Cellular/Molecular

Prolonged Photoresponses and Defective Adaptation in Rods of G$\beta5^{-/-}$ Mice

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Timely deactivation of G-protein signaling is essential for the proper function of many cells, particularly neurons. Termination of the light response of retinal rods requires GTP hydrolysis by the G-protein transducin, which is catalyzed by a protein complex that includes regulator of G-protein signaling RGS9-1 and the G-protein β subunit Gβ5-L. Disruption of the Gβ5 gene in mice (G$\beta5^{-/-}$) abolishes the expression of Gβ5-L in the retina and also greatly reduces the expression level of RGS9-1. We examined transduction in dark- and light-adapted rods from wild-type and G$\beta5^{-/-}$ mice. Responses of G$\beta5^{-/-}$ rods were indistinguishable in all respects from those of RGS9$^{-/-}$ rods. Loss of Gβ5-L (and RGS9-1) had no effect on the activation of the G-protein cascade, but profoundly slowed its deactivation and interfered with the speeding of incremental dim flashes during light adaptation. Both RGS9$^{-/-}$ and G$\beta5^{-/-}$ responses were consistent with another factor weakly regulating GTP hydrolysis by transducin in a manner proportional to the inward current. Our results indicate that a complex containing RGS9-1–Gβ5-L is essential for normal G-protein deactivation and rod function. In addition, our light adaptation studies support the notion than an additional weak GTPase-accelerating factor in rods is regulated by intracellular calcium and/or cGMP.

Key words: **phototransduction; G-protein; transducin; adaptation; RGS; calcium; cGMP**

Introduction

Regulator of G-protein signaling (RGS) proteins stimulate GTP hydrolysis by G-protein α subunits. RGS genes are expressed in unique patterns throughout the brain (Gold et al., 1997), suggesting that their physiological functions are tissue specific. Several members of the RGS family (RGS6, -7, -9, and -11) contain G-protein γ-like (GGL) domains through which they strongly interact with a neuronal G-protein β subunit called Gβ5 (Watson et al., 1996).

Despite the seemingly clear function of RGS proteins *in vitro*, surprisingly little is known about how these proteins might regulate the time course of G-protein signaling in neurons. An exceptional model system for studying signal transduction in real time is the phototransduction cascade of retinal rod photoreceptors, in which the inward current through cGMP-gated ion channels in the plasma membrane can be used to measure the activation and deactivation of the cascade (Pugh and Lamb, 2000). Transduction begins with the absorption of a photon by the G-protein-coupled receptor rhodopsin in the outer segment of the rod. Photoexcited rhodopsin catalyzes the exchange of GDP for GTP on the α subunit of the G-protein transducin (Tα). Each activated Tα binds to the γ subunit of cGMP phosphodiesterase (PDEγ), removing the inhibition by PDEγ of the catalytic subunits of PDE (Hurley and Stryer, 1982). As long as GTP–Tα is bound to PDEγ, the catalytic subunits of PDE are free to hydrolyze cGMP. Because cGMP is responsible for gating the rod membrane cation channels, the decrease of cGMP concentration results in the closure of some of the channels, leading to a decrease in inward current. The reduction in inward current leads to a hyperpolarization of the cell membrane that passively spreads to the synaptic terminal, where it causes a reduction in neurotransmitter release.

Like all G-proteins, Tα requires GTP hydrolysis for deactivation. Upon hydrolyzing GTP, Tα releases PDEγ, which then reinhibits the catalytic subunits of PDE. In photoreceptors, GTP hydrolysis by Tα is speeded by RGS9-1 (He et al., 1998), a short, membrane-associated, and photoreceptor-specific isoform of the RGS9 gene. RGS9-1 stimulates GTP hydrolysis preferentially when GTP–Tα is bound to PDEγ (Tsang et al., 1998), because the Tα–PDE complex has a higher affinity for RGS9 (Skiba et al., 2000). RGS9-1, like other members of its subfamily (for review, see Cowan et al., 2001), contains a GGL domain between the N terminal and the RGS homology domain (Snow et al., 1998; Kovoor et al., 2000; Lishko et al., 2002) that associates with Gβ5. The catalytic activity of RGS9-1 is greatly enhanced by the association of Gβ5-L (Makino et al., 1999), the retina-specific long-splice variant of the Gβ5 gene, as well as a protein anchor that mediates its attachment to the disc membrane (Lishko et al., 2002; Hu et al., 2003).

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Table 1. Kinetic characteristics of control and Gβ5-deficient flash responses

Strains	Integration time (sec)	Dim flash τ_{rec} (sec)	Time-to-peak (msec)	Dominant τ_{rec} (sec)
Gβ5$^{+/+}$	0.26 ± 0.02 (30)	0.17 ± 0.02 (28)	89 ± 3 (30)	0.19 ± 0.01 (21)
Gβ5$^{+/-}$	0.24 ± 0.02 (13)	0.16 ± 0.01 (14)	104 ± 3 (14)	0.19 ± 0.02 (16)
Gβ5$^{-/-}$	2.7 ± 0.2 (26)	2.5 ± 0.2 (26)	196 ± 30 (14)	8.8 ± 0.3 (17)

All values are means ± SEMs. The number of rods used in each measurement is indicated in parentheses. Dim flash τ_{rec} refers to the time constant of the exponential decay fitted to the recovery of the average dim flash response of each cell. The dominant τ_{rec} refers to the slope of the linear relation between time in saturation and log of intensity of the flash (see Results).

