Dynamic and steady-state light adaptation of mouse rod photoreceptors in vivo

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1. Electroretinographic (ERG) methods were used to investigate the effects of background illumination on the responses of mouse rod photoreceptors in vivo. A paired-flash procedure, involving the recording and analysis of the ERG a-wave response to a bright probe flash presented after a brief test flash, was used to derive the rod response to the test flash in steady background light. A related, step-plus-probe procedure was used to derive the step response of the rods to backgrounds of defined strength.

2. Steady background light produced a maintained derived response that was graded with background strength. Determinations of the full time course of the derived weak-flash response in steady background light, and of the effect of background strength on the flash response at fixed post-test-flash times, showed that moderate backgrounds reduce the peak amplitude and duration of the flash response.

3. The response to stepped onset of an approximately half-saturating background (1.2 sc cd m⁻²) exhibited a gradual rise over the first 200–300 ms, and an apparent subsequent relaxation to plateau amplitude within 1 s after background onset. Determinations of normalized amplitudes of the derived response to a test flash presented at 50 or 700 ms after background onset indicated substantial development of background-induced shortening of the test flash response within this 1 s period. These findings indicate a time scale of ~1 s or less for the near-completion of light adaptation at this background strength.

4. Properties of the derived response to a stepped background and to test flashes presented in steady background light are in general agreement with photocurrent data obtained from mammalian rods in vitro and suggest that the present results describe, to good approximation, the in vivo desensitization of mouse rods by background light.

Understanding the mechanisms by which background light desensitizes, or ‘adapts’, the photic responses of retinal neurons is a central goal in vision physiology (for reviews, see Dowling, 1987; Sharpe, 1990; Lamb, 1990). Photocurrent data obtained from single rod photoreceptors of both lower vertebrates and mammals provide strong evidence for the operation of light adaptation in rods, as measured by changes in the sensitivity and kinetics of the response to a test flash (Baylor et al. 1979; Fain et al. 1989; Tamura et al. 1989; Cornwall et al. 1989, 1990; Matthews, 1991; Nakatani et al. 1991; Kraft et al. 1993). However, relatively little direct information is available on the nature of rod light adaptation in vivo. Recent studies in several mammalian species, including mouse, have shown that the technique of ‘paired-flash’ electroretinography can, to a good approximation, determine the full time course of the in vivo, massed rod response to a test flash under dark-adapted conditions (Birch et al. 1995; Lyubarsky & Pugh, 1996; Pepperberg et al. 1996, 1997; Hetling & Pepperberg, 1999; Robson & Frishman, 1999, 2000). Using this method, the rod response to the test flash at defined times is derived from measurements of the response to a bright probe flash that rapidly saturates the rod response, i.e. drives the rod circulating current to zero.

The present study addresses the nature of light adaptation of mouse rod photoreceptors in vivo using electroretinography. We first describe the effects of steady background light on the sensitivity and time course of the derived response to a brief test flash. We then examine properties of the rod response produced by the stepped onset of background light. Preliminary results have been reported in abstract form (Silva et al. 2000).

METHODS

The experiments employed C57BL/6J mice obtained from Jackson Laboratories (Bar Harbor, ME, USA). The mice were maintained on a light–dark cycle (12 h light : 12 h dark; ambient illumination of ~2–19 lux), were 5–16 weeks at the time of experiments, and were dark-adapted for at least 3 h prior to the experiments. All procedures...
were in accordance with protocols approved by the Animal Care Committee of the University of Illinois at Chicago, and with the principles outlined in the Statement for the Use of Animals in Ophthalmic and Vision Research established by the Association for the Research in Vision and Ophthalmology. Mice were anaesthetized under dim red light by intraperitoneal injection of a saline solution containing ketamine and xylazine (0.15 and 0.01 mg (g body weight)$^{-1}$, respectively). Other conditions relevant to the systemic administration of anaesthetic, pupil dilatation and anaesthesia, and corneal lubrication were similar to those described previously (Hetling & Pepperberg, 1999). Corneal moistening was achieved by the periodic addition of distilled water or Natural Tears (Alcon Ophthalmic, Forth Worth, TX, USA). During the experiment, the mouse was positioned on a temperature-regulated heating pad that maintained body temperature at about 38–40°C as determined rectally. Following completion of the experiment the mouse was given an intraperitoneal injection of normal saline to speed rehydration, and its recovery monitored. Following recovery, the animal was returned to the university animal care facility. A given mouse was used in only a single experiment.

A photostimulator modified from that previously described (Hetling & Pepperberg, 1999) was used for the presentation of full-field test and probe flashes as well as background illumination. The apparatus used to produce test and probe flashes, as well as the geometry of the photostimulator and positioning of the animal, and instrumentation for the recording and storage of electoretinographic (ERG) responses, were as previously described (Hetling & Pepperberg, 1999). Photic stimuli were calibrated by photometric measurement (Model 1700 photometer equipped with an SED033 detector, radiant barrel and ZCIE scotopic filter; International Light Inc., Newburyport, MA, USA). Probe flashes used in paired-flash and step-plus-probe experiments were within the range of 350 to 530 scotopic candelas second per square metre (sc cd s m$^{-2}$). Test flashes ranged from 0.11 to 2.6 sc cd m$^{-2}$. Background light was generated by a tungsten–halogen projector lamp (Spindler and Hoyer, Göttingen, Germany) powered by a regulated DC supply (Model 718-10D; Leader, Inc., Hauppauge, NY, USA). The background light passed through a heat filter and was brought to the inner surface of the hemispheric dome photostimulator using a fibre optic light guide (12 mm diameter) with a diffusing convex lens. The background beam was controlled by an electronic shutter (Model 3108, Uniblitz, Rochester, NY, USA) activated either manually or by computer as appropriate to a given experiment. Background strengths ranged up to 50 sc cd m$^{-2}$. Tolerances of all reported background and flash strengths can be taken as ±10%, based on periodic calibrations of the apparatus.

