Slower Rhodopsin Bleaching in Retinal Rods Lacking Transducin

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Introduction

- Vision in dim light begins with the absorption of a photon by rhodopsin, the visual pigment in rod photoreceptors in the retina that initiates a G-protein mediated phototransduction cascade. Upon absorption, the 11-cis-retinal chromophore isomerizes to the all-trans conformation, which prompts structural change in the protein that leads to rhodopsin bleaching through a series of photointermediates.

11-cis retinal Isomerization to all-trans retinal

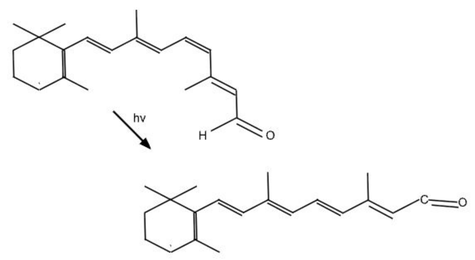

Figure 1

- Meta III, the final intermediate, breaks down into apo-opsin protein and all-trans retinal, allowing a new 11-cis-retinal chromophore to combine with the opsin to regenerate light-sensitive rhodopsin.
- Does the presence of transducin G-protein speed up Meta III decay in Wildtype versus GNAT-/- (transducin knockout) mice?

Methods

- Dark-adapted retinas from Wildtype and GNAT-/- (transducin knockout) mice.
- Cornwall microspectrometer

MSP Schematic

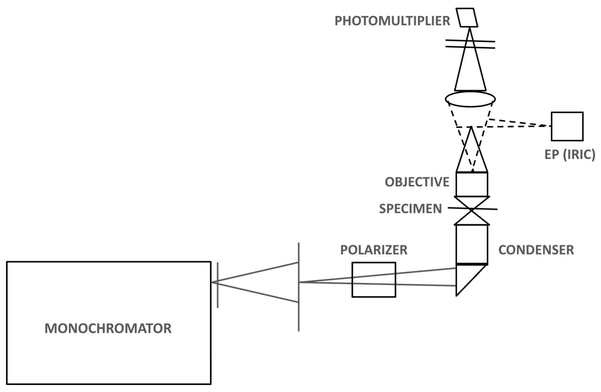

Figure 2

- Adjusted baseline and normalized by rhodopsin. Calculated true Wavelength.

