Increased Visual Sensitivity Following Periods of Dim Illumination

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PURPOSE. We measured changes in the sensitivity of the human rod pathway by testing visual reaction times before and after light adaptation. We targeted a specific range of conditioning light intensities to see if a physiological adaptation recently discovered in mouse rods is observable at the perceptual level in humans. We also measured the noise spectrum of single mouse rods due to the importance of the signal-to-noise ratio in rod to rod bipolar cell signal transfer.

METHODS. Using the well-defined relationship between stimulus intensity and reaction time (Piéron's law), we measured the reaction times of eight human subjects (ages 24–66) to scotopic test flashes of a single intensity before and after the presentation of a 3-minute background. We also made recordings from single mouse rods and processed the cellular noise spectrum before and after similar conditioning exposures.

RESULTS. Subject reaction times to a fixed-strength stimulus were fastest 5 seconds after conditioning background exposure ($79\% \pm 1\%$ of the preconditioning mean, in darkness) and were significantly faster for the first 12 seconds after background exposure ($P < 0.01$). During the period of increased rod sensitivity, the continuous noise spectrum of individual mouse rods was not significantly increased.

CONCLUSIONS. A decrease in human reaction times to a dim flash after conditioning background exposure may originate in rod photoreceptors through a transient increase in the sensitivity of the phototransduction cascade. There is no accompanying increase in rod cellular noise, allowing for reliable transmission of larger rod signals after conditioning exposures and the observed increase in perceptual sensitivity.

Keywords: hypersensitivity, scotopic sensitivity, rod vision, rods, phototransduction

The human visual system functions over an enormous range of light intensities. This is accomplished in part by splitting light sensation into two neural pathways: one driven by rod photoreceptors that respond optimally to dim illumination and the other driven by cone photoreceptors that respond to higher light levels. In addition to the inherent cell-specific sensitivity ranges, there are molecular adaptations that occur within the photoreceptors as they experience changes in photon flux. Classically, when photoreceptors are exposed to constant illumination, their sensitivity is decreased as they respond to further changes in luminance. However, we recently described an alternative adaptive mechanism in which there is rod hypersensitivity following a period of barely saturating illumination.¹ Given the increased sensitivity of the rod photoreceptors, this phenomenon was called “adaptive potentiation” (AP). In mouse photoreceptors, AP occurs maximally after 3 minutes of light exposure, and the increase in sensitivity diminishes with an exponential time constant of ~ 7 seconds. Here, in human subjects, we report a psychophysical manifestation of increased sensitivity under similar light exposure conditions, consistent with the physiological results reported in mice.

Adaptations in the sensitivity of the human visual system can be measured using psychophysical approaches, and forms of background adaptation have been previously studied. For example, increment thresholds rise rapidly with increased

illumination, as the scotopic system adapts to different rates of photon absorption.^{2,3} One estimate showed decreased visual sensitivity when background illumination is introduced,³ although a separate report demonstrated that brief backgrounds may actually increase sensitivity.⁴ Because traditional threshold measurements are relatively time consuming, some salient features of fast light adaptation may be missed. Thus, in the present study, we tracked the sensitivity of the visual system by collecting subject reaction times (RTs) to a single constant stimulus that was repeatedly delivered immediately after conditioning light exposure.

The sensitivity of various sensory systems has been measured using RTs since the studies of Piéron described the relationship between RT and stimulus intensity.^{5,6} The initial findings and many subsequent studies defined a power law relationship between RT and stimulus intensity, in which a declining power function describes the relationship between threshold and asymptotic reaction speed. Reaction times to visual stimuli in the scotopic (rod dominant), mesopic (rod + cone mixture), and photopic (cone dominant) light ranges have been modeled previously,⁷ and physiological evidence has attributed the relationship between stimulus intensity and RTs to the physiological properties of the retina, particularly the photoreceptors.^{8–10}

In this study, scotopic RTs were measured before and immediately after a conditioning background was presented. In

comparing the RTs, we report that the subjects showed faster RTs after conditioning background offset than after a period of maintained darkness. This finding was only true over a specific range of conditioning intensities, above which there was instead a masking of the stimulus following conditioning illumination. Our findings suggest that over this range of conditioning illumination, an adaptive potentiation in human rods, similar to that described in mouse rods, may be operating to increase the sensitivity of the entire human visual system by increasing the sensitivity of rod photoreceptors.