Previous work demonstrated that photoreceptors of mice lacking functional RGS9 genes (RGS9$^{-/-}$) show abnormally slowed recovery of their light responses (Chen et al., 2000; Lyubarsky et al., 2001) and slowed rates of GTP hydrolysis by transducin (Chen et al., 2000). In addition, retinas of RGS9$^{-/-}$ mice do not express Gβ5-L, despite abundant Gβ5-L mRNA. Similarly, disruption of the Gβ5 gene (Gβ5$^{-/-}$) results in very low (<5% normal) levels of RGS9-1, and undetectable levels of other subfamily members RGS6, RGS7, or RGS11 in the retina, despite the abundance of mRNA transcripts for these proteins (Chen et al., 2003). Thus, the Gβ5$^{-/-}$ rods not only lack Gβ5-L but also essentially lack RGS9-1 and other RGS proteins that may be present in photoreceptors at low levels.

The purpose of this study was to quantitatively assess transduction in the Gβ5$^{-/-}$ rods and to compare them with the known characteristics of RGS9$^{-/-}$ responses.

Materials and Methods

Suction electrode recording. Mice were cared for and handled following an approved protocol from the Animal Care and Use Committee of University of California, Davis, and in compliance with National Institutes of Health guidelines for the care and use of experimental animals. Mice were housed in 12 hr cyclic light and were dark-adapted overnight before an experiment. Under infrared light, animals were anesthetized and euthanized, and the retinas removed and stored in L15 solution with 10 mM glucose and 0.1 mg/ml bovine serum albumin on ice. Retinas were then chopped in a chopping dish with a razor blade and placed in the recording chamber. The recording chamber was perfused with a solution containing (in mM): 112.5 NaCl, 3.6 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 10 HEPES, 0.2 EDTA, 20 sodium bicarbonate, 3 disodium succinate, and 10 glucose. The solution was bubbled with 95% O_2–5% CO_2 oxygen and warmed to 35–37°C, and the pH was adjusted to 7.4 with KOH. Small pieces of retina were visualized with a sensitive CCD camera (Stanford Photonics, East Palo Alto, CA) using infrared light. Individual mouse rods were drawn into a glass electrode containing (in mM): 140 NaCl, 3.6 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 3 HEPES, 0.2 EDTA, and 10 glucose. The pH of this solution was also 7.4 at 37°C. The bath and suction electrodes were connected to calomel half-cells by agar bridges, and the bath voltage was maintained at ground potential by an active feedback circuit. The rod membrane current was amplified (Axopatch 1B; Axon Instruments, Foster City, CA) and filtered at 20 Hz with an eight pole Bessel filter. Data was digitized continuously at 200 Hz using NiDAQ (National Instruments, Austin, TX) for IgorPro (Wavemetrics, Lake Oswego, OR). Tissue in the chamber was presented with 10 msec flashes of 500 nm light either in darkness or in the presence of steady, 520 nm light. Light intensity was controlled by calibrated neutral density filters, and at the end of each experiment, the lamp power was measured at 500 and 520 nm light using a silicon photodiode detector (United Detector Technology, Baltimore, MD). We could detect only minimal qualitative morphological differences between wild-type and the Gβ5$^{-/-}$ rods, with the Gβ5$^{-/-}$ rod outer segments seeming slightly shorter and of slightly larger diameter. The resting dark currents of wild-type and Gβ5$^{-/-}$ rods were not significantly different (Table 1).

The average response to a large number (>20) of flashes was considered to be in the linear range if its mean amplitude was <25% of the maximal response amplitude. These dim flash responses were used to estimate the form of the single photon response using the variance-to-mean method, as previously described (Mendez et al., 2000). Briefly, the mean dim flash response was squared and scaled until its rising phase coincided with the rising phase of the ensemble time-dependent variance. Assuming that the predominant source of variance at this early time in the response arises from Poisson fluctuations in the number of photoisomerizations, the scaling factor that brings these two traces into alignment is proportional to $1/n$, where n is the mean number of photoisomerizations per flash. The mean response was then divided by n to yield the form of the single photon response. The small underestimation introduced by the inclusion of cells whose dim flash responses exceeded 15% of the maximal amplitude is within experimental error (SEM).

Integration time was used as a measure of the duration of the incremental flash response and is defined as the time integral of the average linear response divided by its peak amplitude (Baylor and Hodgkin, 1973). The time that a bright flash response remained in saturation was calculated as the time interval between the midpoint of the flash and the time at which the current recovered by 10%.

Adaptation experiments. Adaptation was assessed by comparing the responses of wild-type and Gβ5$^{-/-}$ rods with flashes in darkness and in the presence of steady light. Cells were presented with test flashes in darkness, then in the presence of a background light, and then again in darkness. Cells were kept for analysis if the dark currents measured before and after the steady light did not vary by >20%. We define dim background light as those intensities that turned off between 10 and 49% of the dark current in the steady state.

