A. COVER PAGE

Project Title: Physiological and perceptual examination of vision restoration	
Grant Number: 5R01EY021166-13	**Project/Grant Period:** 02/01/2011 - 01/31/2024
Reporting Period: 02/01/2022 - 01/31/2023	**Requested Budget Period:** 02/01/2023 - 01/31/2024
Report Term Frequency: Annual	**Date Submitted:** 12/16/2022
Program Director/Principal Investigator Information: WILLIAM H MERIGAN , PHD **Phone Number:** (585) 275-4872 **Email:** billm@cvs.rochester.edu	**Recipient Organization:** UNIVERSITY OF ROCHESTER UNIVERSITY OF ROCHESTER 500 Joseph C. Wilson Blvd, RC Box 270140 ROCHESTER, NY 146270140 **DUNS:** 041294109 **UEI:** F27KDXZMF9Y8 **EIN:** 1160743209A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: SUZANNE LANDY 518 Hylan Building Rochester, NY 146270140 **Phone number:** 585-273-1432 **Email:** Suzanne.Landy@rochester.edu	**Signing Official:** SUZANNE LANDY 518 Hylan Building Rochester, NY 146270140 **Phone number:** 585-273-1432 **Email:** Suzanne.Landy@rochester.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. Insert ChrimsonR into macaque ON bipolar cells or RGCs and examine vision restoration using AO imaging of G-CaMP (in RGCs). Although we recently found the first evidence of ChrimsonR-mediated vision in the macaque, we have not yet explored the diversity of RGC responses this can support. Here we will explore spatial resolution and high temporal frequency response of RGC cells. We will also explore the possibility that ON bipolar cells provide a better substrate for optogenetics than RGCs, especially examining ON and OFF responses.

Aim 2. Use controlled fixation psychophysics to examine the spatial, temporal and sensitivity range of perception provided by optogenetic restoration with ChrimsonR. Visual capabilities tested will include basic abilities such as orientation discrimination and contrast sensitivity as well as more specialized discriminations such as motion speed and direction, vernier acuity and shape discrimination. This work will also examine light sensitivity provided by channelrhodopsin.

Aim 3. Psychophysically determine the time course of vision restoration. This series of studies will compare function at restored and non-restored retinal locations to chart any decline in sensitivity (e.g. that due to fading of channelrhodopsin over time), as well as any improved visual function as monkeys become more familiar with the optogenetic-mediated visual experience at restored locations.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : B2-rev.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the next year we will complete testing of restoration of spatial vision thresholds by CatCH in the initial and in the second monkey 849. The first ability tested will be contrast sensitivity restoration by optogenetic treatment. Further studies will then examine optogenetic mediated increment and decrement thresholds, motion sensitivity, orientation thresholds and sensitivity to shape discriminations. These results will help us understand if cortex remains responsive to RGC activity when photoreceptors are lost, and if so what is the time course of plastic responses to the optogenetic prosthesis.

B.2 (B2-rev.pdf)

B. Studies and Results

Aim 1. Insert ChrimsonR into macaque ON bipolar cells or RGCs and examine vision restoration using AO imaging of G-CaMP (in RGCs). We completed a study on the time course of optogenetic therapy using the in vivo retinal imaging of retinal activity that we have used before (Molecular Therapy 2022). on longitudinal study of RGC mediated ChrimsonR restoration of vision. In this study we examined the signal to noise of restored vision in two macaques up to 2 years following loss of photoreceptors. We found that restored RGC responses to drifting spatial gratings was essentially unchanged months to years after loss of photoreceptors, a surprising finding given the many reports of retinal "remodeling" in such cases. Furthermore, there was no imaging evidence of cell loss in the ganglion cell layer, we are currently assessing this longitudinally using imaging in one monkey which at the time of this publication had survived photoreceptor loss for over 2 years. It is now approaching 3 years and there is no imaging evidence of changes to the optogenetic expressing RGCs.

