Form Approved Through 02/28/2023 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

12173153 **Grant Application**

Do not exceed character length restrictions indicated.

PI: **FONG, MING-FAI** Council: 10/2021
4 R00 EY029326-03 IPF:676603 [3K99EY029326-02]
Dual:
IRG: NSS Received: 11/16/2021

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
"Controlling synaptic and intrinsic plasticity underlying visual cortical enhancement"

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PA-20-188 Title: NIH Pathway to Independence Award (Parent K99/R00 Independent Clinical

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Fong, Ming-fai	PhD	eRA Commons User Name

3c. POSITION TITLE
Assistant Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Redacted by
Atlanta, GA 30332

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Wallace H. Coulter Dept. of Biomedical Engineering

3f. MAJOR SUBDIVISION
Engineering

3g. TELEPHONE AND FAX *(Area code, number and extension)*
Redacted by agreement

E-MAIL ADDRESS:
Redacted by agreement

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☒ No ☐ Yes	☒ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
A 3822-01	☒ No ☐ Yes	☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes
5a. Animal Welfare Assurance No. pending

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
11/01/2021	10/30/2024	205221	249000	538407	747000

9. APPLICANT ORGANIZATION
Name Georgia Tech Research Corporation
Address 926 Dalney Street, NW
Atlanta, GA 303329-0415

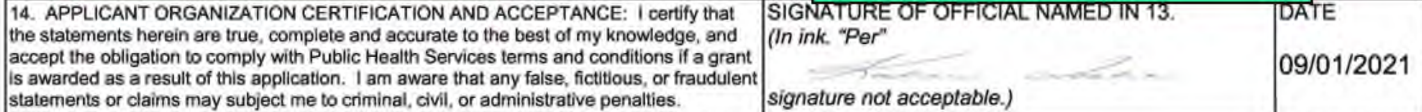
10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
580603146
DUNS NO. 097394084 Cong. District GA05

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Kadian Leslie
Title Contracting Officer
Address 926 Dalney Street NW
Atlanta, GA 30332-0420
Tel: Redacted by agreement
E-Mail

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Kadian Leslie
Title Contracting Officer
Address 926 Dalney Street NW
Atlanta, GA 30332-0420
Tel: Redacted by agreement
E-Mail

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I 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 OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)*

DATE
09/01/2021

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

PROJECT SUMMARY (See instructions):

Amblyopia is widespread form of human visual disability caused by a disparity in visual quality between the two eyes during early postnatal life. This disparity drives ocular dominance plasticity in the visual cortex to favor the stronger eye at the expense of the weaker (amblyopic) eye. Consequently, synapses in the visual cortex downstream of the amblyopic eye are weakened, a process that is difficult to reverse unless treatment is initiated during infancy or early childhood. Recent work in animal models has suggested several strategies for promoting recovery from amblyopia. While the pathophysiology underlying amblyopia has been well studied, the synaptic, cellular, and circuit changes underlying recovery are less clear. This proposal focuses on a treatment strategy that rapidly promotes visual recovery following experimental amblyopia via temporary inactivation of the retinas. A temporary period of retinal inactivation leads of a stable enhancement of visual cortical responses once vision is restored. The previous mentored research focused on understanding how retinal inactivation promotes recovery at through synaptic and cellular plasticity in the primary visual cortex. The current proposal shifts the focus to how recovery manifests in the statistics of neural activity within visual circuits, and whether this recovery can be controlled through reproducing these activity regimes. The long- term objective of this research is to understand how cortical plasticity is engaged to promote recovery and to inform clinical interventions for treating human amblyopia.

RELEVANCE (See instructions):

The proposed research seeks to systematically identify plasticity mechanisms that lead to enhanced visual ability. The work will provide new insights on neural mechanisms of visual augmentation, and may suggest new treatments for visual disabilities such as amblyopia.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location

Organizational Name: Georgia Institute of Technology

DUNS: 097394084

Street 1: Redacted by agreement | Street 2:

City: Atlanta | County: Fulton | State: GA

Province: | Country: USA | Zip/Postal Code: 30332-0535

Project/Performance Site Congressional Districts: GA-005

Additional Project/Performance Site Location

Organizational Name:

DUNS:

Street 1: | Street 2:

City: | County: | State:

Province: | Country: | Zip/Postal Code:

Project/Performance Site Congressional Districts:

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Fong, Ming-fai	eRA Commons User Name	GTRC	Principal Investigator

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project

Human Embryonic Stem Cells ☐ **No** ☐ **Yes**

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: https://grants.nih.gov/stem_cells/registry/current.htm. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 11/01/2021 THROUGH 10/31/2022

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Ming-fai Fong	PD/PI	Institutional Base Salary				11216	3656	14872
Research Technician	Technician					37500	12225	49725
SUBTOTALS →						48716	15881	64597

Category	Amount
CONSULTANT COSTS	
EQUIPMENT *(Itemize)* Immediate equipment needs include mouse stereotax ($12,000), surgical stereoscope ($15,000), isoflurane vaporizer ($5,500), patch clamp amplifier ($21,000), patch clamp digitizer ($11,000), patch clamp acquisition software ($9,000), vibratome ($25,000), *in vivo* head fixed electrophysiology acquisition system ($7,000), and *in vivo* freely moving ephys+behavior acquisition system ($24,500). These items will be purchased during Year 1 to support Aims 1 and 2.	130000
SUPPLIES *(Itemize by category)* Consumables: $5,625 Animals: $5,000	10625
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	$ 205221
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	43779
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$ 249000

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 11/01/2022 THROUGH 10/31/2023

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Ming-fai Fong	PD/PI	Institutional Base Salary				11552	3766	15318
Research Technician	Technician					38625	12592	51217
Postdoctoral Fellow	Postdoc					41826	13635	55461
SUBTOTALS →						92003	29993	121996

Item	Amount
CONSULTANT COSTS	
EQUIPMENT *(Itemize)* An *in vivo* recording and stimulation system will be purchased during Year 2	40000
SUPPLIES *(Itemize by category)* Consumables: $5,115 Animals: $5,000	10115
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	$ 172111
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	76889
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$ 249,000

