Publication IV

H. Heikkinen, F. Vinberg, S. Nymark, and A. Koskelainen. 2011. Mesopic background lights enhance dark-adapted cone ERG flash responses in the intact mouse retina: a possible role for gap junctional decoupling. Journal of Neurophysiology, volume 105, number 5, pages 2309-2318.

Mesopic background lights enhance dark-adapted cone ERG flash responses in the intact mouse retina: a possible role for gap junctional decoupling

H. Heikkinen, F. Vinberg, S. Nymark and A. Koskelainen

J Neurophysiol 105:2309-2318, 2011. First published 9 March 2011; doi:10.1152/jn.00536.2010

Journal of Neurophysiology publishes original articles on the function of the nervous system. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2011 by the American Physiological Society. ISSN: 0022-3077, ESSN: 1522-1598. Visit our website at http://www.the-aps.org/.

J Neurophysiol 105: 2309–2318, 2011.
First published March 9, 2011; doi:10.1152/jn.00536.2010.

Mesopic background lights enhance dark-adapted cone ERG flash responses in the intact mouse retina: a possible role for gap junctional decoupling

H. Heikkinen, F. Vinberg, S. Nymark, and A. Koskelainen
Aalto University School of Science, Department of Biomedical Engineering and Computational Science, Helsinki, Finland

Submitted 15 June 2010; accepted in final form 4 March 2011

Heikkinen H, Vinberg F, Nymark S, Koskelainen A. Mesopic background lights enhance dark-adapted cone ERG flash responses in the intact mouse retina: a possible role for gap junctional decoupling. *J Neurophysiol* 105: 2309–2318, 2011. First published March 9, 2011; doi:10.1152/jn.00536.2010.—The cone-driven flash responses of mouse electroretinogram (ERG) increase as much as twofold over the course of several minutes during adaptation to a rod-compressing background light. The origins of this phenomenon were investigated in the present work by recording preflash-isolated (M-)cone flash responses ex vivo in darkness and during application of various steady background lights. In this protocol, the cone stimulating flash was preceded by a preflash that maintains rods under saturation (hyperpolarized) to allow selective stimulation of the cones at varying background light levels. The light-induced growth was found to represent true enhancement of cone flash responses with respect to their dark-adapted state. It developed within minutes, and its overall magnitude was a graded function of the background light intensity. The threshold intensity of cone response growth was observed with lights in the low mesopic luminance region, at which rod responses are partly compressed. Maximal effect was reached at intensities sufficient to suppress ~90% of the rod responses. Light-induced enhancement of the cone photoresponses was not sensitive to antagonists and agonists of glutamatergic transmission. However, applying gap junction blockers to the dark-adapted retina produced qualitatively similar changes in the cone flash responses as did background light and prevented further growth during subsequent light-adaptation. These results are consistent with the idea that cone ERG photoresponses are suppressed in the dark-adapted mouse retina by gap junctional coupling between rods and cones. This coupling would then be gradually and reversibly removed by mesopic background lights, allowing larger functional range for the cone light responses.

photoreceptor; retina; rod-cone coupling; light adaptation; electroretinogram

THE VERTEBRATE RETINA IS A specialized neural tissue that collects, processes, and transmits information about the spatial, spectral, and temporal features of the light reaching the eye. Through a range of powerful adaptational mechanisms, the retina manages to produce visual information over 10^9-fold range of light-intensities. The grounds for this impressive performance are laid already at the level of photoreceptor cells, which adjust their signal transduction cascade in response to changes in the average illumination level. Visual processing is further optimized by the existence of two main photoreceptor classes: the highly sensitive rods convey visual messages in darkness, while the higher temporal performance and faster adaptation of daylight vision are based on the function of the cones. However, between these two extremes there is a significant range of light conditions at which these two photoreceptor classes are simultaneously active. In this mesopic range, the neural network of the retina receives light responses from two photoreceptor classes with different sensitivities and temporal properties, calling for adaptational "switches" to facilitate transfer from rod- to cone-based vision.

Photoreceptor signals are processed via several parallel pathways in the mammalian retina. In the primary pathways, the rods connect with glutamatergic synapses to depolarizing rod bipolar cells (DBC_r) and cones to several types of depolarizing (DBC_C) and hyperpolarizing (HBC_C) bipolar cells (Kolb and Famiglietti 1974; reviewed by Wässle and Boycott 1991). The primary rod pathway converges to the cone ON and OFF pathways through AII amacrine cells. In addition, mammalian rods connect to the cone pathways at several stages, harnessing them to carry rod-mediated signals. This happens partly by direct gap junctional coupling between the rods and cones in the outer plexiform layer (macaque and rabbit: Raviola and Gilula 1973; cat: Kolb 1977, Nelson 1977; macaque: Schneeweis and Schnapf 1999; mouse: Tsukamoto et al. 2001): light-induced hyperpolarization of surrounding rods spreads into the cone pedicles and is transmitted to DBCs and HBCs via glutamatergic synapses.

In mammalian electroretinogram (ERG), the retinal shift from rod- to cone-dominated action is readily observed when dark-adapted retina is subjected to a steady light. The cone-driven flash responses grow substantially within the first 10 min after switching on a rod-saturating background light (Burian 1954; Armington and Biersdorf 1958; Gouras and McKay 1989; Peachey et al. 1993). This includes growth in the a-wave, the b-wave, and the oscillatory potentials, indicating changes at several levels of retinal processing. Also the threshold of cone-driven responses to sinusoidal flicker stimuli decreases (Goldberg et al. 1983). Similar adaptation can be observed in amphibian retinas, and much of it happens postsynaptically from the photoreceptors (e.g., Frumkes and Wu 1990). However, the up to 100% growth in the mammalian a-wave remains mostly unexplained. In rats, the light-induced growth of the cone photoreceptor component persists in vivo after pharmacological blockade of glutamatergic transmission to second-order neurons (Bui and Fortune 2006), suggesting that it cannot be readily attributed to postreceptoral feedback to cones. It is not clear, though, whether the photoreceptor response growth shown by Bui and Fortune represents true enhancement of cone responses with respect to the dark-

Address for reprint requests and other correspondence: H. Heikkinen, Aalto Univ., School of Science, Dept. of Biomedical Engineering and Computational Science, PO Box 12200, FI-00076 Aalto, Finland (e-mail: hanna.heikkinen@aalto.fi).

adapted state or merely recovery from an initial suppression due to excitation by the background light.

We studied the light-induced growth of preflash-isolated cone ERG flash responses when recorded transretinally across isolated mouse retinas (ex vivo). In this method, the cone-stimulating test flash was preceded by an intense preflash. The fast recovery of the cones' response to the preflash enabled studying their dark- and light-adapted flash responses while the rods remained hyperpolarized and unresponsive. We found that moderate backgrounds expected to induce substantial light adaptation in the phototransduction of rods but not of cones enhanced the cone flash responses with respect to the dark-adapted state. The light-induced growth occurred on a time scale of minutes and was not sensitive to the presence of glutamate agonists or antagonists. Experiments with three structurally independent gap junction blockers suggested that the growth in cone responses could be mediated by a light-induced decrease in gap junctional coupling, likely between rods and cones. The strength of cone response modulation appears to be directly related to the static level of rod response compression by the background light.

MATERIALS AND METHODS

Animals. Transretinal ERG was recorded across isolated retinas of adult C57Bl/6N mice. The use and handling of the animals were in accordance with the Finland Animal Welfare Act 1986 and guidelines of the Animal Experimentation Committee of University of Helsinki, Finland, which approved this study.

