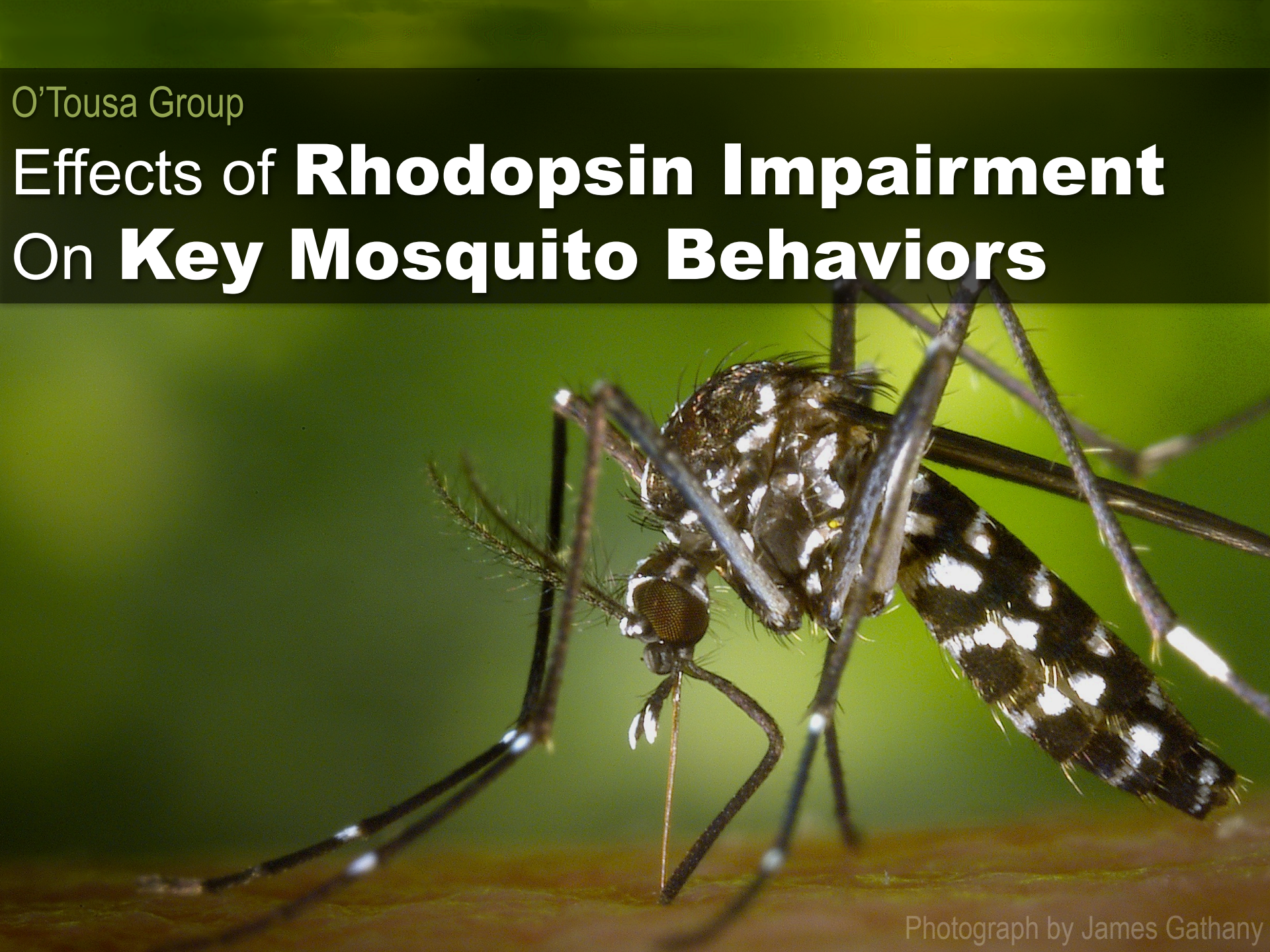
O'Tousa Group

# Effects of **Rhodopsin Impairment** On **Key Mosquito Behaviors**



Photograph by James Gathany

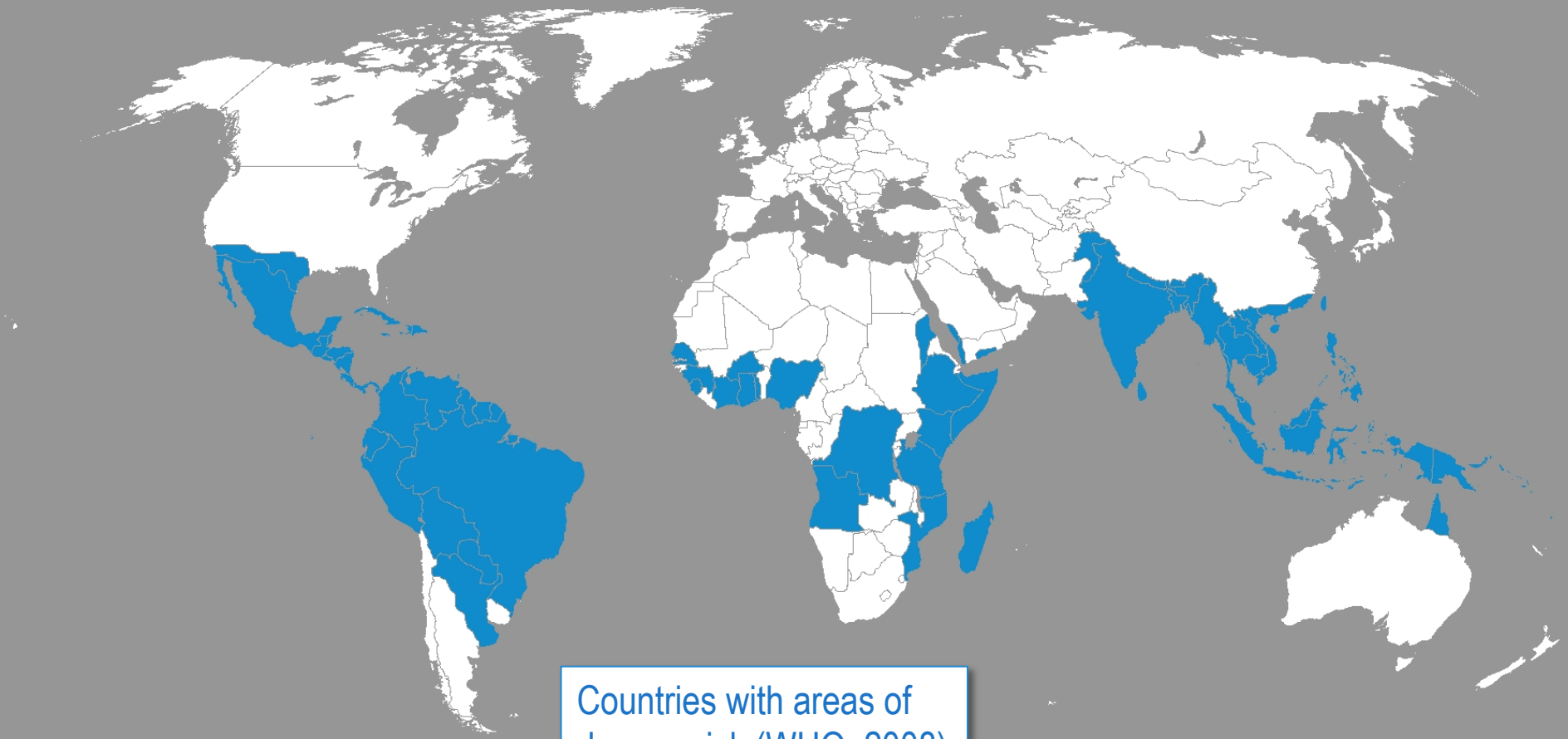
## Research Goal

Determine the importance of the complex mosquito visual system for behaviors related to vector competence.

# Why mosquitoes?

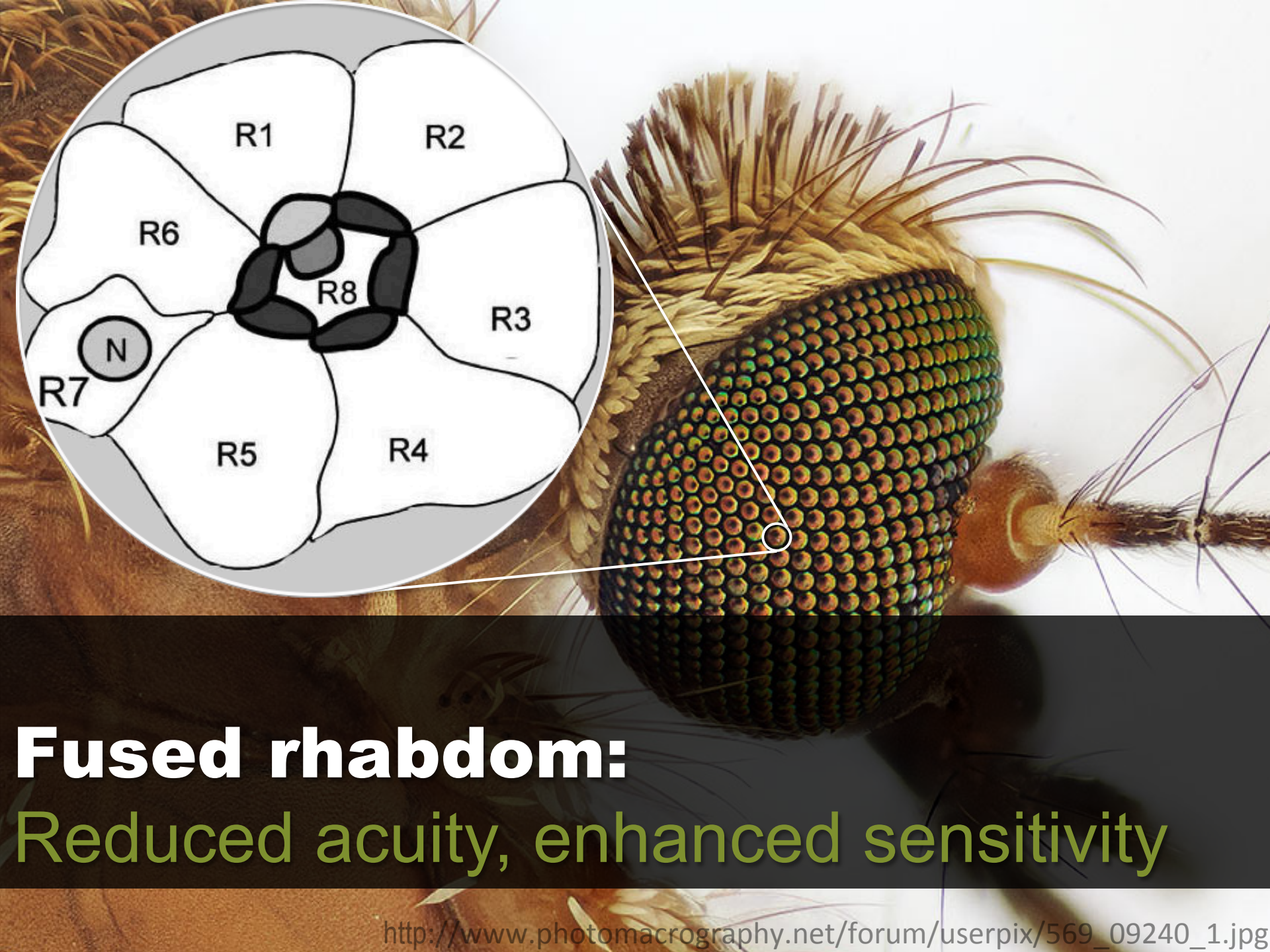
# Why mosquitoes?

