Purpose. In the Gβ5 γ-/− mouse, the electroretinogram (ERG) b-wave is absent, and the R7 subfamily of regulators of G protein signaling (RGS), which includes RGS6, -7, -9, and -11, is downregulated. Mutant mouse strains deficient in RGS7 or -11 were characterized, and the SG711 strain which is deficient in both proteins was examined, to learn whether the loss of some of these RGS proteins causes the absence of the ERG b-wave.

Methods. Antibodies to RGS7 and -11 were generated to determine their expression levels and localizations in retinas with various genetic backgrounds by Western blot analysis and immunohistochemistry, respectively. The implicit times and amplitudes of ERG a- and b-waves were analyzed to examine photoreceptor and bipolar cell functions.

Results. RGS7 and -11 co-localized to the dendritic tips of the ON-bipolar cells. In the SG711−/− mouse, the level of RGS7 protein increased. However, the level of RGS11 protein remained unchanged in the SG77 mutant mouse, where a truncated RGS7 protein was expressed due to the deletion of exon 10. In the SG711 mouse retina, the Gβ5-S protein level was reduced. The ERG b-wave of SG711 mice was markedly delayed. In contrast, RGS11−/− mice showed a moderately delayed b-wave, whereas the RGS7 mutant mice showed normal ERG responses.

Conclusions. The data demonstrate the presence of a delayed ERG b-wave in SG711 mice and a functionally redundant role for RGS11 and -7 at the tips of ON-bipolar cell dendrites. These results suggest that RGS11 or -7 works as the major physiological component involved in the mGluR6 signaling pathway responsible for relaying visual information in the depolarizing bipolar cells (DBC)s.

Recently, several RGS proteins, including RGS7, RGS11, and Ret-RGS1, were postulated to participate in the mGluR6 signaling pathway. RGS functions inside a cell as a negative regulator of a subset of heterotrimeric G-proteins. Members of the RGS protein family contain a conserved RGS domain roughly 120 amino acids in length and can be further classified into subgroups by their sizes as well as sequences outside the RGS domain. RGS9-1 was the first mammalian RGS protein identified to have a physiological function. It is essential for timely deactivation of transducin in photoreceptors in mice and humans. RGS9-1 exists in complex with two other proteins: Gβ5-L (long-splice form of the fifth member of the G-protein β subunit) and RGS9 anchoring protein (R9AP). Together, these three proteins form the transducin GTPase acceleration protein (GAP) complex, the level of which determines the duration of rhodopsin signaling in rods. The stability of the transducin GAP complex depends on a unique interaction between Gβ5-L and the G protein γ-like (GGL) domain of RGS9-1. Three additional RGS proteins contain the hallmark GGL domain: RGS6, -7, and -11. Together with RGS9, they constitute the R7 subfamily of RGS proteins. Unlike RGS9-1, however, these three R7 RGS proteins interact with the short-splice form of Gβ5 (Gβ5-S), which is more broadly expressed in the nervous system. In Gβ5 knockout (Gβ5−/−) mice, all four R7 RGS proteins are destabilized, and the ERG b-wave is absent. In addition to the no-b-wave ERG phenotype, DBCs of Gβ5−/− mice exhibit unique dendritic

6 (mGluR6) signaling pathway responsible for relaying visual information in the depolarizing bipolar cells (DBC)s. In the outer segment of a rod photoreceptor, light activates rhodopsin, which in turn activates the photoreceptor-specific G-protein, transducin. Activated transducin sequesters the inhibitory subunit of a phosphodiesterase and in turn enables its catalytic subunits to hydrolyze cyclic guanosine monophosphate (cGMP), leading to a rapid decline in intracellular cGMP level and to the closure of cGMP-gated cation channels located in the plasma membranes of the outer segment. Channel closure leads to membrane hyperpolarization and decreases synaptic release of glutamate in the OPL. The reduction of glutamate concentration is sensed by dendrites of two types of bipolar cells. In hyperpolarizing bipolar cells (HBCs) ionotropic glutamate receptors are expressed and the cell becomes hyperpolarized in response to the light-induced decrease in OPL glutamate level. In contrast, mGluR6 is expressed in DBCs, and light exposure causes the cell to depolarize. An alternatively spliced form of Gao, Gao1, is necessary for DBC-derived ERG b-wave responses. It is known that activation of mGluR6 by glutamate leads to the closure of a cation channel, which has recently been suggested to be a transient receptor potential-like channel, TRPM1. Akin to the knockout mouse deficient in mGluR6 or Gao, the TRPM1 knockout mouse lacks the ERG b-wave. Unlike the phototransduction cascade, many components involved in the mGluR6 signaling pathway remain unidentified.