The ERG recordings. The method for obtaining isolated cone flash responses was adapted with minor modifications from Heikkinen et al. (2008). The animals were dark-adapted overnight and killed with CO_2 asphyxiation and cervical dislocation. The retinas were detached under dim red light and mounted in a specimen holder distal (photoreceptor) side upwards. The photoreceptors were continuously superfused (4.5 ml/min, 36–38°C) with modified Ringer solution containing (in mM): Na^+ 135.3, K^+ 3.3, Mg^{2+} 2.0, Ca^{2+} 1, Cl^- 124.6, glucose 10, EDTA 0.01, HEPES 10, HCO_3^- 20. The pH was balanced to 7.5–7.6 with 4.8 mM NaOH, and the solution was bubbled with carbogen (95% O_2-5% CO_2). Leibovitz culture medium L-15, 0.72 mg/ml, was added to improve photoreceptor viability. To suppress glial currents, $BaCl_2$ (10 mM) was added in the lower electrode space (Nymark et al. 2005), or 30–70 μM was added to the superfusion solution. DL-AP4 (20–80 μM) and NBQX (10–40 μM) were added to the superfusion during the course of the experiments of Figs. 2–8 to block glutamate-mediated transmission. Meclofenamic acid (MFA), octanol, or carbenoxolone (CBX) were used to block gap junctions in the retina, as indicated in RESULTS. The chemicals were obtained from Tocris (DL-AP4, NBQX, and D-AP5) or Sigma-Aldrich (others).

The transretinal potential was recorded with two Ag-AgCl electrodes across an effective measurement area 0.5 mm in diameter at the central retina. The DC signal was low-pass filtered (Bessel 8 pole with f_c = 1 kHz) and digitized at 10 kHz with 0.25 μV resolution. Homogeneous full-field illumination on the photoreceptor side was provided by a 532-nm diode laser [Power Technology IQ5C(532–100)L74; 130 mW] and a 543.5-nm He-Ne laser (Melles Griot 05 LGR 173; 0.8 mW). The light intensity was controlled for each light source separately with calibrated neutral density wedges and filters. Two-ms pulses of stimulus light (referenced as "test flashes" or "preflashes" in the subsequent text) were generated from the 532-nm laser with a computer-controlled Oriel shutter (model 76992). The background lights were produced with the He-Ne 543.5 nm laser, controlled with a similar shutter.

Conversion of stimuli to number of isomerized pigments in rods and cones. The 532- and 543.5-nm lasers stimulate exclusively rods and the M-cone pigments in the mouse retina, as the UV-pigment absorbance is weakened by 7–8 log-units at these long wavelengths. To convert flash and background intensities (I_F and I_{Bg}, respectively, with subscript denoting the used wavelength) to activated rhodopsins per rod (Rh*, Φ_{rod} and $\Phi_{Bg,\ rod}$) or M-cone opsins per cone (P*, Φ_{cone} and $\Phi_{Bg,\ cone}$), the effective collection areas for rods and the M-pigment-driven responses of the cones at the central retina were calculated for our geometry as in Heikkinen et al. (2008), and scaled to the stimulus wavelengths with the templates of Govardovskii et al. (2000).

In brief, the rod outer segments were considered cylinders with diameter 1.4 μm, length 24 μm (Carter-Dawson and LaVail 1979), and specific absorbance 0.016 μm^{-1} (Nymark et al. 2005) at λ_{max} (498 nm, Lyubarsky et al. 1999). This led to an effective collection area $a_{c,\ rod}$ of 0.73 μm^2 for axially incident light at λ_{max}, with absorption scaled down by 0.69 and 0.5 at the stimulating and background wavelengths 532 and 543.5 nm, respectively.

The collection area for a fictitious 100% M-cone was calculated by approximating the cone outer segments as cylinders with diameter 1.2 μm, length 13.4 μm (Carter-Dawson and LaVail 1979), and specific absorbance 0.016 μm^{-1} at λ_{max} (508 nm, Lyubarsky et al. 1999). This led to a_c = 0.29 μm^2 at λ_{max}, lowered by factors 0.85 and 0.68 at 532 nm and 543.5 nm, respectively. In reality, to come up with the effective collection area for M-pigment-driven cone responses, one had to make estimates about the average percentage of M-cone pigment in the cones of the central retina, as well as the shadowing effect due to the rods in our geometry. Mouse cones coexpress M- and S-pigments with maximal absorption at 508 and 355 nm, respectively. There is a gradient in the dorsal-ventral direction regarding the relative proportions of the pigments within individual cones, as well as the fraction of "S-cones" expressing almost exclusively the short wavelength pigment. Based on the data of Applebury et al. (2000) and the electrophysiological recordings of Nikonov et al. (2006), we estimated that M-pigment consisted 35% of the total cone pigment concentration in the central region of the retina. This led to a corrective factor 0.4 to a collection area calculated for purely M-pigment-containing cones. Additionally, the larger and more numerous rods shadow cones from light entering axially from the distal side of the retina. Based on the electromicroscopic figures of Carter-Dawson and LaVail (1979), we came up with a corrective term based on incoming light having to traverse half of the rod OS layer before reaching the small cone outer segments. This shadowing is maximal at wavelengths near the absorption maximum of the rods, when up to 35% of incoming light gets absorbed before reaching the cones. At the stimulating wavelengths 532 nm and 543.5 nm, this corrective term adds up to 0.75 and 0.86, respectively. Thus the overall effective collection area for mouse M-pigment-driven cone responses at the central retina was calculated as $a_{c,\ M\text{-}cone}$ = 0.078 μm^2, scaled to 0.078 μm^2 and 0.069 μm^2 at the stimulating wavelengths 532 nm and 543.5 nm, respectively.

Isolation of the cone flash responses. Due to the overlapping absorption spectra of murine rods and cones, the distinction between rod and cone flash responses has to be based on the different sensitivities and kinetics of these two cell types. As our aim was to study flash responses originating in the cone photoreceptors at varying levels of dark and light adaptation, we could not apply rod saturating backgrounds, such as in, e.g., Nikonov et al. 2006. Instead, we isolated the cone flash responses with a rod-saturating preflash producing 150,000–260,000 Rh*. The preflash-test stimulus interval (typically 500 ms) was chosen to allow cone responses to recover from the preflash while the rods remained unresponsive (Heikkinen et al. 2008, see also Figs. 2 and 3). The 2-min interval between preflash-test stimulus pairs in darkness was sufficient to allow also rods to regain their sensitivity and kinetics before subsequent stimulation. This way we could record cone responses in a nominally

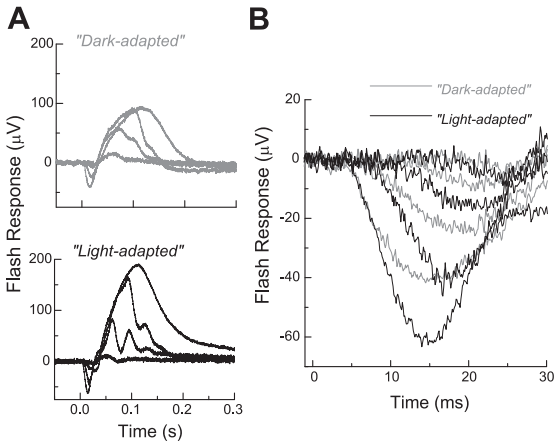

Fig. 1. Continuous background lights enhance cone-driven flash responses. *A*: preflash-isolated cone response families to 2-ms flashes administered at t = 0 s, stimulus strength Φ_{cone} = 18, 250, 1,900, and 26,000 P*, recorded from a dark-adapted retina (*top*) and from the same retina following 10-min adaptation to background light I_{Bg} = 4,800 h$\nu_{543.5nm}$ $\mu m^{-2}s^{-1}$ ($\Phi_{Bg,\ cone}$ = 330 P*s^{-1}, *bottom*). No glutamate agonists/antagonists were present in the superfusion. *B*: the dark- and light-adapted responses of *A* superimposed and drawn on a shorter timescale to reveal changes in the a-wave more clearly.

"dark-adapted" state (the preflash-test stimulus pair was presented on the dark-adapted retina) as well as in a "light-adapted" state (the preflash-test stimulus pair was superimposed on a steady background light). The flash responses shown in the figures are averages of two to five trials, while the amplitude data were collected from individual flash responses.