## Dengue & yellow fever vector *(Aedes aegypti)*



Countries with areas of dengue risk (WHO, 2008)

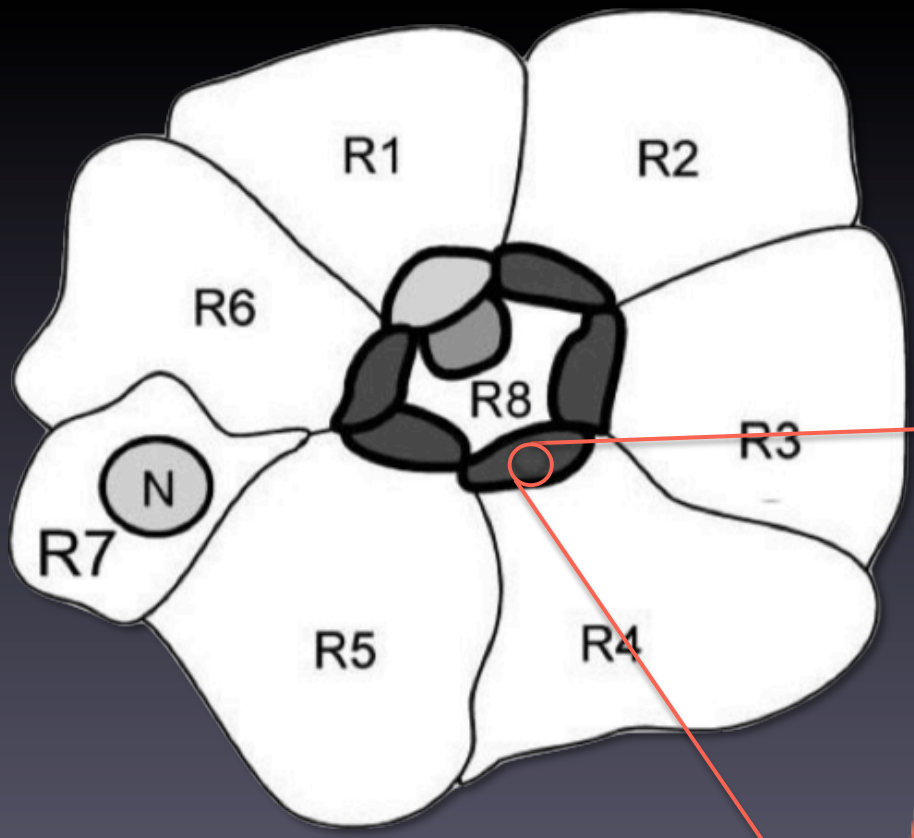
# Why mosquito vision?

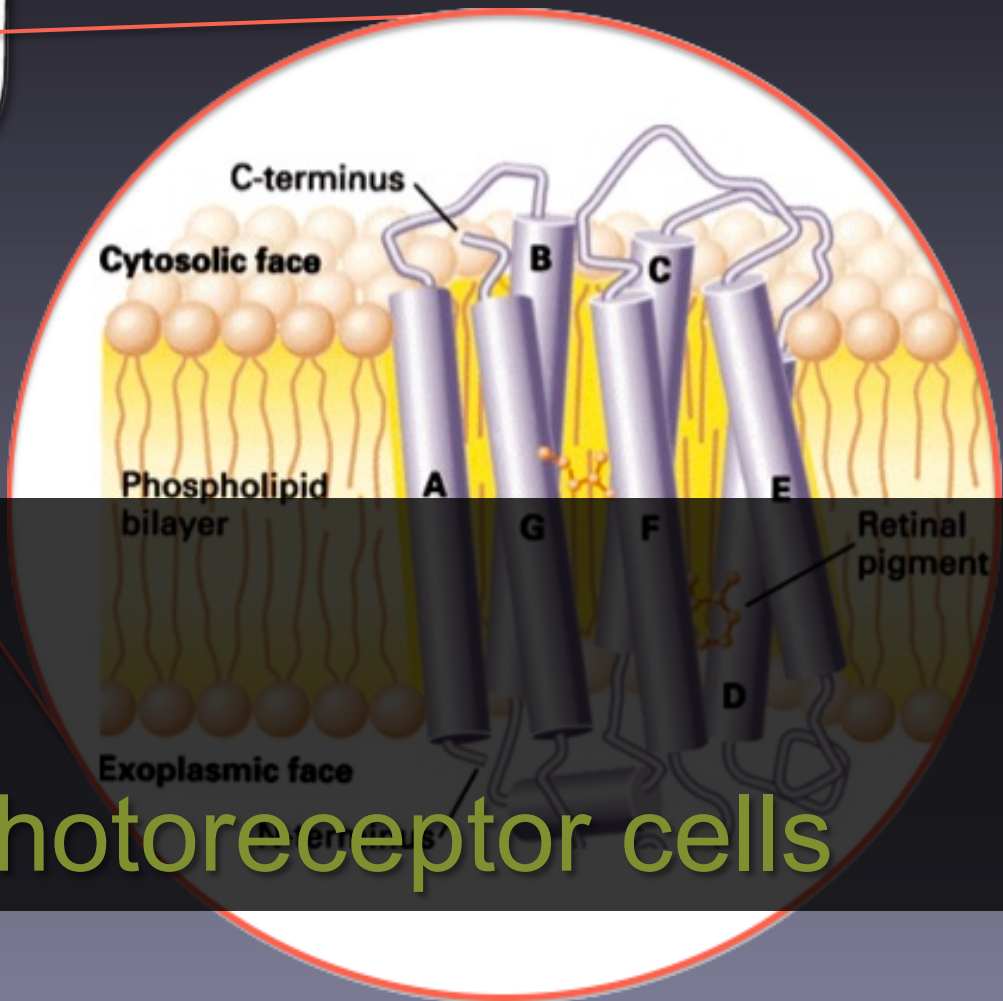



# Fused rhabdom:

## Reduced acuity, enhanced sensitivity

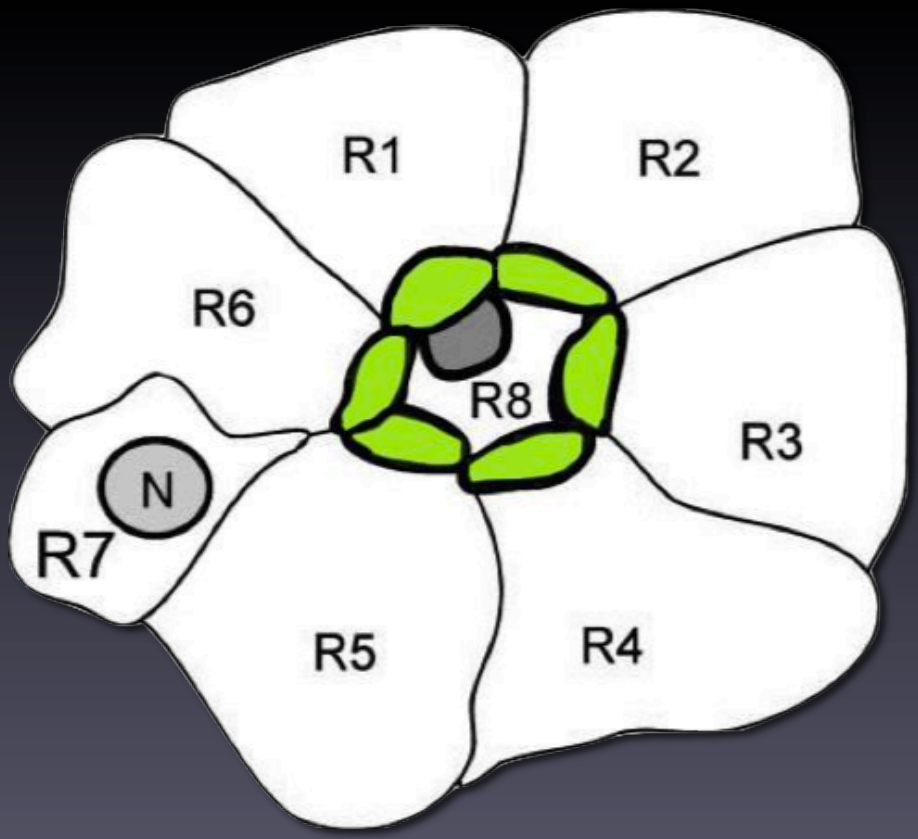
http://www.photomacrography.net/forum/userpix/569_09240_1.jpg



