Subfoveal Choroidal Blood Flow in Response to Light–Dark Exposure

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Purpose. To document the response of subfoveal choroidal blood flow (ChBF) in the human eye induced by light and dark exposures and provide some insight into the mechanism underlying this response.

Methods. In a group of 12 volunteers (age, 25–60 years), ChBF was measured with a confocal laser Doppler flowmeter. Wavelength of the probing laser beam was 785 nm (90 μW at the cornea). ChBF was recorded in room light, in darkness, in room light after dark adaptation, and during strong green light exposure after exposure to room light. After dark adaptation of both eyes, ChBF was also measured in one eye while only the fellow eye was exposed to strong visible light.

Results. Although ChBF was stable during room light condition, it decreased significantly by 15% ($P < 0.01$) during dark adaptation. After 6 minutes of room light following 20 minutes of darkness, ChBF was back to baseline. Strong, diffuse, green light exposure over a field of 40°, as well as the probing laser beam, had no detectable effect on ChBF. No change in ChBF was detected when the fellow eye was illuminated after both eyes had been dark adapted.

Conclusions. The findings did not confirm the presence of an active process of ChBF regulation in response to light exposure in humans. They demonstrate, however, a reversible decrease in ChBF that occurs after a transition from room light to darkness, which could involve a neural mechanism. (*Invest Ophthalmol Vis Sci.* 2000;41:2678–2683)

One of the physiological functions of the high-flow choroidal circulation is the maintenance of a stable temperature environment for the outer retinal layers. This is particularly the case for the macular region.[1] This function is achieved, presumably, through both passive and active mechanisms, the latter involving a reflexive increase in choroidal blood flow (ChBF) in response to light.[2] The precise neural circuitry mediating this light-induced increase in ChBF is unknown. A number of theoretical investigations, however, have concluded that cooling of the retina during strong laser light exposure can occur without active increase in ChBF.[3–5]

The concept of the modulating action of ChBF has been formulated based on measurements in the light-exposed monkey eye of retinal–choroidal temperature[6] and ChBF, the latter by hydrogen clearance,[7] and measurement in humans of the conjunctival temperature during light exposure of the contralateral eye.[7,8] So far, however, studies performed in the measured eye in rabbits,[9] cats[10] and macaque monkeys[11] have failed to detect a response of ChBF to changes in light exposure. The present investigation attempts to document such a response in the human eye, using laser Doppler flowmetry (LDF).

Methods

Subjects

Twelve healthy white volunteers with a mean age of 35 ± 10 years (mean ± SD) participated in this study. They had a visual acuity better than 0.8 (emmetropia or less than 2 D of myopia or hyperopia), clear media, and no history of ocular or systemic disease or therapy. Brachial artery blood pressures (BP) at rest, measured by automatic sphygmomanometry, were in the normal range. Intraocular pressure (IOP) at rest measured by applanation tonometry ranged from 10 to 15 mm Hg. The procedures followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects after the nature and possible consequences of the study were fully explained. Pupil dilation, when mentioned, was achieved with one to two drops of 1% tropicamide.

Choroidal Blood Flow

Because the LDF measurement of ChBF in the region behind the fovea (subfoveal ChBF) has been described in detail,[12] only a brief summary will be given here. In the present study, a confocal LDF system[13] was used with a probing beam in the near infrared (laser diode, 785 nm). Diameter and power of the beam at the cornea were 1.3 mm and 90 μW, respectively. This power conforms to the American National Standards Institute (ANSI) Z 136.1 standards for laser safety. It was kept at this value during all experiments, unless otherwise stated. The light scattered by the red blood cells in the volume sampled by the laser light was collected by a bundle of six optical fibers (core

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diameter of 110 μm each) and guided to an avalanche photodiode. These fibers were arranged on a circle with a diameter of 180 μm, which was imaged at the retina so that the center of the circle coincided with the focus of the incident beam.