RESULTS

Mesopic background lights enhance the preflash-isolated mouse cone flash responses. The preflash-isolated, cone-driven ERG flash responses recorded ex vivo from the dark-adapted mouse retina (Fig. 1, *A* and *B*) are dominated by the positive-going b-wave, that originates in the mGluR6-mediated pathway through the ON-bipolar cells (Knapp and Schiller 1984; Stockton and Slaughter 1989; Masu et al. 1995). The b-wave is preceded by the negative a-wave, which originates in the light-responses of photoreceptors and the OFF-pathway (Bush and Sieving 1994; Shirato et al. 2008). The amplitudes and times to peak of the b-waves are comparable to those observed in corneally recorded ERG immediately after applying a rod-saturating background upon a previously dark-adapted retina (e.g., Peachey et al. 1993, Ekesten et al. 1998). The waveform is distinctly round and lacks strong oscillatory components that originate in the inner retina and are routinely observed in the ERG registered in vivo. A steady background light of I_{Bg} = 4,800 h$\nu_{543.5\ nm}$ $\mu m^{-2}s^{-1}$ arriving from the photoreceptor side induces a prominent growth in the a- and b-waves of the preflash-isolated responses, accompanied by the appearance of oscillatory potentials (Fig. 1, *A* and *B*). This suggests that several stages in the cone-signaling pathway are released to mediate signals under these mesopic conditions, after an initially suppressed state in the dark-adapted retina. When superimposed and plotted on an expanded time scale to reveal the amplitude and time course of the a-wave more clearly (Fig. 1*B*), it appears that despite the notable increase in the a-wave amplitude, the dark- and light-adapted flash responses share a common path during the first milliseconds and diverge only at later times.

The light-induced growth of the cone flash responses is not inhibited by disrupting glutamatergic signaling to second-order neurons. To study the photoreceptor component of the cone-driven responses (Fig. 2*A*), 20 μM DL-AP4 (Slaughter and Miller 1981) and 10 μM NBQX (Yu and Miller 1995) were used to prevent transmission mediated by metabotropic glutamate and AMPA/kainate receptors, respectively. This revealed the negative, longer-lasting photoreceptor flash responses (fast PIII, Fig. 2*A*. Note the longer time course of the response in Fig. 2*A* after the b-wave was eliminated, compared with the response in Fig. 1*B* in which the b-wave masks the later portion of the a-wave). Figure 2*B* plots the preflash-isolated photoreceptor responses superimposed on the response to the preflash only. The prolonged saturation of the rods

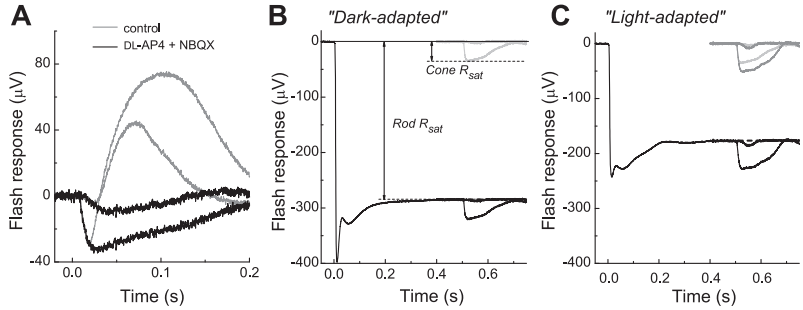

Fig. 2. Light-induced growth of the preflash-isolated cone flash responses persists after pharmacological isolation of the photoreceptor responses. *A*: the cone flash responses (Φ_{cone} = 250 and 26,000 P*) recorded from a dark-adapted retina before and after pharmacological blockage of glutamatergic signaling to metabotrobic and AMPA/kainate receptors (gray and black traces, respectively). Disrupting glutamatergic transmission with 20 μM DL-AP4 and 10 μM NBQX reveals the negative cone photoreceptor responses. *B*: pharmacological isolation of the photoreceptor responses reveals the prolonged rod saturation following the intense preflash (Φ_{rod} = 220,000 Rh* at time t = 0 s). The responses to the test flash (Φ_{cone} = 30 and 38,000 P*, stimulus onset at t = 0.5 s) are superimposed to the saturated rod plateau. The cone responses isolated by subtracting the response to preflash presented alone are depicted with light gray traces. The measures used for saturated amplitudes of both rods and cones are indicated with arrows. *C*: a mesopic background suppressing approximately one-third of the rod response amplitude in the retina of *B* initiates growth in the test flash responses. For comparison, the dark-adapted cone responses to the same stimuli (same responses as in *B*) are drawn (light gray traces) superimposed to the isolated light-adapted test flash responses (dark gray traces).

following the bright preflash can be readily observed after blockade of synaptic transmission, indicating that the test flash response originates mainly in cones.

A steady background of I_{Bg} = 3,300 $h\nu_{543.5nm}$ $\mu m^{-2}s^{-1}$ ($\Phi_{Bg,cone}$ = 230 P*s^{-1} and $\Phi_{Bg,rod}$ = 1,200 Rh*s^{-1}, Fig. 2*C*) enhanced the pharmacologically isolated test flash responses similarly to the a-wave in Fig. 1*B*: the early activation phase of the responses remained unchanged, while the rest of the response was altered. The recovery phase of the background-enhanced responses was typically steeper than in the dark-adapted state, and a positive overshoot appeared in many retinas. In control experiments, the NMDA receptor antagonist D-AP5 (10 μM, 2 retinas, data not shown) had no further effect on the photoresponses. Increasing the concentrations of both DL-AP4 and NBQX fourfold, up to 80 and 40 μM, respectively, did not affect the light-induced growth of the test flash responses, nor did the glutamate agonist L-aspartate (data not shown). The results suggest that the induction of the response growth in the preflash-isolated cone photoreceptor responses does not require glutamate-mediated transmission from photoreceptors to the second-order neurons.

Cone flash responses can be isolated with a rod-saturating preflash also in a moderately light-adapted retina. Our method for isolating cone flash responses relies on the prolonged saturation of rod phototransduction after the preflash (Fig. 2*B*). This method has been widely used for isolating cone flash responses in the dark-adapted ERGs of many species, including mouse (reviewed, e.g., by Weymouth and Vingrys 2008). In the present work, we expand this method to retinas that have been adapted to mesopic backgrounds (Fig. 2*C*). However, the rods adapt to background lights through desensitization, which also decreases the time they remain saturated following a bright flash of fixed intensity. Thus the observed growth in the preflash isolated cone responses could in principle not originate in cones at all, but rather trivially arise from partial recovery of the rods' responsiveness between the preflash and the cone-stimulating flash. Therefore, we investigated how the compromised rod saturation time in the light-adapted retina affected the measured "cone response" to the second stimulus.

Figure 3*A* shows pharmacologically isolated photoreceptor responses to the rod saturating preflash, delivered without a subsequent cone-stimulating flash. The traces portray a typical rod response saturation both in the dark- and moderately light-adapted retina, apparently lasting well over the time our test flash is typically presented. Since the rod photoresponses are about tenfold larger than the cone responses, even a minor fraction of regained rod responsiveness between flashes in the light-adapted retina could account for the observed significant growth in the responses to the test stimulus (Fig. 3*B*). We investigated this possibility by recording responses to the test flash at varying times (Δt) following the preflash (Fig. 3*C*). The gray traces present responses recorded from a dark-adapted retina with 300- to 2,000-ms intervals between the preflash and the test flash. The responses maintain constant amplitude up to $\Delta t > 600$ ms. After this they are progressively contaminated by a presumably rod-originated component that increases gradually with increasing stimulus interval. The responses recorded from the same retina under light-adaptation also maintain fixed amplitude (and thus no apparent component due to rod responsivity) beyond the time of our regular stimulation, 500 ms. Yet the photoresponse to the test stimulus remains larger than in the