We also made physiological measurements of the photocurrents in single mouse rods to quantify changes in cellular noise following conditioning exposures that elicit AP. We investigated single cell noise due to the importance of the signal-to-noise ratio in defining signal transfer at the rod to rod bipolar cell synapse.^{11,12} The rod bipolar cell samples rod neurotransmitter release nonlinearly near threshold; thus, if cellular noise was increased following light exposure, it could prevent a perceptual manifestation despite elevated rod sensitivity. We determined that there is not a significant increase in rod noise during periods of AP. This finding is consistent with the idea that increased single photon amplitudes manifest as increased signal-to-noise ratio in rod photoreceptors, and these signals are transmitted downstream in the visual system.

METHODS

Human Subjects

Eight subjects, aged between 24 and 66 years (four females), participated in this study. If needed, subjects wore either glasses or contact lenses to maintain their best-corrected visual acuity. Visual acuity was self-reported and was 20/20 or better in each subject. Both right- and left-handed subjects were used, and all subjects used his or her dominant hand to respond to stimuli (one left-handed subject). Each subject used his or her right eye for viewing, although one subject was left-eye dominant. Subjects dark adapted for at least 35 minutes while wearing an eye patch in a completely dark room. Testing times ranged from 9 AM to 5 PM in the months of April to June. Subjects were recruited as volunteers, and the informed consent of each subject was obtained before the first testing session. There was no correlation between age groups, under 35 versus over 54, and the observed adaptation effect ($P > 0.3$, t -test). All studies were performed with approval of the Institutional Review Board at the University of Alabama at Birmingham and followed the tenets of the Declaration of Helsinki.

Psychophysical Light Stimulation

A tachistoscope (Iconix 6137; Siliconix, Inc., Santa Clara, CA, USA) was used to combine three light sources within the viewing field (Fig. 1A). A red fixation light-emitting diode ([LED] 650 $\pm$ 20 nm half-width at half-height) was placed 60 cm from the subject's eye, and a white stimulus LED was positioned 22.5 cm (20.4° visual angle) temporal to the fixation light (Fig. 1B) to target an area of high rod density.^{13,14} All stimulus wavelengths were measured using a spectroradiometer (PR-655 SpectraScan; Photo Research, Inc., Chatsworth, CA, USA), with the range reported at half-width at half-height. The stimulus LED was attenuated by neutral density filters, and its spectral emission was set by a 500 $\pm$ 7-nm interference filter. The intensity of the stimulus was adjusted by a potentiometer that controlled the pulse width modulation of an LED at 1 KHz. The intensity of the 5-ms stimulus flash was

calibrated across its range using a photometer (model 350 linear/log optometer; Graseby Optronics, Grass Valley, CA, USA). The flash intensities used ranged from 0.0044 to 0.086 cd/m². Using the measured pupil diameter of 6 mm and a scotopic to photopic td luminosity conversion factor of 3.04 (at 500 nm) yields 0.379 to 7.40 scot td. Multiplying by 8.5 R*/scot td per Kraft et al.,¹⁵ resulted in a stimulus range of 3.2 to 62.8 R*/rod (see x -axis of Fig. 2). Electronic modules controlled the flash duration and stimulus timing (Coulbourn Instruments, Lehigh, PA, USA).

The subject was instructed to fixate on the red LED for the duration of a trial, and eye position was not monitored. No pupil dilation was used for any experiment. In three subjects (two females), changes in pupil size were measured in response to background onset and offset. The conditioning background initially reduced the diameter of the pupil by 16.5% $\pm$ 5% (mean $\pm$ 1 SD) relative to the diameter of the dark-adapted pupil. However, during conditioning background exposure, the pupil recovered to 97.4% $\pm$ 2% of the dark-adapted diameter, and background offset did not expand the pupil. At no time were the measured pupil diameters in any of the subjects larger than the respective measures in the dark-adapted state. We conclude that the light levels that entered the eye throughout the experiment were negligibly affected by changes in pupil diameter.