RESULTS

Response in steady background light

The response to a probe flash presented alone during maintained background illumination provides a measure of the steady-state, light-suppressible circulating current. The inset of Fig. 1 shows probe flash responses obtained in a single experiment that involved the variation of background strength ($I_b$) over the range 0–21 sc cd m$^{-2}$. In all cases, flashes were delivered at least 2 min after background onset. Increasing the strength of the background progressively reduced the amplitude of the probe response from the dark-adapted ($I_b = 0$) value. We may define $A_{b0}$ the maintained derived response to the background, as the difference in amplitude between the probe-alone response obtained in darkness and that obtained in background light:

\[ A_b = A_{mod} - A_{mol}, \]  

(2)

where $A_{mod}$ and $A_{mol}$ are, respectively, amplitudes of the dark- and light-adapted probe-alone responses (cf. eqn (1)). Figure 1 (open symbols) plots values of $A_b/A_{mod}$, the normalized derived response, determined as a function of the logarithm of background intensity ($\log I_b$).

The Fig. 1 data may be considered in relation to the simple hypothesis that background light produces no desensitization of the rod response. Curve 1, re-plotted from Hetling & Pepperberg (1999) (their eqn (2) and Fig. 2C), describes the experimentally observed dependence of the normalized, dark-adapted derived response on test flash strength ($I_{test}$) as determined at the near-peak time of 86 ms after test flash presentation. The curve plots the relation:

\[ A(86)/A_{mod} = 1 - \exp(-k_{86} I_{test}), \]  

(3)

where $k_{86}$, a sensitivity parameter, is equal to $7.0 \text{ sc cd s m}^{-2}$. If the response maintained in background light were due to the superposition of elemental responses identical to those underlying the dark-adapted response to a weak test flash (Hetling & Pepperberg, 1999; Robson & Frishman, 2000), $A_b/A_{mod}$ should be related to the dark-adapted amplitude-intensity function (curve 1 and eqn (3)) by an integration time that links a given background strength $I_b$ with an effective value of $I_{test}$ in eqn (3). That is:

\[ A_b/A_{mod} = 1 - \exp(-k_{86} I_{test}), \]  

(4)

where $\tau_{int}$ is the integration time and where $I_{test} \tau_{int} = I_{test}$. Curve 2 in Fig. 1 plots eqn (4) with $\tau_{int}$ set equal to 235 ms.
Although this curve approximates the behaviour of the experimental data at low values of $A_b/A_{m0}$, the curve rises more sharply than the data at higher background values. This is an indication of light adaptation; that is, with moderate background illumination, the maintained response is smaller than that predicted by the properties of the dark-adapted response. This observation is consistent with results obtained in previous studies of single rod photocurrents (Fain et al. 1989; Tamura et al. 1989; Nakatani et al. 1991; Xu et al. 1997). In addition, the integration time $\tau_{int}$ determined from the dark-adapted derived response (235 ms; cf. Fig. 1 legend) is similar to integration times determined for the dark-adapted flash responses of mouse rods in vitro (reported ranges of 238–270 ms; Sung et al. 1994; Raport et al. 1994; Xu et al. 1997); a somewhat shorter integration time (average value: 209 ms) has recently been reported by Calvert et al. (2000).

Accurate determination of the derived response amplitude using the paired-flash method relies on the rods being rapidly driven to saturation by the probe flash (reviewed by Pepperberg et al. 2000). Desensitization of the transduction pathway in background light can be expected to exert some desensitizing effect on the response to the bright probe flash, i.e. a slowing of the rate of rise of the response (see, for example, Thomas & Lamb, 1999) and thus, potentially, a skewing of determinations based on amplitude measurements at a fixed time in the probe response. To investigate the

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**Figure 1. Normalized amplitude of the derived response in steady background light**

Results obtained with presentation of the probe flash at least 2 min after the onset of background illumination. Each data point indicates the maintained response $A_b$ normalized to the dark-adapted maximal amplitude $A_{m0}$ (cf. eqn (2)) and plotted against the logarithm of background strength ($\log I_b$). Results obtained in 5 experiments; identical symbols represent data from the same experiment. Data positioned at $\log I_b = -4.5$ represent dark-adapted ($I_b = 0$) results. Curve 1 (eqn (3)), replotted from Hetling & Pepperberg (1999), describes the amplitude–intensity relation for the derived flash response at $t = 86$ ms under dark-adapted conditions ($A(86)/A_{m0}$ plotted against the logarithm of test flash strength ($\log I_{test}$)). Curve 2 plots the relation $A_b/A_{m0} = 1 - \exp(-k_{m1}I_b\tau_{int})$ (eqn (4)). Here, the integration time $\tau_{int}$ is defined by the relation $[A(86)/A_{m0}]\tau_{int} = [A(t)/A_{m0}]\Delta t$, where $A(86)/A_{m0}$ is as given by eqn (3), and where $A(t)/A_{m0}$ is the normalized dark-adapted response to a weak test flash (eqn (6) of Hetling & Pepperberg, 1999) with $I_{test} = 0.12$ sc cd s m$^{-2}$. The integration was carried out over the interval $0 \leq t \leq 1000$ ms, yielding $\tau_{int} = 235$ ms. Inset, probe flash responses obtained in a single experiment (single responses). Labels identify background strength in sc cd m$^{-2}$. The lowest trace ($I_b = 0$) is the average of nine probe responses obtained in the absence of the background.
Figure 2. Effect of probe flash attenuation on normalized amplitude of the probe response