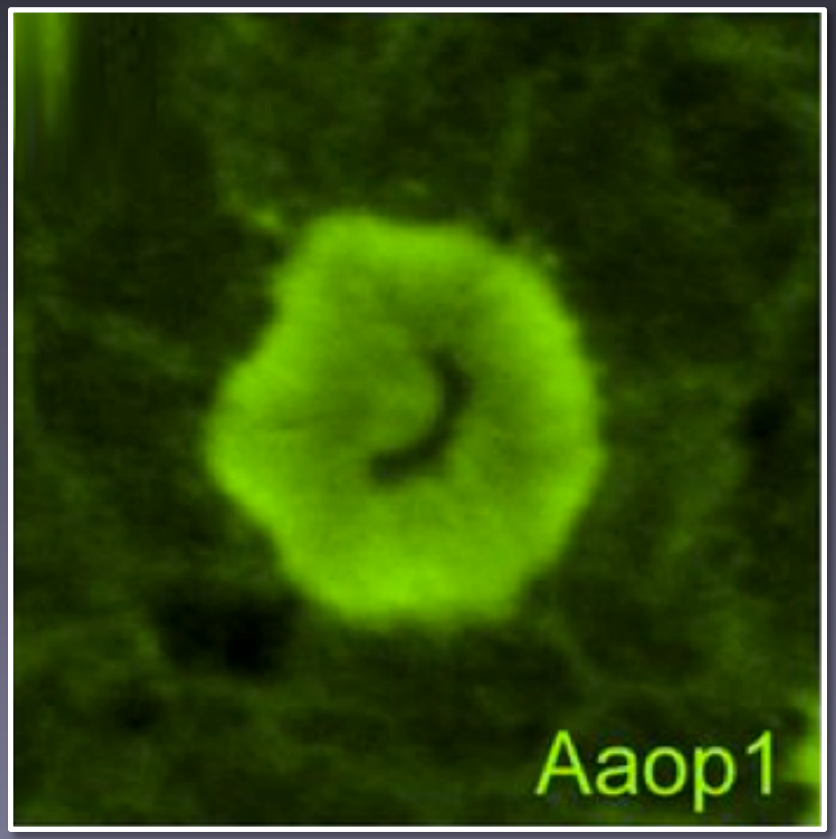




# 10 rhodopsins

## Co-expression in photoreceptor cells

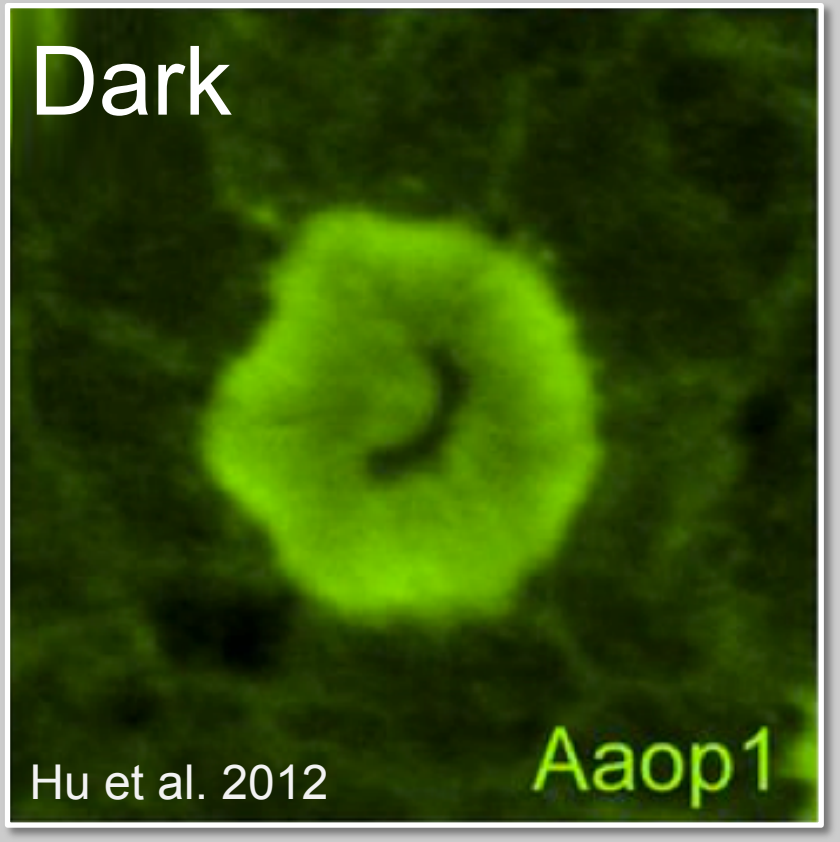


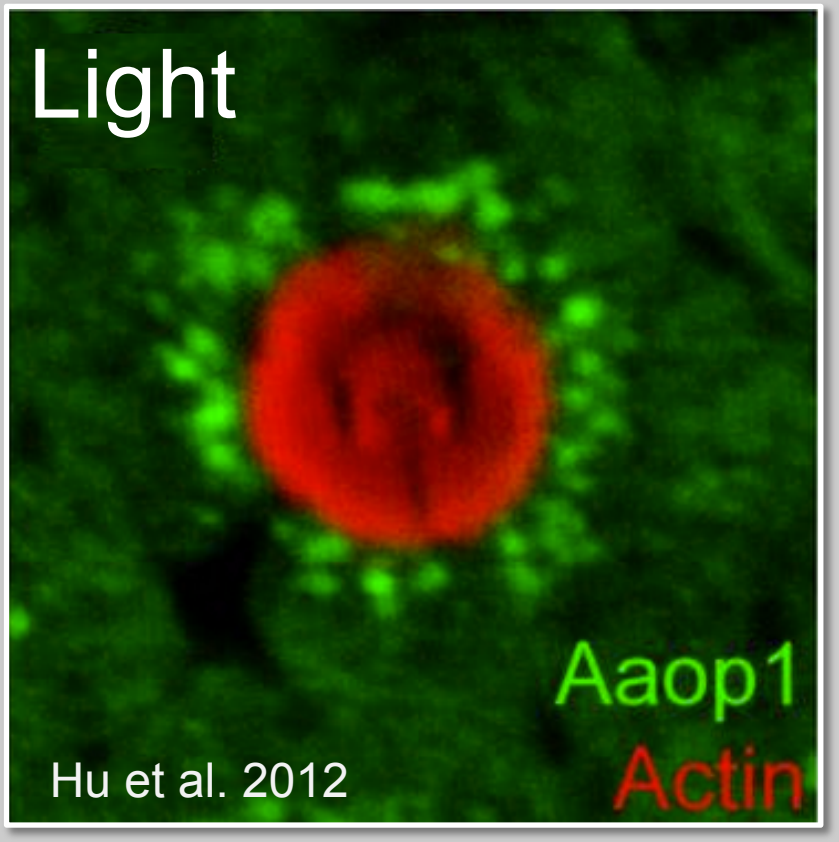

# op1



# Translocation of op1

## Localized to rhabdomere in the dark, cytoplasm in light



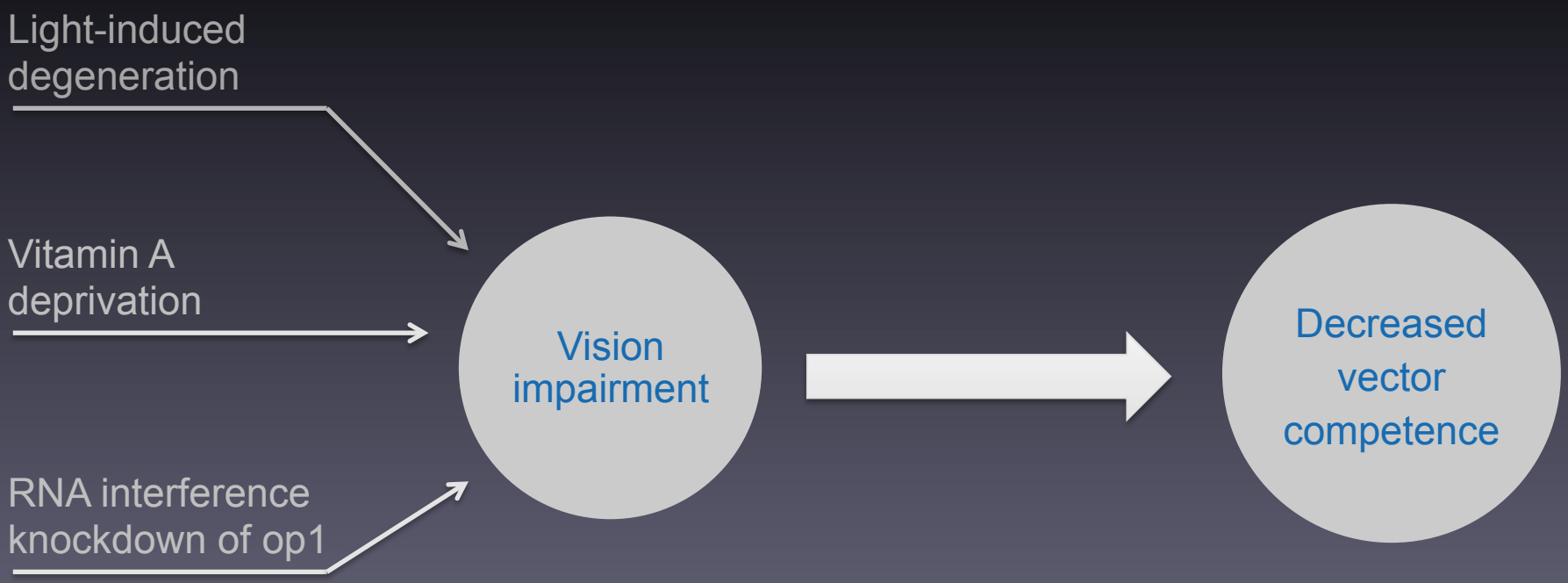
# Research Goal

Determine the importance of the complex mosquito visual system for behaviors related to vector competence.

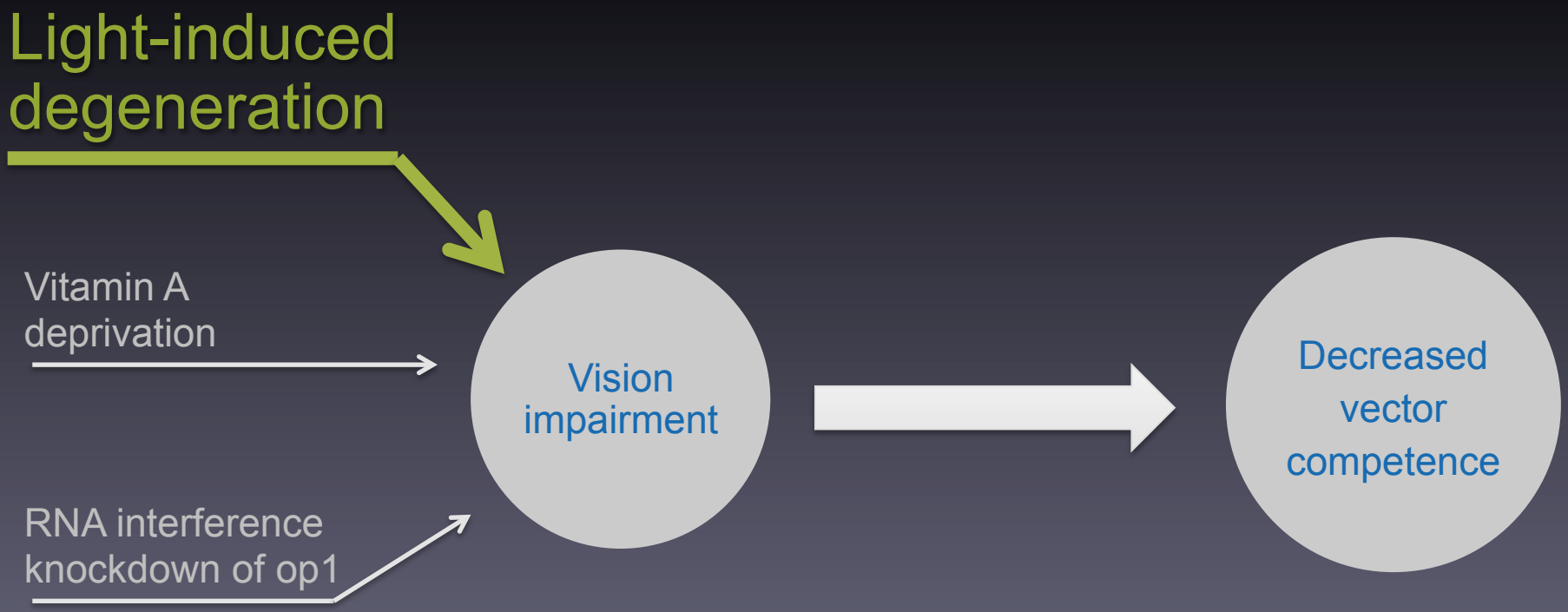
# Project Hypothesis

Rhodopsin impairment will reduce the mosquitoes' ability to execute key behaviors.