Time course of light-activated PDE activity. Time course of light-activated PDE activity [$P^*(t)$] was calculated according to Pugh and Lamb, (1993):

$$P^*(t) = -\frac{1}{n}\frac{d[\ln(1 - r(t)/r_{max})]}{dt},$$

where $r(t)$ is the time course of the response, r_{max} is the maximal response amplitude to a bright flash, and n is the cooperativity of the cGMP-gated channels. We used this equation to calculate $P^*(t)$, assuming $n = 3$ (Haynes et al., 1986; Zimmerman and Baylor, 1986). The initial rate of change of the light-activated PDE activity (dP^*/dt) was determined by linear regression of the initial rate of change of $P^*(t)$ as described in Tsang et al. (1998). The corner frequency of the low-pass filter (20 Hz) may have contributed to the saturation of the rate of change of $P^*(t)$ in response to bright flashes (>1000 photons/μm^2) but did not interfere with the rising phases of the responses over most of the experimental range of flash strengths presented in Figure 2*c*.

Results

Loss of Gβ5 slows recovery of dim-flash responses

To study the effects of inactivating the Gβ5 gene on phototransduction, we used suction electrodes to record the light responses of rods from wild-type mice (Gβ5$^{+/+}$) and rods from Gβ5 hemizygous (Gβ5$^{+/-}$) and homozygous (Gβ5$^{-/-}$) knock-out mice. Representative families of responses from such rods across a wide range of flash strengths are shown in Figure 1. There were no detectable differences in the amplitude or kinetics of wild-type and Gβ5$^{+/-}$ rods (Fig. 1, Table 1), consistent with the normal level of expression of both RGS9-1 and Gβ5-L in these retinas (Chen et al., 2003). However, the responses of Gβ5$^{-/-}$ rods showed a specific defect in the recovery phase of the flash response (Fig. 1). Exponential functions fit to the final falling phases of the average dim flash responses of Gβ5$^{-/-}$ rods had a

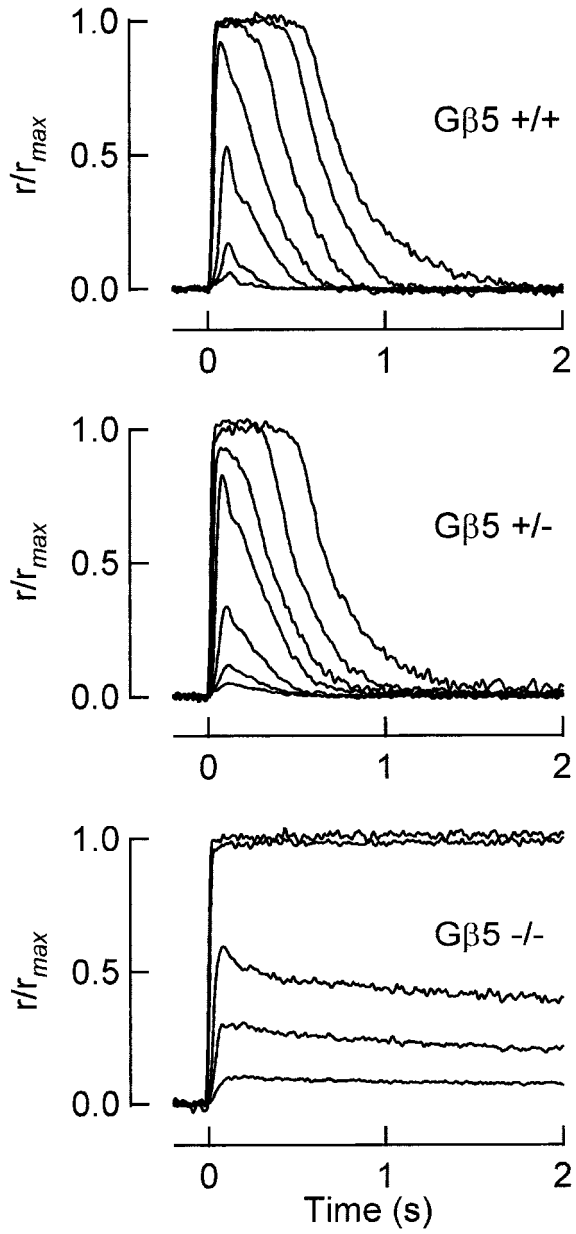

Figure 1. Families of responses to increasing flash strengths from representative wild-type, knock-out, and hemizygote mice. Responses have been normalized (r/r_{max}) by the maximal response amplitudes, which were the following (in pA): 16.3 (+/+), 16.1 (−/−), and 17.7 (+/−). Flash strengths (in photons/μm^2) ranged from 11 to 4546 (+/+), 11 to 8563 (+/−), and 19 to 8277 (−/−).