A and B, representative probe responses obtained in darkness ($I_b = 0$; A) and in the presence of a 5.8 sc cd m$^{-2}$ background (B). Data obtained in a single experiment. Labels indicate probe flash strength in sc cd s m$^{-2}$. C and D, responses from A and B matched for amplitude at 6 ms after probe flash presentation. E, probe response amplitudes plotted against the logarithm of probe flash strength. Results
Table 1. Fitted parameters and referenced integration times for derived responses and inferred elemental responses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 Dark-adapted</th>
<th>2 0.63 sc cd m⁻² background</th>
<th>3 1.2 sc cd m⁻² background</th>
<th>4 2.5 sc cd m⁻² background</th>
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</thead>
<tbody>
<tr>
<td>φ (sc cd s m⁻²)⁻¹</td>
<td>15.47</td>
<td>7.51</td>
<td>4.96</td>
<td>8.73</td>
</tr>
<tr>
<td>α (ms⁻²)</td>
<td>2.32 x 10⁻⁴</td>
<td>2.29 x 10⁻⁴</td>
<td>1.05 x 10⁻⁴</td>
<td>4.52 x 10⁻⁵</td>
</tr>
<tr>
<td>τ₀ (ms)</td>
<td>132</td>
<td>88</td>
<td>119</td>
<td>92</td>
</tr>
<tr>
<td>τₘ₀ (ms)</td>
<td>203</td>
<td>54</td>
<td>45</td>
<td>36</td>
</tr>
<tr>
<td>τₘ₉ (ms)</td>
<td>234</td>
<td>71</td>
<td>65</td>
<td>54</td>
</tr>
</tbody>
</table>

Entries for φ, α and τ₀ show values of eqn (5) parameters for the fitted functions illustrated in Fig. 3. For each condition of background illumination, the referenced integration time τₘ₀ for the elemental response ε(t) (fourth row) and for the derived response (Iₙₒ = 0.12 sc cd m⁻²) (fifth row) were determined with the use of eqn (5) (see text). In all cases, curves were integrated from 0 to 1000 ms.

magnitude of this effect, we examined how defined attenuation of the probe flash affects the normalized probe response amplitude. Figure 2A and B shows probe responses obtained with attenuation of the probe flash in darkness (Fig. 2A) and in the presence of a relatively strong background (5.8 sc cd m⁻²) (Fig. 2B). Under both conditions, the investigated range of attenuated probe flash strengths extended to values well below 350 sc cd s m⁻², the lowest strength used in the paired-flash experiments of the present study.

Two properties of the Fig. 2 results indicate that in background light, the tested probe flashes shut down the rod circulating current essentially as efficiently as in darkness. First, rescaling the dark- and light-adapted responses of Fig. 2A and B to achieve an amplitude match at 6 ms (Fig. 2C and D, respectively) indicates a kinetic similarity of the waveforms over the initial 6 ms of the response, consistent with the absence of substantial intrusion into the a-wave leading edge by post-receptor ERG components. Thus, both in darkness and in background light, the fractional response developed at a given early time in the response to the probe flash (i.e. during the first 6 ms) is largely independent of probe flash strength over the investigated range. The second point concerns the dependence of the probe response amplitude on probe flash strength (Fig. 2E). If background light desensitized the rod response to a probe flash of fixed strength, the decline in probe response amplitude with decreasing probe flash intensity should be more pronounced in background light than in darkness. To test this possibility we determined amplitudes of the response to a range of probe flashes in darkness and in background light (1.2 and 5.8 sc cd m⁻²), and examined the slopes of the resulting amplitude functions. Figure 2E shows that the slopes of these functions are relatively shallow and similar to one another. In particular, the local slope of each function at the highest probe flash strengths (i.e. strengths closest to those used in the paired-flash experiments) is shallow. The data of Fig. 2 thus indicate that the tested backgrounds have at most a modest desensitizing effect on the rod response to the probe flash.

In vitro photocurrent data from mammalian rods indicate that an approximately half-saturating background reduces the normalized peak amplitude of the weak-flash response by severalfold and shortens the time course of the response (Tamura et al. 1989; Nakatani et al. 1991; Kraft et al. 1993). Figure 3A compares results obtained in relatively weak background light with a function shown previously to describe the derived response to a weak test flash under dark-adapted conditions. Dashed curve DA, which describes the normalized derived response to a test flash of 0.12 sc cd s m⁻² in darkness (Hetling & Pepperberg, 1999; their eqn (6), and curve 1 of their Fig. 5), plots the equation:

\[
A(t)/A_{max} = 1 - \exp[-I_{test}(t)];
\]

\[
\epsilon(t) = \phi(1 - \exp[-\alpha(t - t_i)])\exp(-t/\gamma),
\]

where \(A_{max}\) is the prevailing maximal amplitude (for curve DA in Fig. 3, equal to the dark-adapted maximal amplitude \(A_{max}\)) and where the values of the free parameters φ, α and τ₀ are as specified in column 1 of Table 1. (Here, φ is equivalent to the product of the parameters \(k_{max}\) and γ used in eqn (6) of Hetling & Pepperberg, 1999.) In eqn (5) and throughout this paper, the delay \(t_i\) was fixed at 3.1 ms (cf. Hood & Birch, 1993; Cideciyan & Jacobson, 1996; Hetling & Pepperberg, 1999). By analogy with the analysis presented by Hetling & Pepperberg (1999), ε(t) can be viewed as an elemental component of the macroscopic derived response to a test...
flash, i.e. a unit response that is independent of the test flash strength.