The conditioning background was generated by a Kodak slide projector focused on a 15- $\times$ 10-cm white diffuser that was placed in the split optical path 32 cm from the subject's eye (visual field angle: 50° $\times$ 36°). The beam of the projector was attenuated with neutral density filters and a 500 $\pm$ 7-nm interference filter that set the spectral emission. The Coulbourn modules also drove the shutter of the projector and controlled the timing of the conditioning background exposure. The conditioning period used in these psychophysical studies (3 minutes) was chosen to maximize the potential effects of AP, as the mouse physiology suggested that AP is maximally induced with 3 minutes of just-saturating light exposure.¹ The conversion factor of 1 scotopic td = 8.5 R*/sec (Kraft et al.¹⁵) was used to transform the measured conditioning stimulus intensity of 10.28 to 14.49 scot td, which yields a range of 88 to 123 R*/rod $\times$ sec. This is similar to the range of R* activation rates reported in the physiological report and represents $\sim$ 0.03% bleach of the total rhodopsin in a single rod over a 3-minute period.

Collection of Human Reaction Time Data

Subjects were instructed to maintain fixation on the red LED during all trials, as well as during conditioning light exposure or sustained darkness. Subjects were requested to initiate each trial by depressing and holding down a handheld button, and releasing the button as quickly as possible if they perceived the flash. Following a variable preperiod that lasted between 300 and 1600 ms, a 5-ms flash was delivered 20.4° temporal to the fixation light. The time between the flash and the release of the button was defined as the subject RT throughout the study. Following each trial, a brief tone indicated that the subject could initiate another trial. This tone occurred at a variable time between 200 and 800 ms and served to confound subject anticipation of flash presentation (Fig. 1C). Data from the Coulbourn modules was collected using an Arduino Uno board sampling at 2 KHz, and time-stamped RTs were logged into a text file for offline analysis. Acceptable RTs were limited to a range of 200 to 800 ms; RTs faster than 200 ms were considered to be anticipated reactions, and RTs slower than 800 ms were considered missed flashes. At the beginning of each day, the experimenter adjusted intensity of the stimulus LED to a value where the

FIGURE 1. The setup and procedure for psychophysical reaction time measurements. (A) The tachistoscope, with beam splitter, presents two channels of visual stimulation. There was a red LED fixation point and a green stimulus LED 20.4° to the temporal side of the visual field. The conditioning background was illuminated by the slide projector, driven on a 3-minute timer. (B) The subject's view, with fixation and stimulus locations overlaid by the conditioning background. The subject did not initiate any trials while the background was on. The background encompassed both the fixation point and the stimulus. (C) Representation of a single reaction time trial. A trial was initiated by a button press, and after a variable preperiod ranging from 300 to 1300 ms, a 5-ms stimulus flash was delivered. The subject released the button when they detected the flash. The time between the midpoint of the stimulus flash and the button release was logged as the reaction time for that trial. 200 to 800 ms after button release, a 1-KHz tone sounded to indicate that the subject could initiate another trial. This tone was delivered with variable timing to prevent subject rhythm. If the subject did not react to the flash delivery, the trial timed out at 1000 ms, and the tone sounded to instruct the subject release the button and begin another trial.

subject saw greater than ~90% of the stimuli without being asymptotically fast (example in Fig. 2A). The subject then performed four randomly ordered blocks of trials, three tests with conditioning background exposures, and one control in which the subject sat for 3 minutes of darkness, over a 1.5-hour period. Subjects were allowed to rest for 5 to 10 minutes between trial blocks. Each subject returned for three separate experimental days for a total of 12 tests and three control experiments for each individual.

Mouse Single Cell Noise Analysis

Single cells were isolated for recording as previously reported.¹ Briefly, wild-type C57B6/J mice were dark adapted overnight before being killed and enucleated under infrared illumination. Under a dissection microscope, the retina was removed from the eye cup in cold L-15 (Leibovitz, powder with glutamine;

Sigma-Aldrich Corp., St. Louis, MO, USA) and chopped into ~1 mm² sections that were transferred to the recording chamber and then warmed slowly to between 36 and 37°C. Cells were perfused with a solution of Locke's buffer that contained, in millimolar, the following: 120 NaCl, 3.6 KCl, 2.4 MgCl₂, 1.2 CaCl₂, 3 HEPES, 20 NaHCO₃, 0.02 EDTA, and 10 glucose.