# Experimental Overview



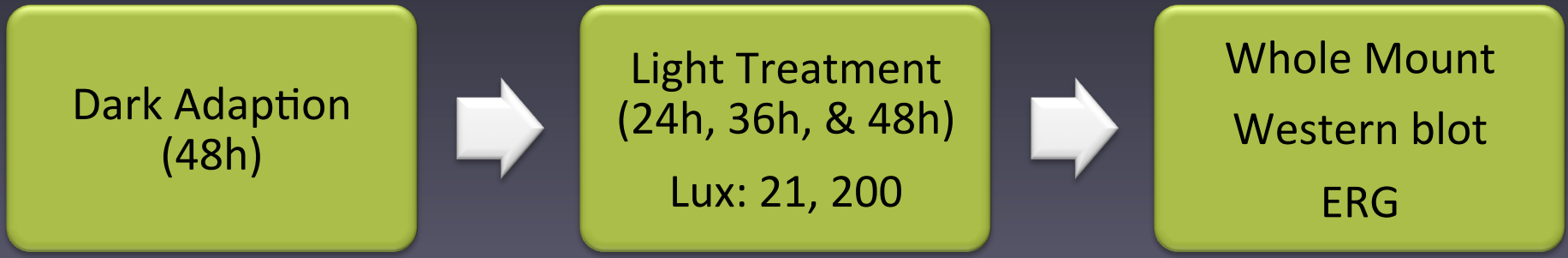
# Experimental Overview



# Light-Induced Degeneration

Hypothesis

# Method of Light-Induced Degeneration



# Molecular Assays: Whole Mount

**Collection**

- Bisect Head
- Fix in 4% paraformaldehyde
- PBS

**Staining**

- Dissect in PBS
- Primary Ab: Rabbit Anti-Op1
- Secondary Ab: HR-GAR
- Phalloidin

**Mount**

- Vectashield
- Fluorescent Microscope

# Molecular Assays: Electroretinogram

**Prep Animal**

- Etherize
- Wax mosquito to coverslip

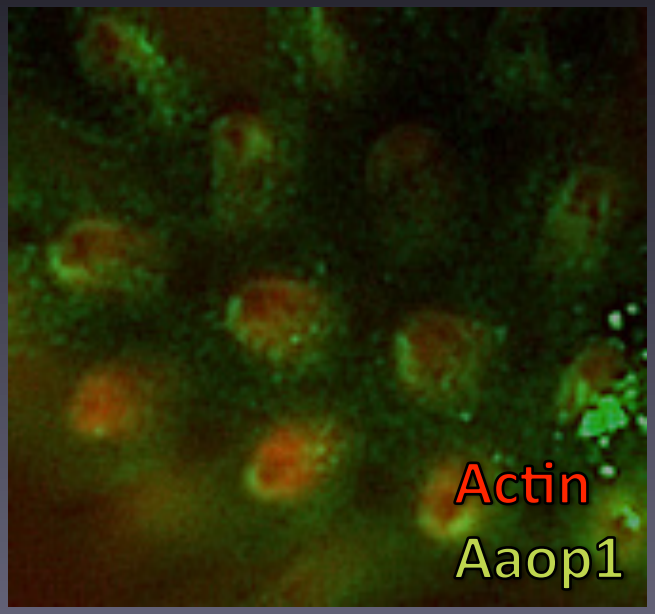
**Set Up Device**

- Glass microelectrodes filled with saline
- Recording electrode positioned with tip puncturing cornea
- Reference electrode placed in other eye

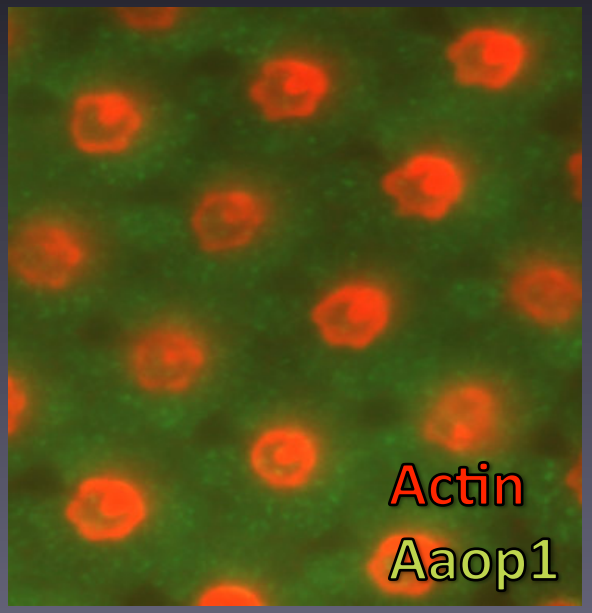
**Run**

- 1-sec light stimulus every 5 seconds
- Light intensity increases by a power of 10