Curve DA may be compared with the open circles in Fig. 3A, which show the derived response to a test flash of 0.30 sc cd s m\(^{-2}\) in the presence of a 0.63 sc cd m\(^{-2}\) background. These data are well described by the continuous curve, which plots eqn (5) with maximal amplitude \(A_{mol}\), and with \(\phi, \alpha\) and \(\tau_o\) evaluated as shown in column 2 of Table 1. With this background, the derived response to the 0.30 sc cd s m\(^{-2}\) test flash had a similar peak amplitude as the function describing the derived response in darkness to a test flash of 0.12 sc cd s m\(^{-2}\). The derived response in background light furthermore showed a somewhat faster recovery to the pre-flash baseline. Figure 3B and C shows normalized derived responses to a 0.98 sc cd s m\(^{-2}\) test flash in the presence of stronger backgrounds (1.2 sc cd m\(^{-2}\) in B; 2.5 sc cd m\(^{-2}\) in C). The derived flash responses determined under these two conditions were similar to one another, and reduced in both peak amplitude and duration from the function describing the dark-adapted response to the same test flash (Fig. 3D).

Figure 3. Derived responses to test flashes presented in darkness and in steady background light. A, ○, normalized response \(A(t)/A_{mol}\) determined with a test flash of 0.30 sc cd s m\(^{-2}\) and a background of 0.63 sc cd m\(^{-2}\). Results obtained in 3 experiments; data points show the means ± s.d. of 1–4 determinations. The continuous curve plots eqn (5) with parameters indicated in column 2 of Table 1. The dashed curve DA, re-plotted from Hetling & Pepperberg (1999), describes the normalized dark-adapted response \(A(t)/A_{mol}\) to a test flash of 0.12 sc cd s m\(^{-2}\) (eqn (5) with parameters indicated in column 1 of Table 1). B and C, ○, normalized responses determined with a test flash of 0.98 sc cd s m\(^{-2}\) and with background strengths of 1.2 sc cd m\(^{-2}\) (B) and 2.5 sc cd m\(^{-2}\) (C). Results in each panel obtained in four experiments; the data indicate the means ± s.d. for 1–6 determinations. Curves in B and C plot eqn (5) with parameters as indicated, respectively, in columns 3–4 of Table 1. D, comparison of functions describing the derived response to a test flash of 0.98 sc cd s m\(^{-2}\). Curves labelled 1.2 and 2.5 sc cd m\(^{-2}\) re-plot the curves shown in B and C, respectively. Curve DA plots eqn (5) evaluated with dark-adapted parameters indicated in column 1 of Table 1 and with a test flash strength of 0.98 sc cd s m\(^{-2}\).
The effect of background light on the kinetics of the derived flash response was further investigated by determining normalized amplitudes at fixed times in the response over a range of background intensities. Open and filled symbols in Fig. 4 show normalized amplitudes determined with post-test-flash times $t = 86$ ms and $256$ ms ($A(86)/A_{\text{moD}}$ and $A(256)/A_{\text{moD}}$, respectively). With use of a test flash of $2.6$ sc cd s m$^{-2}$ (Fig. 4A), derived normalized amplitudes at the two investigated times were near-saturating at weak backgrounds and gradually declined with increasing background strength. Over the investigated range of backgrounds (0.40 to 5.8 sc cd m$^{-2}$), the decline at $256$ ms was steeper than that at $86$ ms, consistent with the Fig. 3 results indicating a shortened time scale of the flash response in background light. Furthermore, the reductions in $A(256)/A_{\text{moD}}$, determined at the two highest backgrounds were similar. Figure 4B shows results obtained at $86$ and $256$ ms with a test flash of $0.11$ sc cd s m$^{-2}$ (Fig. 4B), derived normalized amplitudes at the two investigated times were near-saturating at weak backgrounds and gradually declined with increasing background strength. Over the investigated range of backgrounds (0.40 to 5.8 sc cd m$^{-2}$), the decline at $256$ ms was steeper than that at $86$ ms, consistent with the Fig. 3 results indicating a shortened time scale of the flash response in background light. The fitting of eqn (5) to results obtained for the derived response in darkness and in background light (Fig. 3) specifies the shape of the flash-strength-independent function $e(t)$ under a given background condition. Curve DA in Fig. 5 shows $e(t)$ determined by the fitting of eqn (5) to dark-adapted data (parameters of Table 1, column 1), determined from results previously reported by Hetling & Pepperberg, 1999); for clarity, the peak value of this curve is here set equal to unity. Also plotted in Fig. 5 are the functions $e(t)$ determined by fitting eqn (5) to the present results obtained with backgrounds of 0.63, 1.2 and 2.5 sc cd m$^{-2}$ (Table 1, columns 2–4); in the figure, these light-adapted functions are normalized to the peak value of curve DA. The functions $e(t)$ determined for the three investigated levels of background illumination exhibit peak amplitudes smaller than that of the dark-adapted $e(t)$, and decreasing peak amplitude with increasing background strength. The fitting of eqn (5) to results obtained for the derived response in darkness and in background light (Fig. 3) specifies the shape of the flash-strength-independent function $e(t)$ under a given background condition. Curve DA in Fig. 5 shows $e(t)$ determined by the fitting of eqn (5) to dark-adapted data (parameters of Table 1, column 1), determined from results previously reported by Hetling & Pepperberg, 1999); for clarity, the peak value of this curve is here set equal to unity. Also plotted in Fig. 5 are the functions $e(t)$ determined by fitting eqn (5) to the present results obtained with backgrounds of 0.63, 1.2 and 2.5 sc cd m$^{-2}$ (Table 1, columns 2–4); in the figure, these light-adapted functions are normalized to the peak value of curve DA. The functions $e(t)$ determined for the three investigated levels of background illumination exhibit peak amplitudes smaller than that of the dark-adapted $e(t)$, and decreasing peak amplitude with
increasing background intensity. Each of these $e(t)$ functions can be characterized in terms of a referenced integration time ($\tau_{\text{ref}}$), defined for a given function as the base of a rectangle on the time axis, the area of which is equal to the time integral of the function and the height of which is equal to the peak of the dark-adapted function $DA$. Shown in row 4 of Table 1 are the values of $\tau_{\text{ref}}$ determined for the Fig. 5 functions $e(t)$. Even with the weakest background, $\tau_{\text{ref}}$ is decreased by about fourfold from the dark-adapted value, and further decreases in $\tau_{\text{ref}}$ are evident with increasing background intensity. These data are comparable to referenced integration times for derived responses determined with eqn (5) and with a test flash of 0.12 sc cd s m$^{-2}$ (row 5 of Table 1; see Discussion).

