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Loss of $G_{q/11}$ Genes Does Not Abolish Melanopsin Phototransduction

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Abstract

In mammals, a subset of retinal ganglion cells (RGCs) expresses the photopigment melanopsin, which renders them intrinsically photosensitive (ipRGCs). These ipRGCs mediate various non-image-forming visual functions such as circadian phototransduction and the pupillary light reflex (PLR). Melanopsin phototransduction begins with activation of a heterotrimeric G protein of unknown identity. Several studies of melanopsin phototransduction have implicated a G-protein of the $G_{q/11}$ family, which consists of $Gna11$, $Gna14$, $Gnaq$, and $Gna15$, in melanopsin-evoked depolarization. However, the exact identity of the $G_{q/11}$ gene involved in this process has remained elusive. Additionally, whether $G_{q/11}$ G-proteins are necessary for melanopsin phototransduction in vivo has not yet been examined. We show here that the majority of ipRGCs express both $Gna11$ and $Gna14$, but neither $Gnaq$ nor $Gna15$. Animals lacking the melanopsin protein have well-characterized deficits in the PLR and circadian behaviors, and we therefore examined these non-image forming visual functions in a variety of single and double mutants for $G_{q/11}$ family members. All $G_{q/11}$ mutant animals exhibited PLR and circadian behaviors indistinguishable from WT. In addition, we show persistence of ipRGC light-evoked responses in $Gna11^{–/–}$; $Gna14^{–/–}$ retinas using multielectrode array recordings. These results demonstrate that $G_{q}$, $G_{14}$, $G_{16}$, or $G_{15}$ alone or in combination are not necessary for melanopsin-based phototransduction, and suggest that ipRGCs may be able to utilize a $G_{q/11}$-independent phototransduction cascade in vivo.

Introduction

Intrinsically photosensitive retinal ganglion cells (ipRGCs) comprise a distinct subset of retinal ganglion cells (RGCs) and express the photopigment melanopsin (Opn4) [1]. ipRGCs constitute the sole conduit of light information from the retina to non-image forming visual centers in the brain and are responsible for driving a variety of behaviors [2,3]. These behaviors include circadian photentrainment, which is the process by which the circadian clock is aligned to the environmental light-dark cycle, and the pupillary light reflex (PLR), in which the area of the pupil changes in response to changes in light intensity.

Despite the well-established role for ipRGCs and melanopsin in the regulation of non-image forming visual functions, little is known about the molecular components of melanopsin phototransduction. Previous research has suggested that ipRGCs likely utilize a phototransduction pathway similar to that used in Drosophila microvillar photoreceptors [1,4], in which the activated opsin stimulates a $G_{q/11}$ protein. In Drosophila, the $z$-subunit of the $G_{q/11}$ protein activates phospholipase C- $\beta$ (PLC- $\beta$), which results in the opening of TRP and TRPL channels allowing Na$^{+}$ and Ca$^{2+}$ to flow into the cell resulting in depolarization of the rhabdomere in response to light [5,6].

Homologs of the components of the Drosophila phototransduction pathway are found in mice. Specifically, there are four $G_{q/11}$ genes ($Gnaq$, $Gna11$, $Gna14$, and $Gna15$), four PLC-$\beta$ genes (PLC-$\beta$-1 – $\beta$-4), and seven TRPC channel genes (TRPC-1–7). The tandemly duplicated $Gna15$ and $Gna11$ genes are linked to mouse chromosome 10 [7,8], and $Gnaq$ and $Gna14$ colocalize to mouse chromosome 19 [9]. To date, there have been several electrophysiological studies implicating $G_{q/11}$, PLC-$\beta$-, and TRPC genes in ipRGC phototransduction [4,10,11]. However, there have been no functional studies investigating the identity of the $G_{q/11}$ protein utilized by melanopsin in vivo or any studies of the effects of the loss of any presumptive ipRGC phototransduction genes on behavior. In this study, we sought to determine the identity(ies) of the $G_{q/11}$ protein(s) utilized for melanopsin phototransduction in vivo.