# 24 hours in constant light did not alter photoreceptor structure



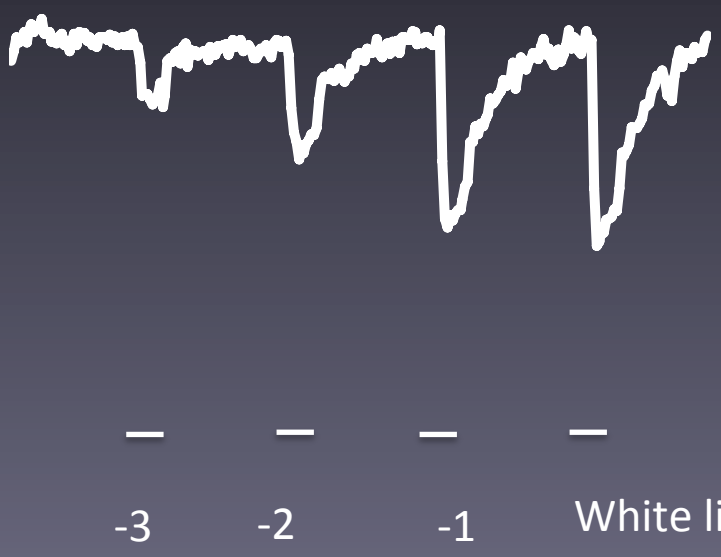

12/12 Light/Dark



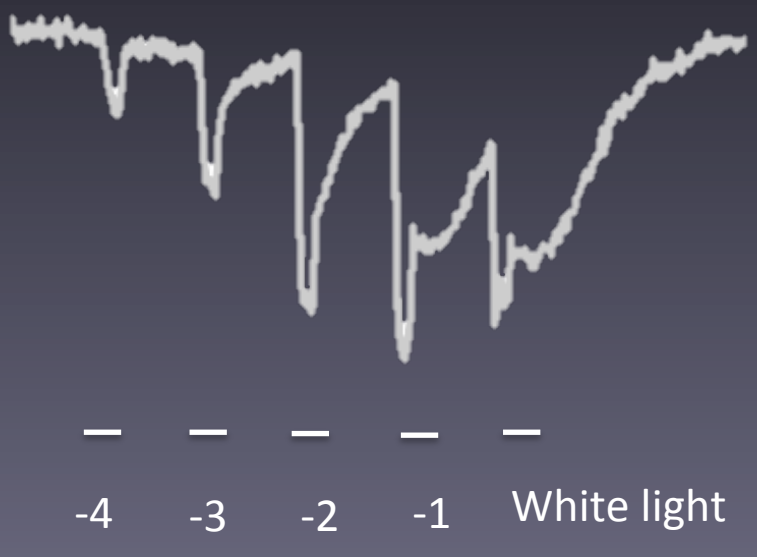

24 Hr Constant Light

# 24 hours in constant light impairs response

12/12 Light/Dark




24 hour Constant Light



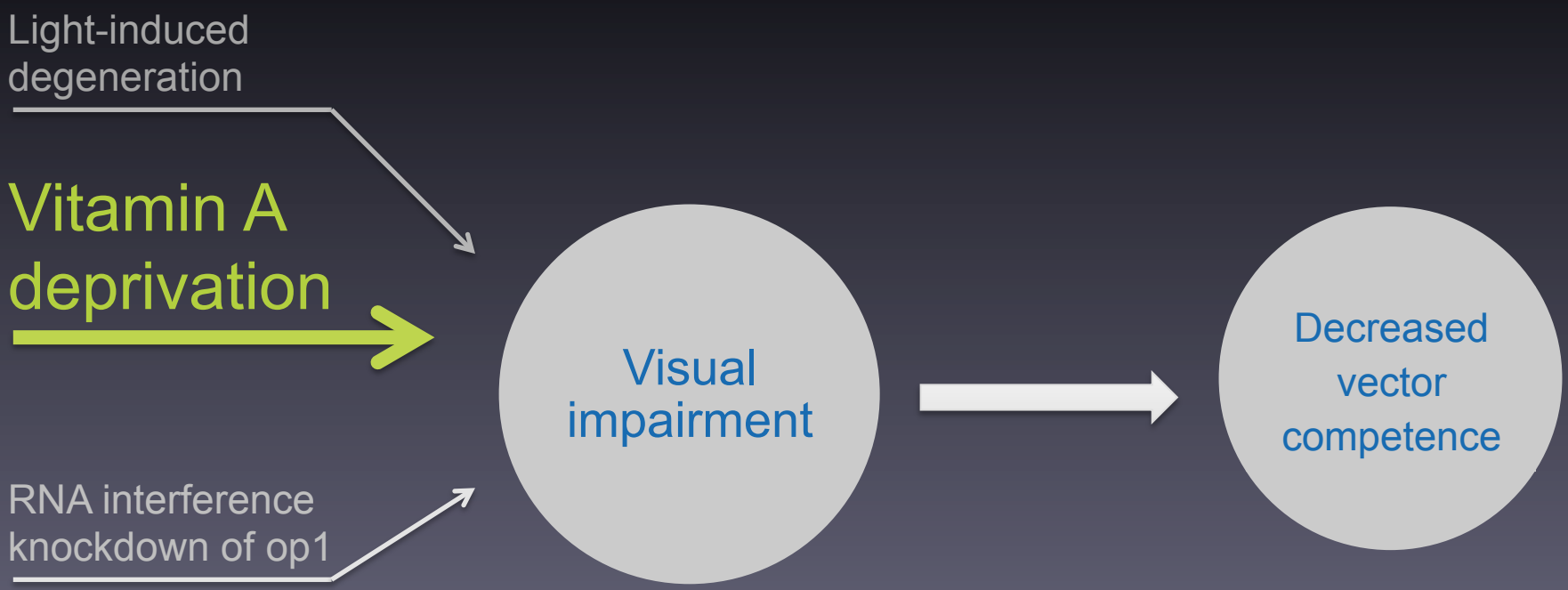

0.0005 mV

1 sec

# Future plans for light-induced degeneration

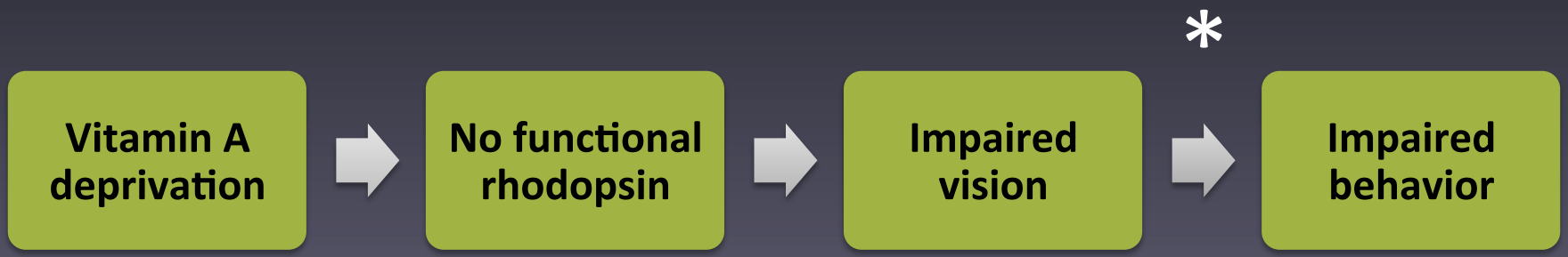
- 36 and 48 hour time points
- Confocal imaging
- Behavioral assays

# Experimental Overview



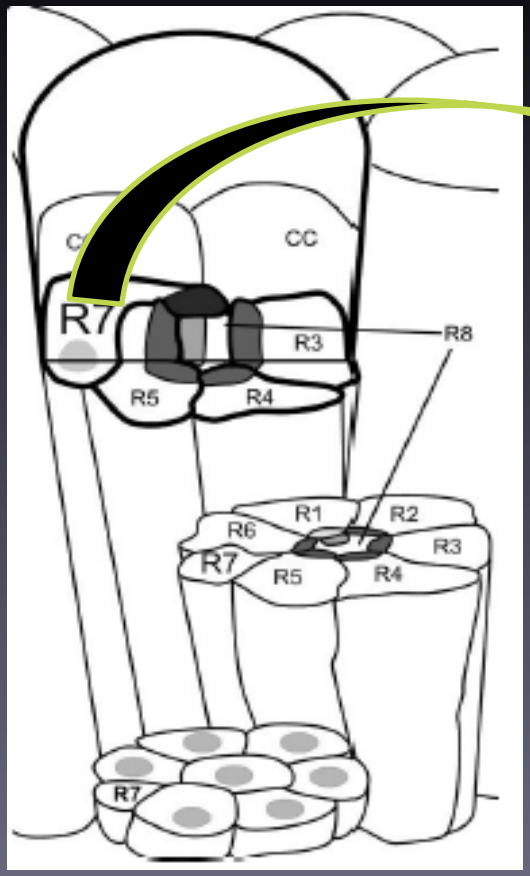
# Rhodopsin impairment from Vitamin A deprivation

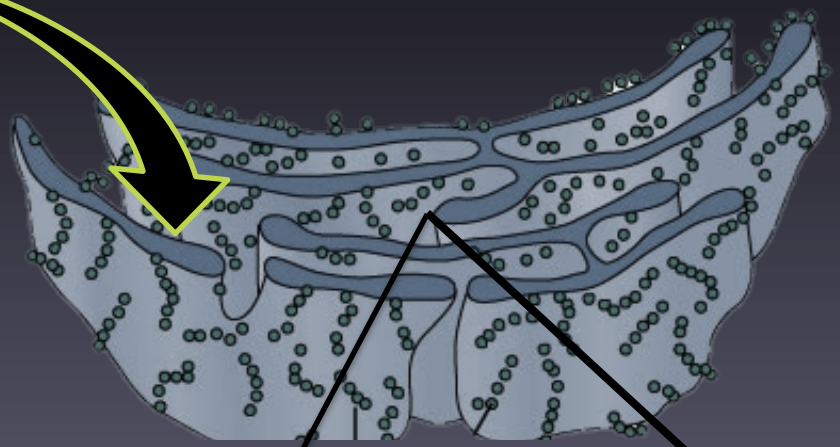
## Hypothesis

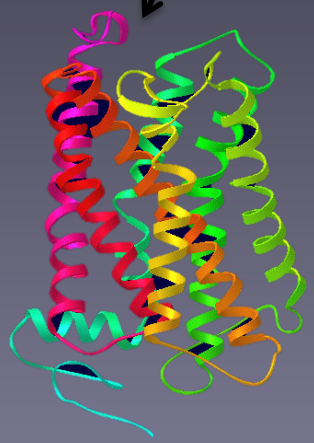



* Crucial role of visual system in behaviors related to disease transmission

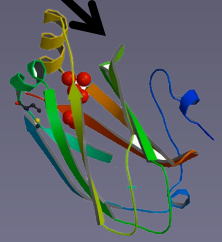
# Opsin and Retinal Within the Photoreceptor Cell