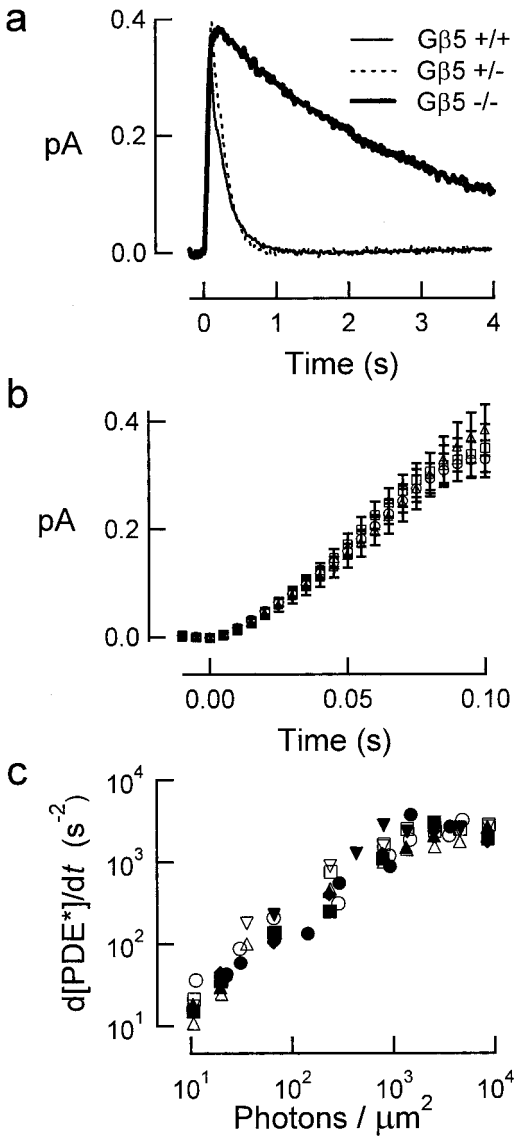

Figure 2. Effect of disrupting Gβ5 on the mean single photon response. *a*, Population mean single photon responses for +/+ (circles; $n = 28$), +/− (triangles; $n = 13$), and −/− (squares; $n = 12$) rods. Average dark currents (in pA) of the cells used in this determination were 11.3 ± 0.5, 13.0 ± 0.9, and 12.1 ± 1.3, respectively. *b*, Mean single photon responses from *a*, shown on an expanded time scale. Error bars represent SEM. *c*, Initial rate of change of light-evoked PDE activity (*d*PDE/*dt*) for four wild-type rods (open symbols) and five Gβ5 $^{-/-}$ rods (filled symbols) (see Materials and Methods).

time constant >10-fold longer than those fitted to wild-type responses (Table 1). These values are very similar to those of dim flash responses in RGS9 $^{-/-}$ rods (Chen et al., 2000) (see Fig. 3) consistent with the complete functional loss of the RGS9-1–Gβ5-L complex in the Gβ5 $^{-/-}$ rods.

As observed previously in RGS9 $^{-/-}$ responses, the defect in the Gβ5 $^{-/-}$ responses was limited to the recovery phases of the responses. There were no significant differences in the sensitivity of these dark-adapted rods: the flash sensitivity [as measured in pA/(photons/μm^2)], single photon response amplitude, and the flash strength needed to elicit a half-maximal response (in photons/μm^2) were also very similar in each group of mice (Table 2). Furthermore, the rising phases of the single photon response in all three lines of mice were indistinguishable (Fig. 2*a*,*b*). This suggests that the loss of the RGS9-1–Gβ5-L protein complex in Gβ5 $^{-/-}$ rods does not affect the activation or the amplification of the G-protein cascade. To further test this idea, we calculated the rate of change of PDE activity from the rising phases of the responses to a wide range of flash strengths using the method developed by Pugh and Lamb (1993) (see Materials and Methods). The light-activated change in PDE activity was similar for rods from each line of mouse (Fig. 2*c*), supporting the idea that there were no changes in the activities or level of expression of any of the proteins involved in the activation stages of the cascade.

Loss of Gβ5 more strongly affects recovery of bright-flash responses than dim-flash responses

In normal rods, increasing the strength of a saturating flash results in responses that remain in saturation for longer times.

Table 2. Sensitivity characteristics of control and Gβ5-deficient rods

Strains	i_o (photons/μm^2)	Single photon response amplitude (pA)	Flash sensitivity [pA/(photons/μm^2)]	I_d (pA)
Gβ5$^{+/+}$	70 ± 5 (27)	0.34 ± 0.04 (28)	0.10 ± 0.01 (30)	11.3 ± 0.5 (34)
Gβ5$^{+/-}$	96 ± 12 (16)	0.41 ± 0.05 (13)	0.10 ± 0.01 (16)	12.6 ± 0.8 (19)
Gβ5$^{-/-}$	80 ± 10 (20)	0.43 ± 0.07 (12)	0.14 ± 0.02 (26)	11.5 ± 0.6 (29)

All values are means ± SEMs. The number of rods used in each measurement is indicated in parentheses. i_o refers to the flash strength that elicited a half-maximal response. I_d refers to the inward current in darkness.