Vision begins at retinal photoreceptors, and encoded information is relayed to bipolar cells at the retinal outer plexiform layer (OPL). The phototransduction cascade responsible for transducing light into neural signals in photoreceptors is G-protein mediated, as is the metabotropic glutamate receptor
defects as the result of an arrest in the formation of the triadic ribbon synapses during OPL development. Transgenic restoration of Gβ5-L in rods of adult Gβ5−/− mice rescued neither the OPL morphologic defects nor the absent ERG b-wave, indicating that both phenotypes come from the loss of Gβ5-S downstream of the photoreceptors.8 However, it remains uncertain whether a signaling defect in the mGluR6 signaling pathway or the reduced triadic ribbon synapses at the OPL, or both, causes the absence of ERG b-wave in Gβ5−/− mice. To gain further insight, we recorded ERG responses from mice carrying targeted mutations in both RGS7 and -11 genes. We found that RGS7 and -11 are co-localized at the tips of DBC dendrites and that both are involved in the generation of ERG b-waves in a functionally redundant manner. Because of the presence of a robust, RGS7-deleted ERG b-wave response when both RGS7 and -11 are mutated, our data suggest that OPL morphologic defects, rather than the prolonged mGluR6/Gq01 signal transduction in DBCs, are the major contributing factor to the loss of ERG b-waves in Gβ5−/− mice.

Methods

Animals

RGS11 and -7 mutant mice, generated by homologous recombination by Lexicon Pharmaceuticals (The Woodlands, TX), are available from the Mutant Mouse Regional Resource Center. In targeting the RGS11 gene, the first four exons were deleted. The homozygous RGS11 knockout (RGS11−/−) mouse has no detectable RGS11 protein and hence is a true null.20 However, in the homozygous RGS7 mutant mouse, the deletion of exon 10 resulted in the production of a transcript encoding a truncated RGS7 protein lacking amino acids S229-Q261. To distinguish it from a true null, we named the homozygous script encoding a truncated RGS7 protein lacking amino acids S229-Q261. The individually targeted mouse line the RGS7 null mouse. The individually targeted mouse genes, the F1 offspring derived from RGS7−/− and SG7 matings were intercrossed. Genotyping of the SG7−/− mouse which carry homozygous mutations in both the RGS7−/− and -11 genes, the F1 offspring derived from RGS11−/− and SG7 mating were intercrossed. Genotyping of the SG7−/− mouse was performed by PCR using tail-snip DNA as templates with 60°C annealing temperature. The 190-bp PCR product of the mutant RGS11 allele was amplified with the primers SG11WT-F: 5′-AGT TAA GGA CAT TGG AGA CCG T and SG11WT-R: 5′-CCG AAG AAA CCG AAA GTG TGT TAG GG. A 750-bp PCR product of the mutant RGS11 allele was obtained with the primers SgNeo3A: 5′-GCA GGC CAT CGT CTA TC and SG11KO: 5′-CTT CCA ATA TTA ACC CTA GC. A mixture of three primers was used to genotype RGS7 locus: SGNeo3A, SG7-c: 5′-GAC TGG TAC TGA CCA AAC CC, and SG7WT: 5′-CCT ACA CCA GAA ACC AAC GC. The presence of 290- and 380-bp products indicated wild-type and targeted alleles, respectively. To generate mice in which cone photoreceptors were marked with EGFP, a transgenic construct pOPE-GFP, which contains a 5.4-kb mouse green opsin promoter amplified according to Akimoto et al.,21 followed by a full-length EGFP cDNA and a 0.64-kb mouse protamine polyadenylation signal, was injected into the F1 embryos of C57BL6 x BALB/c mating. Six founder lines, GGFP1–6, were produced with varying degrees of EGFP expression and distribution in retinal cones. GGFP5 was used in this study because of its high expression level in the retina. The GGFP mice were genotyped by PCR, using primers specific for Gβ5, with the presence of a 750-bp product with the following primers at 58°C annealing temperature: GOP1: 5′-GAG ACA GTT TTC TAC AGC CT and EGFP-e: 5′-TTA CTT GTA CAC CTC GTC. The Gβ5−/− mouse has been described.19 All animals used in the study were pigmented. All studies adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental procedures were performed in accordance with the rules and regulations of the NIH guidelines for research animals and approved by the institutional animal care and use committee of Virginia Commonwealth University.