**Response to stepped background**

Presentation of a probe flash at defined times shortly after background onset allowed determination of the response to the step of light. In each of a series of experimental runs separated by a 2 min period of dark adaptation, a background of fixed strength and fixed duration (1 s) was presented; onset of the background defined time zero in each run. A probe flash was presented at a defined time ($t_{\text{probe}}$) during the 1 s background exposure. The amplitude of the probe-induced response was measured 6 ms after presentation of the probe flash and taken as $A_{\text{m}}(t)$, where $t = t_{\text{probe}} + 6$ ms (cf. eqn (1)). $A(t)$, the derived rod response to the stepped background at time $t$, was then determined

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**Figure 6. Derived response amplitudes at a fixed time ($t = 156$ ms) after background onset**

A, probe responses obtained with presentation of the probe flash at 150 ms after the onset of background light of defined strength. Labels identify the strength of the background in sc cd m$^{-2}$. The illustrated data are raw probe responses not corrected for the response to the background alone. Waveform PA, average of two probe-alone responses. Data obtained in a single experiment. B, waveform BA(150) is a 30 ms segment of the response to a 50 sc cd m$^{-2}$ background alone obtained in the same experiment; the marker above the trace identifies time $t = 150$ ms after onset of the background. Trace PA, probe-alone response. C, normalized amplitude of the derived response to a stepped background at 150 ms after background onset ($A(150)/A_{\text{m}}$), plotted in relation to log background strength. Results obtained in 3 experiments including that described in A; amplitude data not corrected for the contribution of the background alone. O, re-plot of the steady-state data shown in Fig. 1. Data positioned at $\log I_b = -3.0$ represent dark-adapted ($I_b = 0$) results.
with eqn (1). Unless otherwise indicated, step-plus-probe data were normalized to the prevailing amplitude of the dark-adapted probe-alone response (see Methods).

Figure 6 illustrates results obtained with a fixed time of probe flash presentation ($t_{probe} = 150$ ms) following the onset of backgrounds of differing strength. Traces in Fig. 6A show data obtained in a single experiment; the accompanying labels identify the strength of the background in sc cd m$^{-2}$. These data are not corrected for the response to the background alone. However, the rates of change of the background-alone responses at times near 150 ms were relatively small, as illustrated by the pre-probe baselines in Fig. 6A and by the 30 ms trace obtained with the strongest background investigated (50 sc cd m$^{-2}$)(trace BA(150) in B). Filled circles in Fig. 6C show determinations of the normalized derived amplitude at $t = 156$ ms ($A(156)/A_{mod}$) obtained in the experiment of Fig. 6A and in two additional experiments. Over the investigated range of backgrounds, these normalized amplitudes were graded with background strength. With moderate backgrounds the normalized amplitude at 156 ms was somewhat lower than the maintained level determined $\geq 2$ min after background onset (open circles in Fig. 6C; data replotted from Fig. 1), consistent with incomplete development of the response to the stepped background at 156 ms (see below).

Figure 7 illustrates derived responses to stepped backgrounds of defined strength over an approximately 800–900 ms period that immediately followed background onset. Figure 7A shows results obtained with a background of 1.2 sc cd m$^{-2}$, a level found in other experiments to produce an approximately half-saturating steady-state response (cf. Fig. 1). Here, open circles plot normalized amplitudes $A(t)/A_{mod}$ of the step response; the filled circle at the right indicates the steady-state, i.e. plateau, amplitude maintained by this background. Over an initial period of 200–300 ms, the derived response to the stepped background exhibited a gradual rise toward a level representing about 70% of the dark-adapted maximal response. Subsequently there developed an apparent gradual decline toward the plateau level. Attainment of this plateau was near-complete by about 800 ms after background onset.

The data of Fig. 7A are well described by an empirical, delayed Gaussian function that exhibits a peak prior to attaining its asymptotic value (curve in Fig. 7A; see Fig. 7 legend). For comparison, the Fig. 7A data are re-plotted in Fig. 7B to illustrate the fitting of two alternative functions to these data: an exponentially rising function (dashed curve 1) and a second-order damped sinusoidal function (continuous curve 2). Consistent with the occurrence of a peak and subsequent relaxation to plateau level in the experimental data, the Fig. 7A delayed Gaussian function exhibits a smaller root-mean-square (RMS) deviation than does the exponentially rising function of Fig. 7B (RMS deviations of 0.0836 and 0.1048, respectively). The second-order damped sinusoidal function of Fig. 7B yielded an RMS deviation of 0.0832, similar to that provided by the delayed Gaussian function in Fig. 7A. Results obtained in similar experiments employing a background of 0.40 sc cd m$^{-2}$ were consistent with an exponential rise to an asymptote representing $\sim 20\%$ of the maximal excursion (triangles and accompanying curve in Fig. 7C; see legend). The normalized response determined with a background of 2.6 sc cd m$^{-2}$ attained, within 1 s after background onset, a plateau level equal to $\sim 60\%$ of the maximal excursion (circles in Fig. 7C).