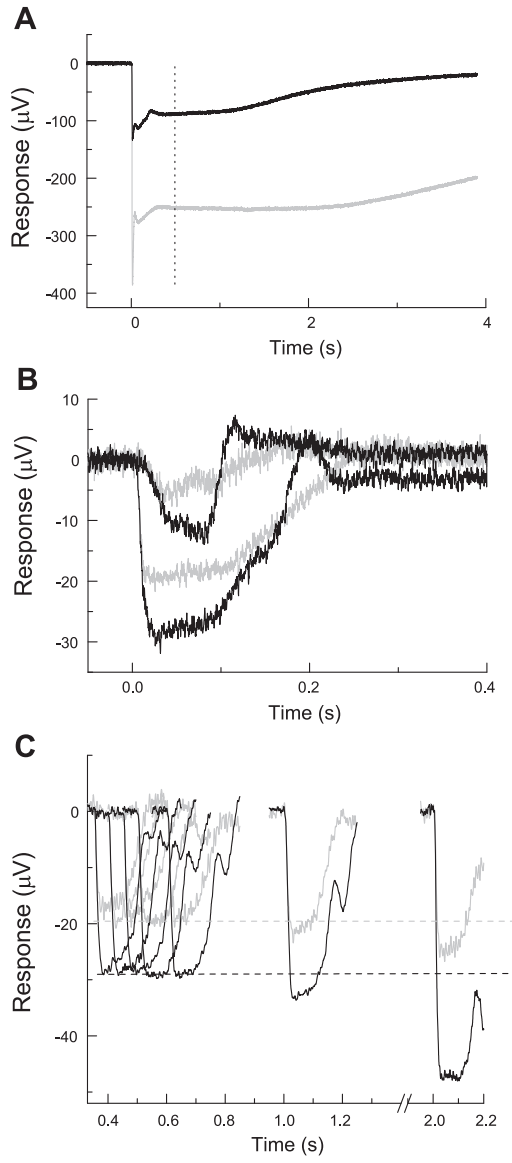

Fig. 3. Recovery of rod responsiveness after the preflash does not explain cone response enhancement at mesopic backgrounds. *A*: the pharmacologically isolated photoreceptor response to the rod-saturating preflash (Φ_{rod} = 180,000 Rh*) presented to the dark-adapted (gray trace) and moderately light-adapted (black trace, $\Phi_{Bg,rod}$ = 1,300 Rh*s^{-1}) retina. No test flash was administered after the preflash, but the usual moment of its delivery (500 ms) in the cone response experiments is depicted with a dashed line. *B*: the responses to test flashes (Φ_{cone} = 290 and 30,000 P*) following the preflashes delivered to the retina of *A* in the dark-adapted (gray) and light-adapted state (black). *C*: the responses to test flashes delivering Φ_{cone} = 26,000 P* were recorded from another retina in a dark-adapted (gray traces) and light-adapted state (black traces, $\Phi_{Bg,rod}$ = 650 Rh*s^{-1}, $\Phi_{Bg,cone}$ = 130 P*s^{-1}) at varying times (300–2,000 ms) following a rod-saturating preflash. The responses in *C* have been digitally low-pass filtered (FFT, f_c = 200 Hz) to avoid clutter in the figure.

dark-adapted state throughout the interstimulus interval range investigated, indicating a true growth in the cone flash responses. Similar results were obtained in all experiments with the same protocol (3 retinas).

The growth in pharmacologically isolated cone flash responses correlates with the steady level of rod response suppression by background light. The light-induced enhancement of the cone flash responses coincides with the mesopic intensity regime at which both rods and cones are active, and develops gradually with increasing background intensity. Figure 4*A* compares the cone responses of one retina under darkness and in the steady state following exposure to 4,600 h$\nu_{543.5nm}$ $\mu m^{-2}s^{-1}$ (320 P*s^{-1}). This background is expected to suppress a large portion of the light-sensitive current in mouse rods but not to affect the cone-circulating current (Nikonov et al. 2006) or to desensitize mammalian cones substantially (Dunn et al. 2007). The cone responses were further enhanced by a 10-fold increase of the background intensity to 3,200 P*s^{-1} (Fig. 4*B*).

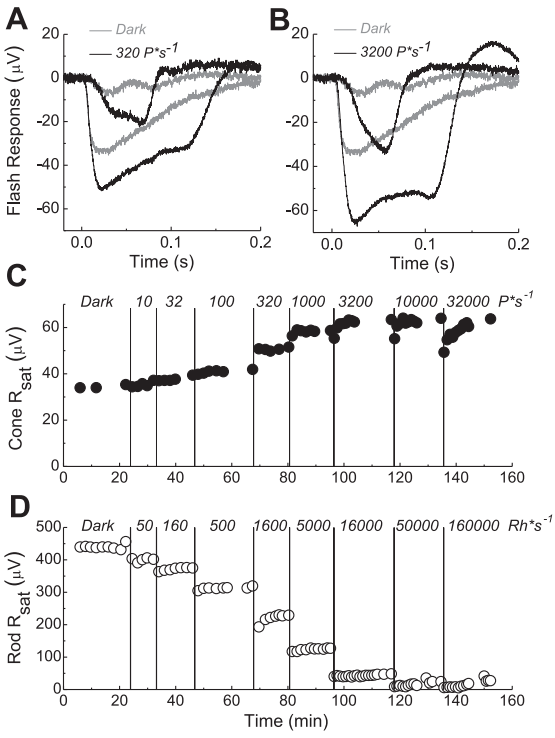

Fig. 4. Background light induces graded growth in the preflash-isolated cone flash responses. *A*: DL-AP4/NBQX-isolated cone responses to flashes producing Φ_{cone} = 250 and 26,000 P* in darkness (gray traces) and following 10-min exposure to background light ($\Phi_{Bg,cone}$ = 320 P*s^{-1}, black traces). *B*: gray traces depict the same dark-adapted cone flash responses as in *A* compared with the those recorded following 10-min adaptation to a 10-fold stronger background ($\Phi_{Bg,cone}$ = 3,200 P*s^{-1}, black traces). *C*: the saturated cone response amplitudes (Φ_{cone} = 26,000 P*) from the same retina as in *A* and *B* during an experiment in which dark-adapted state was followed by backgrounds with intensity increasing stepwise from 150 to 460,000 h$\nu_{543.5nm}$ $\mu m^{-2}s^{-1}$ (10–32,000 P*s^{-1}), as depicted above the amplitude data. *D*: the rod plateau amplitudes, determined 400 ms after the rod-saturating preflash (Φ_{rod} = 150,000 Rh*), during the light adaptation experiment in *C*.

Figure 4, *C* and *D*, emphasizes the graded nature of the dependence of cone response amplitudes on background light intensity. Figure 4*C* shows the amplitudes of the cone responses to a saturating stimulus (Φ_{cone} = 26, 000 P*) in darkness and following application of incremental steady background lights. In this intensity range, the rod light responses are gradually suppressed as the background light-induced phosphodiesterase activity compresses their circulating current. Figure 4*D* shows the rod plateau amplitudes during the same experiment, as determined from the combined photoreceptor response 400 ms after the preflash. The first signs of cone response enhancement were observed at I_{Bg} = 460 h$\nu_{543.5nm}$ $\mu m^{-2}s^{-1}$, corresponding to 32 P*s^{-1} and 160 Rh*s^{-1} in cones and rods, respectively. At stronger backgrounds, the steady state cone response amplitudes increased in a graded manner with increasing background intensity. Maximal steady state amplitude, 170 ± 20% (n = 5, means ± SE) of the dark-adapted value, was reached at background intensities just sufficient to saturate rods. No additional growth was observed upon further increasing the background intensity, but an initial dip of amplitude was evident, followed by recovery to the maximal amplitude. We interpret this transient decrease and subsequent recovery of the responses to represent adaptation originating in cones themselves.

Cone enhancement occurs over the course of several minutes. The data of Fig. 4, *B* and *C*, show how the cone responses are gradually enhanced at increasing backgrounds, with the steady state level of enhancement correlating with the steady level of rod response suppression by each background. Yet there is no instantaneous one to one relation between the rod and cone response amplitudes: the time course of the enhancement is rather slow, taking several minutes to complete after a background is turned on. This observation is in line with the rate of photopic growth in vivo (cf. Peachey et al. 1993).