Aim 2. Use controlled fixation psychophysics to examine the spatial, temporal and sensitivity range of perception provided by optogenetic restoration with ChrimsonR. In two monkeys we have completed testing contrast thresholds over a grid near two locations in the left eye, and both monkeys have received photoreceptor lesions with a 2 photon microscope. Multiple 2 photon exposures each were used to make cone lesions just nasal and temporal of the fixation locus of the left eye of each monkey (Figure 1 shows the lesion made in the second monkey). The extent of visual loss following the lesions was then mapped with controlled fixation testing of contrast thresholds (Figure 2 is again the second monkey). The borders of visual loss appear stable, extending to fill slightly more than a quadrant of vision, and restoration in these regions will be examined at chosen locations in each eye which correspond to regions of good expression of the optogenetic agent, CatCH. CatCH injection will be made in the left eye of the second monkey in the next two weeks and then both monkeys will be used to test the possibility of optogenetic restoration. We have fully developed and tested a second psychophysical procedure, which the first tested monkey was able to be trained on to measure increment and decrement thresholds for 0.1 deg circular stimuli presented on a mid grey background. Optogenetics will be tested initially with the contrast threshold measure and later with increment and decrement thresholds. Both monkeys are also trained to perform psychophysically with broadband normal stimuli (ranging from 350 to over 700 nm) as well as with stimuli filtered by red or blue filters to pass only the wavelengths visible to or not visible to CatCH optogenetic.

Aim 3. Psychophysically determine the time course of vision restoration. This project will proceed in time from our initial studies of optogenetic vision restoration. The parallel imaging study of long-term optogenetic vision restoration described above will inform the longitudinal psychophysics study, which will be done with psychophysics, making it unnecessary to inject the calcium indicator G-CaMP6s.

Significance Our preliminary retinal imaging data suggests that the retinal ganglion cells that lose their photoreceptor input remain viable and responsive to visual stimulation for at least 3 years year after the loss. However, we do not know if these ganglion cell signals are conveyed to visual cortex sufficiently intact to be decoded by cortical neurons. This result will be obtained in the next few months.

Near foveal lesions in second monkey (#849)