We performed single-cell RT-PCR on individual ipRGCs to determine which of the genes were expressed in ipRGCs and if there was heterogeneity in their expression among the ipRGC population. Similar to previous studies, we found that the majority of ipRGCs express both $Gna11$ and $Gna14$, but not $Gnaq$ or $Gna15$. Since loss of the melanopsin protein results in well-characterized deficits in the pupillary light reflex and circadian behaviors, we examined these non-imaging forming visual functions in $Gna11^{–/–}$; $Gna14^{–/–}$ ($Gna11$; $Gna14$ DKO) mice and $Gnaq^{–/–}; Gna11^{–/–}$; $Gna14^{–/–}$ ($Gna11$; $Gna14$; $Gnaq$ DKO) mice as well as several single $G_{q/11}$ gene knockouts [9,12–14]. All genotypes examined exhibited non-image forming visual functions indistinguishable from WT. Furthermore, multielectrode array recordings revealed...
no deficits in ipRGC light responses in Gna11; Gna14 DKO animals. Contrary to previous work, this study indicates that ipRGCs may be able to utilize a Gq/11-independent phototransduction cascade in vivo.

Results

Gna11 and Gna14 are expressed in ipRGCs

Previous reports have shown that Gq/11 genes are expressed in ipRGCs. However, there has been disagreement regarding which Gq/11 genes are actually expressed, with one study reporting heterogeneous expression of each of the four Gq/11 genes and another reporting primarily Gna14 and some Gna11 expression [4,15]. We therefore sought to definitively identify which Gq/11 genes are expressed in ipRGCs. We isolated individual ipRGCs by dissociating retinas of Opn4Cre+/−;Z/EG mice, in which ipRGCs are labeled with GFP, and picking individual ipRGCs with a microneedle. We specifically chose to utilize retinas from P1 and P4 mice since there is GFP labeling of some cones in adult Opn4Cre+/−; Z/EG mice [16]. By RT-PCR, we confirmed that the 32 isolated cells expressed melanopsin (Figure 1A, F-H), and then screened those 32 melanopsin-expressing cells for the four Gq/11 genes (Figure 1B-H). 23 of the 32 ipRGCs expressed both Gna11 and Gna14, and an additional 6 cells expressed either Gna11 or Gna14 (Figure 1F-H). Neither Gnaq nor Gna15 were detected in any of the melanopsin-expressing cells, and 3 melanopsin-expressing cells had no detectable levels of any Gq/11 gene (Figure 1F-H).

Loss of Gq/11 genes does not affect non-image forming visual functions

Mice that lack melanopsin phototransduction due to loss of melanopsin have several well characterized deficits in non-image forming visual behaviors including defects in the PLR at high light intensities and a deficit in circadian period lengthening in response to constant light. Since Gna11 and Gna14 were the only Gq/11 genes identified as being expressed in ipRGCs and nearly all cells tested expressed at least one, we produced Gna11−/−; Gna14−/− (Gna11; Gna14 DKO) mice from previously published single knockouts [14,17,18]. We recorded the pupillary light reflex of 4–6 month old WT (n = 16), Opn4Cre+/−;Z/EG (MKO; n = 7), Gna11−/− (Gna11 KO; n = 4), Gna14−/− (Gna14 KO; n = 5), Gna15−/− (Gna15 KO; n = 7), Gnaq−/−;Gna11−/−;Gna14−/− (Gnaq; Gna11 DKO; n = 9), and Gna11−/−;Gna14−/−;Gna15−/−;Z/EG (Gna11; Gna14; Gna15 DKO; n = 7) at both low and high light intensities (Figure 2). Consistent with previous studies [19], MKOs exhibited deficits at high light intensities. Surprisingly, all mice mutant for Gq/11 genes were indistinguishable from WT animals at both low and high light intensities (Figure 2).