A time series of light responses such as presented in Fig. 4 does not allow accurate determination of the time scale of the cone response enhancement. Tracing the time course during the first seconds of light-adaptation would necessitate repeated stimuli that may themselves add to the effect of the steady background in adapting the rods. We thus traced the time course of cone enhancement with a series of experiments in which individual pairs of preflash and cone stimulus were delivered at variable times following a step of background light; to allow tracking the time course of the cone response growth, the retina was allowed to dark-adapt between subsequent steps. Figure 5 shows the average time course of cone response growth in four retinas in response to a background light sufficient to reduce the rod circulating current by approximately one-half. The average time course can be fitted well with a single exponential with τ = 80 s, but there is considerable variability regarding the steepness of the curve, as is evident from the large scatter in the data (apparent as large SE) near 60 s. No change was ever observed in the cone responses during the first second following the background onset.

Flash trains designed to light-adapt and suppress light responses only in rods induce growth in the cone flash responses. The cone response growth is initiated by relatively dim background lights that are not expected to cause adaptational changes in the cone phototransduction machinery. This suggests that the enhancement of the cone flash responses might be triggered by the adaptive or suppressive changes the

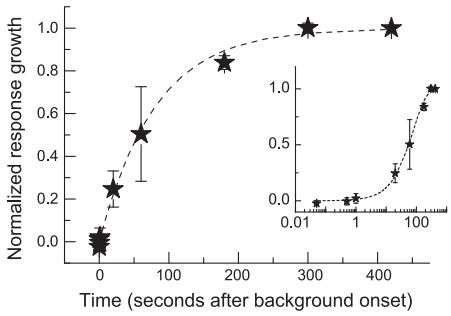

Fig. 5. The cone response growth develops within minutes after application of the adapting light ($\Phi_{Bg,cone}$ = 260 P*s^{-1}, corresponding to 1,300 Rh*s^{-1} in rods). The normalized growth in the amplitude of the saturated, pharmacologically isolated responses relative to the dark-adapted state was probed at variable times following the onset of the background light. For background durations <60 s, each data point was collected as a separate trial in which a background light step was followed by the combination of preflash and cone-stimulating flash. The time on *x*-axis designates the moment of cone-stimulating flash delivery, relative to the onset of the background light step. The retina was dark-adapted between subsequent background light steps. For background durations >60 s, the background light was left on and additional responses were collected at 180, 300, and 500 s after the background onset. The dashed line depicts an exponential with time constant of 80 s fitted to the data averaged from 4 retinas, error bars depicting SE. The *inset* shows the same data on a semi-logarithmic scale.

background light causes in the rods. Or alternately, cone flash responses may be somehow suppressed through rod function in darkness, and released from this suppression by light.

The rods and M-cones of the mouse retina have similar action spectra (Lyubarsky et al. 1999), and their contribution to the ERG signal cannot be distinguished by varying the spectral composition of the stimulus. However, we attempted to light-adapt the rods selectively by using high-intensity flash trains with 1-s interflash intervals: this interval is sufficiently small for the rods to be driven into partial saturation by the flash train. Yet cone flash responses recover between subsequent flashes (Fig. 4). Figure 6 shows the cone and rod amplitudes in a typical flash train experiment. The responses were first recorded in dark-adapted conditions to ensure a steady baseline. A background light $\Phi_{Bg,cone}$ = 230 P*s^{-1} was then applied for ~20 min, leading into typical rod suppression and cone response enhancement. Re-adapting the retina to darkness led to recovery of both rod and cone amplitudes. Then a train of 60 flashes with intensity equal to the cone-isolating preflash, administered with 1-s intervals, was delivered. The flash train transiently suppressed the rod responses by almost 90% and initiated similar growth in the preflash-isolated cone flash responses as the continuous background. The light gray traces in the inset of Fig. 6 show half-saturated and saturated cone responses recorded shortly after ending the flash train stimulation.

Disrupting gap junctional coupling in the retina enhances dark-adapted cone responses similarly to background light. The graded growth of the cone responses at relatively low backgrounds or with rod-suppressing flash trains suggests a link between cone enhancement and rod suppression by the background lights. The hypothetical secondary rod pathway, i.e., gap junctional coupling between rods and cones, provides a possible route for rod influence on the cone system that persists when post-receptoral feedback is inhibited. We explored this possibility with three structurally unrelated compounds that have been used as potent gap junction blockers: CBX (Davidson et al. 1986), octanol (Johnston et al. 1980), and MFA. MFA has recently been shown to reversibly remove electrical and dye coupling between cells connected with Cx43 gap junctions (Harks et al. 2001), dye coupling between retinal cells connected with Cx36, Cx50, and Cx57 gap junctions (Pan et al. 2007), and electrical coupling between amacrine cells connected with Cx36 gap junctions (Veruki and Hartveit 2009, Veruki et al. 2010). Figure 7, *A* and *B*, compares the effects of light and MFA on the dark-adapted cone flash responses. Figure 7*A* presents cone photoresponses in dark-adapted state and following 3- to 5-min adaptation to maintained background light (I_{Bg} = 4,600 h$\nu_{543.5nm}$ μm^{-2}s^{-1}) sufficient to suppress 60% of the rod-saturated response. This background enhanced the cone responses substantially, yet reversibly, as shown in the amplitude data of Fig. 7, *C* and *D*. Figure 7*B* shows the cone responses from the same retina to the same stimuli in the dark-adapted state before and during the application of 50 μM MFA. MFA induced similar growth of the cone flash responses as the previously applied background light. Application of background light did not enhance responses further in the presence of MFA. Both the original dark-adapted cone response amplitudes and the ability to induce response growth

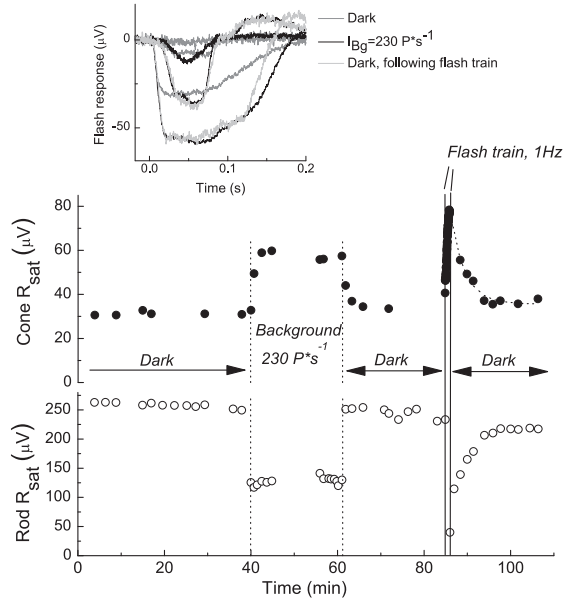

Fig. 6. Rod-suppressing flash trains enhance cone flash responses similarly to background light. Cone (*top*, filled circles) and rod (*bottom*, open circles) response amplitudes during an experiment in which first steady background light ($\Phi_{Bg,cone}$ = 230 P*s^{-1}) and then a 1 Hz train of intense flashes (250,000 Rh*, same as the preflash) were applied to a previously dark-adapted retina in the presence of DL-AP4 and NBQX. The photoreceptors were allowed to regain dark-adapted sensitivity and response amplitudes between the 20-min background and the 1-min flash train. During the gaps in the response amplitude data, responses to low intensity flashes were collected. The *inset* shows the preflash-isolated cone flash responses to Φ_{cone} = 30, 400, and 42,000 P* recorded in darkness (gray traces), and following adaptation to the steady background (black traces) as well as the responses to the two most intense flashes recorded 1–2 min after ending the flash train (light gray traces).

with background lights were gradually recovered when MFA was removed from the superfusion (Fig. 7*C*).

In experiments similar to that shown in Fig. 4, the saturated cone responses maintained their constant steady state amplitudes up to backgrounds producing $\Phi_{Bg,cone}$ = 10 000 P*s^{-1} (Fig. 7*D*). The apparent decrease of the response amplitude at stronger backgrounds is due to desensitization of cone phototransduction: the actual saturated amplitude was not affected, as seen from the amplitudes of responses to yet larger stimuli (Φ_{cone} = 83 000 P*, marked by stars in Fig. 7D). In control experiments (data not shown), application of MFA on light-adapted retinas did not generate growth or substantial waveform changes in the cone responses; instead, it decreased the amplitudes slightly.

