negative peak followed onset of light stimulation, and the c-wave is a slow positive response usually peak at 3-5 sec after light onset. The time course of ERG responses is considerably faster than those observed for ORG light-ON and -OFF kinetics (Fig. 2), indicating these two responses interrogate distinctly different functional pathways. Fig. 6B show data normalized to the maximum values, with continuous curves as fit to a Naka-Rushton equation [eq (1) in Methods]. Measured by half-saturation constant K, the response of OS band also showed significant higher sensitivity ($K = 1.48 \pm 0.40 \text{ cd.s/m}^2, n=6$) than the response for ISez band ($K = 7.81 \pm 1.65 \text{ cd.s/m}^2, p<0.01$). Interestingly, the ORG showed intermediate sensitivity between ERG a-wave and c-wave.

**Impact of phototransduction:**

To investigate the effects of phototransduction on the mouse ORG responses, we examined the OCT intensity of dark-adapted retina, and recorded the light-induced responses after administering sildenafil, a phosphodiesterase (PDE5/6) inhibitor. PDE6 activity in photoreceptor is inhibited in the dark, and light stimulation removes the inhibition and increases the enzymatic activity. Dark-adapted mice treated with sildenafil exhibited reduced OCT intensity changes of both ISez and OS bands (Fig. 7A), whereas sildenafil had no significant effect on dark-adapted ELM-RPE thickness or HB magnitude. Since sildenafil acts on the ERG, reducing PDE6 activity with sildenafil also decreased the sensitivity of the ORG response for both the OS (Fig. 7B, p<0.01) and the ISez (Fig. 7C, p<0.01) bands.
Discussion

In this study, we identified for the first time with a commercial OCT system, differences between fast and slow OCT responses, and the ERG, from the same mouse eye. Light-induced fast responses were largely located at OS and ISez retinal bands, similar to those reported with non-commercial systems. It has been postulated that ORG responses are mediated by light-induced passive osmotic changes that alters cell shape. Here we provide additional support to link ORG signals with the complete phototransduction pathway. Light adaptation, which saturates the rod photoreceptor, significantly reduced the ORG responses (Fig. 5). Sildenafil, a PDE inhibitor that alters sensitivity of phototransduction, also reduced the ORG photosensitivity for both the OS and ISez responses (Fig. 7).

The kinetics of the responses are also consistent with the ORG signals originating from phototransduction mechanisms. The phototransduction machinery is located mainly in the photoreceptor OS, and the ORG response was observed first in the OS region with relatively slower onset kinetics for the ISez band (Fig. 2) in agreement with a previous report. The ORG recovery kinetics for both OS and ISez depended on stimulus intensity (Fig. 2D), similar to the rod photoreceptor dark-adaptation time course recovery after different levels of light stimulation. Also, the ISez response is about 0.7 log unit less sensitive than the OS responses (Fig. 6B). Such differences in the time course and light sensitivity for ISez and OS band could reflect different subcellular organelle and protein composition in these two distinct photoreceptor regions. Comparison of the ORG with the ERG are summarized in Fig. 6. The dark-adapted ERG a-wave mainly reflects rod photoreceptor electrical activity (i.e. light-induced reduction of the dark current).
The higher light sensitivity of the ORG than the ERG a-wave indicated a physiological mechanism for the ORG response subsequent to the ionic changes mediated by the dark-current. The ERG c-wave is generated by RPE and Muller cells in response to potassium concentration changes in the subretinal space produced by the rod photoreceptor light response. The lower sensitivity and slower time course of the ORG OS and Ise responses compared with the ERG c-wave (Fig. 6) suggested a more delayed mechanism for the ORG than those produced by light-induced potassium changes in the subretinal space. It should be noted, however, that OCT-based ORG often only measures from selected regions of the retina, whereas full-field ERG measures summed activity from whole retina. In this study, we did not compare ORG with the ERG b-wave which is mainly mediated by bipolar cells in the inner retina.