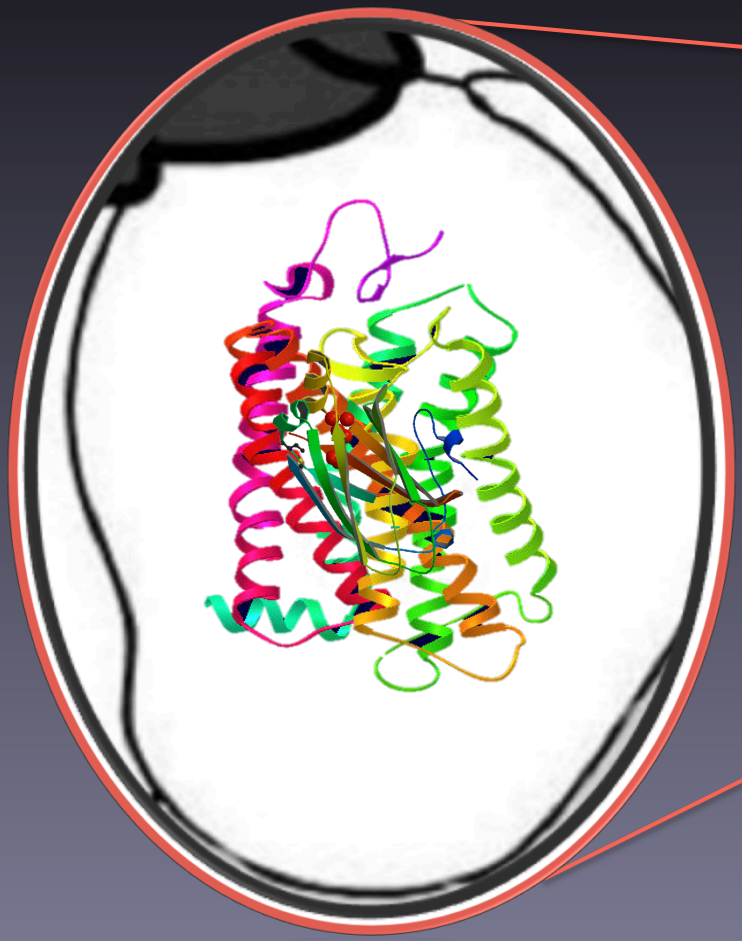


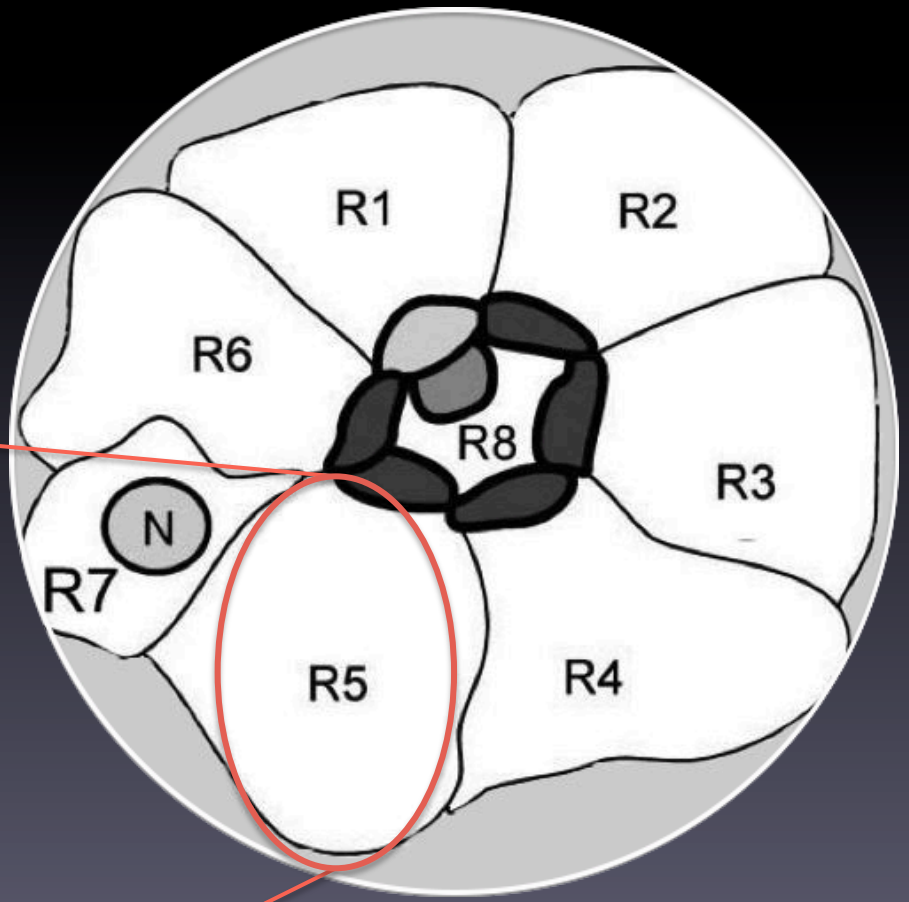
Opsin



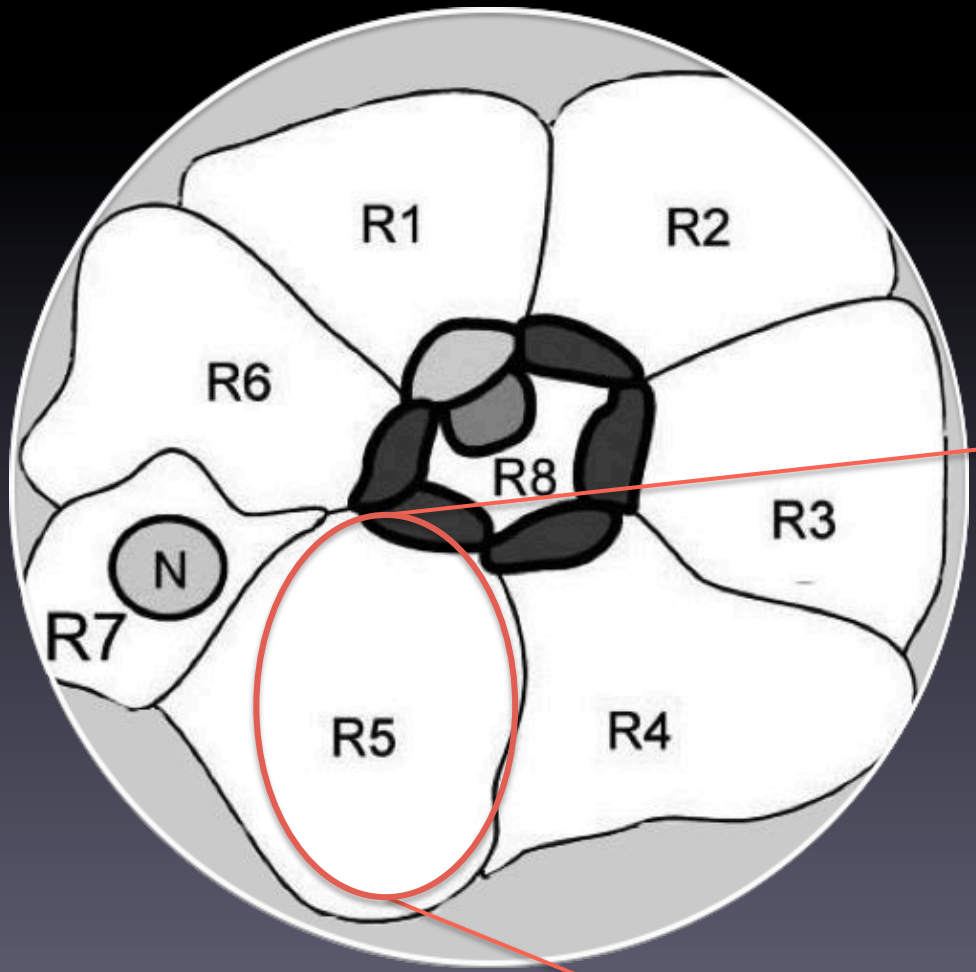
Retinal

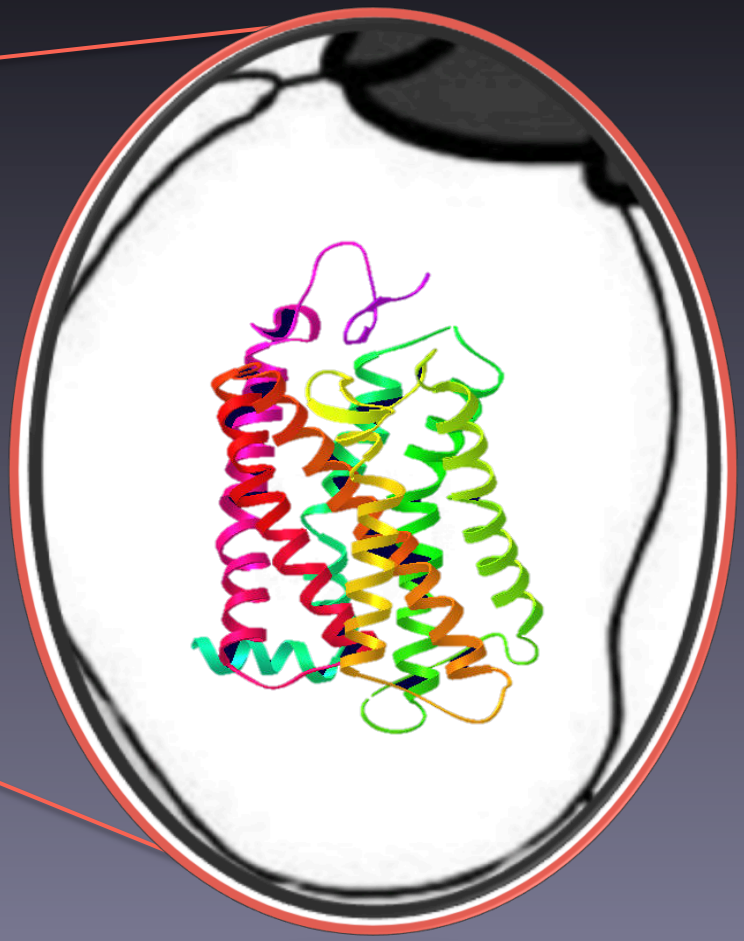
# Wild Type

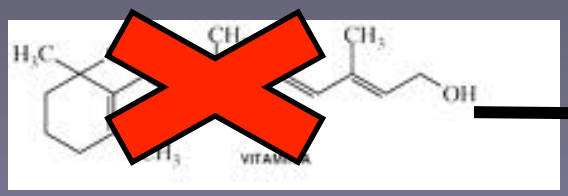


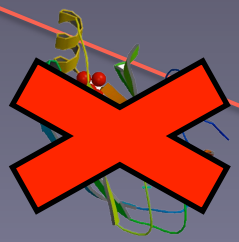


# Vitamin A deprived





# Method of vitamin A deprivation

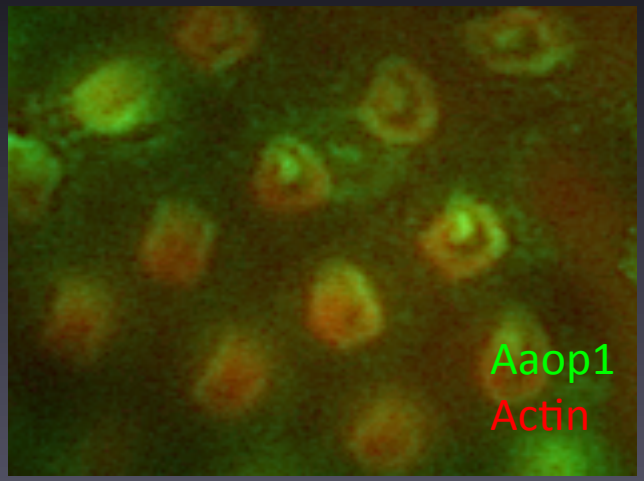
Feed heart powder

Collect 2-7 days following eclosion

Western blot, ERG, whole mount

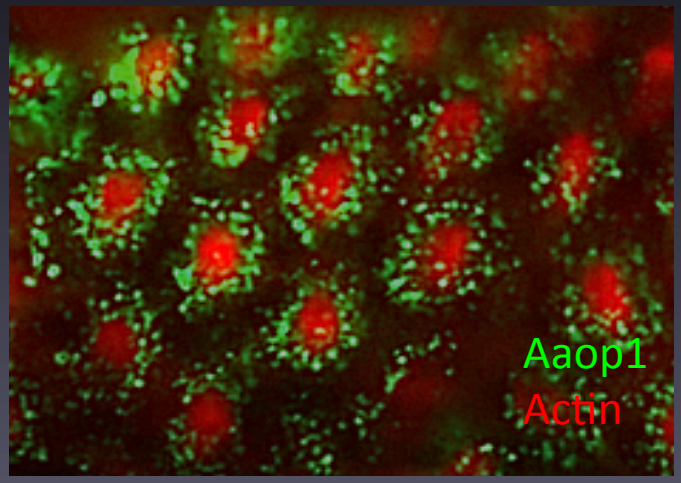
# Vitamin A Deprivation Altered Aaop1 Localization

## Whole mount IF Images- Light Adapted




Liver Powder (wild type)

- Characteristic of red-eyed
- Op1 in rhabdomere and cytoplasm




Heart Powder (Vitamin A Deprived)

- Op1 only in cytoplasm
- Vesicular

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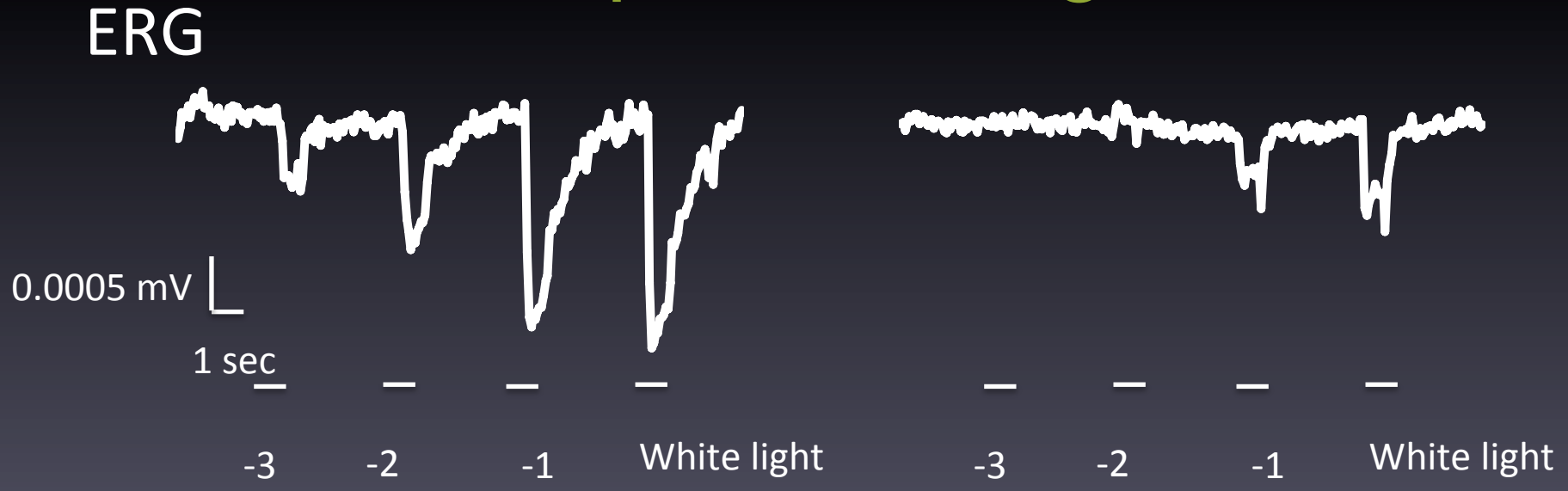
- Dark adaptation needed for verification