Antibodies

A rabbit polyclonal antibody for RGS11 (VCU008) was raised against a synthetic peptide (CGTSTPPAATSSPGADGE) coupled to keyhole limpet hemocyanin and the antibody was affinity purified on the corresponding peptide column (Yenney, Burlingame, CA). Similarly, a rabbit polyclonal antibody for RGS7 (VCU015) was raised and purified against a synthetic peptide (CXTLSKRTLSTLVSQ; GenScript, Piscataway, NJ). Rabbit anti-Gβ5 antibody CT-215 was graciously provided by Mel Simon (California Institute of Technology, Pasadena, CA).20 Goat anti-RGS11 antibody was generously provided by Kirill Marтемянов (University of Minnesota, Minneapolis, MN).20 Rabbit anti-RGS7 antibody 7RC-1 was kindly provided by William Simonds (National Institute of Diabetic and Digestive and Kidney Diseases, Bethesda, MD).22 Rabbit anti-RGS7 antibody H-190 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); rabbit anti-RGS6 antibody IMG-554 from Imgenex (San Diego, CA); rabbit anti-GAPDH antibody from Cell Signaling Technologies (Danvers, MA); and HRP-conjugated secondary antibodies from Santa Cruz Biotechnology.

Immunohistochemistry

The eyeballs were enucleated immediately after the animals were killed by CO2 inhalation. A small incision was made in the cornea, and the eyes were immersion fixed with 4% paraformaldehyde in 1× phosphate-buffered saline (PBS) at 4°C for 10 to 30 minutes. The short fixation time is crucial for OPL staining of RGS7 and Gβ5, as previously noted.29 After removal of the cornea and lens, the resulting eye cups were cryoprotected in cold 30% sucrose in 1× PBS, embedded in a freezing medium (Triangle Biomedical Sciences, Durham, NC), and sectioned at −20°C at 20-μm thickness. The sections were blocked for 1 hour at room temperature with 10% goat serum and 0.3% Triton X-100 in 1× PBS (PBT) and incubated with rabbit primary antibodies at 1:100 dilution for 4 to 8 hours at room temperature. Alexa Fluor-conjugated goat anti-rabbit antibody (1:2000 dilution; Invitrogen, Carlsbad, CA) was used as a secondary antibody for protein localization. For co-localization studies using antibodies raised in rabbit, a rabbit IgG labeling kit (Zzen Tricolor, Z25360; Invitrogen) was used according to the manufacturer’s instructions. Briefly, 1 μg of affinity-purified antibody was incubated with the labeling reagents in a molar ratio of 1:3 for 5 minutes at room temperature, then stopped by adding the blocking reagent and incubated for an additional 5 minutes. The entire mixture was adjusted with 10% goat serum in PBT and then used in staining with appropriate dilutions unique to each primary antibody. Extensive washing (five times, 30 minutes each) was performed to minimize the background signal. Fluorescent images were acquired with a confocal microscope at the shared EM and microscopy facility of the Department of Anatomy and Neurobiology, Virginia Commonwealth University (LSM510Meta; Carl Zeiss Microimaging, Thornwood, NY). Unmodified *.lsm files were archived and viewed in a separate computer (LSM Image Browser program; Carl Zeiss Microimaging). Images were then exported as *.tiff files and opened in image-analysis software (Photoshop; Adobe, San Jose, CA) for cropping. No post hoc image processing, such as contrast and brightness adjustments, was performed during or after image acquisition.