The output photocurrent was sampled at a frequency of 44 kHz (bandwith of 22 kHz) and processed with a software implemented on a computer (NeXT; NeXT Computer, Inc., Redwood City, CA),[14] to determine the ChBF parameters in real time at a rate of 21.5 Hz, using an algorithm based on a photon diffusion theory.[15] These parameters were the velocity, ChBVel (kHz); the number, ChBVol (in arbitrary units, AU), and the relative flux, ChBF (in arbitrary units), of the red blood cells within the sampled tissue region. They are related to each other by the relationship ChBF = $K \times$ ChBVel $\times$ ChBVol, where K represents an instrumental constant. Assuming constant hematocrit, the changes in ChBVol and ChBF were proportional to the changes in actual volume and flow of blood, respectively. The software automatically excluded the Doppler signal during blinks, thereby reducing potential artifacts. Artifacts caused by rapid eye movements were manually removed.[16] The results were stored on a hard disk for immediate monitoring and subsequent data analysis and computation, including average and SD of the flow parameters. The Doppler signal at the output of the detector was also fed into a loudspeaker. Its DC component, which is proportional to the total amount of light reaching the detector, was continuously displayed on the computer monitor.

The flowmeter was mounted on a table equipped with a chin rest and head holder. Seated subjects looked into the instrument, directly at the laser beam. The operator aligned the instrument in such a way that the audio signal reached a maximum. It was maintained in alignment throughout the experiment, by using both this signal and the DC display.

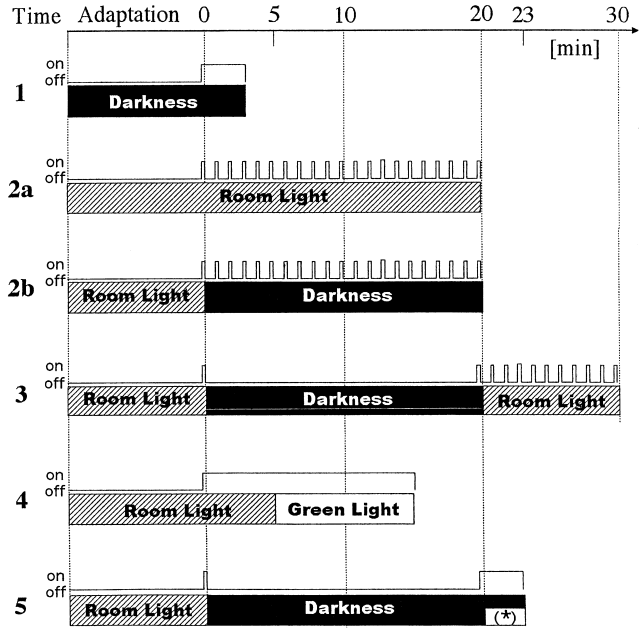

FIGURE 1. Protocol for the various experiments, with light conditions as indicated. The numbers at the left of the picture refer to the various experiments. The label ON indicates when measurements were performed, either intermittently (*narrow vertical rectangles*) or continuously (*broad horizontal rectangles*). Intermittent measurements were performed every 2 minutes, and each lasted 15 seconds. In experiments 1, 2, and 3, both eyes were exposed to darkness or room light before the beginning of the measurements (time 0). In experiment 4, only the measured eye was exposed to the green light. In experiment 5, after 20 minutes of dark adaptation in both eyes, ChBF was measured while the fellow eye was exposed to light (*).

Experiments

In each subject, the various experiments were conducted on different days in the same eye chosen randomly at the beginning of the study. Figure 1 illustrates the timing and characteristics of the retinal illumination and LDF measurements. During each experiment, BP was measured intermittently.

Experiment 1: Effect of the Probing Beam

We investigated in six subjects with dilated pupils whether the probing beam had any effect on the ChBF parameters. After 30 seconds of recordings to verify the alignment, the lights in the room were extinguished, and the power of the probing beam was reduced from 90 to 0.09 μW, a power just sufficient for the subject to see the beam. After 10 minutes of dark adaptation, the power of the laser was switched back to 90 μW (retinal irradiance of ~0.3 W/cm^2) and the flow parameters immediately recorded for 3 minutes, with the room lights off. The data were averaged over segments of 15 seconds and normalized relative to the mean calculated from the first 15 seconds of measurement.

Experiment 2: Effect of Darkness

In eight subjects, after 10 minutes in room light (neon lamp illumination, radiance of approximately 97 μW/cm^2/steradian), the ChBF parameters were measured in the eye with dilated pupil for 15 seconds every 2 minutes during 20 minutes in room light (experiment 2A) and for 15 seconds every 2 minutes during 20 minutes of darkness—that is, no light except the probing laser (experiment 2B). These experiments were conducted on different days. The flow parameters were averaged over each 15-second segment, the first segment serving as baseline for normalization.