We also recorded wheel-running activity in 4–6 month old WT (n = 14), MKO (n = 9), Gna15 KO (n = 7), Gnaq; Gna11 DKO (n = 8), and Gna11; Gna14 DKO (n = 7) mice to measure the daily activity rhythms of these mice (Figure 3). We conducted these measurements under three different conditions: a 12:12 light/dark cycle, constant darkness, and constant light. We also administered a 15-minute light pulse in constant darkness to determine the amplitude of the light-evoked circadian phase shifts in each mouse line. All genotypes were able to photoentrain to the 12:12 light/dark cycle. All mutant lines exhibited a normal circadian period under constant darkness (WT: 23.85 ± 0.36 hours, MKO: 23.68 ± 0.26 hours, Gna13 KO: 23.84 ± 0.08 hours, Gnaq; Gna11 DKO: 23.83 ± 0.10 hours, and Gna11; Gna14 DKO: 24.01 ± 0.24 hours) (Figure 3A, B). All mice phase shifted normally to a light pulse presented at CT15. We observed no deficits in phase delay among any genotypes tested (WT: 1.40 ± 0.78 hours, MKO: 1.45 ± 0.49 hours, Gna13 KO: 1.96 ± 0.84 hours, Gnaq; Gna11 DKO: 1.85 ± 0.74 hours, and Gna11; Gna14 DKO: 1.25 ± 0.65 hours) (Figure 3A,C). Melanopsin knockout animals have well-characterized deficits in circadian period lengthening under constant light [20] that we confirmed here (WT period: 25.09 ± 0.17 hours, MKO period: 23.75 ± 0.20 hours) (Figure 3A, D). In contrast, Gna13 KO (24.99 ± 0.20 hours) and Gna11; Gna14 DKO (24.66 ± 0.42 hours) were indistinguishable from WT mice. While Gnaq; Gna11 DKO animals (24.34 ± 0.5 hours) did show a significantly shorter period than WT animals, the period was still significantly longer than MKO animals (Figure 3A, D).

ipRGC light responses persist in Gna11; Gna14 double knockouts

The lack of behavioral deficits in Gq/11 mutant animals led us to examine whether melanopsin phototransduction is perturbed at the cellular level in these lines. We therefore examined the light responses of ipRGCs in isolated retinas of WT and Gna11; Gna14 DKO mice using multielectrode array (MEA) recordings. We recorded from retinas of postnatal day 3 mice, since it has been shown that outer retinal signaling to ganglion cells is not present at early postnatal ages [21], and thus ipRGCs constitute the only light-responsive ganglion cells at this age [22,23]. Nonetheless, to guarantee that all detected light responses were from ipRGCs, we included a cocktail of synaptic blockers in the Ames’ medium to inhibit all glutamatergic, GABAergic, and glycinegic signaling to ipRGCs. Additionally, we included cholinergic blockers to minimize interference from retinal waves present at this developmental stage [24]. Retinas were dark adapted for at least 20 min and then stimulated with diffuse, uniform light of both low (7 x 10^12 photons/cm² · sec) and high light intensity (7 x 10^13 photons/cm² · sec) for 60 sec at 480 nm, the peak wavelength for melanopsin activation [25,26]. We also stimulated the retinas with bright white light (267 mW/cm²). The retinas were allowed to readapt to dark for 5 min between stimulations. Figure 4A shows representative voltage traces of typical ipRGCs in WT and Gna11; Gna14 DKO mice at both low and high light intensity. We found that Gna11; Gna14 DKO ipRGCs were indistinguishable from the WT controls. ipRGCs in both WT and Gna11; Gna14 DKO mice responded to increasing light intensities with increased spiking (Figure 4B) that reached maximum levels several seconds following light onset. After light offset, ipRGCs continued to spike for as long as 20 seconds (Figure 4A, C, D). These slow dynamics are consistent with previous descriptions of melanopsin-dependent light responses [23,27–29]. These data show that despite the fact that Gna11 and Gna14 are the only Gq/11 genes expressed in ipRGCs, they are not required for melanopsin phototransduction.