Immunoblot Analysis

Retinal extracts (10 μg) were resolved by 12% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 10% dry milk in TBST buffer containing 25 mM Tris (pH 7.5), 137 mM NaCl, and 0.05% Tween-20. For the detection of both forms of Gβ5, CT215 was used at 1:10,000 dilution. To detect RGS7, 7RC-1 or H-190 was used at 1:20,000 and 1:5,000 dilution, respectively. For RGS11, goat anti-RGS11 or VCU008 was used at 1:5000 and 1:2000 dilution, respectively. The anti-GAPDH antibody was used at 1:200,000 dilution and the GAPDH signal was used as an internal lane-loading control. Species-specific secondary antibodies were used at 1:25,000 dilution. The signal was detected by enhanced chemiluminescence with an extended-duration substrate kit (SuperSignal West Dura;
Thermo Fisher Scientific, Rockford, IL), and was captured and quantified using an imaging system with accompanying 1-D image-analysis software (IS440; Eastman Kodak, Rochester, NY).

**Electroretinography**

Before ERG recording, the mice were dark-adapted overnight. They were anesthetized with ketamine/xylazine (150/10 mg/kg; IP), and the pupils were dilated in the dark for a minimum of 10 minutes with topical eye drops of 1% tropicamide and 2.5% phenylephrine (Bausch & Lomb, Tampa, FL). The body temperature was monitored by a rectal probe (Braintree Scientific, Braintree, MA) and maintained at 35°C to 37°C using a plastic heating coil with 43.5°C circulating water. ERG from both eyes was recorded simultaneously (UTAS E-3000 system; LKC Technologies, Gaithersburg, MD) under scotopic conditions, as previously described.23 Typically, the difference in a-wave amplitudes between the two eyes of an animal was <10%. For the study of light sensitivity, responses from 5 to 20 flashes were averaged at different flash intensities. The interstimulus interval (ISI) was changed incrementally from 5 to 180 seconds with increasing stimulus intensity to ascertain the dark-adapted state of the animals. For double-flash recordings, the interval between trials was empirically determined to be >120 seconds. A typical recording session lasted 2 to 3 hours, and booster injections of ketamine/xylazine (one fifth of the initial volume) were given subcutaneously as needed to maintain a steady plane of anesthesia. Because of the length of the recording period, 5 μL of filtered PBS was added every 20 minutes to the surface of the eyes to prevent corneal clouding and cataract formation.

**Determination of the Amplification Factor of Rod Phototransduction**

The amplification factor of rod phototransduction was determined in a data-analysis software (IgorPro; WaveMetrics, Lake Oswego, OR) by fitting the early descending portion of the ERG a-waves initiated by topical eye drops of 1% tropicamide and 2.5% phenylephrine (Bausch & Lomb, Tampa, FL). The body temperature was monitored by a rectal probe (Braintree Scientific, Braintree, MA) and maintained at 35°C to 37°C using a plastic heating coil with 43.5°C circulating water. ERG from both eyes was recorded simultaneously (UTAS E-3000 system; LKC Technologies, Gaithersburg, MD) under scotopic conditions, as previously described.23 Typically, the difference in a-wave amplitudes between the two eyes of an animal was <10%. For the study of light sensitivity, responses from 5 to 20 flashes were averaged at different flash intensities. The interstimulus interval (ISI) was changed incrementally from 5 to 180 seconds with increasing stimulus intensity to ascertain the dark-adapted state of the animals. For double-flash recordings, the interval between trials was empirically determined to be >120 seconds. A typical recording session lasted 2 to 3 hours, and booster injections of ketamine/xylazine (one fifth of the initial volume) were given subcutaneously as needed to maintain a steady plane of anesthesia. Because of the length of the recording period, 5 μL of filtered PBS was added every 20 minutes to the surface of the eyes to prevent corneal clouding and cataract formation.