Because the cGMP and calcium levels both fall to a minimum during the time that the response is saturated, a calcium- and cGMP-independent measurement of the rate limiting, or dominant, time constant of saturating response recovery can be found by plotting the time that responses remain in saturation as a function of the log of the flash strength (Pepperberg et al., 1992; Lyubarsky et al., 1996). This time constant was 0.2 sec for the wild-type rods and 9 sec for the Gβ5$^{-/-}$ rods (Table 1). These results are very similar to results of previous studies of the RGS9$^{-/-}$ mice (Chen et al., 2000) and are consistent with the RGS9$^{-/-}$ mice examined in this study for direct comparison (Fig. 3). Thus, whereas there was a ~10-fold difference in recovery kinetics of the dim flash responses of Gβ5$^{-/-}$ and wild-type rods, there was a 50-fold difference in the time constant of recovery of bright flash responses.

The profound slowing of recovery in the Gβ5$^{-/-}$ bright flash responses was not irreversible. Instead, the response recovery appeared to speed up as the current was restored (Fig. 3*c*). In contrast, saturating responses of wild-type mouse rods recovered with an exponential time constant of 0.2 sec throughout the entire falling phase of the response (data not shown) (Chen et al., 2000), as evidenced by the similarity between the dim flash and bright flash recovery time constants (both 0.2 sec). The acceleration of recovery that we observed in Gβ5$^{-/-}$ rods also occurs in RGS9$^{-/-}$ responses (Fig. 3*c*) (Chen et al., 2000). This suggests that none of the other RGS proteins that are lacking in the Gβ5$^{-/-}$ mice (RGS6, -7, and -11) contribute to the deactivation of Tα in the absence of RGS9. In addition, the residual RGS9-1 (<5%) found in Gβ5$^{-/-}$ retinal homogenates (Chen et al., 2003) does not accelerate transducin GTP hydrolysis under our experimental conditions (see Discussion).

Our previous modeling work suggested that the progressive acceleration of recovery in the absence of RGS9-1 could result if the rate of Tα deactivation was regulated by calcium or cGMP (Chen et al., 2000) (see also Discussion). We therefore investigated the effect of sustained reduction in calcium and cGMP on the kinetics of the responses of Gβ5$^{-/-}$ rods, such as occurs during light adaptation.

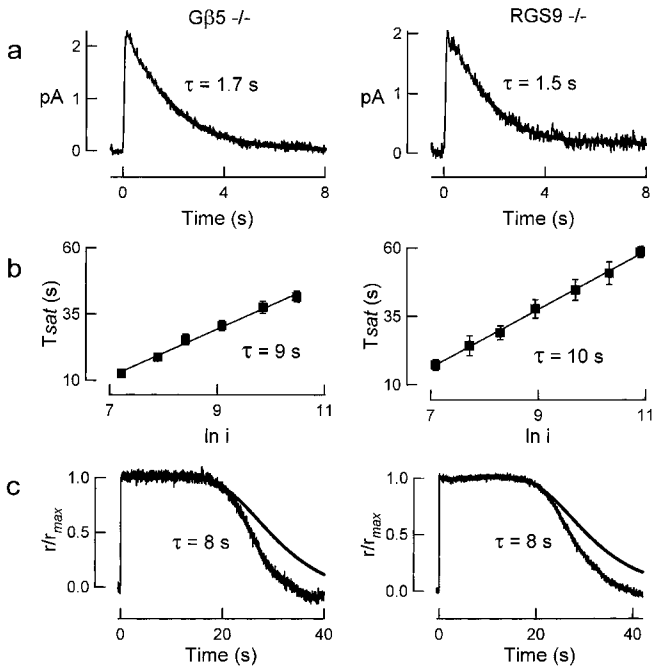

Figure 3. Comparison of Gβ5$^{-/-}$ rods (left) and RGS9$^{-/-}$ rods (right). *a*, Average dim flash responses of representative Gβ5$^{-/-}$ and RGS9$^{-/-}$ rods. Falling phases were fitted with single exponential functions (bold) with time constants (τ) as indicated. Dark currents were 17.1 pA (Gβ5$^{-/-}$) and 9.7 pA (RGS9$^{-/-}$). Flash strengths (in photons/μm^2) were 5.7 (Gβ5$^{-/-}$) and 9.4 (RGS9$^{-/-}$). *b*, Time that bright flash responses remained in saturation (T_{sat}) as a function of the natural log of the flash strength (ln *i*) (in photons/μm^2). Each point in the Gβ5$^{-/-}$ plot is the average of 12–14 cells, and each point in the RGS9$^{-/-}$ plot is an average of three to four cells. Error bars reflect SEM. Mean time constants were 8.8 ± 0.3 sec (Gβ5$^{-/-}$ rods; n = 17) and 9.8 ± 0.6 sec (RGS9$^{-/-}$ rods; n = 4). *c*, Saturating flash responses for representative Gβ5$^{-/-}$ (left) and RGS9$^{-/-}$ (right) rods. For both cells, the recovery accelerates as the current returns. Dark currents (in pA) and flash strengths (in photons/μm^2) for these examples were 9.5 pA and 2255 photons/μm^2 (Gβ5$^{-/-}$) and 14.4 pA and 2095 photons/μm^2 (RGS9$^{-/-}$). Response amplitudes were normalized by the dark current.