Experiment 3: Effect of Room Light in the Tested Eye after Dark Adaptation

After a 30-second baseline measurement in room light adaptation, subjects (n = 6) underwent dark adaptation for 20 minutes The room light was then switched on, and recording segments of 15 seconds' duration were performed in the dilated eye every 2 minutes for 10 minutes. The ChBF parameters were averaged over each segment and normalized relative to baseline values.

Experiment 4: Effect of Strong Visible Light in the Tested Eye after Room Light Adaptation

In six subjects, after 10 minutes of room light adaptation, the choroidal flow parameters were continuously measured in this light for 5 minutes and then for 10 minutes during diffuse light exposure delivered in Maxwellian illumination over a 40° field centered at the fovea. This light originated from an LED with an emission spectrum centered at 524 nm and a Δλ of 60 nm at half height. This source provided a retinal irradiance of 5.2 μW/cm^2, a value equal to the maximum permissible level for approximately 33-minute exposure. The baseline value in each

subject was the average of the first 100 seconds in room light before the diffuse exposure. In one subject, we also exposed the tested eye to a diffuse near-infrared illumination of the fundus of 30° centered at the fovea for 3 minutes. This light was supplied by a diode laser with an emission at a wavelength of 826 nm. Retinal irradiance was 180 $\mu W/cm^2$, a value below the maximum permissible levels of approximately 200 and 800 $\mu W/cm^2$ allowed for continuous exposure for infinite time at wavelengths between 700 and 900 nm, respectively, in accordance with the ANSI Z 136.1 standards.

Experiment 5: Effect of Light in the Fellow Eye after Dark Adaptation of Both Eyes

In eight subjects with both eyes dilated, after a 30-second baseline measurement in room light, subjects were dark adapted for 20 minutes and, with no light other than the probing laser beam, the flow parameters were measured thereafter for 30 seconds in one eye. Then, only the fundus of the fellow eye was diffusely illuminated (Maxwellian view) over a field of approximately 40° with a retinal irradiance of 4.4 $\mu W/cm^2$ while LDF measurements were pursued in a continuous manner for 3 minutes (four subjects) and 5 minutes (four subjects). This illumination was provided by a 20-W halogen lamp and lens inserted into an opaque tube that was placed directly in front of the eye. Care was taken to prevent any of this light from reaching the eye measured by LDF by placing a black screen between the two eyes, perpendicular to the subject's face.

Statistics

Mean changes in the flow parameters were assessed for significance using analysis of variance (ANOVA) and regression analysis. A significance level of 0.05 was used.

Results

Experiment 1

Linear regression of the choroidal flow parameters versus time demonstrated no significant changes in the flow parameters during the 3 minutes of measurements, with the probing beam the only light presented to the subjects. No statistically significant change could be observed in any of the 15-second segments compared with the first segment of measurement.

Experiment 2

Figure 2 shows the results of experiments 2A and 2B. No significant change (both by linear regression and ANOVA) was observed in the flow parameters during the 20 minutes in room light. During the 20 minutes of dark adaptation, however, a log regression analysis revealed a significant decrease in the flow parameters that amounted to 2.8% ($r = 0.62$, $P < 0.05$) for ChBVel, 11% ($r = 0.75$, $P < 0.01$) for ChBVol, and 15% ($r = 0.87$, $P < 0.001$) for ChBF.

Experiment 3

A linear regression of the flow parameter measured intermittently (every 2 minutes) during the 10 minutes of exposure to light after the 20 minutes of dark adaptation (Fig. 3) showed significant increases in ChBVol ($r = 0.96$, $P < 0.01$) and ChBF ($r = 0.967$, $P < 0.001$). Significantly lower values in ChBF compared with baseline were found immediately (−14% ± 8%; $P = 0.003$, ANOVA) at 2 minutes ($P = 0.026$) and 4 minutes ($P = 0.04$) after turning on the light. However, there was no difference at 6 minutes and thereafter.