Wavelength Calibration

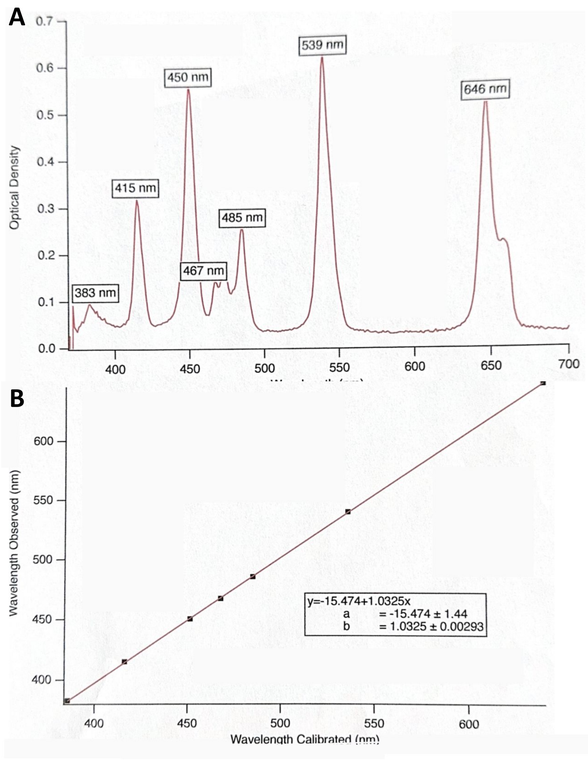

Figure 3

Results

WT OD decreases with longer bleach

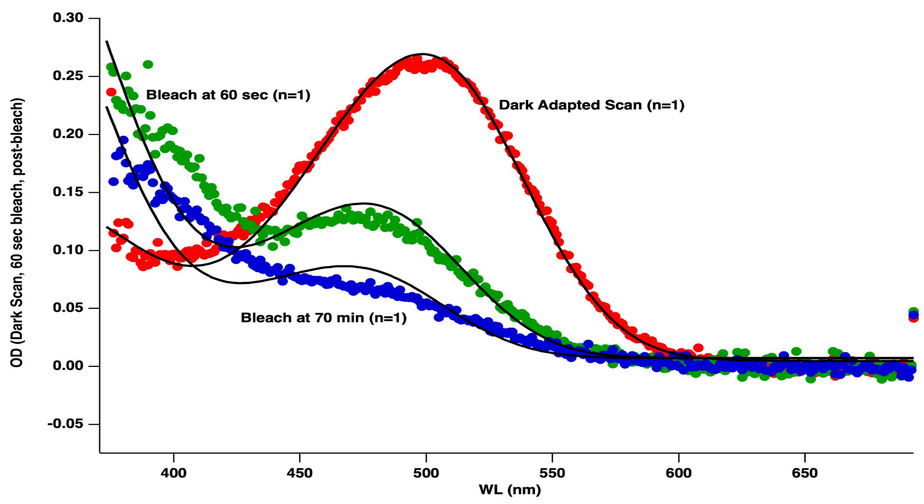

Figure 4: Spectra before (dark adapted) and after a 60 second exposure to bright light.

Greater Meta III Formation at 472 nm Post-Bleach GNAT-/- vs Wildtype

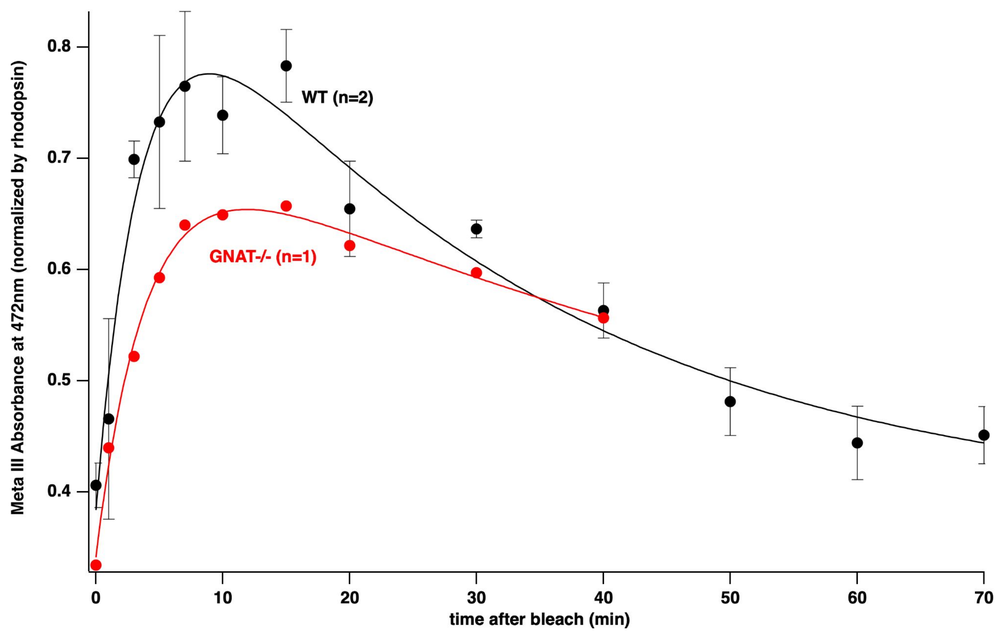

Figure 5: Measured Meta III formation at 472 nm for Wildtype (black) and GNAT -/- (red) samples. Wildtype line is an average of two samples (third sample considered null). Fitted with biexponential function.

