Novel Form of Adaptation in Mouse Retinal Rods Speeds Recovery of Phototransduction

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ABSTRACT Photoreceptors of the retina adapt to ambient light in a manner that allows them to detect changes in illumination over an enormous range of intensities. We have discovered a novel form of adaptation in mouse rods that persists long after the light has been extinguished and the rod's circulating dark current has returned. Electrophysiological recordings from individual rods showed that the time that a bright flash response remained in saturation was significantly shorter if the rod had been previously exposed to bright light. This persistent adaptation did not decrease the rate of rise of the response and therefore cannot be attributed to a decrease in the gain of transduction. Instead, this adaptation was accompanied by a marked speeding of the recovery of the response, suggesting that the step that rate-limits recovery had been accelerated. Experiments on knockout rods in which the identity of the rate-limiting step is known suggest that this adaptive acceleration results from a speeding of G protein/effector deactivation.

KEY WORDS: photoreceptors • GTP-binding proteins • kinetics • phototransduction • knock-out mice

INTRODUCTION

Nearly all sensory neurons adapt to prolonged stimuli, usually by decreasing the amplitude of the receptor potential and thereby changing the amount of neurotransmitter released onto the second-order neuron. Retinal photoreceptors signal the presence of light across a wide range of light intensities by greatly reducing their sensitivity and shortening the time course of their responses as the intensity of the background light increases. Both of these processes help to attenuate the response to continuous illumination.

The biochemical cascade that underlies the response to light is initiated by photoexcited rhodopsin, which drives amplification by activating many copies of the heterotrimeric G protein, transducin (G_T). G_T in turn activates the effector, cGMP phosphodiesterase (PDE) for as long as G_T remains in its active, GTP-bound state. The decrease in cGMP produced by the light-activated PDE activity causes cGMP-gated cation channels in the plasma membrane to close, leading to a decrease in inward current and a hyperpolarization of the cell. Recovery of the light response requires deactivation of the cascade components, including phosphorylation of, and arrestin binding to, activated rhodopsin (Rh*) as well as hydrolysis of GTP by G_T. At the same time, guanylate cyclase synthesizes more cGMP, and the return of the cGMP concentration to its dark level restores the inward dark current by reopening the channels.

These primary cascade reactions are altered during light adaptation in a manner that causes decreased sensitivity and speeded response kinetics. These two hallmarks of light adaptation arise from the concerted actions of calcium on many cascade components (for review see Fain et al., 2001). The fall in intracellular calcium during steady background light speeds rhodopsin deactivation, increases the rate of cGMP synthesis by guanylate cyclase, and decreases the sensitivity ($K_{1/2}$) of the channels for cGMP. In addition, the increased steady-state PDE activity that results from the presence of background light contributes to the reduction in sensitivity and a speeding of response kinetics as a result of increased cGMP turnover (Hodgkin and Nunn, 1988; Nikonov et al., 2000). All of these calcium-dependent and -independent mechanisms require the presence of light, and seem to disappear quickly after PDE activity and calcium have returned to their dark levels. It is unclear whether other processes can regulate transduction on a longer time scale.

We have identified a novel form of light adaptation in intact mouse rods. It persists long after the adapting light has been extinguished, and is not associated with a change in cascade gain. Rather, our data indicate that it arises from a long-lasting acceleration of response recovery.

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Abbreviation used in this paper: PDE, phosphodiesterase.

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MATERIALS AND METHODS

Suction Electrode Recording

Mice were cared for and handled following an approved protocol from the Animal Care and Use Committee of the University of California, Davis and in compliance with NIH guidelines for the care and use of experimental animals. Wild-type mice in this study were either adult C57BL/6 mice (Charles River) or a Sv129/C57BL/6 outbred strain of the same genetic background as the RGS9 knockout mice. Prior to an experiment, animals were dark adapted overnight (12 h, minimum). Under infrared light, the animal was anesthetized and sacrificed, and the retinas dissected and stored on ice in L-15 solution with 10 mM glucose and 0.1 mg/ml bovine serum albumin (Sigma). For recording, the retina was chopped with a razor blade and placed in a recording chamber, which was perfused with a solution containing 112.5 mM NaCl, 3.6 mM KCl, 2.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM HEPES, 20 mM $NaHCO_3$, 3 mM Na_2 succinate, and 10 mM glucose, at 35–37°C. Prior to recording, the pH of the solution at 36°C was adjusted to 7.4 with NaOH and the osmolarity adjusted to 290 milliosmolar. Individual cells were visualized under infrared light, using a CCD camera (Stanford Photonics). Capillary glass (WPI) was heated, pulled, and polished to form suction electrodes with tip diameters of ~1–2 μm, and filled with a solution containing 140 mM NaCl, 3.6 mM KCl, 2.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 3 mM HEPES, and 10 mM glucose. The solution was warmed to 37°C, and the pH brought to 7.4 with NaOH. Individual rods were drawn into the electrode using gentle suction in order to record the inward current of the outer segment. The recording electrode and the bath solution were connected to calomel half cells by agar bridges, and the voltage of the bath solution was maintained at 0 mV by an active bath-clamp circuit. The membrane currents were recorded, amplified by a current-to-voltage converter (Axopatch 1B; Axon Instruments, Inc.) and low-pass filtered (8-pole Bessel; Frequency Devices) using 20 Hz corner frequency. Data was digitized at 200 Hz using IGOR-National Instruments acquisition software (IgorPro for NIDAQ for Windows; Wavemetrics) and analyzed off-line.