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	11/01/2023	10/31/2024

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Ming-fai Fong	PD/PI	Institutional Base Salary				11899	3879	15778
Research Technician	Technician					48625	15852	64477
Postdoctoral Fellow	Postdoc					43396	14147	57543
SUBTOTALS →						103919	33878	137797

CONSULTANT COSTS	
EQUIPMENT *(Itemize)* Small equipment purchases during Year 3 to support Aim 3	10000
SUPPLIES *(Itemize by category)* Consumables: $8277 Animals: $5,000	13277
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	$ 161075
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	87925
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$ 249,000

Obtained via FOIA by White Coat Waste Project (WCW)

Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	64597	121996	137797		
CONSULTANT COSTS					
EQUIPMENT	130000	40000	10000		
SUPPLIES	10625	10115	13277		
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	205221	172111	161075		
F&A CONSORTIUM/ CONTRACTUAL COSTS	43779	76889	87925		
TOTAL DIRECT COSTS	249000	249000	249000		
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD					$ 747000

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Budget Justification:

KEY PERSONNEL: $34,666 over 3 years

PI: Ming-fai Fong, Ph.D. EFFORT

Dr. Fong will lead the project including the design, execution, analysis, interpretation, and publication of the research. She will also train all personnel involved in the project. This will be Dr. Fong's primary research focus over the funding period. Georgia Tech covers Dr. Fong's salary during the academic months, so only summer salary is requested. Projected annual inflation is budgeted at 3%

OTHER PERSONNEL: $209,972 over 3 years

Research Technician or equivalent: $124,750 EFFORT

The research technician will assist with execution of the project research. Specific responsibilities include ordering materials and supplies, coordinating equipment installation, overseeing animal care, maintaining inventories and drug logs, performing surgical procedures, collecting and analyzing data, performing histological analyses, and organizing data for future public dissemination. This project will be the technicians's primary focus over the funding period. Salary based on Georgia Tech payscale for research technicians and projected annual inflation is budgeted at 3%

Postdoctoral Fellow or equivalent: $85,222 EFFORT

The postdoctoral fellow will assist with the design, execution, analysis, and publication of the research. Specific responsibilities include performing surgical procedures, collecting and analyzing data, drafting manuscripts. This project will be the postdoc's primary research area focus over the funding period. Dr. Fong's startup funds may be used for salary during the Year 1, and to pay remaining salary for full-time work in Years 2-3 as necessary. Salary based on NIH NRSA stipend payscale for FY2021 at 0-2 years of experience, with projected annual inflation is budgeted at 3%.

OTHER DIRECT COSTS:

Equipment: **Year 1: $130,000; Year 2: $40,000; Year 3: $10,000**

Immediate equipment needs include mouse stereotax ($12,000), surgical stereoscope ($15,000), isoflurane vaporizer ($5,500), patch clamp amplifier ($21,000), patch clamp digitizer ($11,000), patch clamp acquisition software ($9,000), vibratome ($25,000), *in vivo* head fixed electrophysiology acquisition system ($7,000), and *in vivo* freely moving ephys+behavior acquisition system ($24,500). These items will be purchased during Year 1 to support Aims 1 and 2. Other necessary equipment such as air tables, fridges/freezers, and water purification systems will be purchased from Dr. Fong's startup package, and some equipment will be loaned from other investigators including a patch microsope, pipet puller, and cryostat. We anticipate purchase of an *in vivo* recording and stimulation equipment during Year 2 and small equipment purchases during Year 3 to support Aim 3.

Materials and Supplies: **Year 1: $ 10,625; Year 2: $ 10,115; Year 3: $ 13,277**

Consumables: $5,625 (Year 1), $5,115 (Year 2), $,8,277 (Year 3)
Animals: $5,000/year
Any addition M&S needs will be purchased from Dr. Fong's startup funds.

Fringe Benefits: **$79,752 per year**

Fringe Benefits are calculated at 32.6% for faculty and staff.

Total Direct Costs <u>$538,407</u>

Indirect Costs: <u>$208,593</u>

Georgia Tech facilities and administration costs are calculated at the approved rate of 58.2% on a MTDC basis. Facilities & Administrative (F&A) rates have been established and approved by the Office of Naval Research (ONR).

Total Direct and Indirect Costs: <u>$747,000.</u>

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

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Fong, Ming-fai

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge, MA	BS	06/2005	Mechanical Engineering
Emory University, Atlanta, GA	PhD	08/2014	Neuroscience
Massachusetts Institute of Technology, Cambridge, MA	Postdoc	09/2021	Brain & Cognitive Sciences

A. Personal Statement

My research interests lie in how sensory experience is encoded in the mammalian central nervous system, and how neural circuits can be engaged for rehabilitation from neurological forms of sensory impairment. My recent work has focused on a widespread human visual disability called amblyopia, which can be recapitulated and studied in animal models. Inspired by foundational research in synaptic plasticity, I have identified plasticity-based strategies for promoting recovery from visual impairment in amblyopic mice and cats. Looking forward, my lab will investigate how transient changes in synaptic and neuronal activity drive long-lasting plasticity in neural circuits to alter perceptual capacity. Further, we will develop therapeutic interventions for sensory and motor disabilities ranging from neural prosthetics that can provide surrogate sensory information to the brain, to low-cost portable treatments for practical implementation in resource-poor communities. In the long term, I aspire to investigate plasticity-based therapies for a variety of sensory/motor disabilities and neurological disorders. I plan to complete this work in partnership with students and research staff from diverse backgrounds while helping to train the next generation of scientists and engineers.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 -	Assistant Professor, Georgia Tech and Emory University, Department of Biomedical Engineering, Atlanta, GA
2014 - 2021	Postdoctoral Scholar, Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge, MA
2016 - 2017	Visiting Lecturer, Wellesley College, Neuroscience Department, Wellesley, MA
2008 - 2014	Graduate Student, Emory University and Georgia Institute of Technology, Departments of Physiology and Biomedical Engineering, Atlanta, GA
2006 - 2008	High Schoolar Teacher, American School Foundation of Guadalajara, Department of Science, Guadalajara
2005 - 2006	High School Teacher, Teacher, Instituto Tecnológico y de Estudios Superiores de Monterrey, Departments of Computer Science, Mathematics, and Science, Monterrey

