MRI of Retinal Free Radical Production With Laminar Resolution In Vivo

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PURPOSE. Recent studies have suggested the hypothesis that quench-assisted 1/T1 magnetic resonance imaging (MRI) measures free radical production with laminar resolution in vivo without the need of a contrast agent. Here, we test this hypothesis further by examining the spatial and detection sensitivity of quench-assisted 1/T1 MRI to strain, age, or retinal cell layer-specific genetic manipulations.

METHODS. We studied: adult wild-type mice; mice at postnatal day 7 (P7); cre dependent retinal pigment epithelium (RPE)-specific MnSOD knockout mice; doxycycline-treated Sod2flox/flox mice lacking the cre transgene; and α-transducin knockout (Gnat1−/−) mice on a C57Bl/6 background. Transretinal 1/T1 profiles were mapped in vivo in the dark without or with antioxidant treatment, or followed by light exposure. We calibrated profiles spatially using optical coherence tomography.

RESULTS. Dark-adapted RPE-specific MnSOD knockout mice had greater than normal 1/T1 in the RPE and outer nuclear layers that was corrected to wild-type levels by antioxidant treatment. Dark and light Gnat1−/− mice also had greater than normal outer retinal 1/T1 values. In adult wild-type mice, dark values of 1/T1 in the ellipsoid region and in the outer segment were suppressed by 13 minutes of light. By 29 minutes of light, 1/T1 reduction extended to the outer nuclear layer. Gnat1−/− mice demonstrated a faster light-evoked suppression of 1/T1 values in the outer retina. In P7 mice, transretinal 1/T1 profiles were the same in dark and light.

CONCLUSIONS. Quench-assisted MRI has the laminar resolution and detection sensitivity to evaluate normal and pathologic production of free radicals in vivo.

Keywords: oxidative stress, rods, MRI, free radicals, retina

Rod cell oxidative stress, the continual generation of free radicals exceeding a cell’s quenching capacity, has been implicated in the pathogenesis of currently untreatable, degenerative diseases of the retina, including retinitis pigmentosa and diabetic retinopathy.1–16 However, clinical benefits from antioxidant treatment have not been realized, perhaps because a one-size-fits-all approach may be too simple and also because we cannot assess antioxidant treatment efficacy in vivo on rod cells. Conventional methods, such as optical coherence tomography (OCT) and electrophysiology (ERG), do not measure rod cell free radical production. Recent methods have examined redox-responsive probes to evaluate oxidative stress from specific reactive oxygen species in vivo, but these approaches are problematic because they (1) modify the redox environment by the presence of the reporter probes, (2) require careful consideration of the biologic time course of the probe, (3) require intravitreal injections, and (4) measure fundus oxidative stress without laminar resolution.17–20 Thus, the use of these methods for longitudinal studies and in the clinic is unlikely, limiting objective evaluation of antioxidant treatment efficacy over time within rod cells.

Recently, we, and others, unmasked a contribution of continuously produced free radicals to the spin-lattice relaxation rate (1/T1) magnetic resonance imaging (MRI) signal by collecting data in the absence and presence of a quenching condition ("quench-assisted MRI").21,22 Quench-assisted 1/T1 MRI measured outer retina oxidative stress following pharmacological manipulation.21 Furthermore, quench-assisted MRI reliably reported the in vivo suppression of free radical production in healthy outer retina in response to a light quench.21 For example, light quench-assisted MRI readily distinguished between the expected differences in the light response of rods of 129S6 wild-type mice versus cones of R91W/Nrt−/− mice.21 These results suggest that light-evoked suppression of outer retinal 1/T1 signal is linked with reduced leakage of free radicals from mitochondria.9,23

Here, we extend the above studies by testing the spatial and detection sensitivity of quench-assisted MRI to strain, age, or retinal cell layer-specific genetic manipulations. The following groups were evaluated: (1) a common wild-type mouse strain, the C57Bl/6 mouse, (2) immature mice before outer retinal photosensitivity develops, postnatal day 7 mice (P7), (3) mutant mice missing mitochondrial superoxide dismutase (SOD) from only the retinal pigment epithelium (RPE) layer (and their controls), and (4) mice lacking rod specific α-transducin (Gnat1). α-transducin is an essential signaling...
element for rod phototransduction. In general, because Gnat1−/− mice have inoperant rod phototransduction without retinal degeneration, they allow for the exploration of cone responses to light, as well as rhodopsin regeneration via the visual cycle. In this study, quench-assisted MRI outcomes in vivo in these groups matched results generated from previous studies using ex vivo methods. Overall, our results provide further support for the hypothesis that quench-assisted MRI has the cellular and subcellular spatial resolution, and detection sensitivity, for evaluating normal and pathologic production of retinal free radical production in vivo.