Rods were presented with 10-ms flashes of 500-nm light or steady adapting light of 520 nm. The intensity of the light was measured at each wavelength after each experiment using a silicon photodiode (United Detector Technology) and the intensity was controlled using calibrated neutral density filters.

The time that a bright flash response remained in saturation was defined as the time interval between the midpoint of the flash and the time at which the response recovered by 10%.

Adaptation Protocol

Cells were exposed for indicated times to steady light that was just bright enough to close all of the channels in the outer segment. The adapting light intensities (in photons/μm^2s) ranged from 2,896–9,672 for wild-type rods and from 220–517 for RGS9 knockout rods. Cells were considered for analysis only if the dark current values after light exposure were within 90% of initial dark current values. For those experiments in which we examined the subsaturating responses before and after light adaptation, the traces used for comparison were averages of five responses. After the adapting light, all five subsaturating responses were obtained in less than 1 min of the return of the dark current (mean ± SEM across experiments: 46 ± 9 s, $n = 5$). The duration of these subsaturating responses was measured as the time integral of the average flash response, divided by the peak amplitude.

Determination of the Dominant Time Constant of Recovery after Adapting Light

Allowances for the time-dependent changes in saturation times were required to determine accurately the dominant time constant of recovery (τ_D) following light exposure. For wild-type rods, the saturation time (T_{sat}) for a bright flash response as a function of time (t) after the adapting light was turned off was described by:

$$T_{sat}(t) = T^o_{sat} + Ae^{-t/\tau_{ADAPT}},$$

where T^o_{sat} is the initial/final time in saturation of the response, τ_{ADAPT} is the time constant for the return to the dark-adapted state, and A is a constant. For all cells, t = 0 was the time at which the current had returned to its initial (dark) value. Using measured $T_{sat}(t)$, t, and T^o_{sat} values, and assuming τ_{ADAPT} = 80 s (see RESULTS), we solved for A in each cell. We then used A to calculate T_{sat} at a specific time (t). In this manner, we normalized saturation times of consecutive responses to the same instant in time. In wild-type rods, the dark current recovered within 1–3 s after the adapting light was turned off; in RGS9 knockout rods, tens of seconds were required for the dark current to recover. Because prolonged saturation times could affect the adaptation state of the cell, only flash strengths producing responses which were in saturation for less than 30 s were used. The shortening of τ_D occurred in all cases, regardless of the order in which the flashes were given. Throughout, error bars indicate SEM.

Estimation of Percent Bleach

The effective collecting area (A_c) of a mouse rod was calculated as (Eq. 14 in Baylor et al., 1979b):

$$A_C = \frac{\pi d^2 l}{4} Q_{isom} f 2.303 \alpha,$$

where d is the diameter of the outer segment, l is its length, Q_{isom} is the quantum efficiency of isomerization (0.67; Dartnall 1972), f is the factor (0.5) that allows for the imperfect absorption of unpolarized light perpendicular to the longitudinal axis of the outer segment, and α is the specific axial pigment density (0.016 μm^{-1}; Harosi, 1975). For a mouse rod with d = 1.25 μm and l = 15 μm, A_c = 0.23 μm^2. This is very similar to experimental estimates obtained in the analysis of single photon responses (unpublished data).

The mean number of Rh*/flash was calculated by multiplying the flash strength (photons/μm^2) by A_c. The percent of rhodopsin bleached by the adapting light (% *bleach*) was calculated as:

$$\%\ bleach = \frac{I t_{dur} A_C}{Rh_{tot}} 100,$$

where I is the adapting light intensity (in photons/μm^2 s), t_{dur} is the duration of the adapting light (usually 180 s), and Rh_{tot} is the number of rhodopsin molecules in a mouse rod, which we assumed conservatively to be 10^7.

RESULTS

Characterization of a Novel Form of Adaptation

To study long-term adaptive changes in rods, we used suction electrodes (Baylor et al., 1979a) to record the responses to bright test flashes before and after exposure to a bright adapting light. These test flashes were