Honors

2018 - present	Pathway to Independence Award, NIH National Eye Institute
2015 - 2018	Picower Institute Innovation Fund, JBP Foundation
2013 - 2014	Scholarly Inquiry and Research and Emory Predoctoral Fellowship, HHMI and Emory College
2010 - 2013	Graduate Research Fellowship, National Science Foundation

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2010 - 2012	Scholars Program in Interdisciplinary Neuroscience Research Fellowship, Emory Neuroscience Initiative
2008 - 2010	Integrative Graduate Education and Research Traineeship at Georgia Tech and Emory, National Science Foundation
2018	Picower Postdoctoral Fellowshop, JPB Foundation
2014	Graduate Career Award Finalist, Emory University GDBBS
2013	Outstanding Scientific Achievement Award, Emory University Neuroscience Program

C. Contribution to Science

1. **Amblyopia and visual cortical plasticity:**
Amblyopia is a widespread neurodevelopmental disorder wherein vision is impaired in at least one eye due to improper brain development. My postdoctoral work has focused on the synaptic pathophysiology underlying amblyopia, and strategies for treating amblyopia using synaptic plasticity. In one study, I identified synaptic receptors and cell types that uniquely contribute to amblyopia pathology compared to other forms of visual cortical plasticity (Fong et al., 2020). My most significant work has been a series of preclinical studies demonstrating that silencing activity in one or both retinas can promote recovery from deprivation amblyopia. I have conducted this work in two species (mice and cats) with different disease severity and ages (Fong et al., 2016; Duffy et al., 2018; Fong et al., 2021). This body of work provides support for a novel, portable treatments for human amblyopia, and suggests that leveraging homeostatic synaptic plasticity may be a useful approach for other disorders of the nervous system.

    a. **Fong M-f**, Mitchell DE, Duffy KR, Bear MF. Rapid recovery from the effects of early monocular deprivation is enabled by temporary inactivation of the retinas. Proc Natl Acad Sci U S A. 2016 Dec 6;113(49):14139-14144. PubMed Central PMCID: PMC5150384.

    b. **Fong M-f**, Finnie PS, Kim T, Thomazeau A, Kaplan ES, Cooke SF, Bear MF. Distinct Laminar Requirements for NMDA Receptors in Experience-Dependent Visual Cortical Plasticity. Cereb Cortex. 2020 Apr 14;30(4):2555-2572. PubMed Central PMCID: PMC7174998.

    c. Duffy KR, **Fong M-f**, Mitchell DE, Bear MF. Recovery from the anatomical effects of long-term monocular deprivation in cat lateral geniculate nucleus. J Comp Neurol. 2018 Feb 1;526(2):310-323. PubMed PMID: 29023717.

    d. **Fong M-f**, Duffy KR, Leet MP, Candler CT, Bear MF. Correction of amblyopia in cats and mice after the critical period. bioRxiv [Preprint]. 2021. DOI: 10.1101/2021.05.03.442423

2. **Homeostatic synaptic plasticity:**
Homeostatic plasticity is a family a mechanisms that act to compensate for chronic disruptions to neural activity. The most widely studied form of homeostatic plasticity is synaptic scaling, a phenomenon believed to occur when all synapses onto a neuron multiplicatively strengthen (or weaken) in response to reductions (or elevations) in firing rate. Work I conducted as a graduate student challenges both the causal nature of changes in firing rate on synaptic scaling, as well as multiplicative nature of the plasticity. Historically, it has been difficult to decouple the effects of spiking activity and neurotransmission on plasticity. I collaborated to develop a closed-loop system for precisely controlling firing rate to a setpoint even when neurotransmission was blocked (Newman et al., 2015; Fong et al., 2015). Using this technology, I discovered that synaptic scaling persisted during blockade of AMPA-type neurotransmission even when spiking was maintained at normal levels (Fong et al. 2015). Using pharmacological approaches, I confirmed the requirement for reduced AMPAergic transmission, rather than reduced spiking, in synaptic scaling (Fong et al., 2015). These findings challenged the dogma that synaptic scaling is a firing rate-dependent process. Further, mathematical analysis of the experimental data, in conjunction with independently-collected data sets from other laboratories, revealed that the increase in synaptic strengths did not follow a purely multiplicative rule; rather, stronger synapses strengthened more and weaker synapses strengthen less (Hanes et al., 2020). This "divergent" plasticity challenged the notion that synaptic scaling is a simple multiplicative process.

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a. **Fong M-f**, Newman JP, Potter SM, Wenner P. Upward synaptic scaling is dependent on neurotransmission rather than spiking. Nat Commun. 2015 Mar 9;6:6339. PubMed Central PMCID: PMC4355957.
b. Newman JP, **Fong M-f**, Millard DC, Whitmire CJ, Stanley GB, Potter SM. Optogenetic feedback control of neural activity. Elife. 2015 Jul 3;4:e07192. PubMed Central PMCID: PMC4490717.
c. Hanes AL, Koesters AG, **Fong M-f**, Altimimi HF, Stellwagen D, Wenner P, Engisch KL. Divergent Synaptic Scaling of Miniature EPSCs following Activity Blockade in Dissociated Neuronal Cultures. J Neurosci. 2020 May 20;40(21):4090-4102. PubMed Central PMCID: PMC7244191.

3. **Closed-loop control of neural activity:**

The advent of voltage clamp over 50 years ago has allowed generations of researchers study currents across a cell membrane without the confounds of a fluctuating membrane potential (e.g. action potential firing). During graduate school, I collaborated on project aimed at clamping network activity that would enable researchers to examine system dynamics independent of a fluctuating network firing rate. This team project yielded new developments in software and hardware (Newman et al., 2012; Tchumatchenko et al., 2013; Newman et al., 2015). We built a system that used multisite electrical recording to continuously monitor spiking activity, and delivered electrical or optogenetic stimulation contingent upon recorded activity in order to maintain firing rate at a setpoint (Newman et al., 2012; Newman et al., 2015). These new tools allowed us to conduct experiments that previously were not possible, such as restoring to normal spike rates during pharmacological manipulations that typically create aberrant circuit activity, or precisely altering spiking activity during ongoing sensory stimulation (Fong et al., 2015; Newman et al., 2015).