Adaptation of wild-type and Gβ5$^{-/-}$ rods

Continuous illumination causes photoreceptors to adapt; that is, they undergo a drastic decrease in sensitivity and a speeding of the incremental response kinetics (Baylor and Hodgkin, 1973; Fain et al., 1989). Both of these characteristics decrease the steady-state response to continuous illumination. Because the recovery kinetics change with the amount of inward current in the Gβ5$^{-/-}$ rods (see above), we chose to focus our studies to a narrow range of light intensities that caused similar changes in the circulating currents in wild-type and Gβ5$^{-/-}$ rods. The average percentage of dark current turned off by the background light was similar for both wild-type and knock-out mice. Assuming that the knock-out and wild-type rods have channels with similar sensitivities for cGMP and have similar Na^+–Ca^{2+}, K^+ exchanger activities, both groups experienced on average the same fall in intracellular messengers cGMP and Ca^{2+}.

During dim continuous illumination that turned off approximately one-quarter of the dark current (wild-type rods, 24 ± 6%, n = 7; Gβ5$^{-/-}$ rods, 29 ± 5%, n = 7), the incremental flash sensitivity of both wild-type and Gβ5$^{-/-}$ rods was reduced to similar extents (wild-type rods, mean ± SEM, 71 ± 12%; n = 7; Gβ5$^{-/-}$ rods, 56 ± 4; n = 7). However, there was a striking difference in the effect of light adaptation on the time course of the response. In the presence of dim background lights, the integration times of the wild-type dim-flash response (n = 7) shortened slightly, on average to 89 ± 9% of their dark values (Fig. 4), with a few of these cells having no detectable change in integration time (*B*). In contrast, the integration times of all of the knock-out rods (seven of seven cells) significantly increased in the presence of background lights to an average of 167 ± 12% of their dark values (n = 7) (Fig. 4). Similar results were also ob-