As demonstrated in previous studies, slow responses can be detected in outer retina on both ELM-RPE thickness and the photoreceptor tip-HB magnitude. As might be expected, light-induced changes on both ELM-RPE thickness and HB magnitude on short time scale (within minutes) were only a small fraction of the response amplitudes exhibited for fully (over hours) dark-adapted and light-adapted mouse retina (Fig. 5). Due to the low signal-to-noise ratio for ELM-RPE thickness and HB biomarkers, it is not possible to accurately measure the kinetics of these OCT signals for each mouse eye examined. However, the averaged signal from all recorded mice showed a similar time course as those of the ORG responses (Fig. 2 and Supplemental Figs S2 and S3), suggesting a common mechanism (i.e., water redistribution between photoreceptor and subretinal space) for ORG and the early changes in these slow OCT biomarkers. Also, the different mechanisms mediating initial and long-term responses of slow OCT
biomarkers are supported by a comparison of responses from B6J and S6 mice. These two strains of mice exhibit large differences in long-term responses that attribute to their difference in mitochondria activity. On the other hand, the phototransduction machinery is likely very similar in these two strains of mice. Consequently, both the ORG and the initial responses of these slow OCT biomarkers for these two mice were also very similar (Fig. 2C and 3B).

The mechanisms involved in generating the ORG response are an active area of investigation. It has been postulated that the ORG responses are mediated by an osmolality increase of the photoreceptor initiated from light-triggered dissociation of transducin from a trimer into α and βγ subunits. This hypothesis predicts water flow into the photoreceptor under light adaptation. However, this interpretation appears to contradict a large body of work since the mid-1990’s involving a variety of techniques and model systems showing that light induces accumulation of water in subretinal space. Based on the common observation that excitation induces neurons to swell and decrease light scattering, we propose following signal pathway:

Light \rightarrow \text{photoreceptor hyperpolarization} \rightarrow \text{osmolality decreases} \rightarrow \text{outer flow of water to subretinal space} \rightarrow \text{photoreceptor shrink} \rightarrow \text{OCT intensity (scattering) increase}

Pfäffle et al provided a mathematic model of how reduction of photoreceptor volume (shrinking) leads to elongation of cell length.

On the other hand, as reviewed in a recent publication, long-term responses of slow OCT biomarkers (ELM-RPE thickness and HB) are correlated with metabolic demands in outer retina region. Specifically, the slow responses of the ELM-RPE thickness and HB bands are contributed by water production from mitochondria.
metabolism and water removal by the fluid transporter on the RPE. The ORG responses (OS and Isez) reflect passive changes in the outer retina triggered by phototransduction, whereas the slow OCT biomarkers require active contribution of mitochondria metabolism and have a slower time course.

In sum, commercial OCT imaging can reveal light induced change in the outer retina with both fast and slow kinetics. Passive cellular water movement seems to dominate the ORG response whereas active modulation of the subretinal space hydration in the dark contributes to the slower OCT response. In any event, our results raise the possibility that the ORG and long-term OCT biomarkers are useful complementary non-invasive tools to probe direct photoreceptor activity and metabolic regulation, respectively; and a combination of the measurements would provide a more comprehensive view of outer retina function than currently available from ERG measurements, which could have clinical utility in evaluating visually impaired patients.

Materials and Methods

Animal: All procedures involving animals were conducted under an approved National Institutes of Health (NIH, Bethesda, MD, USA) animal care protocol by the National Eye Institute Animal Care and Use Committee and ARRIVE guidelines. All methods were performed in accordance with the relevant guidelines and regulations. Adult wild-type mice (C57Bl/6J and 129/S6), 3 to 6 months old, of either sex were used in this study. The mice were kept in regular animal housing under a 50 lux 14:10 hour light/dark cycle.
**OCT imaging and analysis:** OCT images were captured using an Envisu UHR2200 system (center wavelength 870 nm) (Bioptigen, Durham, NC). Mice were anesthetized with ketamine (100 mg/kg) and xylazine (6 mg/kg), and the eye was positioned with the optic nerve head (ONH) in the center of the OCT scan. To capture kinetics of ORG response, 2000 B-scans (1000 A-scan for 1.4 mm) were captured in 88 seconds and saved into one OCT files. Initiation of the OCT scan was triggered by a footswitch which was simultaneously synchronized to activate light stimulation after a prescribed delay using a Raspberry Pi microcomputer. 8-bit OCT images were used in this study, and intensity of OCT bands are expressed 8-bit scale as arbitrary unit (a.u.). To calculate the OCT intensity of retinal bands, 20 B-scans were averaged, and OCT images from 350 µm to 630 µm away from optic nerve head were analyzed. OCT transretinal intensity profiles were calculated for each region and averaged across the image after aligning the ELM peaks. ELM-RPE thickness was measured from peak of ELM layer to basal side of RPE layer. Magnitude of the hyporeflective band (HB) between photoreceptor tip (OS) and RPE layer follows previously described methods.