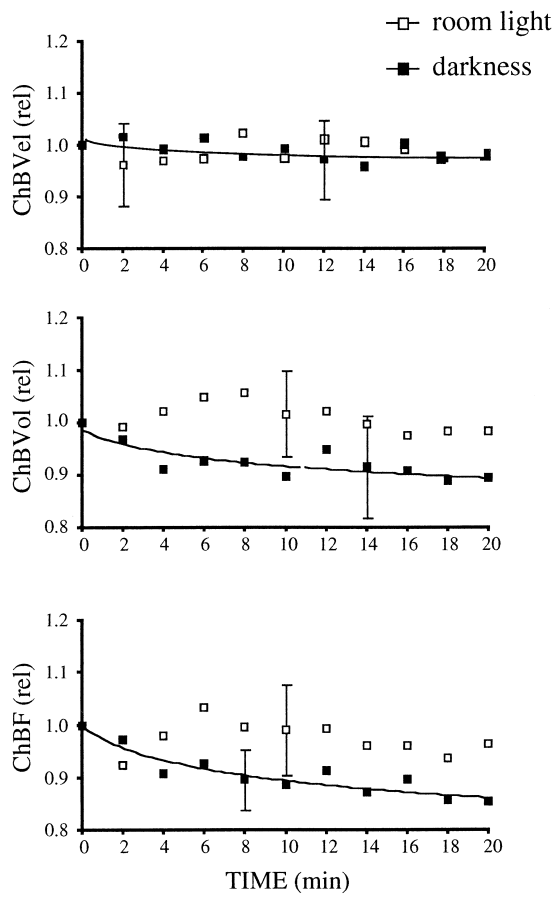

Figure 2. Time course of the group means of ChBVel, ChBVol, and ChBF obtained from one eye of eight subjects during 20 minutes at room light (□, experiment 2A) and during darkness (■, experiment 2B). Measurements were performed intermittently (every 2 minutes). No significant changes were detected at room light. A log regression (*continuous line*) revealed significant decreases in all the flow parameters during the 20-minute exposure to darkness. These decreases amounted to 2.8% ($r = 0.62$, $P < 0.05$) for ChBVel, 11% ($r = 0.75$, $P < 0.01$) for ChBVol and 15% ($r = 0.87$, $P < 0.001$) for ChBF. Typical error bars are ±1 SD.

Experiment 4

Ten minutes of diffuse illumination in green (six subjects) and near-infrared light (one subject) over a field of approximately 40° preceded by 5 minutes in room light demonstrated no significant changes in the flow parameters by ANOVA or regression analysis.

Experiment 5

ChBF after 20 minutes of darkness was an average of 15% below the baseline value. In the four subjects in whom measurements were pursued for 3 minutes of diffuse illumination in the fellow eye, ChBF did not change significantly. In the four subjects in whom the illumination of the fellow eye lasted 5 minutes, there was a significant further decrease in ChBF of