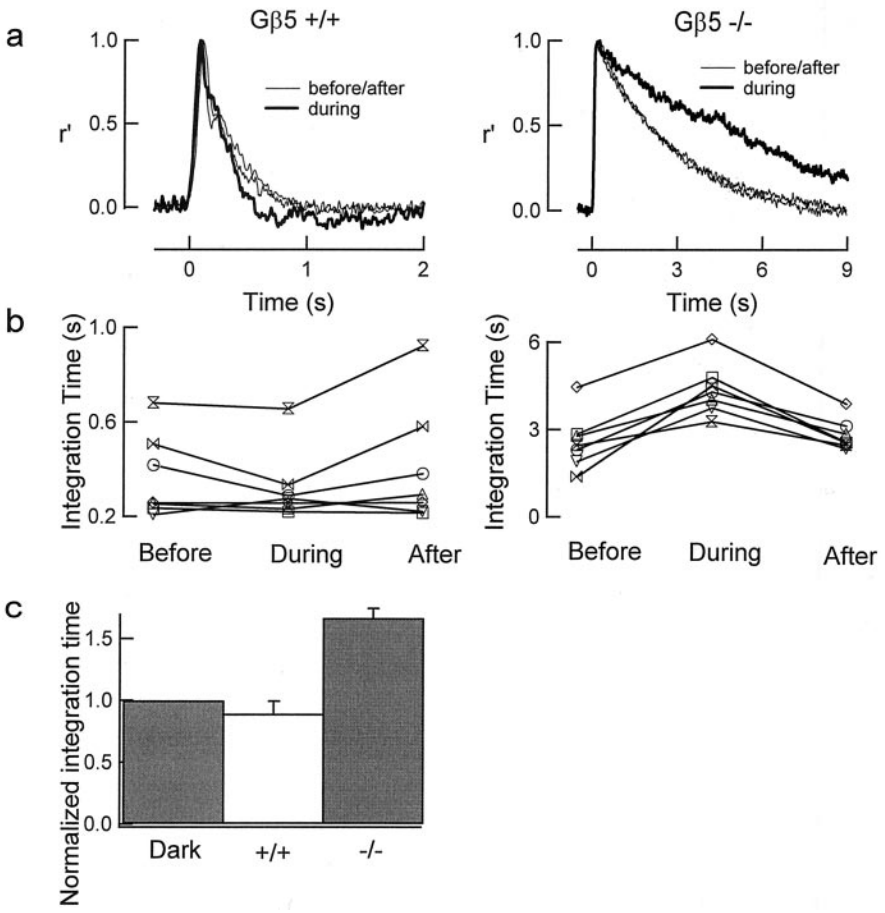

Figure 4. Impaired adaptation of dim flash responses in Gβ5 $^{-/-}$ rods. *a*, Average dim flash response of representative Gβ5 $^{+/+}$ (left) and Gβ5 $^{-/-}$ (right) rods before, after, and during steady light exposure. The intensities of the background light were 30 photons/μm ^{2}/sec (Gβ5 $^{+/+}$) and 7.2 photons/μm ^{2}/sec (Gβ5 $^{-/-}$), which turned off 10 and 16% of the original dark current, respectively. The Gβ5 $^{+/+}$ integration times shortened slightly in the presence of background light, whereas the Gβ5 $^{-/-}$ integration times slowed significantly in the presence of background light. Dim flash responses were normalized (*r*′) by peak amplitude for comparison of response durations (integration time) (see Materials and Methods). Dark currents (in pA) were 15.2 (Gβ5 $^{+/+}$) and 16.0 (Gβ5 $^{-/-}$). In this example, flash strengths were 10 photons/μm ^{2} in the presence and absence of background light (Gβ5 $^{+/+}$), and 10 photons/μm ^{2} in darkness and 19 photons/μm ^{2} in background light (Gβ5 $^{-/-}$). *b*, Integration times from Gβ5 $^{+/+}$ (left) and Gβ5 $^{-/-}$ (right) dim flash responses before, during, and after exposure to steady light. The background light intensities ranged from 30 to 1340 photons/μm ^{2}/sec for the wild-type rods, turning off 10–49% of the original dark current. The background intensities for the Gβ5 $^{-/-}$ rods ranged from 5.9 to 25 photons/μm $^{-2}$/sec, turning off 16–40% of the original dark current. *c*, Average fractional change in integration times during light adaptation for the cells in *b*. The integration time in the dark is the average of the integration times before and after background light. Error bars represent SEM.

served with the RGS9 $^{-/-}$ rods in the presence of background lights ($n = 2$; data not shown). We interpret this to indicate that in the absence of the RGS9-1–Gβ5-L complex, the suppression of current by background light further slows GTP hydrolysis in a manner that interferes with the speeding up of the incremental response that usually occurs during adaptation (see Discussion).

In addition to accelerating the incremental dim flash response, the time that a bright flash response remains in saturation is also normally shorter in the presence of background light (Baylor and Hodgkin, 1973; Fain et al., 1989; Matthews, 1995) (Fig. 5*a*). This form of adaptation is also observed in mouse rods; in our experiments, on average, the saturation time of a wild-type response shortened to 68 ± 8% of the dark value in the presence of dim background lights that turned off 24 ± 6% of the dark current ($n = 7$) (Fig. 5*c*). A similar decrease of saturation times (to 63 ± 6% of the dark value) was also seen for the knock-out rods in the presence of background light that turned off comparable current levels (28 ± 3%; $n = 5$). In addition, the presence of background light had no effect on the dominant time constant of recovery from saturating flashes in wild-type rods, nor in Gβ5 $^{-/-}$ rods (data not shown), consistent with previous reports (Lyubarsky et al., 1996; Calvert et al., 2002). Thus, unlike the dim incremental responses that show slowed deactivation during steady light, bright flash responses adapt normally and show no change in the dominant time constant of recovery, indicating that this adaptation mechanism is unaffected by the loss of the RGS9-1–Gβ5-L complex.

Discussion

Our experiments support the notion that the RGS9-1–Gβ5-L complex is essential for normal, rapid recovery of rod photoresponses. Although Western blots of retinal homogenates suggest that Gβ5 $^{-/-}$ rods express ~5% normal levels of RGS9-1 (Chen et al., 2003), the similarities between the responses of the RGS9 $^{-/-}$ rods and the Gβ5 $^{-/-}$ rods lead us to conclude that there is no functional expression of RGS9-1 in the outer segments of the Gβ5 $^{-/-}$ rods. Thus, just as Gβ5-L is not expressed in RGS9 $^{-/-}$ rods, RGS9-1 is functionally absent without Gβ5-L. This supports the hypothesis that RGS9-1 and Gβ5-L are obligate binding partners (Makino et al., 1999) whose activity and targeting are inextricably linked (Chen et al., 2000; Kovoor et al., 2000; Witherow et al., 2000; Chen et al., 2003).

The Gβ5 $^{+/-}$ rods yielded responses that were not significantly different from those of wild-type rods, consistent with the observation that retinas of Gβ5 $^{+/-}$ mice contain normal levels of both Gβ5-L and RGS9-1 protein (Chen et al., 2003). It has been proposed that the abundance of RGS9-1 in cones may contribute to their faster recovery kinetics (Cowan et al., 1998). This attractive idea has still to be supported, and future experiments will test this idea in rods by expressing intermediate and excess levels of RGS9-1–Gβ5-L and determining whether there are corresponding changes in the recovery rates of the flash responses.