Other Gq/11 genes are up-regulated in single and double Gq/11 knockouts

Since Gna11 and Gna14 were the only Gq/11 genes detected in ipRGCs, we were surprised that Gna11; Gna14 DKO mice did not recapitulate any of the phenotypes observed in melanopsin knockout animals. To test whether removal of one or two Gq/11 genes results in upregulation of other Gq/11 family members, we performed quantitative RT-PCR on RNA extracted from the retinas of mutant mice (Figure 5). We measured the mRNA levels of Gnaq, Gna11, Gna14, and Gna15 in WT, Gna14 KO, Gna15 KO, Gnaq; Gna11 DKO, and Gna11; Gna14 DKO mice. We found that all animals had levels of Gnaq mRNA that were indistinguishable from WT (Figure 3A). It is important to note that in Gnaq; Gna11 DKO mice, Gnaq is conditionally knocked-out in ipRGCs (Gnaq0.26).
Gna11-/-; Opn4Cre/+; therefore, we did not expect a significant reduction in whole retinal Gnaq mRNA in these mutants. Gna14 KO and Gna15 KO s exhibited normal levels of Gna11 mRNA, while, Gnaq; Gna11 DKO s, and Gna11; Gna14 DKO s had undetectable levels (Figure 5B). Levels of Gna14 mRNA were reduced in Gna14 KO and Gna11; Gna14 DKO s, but increased in

Figure 1. Gna11 and Gna14 are expressed in ipRGCs, often in combination. A–E: Representative images of RT-PCR analysis of single ipRGCs for Opn4, Gnaq, Gna11, Gna14, and Gna15. All representative gels show RT-PCR analysis of single ipRGCs taken from P1 Opn4Cre/+; Z/EG mice. Each lane represents one cell, the positive control is whole retinal RNA, and the negative control is water. F–G: Summary of expression of Gq/11 family members in the 16 ipRGCs obtained from P1 and P4 Opn4Cre/+; Z/EG mice. All cells expressed melanopsin. 15 cells expressed Gna11, 10 of which also expressed Gna14. H: Venn diagram showing the distribution of Gq/11 family member expression in all 32 ipRGCs sampled.

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Gnaq; Gna11 DKO (Figure 5C). Gna14 mRNA levels are not abolished in Gna14 KO and Gna11; Gna14 DKOs because the Gna14 KO line was created by knocking a neo cassette into exon 3 of the gene. This removes part of exon 3 and results in a frameshift. Thus, while, mRNA is still produced from the Gna14 locus in Gna14 KO, it encodes a nonsense protein. Additionally,
Figure 3. \(G_{\alpha 11}\) mutant lines exhibit circadian behaviors indistinguishable from WT. A. Representative actograms of wheel running activity from WT (14 animals), MKO (9 animals), \(Gna15\) KO (7 animals), \(Gnaq;Gna11\) DKO (8 animals), and \(Gna11;Gna14\) DKO (7 animals) mice under a 12:12 LD cycle, constant darkness, and constant light. The white background indicates light, grey background indicates darkness, and the yellow asterisk indicates a 15-minute light pulse at circadian time (CT) 15. All mice photoentrained to the LD cycle. B. Quantification of free-running period under constant dark conditions. All animals exhibited circadian periods indistinguishable from WT. One-way ANOVA with Tukey post-hoc analysis. Error bars represent s.e.m. C. Quantification of phase shifting to a 15-minute light pulse given at CT 15. All animals exhibited phase shifting indistinguishable from WT. One-way ANOVA with Tukey post-hoc analysis. Error bars represent s.e.m. D. Quantification of free running period under constant light. As previously reported, MKO mice exhibited reduced lengthening of their circadian period under constant light conditions. \(Gnaq;Gna11\) DKO exhibited a slight reduction in the lengthening of their circadian period in constant light, and their period length was significantly different from both WT and MKO. \(Gna15\) KO and \(Gna11;Gna14\) DKO exhibited lengthened periods that were indistinguishable from WT. One-way ANOVA with Tukey post-hoc analysis. Error bars represent s.e.m.