a. Newman JP, Zeller-Townson R, **Fong M-f**, Arcot Desai S, Gross RE, Potter SM. Closed-Loop, Multichannel Experimentation Using the Open-Source NeuroRighter Electrophysiology Platform. Front Neural Circuits. 2012;6:98. PubMed Central PMCID: PMC3548271.
b. Tchumatchenko T, Newman JP, **Fong M-f**, Potter SM. Delivery of continuously-varying stimuli using channelrhodopsin-2. Front Neural Circuits. 2013;7:184. PubMed Central ID: PMC3853882.
c. **Fong M-f**, Newman JP, Potter SM, Wenner P. Upward synaptic scaling is dependent on neurotransmission rather than spiking. Nat Commun. 2015 Mar 9;6:6339. PubMed Central PMCID: PMC4355957.
d. Newman JP, **Fong M-f**, Millard DC, Whitmire CJ, Stanley GB, Potter SM. Optogenetic feedback control of neural activity. Elife. 2015 Jul 3;4:e07192. PubMed Central PMCID: PMC4490717.

4. **Locomotive robotics:**

My undergraduate thesis focused on mechanical design of a bipedal walking robot. I worked on a team to build a passive-dynamic walker, which exploited a unique mechanical design to walk down a shallow slope with a naturalistic gait despite no actuated joints. We then introduced a single actuator at the hip, controlled by a reinforcement learning algorithm, allowing the robot to walk on various terrains. The controller enabled the robot to "teach" itself to walk, and converged upon a stable solution within 30 minutes with remarkably low energy cost (Tedrake et al., 2004). An energetically efficient robot that can teach itself to walk is of broad appeal across many sectors, and may have particularly interesting applications in healthcare robotics and prosthetic devices.

a. Tedrake R, Zhang TW, **Fong M-f**, Seung HS. Actuating a simple 3D passive dynamic walker. IEEE International Conference on Robotics and Automation. 2004. DOI: 10.1109/ROBOT.2004.1302452

5. **STEM education:**

I have been involved in teaching and mentoring for 15 years. I am particularly interested in education of underrepresented groups and outreach to underserved communities. After college, I began my career as a high school computer science teacher in Mexico. After graduate school, I taught a sustainable design course focused on clean water initiatives in Panama. Concurrent with my postdoctoral training, I worked as a part-time visiting lecturer in the Neuroscience Department at Wellesley College, a women's liberal arts school. One of the courses I developed and taught at Wellesley was a neuroengineering seminar, which culminated with a design project where each student developed a startup company pitch and wrote a white

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paper. I have also directed and taught college-level courses at Emory and MIT. Over 500 students have passed through my classroom, and I remain as a resource for former students looking for career or life advice. In the laboratory, I have been the primary mentor for 13 undergraduate students, including 10 females, 6 underrepresented minorities, 2 first-generation college students, and 1 individual with disabilities. All of my research trainees learned to interface electronics with living neural tissue. Currently, 7 are pursuing postgraduate degrees in STEM, 2 are working in STEM positions in industry, 1 is working in management, 1 is working in secondary STEM education, 1 is an undergraduate in electrical engineering and computer science, and 1 is conducting postdoctoral training in neuroprosthetics. I have also mentored several high school students and research technicians in the laboratory, all of whom are currently pursuing undergraduate or graduate degrees in science.

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RESOURCES

Scientific Environment

Dr. Fong will be joining the Coulter Department of Biomedical Engineering (BME), a joint department between the Georgia Institute of Technology and Emory University. As an assistant professor in BME, Dr. Fong will be a faculty member at both Georgia Tech and Emory, providing her access to students, colleagues, collaborations, and facilities at both universities. As a vision science and neurobiology researcher, she will have the opportunity to take advantage for the strong research and clinical communities within the Atlanta area. These resources will aid successful execution of the proposed research and in Dr. Fong's early independent career development.

Laboratory:
The Fong Lab will be physically located in Redacted by agreement at the Georgia Institute of Technology. Dr. Fong has been allocated 600 square feet of dedicated newly renovated laboratory space and 450 square feet of space shared with 3 investigators using similar equipment and approaches (Redacted by agreement. The dedicated space will contain several benches and three sinks to support wet lab work (e.g. generating brain slices, making solutions), dry lab work (e.g. electronics fabrication), and histology. This space will also contain a dedicated *ex vivo* electrophysiology rig and a shared perfusion hood. The shared space contains 4 small suites for surgery, *in vivo* electrophysiology, and behavior. At the start of her appointment, half of the shared space will be allocated to Dr. Fong's research program: one room with a survival surgery station and a rig for head-fixed electrophysiology with visual stimulation, and one light-tight and sound-isolated room for freely-moving electrophysiology, behavior, visual stimulation, and light-sensitive visual assays. The light-tight room will have no windows, and all other lab spaces will have black-out curtains available. Both rooms will have white and red/infrared lighting options. The surgical room will have a sink, washable walls, and stainless steel surfaces. Large appliances (e.g. fridges, freezers) will be housed in a corridor adjacent to the Fong Lab on an emergency power circuit.

Office: Dr. Fong will have a private office (120 square feet) across the hall from her research laboratory and next door to other BME faculty conducting neuroscience and vision research. Dr. Fong's staff and trainees will have desk and computer work space in office bays directly adjacent to the Fong lab, intermingled with other neuroscience staff/trainees working on the same floor. In addition, there are conference rooms with multimedia equipment for lab meetings.

Institution/Community: As a neuroscience and vision researcher within the Georgia Tech / Emory BME department, Dr. Fong will enjoy access to multiple resources that will aid in the current proposal as well as her career development as an early investigator. The current proposal is multidisciplinary, involving neurobiology, vision science, and engineering, and is motivated by clinical considerations. The community where Dr. Fong will be working excels in all these areas. Specific groups and associated resources available to Dr. Fong are described below.