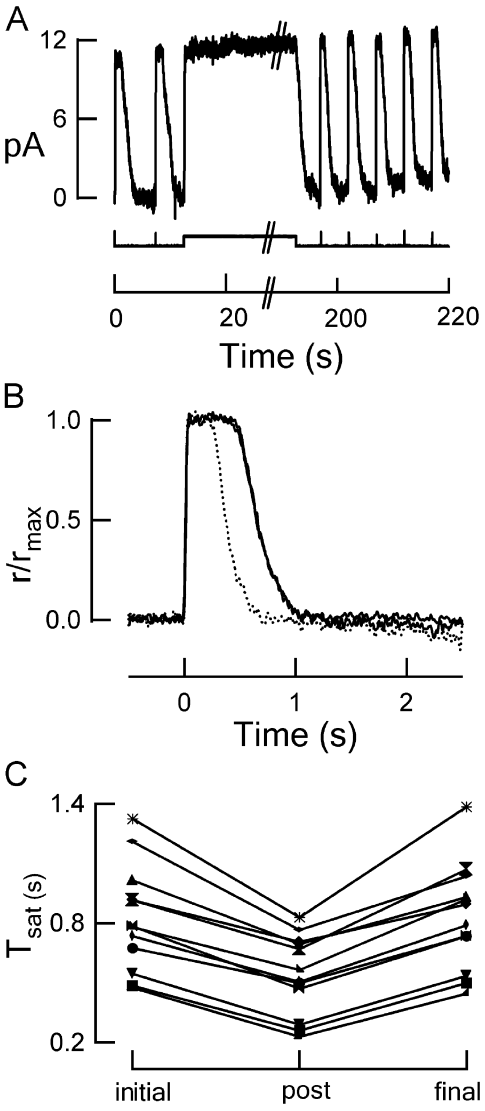

FIGURE 1. Previous light exposure causes shortening of saturating responses in darkness. (A) Suction electrode recording of a wild-type mouse rod. Test flashes (ticks in light monitor, lower trace; 2,180 photons/μm^2) were delivered to the rod before and after 3 min of steady, saturating light (5,750 photons/μm^2 s). (B) Responses from a representative cell before (solid trace), immediately after (dashed trace), and several minutes after (solid trace) the adapting light (7,055 photons/μm^2 s). The initial and final traces superimpose. Dark currents of this cell (in pA) for the initial, post, and final traces were 12.8, 11.7, and 13.0, respectively. Flash strength was 2,076 photons/μm^2. (C) Time spent in saturation (T_{sat}) for individual cells before (initial), immediately after (post) and several minutes after (final) three minutes light exposure. Intensities of background light ranged from 2,896–9,672 photons/μm^2 s, which just saturated the cells.

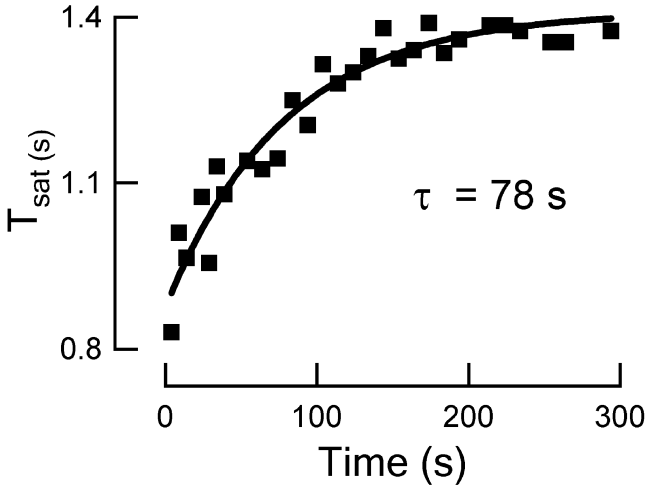

FIGURE 2. Time in saturation of a bright flash response (2,180 photons/μm^2) changed over time following the adapting light. Time 0 represents the time the current returned to baseline following adapting light offset. Adapting light (5,750 photons/μm^2 s) was applied for 3 min.

sufficiently bright to close all of the channels on the rod's plasma membrane, and therefore generated responses that saturated. After initial test flashes, wild-type rods were exposed to steady, saturating light ("adapting light") for 3 min (~2.4% cumulative bleach). When the adapting light was turned off, the inward current rapidly recovered, returning to the original dark level within only a few seconds, at which time additional test flashes were given (Fig. 1 A). A test flash delivered as soon as the dark current had returned evoked a response that remained in saturation for a much shorter time (0.64 ± 0.03 of the initial saturation time; mean ± SEM; n = 13 rods). This shortening was observed in all cells examined (Fig. 1 C) and was not associated with any significant change in the dark current (1.04 ± 0.03 of the initial dark current; n = 13), suggesting that the shortening did not arise from residual PDE activity or a persistent change in internal calcium concentration. The same flash delivered several minutes later evoked a response that was indistinguishable from those recorded before the adapting light, indicating that the effect was reversible over time (Fig. 1 B).

To determine the time course with which this adaptive effect decayed, we delivered the test flashes repeatedly following the adapting light and measured the saturation times of the evoked responses. Representative results from one such experiment are plotted in Fig. 2. The return to the dark-adapted state could be fit by a single exponential function with an 80-s time constant (τ_{ADAPT} = 81 ± 15 s; n = 8).

To characterize the light dependence of this form of adaptation, we varied the duration and intensity of the adapting light and observed the degree of shortening of the responses to saturating test flashes. We found that exposure to saturating steady light for 1 min (~0.8% cumulative bleach) also resulted in response shortening of the same magnitude (0.69 ± 0.03 of original, n = 4) as

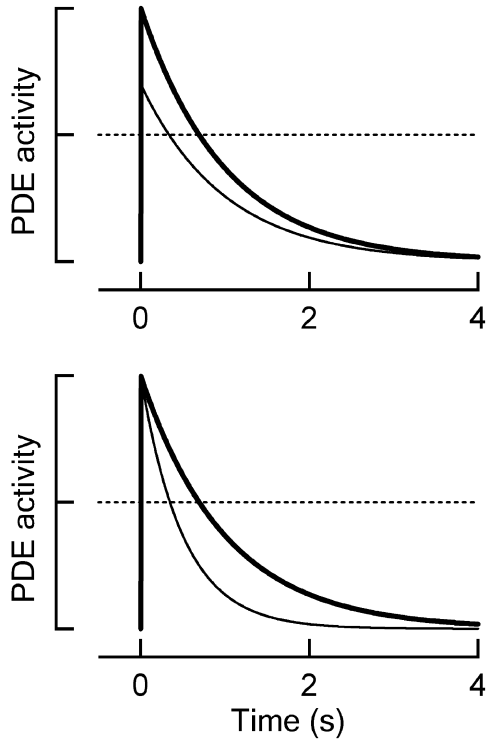

FIGURE 3. Two mechanisms could account for response shortening. (Top) A decrease in transduction gain would reduce light-activated PDE activity and produce a response that remained in saturation for a shorter time than the initial response (thick trace). The maximal response amplitude is attained when all of the channels in the outer segment are closed (represented by dashed line), and beyond this point, differences in PDE activity result in different saturation times. (Bottom) In the second possible mechanism, the amplitude of the light-activated PDE activity is unaffected by adaptation (thin trace), but recovers faster, resulting in a response with a shorter saturation time.