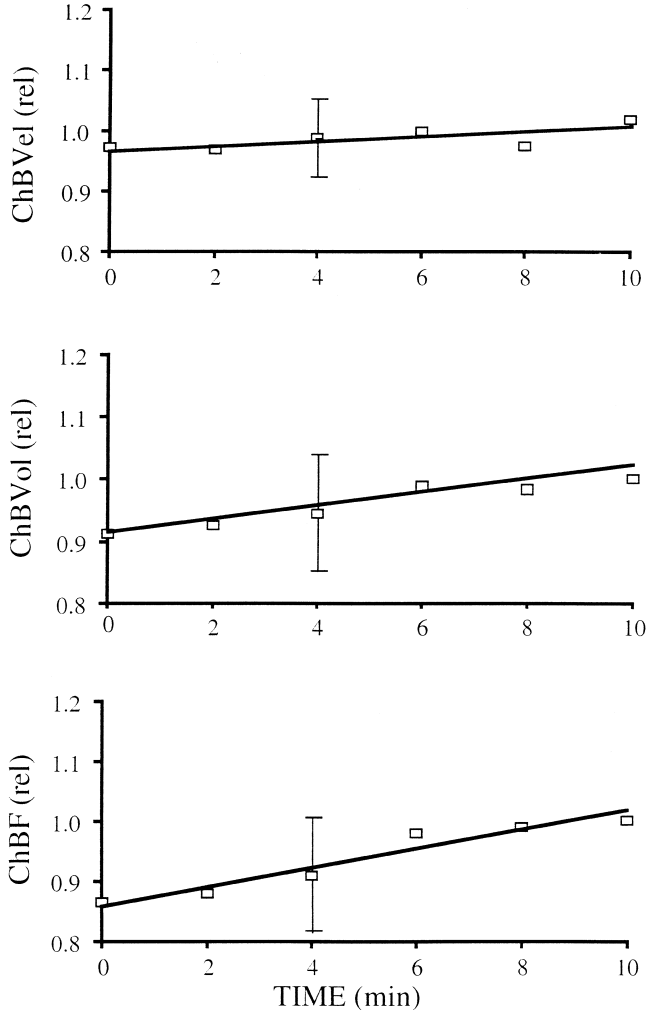

FIGURE 3. Time course of the group means of ChBVel, ChBVol, and ChBF obtained from six healthy volunteers (one eye in each) during 10 minutes of room light exposure after 20 minutes of dark adaptation (experiment 3). Intermittent measurements (every 2 minutes) showed significant increases by linear regression (*solid line*) in ChBVol ($r = 0.96$, $P < 0.01$) and ChBF ($r = 0.967$, $P < 0.001$). ChBF was significantly lower ($-14 \pm 8\%$) at the end of darkness (time 0 minutes) compared with baseline (ANOVA, $P < 0.003$). It was not different from baseline after 6 minutes of room light exposure. The typical error bars are ± 1 SD.

4%. In all experiments there was no significant change in BP based on both ANOVA and regression analysis.

DISCUSSION

The validity of LDF to measure changes in ChBF in the foveal region has been demonstrated.[12] The response of ChBF to changes in ocular perfusion pressure,[17,18] arterial blood oxygen and carbon dioxide tensions,[19] and Valsalva[12] has been documented. Furthermore, the sensitivity of ChBF measurements, defined as the minimum change that can be detected based on 11 subjects, was found to be approximately 6%.[18] Because most of the results of this study are based on six or eight subjects, a sensitivity of less than 9% for ChBF can be expected.

Measurements of ChBF using a fundus camera-based LDF system strongly suggested that the LDF signal originates predominantly from the choriocapillaris, rather than from the larger choroidal vessels behind this layer or the capillaries of the macular region of the retina.[12] In the present study, the relative contribution of light scattered by the blood in the choriocapillaris compared with that scattered from the larger vessels should be even stronger because the probing beam and detecting aperture were confocally focused at the photoreceptors. With regard to the contribution from retinal capillaries, a recent study performed with this instrument demonstrated no detectable change in ChBF in response to 100% oxygen breathing, confirming the absence of contribution from the retina.[19]

Our investigation is the first to report on ChBF measurements in response to light and dark exposures in a group of volunteers. Experiment 1 showed no effect of the laser beam during the 3 minutes of recording that were performed after 10 minutes of dark adaptation (no laser beam). Therefore, we can legitimately assume that the changes in the flow parameters we detected in the other experiments represent responses induced by the various conditions of light–dark exposure.

Exposing room light–adapted eyes to strong, blurring light did not produce any detectable change in ChBF (experiment 4), as previously found in rabbits, in which ChBF was measured by hydrogen clearance.[9] We also did not detect any significant blood flow change when the fellow eye was exposed to strong light (experiment 5). These results are at variance with previous reports in monkeys and humans.[7,8]

Previous studies have advocated both a passive and active role of the choroidal circulation in the maintenance of a stable temperature in the macula.[2,6,8] The observation that when the IOP was increased the retinal–choroidal temperature in the macula of anesthetized monkeys decreased at room light but increased when the eye was exposed to a 7.5-V light source[2,6] led to the hypothesis that ChBF passively dissipates the heat induced by light. In addition, in monkeys, illuminating one eye increased ChBF in the posterior pole of the contralateral eye.[7] This type of illumination also increased the temperature of the posterior pole and conjunctiva of the contralateral eye.[20] The temperature effect on the conjunctiva was also detected in human volunteers.[8] Based on these findings, an active mechanism was postulated with the function of regulating ChBF in response to light. However, the neural circuit that mediates this putative process has not been identified so far by anatomic or functional studies in any species other than pigeon.[21]

The low sensitivity of the LDF technique cannot explain our failure to show an effect of intense light exposure on ChBF. In monkeys, the changes in ChBF were on the order of 70%, corresponding to temperature increases of as much as 0.8°C (Fig. 4 of Reference 8). In humans, the average changes in ocular tissue temperature were reported to be 0.27°C,[9] which would be expected to cause an approximately 20% change in ChBF. Such a change is well within the detection capability of our LDF technique, particularly for our group of subjects, all of whom had excellent target fixation. Indeed, a study in a similar group of volunteers demonstrated significant increases in ChBF of 12% during the breathing of carbogen.[19] We cannot rule out, however, that in humans the change in ChBF associated with an increase of 0.27°C in ocular tissue temperature is less than expected from extrapolating the results of the experiments in the monkey eye[7] and too small to be detected by our LDF method.