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**Determination of the Pepperberg Constant**

The recovery phase of rod phototransduction was determined by double-flash techniques as previously described.27 The relationship between ISI (in milliseconds) and 1 – R(t)/Rmax, where R(t) is the a-wave amplitude when t = ISI and the Rmax is the amplitude when ISI = 2000, was determined. The test (first) flash intensity ranged from −0.81, −0.4, −0.03, and 0.38 log cd m−2 and the probe (second) flash intensity was fixed at 1.63. By plotting 1 – R(t)/Rmax versus ISI and ensemble-fitting the data in the analysis software (IgorPro; WaveMetrics) using a customized exponential function: f(x) = 1 – exp(−a·exp(b·x)), where a is a coefficient, b is the time at which there is no recovery, and t is an exponential decay time constant, the time required for 50% recovery is determined for each of the four test flash intensities and used in the Pepperberg plot in which the ISI at 50% recovery (ordinate) vs. the natural log of photoisomerizations (R′) per flash per rod per second (abscissa) was drawn. The slope of the linear regression line revealed the dominant recovery time constant (τr). The number of R′ produced in each test flash was calculated according to the empirically determined conversion factor.

**Figure 1.** The level of R7 RGS proteins in retinas of various genetically modified mouse lines. (A) Western blot analysis of retinal extracts derived from wild-type (lane 1) and GB5+/− (lane 2) mice probed with the following antibodies: CT215 (anti-GB5), VCU008 (anti-RGS11), 7RG-1 (anti-RGS7), and IMG554 (anti-RGS6). (B) Western blot analysis showing the level of R7 RGS proteins in retinal extracts derived from wild-type (lane 1), SG7 (lane 2), RGS11−/− (lane 3), and SG711 (lane 4) mice. Arrows: GAPDH signal used as an internal loading control.
by the targeted mutation of the other. As seen in Figure 3, whereas the overall concentration of Gβ5-S remained unchanged in the RGS11−/− retinal extracts, the RGS7 level increased an average of 60% in the RGS11−/− mice. In contrast, we did not observe detectable changes in the RGS11 level in the SG7 mice (Fig. 1B). The changes in RGS7 levels were specific because the level of Gβ6S was not affected in either the RGS11+/− or the SG7 mice, even though, similar to other R7 RGS members, it was downregulated below detection level in the Gβ5−/− mice (Fig. 1A). These data support a selective, compensatory upregulation of RGS7 in retinas of RGS11−/− mice, which would be predicted for proteins with redundant functions. Failure to observe an increase in RGS11 in SG7 mice may be explained by the incomplete genetic knockout in SG7 mice.

The Decrease of Gβ5-S Level in the SG711 Mice

RT-PCR analysis of RNA derived from homozygous RGS7 mutant mouse retinas revealed RGS7 transcripts with exon-10 deletion, which leads to a loss of 33 amino acids (S229-Q261) with the first 27 residues in the interdomain and the last 6 residues in the GGL domain required for interaction with Gβ5-S. This truncated RGS7 protein was not stable when ectopically expressed in insect H5 cells (data not shown). However, in the retina, the truncated RGS7 were detectable and appeared as multiple bands in the immunoblots (Figs. 1, 4). Most of the truncated RGS7 bands migrate faster than the wild-type RGS7 when longer electrophoretic time was used

**FIGURE 2.** Co-localization of RGS7 and -11 at the tips of DBC dendrites. A maximum projection transformation of a 3.2-μm z-stacked confocal image of the OPL of a GGFP5 mouse retinal section is shown. The retinal section was stained with two rabbit polyclonal antibodies, H190 (anti-RGS7) and VCU008 (anti-RGS11), in conjunction with the rabbit IgG labeling kit. Most cone terminals in the GGFP5 retina are visible under direct EGFP fluorescence. Both RGS7 and -11 are expressed in dendritic tips of DBCs and virtually all tips express both proteins. Scale bar, 5 μm.