The more commonly used gap junction antagonist CBX (100 μM) also prevented light-induced enhancement of cone responses. However, it decreased cone flash sensitivity ~10-fold, slowed their photoresponses, and diminished the response amplitudes by more than 50%. This is consistent with recent reports that besides its potency on blocking gap junctions, CBX exerts several nonspecific inhibitory effects on neuronal signaling, including retinal function (Tovar et al. 2009). The third structurally unrelated gap junction blocker, octanol (1 mM), enhanced dark-adapted cone flash responses similarly to MFA, with no further growth when background light was applied. Neither octanol nor MFA affected the dark-adapted cone sensitivity differently from the light-induced enhancement. Also, rod response compression with background light was similar in the presence of these two compounds, although octanol reduced the rod (but not cone) response amplitudes somewhat. The effects of the three gap junction blockers on the dark- and light-adapted cone response amplitudes, normalized to the dark-adapted amplitude before their administration, are shown in Fig. 8.

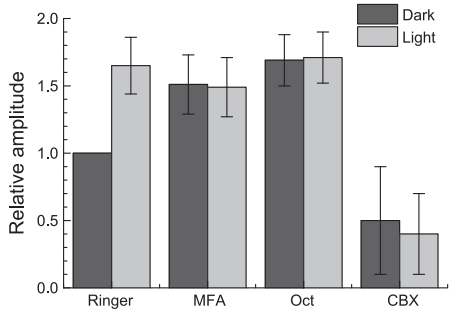

Fig. 8. The saturated cone response amplitudes (Φ_{cone} = 26,000 P*) in darkness and under background light (2,600–3,200 P*s^{-1}, following 8–10 min adaptation) in Ringer solution (5 retinas) and after administration of 3 pharmacological gap junction blockers: MFA, 50 μM, 5 retinas; octanol, 1 mM, 3 retinas; and carbenoxolone (CBX; 100 μM, 3 retinas). Values normalized to the amplitude in darkness before exposure to background light or gap junction blockers. The error bars depict SE between the retinas.

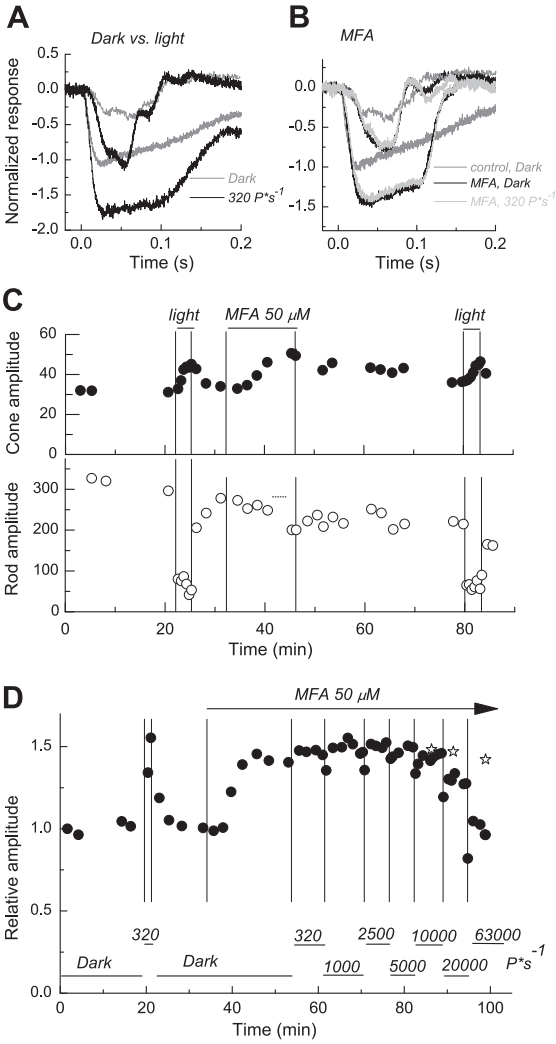

Fig. 7. Meclofenamic acid (MFA) enhances dark-adapted cone photoresponses and prevents further enhancement by background lights. *A*: DL-AP4/NBQX-isolated cone responses to Φ_{cone} = 250 and 26,000 P* in darkness (gray) and after 3–5 min of exposure to background $\Phi_{Bg,cone}$ = 320 P*s^{-1}. *B*: responses from the same retina to the same stimuli in darkness after recovery from exposure to the background light (gray), 20 min after applying 50 μM MFA (black), and following 8 min of subsequent adaptation to background light $\Phi_{Bg,cone}$ = 320 P*s^{-1} (light gray). *C*: the saturated cone response amplitude (black circles in the *top* panel, Φ_{cone} = 30,000 P*) in darkness, during a short exposure to a moderate background of $\Phi_{Bg,cone}$ = 260 P*s^{-1} and subsequent dark-adaptation, followed by administration and washout of 50 μM MFA. At 80 min, the reversibility of the MFA-generated enhancement was tested by reapplying the background light. The *bottom* presents the saturated rod response amplitudes (open circles) during the same experiment. The dotted line above the rod data (at ~41–50 min) indicates a period during which small stimulus cone response data was collected with lowered interstimulus intervals, temporarily decreasing the rod amplitudes. The rod response amplitudes during this higher frequency sequence are not included in the figure. *D*: the normalized cone response amplitude (circles: Φ_{cone} = 26,000 P*; stars: 83,000 P*) in darkness, during (at around 20 min) and after short exposure to a moderate background of $\Phi_{Bg,cone}$ = 320 P*s^{-1} in Ringer solution. MFA (50 μM) was added after recovery of dark-adapted amplitudes, as indicated by the arrow above data. The retina was then exposed to incremental adapting backgrounds ranging from 320 to 63,000 P*s^{-1}, as depicted below the data.

DISCUSSION

The light-induced growth in photopic ERG is a robust phenomenon that has been known and studied for several decades, although its cellular and molecular origins have remained unresolved. It has also been unknown whether the growth represents enhancement of the cone responses with respect to the dark-adapted state or adaptation/repolarization after an initial suppression by the photopic background. Our results indicate that much of the photopic growth arises as a true enhancement of the responses compared with the dark-adapted state. It also appears that in the mouse retina, its graded appearance correlates with the level of rod stimulation by the background.

Origins of the light-induced growth in cone flash responses. The persistence of light-induced growth in the cone photoresponses after repressing glutamatergic synaptic transmission to higher-order neurons indicates adaptive changes in the cone impulse response, as recorded with the ERG. This may involve either adaptation in the cone phototransduction machinery or in the way its impulse response is transformed into the membrane voltage response and how it is reflected in the inner segment currents (which indirectly give rise to the ERG signal). The response growth appears already at relatively dim backgrounds and can also be induced by relatively low frequency flash trains (Figs. 4 and 6) so that modulation of the cone phototransduction process itself as a key factor seems unlikely. The role of interphotoreceptor coupling in this phenomenon has not been explicitly addressed before the present study, but also some earlier data suggest that rod-cone interaction may be involved in it. In human multifocal ERG, the growth of the photopic ERG upon introducing rod-suppressing background light is strongest in the rod-dominated peripheral retina (Kondo et al. 1999). Also, in the rodless ($Nrl^{-/-}$ and $Rho^{-/-}$) mouse models and in the $Gnat1^{-/-}$ mouse lacking rod phototransduction, the photopic growth is significantly altered compared with wild-type animals (Tanikawa et al. 2004; Cameron and Lucas 2009). The current results suggest that cone flash responses of the mouse retina are indeed suppressed in darkness, and support the idea that this suppression is gradually removed as rods are progressively saturated by light.