# Vitamin A Deprivation Altered Response to Light



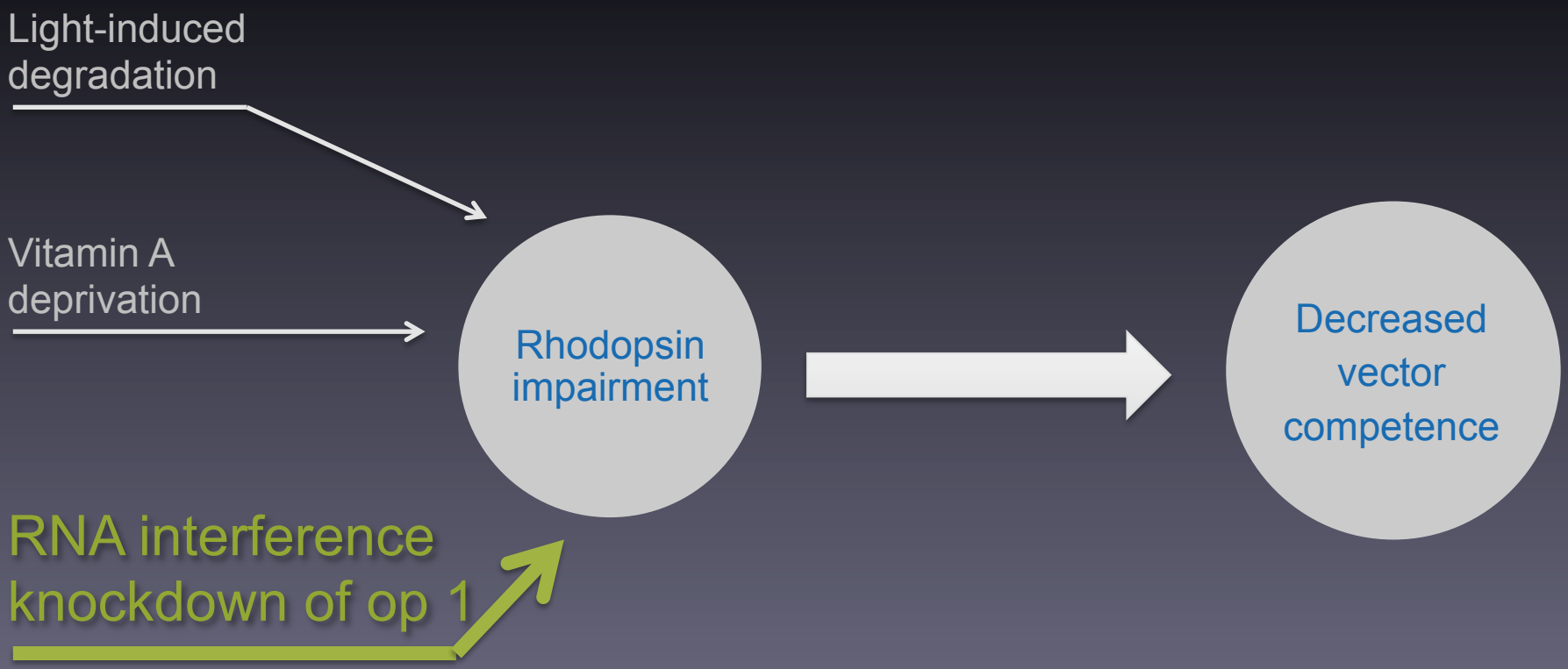

## Liver Powder (wild type)

- Proportional response to all light signals
- Clean response
- Standard recovery

## Heart Powder (Vitamin A Deprived)

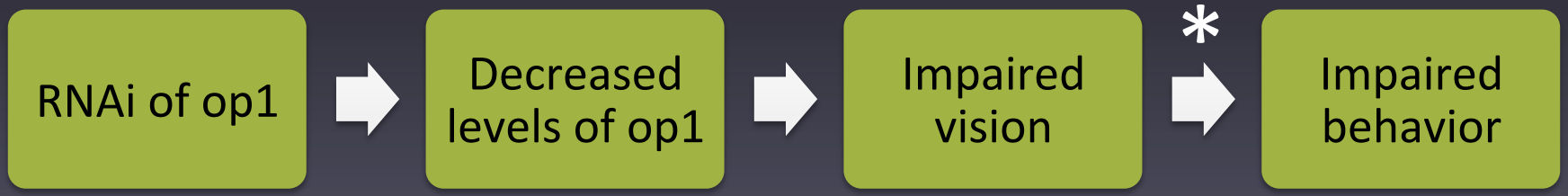
- More light required for initial response
- Staggered response
- Irregular recovery