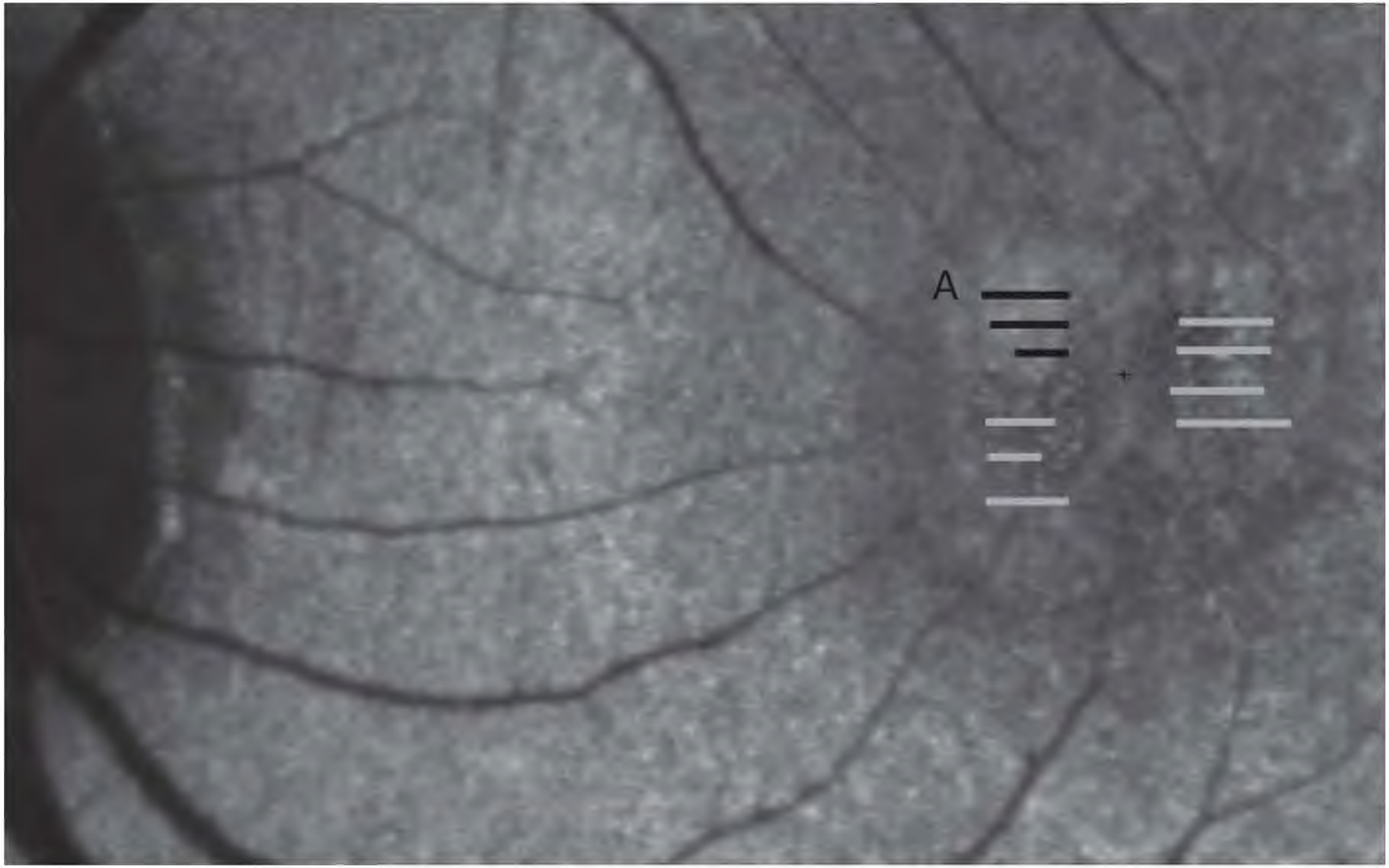

Figure 1. IRHRA of near foveal lesions in the second monkey (849) illustrating with OCT overlays regions of clear photoreceptor lesions marked with bars showing the severity of lesion at each OCT plane; light grey for mild lesion, black for dense lesion. Fixation locus is near the cross, but there remains slight uncertainty about its vertical position. The substantial lesion near A is being tested psychophysically.