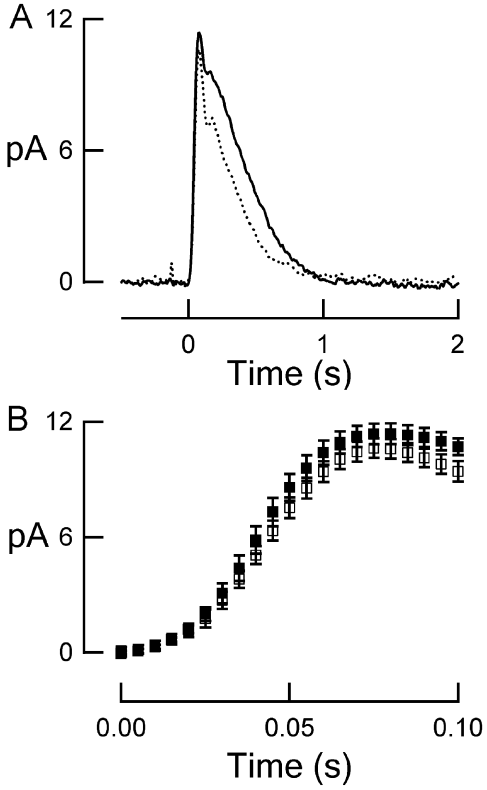

FIGURE 4. Adaptation produced no change in the gain of transuction. (A) Average responses (each to five flashes) of a representative cell before (initial, solid trace) and shortly after (post, dashed trace) the adapting light. (B) Expanded time scale, showing rising phases of responses in A. Closed squares, initial; open squares, post. Flash strength was 56.0 photons/μm^2. The adapting light (7,016 photons/μm^2 s) was applied for 3 min. Error bars reflect SEM.

the 3-min exposures (above). Steady illumination for nine minutes (~7.2% cumulative bleach) did not cause additional shortening (0.70 + 0.04 of original, $n = 3$). The adaptation evoked by both 1- and 9-min protocols also decayed with an 80-s time constant ($n = 4$, 1-min protocols; $n = 2$, 9-min protocols), suggesting that the mechanism of action was the same. In contrast, exposure to steady saturating light for shorter times (10–30 s), or to subsaturating steady light (0.09–0.16% cumulative bleach) shortened the time in saturation to a lesser extent and less reliably. We therefore limited our characterization to the 3-min adaptation protocol.

The shortened saturation time of the responses after steady light could arise from two distinct categories of mechanisms (Fig. 3). First, the gain of transduction could be reduced (Fig. 3, top). For example, a reduction in the number of available rhodopsin molecules, a decrease in G_T concentration, or a reduction in light-activated PDE activity would manifest itself in this manner. A second category of mechanism that could produce this effect is a speeding of response recovery (Fig. 3, bottom). In mouse rods, flash responses normally recover along a time course well-fitted by a single exponential function (τ ~0.2 s) that reflects the time constant of the slowest, or rate-limiting step in deactivation of the phototransduction cascade (Lyubarsky and Pugh, 1996; Chen et al., 2000). Speeding this rate-limiting step would in theory also shorten the saturation time of a bright flash response. To differentiate between these two categories of mechanisms we assessed the rod's gain and recovery kinetics before and after exposure to the adapting light.

Decrease in Gain Is not Responsible for the Response Shortening

One way to assess the gain of the cascade is by measuring the flash sensitivity of the cell. Classically, flash sensitivity during adaptation is defined as the mean dim flash response amplitude normalized by the flash strength. Because this requires averaging responses to a large number of dim flashes delivered over time (more than 100 s), any change in the dim flash sensitivity

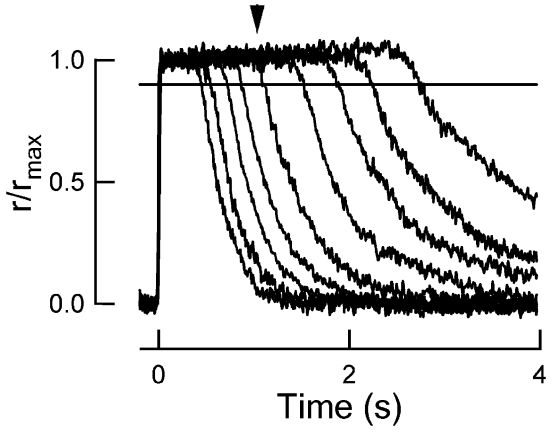

FIGURE 5. Response saturation times increased with increasing flash strengths. A family of saturating responses from a representative wild-type cell to flashes ranging in strength from 519 photons/μm^2 to 74,968 photons/μm^2, in roughly twofold increments. The form of the response is invariant across flash strengths, as described previously (Pepperberg et al., 1992). At flash strengths between 5,000 and 10,000 photons/μm^2, the form of the response changed (arrow; in this cell 5,630 photons/μm^2). This is also the flash strength at which the Tsat relation typically deviates from linearity (see text). The time in saturation was measured from the midpoint of the time of the flash and the time at which the response had recovered to 90% of its maximal amplitude. Traces represent the average of two responses to a particular flash strength. Dark current for this cell was 17 pA.