To date, electrical coupling between mammalian rods and cones has been functionally shown in cat and macaque (Nelson 1977; Schneeweis and Schnapf 1999), but only indirectly in mouse, through the appearance of small gap junction-like contacts between rod spherules and cone pedicles in electron microscopic graphs (Tsukamoto et al. 2001). We have shown that two pharmacological agents that block gap junctions (i.e., MFA and octanol) increased cone ERG response size in dark-adapted retinas to a similar extent as that of mesopic background light and prevented additional enhancement by background light. Carbenoxolone, which also blocks gap junctions, decreased cone ERG response size in dark-adapted retinas and prevented enhancement of cone ERG response size by background illumination. As with any pharmacological approach, we cannot conclusively rule out all nonspecific effects of the compounds used as gap junction blockers. However, the finding that all three of the structurally different gap junction blockers appear to prevent the light-induced growth speaks in favor of the effect being mediated through their common effect on gap junctions.

Concurrently, a number of facts speak against feedback or other contribution from the postreceptoral neurons. The glutamatergic transfer of photoreceptor signals was pharmacologically prevented, or at least greatly suppressed in our experiments. Thus a light-induced feedback stemming from the basic glutamatergic routes of the retina seems unlikely. Specifically, the horizontal cell light responses of the macaque retina are abolished by a lower dose (5 μM) of NBQX (Dunn et al. 2007). Also, in the rabbit retina, the light responses of A-type horizontal cells are completely eliminated in a HEPES-buffered superfusate lacking bicarbonate (Hanitzsch and Küppers 2001), which treatment also failed to prevent light-induced cone response growth in our control experiments (data not shown). Due to the homogenous full-field stimuli used in this study, cone-cone coupling should not contribute to the observed light-induced growth. Furthermore, the background intensities sufficient to initiate release of cone suppression are too low to be expected to stimulate the melanopsin-containing ganglion cells (ipRGCs) in the inner retina (Berson et al. 2002; Tu et al. 2005; Do et al. 2008). Specifically, the threshold for an electrical light response in the ipRGCs has been found to be $\sim$1,000–1,500 hν $\mu m^{-2}s^{-1}$ at 480 nm (Do et al. 2008), which corresponds to $>$8,000 hν $\mu m^{-2}s^{-1}$ at 543.5 nm in our recording geometry (in which almost one-half of the incoming light is absorbed by rod outer segments before reaching the inner retina). The cone response growth is observed already at much lower intensities and is almost maximal at this light level.

Rod-cone coupling and modulation of the cone flash responses in the mesopic range. There are several ways in which closing the gap junctions between rods and cones could affect the ERG light-response originating in the cones, but the most straightforward explanation lies on direct electrical coupling between the cells. Gap junctions between rod terminals and cone pedicles enable rods to connect to cone bipolar cells in mammals. Due to the higher sensitivity of rods, cone light responses are superimposed on hyperpolarization spreading through rod-cone gap junctions (Nelson 1977; Schneeweis and Schnapf 1999). There is little difference in the absorption spectra of the rhodopsin and M-cone pigment in the mouse retina, so that rods are strongly hyperpolarized every time the cones activate in the dark-adapted retina. Closing rod-cone gap junctions at rod-saturating light levels would serve to remove this hyperpolarization and allow cone-originated signals the full functional range of synaptic transmission.

It is worth to note here that the process of cone response growth is rather slow, initiated seconds after the background onset and requiring minutes to reach full effect (see Fig. 5). Thus it can be induced only with relatively long-lasting light stimulation and cannot be driven by the short-lived hyperpolarization induced in rods by the single bright preflashes used in our recording protocol. We also point out that we observe correlation between the steady state level of rod amplitude suppression and cone response amplitude growth. This does not mean that cone response growth should be directly linked to the rod membrane potential; given the relatively slow time scale of the phenomenon, it actually seems unlikely. Because background illumination-induced cone response size enhancement takes several minutes to reach full effect (Fig. 5), but transjunctional voltage-induced changes in gap junctional conductance typically take hundreds of milliseconds to seconds to

occur (Harris 2001), it seems likely that cone response enhancement by mesopic backgrounds does not result from a direct rod hyperpolarization-induced decrease in rod-cone gap junctional conductance, but rather that the hyperpolarization of rods initiates a more slowly acting mechanism that results in cone response enhancement. Thus, it seems plausible to search for the molecular mechanism(s) in the light-induced metabolic changes within the rods. While the present work does not extend to clarifying the precise molecular pathway, one possible route is discussed below.

About the molecular mechanisms behind cone response suppression/enhancement. The level of rod-cone coupling in some teleosts and mammals has been shown to be regulated by the circadian clock in the retina (Ribelayga et al. 2008): rod signals are allowed to mix with the cone pathway at night, while the connection is closed during the day time. In addition, evidence suggests that there is a circadian clock in rods and cones that can be acutely affected by rod hyperpolarization and that the actions of the retinal clock are independent of glutamatergic transmission (Iuvone et al. 2005).

We found mesopic background lights to modulate cone ERG flash responses in a highly reversible manner, during a time scale of minutes. The degree of modulation of the cone responses was related to the background light intensity, and similar modulation was initiated by substances commonly used to decrease gap junctional coupling between adjoining cells. These findings are consistent with the idea that gap junctional coupling between rods and cones is modulated by background light, implying a relatively fast and reversible modulation of the connectivity between rod and cone pathways by light. Our experiments were typically started 2–3 h before the lights were switched on during the 12/12-h light cycle our mice were housed under. Thus, in principle, our findings are in concordance with the idea of circadially regulated rod-cone coupling. It would seem intuitively plausible that also during nighttime, the gap junctional conductance could be modulated by light-exposure. However, we did not specially investigate the effect of circadian rhythm on the phenomenon.

In a very recent work, Ribelayga and Mangel (2010) show that circadian clock modulates the extent of dye coupling between rods and cones in the rabbit retina through dopamine acting on D_2 receptors on the rod plasma membrane. The exact molecular route of how activation of the dopamine receptors leads to decoupling of the gap junctions is not yet known in mammals. In teleosts, the process has been reported to advance through cAMP-dependent protein kinase A activity and phosphorylation of the connexin proteins (Li et al. 2009). The cAMP-level in the mammalian photoreceptors can be regulated independently by exogenous dopamine or light (Cohen and Blazynski 1990, Nir et al. 2002). This allows for the enticing hypothesis that similar molecular mechanisms within the rods operate behind circadian and light-induced regulation of rod-cone coupling even though the triggering signal may be different for the two phenomena.

ACKNOWLEDGMENTS

We thank Teemu Turunen and Marja Pitkänen for assistance in conducting part of the experiments.

Present address of Dr S. Nymark: Dept. of Physiology and Biophysics, Boston University School of Medicine, Boston, MA 02118.

GRANTS

This project was supported by the Academy of Finland (Grants 111866 and 128081) and International Graduate School in Biomedical Engineering and Medical Physics.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

Applebury ML, Antoch MP, Baxter LC, Chun LLY, Falk JD, Farhangfar F, Kage K, Krzystolik MG, Lyass LA, Robbins JT. The murine cone photoreceptor: A single cone type expresses both S and M opsins with retinal spatial patterning. *Neuron* 27: 513–523, 2000.

Armington JC, Biersdorf WR. Long term light adaptation of the human electroretinogram. *J Comp Physiol Psychol* 51: 1–5, 1958.

Barnes S, Merchant V, Mahmud F. Modulation of transmission gain by protons at the photoreceptor output synapse. *Proc Natl Acad Sci* 90: 10081–10085, 1993.

Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073, 2002.

Bloomfield SA, Dowling JE. Roles of aspartate and glutamate in synaptic transmission in rabbit retina. I. Outer plexiform layer. *J Neurophysiol* 53: 699–713, 1985.

Bui BV, Fortune B. Origin of electroretinogram amplitude growth during light adaptation in pigmented rats. *Vis Neurosci* 23: 155–1567, 2006.

Burian HM. Electric responses of the human visual system. *Arch Ophthalmol* 51: 509–524, 1954.

Bush RA, Sieving PA. A proximal retinal component in the primate photopic ERG a-wave. *Invest Ophthalmol Vis Sci* 35: 635–645, 1994.

Cameron MA, Lucas RJ. Influence of the rod photoresponse on light adaptation and circadian rhythmicity in the cone ERG. *Mol Vis* 15: 2209–2216, 2009.