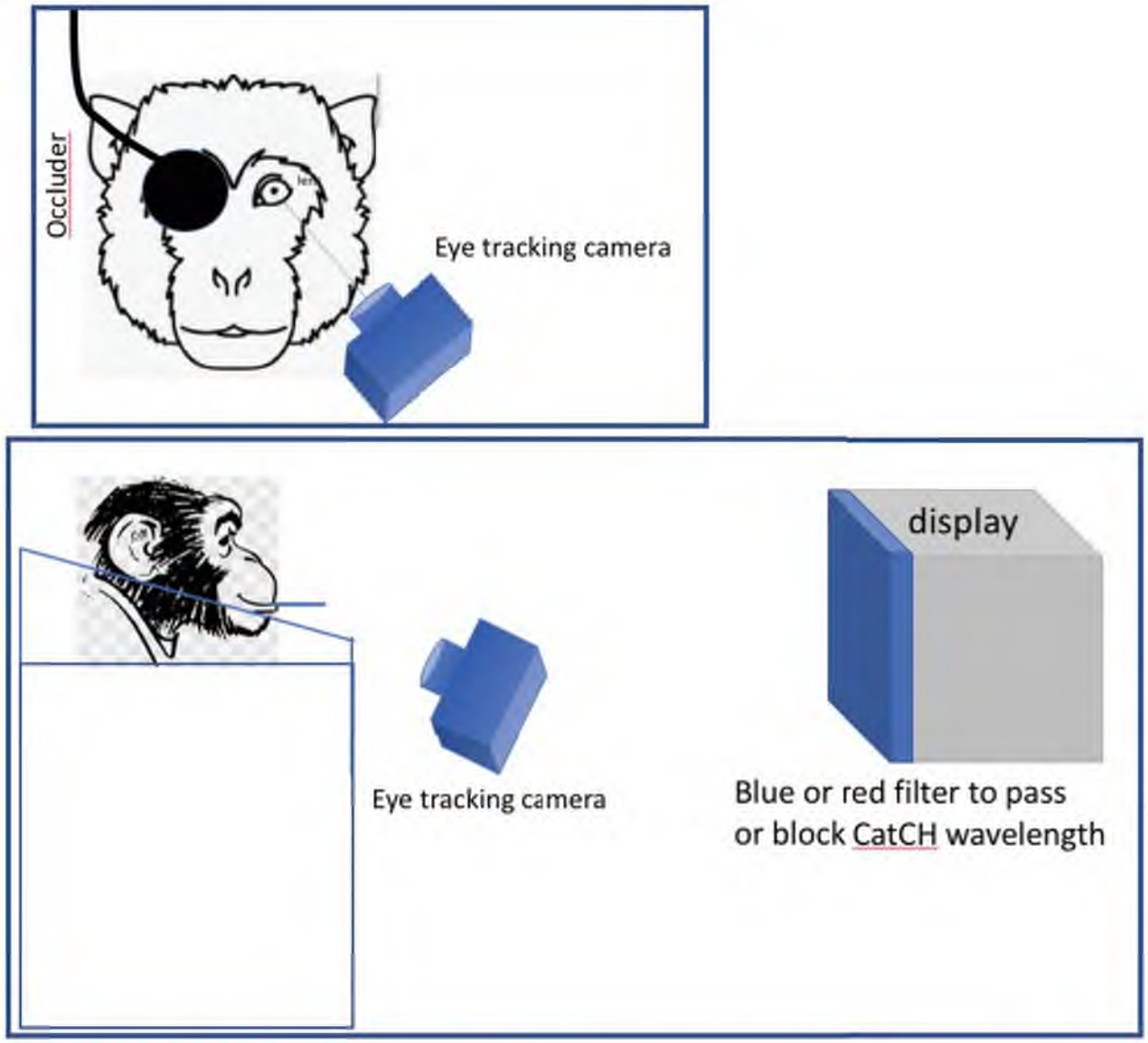

Figure 2. Behavioral (psychophysical) test setup. The monkey views the stimulus monocularly. Fixation is monitored with a video image. On test conditions vision is compared using either a red filter that passes visible light but blocks CatCH or a blue filter that passes the wavelengths Catch is sensitive to.

B4. Training opportunities

Graduate student For the past year, Redacted by , an optics graduate student, has been working with Dr. Merigan and Redacted by on both the major psychophysics project that is the subject of this grant and on a short-term project to develop optical stimulation of single identified retinal ganglion cells. He meets regularly with the PI and continues to work with a major user of the 2 photon system, the faculty member Redacted by agreement Redacted by as well as our optical engineer, Redacted by agreement to tailor the two-photon system (which has received a new TiSapphire laser to replace the prior laser which was failing) for creating highly selective lesions of photoreceptors in the macaque for vision restoration.

Undergraduates

Redacted by agreement is now assisting Redacted by and learning adaptive optics imaging from him. The two of them will assist with further testing of behavioral thresholds for spatial stimuli once fixation issues are stabilized.

Redacted by a sophomore in the Department of Brain and Cognitive Sciences is now working closely with Redacted by agreement the technician doing the training and Redacted by the programmer modifying the program to plot daily fixation calibration and fixation during the testing to measure and modify residual problems in the fixation calibration.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Godat T, Cottaris NP, Patterson S, Kohout K, Parkins K, Yang Q, Strazzeri JM, McGregor JE, Brainard DH, Merigan WH, Williams DR. In vivo chromatic and spatial tuning of foveolar retinal ganglion cells in Macaca fascicularis. PloS one. 2022;17(11):e0278261. PubMed PMID: 36445926; PubMed Central PMCID: PMC9707781; DOI: 10.1371/journal.pone.0278261.