**RESULTS**

**Coexpression of RGS7 and -11 in the Dendritic Tips of DBCs**

We and others have reported that RGS7 and -11 proteins localize to the tips of DBC dendrites.10 However, the degree to which they co-localize in individual dendritic tips has not been assessed. Using the rabbit IgG labeling kit (Zeon; Invitrogen), we investigated the distribution patterns of RGS7 and -11 in retinal OPL using two well-characterized rabbit antibodies against RGS7 (7RC-1)20,22 and RGS11 (VCU008, see the Methods section). The specificity of the two antibodies was verified by using retinal extracts derived from wild-type, Gβ5−/−, and respective gene-targeted mice (Fig. 1). Immunofluorescence with these two antibodies revealed that RGS11 and -7 are co-localized to the tips of bipolar cell dendrites (Fig. 2). Because RGS7 and -11 both require interaction with Gβ5-S for stability,19 the co-localization suggests a possible redundant function for these two R7 RGS proteins at the tips of DBC dendrites.

**Upregulation of RGS7 in the RGS11 Knockout Retinas**

Because the localization of RGS7 and -11 overlaps in the OPL, we examined how the level of each RGS proteins is influenced...
We have frequently obtained more than one band of RGS7 by immunoblots, and similarly we have found more than one RGS7 transcript by RT-PCR. The relationship between the multiple transcripts and the proteins is still under investigation.

To compare the levels of the normal and mutant RGS7 in wild-type and SG7 mice, we took into account the ensemble signals from all bands and found an average 50% reduction in the level of truncated RGS7 in SG7 mice when compared with full-length RGS7 in wild-type, suggesting that the mutant RGS7 protein is also less stable in the retina. These results did not depend on the nature of the antibody used since similar results were obtainable with an independent, commercially available RGS7 antibody, H-190, and a custom-made antibody named VCU015, which targets a C-terminal peptide of mouse RGS7 (data not shown). Thus, these results support the conclusion that RGS7 is an unstable, truncated protein in retinas of SG7 mice.

We next examined how the level of retinal Gβ5-S protein changes when both RGS7 and -11 genes are targeted. Figure 5 shows that the Gβ5-S protein level decreased by ~30% in SG711 mice. However, the reduction of Gβ5-S in the SG711 retina is not the result of the elimination of Gβ5-S in the OPL as it can still be found at the dendritic tips of DBCs in SG711 mice (data not shown). We reason that the truncated RGS7 protein lacking S229 to Q261 can still weakly associate with Gβ5-S to protect it from degradation.

**Comparable Phototransduction Properties in the Control and Mutant R7 RGS Mice**

The abundant expressions of all members of R7 RGS proteins in the retina indicate their involvement in various retinal responses. To assess whether the loss of RGS11 protein or the truncation of RGS7 protein affects phototransduction, we recorded and analyzed ERG a-waves from these mice and determined the two major photoreceptor-derived properties, the amplification factor and the dominant recovery time constant (Table 1). We found similar maximum a-wave amplitude, amplification factor, and dominant recovery time constant (the Pepperberg constant) among the different genotypes. Therefore, we conclude that photoreceptor functions are normal when both RGS7 and -11 are targeted. This finding is consistent with the fact that RGS9-1 is the major R7 RGS protein in photoreceptors.

![Figure 4](image_url)

**Figure 4.** Exon10 deletion of RGS7 reduced both the size and the level of RGS7 in SG7 mouse retinas. (A) A representative immunoblot showing the level of the truncated mutant RGS7 protein in SG7 versus WT mouse retinas. (B) Quantification of the level of reduction in RGS7 using GAPDH signal as a normalization factor \( n = 8 \). A significant 50% decrease of overall RGS7 level was found in the SG7 retinas, whereas the level of RGS11 remained unchanged (data not shown).