**Flash response shortly after background onset**

The Fig. 7 results indicate near-complete development of the response to a moderate background (1.2 sc cd m$^{-2}$) within 1 s after background onset. In the experiments of Fig. 8 we investigated the kinetics of the response to a test flash presented at varying times during this 1 s period, i.e. we examined the developing effect of background light on the derived response to a superimposed test flash. In each of three experiments, paired-flash trials with a fixed-strength test flash (0.98 sc cd s m$^{-2}$) and a test-probe interval of either 20 or 250 ms were carried out under dark-adapted conditions (left-hand pair of bars); with test flash presentation at 50 ms after onset of the 1.2 sc cd m$^{-2}$ background (middle pair of bars); and with test flash presentation at 700 ms after background onset (right-hand pair of bars). Each pair of bars shows amplitudes of the derived response to the test flash determined with test-probe intervals of 20 and 250 ms (open and shaded bars, respectively). Derived amplitudes with these test-probe intervals were determined at 26 ms and 256 ms, respectively, after test flash presentation (cf. eqn (1)), and normalized to the prevailing probe-alone response (see figure legend).

Values of normalized amplitude of the dark-adapted response at 26 ms exhibited a relatively large standard deviation, due probably to the coincidence of probe flash presentation with large test-flash-induced oscillatory potentials under this dark-adapted condition. However, under the three conditions investigated, there was no significant difference among the amplitude data obtained at 26 ms, indicating little if any effect of the background on the normalized test flash response at this early time in the response. By contrast, with test flash presentation at 700 ms after background onset, the normalized amplitude at 256 ms was significantly smaller ($P < 0.005$) than that determined for the dark-adapted response. In addition, the 256 ms data obtained with presentation of the test flash at 700 ms differed significantly ($P < 0.05$) from those obtained with test flash presentation at 50 ms. Thus, background-induced shortening of the time scale of the test flash response, an effect evident under steady-state conditions (Figs 3–4 and Table 1), develops within 1 s after onset of a moderate background.

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Figure 7. Time course of the derived response to a stepped background

A, normalized derived amplitudes $A(t)/A_{m0}$ determined at varying times after onset of a 1.2 sc cd m$^{-2}$ background ($\bigcirc$). Results obtained in 6 experiments; data points indicate the means ± s.d. of results obtained from 1–7 determinations. The filled circle at the right is the mean ± s.d. for 12 determinations of the steady-state derived response at ≥ 2 min after background onset. The continuous curve plots the relation, $A(t)/A_{m0} = \{1 - \exp[-\theta(t - t_0)]\}[a_1 + (1 - a_1)\exp(-t/\tau)]$, where $\theta = 6.29 \times 10^{-5}$ ms$^{-2}$, $t_0 = 3.1$ ms, $a_1 = 0.53$ and $\tau = 235$ ms. B, experimental data re-plotted from A. Dashed curve 1 plots the relation, $A(t)/A_{m0} = b_1[1 - \exp(-b_2t)]$, where $b_1$ and $b_2$ are free parameters equal to 0.61 and 8.86 × 10$^{-3}$ ms$^{-1}$, respectively. Continuous curve 2 plots the relation $A(t)/A_{m0} = c_1 - [c_3\exp(-c_4t)\cos(c_5t)]$, where $c_1 = 0.572$, $c_2 = 0.589$, $c_3 = 5.22 \times 10^{-3}$ ms$^{-1}$ and $c_4 = 8.76 \times 10^{-3}$ ms$^{-1}$. C, normalized derived amplitudes determined with backgrounds of 0.40 sc cd m$^{-2}$ (triangles) and 2.6 sc cd m$^{-2}$ (circles). Steady-state data are shown by filled symbols at the right. Results with the 0.40 sc cd m$^{-2}$ background were obtained in two experiments (2–3 determinations represented by each data point); those with the 2.6 sc cd m$^{-2}$ background were obtained in three experiments (2–5 determinations represented by each data point). The illustrated curve accompanying the results obtained with the 0.40 sc cd m$^{-2}$ background plots the relation, $A(t)/A_{m0} = d_1[1 - \exp(-d_2t)]$, where $d_1 = 0.253$ and $d_2 = 5.27 \times 10^{-3}$ ms$^{-1}$. 

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DISCUSSION

In paired-flash and step-plus-probe ERG experiments we have examined the sensitivity and kinetics of flash responses in steady background light, as well as responses to stepped backgrounds of defined strengths. This study extends a previous investigation of paired-flash ERGs in mice under dark-adapted conditions (Hetling & Pepperberg, 1999). Evidence presented in that earlier study argued against a substantial cone contribution to paired-flash-derived responses obtained in darkness. Several considerations furthermore imply at most a small contribution of cone-dependent processes to the effects of background light described here. The first of these concerns the strength of background illumination required to diminish the a-wave response of mouse cones from that measured under dark-adapted conditions. Lyubarsky et al. (1999) found that backgrounds producing 3000–6000 photoisomerizations rod\(^{-1}\) s\(^{-1}\) largely preserve the cone-mediated ERG a-wave, while suppressing almost entirely the a-wave response of the rod photoreceptors (also cf. Schneeweis & Schnapf, 1999). Taking 1 sc cd m\(^{-2}\) as equivalent to 100 photoisomerizations rod\(^{-1}\) s\(^{-1}\) in the mouse eye (Hetling & Pepperberg, 1999), all but the highest backgrounds used in the present study were well below the cone-decrementing backgrounds determined by Lyubarsky et al. (1999), yet significant desensitization was observed. For example, a background of 1.2 sc cd m\(^{-2}\) (representing an estimated 120 photoisomerizations rod\(^{-1}\) s\(^{-1}\)) produced substantial reductions in the baseline level of circulating current and in both the size and duration of the derived response to a test flash (e.g. Figs 1, 3, 7 and 8).

