Turelli, W.R. Harcombe, K.T. Reynold, and A.A. Hoffmann 2007, PloS Biol. 5: e114; Weeks, A.R., R. Velten, and R. Stouthamer 2003, Proc. R. Soc. Lond. B. 270: 1857-1865; Werren, J.H., L. Guo, and D.W. Windsor 1995, Proc. R. Soc. Lond. B. 262: 174-204; Werren, J.H., 1997, Annu. Rev. Entomol. 42: 587-609; Werren, J.H., and D.M. Windsor 2000, Proc. Biol. Sci. 267: 1277-1285; Zchori-Fein, E., and S.J. Perlman 2004, Mol. Ecol. 13: 2009-2016; Zhou, W., F. Rousset, and S. O'Neill 1998, Proc. R. Soc. Lond. B. 265: 509-515.

Light-induced retinal degeneration in *Drosophila* with green fluorescent protein (GFP) attached to rhodopsin.

Shah, Chintan[1], Nihar Shah[1], Katelyn Anderson[1], George Denny[2], Barbara Nagel[3], Jan Ryerse[3], and William S. Stark[1]. [1]Department of Biology, [3]Department of Pathology and Research Microscopy Core, Saint Louis University, St. Louis, MO 63103; [2]Washington University School of Medicine, St. Louis, MO 63110; e-mail starkws@slu.edu.

Quite by serendipity, this laboratory noticed that *w*; *cn bw*; Rh1-GAL4 + UAS-Rh1GFP/TM2 *Drosophila* had degeneration of R1-6 receptors; this white-eyed stock has a transgene with GFP-labeled R1-6 rhodopsin (Rh1-GFP) driven into R1-6 by the promoter of the Rh1 gene (ninaE), so we will nickname this stock "Rh1-GFP." We utilized mostly time-tested optical ("pseudopupil") techniques summarized by Stark and Thomas (2004).

Dark-reared flies have a very obvious pseudopupil darkening, our way to visualize the amount of rhodopsin to metarhodopsin conversion (Figure 1 A *vs*. B); also R1-6's GFP was clear in the fluorescent deep pseudopupil (Figure 1 C), and R1-6's GFP-labeled rhabdomere tips were nicely imaged with optical neutralization of the cornea (Figure 1 D).

Contrast this with flies maintained 5 days in constant room light: there is a substantial decrease in the pseudopupil darkening (Figure 1 E *vs*. F); the fluorescent deep pseudopupil is hazy (Figure 1 G), and the fluorescent rhabdomere tips seen under oil immersion have missing profiles (Figure 1 H). White-eyed control *Drosophila*, without the Rh1-GFP transgene, had never shown any indications of light-induced damage in decades of research by this laboratory.

We used optical neutralization and the confocal microscope to verify the expectation that vitamin A deprived flies do not have fluorescent rhabdomere tips (Figure 1 I), while vitamin A replete flies show R1-6 GFP label (Figure 1 J). We aged vitamin A deprived flies for 8 days in constant room light, then put them in the dark on carrot juice; their GFP-labeled R1-6 rhabdomere tips look beautiful in the confocal microscope (Figure 1 K). We aged vitamin A deprived flies 5 days in constant light, then put them in the dark with carrot juice and obtained substantial recovery in the pseudopupil darkening (Figure 1 L *vs*. M); also R1-6 showed tidy GFP fluorescence in the deep pseudopupil (Figure 1 N). The work with vitamin A deprivation and replacement therapy shows that light is not damaging when Rh1-GFP is greatly reduced.

We present a light micrograph of Rh1-GFP flies maintained 23 days in room light showing cells in the process of degeneration and missing rhabdomeres (Figure 1 O). Control white-eyed flies (without Rh1-GFP), also maintained in room lighting for this same duration, did not have any signs of degeneration (not shown).