Non-compliant Publications Previously Reported for this Project

Public Access Compliance	Citation
Non-Compliant	Cheong. In Vivo Functional Imaging of Retinal Neurons Using Red and Green Fluorescent Calcium Indicators. Advances in experimental medicine and biology. 2018.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	Merigan, William H	PHD	PD/PI	EFFORT					NA
	N	Redacted by agreement	PHD,MA,BS	Co-Investigator						NA
	N		OTH,PHD	Faculty						NA
	N		PHD,MS,BS	Staff scientist (Doctoral level)						NA
	N		PHD	Graduate Student (research assistant)						NA
	N		AAS	Non-Student Research Assistant						NA
	N		MS	Technician						NA
	N		BS	Technician						NA
	N		BS	Technician						NA
	N		MS	Programmer						NA

Glossary of acronyms:
S/K - Senior/Key
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RS - Reentry Supplement
DS - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support **Has there been a change in the active other support of senior/key personnel since the last reporting period?** Yes File Uploaded: other support combined.pdf
D.2.d New Other Significant Contributors **Are there, or will there be, new other significant contributors?** No
D.2.e Multi-PI (MPI) Leadership Plan **Will there be a change in the MPI Leadership Plan for the next budget period?** NA

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

For New and Renewal Applications – DO NOT SUBMIT UNLESS REQUESTED
PHS 398 OTHER SUPPORT

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Merigan, W.
Commons ID: eRA

Other Support – Project/Proposal

ACTIVE

*Title: Physiological and perceptual examination of vision restoration

Major Goals: The goal of this research is examine optogenetically-restored vision in non-human primates to guide future restoration of vision in blind humans using in-vivo adaptive optics imaging and controlled fixation psychophysical methods in macaque to examine visual responses provided by ganglion cell and ON bipolar cell optogenetics.

*Status of Support: Active

Project Number: R01 EY02116

Name of PD/PI: Merigan, W.

*Source of Support: NIH/NEI

*Primary Place of Performance: University of Rochester

Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/2019 – 01/2024

* Total Award Amount (including Indirect Costs): $3,638,823

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
9. 2019	EF FO RT calendar
10. 2020	calendar
11. 2021	calendar
12. 2022	calendar
13. 2023	calendar

*Title: High Resolution Mapping of Foveal Ganglion Cell Receptive Fields in the Living Primate Eye

Major Goals: The goal of this project is to provide a comprehensive survey of the foveal ganglion cell classes that mediate primate foveal vision.

*Status of Support: Active

Project Number: R01 EY031467

Name of PD/PI: Williams, D.R./Merigan, W.

*Source of Support: NIH/NEI

*Primary Place of Performance: University of Rochester

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

Name of Individual: Merigan, W
Commons ID: eRA Commons

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021 – 11/2025

* Total Award Amount (including Indirect Costs): $2,823,077

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar
2. 2022	calendar
3. 2023	calendar
4. 2024	calendar
5. 2025	calendar

*Title: Examining the effects of retinal cell loss on downstream visual brain areas

Major Goals: The goal of this project is to examine the effects of retinal cell loss on functional response properties, response statistics, and functional connectivity among neurons in the LGN.

*Status of Support: Active

Project Number: R21 EY031052

Name of PD/PI: Briggs, F.

*Source of Support: NIH/NEI

*Primary Place of Performance: University of Rochester

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021 –12/2022

* Total Award Amount (including Indirect Costs): $423,500

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar
2. 2022	calendar

*Title: Accelerating photoreceptor replacement therapy with in-vivo cellular imaging of retinal function

Major Goals: The goal of this project is to use a novel functioning imaging technique that will be applied to evaluate photoreceptor replacement therapy in the fovea. Success in restoring vision at this location will provide strong evidence that this type of therapy could restore usable vision in patients and regenerative therapies should move rapidly into clinical trials.

*Status of Support: Active

Project Number: U24 EY033275

Name of PD/PI: McGregor, J.

*Source of Support: NIH/NEI

*Primary Place of Performance: University of Rochester

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2021 – 07/2025

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

Name of Individual: Merigan, W
Commons ID: eRA Commons User Name

* Total Award Amount (including Indirect Costs): $5,717,798

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar
2. 2022	calendar
3. 2023	calendar
4. 2024	calendar
5. 2025	calendar

*Title: Single Retinal Ganglion Cells and Sensation

Major Goals: The goal of this project is to establish a new experimental paradigm that can reveal how the individual computations performed by the retina impact vision.