Tau2 Value Consistent between GNAT-/- and Wildtype Samples

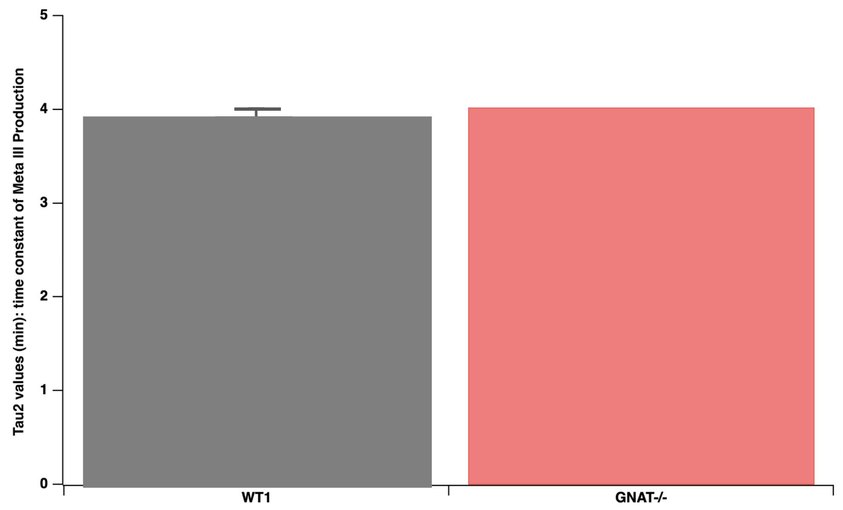

Figure 6: Tau2 values (Meta III production) for all samples appear to be consistent.

Tau2 Value Greater for Wildtype samples vs GNAT-/-

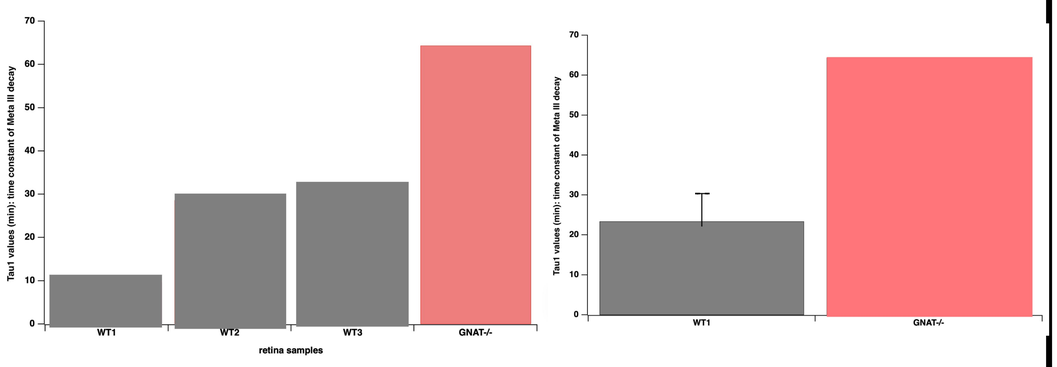

Figure 7a and 7b: Tau1 (Meta III decay) values for WT2 and WT3 appear to be pretty similar, while Tau1 value for WT1 seems to be an outlier, thus is excluded from final data. Tau1 values for Wildtype and GNAT -/- are considerably different. Thus, rate of decay of Meta III is different between samples, indicating the effect of transducin on Meta III decay.

Conclusions

- Meta III production (Tau1) were fairly similar, but Meta III decay (Tau1) was approximately 3 times faster in Wildtype (30 sec ± 6.4, n=2) than in GNAT-/- rods (60 sec).
- Observed to be less Meta III formation in GNAT-/- samples, compared to in Wildtype samples.

Discussion

- This finding indicates that the presence of transducin in intact rods accelerated Meta III decay into opsin and all-trans retinal. This allowed for faster regeneration of rhodopsin and more rapid recovery of sensitivity after exposure to bright light.
- If the all-trans retinal is not released, rhodopsin, which is needed for detecting photons, will not be regenerated. Thus, photoreceptors remain desensitized for longer periods of time, impacting vision in the dark after exposure to bright light.
- When transducin is present, it induces the transition between Meta III to Meta II through a direct bimolecular interaction between transducin and Meta III. The transition allows the retinylidene bond to be hydrolyzed, freeing the all-trans retinal to enter the regeneration process,
- Although three Wildtype samples were measured and the procedure followed was identical, one sample had a Tau1 value much lower than the other two Wildtypes, and thus was not included in the final data.
- Bleach light misalignment could contribute to the discrepancy between the wildtype absorbance curves. However, any light exposure during dark adaptation could directly alter Meta III decay by leading to less Meta III formation.

References

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