**Materials and Methods**

All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and Institutional Animal and Care Use Committees of both the Wayne State University and the University of Florida. Animals were housed and maintained in 12-hour:12-hour light-dark cycle laboratory lighting, unless otherwise noted.

**Groups**

The following mix of male and female mice, all on a C57Bl/6 background, were used: wild-type adult mice (2–3 mo. of age) or P7 mice (Jackson Laboratories, Bar Harbor, ME), and adult Gnat1−/− mice (kind gift of Dr. Janis Lem). The RPE-specific MnSOD knockout mice (2–3 months of age) were generated as previously described after seven generations of breeding with C57Bl/6 wild-type mice. Briefly, experimental mice in which exon 3 of Sod2 was flanked by foxP sites were also transgenic for PVM2D2cre and tetO-PcMVcre, so that cre recombinase was expressed only in the RPE. Pups of this genotype (Sod2foxPfoxVM2D2cre) were induced to express cre recombinase by feeding doxycycline-laced chow to nursing dams for 2 weeks after delivery (P1–P14). Two control groups were used: (1) doxycycline-treated Sod2foxPfox mice lacking the cre transgene and (2) C57Bl/6 wild-type mice (2–3 months of age). Magnetic resonance imaging examination occurred 6 to 8 weeks after cessation of doxycycline treatment.

Dark-adapted RPE-specific MnSOD knockout mice were studied either untreated, or with pretreatment by a combination of two antioxidants, methylene blue (MB, an alternate electron transporter that effectively inhibits superoxide generation by mitochondria), and α-tocopherol (LPA, a potent free radical scavenger). Approximately 24 hours before an MRI examination, mice were injected with 1 mg/kg MB (dissolved in saline) intraperitoneally (IP). The next day, approximately 1 hour before the MRI examination, each MB-treated mouse was also treated with 50 mg/kg LPA (dissolved in saline and pH adjusted to ~7.4) IP.

**Magnetic Resonance Imaging**

The general mouse preparation for high resolution MRI is well established in our laboratory. All animals were maintained in darkness for at least 16 hours before and during the dark phase of the MRI examination. In all groups, immediately before the MRI experiment, animals were anesthetized with urethane (36% depth solution IP; 0.083 mL/20 g animal weight, prepared fresh daily; Sigma-Aldrich Corp., St. Louis, MO, USA). Adults were treated topically with 1% atropine to ensure dilation of the iris during light exposure followed by 3.5% lidocaine gel to reduce sensation that might trigger eye motion, and to keep the ocular surface moist. P7 mice did not undergo a canthotomy, but we have previously shown that ~500 lux room light is sufficient to activate retinal melanopsin in vivo.

In all cases, high resolution 1/T1 data (details below) were acquired on a 7 T system (ClinScan; Bruker Corporation, Billerica, MA, USA) using a receive-only surface coil (1.0 cm diameter) centered on the left eye. Each 1/T1 data set takes 15 minutes to collect. 1/T1 data were collected first in the dark and then at 13 minutes and 29 minutes (midpoint of acquisition) after turning on the light. Unfortunately, our current data acquisition time ability precludes examination of 1/T1 responses at earlier time points. The end of a fiber optic bundle was attached to a light source (Mark II Light Source; Prescott’s, Inc., Monument, CO, USA) placed caudal to the eye, projecting onto a white screen ~1 cm from eye, similar to that previously described. We exposed the eye to 0 (i.e., dark) or ~550 lux (confirmed outside the magnet before and after each MRI run using a Traceable Dual-Range Light Meter (Control Company, Friendswood, TX, USA) placed against a 1-cm diameter aperture; measured this way, room lighting is ~500 lux). In all cases, animals were humanely euthanized as detailed in our Division of Laboratory Animal Resources (DLAR)-approved protocol.