There are other factors, however, that make it difficult to reconcile our results with previous findings.[7,8] One is the variability of the effect of light on the temperature and, consequently, on ChBF. We cite previous investigators[8] p1606 who stated that the light-induced temperature reflex in monkeys and humans "was not detectable on every attempt. In addition, the peak amplitude of the ocular surface temperature recorded varied from subject to subject and sometimes from trial to trial in the same subject." This variability is reflected in the large SEM of the temperature data. For example, the SD of the peak amplitude of the temperature variation in humans was equal to 1.71, for a mean of 0.27, corresponding to a coefficient of variation of more than 600%. Further, in both monkeys and humans, "repeated stimulation often resulted in a diminished or abolished response," that leads to the argument that "possibly, the receptors responsible for mediating the reflex become less responsive with multiple exposure, or some other factors involved in the effector mechanism is either depleted or saturated." Our measurements were performed on different days and, according to the remarks just quoted, this should have warranted maximal response. The absence of a response in our subjects suggests, therefore, that other experimental conditions, yet to be identified, may have contributed to previous results. Clearly, more work is needed to determine the factors that must be controlled to evoke the light temperature reflex in a reproducible and consistent manner.

Experiments 2 and 3 demonstrated a 15% decrease in ChBF when eyes adapted to room light were dark exposed and the reverse when dark-adapted eyes were exposed to room light. The change in ChBF was mainly due to the change in ChBVol, suggesting a local vasoconstriction (in darkness) and vasodilatation (in light) of the choriocapillaris behind the fovea. What are the determinants of the decrease in ChBF during dark adaptation? Previous studies have reported increases in IOP of 2 to 6 mm Hg in nonmidriatic eyes during 1 hour of dark adaptation.[22] We do not know by how much the IOP may have changed during the 20 minutes of measurement in our subjects but can assume values less than these. Based on the results of previous work, demonstrating that ChBF in normal subjects autoregulates in response to moderate increases in IOP,[23]such increases should have no significant influence on ChBF.

There is the possibility that the following two opposing processes simultaneously occur during dark adaptation: an increase in ChBF in response to the increase in oxygen consumption by the photoreceptors and a decrease in ChBF due to a decrease in heat production. A decrease in ChBF is observed if the latter process predominates. An increase in ChBF in response to an increase in oxygen would require the choroidal circulation to be sensitive to the decrease in outer retinal Po_2 resulting from the increased oxygen consumption. Measurements in humans, however, have shown no significant effect of arterial Po_2 on ChBF.[19] Furthermore, in cats and monkeys, ChBF was found to be insensitive to the state of adaptation.[10,11] Regarding a decrease in ChBF, it is difficult to explain why a decrease in heat production during darkness (experiment 2) would reduce ChBF, when an increase in this production has no effect on ChBF (experiments 1 and 4).

Flügel et al.[24] have demonstrated the presence of an intrinsic, nitrergic nerve cell plexus that is specifically localized in human eyes in the temporal–central portion of the choroid, which could be of functional significance. This system could play a role in the regulation of ChBF during darkness by decreasing local NO production. Future studies comparing the responses to light exposure of ChBF in the peripheral and macular regions of the fundus may help answer this question.

In conclusion, this study could not confirm the presence of an active mechanism that modulates subfoveal ChBF to maintain retinal temperature in response to light, at least at the exposure levels tested in this investigation. Our results are consistent with the notion that ChBF stabilizes retinal temperature effectively and passively.

Obviously, this mechanism is ineffective in dissipating the heat at levels that produce irreversible thermal damage to the photoreceptors. We report for the first time, however, a reversible decrease in ChBF during dark adaptation that is presumed to be of neural origin.

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