# Experimental Overview



# RNAi knockdown of op1

**Hypothesis**




* Crucial role of visual system in behaviors related to disease transmission

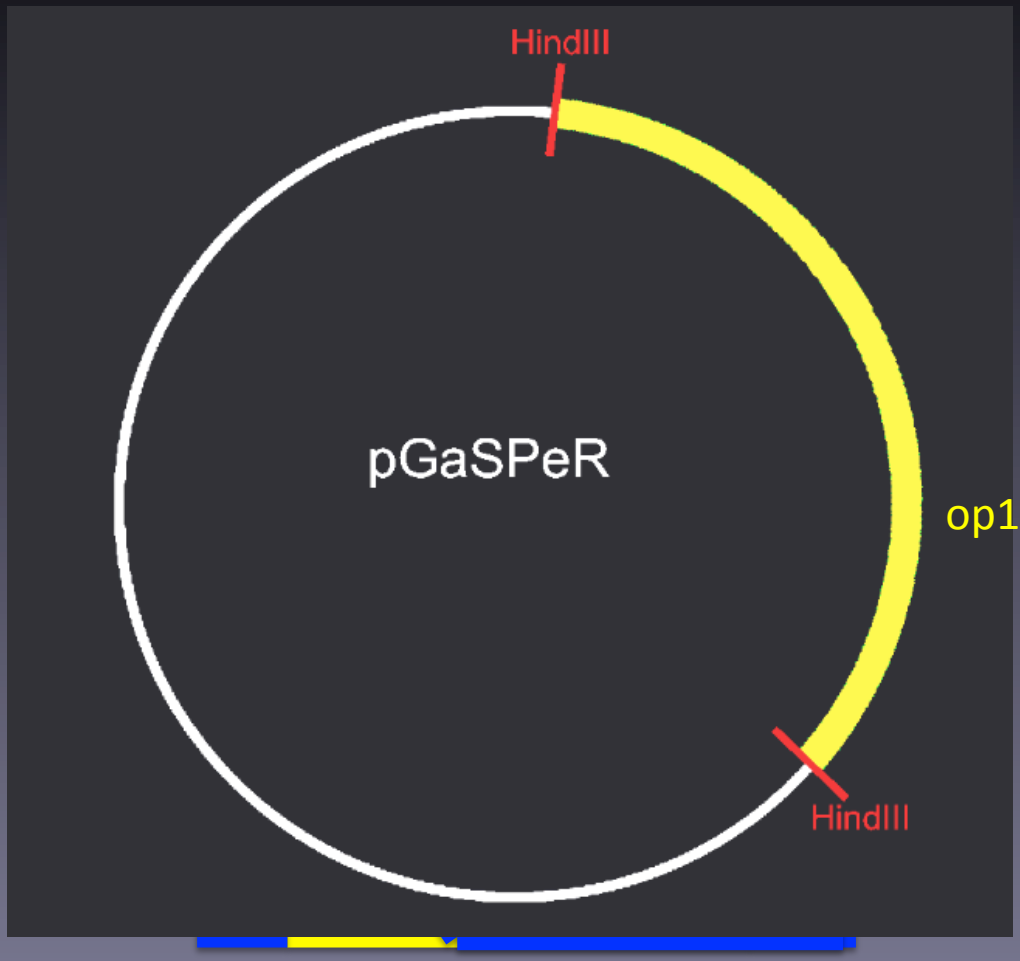
# Methods for RNAi knockdown of op1

Generate RNAi

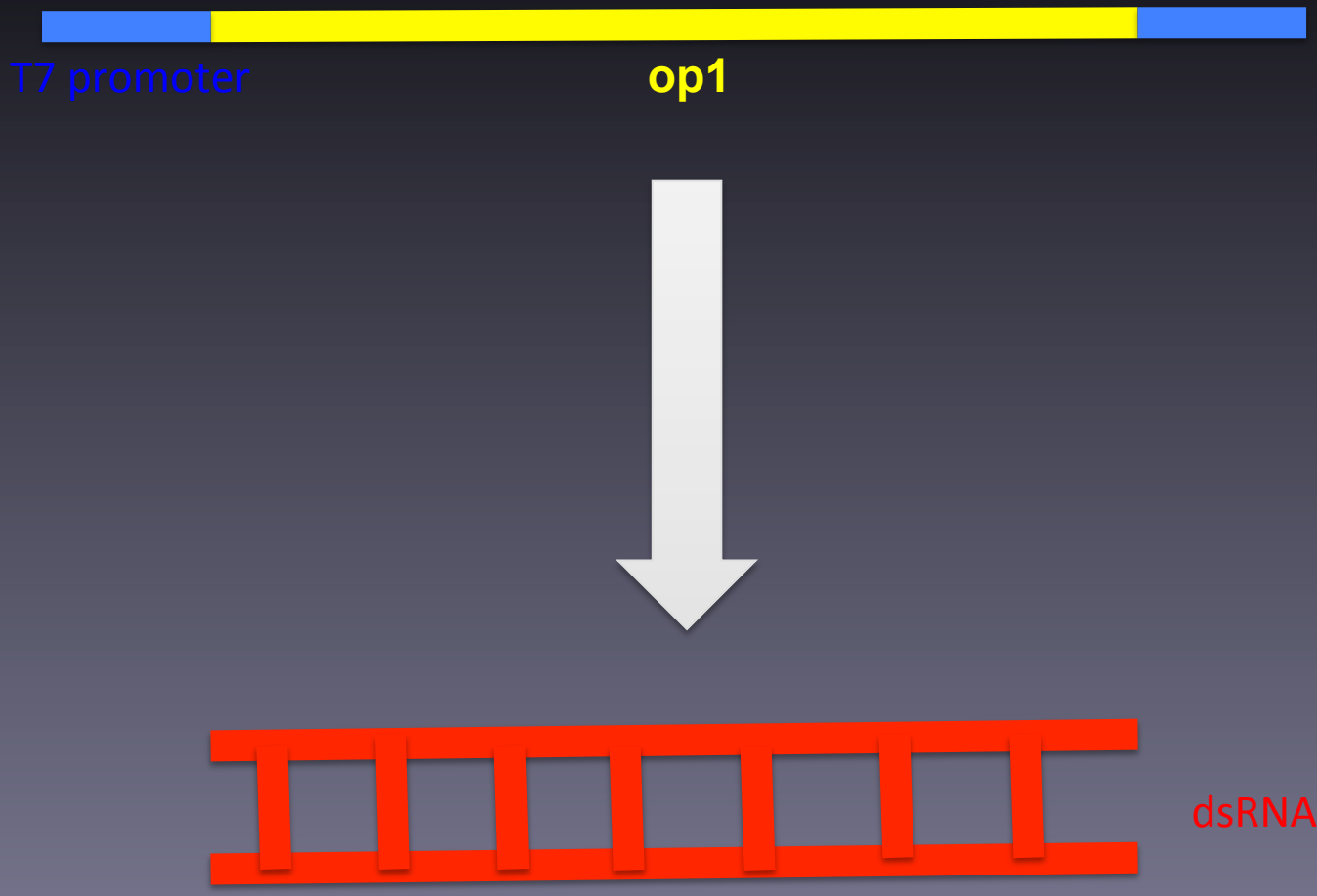
Feed mosquitoes

Western blot, ERG, whole mount

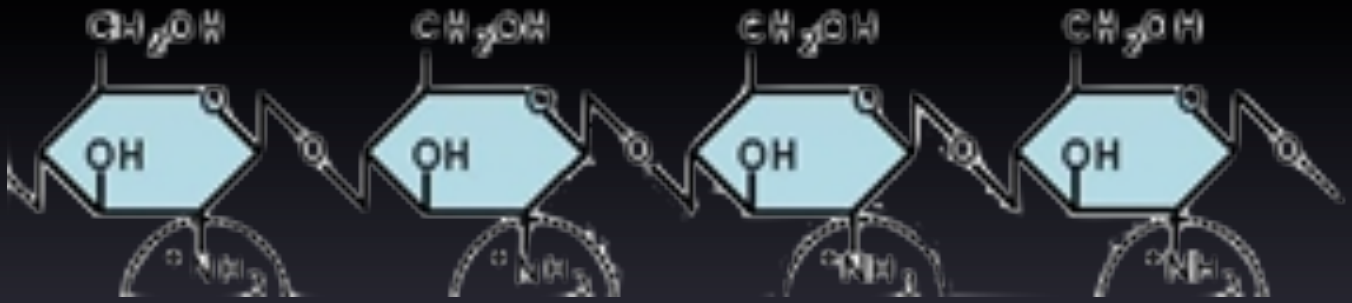
# Generating double-stranded RNA



# Generating double-stranded RNA



# Chitosan

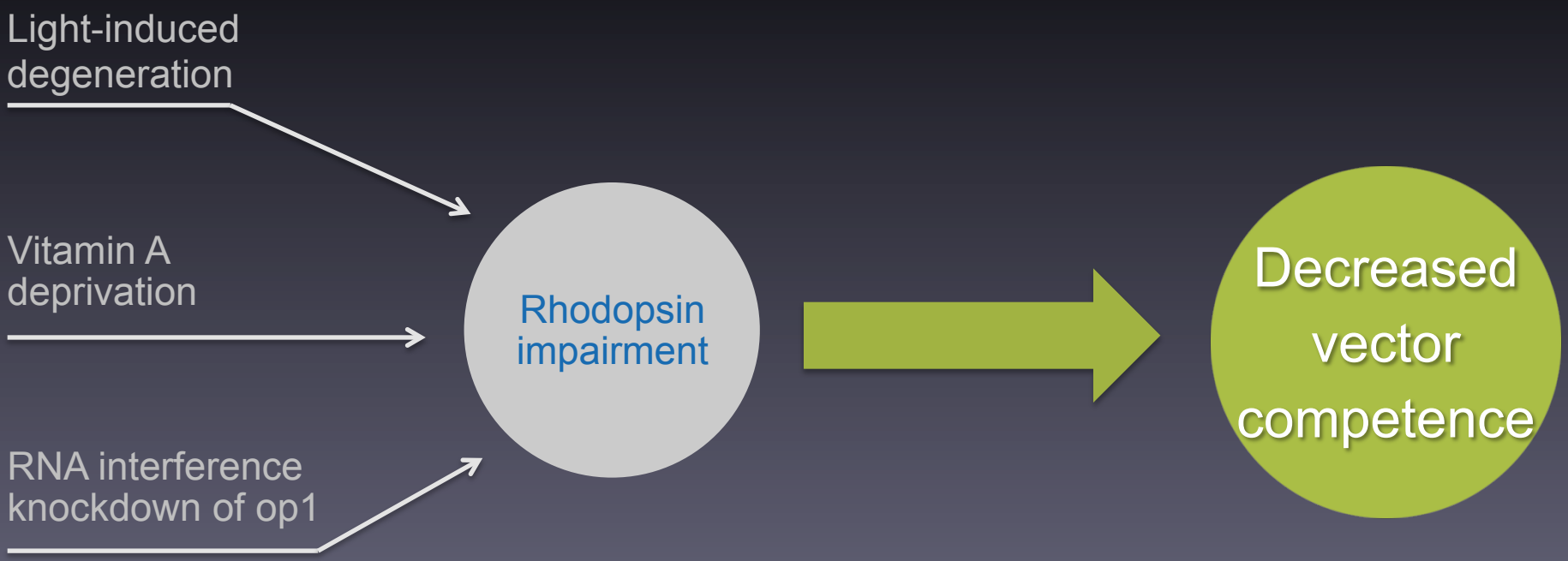


Zhang, 2010

# Progress in RNAi knockdown of op1

- Isolated op1
- Generated 16.7-16.9 ng/μL dsRNA per reaction
- Synthesized dsRNA/chitosan nanoparticles
- Began feeding 3$^{rd}$ instar larvae

# Experimental Overview

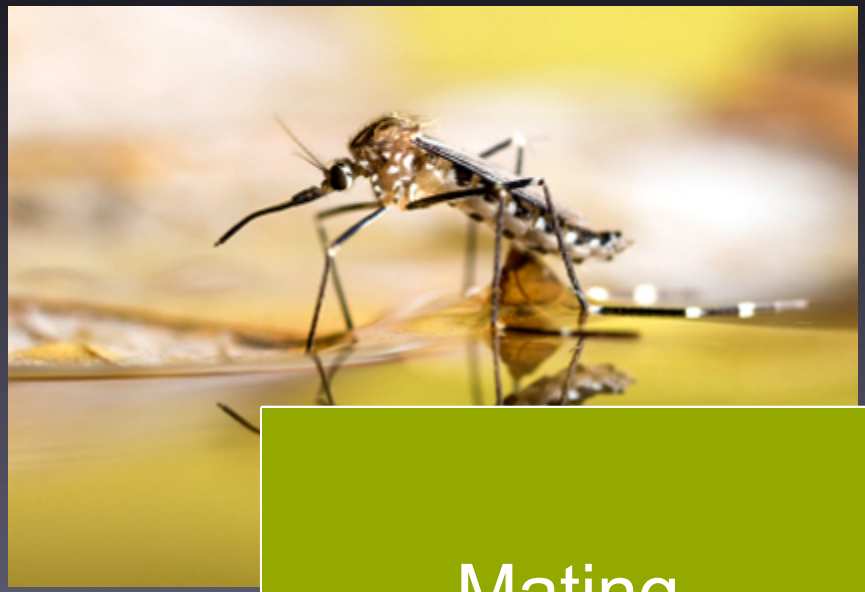


# Behavioral Assays

Blood Feeding

Sugar Feeding

# Behavioral Assays



Mating

Oviposition

# Designing Assays

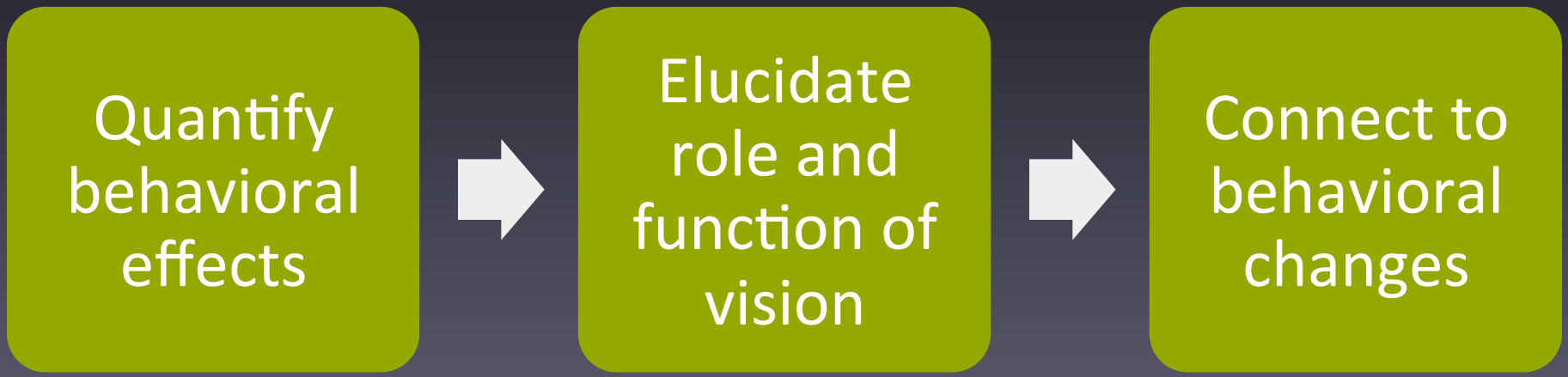
METHODS

- Visual Recording (infrared camera)
- Five replicates
- Setting controls

| Behavior | Time Period | Set-up | Observation Parameters |
|---|---|---|---|
| Blood Feeding | 10 minutes | Mated, non-blood fed female per tube | Time to find host, duration of meal, flight pattern |
| Sugar Feeding | 3 hours | Bucket of 5-10 mosquitoes, flower as sugar source | Freq. of feeding, duration, time to find sugar source |
| Mating | 1 hour | One male and female per tube | Duration of mating, frequency, time to find each other/mate |
| Oviposition | 3 hours | Bucket with cup of water, blood-fed mated female | Where they lay eggs, how long it takes, number of eggs |

# Overall Significance

- Hypothesis




- Determining targets
- Negatively affect vector competence for disease control

# Acknowledgements

We would like to thank the O'Tousa Lab: Dr. O'Tousa and Dr. Whaley for their mentoring time, and our wonderful TAs Matt Leming, Carson Kirkpatrick, Young Moon, and Manny Rocha.

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