The 1/T1 MRI procedure has been described in detail previously. Retinal partial saturation T1 data were acquired using a dual coil mode on a 7 T Bruker ClinScan system: Several single spin-echo (time to echo [TE] 13 ms, 7 × 7 × 7 mm², matrix size 160 × 320, slice thickness 600 μm, in-plane resolution 21.9 μm) images were acquired at different repetition times (TRs) in the following order (number of images collected per time between repetitions in parentheses): TR 0.15 seconds (6), 3.50 seconds (1), 1.00 seconds (2), 1.90 seconds (1), 0.55 seconds (4), 2.70 seconds (1), 0.25 seconds (5), and 0.50 seconds (3). In other words, to compensate for reduced signal to noise ratios at shorter TRs, progressively more images were collected as the TR decreased. During an MRI session, animals were studied in an alternating order between controls and experimental mice.

**MRI Data Analysis**

In each adult animal, we confirmed ocular dilation based on the iris position on the MRI data. Retinal 1/T1 MRI data from the central retina (±1 mm from the center of the optic nerve) were analyzed as previously described. Single images acquired with the same TR were first registered (rigid body) and then averaged. These averaged images were then co-registered across TRs. The central retinal regions-of-interest were analyzed by calculating 1/T1 maps by fitting to a three-parameter T1 equation \( T1 = a + b\exp(-c^TR) \), where a, b, and c are fitted parameters) on a pixel-by-pixel basis using R (version 2.9.0; R Development Core Team, Vienna, Austria) scripts developed in-house, and the minpack.lm package (version 1.1.1, Timur V. Elzhov and Katharine M. Mullen minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK. R package version 1.1–1). The reciprocal (1/\( T1_1 \)) values directly reflect paramagnetic free radical levels. Central intraretinal 1/T1 profiles were obtained as detailed elsewhere. Transretinal profiles from the superior and inferior retina were averaged.

As in our previous study, day-to-day variability was minimized in the MRI experiments by collecting data from at least three control mice studied alternatively with at least three experimental mice. Correction factors were calculated at each retinal depth to adjust the mean control 1/T1 values of the same-day controls to a reference set of control 1/T1 values. These depth-specific correction factors were then...
applied to the experimental data from that day; the Y-axis is labeled as “Adjusted 1/T1.” Because P7 mice data were not collected in this fashion, their Y-axis represents 1/T1 and their data are presented on a separate graph.

In each mouse, retinal thicknesses (um) were objectively determined from MRI data using the “half-height method” wherein a border is determined via a computer algorithm based on the crossing point at the midpoint between the local minimum and maximum, as detailed elsewhere.32,34 The distance between two neighboring crossing-points thus represents an objectively defined thickness. Thickness values were then normalized with 0% depth at the presumptive vitreoretinal border and 100% depth at the presumptive retinal-choroid border. As previously discussed, the dark-to-light transition produces a significant increase in choroidal but not retinal thickness.35–37 To minimize the impact of the light-evoked choroidal expansion on the present analysis, and to allow for comparisons between groups and conditions, 1/T1 transretinal profiles in dark and light in each mouse were spatially normalized to the anatomical thickness value in the dark.38

**Spectral-Domain OCT (SD-OCT)**

One wild-type mouse and one RPE-specific MnSOD knockout mouse treated with antioxidants were randomly chosen for OCT examination (Envisu R2200 VHR SDOIS System; Bioptigen, Inc., Durham, NC, USA). Mice were anesthetized with ketamine and xylazine for the examination. Optical coherence tomography was not performed in the Gnat1−/− group because (1) the MRI retinal thickness measurements are not significantly different from that in the wild-type mice (Fig. 1),35,39 and (2) with only five mutant mice available to be examined by MRI, we did not want to risk possible anesthetic deaths. Instead, OCT measurements of wild-type mice were used in the 1/T1 transretinal profile of Gnat1−/− mice because of a previous report that mice lacking Gnat1 and arrestin1 had nearly normal laminar structure.40 Optical coherence tomography was also not performed in P7 mice because they did not have a clear optical path due to closed eyelids and the presence of an intact hyaloidal circulation.30

**Statistical Analysis**

As in our previous studies, promising MRI transretinal profile location ranges of significant differences were initially suggested using a one-tailed unpaired t-test at different locations of the adjusted 1/T1 transretinal profiles; for these “unofficial” comparisons, no tests for normality of data were performed.38,39,41,42 If the t-test suggested significance (P < 0.05) over a select location range, a more formal statistical analysis using a generalized estimating equation (GEE) approach was used.38,39,41–43 The GEE method is a more powerful two-tailed method that performs a general linear regression analysis using contiguous locations in each subject and accounts for the within-subject correlation between contiguous locations even if the normality assumption is not validated.43,44 Differences on GEE analysis were considered statistically significant at P < 0.05. Data are presented as mean ± standard error of the mean (SEM).