![Figure 5](image_url)

**Figure 5.** Downregulation of Gβ5-S in SG711 mouse retinas. (A) Representative immunoblots showing the level of Gβ5-S and -L in serially diluted retinal extracts (10, 5, and 2.5 µg protein/well from high to low) derived from WT and SG711 mice. (B) Quantification of Gβ5-S versus Gβ5-L signals between WT and SG711 mice \( n = 8 \). A significant ~30% decrease in Gβ5-S level is found in SG711 mice.
Despite the normal photoreceptor functions, we noticed a clear difference in ERG responses between the control, RGS11+/−/− and SG711 mice. The ERG b-wave implicit time significantly increased in the RGS11+/−/− and SG711 mice, whereas the overall b-wave amplitudes remained similar among the four genotypes (Figs. 6, 7; Table 1). Notably, the rise time of the ERG b-wave in the RGS11+/−/− mice was delayed at all flash intensities tested when compared with the wild-type control (Fig. 7A). In addition, further delays in the ERG b-wave implicit time were observed in the SG711 mice, especially when flash strength was above 1 cd s m−2, an intensity that elicits mixed rod/cone responses (Fig. 7A). Because we found

<table>
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<th>Wild-Type</th>
<th>SG7</th>
<th>RGS11+/−/−</th>
<th>SG711</th>
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<td>a-Wave amplitude, μV*</td>
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<td>51 ± 6 (n = 6)</td>
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<td>946.4 ± 50.6 (n = 13)</td>
<td>881.9 ± 84.8 (n = 13)</td>
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* Elicited by a flash intensity of 245 cd s m−2.
† Mean ± SEM (sample size).
‡ The amplification factor of phototransduction, obtained by curve-fitting the descending arms of a-waves according to the Lamb and Pugh24 model.
§ The dominant recovery time constant determined by Pepperberg plots.
¶ P < 0.05 as compared to wild-type, Tukey HSD test.
# P < 0.05 as compared to wild-type, SG7, and RGS11+/−/−; Tukey HSD test.

**Prolonged ERG b-Wave Implicit Time in the RGS11+/−/− and SG711 Mice**

An elastic sequence in ERG responses between the control, RGS11+/−/−, and SG711 mice. The ERG b-wave implicit time significantly increased in the RGS11+/−/− and SG711 mice, whereas the overall b-wave amplitudes remained similar among the four genotypes (Figs. 6, 7; Table 1). Notably, the rise time of the ERG b-wave in the RGS11+/−/− mice was delayed at all flash intensities tested when compared with the wild-type control (Fig. 7A). In addition, further delays in the ERG b-wave implicit time were observed in the SG711 mice, especially when flash strength was above 1 cd s m−2, an intensity that elicits mixed rod/cone responses (Fig. 7A). Because we found
deletion may affect GGL domain function, which is essential for Gβ5 interaction and protein stability.\textsuperscript{17,19} Second, we found that the truncated RGS7 protein is less stable with approximately 50\% of the wild-type level in retinas of SG7 mice (Fig. 4). Together, these data suggest that deletion of the 33 amino acids results in a partial loss-of-function RGS7 protein in vivo. This loss-of-function hypothesis is consistent with the measurable behavioral phenotypes of SG7 mice (available online from the Jackson Laboratory, Bar Harbor, ME at http://www.informatics.jax.org/external/ko/lexicon/1011.html), which includes hypoactivity and decreased exploratory responses in the open field. Of interest, RGS11\textsuperscript{-/-} mice show no phenotype in the open-field test.