1. *BME Department*: The BME department offers Dr. Fong unparalleled access to colleagues, facilities, and collaborations at the very forefront of biotechnology and clinical research. In 2020, *US News and World Report* named Georgia Tech / Emory BME as the #2 Undergraduate and Graduate programs for Biomedical Engineering. The BME department has featured Dr. Fong in student recruitment materials, and as a result Dr. Fong has been contacted by multiple students from these top-ranked programs who are interested in joining her lab. BME trainees who join the Fong lab will be able to help further research described in this proposal. Dr. Fong's startup package includes funding for multiple graduate and/or MD-PhD students. The Fong Lab will occupy a floor shared by six other faculty members, including a total of 7,300 sq. feet of lab space and 2,800 sq. feet of office space for graduate students and postdoctoral scholars. The research will also use several facilities in the Redacted by agreement, most notably the animal facility (described in more detail below), the microscopy core facility, and the neuro design suite.
2. *Neuroengineering community*: The Fong lab will be physically situated in Georgia Tech's Laboratory Redacted by agreement where equipment, ideas, lab space, and approximately 100 students, staff and postdocs facilitate neuroengineering research between labs of multiple faculty

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including Redacted by agreement

Redacted by agreement. The environment facilitates open collaboration and allows investigators to leverage expertise and equipment of neighboring labs. Other faculty closely affiliated with NeuroLab include Redacted by agreement

Redacted by agreement both of whom share Dr. Fong's interest in closed-loop optogenetic stimulation and can provide advice from both the technical and clinical perspectives relevant to the current proposal. Finally, Dr. Fong will be a faculty member on the Computational Neuroscience Training Grant between Emory and Georgia Tech. This allows Dr. Fong access to students with specialized training in quantitative neurobiology methods, which will be an asset for projects involving computation and data analysis.

3. *Neurobiology community*: Georgia Tech and Emory both have strong neuroscience communities. At Georgia Tech, there is a weekly GT Neuro Seminar Series, which Dr. Fong will co-organize as her university service. Organizing this series will allow Dr. Fong to interact directly with GT Neuro faculty members not only in BME, but also in Psychology, Biological Sciences, Math, Electrical Engineering, Computer Science, and Mechanical Engineering. It will also give her the opportunity to invite external speakers to Georgia Tech, allowing Dr. Fong to meet directly with leaders in her field. At Emory, there is also a thriving neuroscience community. The School of Medicine is home to a number of faculty who will be excellent colleagues and mentors including Redacted by agreement Redacted by Emory is also home to the Yerkes National Primate Center where investigators study various neurodevelopmental and neurological disorders, sensory and motor neurobiology, and higher level cognition. There is also a strong cohort of neuroscientists within the Emory Biology department, including Redacted by agreement Dr. Fong has strong relationships with these faculty members from her time as an Emory graduate student. These connections will present the opportunity to establish collaborations and exchange ideas through formal or informal avenues. Overall, Dr. Fong and her lab will have significant interaction with the broad neuroscience community at Georgia Tech and Emory.
4. *Vision research community*: Atlanta has a strong vision research community that will support Dr. Fong's in her current and future aspirations as an independent investigator. As a vision researcher, Dr. Fong be part of the Atlanta Vision Research Community (AVRC), an NEI-funded multi-institutional network that provides scientific resources and support for vision researchers at the Emory Eye Center, the Emory School of Medicine (including departments such as BME and Ophthalmology), the Atlanta Veteran's Affairs (VA) Medical Center, Georgia Institute of Technology, Georgia State University, and Morehouse School of Medicine. One of the stated goals in the AVRC mission is to provide support to new faculty and help early investigators like Dr. Fong to obtain R01 support. Core facilities available to AVRC investigators include a structural biology and imaging core (SBI), a functional genomics and proteomics core (FG&P), and a bioanalysis core. Of particular interest to the current proposal is the FG&P core which includes (a) ocular rodent microsurgery resources available for assistance with optic nerve crush, subretinal, intravitreal, or other intraocular and transscleral drug and DNA/RNA construct delivery, and (b) ocular functional and structural phenotyping resources that provide non-invasive longitudinal assessments following ocular treatments. The former will be useful for learning more about drug delivery methods for inactivating the retina. Included as part of the latter core are multiple portable electroretinography (ERG) and ocular coherence tomography (OCT) systems, which can be lent to Dr. Fong to use within her lab. These will provide an invaluable tool for Dr. Fong to assess functional and structural eye health Proprietary Info During her recruitment, Redacted by agreement Redacted by agreement invited Dr. Fong to join AVRC. The Fong lab is also located Redacted by agreement s, an expert in OCT. Finally, Redacted by agreement has been an excellent resource for Dr. Fong during her recruitment, and continues to support Dr. Fong during her transition.
5. *Clinical community*: While clinical research is not part of the proposal, the work is motivated by the neurodevelopmental visual disability, amblyopia, so will benefit from input from the strong clinical community. The BME Department at GT and Emory has full access to the Emory School of Medicine, including collaborations or informal consults with clinical colleagues. Dr. Fong has also met with several researchers in the Emory Department of Ophthalmology who provided valuable input about patient considerations in visual treatments. In addition to the clinical community at Emory, the

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Atlanta VA is home to several researchers with whom Dr. Fong shares interests who are part of the Center for Visual & Neurocognitive Research (CVNR). The CVNR promotes evidence-based and creative rehabilitation practices, and has invited Dr. Fong to present her research at their biweekly seminar as soon as she relocates to Atlanta. This will be a great opportunity to get feedback and establish formal or informal collaborations.

Animal: Georgia Tech maintains an excellent animal care facility called the Physiological Research Laboratory (PRL). The PRL is directed by Redacted by agreement a trained veterinarian with a graduate degree in neuroscience, and the facility employs other highly-trained veterinarians who assure the welfare of research animals. This facility is located in Redacted by agreement a building Redacted by agreement. The animal facilities in the PRL are visited regularly and accredited by both AAALAC and USDA. Animals are housed in a state-of-art facility with humidity, temperature, air flow, and lighting controls, as well as full-time veterinary and animal husbandry care. In addition, the PRL contains significant resources for training research staff in animal procedures and in providing materials and support for surgical procedures. There are also dedicated procedure rooms for surgeries and experiments that can be used by any investigator, and will be available to Dr. Fong should renovations to her lab space be delayed due to supply chain shortages in building materials associated with the COVID-19 pandemic. The PRL has dedicated, separated breeding and housing areas, including an infrared-illuminated room for reverse light cycle housing.