Earlier, Stark (2005) showed that white-eyed *Drosophila* with GFP driven into R1-6 had completely normal structure, rhodopsin-metarhodopsin conversions, and electrophysiology. While this may seem contradictory, GFP, in that case, was not attached to Rh1; flies were the F1 from a

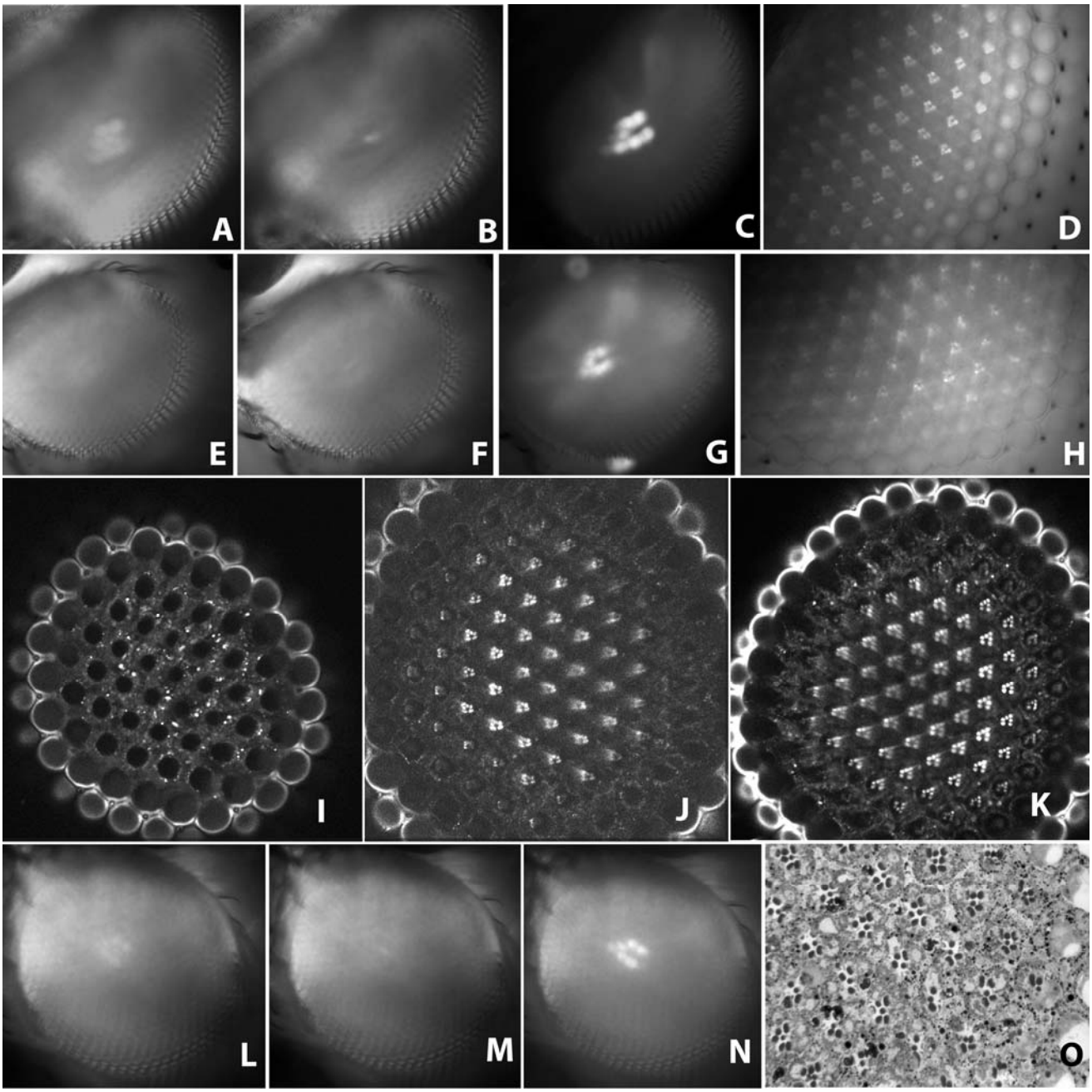

Figure 1.