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as indicated by the reduced mRNA levels, the mutant transcript is degraded. Gna15 mRNA was undetectable in Gna15 KOs, but increased in Gna14 KOs, Gnaq; Gna11 DKOts, and Gna11; Gna14 DKOts (Figure 5D). These data indicate there is upregulation of other Gq/11 genes in some Gq/11 knockout lines; however, it remains unknown whether the upregulation occurs in ipRGCs and if such upregulation would be sufficient to drive melanopsin phototransduction.

Discussion

In this study, we provide the first investigation of the melanopsin phototransduction pathway in vivo. We determined that genetic inactivation of the Gq/11 proteins that are normally expressed in ipRGCs does not abolish melanopsin-dependent behaviors or electrophysiological responses. Specifically, we found that no tested Gq/11 knockout line exhibited the behavioral deficits observed in melanopsin knockout mice. All tested Gq/11 mutant lines exhibited circadian behaviors and pupillary light reflexes that were indistinguishable from WT mice. Additionally, using single-cell RT-PCR for Gq/11 genes in ipRGCs, we found only expression of Gna11 and Gna14, often expressed together. However, using multielectrode array we detected no changes in intrinsic light responses of ipRGCs in Gna11; Gna14 DKO compared to WT controls.

Previous reports have shown expression of Gq/11 genes in ipRGCs although there were inconsistencies as to which Gq/11 genes were detected [4,15]. Specifically, in Graham et al. the authors used single cell RT-PCR and determined that expression of all four Gq/11 genes can be detected in ipRGCs, although expression was heterogeneous among the cells sampled and Gna14 was detected in the majority of cells [4]. Siegert et al. examined ipRGCs as a population and reported expression of Gna11 and Gna14 [15], which is consistent with our findings here. However, neither of these studies investigated the function of ipRGCs in the absence of any of these specific genes and in fact Siegert et al. observed the expression of other heterotrimeric G proteins [15].

Electrophysiological investigations of ipRGC phototransduction have supported the involvement of the Gq/11 pathway. Specifically, Xue et al. showed that melanopsin phototransduction is substantially reduced in the absence of Plc-β4 [10]. In agreement with work from Perez-Leighton et al., Xue and co workers additionally showed that loss of both Trpc6 and 7 virtually abolished the melanopsin-dependent photoresponse suggesting that Trpc6 and 7 function in a combinatorial fashion [10,11]. Since Gq/11 family members are defined based on their ability to activate PLC, it is reasonable to predict that if Plc-β is a critical component of melanopsin phototransduction then there must also be a member of Gq/11 family involved. This prediction was supported with the use of pharmacological inhibitors of the Gq/11 family on dissociated ipRGCs [4]. However, here, we show that in vivo, mice mutant for Gq/11 family members do not exhibit the behavioral deficits indicative of a loss of melanopsin-dependent light responses.

Several possibilities exist to explain these discrepancies. One is that Gq/11 signaling is not required for melanopsin phototransduc-
Siegert et al. observed expression of other heterotrimeric G proteins [15] and thus melanopsin could activate a G_i or G_o protein, as has been observed in vitro [30], the dissociation of which could result in the beta/gamma subunit activating PLC-b4 as has been observed with PLC-b1 and 3 [31]. Another possibility is that there is compensatory upregulation from other remaining Gq/11 family members in the tested mutant lines. Our data supports this possibility since Gna14 and Gna15 were upregulated in Gnaq; Gna11 DKOs. Also, Gna15 was upregulated in Gna14 knockouts; although, the increase in Gna15 expression was not significant in Gna11; Gna14 DKO mice. Normalized to levels of 18S RNA. (N = 3 mice for each; 2 retinas per RNA sample). * indicates P < 0.05 by one-way ANOVA with Tukey post-hoc analysis. Error bars represent s.e.m.