A second point concerns the results obtained with the background of 50 sc cd m\(^{-2}\), representing an estimated 5000 photoisomerizations rod\(^{-1}\) s\(^{-1}\) (i.e. a strength within the range found by Lyubarsky et al. (1999) to suppress the rod response and isolate the cone a-wave). As shown by Fig. 6A, a bright probe flash presented on this background elicited an a-wave response of about 30 µV, a value similar to that previously measured and theoretically predicted for cone a-wave amplitudes (Lyubarsky et al. 1999). This apparent cone-mediated response is small, representing at peak only ~12% of the peak amplitude of the dark-adapted probe-alone response. Third, ERG and in vitro data indicate that subsaturating flash responses of mammalian cones exhibit times to peak of ~60 ms and times to half-recovery of less than 100 ms (Schnapf et al. 1990; Hood et al. 1996; Schneeweis & Schnapf, 1995, 1999; Lyubarsky et al. 1999; Friedburg et al. 1999), i.e. kinetics considerably faster than those of derived responses obtained in the present experiments with moderate backgrounds (Figs 3 and 4). These considerations, as well as the correspondence of the present in vivo data with properties of mammalian rod light adaptation observed in vitro (see below), imply that the ERG-derived responses investigated here are largely rod mediated.

FIGURE 8. Normalized amplitudes of the derived response to a test flash

The test flash was presented in darkness (left-hand pair of bars), at 50 ms and at 700 ms after background onset (centre and right-hand pairs of bars, respectively; B50T and B700T symbolize the 50 or 700 ms interval between background onset and test flash presentation). The test flash strength was in all cases 0.98 sc cd s m\(^{-2}\); the background strength was 1.2 sc cd m\(^{-2}\). Under each condition, paired-flash trials were conducted in which the interval between the test and probe flashes (test-probe interval) was 20 ms (open bars) or 250 ms (shaded bars). The response to the background plus test flash (or, under dark-adapted conditions, to the test flash alone) was computationally subtracted from the raw response obtained with probe flash presentation. The resulting probe response, and the background-corrected response to the probe flash delivered alone, were analysed for amplitude at 6 ms after probe flash presentation to yield the normalized amplitude of the derived response to the test flash at 26 or 250 ms after test flash presentation. The illustrated data indicate the means ± S.D. of results obtained in 3 experiments (2–6 determinations for a given histogram bar). For the case of a test-probe interval of 250 ms, the results obtained with test flash presentation 700 ms after background onset differ significantly (*) from those obtained with test flash presentation in darkness and at 50 ms after background onset (P < 0.005 and P < 0.05, respectively).
mammals including rat, that the halving of flash sensitivity occurs with backgrounds in the range of 31–42 photoisomerizations rod\(^{-1}\) s\(^{-1}\). Somewhat higher values for this criterion background strength (about 100–120 photoisomerizations rod\(^{-1}\) s\(^{-1}\)) have been reported for primate rods (Baylov et al. 1984; Kraft et al. 1993). The present results provide evidence for a comparable desensitization of mouse rods in vivo under similar conditions of background illumination. For example, Fig. 3A indicates an approximate match, at peak, of the normalized derived response obtained in darkness with a 0.12 sc cd m\(^{-2}\) test flash (dashed curve) and that obtained with a 0.30 sc cd m\(^{-2}\) test flash in weak background light (0.63 sc cd m\(^{-2}\)) estimated to produce about 63 photoisomerizations rod\(^{-1}\) s\(^{-1}\). Furthermore, as shown in Fig. 4B (open circles), the normalized derived response amplitude determined with a fixed test flash (0.11 sc cd m\(^{-2}\)) and at a fixed near-peak time (t = 86 ms) is approximately halved by a 1.2 sc cd m\(^{-2}\) background, i.e. a background producing about 120 photoisomerizations rod\(^{-1}\) s\(^{-1}\).

A further aspect of light adaptation evident from in vitro data from both mammals and amphibians concerns the dependence of the steady-state maintained response on background strength; the in vitro data indicate a rise more gradual than that predicted by a saturating exponential function (Fain et al. 1989; Tamura et al. 1989; Nakatani et al. 1991; Kraft et al. 1993; Xu et al. 1997). Figure 1 shows a similarly gradual rise of the maintained derived response with increasing background strength (also cf. Lyubarsky et al. 1999). This behaviour is consistent with representation of the measured (i.e. macroscopic) response as the summation of elemental responses, and the shortening in background light of an effective integration time associated with this elemental response (Table 1; and see below).

A background-induced reduction of integration time is evident also in the shortening of \(\tau_{ref}\), the referenced integration time obtained from determinations of the full time course of the derived response in background light (Table 1, fifth row). A decrease in integration time is furthermore consistent with the observation that desensitization of the rod derived response is relatively pronounced during the response’s falling, i.e. recovery, phase (Figs 3 and 4). For example, Fig. 4A shows that for a given background strength, desensitization as measured at 256 ms after presentation of a 0.98 sc cd m\(^{-2}\) test flash is greater than that determined at the near-peak time of 86 ms. This effect of background light on response kinetics resembles that previously described for rod photocurrent responses in vitro (Tamura et al. 1989; Nakatani et al. 1991; Kraft et al. 1993) and for paired-flash derived responses of human rods in vivo (Pepperberg et al. 1997; Pepperberg & Birch, 1999).