Only cells with at least 10-pA saturating responses and a seal-resistance increase of at least 2:1 were used for noise analysis. Prior to collection of noise data, single photon response amplitudes were estimated by stimulating a cell with 50- to 60-dim stimulus flashes and relating the variance of the signal to the mean of the response.¹⁶ Light responses were sampled at 1-KHz and low-pass filtered at 300 Hz (Axopatch 1-A amplifier; Molecular Devices, LLC, Sunnyvale, CA, USA). Light responses before, during, and after periods of AP were recorded to measure AP amplitude and postconditioning noise. Records for fast Fourier transform (FFT) noise analysis were

FIGURE 2. Relationship between reaction time and stimulus intensity. (A) Reaction times plotted against the stimulus intensity for an individual subject over three different days. Reaction time-stimulus curves were tested each day to ensure the test stimulus elicited RTs that were in the right range, as indicated by the *black rectangle*. The *red curve* is a power fit to the data of the form $f(x) = a(x^b) + c$, with constants: $a = 0.073$, $b = -1.06$, and $c = 349$, $R^2 = 0.762$. (B) Plotting the average RTs of all nine subjects across all testing days. The *red curve* is a power fit to the data of the form $f(x) = a(x^b) + c$, with constants: $a = 29.8$, $b = -0.31$, and $c = 288$, $R^2 = 0.938$. Error bars represent ± 1 SD. For comparison with luminous intensity, a second axis showing the number of photoisomerizations per rod per flash is given (see Methods section).

taken for 6.0 seconds, which allowed analysis of both the flash response and the corresponding noise (see Fig. 6A). After recording dark and AP noise, an intense light step was used to completely close all rod membrane channels, and measurements were made of the instrument noise inherent to the recording setup.¹⁷ Continuous stretches of noise of between 2048 and 4096 ms in length were subjected to FFT analysis. Fast Fourier transform noise analysis was performed on 15 cells, and the individual FFT spectra were averaged together. All noise analysis was performed using a computing language (MATLAB 2014a; MathWorks, Natick, MA, USA).

RESULTS

Calibration of Stimulus Intensity Versus Reaction Time in Human Subjects

Reaction times were collected across a range of stimulus intensities to ensure that we could reproduce the power function described by Piéron's law. Figure 2A represents data from a representative individual subject for three different

FIGURE 3. Reaction times depend on conditioning background light intensities. (A) An individual subject collected RTs before the onset of a conditioning background (negative time) and immediately after the conditioning background was extinguished (time zero, relative to background offset). At a background intensity of 0.129 cd/m^2 , there was a masking of the stimulus that persisted for ~ 20 seconds, as the subject did not appear to perceive the test stimulus flashes. (B) When the conditioning background intensity was 10-fold dimmer (0.013 cd/m^2), the subject exhibited an initial increase in RT that quickly returned to prebackground levels.

sessions. There is a clear power fit for this individual (red trace). The boxed values in Figure 2A represent the range for the stimulus intensity that was set for each subject at the beginning of each day; in this subject's case, the flash stimulus was estimated to produce approximately 3 R^*/rod . In this range, the subject misses very few flashes, but is not yet asymptotically fast; this allows subject RTs to get faster or slower following conditioning illumination. The average RTs for all subjects are plotted in Figure 2B, and a power fit approximates the data well [power fit: $f(x) = a(x^b) + c$, constants $a = 29.8$, $b = -0.31$ and $c = 288$, $R^2 = 0.938$]. A study comparing the components of visually evoked potentials and RTs concluded that there are likely two independent physiological processes that govern RT.⁹ The reaction time-stimulus intensity relationship described by Piéron's law and shown in Figure 2 appears to originate in the retina, while the variability between the RTs of subjects is likely more complex and may have origins in the ocular media or cortical processing connecting visual and motor tasks. Thus, our experimental setup allows us to use measurements of RT to quantify the sensitivity of the visual system.