**RESULTS**

**Elevated 1/T1 Values in RPE-Specific MnSOD Knockout Mice**

Dark-adapted RPE-specific MnSOD knockout mice had greater (P < 0.05) 1/T1 values in all layers of the outer retina than the doxycycline-treated Sod2flox/flox mice lacking the cre transgene control group (Fig. 2A), and supernormal 1/T1 levels in the RPE, outer segments, and outer nuclear layers compared to the wild-type control mice (Fig. 2B). MB-LPA treatment significantly (P < 0.05) corrected these supernormal values to levels of both control groups, indicating that the outer retinal...
supernormal 1/T1 values resulted from excessive free radical production. Inner retina had a small but still significant elevation in 1/T1 ($P < 0.05$) over control values that were not correctable by antioxidant treatment suggesting either a non–free radical etiology or lack of detection sensitivity. Comparing the two control groups revealed a significant decrease in 1/T1 ($P < 0.05$) in the outer retina (76%–92% depth, data not shown); more work is needed to clarify this difference. The retina’s laminar architecture was normal on visual inspection of a representative OCT image (Fig. 2B); quantitatively, whole retinal thickness MRI measurements in all groups were unremarkable (Fig. 1A).

**Light Evoked Reduction of 1/T1 in Adult Wild-Type Mice**

As shown in Figure 3A, adult C57Bl/6 wild-type mice demonstrated in vivo light-evoked reductions ($P < 0.05$) in 1/T1 of rod cells, compared to that in the dark. By 13 minutes of light, 1/T1 was significantly ($P < 0.05$) reduced at 80% to 100% depth into the retina (i.e., the presumptive ellipsoid and outer segment regions that contain $\sim75\%$ of the retina’s mitochondria$^{45}$); by 29 minutes of light significant ($P < 0.05$) reduction of 1/T1 was evident over a larger extent of outer retina (60%–100% depth) including the presumptive outer nuclear layer. In addition, 13 minutes of light lowered ($P < 0.05$) 1/T1 significantly at 28% to 40% depth, which corresponded to the inner nuclear layer on OCT. This change was not observed ($P > 0.05$) by 29 minutes of light. These results were not related to duration of anesthetic because, as noted previously, prolonging the duration of dark did not decrease outer retinal 1/T1 values (data not shown).$^{21}$

**Light Evoked Reduction of 1/T1 in Gnat1$^{-/-}$ Mice**

In dark-adapted Gnat1$^{-/-}$ mice, unlike in wild-type mice, light reduced ($P < 0.05$) outer retinal 1/T1 to similar extents by 13 minutes (64%–100% depth) and 29 minutes (60%–100% depth) (Fig. 3B). In addition, outer retina-specific 1/T1 values were visually higher than that of wild-type mice regardless of dark or light adaptation. Light produced an increase ($P < 0.05$) in inner retina 1/T1 by 13 (0%–12% depth) and 29 minutes (0%–16% depth) (Fig. 3B). Whole retinal thickness values on MRI

![Figure 2](image-url)
examination were not different ($P > 0.05$) between wild-type and Gnat1⁻/⁻ mice (Fig. 1B).

