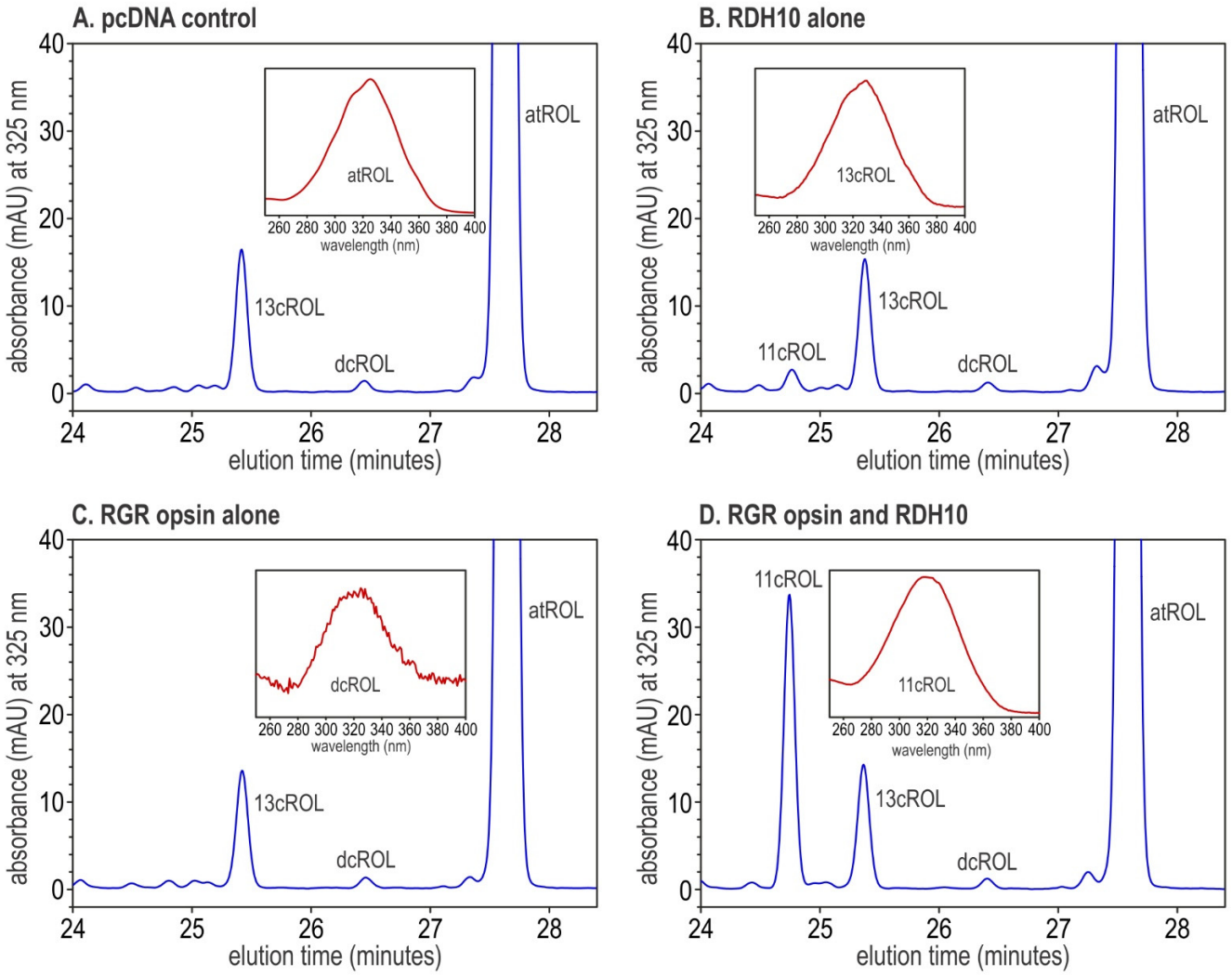
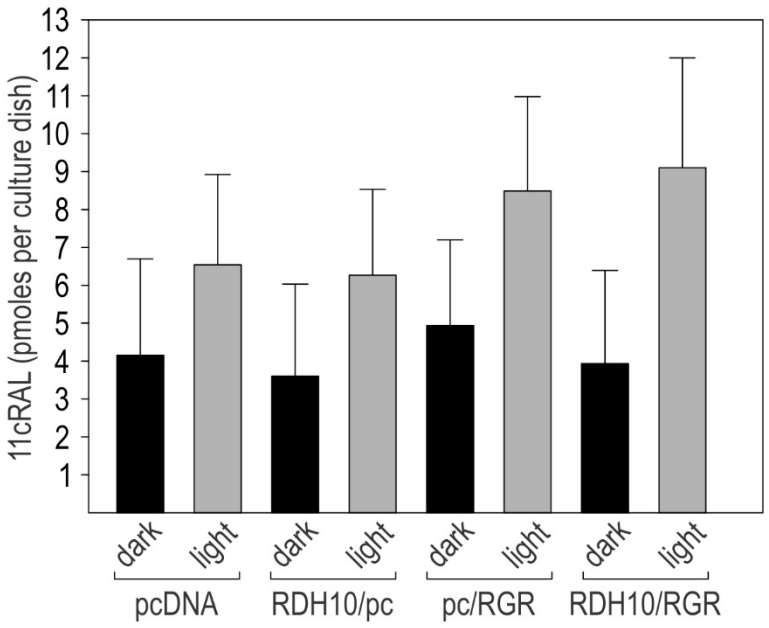
Supplementary Figures and Legends

NEURON-D-19-00210

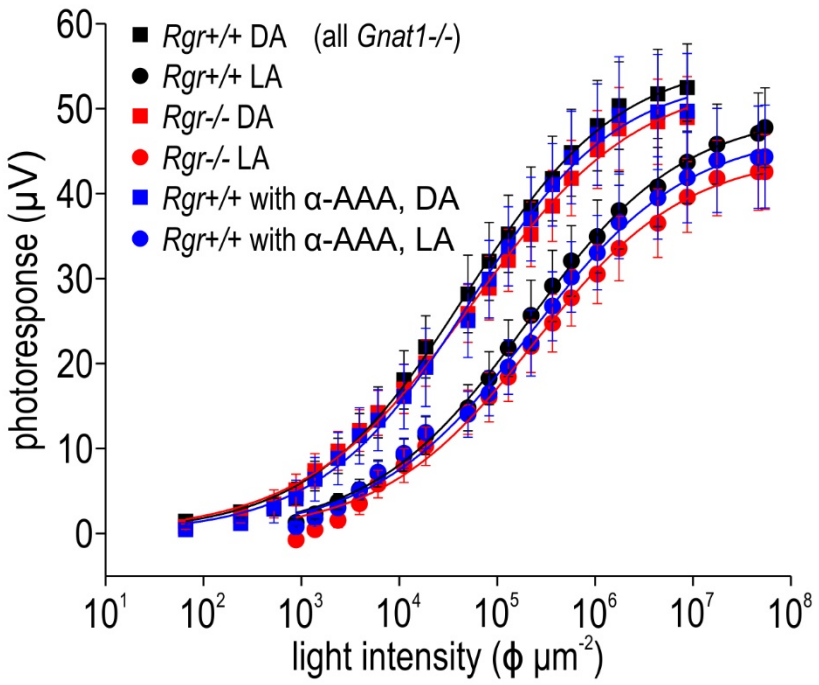
'Light-driven Regeneration of Cone Visual Pigments through a Mechanism Involving RGR opsin in Müller glial cells'



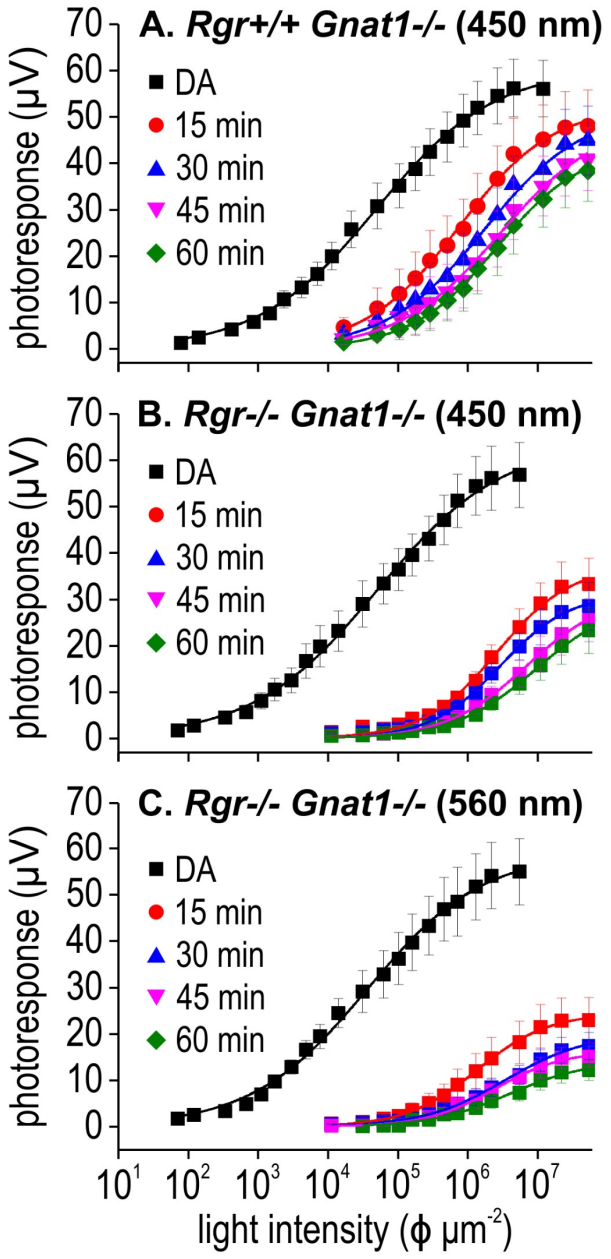
Supplemental Figure S1, related to Figure 1. HPLC chromatograms showing production of 11cROL in blue light exposed cells expressing RGR opsin and Rdh10. Representative HPLC chromatograms and eluted peak spectra are shown for 470-nm light exposed cells from the experiments described in Figure 1. The chromatograms (blue lines) show eluted peaks from media extractions of HEK-293T cells transfected with non-recombinant plasmid (pcDNA3.1) **(A)**, Rdh10 **(B)**, RGR **(C)** or RGR with Rdh10 **(D)**. Peaks in order of elution time are 11cROL (24.7 min), 13-*cis*-retinol (13cROL) (25.3 min), 9,13-di-*cis*-retinol (dcROL) (26.4 min), and atROL (27.6 min) based on standard elution times and matched absorption spectra (insets with red lines). Note the much larger 11cROL peak when RGR and Rdh10 were both expressed in light-exposed cells.



Supplemental Figure S2, related to Figure 1. Production of 11cRAL by RGR- and Rdh10-expressing cells from atROL. HEK-293T cells were transfected with non-recombinant pcDNA3.1, pcDNA3.1-Rdh10, and/or pcDNA3.1-RGR. After two days in culture to allow for expression, the cell plates (~80% confluent) were exposed to narrow-bandwidth light of 470 ± 10 nm at 0.2 W/m^2 for 30 minutes, or kept in the dark. The media above the cells were analyzed for retinoid content. Levels of 11cRAL are shown for each transfection and light-exposure conditions. No significant RGR-, Rdh10- or light-dependent synthesis of 11cRAL was observed ($p > 0.05$). Each bar represents n = 3 plates of transfected cells. Data represent mean ± SD, one-way ANOVA with Tukey's post hoc analysis.