Computing: The Neurolab contains approximately 100 special purpose and general purpose computers, high capacity data servers, and access to high capacity computing cores and GPUs for data processing. The university maintains site licenses for all necessary research tools including Matlab, Mathematica, Adobe Creative Suite, and other computational and engineering software. In addition, the BME department employs two full-time IT specialists who can set up public or private backup data servers at no cost to individual investigators. Finally, the Georgia Tech Partnership for an Advanced Computing Environment (PACE) provides faculty such as Dr. Fong with sustainable, leading-edge high performance computing environments and the Georgia Tech College of Engineering can provide server rooms to host computational systems, including multi-GPU configurations. Together, these resources will be valuable for the collection, storage, analysis, and dissemination of data for this proposal.

Other: There is a dedicated machine shop in the same floor/wing as the Fong lab. The shop is outfitted with a CNC mill, lathe, drill press, 3D printer, as well as tools for microfabrication and soldering. With respect to the current proposal, these tools will be useful to making custom parts for surgical and recording apparatuses, as well as assembling electrodes and performing impedance testing.

Special Facilities

Biohazards: The Fong lab was renovated with Biosafety Level (BSL) 2 compliance in mind, and will adhere to all BSL2 requirements of federal, state, and municipal regulations and with the guidelines outlined in the CDC/NIH *"Biosafety in Microbiological and Biomedical Laboratories"* issued by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). Laboratory spaces and practices therein will be in accordance with Biosafety Level 2 containment, and the lab will be inspected several times a year to ensure full compliance. A fume hood is located within the Fong lab and a shared biosafety cabinet is available within the Neurolab for Dr. Fong's use.

Proprietary Info

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Viral constructs: Viral agents are stored inside a designated area in freezers that are marked with the universal biohazard sign. All viral materials stored in freezers will be properly labeled with the identity, source and quantity of the viral agents, the date of receipt and the initials or name of the receiver. As with select agents, proper lab safety and PPE is always used when handling virus, and standard operating procedures for disposal are followed. All Fong lab personnel will undergo recombinant DNA training through EHS, and be trained directly by Dr. Fong on lab-specific procedures.

Career Development

Georgia Tech and Emory are committed to Dr. Fong's success. Both universities as well as the BME department are committed to supporting junior faculty and provide numerous professional development and mentorship activities through the department and universities.

Institutional and Collegial Support: Both Georgia Tech and Emory University provide training for faculty, post docs and students covering responsible conduct of research, grant writing and laboratory/safety training related topics. There also are numerous opportunities for career enrichment through the Petite Institute Bioengineering Seminars, Distinguished Lecture Series, Suddath Symposiums, and numerous faculty development workshops through the College of Engineering. In particular, the Georgia Tech College of Engineering holds a monthly training series for junior faculty. Topics include grantsmanship (e.g. Getting your first R01, applying for NSF CAREER Award and other grants), graduate student mentoring (e.g. recruitment, NSF/F32 applications), best practices in running a successful research lab, strategies to get national visibility for your lab, making teaching a meaningful experience for you and our students, how to identify collaborators and nuances in features of a symbiotic collaboration, and what committees or service opportunities should one seek out (or avoid!) as a junior hire. Finally, there are opportunities to help entrepreneurs learn how to fund and commercialize their technology, including training through the Petit Entrepreneurship Academy.

Departmental Support: The BME department will create a mentorship committee for Dr. Fong to help her successfully navigate pre-tenure years. This committee will include 3-4 faculty mentors with whom she will meet bi-weekly. Topics covered in meetings will include establishing a lab culture, training students, writing grants, networking, and teaching. Dr. Fong also has the commitment of other faculty within NeuroLab Redacted by agreement) to advise on animal protocols, student mentorship, navigating building-specific policies, and other topics that may arise. Beyond individualized mentorship, the BME department also holds monthly launch committee meetings that include the junior faculty, the department chair, the senior associate chair, and several senior faculty members. The launch committee provides support and guidance to new junior faculty members as they begin their career and provides junior faculty the opportunity to learn best practices and ideas to promote academic success, personal growth, and work satisfaction. The committee focuses on areas that are essential for a new hire to be successful, such as: establishing mentoring relationships, getting a research lab up and running, funding, managing lab personnel, integration into the university, teaching, and service. Finally, the BME department is specifically committed to supporting women in science and engineering. Redacted by agreement runs a monthly series for female faculty, which is fully funded by the BME department. This allows investigators like Dr. Fong to get additional informal mentorship from other female faculty on topics such as setting career goals, adjusting to the balance of a faculty position, and other topics relevant to early investigators.

Logistical support: The BME department has provided Dr. Fong with a grants support specialist and a financial officer to assist with grants submission, grants administration, and startup fund usage. The BME Human Resources specialist is also assisting Dr. Fong with advertising and hiring personnel. The BME department employs numerous administrative specialists who can be deployed based on faculty needs.

Financial support: Dr. Fong will have a minimum of EFFOR of her effort protected for research. Her salary

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K99 Progress Report

Major Goals

Aim 1 (K99): Identify homeostatic synaptic plasticity mechanisms in V1 engaged by retinal inactivation. Temporary inactivation of the retinas promotes recovery from the consequences of monocular deprivation, but how retinal silencing rejuvenates synapses in V1 to facilitate recovery remains unclear. To address this gap in knowledge, it will be necessary to identify the V1 plasticity mechanisms driven by retinal inactivation. I ***hypothesize*** that retinal inactivation drives homeostatic scaling of specific synapse classes, as well as an increase in the propensity for synapses to undergo long-term potentiation. To test this hypothesis, I will measure spontaneous and evoked synaptic strength, as well as capacity for potentiation or depression, in acute slices of mouse V1. Recordings will occur at multiple time points following onset of retinal inactivation.