The shortcoming of using a partial loss-of-function mutant such as the SG7 mouse is that the incomplete deletion limits the conclusions that can be drawn regarding the role of RGS7. For example, we found that SG7 mice generated in this study do not exhibit an obvious weight defect during early postnatal development (data not shown) as reported for Gβ5\textsuperscript{-/-} mice.\textsuperscript{19} Because the SG7 mouse is not a null, we cannot conclude whether the weight defects of Gβ5\textsuperscript{-/-} mice are the consequence of simultaneous loss of RGS7 and -11. Similarly, we find it difficult to determine the exact contribution of RGS7 in the generation of ERG b-wave because there is no significant difference in response kinetics between SG7 and control mice. Therefore, efforts to generate and characterize a true RGS7 null mouse line are necessary for a firmer understanding of RGS7 function. Despite this gap, the significant differences in ERG responses in RGS11\textsuperscript{-/-} and SG711 mice when compared with control mice (Figs. 6, 7) strongly support the involvement of both RGS11 and -7 in the generation of a normal ERG b-wave.

**Functional Redundancy of RGS7 and -11**

We and others have reported the expression of various RGS proteins including RGS7, RGS11, and Ret-RGS1 in DBCs.\textsuperscript{8–10} The lack of an ERG b-wave in Gβ5\textsuperscript{-/-} mice, in which all R7 RGS proteins are downregulated, suggests that Gβ5/S/RGS7 and Gβ5/S/RGS11 may be the physiological GAP for Goa1 in DBCs. We found that RGS7 and -11 coexist at the OPL (Fig. 2), pointing to functional redundancy of these two proteins at the tips of DBC dendrites. Because both RGS7 and -11 depend on their interaction with Gβ5/S for stability, one would expect a compensatory change in protein levels when either RGS is inactivated. Indeed, we found that the RGS7 protein level increased significantly in the RGS11\textsuperscript{-/-} retina (Figs. 1, 3). Unfortunately, we could not test the converse, because the SG7 mouse is not a null. Despite this limitation, we provide strong support for functional redundancy between RGS7 and -11 in the ERG responses of these mice. The ERG b-wave responses of RGS11\textsuperscript{-/-} and SG711 mice showed a significantly delayed rise time, even though the SG7 mice showed no difference from the WT controls. Furthermore, our data suggest that RGS11 is the major RGS protein in the dendritic tips of DBCs because the delay in ERG b-wave is seen at all flash intensities tested in RGS11\textsuperscript{-/-} mice, despite a compensatory increase in RGS7 (Figs. 1, 3).

**Mechanism of RGS Modulation of ERG b-Wave**

In the dark, mGluR6 at the dendritic tips of DBCs is nearly saturated by glutamate\textsuperscript{30} and the ERG b-wave reflects the light-induced reopening of cation channels in DBCs.\textsuperscript{31} Thus, the observed increase in implicit time of the ERG b-wave of RGS11\textsuperscript{-/-} mice suggests a defect in the shutoff phase of the mGluR6/Goa1 signaling pathway in DBCs. This unique ERG phenotype was further augmented in SG711 mice (Figs. 6, 7), in which only the truncated and partial loss-of-function RGS7
protein was present in the OPL. Because mutant RGS7 by itself had no effect, but exacerbated the effect of RGS11 inactivation on ERG b-wave responses, we conclude that both RGS7 and -11 are involved in the mGluR6/Gαo1 signaling pathway in DBCs and that they each function in the deactivation of Gαo1. However, it is noteworthy that the time course of the ERG b-wave in S711 mice (in milliseconds) was still much faster than the GTP turnover rate of Gαo1 in (seconds) assayed in vitro. Because several members of the RGS family are capable of accelerating GTP hydrolysis by Gαo1 in vitro, 10, 17, 52 the specific impact of RGS7 and/or -6 proteins on Gαo1 individually or in combination with other relevant RGS knock-out mice. Although an explanation for the missing ERG b-wave in Gα5−/− mice remains elusive, this study clearly identified an overlapping expression pattern of RGS7 and -11 at the OPL and altered ERG responses in RGS11−/− and S711 mice. Together, these data firmly establish a redundant involvement of RGS7 and -11 in light-evoked responses of retinal ON-bipolar cells.

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