Carter-Dawson LD, LaVail MM. Rods and cones in the mouse retina. *J Comp Neurol* 188: 245–262, 1979.

Cohen AI, Blazynski C. Dopamine and its agonists reduce a light-sensitive pool of cyclic AMP in mouse photoreceptors. *Vis Neurosci* 4: 43–52, 1990.

Davidson JS, Baumgarten IM, Harley EH. Reversible inhibition of intercellular junctional communication by glycyrrhetinic acid. *Biochem Biophys Res Commun* 134: 29–36, 1986.

Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, Yau KW. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature* 457: 281–287, 2008.

Dunn DA, Lankheet MJ, Rieke F. Light adaptation in cone vision involves switching between receptor and post-receptor sites. *Nature* 449: 603–606, 2007.

Ekesten B, Gouras P, Moschos M. Cone properties of the light-adapted murine ERG. *Doc Ophthalmol* 97: 23–31, 1998.

Frumkes TE, Wu SM. Independent influences of rod adaptation on cone-mediated responses to light onset and offset in distal retinal neurons. *J Neurophysiol* 64: 1043–1054, 1990.

Goldberg SH, Frumkes TE, Nygaard RW. Inhibitory influence of unstimulated rods in the human retina: evidence provided by examining cone flicker. *Science* 221: 180–182, 1983.

Gouras P, McKay CJ. Growth in amplitude of the human cone electroretinogram with light adaptation. *Invest Ophthalmol Vis Sci* 30: 625–630, 1989.

Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K. In search of the visual pigment template. *Vis Neurosci* 17: 509–528, 2000.

Hanitzsch R, Küppers L. The influence of HEPES on light responses of rabbit horizontal cells. *Vision Res* 41: 2165–2172, 2001.

Harris AL. Emerging issues of connexin channels: biophysics fills the gap. *Q Rev Biophys* 34: 325–472, 2001.

Harks EG, de Roos AD, Peters PH, de Haan LH, Brouwer A, Ypey DL, van Zoelen EJ, Theuvenet AP. Fenamates: a novel class of reversible gap junction blockers. *J Pharm Exp Ther* 298: 1033–1041, 2001.

Heikkinen H, Nymark S, Koskelainen A. Mouse cone photoresponses obtained with electroretinogram from the isolated retina. *Vision Res* 48: 264–272, 2008.

Iuvone PM, Tosini G, Pozdeyev N, Haque R, Klein DC, Chaurasia SS. Circadian clocks, clock networks, arylalkylamine N-acetyltransferase,and melatonin in the retina. *Progr Retin Eye Res* 24: 433–456, 2005.

Johnston MF, Simon SA, Ramon F. Interaction of anaesthetics with electrical synapses. *Nature* 286: 498–500, 1980.

Knapp AG, Schiller PH. The contribution of ON-bipolar cells to the electroretinogram of rabbits and monkeys. *Vision Res* 24: 1841–1846, 1984.

Kolb H, Famiglietti EV. Rod and cone pathways in the inner plexiform layer of cat retina. *Science* 186: 47–49, 1974.

Kolb H. The organization of the outer plexiform layer in the retina of the cat: electron microscopic observations. *J Neurocytol* 6: 131–153, 1977.

Kondo M, Miyake Y, Piao CH, Tanikawa A, Horiguchi M, Terasaki H. Amplitude increase of the multifocal electroretinogram during light adaptation. *Invest Ophthalmol Vis Sci* 40: 2633–2637, 1999.

Kothmann WW, Massey SC, O'Brien J. Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. *J Neurosci* 29: 14903–14911, 2009.

Li H, Chuang AZ, O'Brien J. Photoreceptor coupling is controlled by connexin 35 phosphorylation in zebrafish retina. *J Neurosci* 29: 15178–15186, 2009.

Lyubarsky AL, Falsini B, Pennesi ME, Valentini P, Pugh EN Jr. UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for coexpression of cone photopigments. *J Neurosci* 19: 442–455, 1999.

Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R, Takada M, Nakamura K, Nakao K, Katsuki M, Nakanishi S. Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell* 80: 757–765, 1995.

Nelson R. Cat cones have rod input: a comparison of the response properties of cones and horizontal cell bodies in the retina of the cat. *J Comp Neurol* 172: 109–135, 1977.

Nikonov SS, Kholodenko R, Lem J, Pugh EN Jr. Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. *J Gen Physiol* 127: 359–374, 2006.

Nir I, Harrison J, Haque R, Low MJ, Grandy DK, Rubinstein M, Iuvone PM. Dysfunctional light-evoked regulation of cAMP in photoreceptors and abnormal retinal adaptation in mice lacking dopamine D4 receptors. *J Neurosci* 22: 2063–2073, 2002.

Nymark S, Heikkinen H, Haldin C, Donner K, Koskelainen A. Light responses and light adaptation in rat retinal rods at different temperatures. *J Physiol* 567: 923–938, 2005.

Pan F, Mills SL, Massey SC. Screening of gap junction antagonists on dye coupling in the rabbit retina. *Vis Neurosci* 24: 609–618, 2007.

Peachey N, Goto Y, Al-Ubaidi MR, Naash M. Properties of the mouse cone-mediated electroretinogram during light adaptation. *Neurosci Lett* 162: 9–11, 1993.

Raviola E, Gilula NB. Gap junctions between photoreceptor cells in the vertebrate retina. *Proc Natl Acad Sci* 70: 1677–1681, 1973.

Ribelayga C, Cao Y, Mangel SC. The circadian clock in the retina controls rod-cone coupling. *Neuron* 59: 790–801, 2008.

Ribelayga C, Mangel SC. Identification of a circadian clock-controlled neural pathway in the rabbit retina. *PLos One* 5: e11020, 2010.

Robson JG, Saszik SM, Ahmed J, Frishman LJ. Rod and cone contributions to the a-wave of the electroretinogram of the macaque. *J Physiol* 547: 509–530, 2003.

Schneeweis DM, Schnapf JL. The photovoltage of macaque cone photoreceptors: adaptation, noise, and kinetics. *J Neurosci* 19: 1203–1216, 1999.

Shirato S, Maeda H, Miura G, Frishman LJ. Postreceptoral contributions to the light-adapted ERG of mice lacking b-waves. *Exp Eye Res* 86: 914–928, 2008.

Slaughter MM, Miller RF. 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science* 211: 182–185, 1981.

Stockton RA, Slaughter MM. B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol* 93: 101–122, 1989.

Tanikawa A, Bush RA, Takada Y, Mears AJ, Swaroop A, Sieving PA. Functional rods are required for photopic ERG amplitude increase during light adaptation: study Of Nrl−/− and Rho−/− mice. *Invest Ophthalmol Vis Sci* 45: E-abstract 81, 2004.

Tovar KR, Maher BJ, Westbrook GL. Direct actions of carbenoxolone on synaptic transmission and neuronal membrane properties. *J Neurophysiol* 102: 974–978, 2009.

Tsukamoto Y, Morigiwa K, Ueda M, Sterling P. Microcircuits for night vision in mouse retina. *J Neurosci* 21: 8616–8623, 2001.

Tu DC, Zhang D, Demas J, Slutsky EB, Provencio I, Holy TE, Van Gelder RN. Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. *Neuron* 48: 987–999, 2005.

Veruki ML, Hartveit E. Meclofenamic acid blocks electrical synapses of retinal AII amacrine and ON-cone bipolar cells. *J Neurophysiol* 101: 2339–2347, 2009.

Veruki ML, Oltedal L, Hartveit E. Electrical coupling and passive membrane properties of AII amacrine cells. *J Neurophysiol* 103: 1456–1466, 2010.

Weymouth AE, Vingrys AJ. Rodent electroretinography: methods for extraction and interpretation of rod and cone responses. *Prog Ret Eye Res* 27: 1–44, 2008.

Wässle H, Boycott BB. Functional architecture of the mammalian retina. *Physiol Rev* 71: 447–480, 1991.

Yu W, Miller RF. NBQX, an improved non-NMDA antagonist studied in retinal ganglion cells. *Brain Res* 692:190–194, 1995.