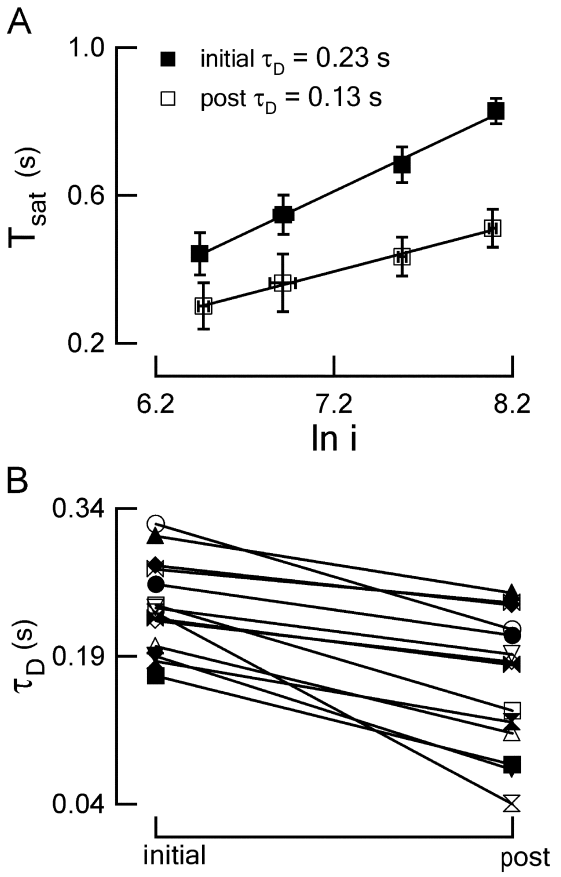

FIGURE 6. Adaptation shortens the dominant time constant of recovery. (A) Average dominant time constant of recovery (τ_D) of 14 rods, before (initial) and immediately after (post) several minutes of the adapting light. Each point is the average of data points from 8–14 cells. Error bars represent SEM. (B) Average dominant time constant of recovery (τ_D) from individual wild-type rods, before (initial) and immediately after (post) adapting light. Adapting light intensities ranged from 2,896–9,672 photons/μm^2 s, which was sufficiently bright to just saturate the cell.

would fade with the decay of adaptation (τ_{ADAPT} ~80 s, see above). We therefore assessed sensitivity by a faster, though less traditional, method. We compared the average response to moderately bright subsaturating flashes before and after the steady light exposure. Although at this flash strength (~13 Rh*/flash on average) there are still small fluctuations in response amplitude that arise from fluctuations in the number of photoisomerizations per flash, the number of trials required to obtain a reliable average is far less and could be obtained within 50 s of the offset of the adapting light, when the adaptation should still have been robust (see MATERIALS AND METHODS). On average, the amplitudes of responses to these flashes did not change significantly after light exposure (0.95 ± 0.03 of the initial amplitude; $n = 5$; Fig. 4 A). Thus, there appeared no measurable change in flash sensitivity, suggesting that mechanisms such as reduction in quantum catch or increased steady-state PDE activity are not responsible for this form of adaptation.

Flash sensitivity, as measured by the peak amplitude of the flash response, reflects primarily the gain of transduction, but strictly speaking also is affected by the speed with which deactivation proceeds (e.g., Sagoo and Lagnado, 1997). Therefore, as a way to isolate gain, we compared the rising phases of these subsaturating responses before and after bright light exposure. If the shortening of the saturation time arose from a decrease in the gain, or amplification, of the cascade, we would expect the rising phases of the early part of the response to be different for flashes given before and immediately after the background illumination. The rising phases of the responses before and after the adapting light were not significantly different (Fig. 4 B; $n = 5$), supporting the idea that steady light exposure did not reduce the gain of transduction, such as might occur if G_T had translocated to the inner segment.

Decreased Time in Saturation Results from a Speeding of Recovery

The recovery phase of a rod's response can be well-fitted by a falling single exponential function, suggesting that a first-order process underlies the time course of recovery. The time constant of this exponential recov-

ery (~0.2 s) is invariant across a wide range of flash strengths (~1–1,000 Rh*/flash; Chen et al., 2000; Calvert et al., 2001; Krispel et al., 2003). A convenient measure of the rate-limiting, or dominant, time constant of recovery (τ_D) independent of calcium feedback mechanisms can be obtained from bright flash responses. As expected, bright flashes of increasing strength produce responses that remain saturated for increasing times (Fig. 5). In mouse rods, for flash strengths that produce on average up to ~1,000 Rh*/flash or 1 Rh*/disc face ($\ln i < 8.5$), the form of the saturated response is invariant and the dependence of saturation times on the log of the flash strength (what we will refer to as the "Tsat relation") is linear with a slope of ~0.2 (Chen et al., 2000; Calvert et al., 2001; Mendez et al., 2001; Krispel et al., 2003; Fig. 6). At higher flash strengths, the time in saturation gets progressively longer, and the relation exhibits a second, steeper component, presumably due to depletion or saturation of a component required for recovery (Lyubarsky and Pugh, 1996). If the mechanism that underlies the adaptive effect arises from a speeding of this rate-limiting step (Fig. 3, bottom), then τ_D should be shorter following the adapting light.

To measure τ_D accurately, it is necessary to measure the responses of a rod to at least three saturating flash strengths. Because the adaptation slowly fades over time (τ_{ADAPT} = 80 s; see above), consecutive responses will display the adaptation to lesser extents, interfering with the determination of τ_D. Thus, we used τ_{ADAPT} to normalize the time in saturation of each flash response relative to the same instant in time (see MATERIALS AND METHODS). This analysis revealed a robust speeding of the recovery kinetics following the adapting light (Fig. 6). On average, τ_D shortened to 0.66 ± 0.04 of the dark value (n = 14), and on all cells tested, τ_D returned to its original value at the end of the experiment (1.04 ± 0.06; n = 7).