FIGURE 4. Individual RTs from the subject shown in Figure 2A in both control (A) and test (B) conditions. (A) Individual RT trials are plotted prior to the control period of sustained darkness (negative time) and immediately after the control period (time zero, relative to the end of 3-minute control period). Three different sessions of RTs (1 block/day) were normalized to the mean of the pre-darkness RT trials for each block. (B) Data plotted as in (A) with three different sessions of RTs (three blocks/day), normalized to the mean of the prebackground RT grouping for each block. In the test condition, the subject experienced 3 minutes of a conditioning background (1.29×10^{-3} cd/m²) before resuming RT trials. Note that the RTs immediately postbackground are consistently faster than those prebackground.

Higher Intensity Backgrounds Mask the Dim Stimulus

The conditioning stimulus caused an increase or decrease of the RT depending on its brightness. After a 3-minute exposure to a background intensity of 0.124 cd/m², subjects could not perceive the dim test stimulus for a brief period of time (Fig. 3A, “misses”). At a background of 0.013 cd/m², there was an initial increase in RT before the subject returned to baseline (Fig. 3B). These controls indicate that there is an upper limit to the conditioning exposure intensity that induces AP and brighter lights result in the more typical form of photoreceptor adaptation that manifests as a masking of the dim stimulus.

Conditioning Backgrounds Decrease Human Reaction Times for a Short Period

After decreasing the background intensity below those reported in Figure 3, we tested RTs with dim backgrounds that did not mask the stimulus. The reaction time data for a

FIGURE 5. Average RTs for eight subjects in both control and test conditions. (A) The average RTs from multiple subjects are plotted relative to the onset of the control period (negative time) and immediately after the 3-minute period of sustained darkness (time zero, relative to the end of 3-minute control period). There is no obvious trend when comparing pre- and postdarkness RTs. $n = 19$ trials in the control conditions. (B) Data are plotted as in (A) before and after a 3-minute conditioning period. An immediate decrease in reaction time is observed that persists for ~ 15 seconds. The black line fit is a single exponential that with a decay time constant of 7.5 ± 1.3 seconds. $n = 54$ trials in the test condition. Error bars represent SEM.

representative individual subject are plotted in Figure 4. Due to day-to-day variability, the RTs for both the control and test conditions were normalized to the mean of all RTs collected during the preconditioning periods for each day (negative time). As a control for the test condition in which a steady background was presented, the subject was instructed to fixate the red LED in sustained darkness for 3 minutes. There is no RT change in the control condition (Fig. 4A), as the normalized RTs vary uniformly around 1.0. In the test condition, where the subject experienced 3 minutes of conditioning light with an intensity range of 9.2×10^{-4} cd/m² to 1.29×10^{-3} cd/m² (Fig. 4B), RTs were faster immediately following the conditioning light, after which the subject's RTs returned to the mean of the preconditioning period.

The population data for all eight subjects illustrates the robust differences between the control and test conditions. In the control condition (Fig. 5A), subjects showed no systematic

FIGURE 6. Spectral analysis of individual mouse rod photoreceptor noise reveals no significant increase in continuous noise over the range of rod bipolar sampling. **(A)** An example of physiological data collected from a single mouse rod photoreceptor before (*black trace*) and immediately after (*red trace*) exposure to a 3-minute conditioning light exposure. The same 2-ms test flash was delivered at time zero before and after the conditioning exposure, demonstrating the increased magnitude of the light response due to adaptive potentiation. *Boxed area* indicates data used in FFT noise analysis. **(B)** Fast Fourier transform spectral analysis of mouse rod recordings ($n = 15$ cells). The rod to rod bipolar cell synapse has been proposed to act as a bandpass filter of rod membrane voltage changes over the 2- to 5-Hz range (*shaded area*). The noise collected in darkness (*black points*) and noise collected immediately after conditioning exposures (*red points*, AP noise) were not significantly different in 10/13 data points in the rod bipolar sampling range. The *blue* data represents the instrument noise of the system, in which the rod is exposed to an intense saturating light that closes all the rod membrane channels.

changes in RT when no background exposure occurred; instead, subject performance appeared to have deteriorated over time. We attribute this to subject fatigue and a lack of visual stimulation during the 3-minute period of darkness. For the test condition (Fig. 5B), all subjects showed significantly faster RTs following the conditioning background exposure, suggesting that the test flash appeared brighter and was easier to detect. When comparing population data between control and test conditions at equivalent time points after background shutoff, the test RTs were significantly different for the first 12 seconds after the background illumination (each time point: $P < 0.05$; two-tailed t -test). The average RT returned to dark-adapted speeds with a profile that was well fit by a single exponential curve with a time constant of 7.3 ± 1.3 s, similar to the time constant of recovery observed in mouse photoreceptors exhibiting AP (6.8 ± 0.7 s, Fig. 2 in Ref. 1).