Bright flash responses of both Gβ5 $^{-/-}$ and RGS9 $^{-/-}$ rods initially recovered slowly, but the recovery accelerated as the current returned. This was previously found to be consistent with a simple model whereby the rate of transducin deactivation varied linearly with the inward current and led to the hypothesis that RGS9 $^{-/-}$ rods contained an additional calcium- or cGMP-dependent factor with weak GTPase-accelerating activity (Chen et al., 2000). This same model also fits well to response families of Gβ5 $^{-/-}$ rods (data not shown), further supporting the notion that, in the absence of RGS9-1–Gβ5-L, another factor can speed GTP hydrolysis in the dark. Because Gβ5 $^{-/-}$ retinas also do not

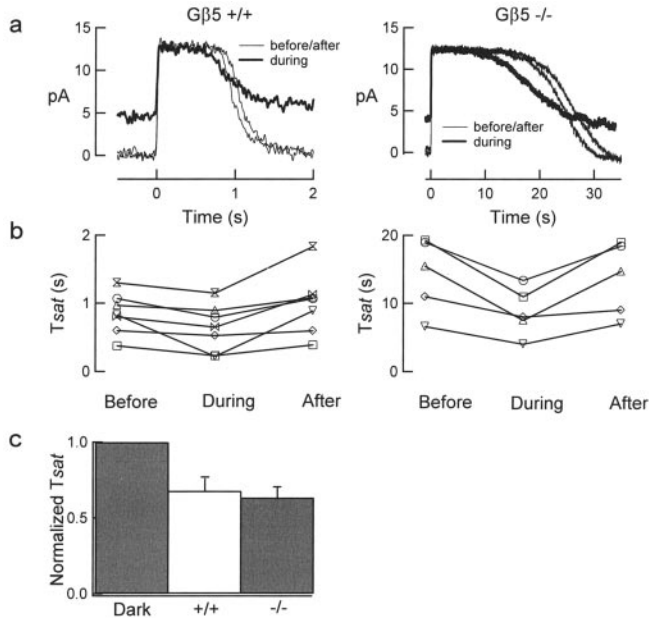

Figure 5. Adaptation of bright-flash responses in Gβ5 $^{-/-}$ rods. *a*, Saturating responses of representative Gβ5 $^{+/+}$ (left) and Gβ5 $^{-/-}$ (right) rods before, after, and during background light exposure. The intensities of the background lights (in photons/μm^2/sec) were 340 (Gβ5 $^{+/+}$) and 17 (Gβ5 $^{-/-}$), and the flash strengths (in darkness and in the presence of the background light) were 2452 (Gβ5 $^{+/+}$) and 788 (Gβ5 $^{-/-}$) photons/μm^2. The time that both Gβ5 $^{+/+}$ and Gβ5 $^{-/-}$ responses remained in saturation shortened in the presence of background light. *b*, Time spent in saturation (T_{sat}) for Gβ5 $^{+/+}$ (left) and Gβ5 $^{-/-}$ (right) rods before, during, and after exposure to background light. The background light intensities ranged from 30 to 1340 photons/μm^2/sec for wild-type rods, turning off 10–49% of the original dark current. The background intensities for Gβ5 $^{-/-}$ rods ranged from 7.1 to 25 photons/μm^2/sec, turning off 16–37% of the original dark current. *c*, Average fractional change in time spent in saturation during adaptation for the cells in *b*. The time in saturation in the dark is the average of the saturation times before and after background light. Error bars represent SEM.

express RGS6, -7, and -11 (Chen et al., 2003), these less abundant retinal RGS proteins cannot be the factor.

Additional evidence for the calcium and/or cGMP dependence of this factor is the slowing of the incremental response of Gβ5 $^{-/-}$ rods in the presence of steady light. Normally, light adaptation speeds the kinetics of incremental flash responses (Baylor and Hodgkin, 1973; Fain et al., 1989; Pugh et al., 1999). The mechanisms responsible for the speeding of the adapted dim incremental response are not entirely known (Baylor and Hodgkin, 1973; Fain et al., 1989; Pugh et al., 1999), although recent evidence suggests that the increased steady-state PDE activity in the presence of steady light can account for a great deal of the kinetic changes (Nikonov et al., 2000). Here, we showed that the Gβ5 $^{-/-}$ responses do not speed up in the presence of background light, indicating that the usual mechanisms—such as the increased steady-state PDE activity—are swamped out by slowed deactivation mechanisms. Somehow, continuous light that lowers calcium and cGMP levels further slows deactivation (and thus GTP hydrolysis) in RGS9 $^{-/-}$ and Gβ5 $^{-/-}$ rods.

Saturating responses of wild-type rods shortened in the presence of background light as expected. In amphibian rods, the shortening of the time in saturation requires a fall in intracellular calcium at or near the time of the flash (Matthews, 1997). The fall in calcium that accompanies light adaptation exerts numerous effects on the cascade, all of which might be expected to make the response come out of saturation sooner. The shortening of the time in saturation has little (Calvert et al., 2002) or no effect (Lyubarsky and Pugh, 1996; Lyubarsky et al., 1996) on the dominant time constant of recovery in amphibians. Likewise, in our experiments on wild-type mouse rods, we also observed that the dominant time constant was not significantly different between darkness and in the presence of background lights. Saturating responses from Gβ5 $^{-/-}$ rods also shorten in the presence of background lights, suggesting that this aspect of adaptation is functioning normally in knock-out rods.

The similarity of Gβ5 $^{-/-}$ and RGS9 $^{-/-}$ responses during adaptation further supports the idea that both RGS9-1 and Gβ5-L are necessary for proper function. Our experiments have also strengthened the hypothesis that an additional weak GTPase-accelerating factor exists in rod photoreceptors and operates in the dark when both calcium and cGMP levels are high. Future experiments will further investigate the identity and biochemical regulation of this putative factor.

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