The identity of the rate-limiting step underlying recovery of the light response and τ_D has not been known, but must be either the deactivation of rhodopsin or deactivation of G_T/PDE (Lyubarsky et al., 1996; Nikonov et al., 1998). To determine which of these steps is speeded during adaptation, we used a knockout mouse line in which G_T/PDE deactivation is known to be rate limiting (RGS9 knockout; Chen et al., 2000).

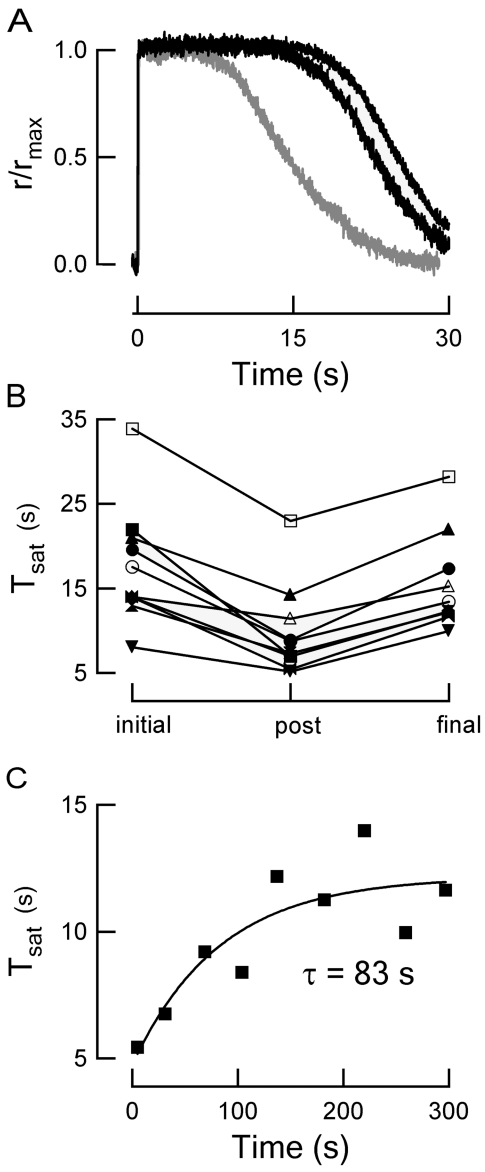

FIGURE 7. Long-lasting adaptation in RGS9 knockout rods. (A) Responses from a representative RGS9 knockout rod before (black), immediately after (gray), and several minutes after (black) the adapting light. In this case, the final trace was slightly faster than the initial trace. Dark current (in pA) was 11.7, 12.7, and 11.3, respectively. Flash strength was 647.6 photons/μm^2. Adapting light (286.4 photons/μm^2 s) was applied for 3 min. (B) Time spent in saturation for individual cells before (initial), immediately after (post), and several minutes after (final) a 3-min light exposure. Intensities ranged from 286 to 517 photons/μm^2 s, which was sufficient to just saturate the cell. (C) Time spent in saturation of each response as a function of the time at which the flash was given. Time 0 is the time at which the current returned to baseline following background light removal.

Acceleration of Recovery Persists in RGS9 Knockout Rods

The RGS9-1–Gβ5 L complex catalyzes the hydrolysis of GTP to GDP by G_T (He et al., 1998; Makino et al., 1999). Physiological studies have shown that rods lacking RGS9-1–Gβ5 L recover much more slowly than wild-type rods, and have a τ_D of ~10 s (Chen et al., 2000; Krispel et al., 2003). Biochemical studies have likewise shown that retinal homogenates from mice lacking these proteins show slowed rates of GTP hydrolysis by G_T, leading to the conclusion that the rate-limiting step of response recovery in these knockout rods is G_T/PDE deactivation (Chen et al., 2000). Thus, RGS9 knockout rods are a useful tool to test whether G_T/