Progress: ~90% complete (Projected completion timeline: Present - January 2022)

Proposed Revisions: Addition of intrinsic plasticity measures and excitatory-inhibitory balance measures

Aim 2 (K99/R00): Monitor changes in neural activity patterns and excitability within V1 cells and circuits driven by retinal inactivation. Preliminary data reveal that binocular inactivation temporarily suppresses visually-driven V1 activity, but subsequently leads to a striking enhancement of spontaneous and evoked activity just 24 hours later as retinal activity and visual experience are gradually restored. To understand the nature of this rapid potentiation, we must identify when and where neural activity changes are occurring. This aim tracks the time course and laminar specificity of V1 activity changes at a cellular and network level. I ***hypothesize*** that retinal inactivation will reduce V1 activity, but that spiking will gradually recover, in part due to homeostatic increases in cellular excitability. Due to increased thalamic bursting, correlated spiking will return first to cells in thalamo-recipient cortical layer 4, and gradually propagate in a feedforward manner to other layers. Activity through the cortical column will be significantly potentiated upon the restoration of visual experience. I will test these hypotheses in mice using a combination of multichannel electrophysiology, calcium imaging, and patch clamp recordings *in vivo* to monitor changes in spiking and excitability before, during, and after binocular inactivation.

Progress: ~50% complete (Projected completion timeline: Present - March 2023)

Proposed Revisions: Move intrinsic plasticity measures to Aim 1. Eliminate acute electrophysiology and expand chronic electrophysiology measures.

Aim 3 (R00): Drive retinal inactivation-associated changes in excitability and plasticity through closed-loop V1 stimulation. Binocular retinal inactivation dramatically alters V1 activity, but the causal role this altered activity plays in subsequent plasticity remains unclear. I ***hypothesize*** that re-creating the initial V1 activity conditions associated with retinal inactivation will be sufficient to drive changes in circuit excitability and synaptic plasticity, even when retinal activity and visual experience are normal. To test this hypothesis, I will design a closed-loop optogenetic stimulation system to control firing rate and cell-to-cell correlations within the cortical circuit in mice. I will use this system to reproduce the V1 spiking statistics observed in Aim 2 without manipulating retinal activity conditions. I predict that just a few hours of closed-loop V1 stimulation will be sufficient to drive the same synaptic/cellular plasticity (Aim 1) and changes in circuit excitability (Aim 2) observed following binocular retinal inactivation.

Progress: 1% complete (Projected completion timeline: September 2022 - October 2024)

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Accomplishments

Probing metaplasticity following retinal inactivation (Aim 1.1) - 75% complete

- Major Activities: I learned to conduct extracellular *ex vivo* recordings from acute slices of visual cortex, as well as to probe for long-term potentiation (LTP) and long-term depression (LTD).
- Specific Objectives: I asked whether retinal inactivation changed the capacity for V1 slices to undergo LTP and LTD. My hypothesis was that the capacity for plasticity would shift to favor LTP.
- Significant Findings: I was able to elicit LTD using low-frequency stimulation, but was unable to elicit LTP in layer 4 (L4) of wildtype animals. This was no different in animals that had retinas inactivated. In the experiments that I conducted to learn the LTD and LTP experimental techniques, I observed an elimination of L4 LTD in animals lacking NMDA receptors in layer 4 (Fong et al., 2020)
- Key Outcomes: Contrary to my hypothesis, there was no shift in the capacity for plasticity in L4 due to retinal inactivation. The worldwide silicon shortage associated with the pandemic made stimulating electrodes scarce and in short supply within the lab, so I did not complete the same assessment for L2/3. However, I was able to successfully transfect feedforward inputs onto L2/3 cells with a channelrhodopsin-2 (ChR2) in L4 cells using the Scnn1a-Cre driver, and am I currently conducting experiments to test LTP and LTD using optogenetic rather than electrical stimulation. Although, the kinetics of ChR2 are not fast enough to reach the highest stimulation frequencies that I planned to use with electrical stimulation, but is sufficient for LTD and should still reveal is there is a stimulation-frequency dependent shift. I am currently blind to the treatment conditions (Proprietary Info), but will unblind myself after I have completed the analysis.

Probing for homeostatic synaptic plasticity following retinal inactivation (Aim 1.2, revised) - 85% complete

- Major Activities: I learned to conduct whole-cell voltage clamp recordings to assess synaptic strengths in acute slices of visual cortex using multiple approaches. I also used my "remote work" time during the pandemic-related campus closures to perform computational assessment of synaptic scaling in these data sets.
- Specific Objectives: I asked whether retinal inactivation would drive homeostatic increases in synaptic strength in V1 cells in L2/3 and L4. I hypothesized that synaptic scaling would be observed in L2/3, as has been reported following dark exposure, but no change in L4.
- Significant Findings: Retinal inactivation drives a homeostatic increase in synaptic strength in L2/3 consistent with synaptic scaling. In L4, there is no change in the average synaptic strength, though there is a broadening in the distribution suggesting an nuanced plasticity at play.
- Key Outcomes: Consistent with my hypothesis, I observed synaptic scaling in L2/3 neurons 48 hours after retinal inactivation. At the same time point, there was a broadening of the distribution of synaptic strengths in L4, suggesting the possibility of "divergent" (rather than the classical "multiplicative") synaptic scaling. My analysis of divergent synaptic scaling in a previous data set has published (Hanes et al., 2020), and I plan to use the same analysis for this data set. There were no synaptic changes detected in the first few hours following retinal inactivation. Experiments on inhibitory cells are in progress and remain blind to treatment condition.
- Notes on revision: Based on our observations in inhibitory cell types using calcium imaging (Aim 2.2), I am also now examining a potential role for inhibition in retinal inactivation-driven V1 plasticity.

Probing for homeostatic intrinsic plasticity following retinal inactivation (formerly Aim 2.3 - now Aim 1.3) - 100% complete

- Major Activities: In the process of conducting whole-cell recordings to assess synaptic strength, I had the opportunity to assess passive intrinsic properties in the same animals. This provided an alternative method to those originally proposed in Aim 2.3, and I elected to use this to address the Aim 2.3 hypothesis in order to reduce the number of animals and focus Aim 2 more on network plasticity.
- Specific Objectives: I asked whether retinal inactivation would drive homeostatic changes in intrinsic cellular excitability in V1 cells in L2/3 and L4. Based on previous work on monocular retinal inactivation, I hypothesized we might see increases in excitability in L2/3 cells.
- Significant Findings: I did not observe any changes in passive membrane properties in L2/3 as I had expected. However, I did observe a hyperpolarizing shift in membrane potential in L4 during the early hours following retinal inactivation.