*Status of Support: Active

Project Number: Private Source

Name of PD/PI: Williams, D.R.

*Source of Support: Private Source

*Primary Place of Performance: University of Rochester

Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/2022 – 03/2025

* Total Award Amount (including Indirect Costs): $2,462,575

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	EFFORT calendar
2. 2023	calendar
3. 2024	calendar

*Title: High-Resolution Functional Imaging of the Retina

Major Goals: Our unique capabilities for cellular-scale two-photon excited fluorescence ophthalmoscopy in the living mouse and monkey eye will be greatly enhanced to provide non-invasive measures of cellular metabolic activity and inner retinal function.

*Status of Support: Current

Project Number: R01 EY022371

Name of PD/PI: Imaging Core – Hunter, J.J.

*Source of Support: NIH/NEI

*Primary Place of Performance: University of Rochester

Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/2022 – 5/31/2026

* Total Award Amount (including Indirect Costs): $1,846,511

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

Name of Individual: Merigan, W
Commons ID: eRA Commons User Name

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
9. 2022	EFFORT calendar
10. 2023	calendar
11. 2024	calendar
12. 2025	calendar

PENDING

***Overlap** (summarized for each individual): None.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: DocuSigned by: William Merigan

Date: December 15, 2022

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E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
$45,000	FRANCE

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

There have been two major issues that have produced a delay in this project.
1. The long-term technician of Dr. Merigan, Redacted by , was extremely skilled at handling and training and testing macaque monkeys. Unfortunately, she refused the covid vaccination and was not allowed to be on the university premises after 3 November 2021. She did assist with remote training for the new hire through March 2022, writing papers, and preparing "how to" manuals for the lab . Dr. Merigan's replacement technician, Redacted by was hired after a very extensive search, in the first week of January 2022. Her ability to start monkey testing was initially delayed for approximately 6 weeks while she passed several on-line training and testing packages designed to prepare her to function as a primate technician. The first few months of her work in the laboratory were devoted to learning to test the two monkeys used in the first phase of the study. An additional challenge is that the monkeys needed time to adapt to a new technician, and in the past few months she has gained their trust.
2. One critical aspect of the study is producing highly focal lesions of photoreceptors, sparing RPE and superficial retina. Such lesions are made with a tiSappire 2-photon laser and in June 2021, shortly after the first lesion was made in monkey 1 (839) on August 11, 2021 the laser became noticeably degraded, showing rapid fluctuations of power which made it impossible to use for producing lesions. For this reason, a 2 photon lesion on monkey 2 (849) could not be done until June 15, 2022 after a comparable new laser was installed.
All aspects of the technician support and instrumentation are now restored and we hope to have initial evidence of potential optogenetic restoration within a few weeks.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	UEI	Congressional District	Address
Primary: UNIVERSITY OF ROCHESTER	F27KDXZMF9Y8	ny-025	UNIVERSITY OF ROCHESTER ORPA 518 Hylan Bldg., Box 270140 ROCHESTER, NY 146270140

G.9 FOREIGN COMPONENT

Organization Name: UNIVERSITE PARIS 6 PIERRE ET MARIE CURIE
Country: FRANCE
Description of Foreign Component:
Inst. of higher Education, Redacted by is listed as a subcontract on this project.

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

Yes

Estimated unobligated balance: $281,060

G.10.b Provide an explanation for unobligated balance:
Departure of 2 postdocs to industry, Sr. Lab Tech of 10 plus years left the University due to mandatory COVID vaccine rule, hiring has been difficult however a new lab tech was hired at the beginning of this budget year to take over the animal training. A critical laser failed and had to be replaced and installed into the AOSLO.

G.10.c If authorized to carryover the balance, provide a general description of how it is anticipated that the funds will be spent
I will be adding additional personnel and increasing the grad student's effort, in the next year to increase productivity on this project before the renewal.

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No