**P7 Wild-Type Mice**

In contrast to the light-evoked outer retina changes observed in adult mice, P7 mice (which have active inner, but not outer, retinal photosensitivity $^{30}$) did not demonstrate any light-dependent changes in inner or outer retina $1/T1$ values ($P > 0.05$) (Fig. 4). While the data collection protocol for the P7 mice prevented direct quantitative comparisons with adult wild type data, visual inspection suggests that the maximum $1/T1$ transretinal value was lower than that in wild-type adults and the overall shape of the transretinal $1/T1$ profile was flatter than in the adult (see Methods section). As expected, whole retinal thickness values were different ($P < 0.05$) between adult wild-type mice (225 ± 3 μm; $n = 10$) and P7 mice (254 ± 6 μm; $n = 4$).$^{30}$

**DISCUSSION**

Here, we take advantage of nearly a decade of research by our lab and others validating MRI’s ability to resolve differences in function between different retinal layers in vivo based on $1/T1$ (and other MRI contrast mechanisms); retinal-layer assignments are supported by aligning transretinal functional MRI profiles against representative OCT images.$^{21,46}$ The present results support our previous suggestion that quench-assisted MRI is a reliable readout in vivo of total free radical burden in the outer retina.$^{21}$ Quench-assisted MRI changes in the inner retina are currently more challenging to interpret because they may represent (1) a direct change in free radical production, (2) an indirect downstream neuronal effect linked with changes in the outer retina, and/or (3) be influenced by hemodynamic-linked factors since the inner retina is vascularized.

It is important to consider whether or not quench-assisted MRI could reflect functional changes in factors other than free radicals, such as $pO_2$, $pH$, or hemodynamics. For example, it is well established that light increases $pO_2$ of rod cells (compared to that in the dark).$^{23,47}$ However, if paramagnetic oxygen were the dominant $1/T1$ contrast mechanism measured herein,$^{48,49}$ the known increase in $pO_2$ in the outer retina with light would increase $1/T1$, but instead rod cell $1/T1$ decreases in the light consistent with reduced mitochondrial activity.$^{25,47}$ In addition, $1/T1$ per se is not particularly sensitive to $pH$ in vivo.$^{50}$ A hemodynamic etiology is unlikely because the outer retina is avascular. Free radicals thus represent the most cogent mechanism for understanding quench-assisted MRI results in all conditions studied to-date.$^{21}$

**Quench-Assisted MRI of Outer Retinal Oxidative Stress In Vivo**

In our previous study, systemic injection of low dose sodium iodate was used to produce oxidative stress in RPE and rod layers, based on previous results obtained ex vivo.$^{21}$ Quench-assisted MRI indeed measured in vivo oxidative stress only in the outer retina.$^{21}$ In this study, we examined the RPE-specific
oxidative stress in with the presence of oxidative stress. The mechanism causing free radical production upon photoactivation. In any event, these data support the spatial and detection sensitivity of this study. However, we speculate that the oxidative stress in this will be a topic of future investigation.

Gnat1 mice demonstrated retinal oxidative stress based on previous data from a lucigenin superoxide assay ex vivo. Here, we observe a greater than normal 1/T1 in the outer retina in both dark and light adapted Gnat1 mice consistent with the presence of oxidative stress. The mechanism causing oxidative stress in Gnat1 mice is unclear and not the focus of this study. However, we speculate that the oxidative stress in Gnat1 mice in the light may result, at least in part, from the reported build-up of b-retinoids, which can generate excess free radical production upon photoactivation. In any event, these data support the spatial and detection sensitivity of quench-assisted MRI to outer retinal oxidative stress.

Strain Sensitivity of Light Quench-Assisted MRI In Vivo

Previously, we found that rod dominant 129S6 adult wild-type mice evaluated at 29 minutes of light had rod cell 1/T1 that was reduced at 84% to 100% depth into the retina, compared to that in the dark. In the present study we examined another common mouse strain, the C57Bl/6 mice at two time points of light exposure. The C57Bl/6 mice, light reduced outer retinal 1/T1 but with a different spatiotemporal profile than that of 129S6 mice. In particular, in C57Bl/6 mice, the spatial extent of 1/T1 reduction by 13 minutes of light exposure (80%-100% depth) was similar to that after 29 minutes of light (the only time point studied in 129S6 mice) in 129S6 mice (84%-100% depth). By 29 minutes of light, neither strain showed inner retinal changes in 1/T1. Together, the above considerations suggest that light quench-assisted MRI is sensitive to strain differences in spatiotemporal rod cell responses to light.