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Figure 5. Gq/11 family members are upregulated in the retinas of some Gq/11 mutant lines. A–D: Expression levels of Gnaq, Gna11, Gna14, and Gna15 in the retina relative to WT in Gna14 KO, Gna15 KO, Gnaq; Gna11 DKO, and Gna11; Gna14 DKO mice. Normalized to levels of 18S RNA.

It is important to note that ipRGCs are not a homogeneous population and ipRGC subtypes (termed M1–M5) have stereotyped yet distinct electrophysiological light responses. Thus, it is possible there is variability in the components of the melanopsin phototransduction cascade among ipRGC subtypes. The study showing that ipRGCs have a severe reduction in their intrinsic light responses in mouse lines mutant for Plc-b4^{−/−} and Trpc6/7^{−/−} ipRGCs [10] only examined the M1 ipRGC subtype, and in Trpc6 mutant mice, both M1 and M2 ipRGCs show some deficits in melanopsin-dependent light responses [11]. While M1 ipRGCs are the predominant subtype mediating circadian behaviors, non-M1 ipRGCs may contribute to the PLR [16]. It remains unknown whether the intrinsic responses of other ipRGC subtypes are affected in Trpc6/7 and Plc-b4^{−/−} mice.

Because we picked single cells for RT-PCR at a developmental time, we could not be certain whether we were picking M1 or non-M1 ipRGCs. A careful analysis of the
phototransduction in M1 versus non-M1 ipRGCs has interesting functional and evolutionary implications.

Materials and Methods

Ethics Statement
All protocols, animal housing, and treatment conditions were approved by the Johns Hopkins University Animal Care and Use Committee (JACUC).

Animal Models
All mice were of a mixed background (C57BL/6;129SvJ). Melvin Simon at University of California San Diego generously provided Gnaqflx/flx; Gna11−/− and Gna14−/− animals [13,14,17], and Thomas Wilkie at University of Texas Southwestern generously provided Gnaq15−/− animals [12]. Gnaqflx/flx; Gna11−/− mice were crossed into our Opn4Cre/+ line to produce Opn4Cre/+; Gnaqflx/flx; Gna11−/− mice. Gnaqflx/flx; Gna11−/− mice were also crossed with Gna14−/− mice to produce Gna11−/−; Gna14−/− animals.

Single Cell RT-PCR
Single ipRGCs were isolated from Opn4Cre/+; Z/EG mice following the protocol described in [32]. Reverse transcription of the RNA from single cells from P1 and P4 retinas, and amplification of the cDNA was performed as described in [32]. The following primers were designed to amplify from the 3′ end of the transcript and used to detect phototransduction components in the resulting amplified cDNA obtained from single ipRGCs: Melanopsin (F: CTTTGCTGGATACCTGCACA; R: CAGGCACCTTGGAAGTCTTA), Gnaq (F: GTTGCGATCCCGACTACAGG; R: GTGTCAGTCCGCAAGACATT), Gna11 (F: GTACCGGTGGGAGTCTTA), Gna14 (F: CCATTCGACCTGGAAAACAT; R: CGATCGAGTATGAA), and 18S RNA (F: CGCCGCTAGAGGTAG; R: CAGCCAAACACAAAGCGGATA), Gna15 (F: TGACCGAGTATGACAGTGTC; R: CAGGTTGATCTCGCACA).