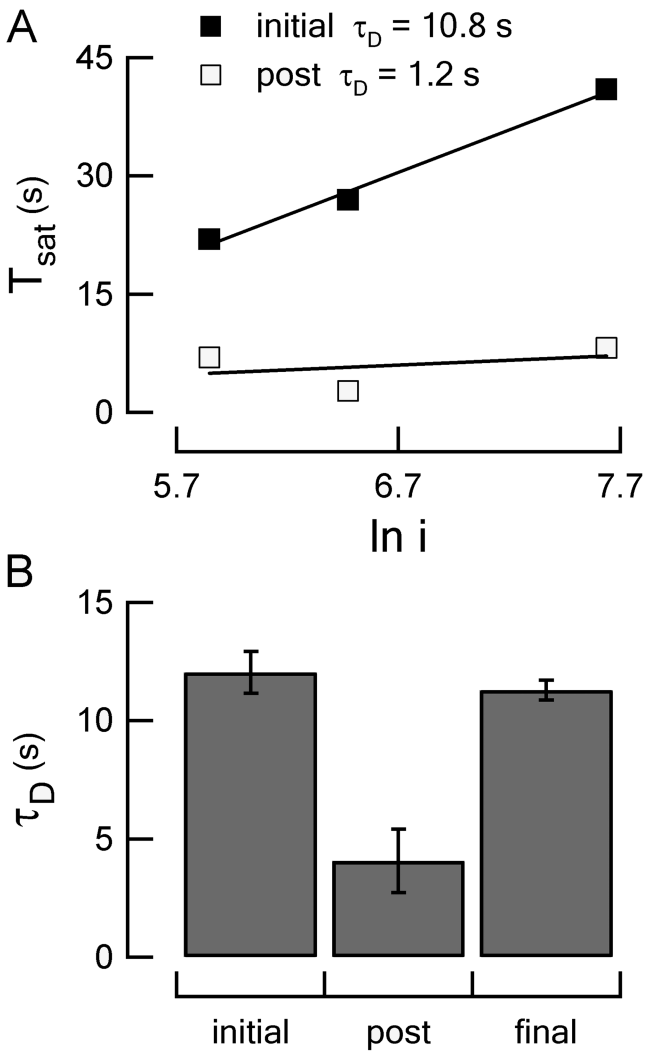

FIGURE 8. Adaptation speeds the rate-limiting recovery step in RGS9 knockout rods. (A) Dominant time constant of recovery (τ_D) of a representative RGS9 knockout rod before (initial, closed squares) and immediately after (post, open squares) several minutes after background light. Adapting light intensity was 286 photons/μm^2 s and was applied for 3 min. (B) Average τ_D values before (initial; $n = 4$), immediately after (post; $n = 4$), and several minutes after (final; $n = 3$). Error bars represent SEM.

PDE deactivation might be speeded by our adaptation paradigm.

Indeed, responses of RGS9 knockout rods remained in saturation for much shorter times after the adapting light (Fig. 7; 0.55 ± 0.05 of the initial saturation time; $n = 10$). As with the wild-type rods, there was no associated change in dark current (1.03 ± 0.05 of the initial dark current; $n = 10$). The light intensities required to saturate RGS9 knockout rods (~0.1% bleach) were ~20-fold lower than those for wild-type rods because the integration time of the knockout responses was much longer. This suggests that the induction of the adaptive effect does not depend directly on the extent of rhodopsin activation. As in wild-type rods, the adaptation of RGS9 knockout responses faded slowly in darkness (τ_{ADAPT} = 83 ± 18, $n = 5$; Fig. 7 C). This suggests that the underlying mechanism speeding recovery in both wild-type and RGS9 knockout rods is the same.

Like the adaptation observed in wild-type rods, the shortening of the time in saturation in RGS9 knockout rods was accompanied by a speeding of the rate-limiting step of recovery (Fig. 8). The dominant time constant of recovery (τ_D) in the RGS9 knockout rods shortened from the initial value of 12.0 ± 0.9 s to 4.1 ± 1.3 s following the adapting light ($n = 4$). On all cells examined, τ_D returned to its initial value (11.3 ± 0.4 s; $n = 3$) over time, as observed in wild-type rods (see above). This result indicates that this adaptation does not arise from a speeding of rhodopsin deactivation because this is already short relative to G_T/PDE deactivation in the RGS9 knockout rods. Rather, it suggests that the adaptation arises from an acceleration of G_T/PDE deactivation.

DISCUSSION

Novel, Long-lasting Adaptation in Mouse Rods

It is well-known that background light decreases the rod's sensitivity and speeds the kinetics of an incremental response. For bright flashes, the saturation time of a bright flash response decreases in the presence of background light (Fain et al., 1989). This shortening of the time in saturation in the presence of background light is dependent on the intracellular calcium concentration at or near the time of the flash (Matthews, 1995, 1997). Our experiments are fundamentally different because all of the flashes were delivered in darkness, and at a time when the current, and thus the calcium concentration, was at its dark level. This form of adaptation is novel because it persists for many tens of seconds in the absence of background light. The time course with which the adaptation fades (τ_{ADAPT} ~80 s) is considerably slower than the return of the two most abundant second messengers, calcium and cGMP, which either precede the return of the current (cGMP) or follow the change in current with brief delay (calcium; Gray-Keller and Detwiler, 1994). Therefore, the expression of this new adaptation is not dependent on the calcium or cGMP concentrations near the time of the flash.

Although to our knowledge this is the first report of a long-lasting acceleration of the rate of recovery, indications of similar mechanisms that can speed the recovery of the light response in a calcium-independent and long-lasting manner have been suggested by a study on toad rods (Coles and Yamane, 1975) and a study on truncated frog rod outer segments (Kawamura and Murakami, 1989). Although the design and methodology

of these experiments were fundamentally different, their results in hindsight suggest that similar mechanisms may indeed be important in rods from many different species.

Induction and Expression of Novel Adaptation

In our experiments, maximum response shortening required only a minute of light sufficiently bright to saturate the rod and therefore reduce free cGMP and calcium levels to a minimum during this time. Therefore although the expression does not require a change in calcium or cGMP, the induction of adaptation (i.e., whatever occurs during the 1-min exposure of saturating light) may require changes in one or both of these second messengers.

The expression of adaptation is saturable once induced; e.g., the extent of adaptation after 9 min of adapting light is not greater than that following only 1 min of adapting light. What changes occur in the outer segment on this short time scale? Although prolonged light exposure is known to induce light-dependent translocation of the proteins transducin and arrestin, the movement is not complete for many minutes. Furthermore, the return of these proteins to their original compartments is much slower (hours for transducin, Sokolov et al., 2002; ~15 min for arrestin, McGinnis et al., 2002) than the time course with which our adaptation fades (τ_{ADAPT} ~80 s). In addition, the fading of adaptation is considerably faster than the time course of pigment regeneration in mouse rods (hundreds of seconds; Kennedy et al., 2001) and other mechanisms that set the time course of human dark adaptation (Thomas and Lamb, 1999). Instead, the rapid induction and persistent expression of the adaptation seems more consistent with a posttranslational modification.