cross between w^-/w^-; Sp/CyO; Rh1Gal4 p(17)/Rh1Gal4 p(17) (Rh1 promoter driving Gal4 in a rosy plasmid homozygous on the third chromosome) and w^-/w^-; UAS GFP/UAS GFP; UAS GFP/UAS GFP (homozygous for UAS GFP on second and third chromosomes). This suggests that light absorbed by either the rhodopsin or the attached GFP in Rh1-GFP *Drosophila* causes damage, but not light absorbed by GFP that is not linked to rhodopsin. However, it is conceivable that light absorbed by the Rh1 that is not attached to GFP in Rh1-GFP flies is what causes damage; we presume that the native ninaE gene, together with its promoter, is still expressing Rh1 (in addition to the Rh1-GFP expressed in the transgenics).

This laboratory (Selimovic *et al*., 2010) showed that room light decreases rhodopsin in *Drosophila* photoreceptors. We still believe those published quantitative measurements. However, we did present a confocal image of the Rh1-GFP stock we used in this study with very weakly fluorescent rhabdomere tips in flies that had been maintained in the light for 6 days. Now we understand that, in addition to light-induced decreases in rhodopsin, degenerative changes were also contributing to the weak fluorescence we observed.

One question remains. Does Rh1-GFP, as well as Rh1 without GFP attached, contribute to the pseudopupil darkening (Figure 1 A *vs*. B)? Recall that the pseudopupil darkening was our way to visualize the photoconversion of rhodopsin to metarhodopsin. In other words, we wonder whether the attachment of GFP to Rh1 interferes with conversion of rhodopsin to metarhodopsin.

Acknowledgments: Funding was from SLU's Beaumont and Presidential funds. We thank Prof Joseph O'Tousa of University of Notre Dame for providing *w*; *cn bw*; Rh1-GAL4 + UAS-Rh1GFP/TM2. Imran Shaikh helped maintain our *Drosophila* stocks.

References: Selimovic, A., *et al*., 2010, Dros. Inf. Serv. 93: 1-2; Stark, W.S., 2005, Invest. Ophthalmol. Vis. Sci. 46 (on line at http://www.abstractsonline.com/viewer/viewAbstract.asp?CKey={DE1F1B1C-574A-451F-A628-89C4164C2BD7}&MKey={74423071-0FB2-42B6-B3C9-3787D20BDD73}&AKey={01DBD563-E053-4A16-A83F-48E737512973}&SKey={F25B894D-8AD1-``````````475C-9042-FEF560594F56}); Stark, W.S., and C.F. Thomas 2004, Molec. Vision 10: 943-955 (on line at http://www.molvis.org/molvis/v10/a113).

A comparison between the effect of aqueous and methanolic extract of *Decalepis hamiltonii* on the level of alcohol tolerance in *Drosophila melanogaster*.

Jahromi, Samaneh Reiszadeh, Mohammad Haddadi, T. Shivanandappa, and S.R. Ramesh. Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore-570006, Karnataka , India .

The oxidative damage of biological molecules is an important event in the development of a variety of human diseases. Antioxidants, especially natural ones, have potential applications in prevention or cure of such diseases. *Decalepis hamiltonii* (family: Asclepiadaceae) has been shown to possess potent antioxidant properties.

The present study was carried out to check whether the root extract of *D. hamiltonii* has any neuroprotective potential. For this purpose, Oregon K strain flies were divided into three groups, *viz*., control, *D. hamiltonii* aqueous extract – fed, and *Decalepis hamiltonii* methanolic extract – fed ones. The exposure chamber was made for each group in which 8 flies of same sex were transferred by aspiration. Cotton stubs were coated with 0.5 ml ethanol and subsequently inserted into exposure vials. Numbers of stationary flies were recorded for each minute and the time required for sedation of 50% flies was documented.

Present study revealed that the flies fed on *Decalepis hamiltonii* aqueous extract containing media have relatively 30 percent higher ST50 (50% sensitivity) value compared to control and *Decalepis hamiltonii* methanolic extract – fed ones.

It can be concluded that antioxidant properties of this plant extract, especially aqueous one, can give higher degree of protection to the flies against oxidative stress induced by ethanol.