Pulpiomometry
Pulpiom experiment were performed on unanesthetized mice that were restrained by hand. WT (16 animals), MKO (7 animals), Gna11 KO (4 animals), Gna14 KO (5 animals), Gnaq; Gna11 DKO (9 animals), Gnaq; Gna11 DKO (7 animals), Gnaq; Gna15 KO (7 animals), Gnaq; Gna15 DKO (9 animals), and Gnaq; Gna11; Gna14 DKO (7 animals) were kept on a 12 hour:12 hour light:dark cycle and given at least 30 minutes to dark-adapt between stimulations. All experiments were performed during the animals’ day (ZT2-10). The contralateral eye was stimulated with 474-nm LED light for 2 min, after being in constant darkness for 18 days. Following each animal was exposed to a light pulse (500 lux; CT15) for 15 min, after being in constant darkness for 4 days. Following constant darkness, all mice were also placed in constant light (500 lux) for 18 days.

Wheel Running Behavior
Mice were placed in cages with a 4.5-inch running wheel, and their activity was monitored with VitalView software (Mini Mitter), and cages were changed at least every 2 weeks.

WT (14 animals), MKO (9 animals), Gna15 KO (7 animals), Gnaq; Gna15 DKO (8 animals), and Gnaq; Gna14 DKO (7 animals) mice were placed in 12:12 LD for 17 days followed by constant darkness for 26 days. For phase-shifting experiments, each animal was exposed to a light pulse (500 lux; CT15) for 15 min, after being in constant dark for 18 days. Following constant darkness, all mice were also placed in constant light (500 lux) for 18 days.

Quantification of circadian behavior
All free-running periods were calculated with ClockLab (Actimetrics) using the onsets of activity on days 10–17 of constant darkness similar to [3]. Phase shifts were calculated similar to [3] and described as follows: an onset for the day after the light pulse was predicted based on the onsets of the previous 7 days. Phase shifts were then determined based on the difference between the predicted onset and the shifted onset on the day after the light pulse. For all animals, the free-running period in constant light was measured with ClockLab (Actimetrics) using the onsets of activity on days 3–10 of constant light. Some animals (2 WT, 1 Gna15 KO, 2 Gnaq; Gna11 DKO, 1 Gna11; Gna14 DKO, and 1 MKO) reduced their activity so much that an accurate period could not be measured and they were thus excluded.

Multielectrode Array Recordings
Multielectrode array recordings, light stimulation, and data analysis were performed as described in [11]. Briefly, retinas were dissected from P3 pups from WT and Gna11; Gna14 DKO animals and mounted ganglion cell side down on the array. Retinas were superfused with Ames’ Medium (Sigma) and synaptic blocker cocktail oxygenated with 95%/5% Oxygen/CO2. Synthetic blocker cocktail consisted of: 250 µM DL-2-amino-4-phosphonobutyrate; 10 µM 6,7-dinitroquinoxaline (DNQX, 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid); 0.3 µM strychnine, 50 µM picrotixin, and 10 nM (-)-epibatidine dihydrochloride. All reagents were purchased from Tocris (Ellesville, MO, USA). Spike sorting was performed using MC-Rack v 4.00 software (Multi Channel Systems) and analyzed offline with Offline Sorter v 2.8.6 software (Plexon Inc, Dallas, TX, USA).

Q-RT-PCR
Retinas were dissected from WT, Gnaq; Gna14 KO, Gnaq; Gna11 DKO, and Gnaq; Gna11; Gna14 DKO (N = 3 mice for each; 2 retinas per RNA sample). RNA was extracted from the retinas using an RNaseasy mini kit (Qiagen; cat# 74104), and the levels were normalized to 18S RNA. The following primers were used: Gnaq (F: AATCTAGTTATCTCCACCTAGTCG; R: GGTTCAAGTCCGCAAAGTACCT), Gna11 (F: TCTCGAACCTACACTTGTC; R: GGTTCAAGTCCGCAAAGTACCT), Gna15 (F: ACCTCGGTCATGCTTCTTCC; R: CGGATCATGTCGAAATGTC; R: TTGGGAAATGCTTGGCT).

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Author Contributions
Conceived and designed the experiments: KSC. Performed the experiments: KSC TMS. AC R JMT. Analyzed the data: KSC TMS. Contributed reagents/materials/analysis tools: PK. Wrote the paper: KSC TMS.
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