Cellular Noise in Single Mouse Rods May Not Compromise Enhanced Rod Signaling During AP

The original report of AP demonstrated that rod signals are increased in amplitude in the linear range of rhodopsin activation as well as for saturating light responses.¹ Here we quantify cellular noise in single mouse rods following conditioning light exposures that elicit AP. Noise analysis was performed on a stretch of data following a stimulus flash before and immediately after a conditioning exposure elicited AP. In Figure 6A, a saturating flash before (black trace) and after (red trace) clearly demonstrates the potentiation of the rods' light response amplitude. Noise was analyzed by performing an FFT on the data before and immediately after the conditioning exposure. Dark noise was not significantly different when comparing postflash continuous noise to the continuous noise of an unstimulated rod (data not shown). Continuous cellular noise was isolated from the instrument noise of the system by recording while the cell was exposed to an intense steady light that closed all of the light-sensitive rod membrane channels (Fig. 6B, blue points). The data from the FFT analysis is plotted in Figure 6B, and it demonstrates that there is no significant difference between the dark noise measured before conditioning exposure (black data points) and the noise during periods of AP (red data points) over the sampling range of the rod bipolar cell (2–5 Hz, gray-shaded area; 10/13 data points, $P > 0.05$, Wilcoxon rank sum test). These results suggest that larger single photon events during AP will not be obscured by elevated cellular noise. Therefore, the increase in rod sensitivity and larger rod signals generated during AP are likely to be transmitted through the retina and may contribute to enhanced visual sensitivity.

DISCUSSION

This study was undertaken to explore the possibility that specific conditions of light exposure, known to increase rod photoreceptor sensitivity in mice, could have a similar effect in humans when measured at the perceptual level. Our results suggest that conditioning light exposures can indeed decrease human RT, similar to the rod hypersensitivity found in mice. In a subset of RT measurements, subject pupil size was recorded before, during, and after conditioning light exposure. After the conditioning background was extinguished, pupil diameter remained stable and did not increase beyond the pupil diameter measured following dark adaptation. Thus, the effect of the adaptation on RT may actually be greater than that observed here, as the pupil diameter did not immediately recover to the fully dark-adapted diameter. A reduction in pupil size following conditioning illumination would reduce the amount of light entering the eye, resulting in slower RTs. The opposite result is seen, indicating that the effect may be even larger with a maximally dilated pupil. We suggest that the increased sensitivity of the rod photoreceptors actually serves to increase the overall sensitivity of the visual system in scotopic conditions.

To further relate the results presented here to the physiological findings that were previously reported, we compare the apparent change in stimulus intensity to the increased sensitivity seen in single rods. Following conditioning exposures the subjects respond faster, as if the stimulus were more intense, despite the stimulus strength remaining fixed. Accelerated RTs can be equated to more intense stimulus strength according to the power fits in Figures 2A and 2B. For instance, in the power fit in Figure 2A, a 25% reduction in RT from within the starting point (box in Fig. 2A) corresponds to $\sim 70\%$ increase in apparent stimulus intensity.

This increase is larger than that observed in single cells, although the intact structure of the photoreceptor-RPE complex and the summed rod response in the intact retina may result in more robust AP in situ.