The light-evoked ORG onset responses were fit to an exponential decay function. Normalized intensity-response data were fit with a Naka-Rushton equation:

\[
R = \frac{I^n}{I^n + K^n} \quad \text{(1)}
\]

where I is the light intensity, n is the Hill coefficient, and K is the half saturation constant. Data fitting were performed with Prism 9 (GraphPad Software, San Diego, CA). Data are presented as mean ± SEM.

**Light stimulation:** Light stimuli were provided by a LED ring light (World Precision Instruments, Sarasota, FL) mounted on the OCT bore. A white plastic half
ping-pong ball diffuser was attached onto the OCT bore to provide full-field uniform light stimulation at the mouse’s eye. The timing and duration of the light stimulation was controlled by a Raspberry Pi using a custom program. Neutral density films were added between the LED light source and ping-pong ball to adjust for light intensity. Light intensities were calibrated with Flame-S Spectrometer (Ocean Optics, Inc., Largo FL) with the probe mounted at the same position as the mouse’s eye.

**Electroretinogram (ERG) recording:** ERG responses were recorded following published protocols with an Espion E2 Visual Electrophysiology System (Diagnosys, Lowell, MA, USA). After an over-night dark adaptation, mice were anesthetized with ketamine (100 mg/kg) and xylazine (6 mg/kg), and their eyes were dilated with a drop of tropicamide (1%) and phenylephrine (0.5%). The body temperature of the mouse was maintained at 37°C by a heating pad. Responses from both eyes were recorded with gold wire loop electrodes with band pass filtering from DC to 300 Hz. Steps of light with intensity ranged from 0.01 to 1000 cd/m² were delivered to mouse eye by the attached ColorDome. Inter-stimulus intervals ranged from 1 to 5 min depending on stimulus intensity. A-wave amplitudes were measured from baseline to negative trough, while slowly developing c-wave amplitudes were measured from the baseline to the wave peak, usually occurring around 3-5 sec after light onset.

**Effect of sildenafil:** Mice were treated with sildenafil according to a previously published protocol. Briefly, dark-adapted mice were given an intraperitoneal injection of 29 mg/kg sildenafil, a dose that produces a transient impairment in retinal electrophysiology. OCT images were acquired 1 hour after sildenafil injection. Saline-injected mice were served as controls.
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Data Availability statement