MnSOD knockout mouse, a nonpharmacologic model thought to specifically produce oxidative stress in RPE. We examined two controls for this mutant mouse: doxycycline-treated Sod2flox/flox mice lacking the cre transgene on a C57Bl/6 background and C57Bl/6 wild-type controls. Retinal pigment epithelium-specific MnSOD knockout mice had a quenchable, supernormal 1/T1 signal in the RPE indicative of oxidative stress. Somewhat surprisingly, the presumptive outer nuclear segments of these mice also showed evidence of oxidative stress; this will be a topic of future investigation.

Gnat1 mice demonstrated retinal oxidative stress based on previous data from a lucigenin superoxide assay ex vivo. Here, we observe a greater than normal 1/T1 in the outer retina in both dark and light adapted Gnat1 mice consistent with the presence of oxidative stress. The mechanism causing oxidative stress in Gnat1 mice is unclear and not the focus of this study. However, we speculate that the oxidative stress in Gnat1 mice in the light may result, at least in part, from the reported build-up of b-retinoids, which can generate excess free radical production upon photoactivation. In any event, these data support the spatial and detection sensitivity of quench-assisted MRI to outer retinal oxidative stress.

Light Quench-Assisted MRI of Immature Retina In Vivo

In this study, light quench-assisted MRI was also used to confirm the hypothesis that developing and photo-insensitive rod outer segments at P7 will show similar outer retinal 1/T1 values in dark and light. The 1/T1 profile at P7 (Fig. 4) appears relatively flat compared to the adult profile (e.g., Fig. 3A) suggesting little intraretinal differences between inner and outer retina in continuous production of free radicals during development also consistent with immature outer retina. Interestingly, by P7 inner retina intrinsically photosensitive retinal ganglion cells (ipRGCs) are mature enough to detect light, even in the presence of fused eyelids, a tunica vasculosa lentis, and a hyaloidal circulation. However, no light induced reduction in 1/T1 in the inner retina was measured with light quench-assisted MRI (Fig. 4). We speculate that this is at least in part due to a 104-fold lower membrane melanopsin density, compared to that of rod pigment, resulting in a relatively lower photo-catch. The lack of light induced free radical quench in the outer retina of the P7 mouse model provides good negative control data that supports light quench-assisted MRI sensitivity to outer retina phototransduction and visual cycle activity. These results set the stage for a more detailed future developmental study that compares, at different levels of maturity, light quench-assisted MRI outcomes to ERG metrics, MRI measures of rod function, and optokinetic tracking data.

Light Quench-Assisted MRI in the Absence of an Essential Component of Traditional Rod Phototransduction In Vivo

Gnat1 mice demonstrated a large and robust light suppression of 1/T1 in the outer retina relative to that in the dark with a faster spatiotemporal pattern than in wild-type C57Bl/6 mice (Fig. 5). Although Gnat1 mice have fully functional cone photoreceptors, these do not seem to be contributing to the Gnat1 mice light response for the following reasons. First, as shown in our previous study of cone-only Retne mice, light does not change outer retinal 1/T1 values in the
dark and light, consistent with the fact that cones do not saturate in the light. Second, the response of 1/T1 in the outer retina to light in Gnat1−/− mice most resembled that in rod-dominated mouse retina and not that from R91/Wn−/− cone-only mice. Third, numerically, cones comprise only ~3% depth of photoreceptors in mice with a C57Bl/6 background.64

Gnat1−/− mice experience normal rhodopsin bleaching and this will lead to an increase in the production of ATP (and thus a presumed increase in mitochondrial leakage of free radicals) which will lead to an increase in the production of ATP (and thus a presumed increase in mitochondrial leakage of free radicals) and also trigger an increase in free radicals due to visual cycle activation.23 Thus, opposing mechanisms modify the net production of free radicals when turning on the light. These opposing mechanisms likely strike a different balance in rods from wild-type and Gnat1−/− mice, but more work is needed to unravel exact mechanisms.

Summary

This study provides additional evidence to support the use of quench-assisted MRI to close the technology gap in measuring outer retinal free radical production in vivo.21 We anticipate that light quench-assisted MRI will be translatable to humans in the near future, based on recent high resolution MRI of the human retina obtained without sedation using a cued-blinking procedure. In addition, because of the specific topography of human retinas, the contribution of cones will have to be studied in more detail using quench-assisted MRI, such as their sensitivity to ultraviolet illumination, in addition to light related rod, cone, and RPE processes presented here and elsewhere.21

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