Supplemental Figure S3, related to Figure 4. Light adaptation in *Rgr+/+ Gnat1-/-*, *Rgr-/- Gnat1-/-* and *Rgr+/+ Gnat1-/-* incubated with α-AAA. Cone photoreceptor responses in dark adapted (squares) and light adapted (circles) retinas recorded immediately after the onset of a continuous 505-nm light (9.1 x 10^6 photons (ϕ) μm^{-2} s^{-1}). Responses have been plotted as a function of the number of effective photons in the flash (as in Figs. 5A—5C) from mouse M-cones in *Rgr+/+ Gnat1-/-* (n = 9), *Rgr-/- Gnat1-/-* (n = 8) and *Rgr+/+ Gnat1-/-* incubated with α-AAA (n = 5). The curves are best fits to the Hill equation (Eqn. 1) with the following parameter values. In *Rgr+/+ Gnat1-/-* retinas, V_{max} = 55.00, $I_{1/2}$ = 4.7 x 10^4, n = 0.55 for dark adapted recordings; V_{max} = 49.6 and $I_{1/2}$ = 2.0 x 10^5, n = 0.54 for light adapted recordings. In *Rgr-/- Gnat1-/-* retinas, V_{max} = 53.8, $I_{1/2}$ = 5.4 x 10^4, n = 0.51 for dark adapted recordings; V_{max} = 44.7 and $I_{1/2}$ = 2.3 x 10^5, n = 0.55 for light adapted recordings. In *Rgr+/+ Gnat1-/-* retinas that have been incubated in α-AAA, V_{max} = 54.0, $I_{1/2}$ = 5.1 x 10^4, n = 0.57 for dark adapted recordings; V_{max} = 47.3 and $I_{1/2}$ = 2.2 x 10^6, 0.53 for light adapted recordings.



Supplemental Figure S4, related to Figure 7. Intensity-response relationship of M-cones in *Rgr+/+ Gnat1-/-* in continuous 450-nm light, *Rgr-/- Gnat1-/-* in continuous 450-nm light and *Rgr-/- Gnat1-/-* in continuous 560-nm light. Responses were recorded and plotted as in **Figs. 5A—5C** for dark adapted and at 15, 30, 45 and 60 min of exposure to a continuous background light. The intensities of the 450-nm and 560-nm backgrounds were set to the same value of 9.1 x 10^6 photons (ϕ) μm^{-2} s^{-1} have been plotted as a function of the number of photons in the flash for *Rgr+/+ Gnat1-/-* in continuous 450-nm light (n = 7, **A**), *Rgr-/- Gnat1-/-* in continuous 450-nm light (n = 7, **B**) and *Rgr-/- Gnat1-/-* in continuous 560-nm light (n = 7, **C**). The curves are best fits to the Hill equation (Eqn. 1) with the following parameter values **(A)** V_{max} = 60.1, $I_{1/2}$ = 3.1 x 10^4, n = 0.55 for dark adapted recordings; V_{max} = 52.5 and $I_{1/2}$ = 3.9 x 10^5, n = 0.67 for 15 min; V_{max} = 51.5, $I_{1/2}$ = 8.8 x 10^5, n = 0.65 for 30 min; ; V_{max} = 49.4 $I_{1/2}$ = 1.4 x 10^6, n = 0.64 for 45 min and ; V_{max} = 43.6, $I_{1/2}$ = 1.3 x10^6, n = 0.74 for 60 min recordings in *Rgr+/+ Gnat1-/-* in continuous 450-nm light. **(B)** V_{max} = 64.0, $I_{1/2}$ = 5.0 x 10^4, n = 0.48 for dark adapted recordings; V_{max} = 37.3 and $I_{1/2}$ = 2.6 x 10^6, n = 0.82 for 15 min; V_{max} = 31.3, $I_{1/2}$ = 2.8 x 10^6, 0.87 for 30 min; V_{max} = 31.0, $I_{1/2}$ = 6.8 x 10^6, n = 0.75 for 45 min and V_{max} = 30.0, $I_{1/2}$ = 9.6 x 10^6 n = 0.73 for 60 min recordings in *Rgr-/- Gnat1-/-* in continuous 450-nm light. **(C)** V_{max} = 59.2, $I_{1/2}$ = 3.6 x 10^4, n = 0.52 for dark adapted recordings; V_{max} = 24.4 and $I_{1/2}$ = 1.4 x 10^6, 0.85 for 15 min; V_{max} = 20.3, $I_{1/2}$ = 3.4 x 10^6, n = 0.74 for 30 min; ; V_{max} = 16.3, $I_{1/2}$ = 3.4 x 10^6, n = 0.88 for 45 min and ; V_{max} = 14.1, $I_{1/2}$ = 4.9 x 10^6, n = 0.78 for 60 min recordings in *Rgr-/- Gnat1-/-* in continuous 560-nm light.