**Time course of approach to steady-state light adaptation**

The present step-plus-probe results show that within the range of investigated backgrounds (0.40 to 2.6 sc cd m\(^{-2}\)), development of the response to a step of light is essentially complete within 1 s after background onset. With weak background light (0.40 sc cd m\(^{-2}\)), the normalized step response \(A(t)/A_{ref}\) rises in monotonic exponential fashion (Fig. 7C). However, with a roughly half-saturating background (1.2 sc cd m\(^{-2}\)), the data suggest the occurrence of a peak followed by a subsequent relaxation toward steady-state level (Fig. 7A and B). Evidence of a peak followed by relaxation to a steady-state plateau has similarly been observed in photocurrent studies of both mammalian and amphibian rods in vitro, with step onset of a background of moderate strength (Tamura et al. 1989; Fain et al. 1989; Nakatani et al. 1991; Xu et al. 1997). A further point of similarity between the present results and previous in vitro findings concerns the photoisomerizing strength of a half-saturating background. Equating 1 sc cd m\(^{-2}\) with 100 photoisomerizations rod\(^{-1}\) s\(^{-1}\), the background strength found here to be roughly half-saturating (1.2 sc cd m\(^{-2}\)) corresponds with \(~120\) photoisomerizations rod\(^{-1}\) s\(^{-1}\). This value is remarkably close to that derived from the mouse rod photocurrent data of Xu et al. (1997), who report a half-saturating background strength of about 560 photons \(\mu m^{-2} s^{-1}\) and a rod collecting area of \(0.22 \mu m^2\) (560 \(\times\) 0.22 = 123 photoisomerizations rod\(^{-1}\) s\(^{-1}\)).

In the step-plus-paired-flash experiments of Fig. 8 we examined rod incremental sensitivity during the transition from dark- to light-adapted state produced by an approximately half-saturating background. With the use of a 20 ms interval between the test and probe flashes, i.e. with analysis of the test flash response at 26 ms after its initiation, normalized derived amplitudes determined with test flash presentation at 50 and 700 ms after background onset differed relatively little from those obtained under dark-adapted conditions. By contrast, with use of a 250 ms interval between the test and probe flashes (analysis of the test flash response at 256 ms), the derived response amplitude determined with test flash presentation 700 ms after background onset was significantly smaller than those determined in darkness or with test flash presentation at 50 ms after onset of the background. We interpret this result as evidence for the substantial development of light adaptation over an interval spanning 50–700 ms after onset of the background under investigation.

The apparent peak and subsequent relaxation of the step response (Fig. 7), as well as the Fig. 8 results, can be explained in terms of background-induced changes in the elemental response \(e(t)\) inferred from the experimental data. As determined under steady-state conditions of...
background illumination, both $e(t)$ and the derived response based on this function exhibit referenced integration times ($\tau_{int}$) considerably smaller than those determined under dark-adapted conditions (Fig. 5 and Table 1). It is reasonable to hypothesize that with increasing time after background onset, light-adaptation mechanisms operating within the rod progressively reduce the peak size and duration of the elemental response, with resulting reductions in the macroscopic summed response. The apparent peak at about 200–300 ms in the step response to a moderate background, the subsequent decline in this response to plateau level over a period of several hundred milliseconds, and the shortening of the test flash response within 1 s after background onset (Figs 7 and 8) implicate a time scale of ~0.1–1 s as that required for essentially full development of the light-adaptation process initiated by onset of a moderate background.

**Dependence of desensitization on background strength**

A noteworthy feature of the present data is the similarity observed in the extent of fractional, or normalized, desensitization produced by relatively strong backgrounds of differing strength. That is, average values of the normalized amplitude $A(256)/A_{max}$ obtained with a 2.6 sc cd s m$^{-2}$ test flash exhibited only a modest decrease as background strength was increased from 1.2 to 5.8 sc cd m$^{-2}$ (Fig. 4A). Furthermore, full normalized derived responses to a 0.98 sc cd s m$^{-2}$ test flash with the 1.2 and 2.5 sc cd m$^{-2}$ backgrounds were similar (Fig. 3). In addition, determinations of the integration time $\tau_{int}$ for both the derived response and for the inferred elemental response $e(t)$ at fixed test flash strength indicated a marked decrease from the dark-adapted values by the weakest of the investigated backgrounds (0.63 sc cd m$^{-2}$), but relatively little change in $\tau_{int}$ with increases in background strength from 0.63 to 2.5 sc cd m$^{-2}$ (Table 1). Paired-flash ERG results previously obtained for the derived response of human rods similarly imply that the progressive decrease in the normalized sensitivity produced by increasing background strength is largely complete with backgrounds that roughly halve the maximal excursion of the response (Pepperberg et al. 1997; their Fig. 7B). Light adaptation is thought to involve the modulation of multiple ‘upstream’ transduction reactions, i.e. reactions that precede closure of the rod’s cGMP-gated channels (e.g. Matthews et al. 1988; Nakatani & Yau, 1988; Tamura et al. 1991; Lagnado & Baylor, 1994; Gray-Keller & Detwiler, 1994, 1996; Younger et al. 1996; McCarthy et al. 1996). There is no doubt that increasing background intensity progressively diminishes absolute rod sensitivity measured, for example, as the absolute peak amplitude of the incremental response to a fixed test flash. However, the similarity observed here among normalized measures of the rod flash response with backgrounds of 1.2 sc cd m$^{-2}$ and higher suggests that the processes responsible for upstream modulation may be brought to essentially full activity by backgrounds that are roughly half-saturating. On this view, further decreases in absolute sensitivity by brighter backgrounds largely reflect response compression, i.e. the approach to closure of all of the cGMP-gated channels and resulting saturation of the response, rather than further modulation of the upstream reactions.