Conditioning exposures affect both the peak amplitude of the flash response and flash response duration.^{1,18} Krispel et al.¹⁸ demonstrated the reduction in response duration, but their stimulus timing and selection criteria precluded them from observing the enhanced amplitudes. Due to the brief nature of our stimulus (5 ms) and long interstimulus intervals, the acceleration of light response recovery is unlikely to affect the RT to a single flash. However, the response to paired or flickering stimuli might be enhanced by a decrease in the rod integration time. Indeed, rod flicker sensitivity increased following exposures to saturating illumination in mice (Burns, et al. *IOVS* 2013;54:ARVO E-Abstract 2457). Thus, the increase in rod photoreceptor sensitivity (AP) is likely playing a more prominent role than acceleration of the photoreponse recovery in this study.

The rod to rod bipolar synapse is important in a discussion of the noise results reported here. Glutamate release at the rod synapse does not track low frequency (<1 Hz) changes in rod membrane voltage, and higher frequency noise in the 5- to 10-Hz range is filtered out at the rod bipolar cell synapse.^{19,20} Thus, the rod to rod bipolar synapse acts as a bandpass filter, with optimal transmission between 2 and 5 Hz. Our recordings of continuous rod noise reveal only an insignificant increase in noise during AP epochs in the 2- to 5-Hz range. Additionally, the rod bipolar synapse samples small and large changes in the polarization state of the rod differently (i.e., nonlinear sampling).¹¹ Such a nonlinearity discards continuous rod noise along with small single photon response (SPR) fluctuations in membrane voltage. During AP, the increase in the SPR amplitude and the unchanged level of continuous noise can explain how more SPRs can be transmitted to the rod bipolar cells. Thus, we hypothesize that more robust signal transmission at the rod bipolar synapse would allow increased rod sensitivity to be propagated downstream in the retina.

Our experiments targeted an area 20° temporal from the fovea, where rod densities are high, in order to invoke a maximum rod-driven effect.^{13,14} If the rods in the human subjects are indeed more sensitive following the conditioning period, they may be influencing cone signaling in the interconnected network of photoreceptors. Rod-rod, rod-cone, and cone-cone interactions have been described and functionally tested in macaques.^{21,22} These studies concluded that there were variable degrees of coupling between different photoreceptors and that coupled photoreceptors could significantly alter the responses in the neighboring cells. In the original report of AP, increased sensitivity was seen in rod photoreceptors that were completely isolated from the neighboring photoreceptors.¹ This finding, along with the mechanisms described in that paper, points to the origin of sensitivity increases being entirely present in the rod photoreceptor outer segment. This finding does not preclude the possibility that signals produced in the outer segments of more sensitive rods could be shared with neighboring photoreceptors through gap junction coupling. Rod-cone interactions have also been extensively measured psychophysically, and a series of experiments have shown how the rod and cone systems interact at mesopic illumination to determine temporal, spatial, and threshold sensitivities.²³⁻²⁷ Finally, rod and cone signals are also transmitted through the same cellular pathways (cone bipolar cells), so there are additional sites where the signals from the two cell types can interact downstream of the photoreceptors, and we cannot rule out such interactions in light adaptation and faster RTs. Cones are likely not directly signaling the stimulus flashes following the

conditioning illumination, as the stimulus intensity and conditioning background ranges that we used both fall below measured estimated cone thresholds (~ 0.01 cd/m²).^{28,29} To entirely preclude or demonstrate a cone response contribution to the accelerated RT observed, the spectral sensitivity of the AP and reaction times would have to be measured and compared to the rhodopsin absorption spectra.

In addition to the ON ganglion cell signaling pathway, histological and physiological evidence has shown rods synapsing directly with cone OFF bipolar cells and driving OFF ganglion cell responses.^{30,31} Thus, part of the reason why RTs are faster following conditioning illumination could be due to the lingering OFF afterimage being transmitted through the OFF channels of the retina. This afterimage could manifest as a less noisy, “darker” background upon which the subject could detect a dim stimulus flash more readily. Whether this mechanism is acting in concert with the ON signaling pathway cannot be distinguished in the present psychophysical experiments.

CONCLUSIONS

The physiological adaptation described in mouse rod photoreceptors (AP) appears to be propagated to the perceptual level, as measured by human RTs. There does not appear to be any corresponding increase in photoreceptor cell noise during periods of AP; thus, the signal transfer at the rod to rod bipolar cell synapse may also be enhanced. However, the precise mechanism of how enhanced rod sensitivity contributes to downstream changes in perception is currently unknown.

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