All data is included in the manuscript and/or supporting information.
References


Figure 1: Examples of the light-induced changes observed in the mouse retinal OCT image. (A) Averaged (20x) B-scan OCT images captured at baseline in the dark (left panel) and 2 min after light stimulation (middle panel). Mean depth intensity profiles across retina are shown on right panel. NFL: nerve fiber layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; ELM: external limiting membrane. (B) Pseudocolor difference image of the images shown in A. Light induced increases in the intensity of the two outer retinal layers are evident, whereas the other parts of retina showed relatively little change to light stimulation. Mean depth intensity profiles across retina are shown on the right panel as black trace, with peaks for ISez and OS retinal layers. (C) Magnified view of the outer retina region of the OCT image, from the area marked by yellow square in A, with mean depth intensity profile differences shown on right side. ELM-RPE thickness is measured as distance between ELM peak and basal side of RPE layer. Hyporeflective band (HB) is defined as intensity dip in the region between OS and RPE.
Figure 2: Kinetics of the light-induced OCT responses. (A) Onset kinetics of ISez and OS band. Each data time point was measured from an averaged B-scan images after light exposure, and continuous curves are fit to an exponential equation. The ISez signal exhibited slight slower development compared with OS signal. The light stimulus time course of is shown as the bottom trace. (B) The fitted time constants for ISez and OS responses show significant kinetic differences ($p < 0.05$) for bright light stimulation (Bright: $1.0 \times 10^4$ cd/m$^2$ (n=6); Dim: $2.2 \times 10^2$ cd/m$^2$ (n=6)). (C) Peak amplitudes for light-induced intensity increase of the ISez and OS bands elicited from C57B6J (n=6) and 129S6 (n=5) mice. (D) Time course of the Off kinetics of ISez and OS band to an 80 sec
pulse of light at two intensities (Bright (n=4): 1 x 10^4 cd/m^2; Dim (n=5): 219 cd/m^2).

Duration of light stimulation is shown as the bottom trace.
Figure 3: Light-induced changes in ELM-RPE thickness. (A) ELM-RPE thickness measured for each mouse eye before (baseline in dark) and after 80-sec bright light stimulation. There is a significant increase (P<0.01, n=7) of ELM-RPE thickness after light stimulation on C57Bl6J mice. (B) Comparison of mean amplitudes of light-induced
ELM-RPE thickness changes elicited from C57BL/6J and 129S6 mice. (C) Time course of off kinetics for ELM-RPE thickness changes from the baseline (values measured before onset of light stimulation). Duration of light stimulation (Bright (n=4): 1 x 10^4 cd/m^2; Dim (n=5): 219 cd/m^2) is shown as the bottom trace. *, significant (p<0.05) difference for values measured after bright and dim light stimulation.
Figure 4: Light-induced changes in the photoreceptor tip hyporeflective band (HB) magnitude of C57/Bl6J mice. (A) HB magnitudes measured for each mouse eye before (baseline in dark) and after 80-sec bright light stimulation. There is a significant increase (P<0.01, n=7) of HB magnitudes after light stimulation. (B) Time course of off kinetics for HB magnitudes. Duration of light stimulation (Bright (n=4): 1 x 10^4 cd/m^2; Dim (n=5): 219 cd/m^2) is shown as the bottom trace. *, significant (p<0.05) difference for values measured after bright and dim light stimulation.
Figure 5: Effect of light- and dark-adaptation on the light-induced OCT responses on C57BL/6J mice. (A) Responses of ISez and OS bands to 80-sec bright light stimulation for dark- and light-adapted mice. (B) Changes in ELM-RPE thickness and HB magnitude to 80-sec bright light stimulation. (C) ELM-RPE thickness of dark- and light-adapted mice before (baseline) and after 80-sec bright light stimulation. (D) HB magnitudes of dark- and light-adapted mice before (baseline) and after 80-sec bright light stimulation. Dark-adapted: n=5; Light-adapted: n=5. **, p<0.01; ***, p<0.001; ****, p<0.0001.
**Figure 6**: Comparison of the intensity-response the OCT ISez and OS band, with the a- and c-wave of the ERG responses. (A) Example of ERG traces elicited by a light step stimulation. Intensities of light stimulation are shown on left side panel. (B) Intensity-response relations for ERG a- and c-wave and OCT ISez and OS band. Data were
normalized to their respective peak amplitudes. Continuous cures are fitting to a Naka-Ruston equation (OCT responses: n=6; ERG responses, n=5).
**Figure 7**: Effect of sildenafil on the light-induced outer retinal OCT responses. Effect of sildenafil on baseline band intensity of ISez and OS on the dark-adapted mouse retina (A) (Control: n=5; Sil: n=9; saline: n=9). Effect of sildenafil on intensity-response relation for ISez band (B) and OS band (C). Continuous curves are fitting to a Naka-Ruston equation. Controls are replotted from data shown in Fig. 6.