Acceleration of τ_D by this Adaptation

The decreased time that bright flash response remained in saturation was accompanied by a speeding of the dominant time constant of recovery. The dominant time constant of recovery (τ_D) has been considered the metric of choice in studying the rate-limiting step because it is independent of calcium feedback mechanisms (Pepperberg et al., 1992; Burns et al., 2002) and must reflect either rhodopsin deactivation or the shutoff of G_T/PDE (Lyubarsky et al., 1996; Nikonov et al., 1998).

Identification of the rate-limiting step for recovery of the photoresponse has been a long-standing quest for the fields of phototransduction and G protein signaling. To our knowledge, ours is the first demonstration of any experimental manipulation that has ever reversibly shortened τ_D in rods. Both wild-type and RGS9 knockout rods displayed faster τ_D values after the adapting light, and in both cases the adaptation faded with a time constant of ~80 s. This suggests that the same adaptation mechanism is at work in both wild-type and RGS9 knockout rods. Because G_T/PDE deactivation is known to be rate-limiting in the RGS9 knockout, we conclude that this is the step accelerated by our adaptation protocol. Therefore, our results suggest that bright light exposure causes a reversible acceleration of G_T/PDE deactivation via some mechanism that can operate in the absence of the RGS9-1–Gβ5 complex.

The similar features of adaptation in wild-type and RGS9 knockout rods suggests that G_T/PDE decay normally rate-limits recovery of the flash response in wild-type rods. This was also suggested by Sagoo and Lagnado (1997) for truncated salamander rods, in which rhodopsin deactivation was slowed by omission of ATP (preventing phosphorylation) and transducin deactivation was slowed with the inclusion of the nonhydrolyzable analogue, GTPγS. In those experiments, slowed rhodopsin deactivation increased the peak amplitude of the response, while slowed GTP hydrolysis affected only the later recovery phase. These differential effects on the amplitude and time course were used to argue that rhodopsin deactivation must be nearly complete by the peak of the response, and therefore that GTP hydrolysis was the slower step. However, experiments on mouse rods lacking arrestin have suggested that this cannot be wholly true for mouse rods, since arrestin knockout rods show a defect only very late in the recovery phase of the response (Xu et al., 1997).

Other dim flash experiments on truncated rods suggested that the time course of rhodopsin activity closely followed the time course of the flash response ("GTP jump experiment" of Rieke and Baylor, 1998), suggesting that rhodopsin deactivation must be rate-limiting for recovery. Others studying bright flashes have concluded that τ_D reflects rhodopsin decay (Pepperberg et al., 1992), while still others have argued that the inability of calcium to modulate τ_D (and the presumed dependence of rhodopsin deactivation on calcium levels) indirectly implicates G_T/PDE decay as rate-limiting (Lyubarsky et al., 1996; Nikonov et al., 1998).

Although our experiments used the dimmest possible flashes to assess adaptation quickly, we were not able to assess the kinetics of the single photon response after the adapting light because of the long times required to obtain enough dim flash responses for a reliable average. Therefore we cannot be certain that G_T/PDE deactivation also limits the time course of the single photon response in dark-adapted rods. However, we did calculate the duration (see MATERIALS AND METHODS) of the average response to subsaturating flashes before and immediately after the adapting light exposure. The response duration was found to be shorter after exposure to the adapting light (0.85 ± 0.04 of the initial duration, $n = 5$). This suggests that the persis-

tent adaptation speeds not only recovery from saturating responses, but also the recovery of dimmer, sub-saturating responses.

We believe that the adaptive acceleration of the rate-limiting step is the simplest explanation for our data. However, strictly speaking, we cannot rule out some other novel mechanism by which very bright saturating flashes recover faster than dimmer saturating flashes, causing an apparent decrease in τ_D. For example, if the adaptation protocol shortened wild-type responses so dramatically that calcium had insufficient time to fall to a minimum (and thus that calcium feedback mechanisms were not uniform across flash strengths), this could cause an anomalous decrease in τ_D. In our experiments, brighter flashes that keep responses in saturation for a longer time cannot be used to test this notion further because they generated responses that were no longer form-invariant (see Fig. 5) and produced the second, steeper component of the Tsat relation that likely results from depletion of key deactivation regulator (see RESULTS).

It is therefore important to consider how quickly calcium drops to a minimum during a saturating response. Although calcium imaging in mouse rods has suggested both a fast (τ = 154 ± 31 ms) and a slow component (τ = 540 ± 80 ms) of calcium decline (Woodruff et al., 2002), measurements of the decline of the Na/Ca^{2+}, K^+ exchange current suggest that the extrusion of calcium is faster (85 ± 10 ms, Burns et al., 2002; 105 ± 13 ms, Calvert et al., 2001). In the experiments presented here, wild-type rods displayed an exchange time constant of 111 ± 7 ms (n = 5; unpublished data). In our adapted wild-type rods, the dimmest saturating flashes elicited responses that remained in saturation for more than 300 ms (Fig. 6), much longer than most estimates for the time constant of calcium extrusion. Furthermore, the responses of RGS9 knockout rods remained in saturation for several tens of seconds, much longer than even the longest proposed time constant for calcium decline. In these cells, therefore, the effect on the response recovery cannot be attributed to the lack of maximal activation of known calcium feedback mechanisms.

How is the acceleration of τ_D beneficial for the animal and what is its role in vision? Although the answer to this question must await behavioral or psychophysical experiments, we propose that this form of adaptation could help the visual system smoothly transition between slow, rod-dominated vision and faster, cone-dominated vision across the wide range of mesopic light intensities.

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