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- Key outcomes: The hyperpolarizing shift in membrane potential was unexpected, as it actually represents a *decrease* in excitability. However, my interpretation is that L4 undergoes a Hebbian (rather than homeostatic) intrinsic plasticity immediately following retinal inactivation. This plasticity is no longer observed by 48 hours, suggesting that homeostatic mechanisms have reversed the plasticity. However, the early reduction in excitability in L4 cells may trigger the subsequent increase in synaptic strength observed in the downstream L2/3 cells. No intrinsic changes were observed at 48h.
- Notes on revision: The original proposal suggested performing these assessment using *in vivo* (Aim 2.3), rather than in slices. Although I learned the *in vivo* technique, the automated targeting of specific cells types proved not to be reliable. Manual patching was possible, but because of motion artifacts it was different to obtain long-duration access to cells for running all intrinsic excitability tests. Further, the duration of the experiments did not allow for littermate controls to be used. While it would have been possible to modify these experiments to use more age-matched non-littermate animals and assess different excitability properties separately in different experiments, I felt this would result in a significant increase in the number of animals and time required for these experiments. Since intrinsic properties were easy to collect in ongoing *ex vivo* slice experiments, I elected to use that method instead and re-focus Aim 2 to more tractable experiments that would better advance the overall goals of the proposal.

Characterizing the effect of retinal inactivation across V1 and LGN in the early visual pathway using in-vivo electrophysiology (Aim 2.1, revised) - 10% complete

- Major Activities: I learned to perform chronic and acute silicon probe recordings in awake mice, although have not yet performed the blinded experiment Proprietary Info. During pandemic-related closures, I created an analysis pipeline for the eventual data that will be collected for the experiment. I also performed pilot experiments in the LGN to show dramatic changes in activity driven by retinal inactivation.
- Specific Objectives: I sought to monitor how activity changes across all layers of V1 during and after retinal inactivation. Revised proposal also includes LGN recordings within the same experiment using a single high-density probe that spans both V1 and LGN.
- Significant Findings: In pilot experiments, I have observed an increase in LGN bursting activity during the period of retinal inactivation and increase in evoked V1 spiking activity after 24 hours after retinal inactivation.
- Key Outcomes: Because littermate saline control experiments have not been performed, I am not making any first conclusions at the moment. Completion of these experiments will be a major thrust of the early R00 period.
- Notes on revision: Since the original proposal was submitted, it has now become possible to chronically implant high-density silicone probes. This eliminates the need for me to perform separate acute experiments using high-density probes from chronic experiments using classic probes. For all future experiments on this project, I will therefore use chronically implanted high-density probes. Another benefit is that these newer probes can span both V1 and LGN, so we will be able to track activity in both regions rather than just in V1. Based on these revisions, the overall wording of this sub-aim has been modified accordingly, although the objective remains the same.

Characterizing the effect of retinal inactivation within layers and across cell-types of V1 using in-vivo calcium imaging (Aim 2.2, revised) - 90% complete

- Major Activities: I learned to conduct chronic, in-vivo 2-photon calcium imaging experiments in genetically-defined cell-types expressing the indicator, GCaMP6f.
- Specific Objectives: I sought to examine how activity is altered within layers of V1 following retinal inactivation. I hypothesized that there would immediately increase in spontaneous and evoked activity in L4, and do so more gradually in L2/3.
- Significant Findings: Perhaps unsurprisingly, activity within excitatory cells in L4 and L2/3 decreased immediately after retinal inactivation. More surprisingly, activity was not enhanced at any point during the course of a week. Because of this surprise, I conducted pilot experiments in inhibitory cells types. I found that inhibitory cells also show reduced activity.
- Key Outcomes: The reduction in calcium activity following retinal inactivity seemingly contradicts my previous observations using electrophysiology that V1 activity is enhanced. One potential explanation

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is that my electrophysiological recordings sample from other neuronal compartments (e.g. dendrites), while calcium imaging is concentrated at the soma. In fact, my observation that resting membrane potential is initially reduced in L4 cells could account for a reduction in calcium activity at the soma, even if the dendrites are experiencing elevated excitation as detected by electrophysiology. In addition, the reduction in activity within inhibitory cells might suggest that there could still be a shift in E/I ratio to favor excitation, which has now been added to Aim 1.2. Overall, there are still limitations in the interpretability of calcium imaging data due to the slow indicator kinetics. Therefore, with the new possibility to do chronic high-density electrophysiology recordings (Aim 2.1) where many units within a layer can be sampled and sorted, I feel that those experiments may help resolve some of these uncertainties.

- Notes on revision: I have added monitoring inhibitory cell experiments to this aim. The additional work is balanced by the simplification of former Aim 2.3 (now Aim 1.3).

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Training and Professional Development

The K99 project has allowed me to learn numerous skills, including ex vivo electrophysiological recordings paired with electrical or optogenetic stimulation, as well as *in vivo* recordings using calcium imaging, patch clamp, and oversampled unit electrophysiology. I had the opportunity to learn from my mentor Redacted by agreement as well as a group of scientific and professional advisors, including Redacted by agreement Redacted by agreement

During the early portion of the K99 project, also had the opportunity to participate in numerous neurobiology and vision conferences (ARVO, SFN, GRCs on neuroplasticity and excitatory synapses), and made connections that garnered me invitations to speak at academic institutions. These included 2 international speaking opportunities, 3 domestic speaking opportunities, and 1 session moderator opportunity. I also gave poster abstracts and got feedback from both clinicians and scientists. These networking opportunities allowed me to make new connections with leaders in my field, and I benefited especially from making connections with

PII

I applied for numerous faculty positions during the K99 award period. I received 8 video interviews, 6 invited

PII

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Dissemination

During the first half of the K99 award period, I presented research at multiple conferences and academic institutions. I also mentored a local high school student and presented research at the MIT Museum "Teen Science Cafe". The second half of the K99 award period was strained by the COVID-19 pandemic. I

circumstances permit. I recently had a paper on the K99 research accepted at *eLife* (open access), and I am in the process completing data analysis for another likely manuscript. I plan to make the manuscript available on bioRxiv and disseminate a link to the preprint through social media tools.

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Goals for next year

Research
My immediate goal is set up my new lab to finish the slice physiology experiments (Aims 1.1-1.2) within 6 months, and complete the chronic high-density electrophysiology experiments (Aim 2.1) with 18 months. During the upcoming year, I also plan to set up equipment infrastructure for simultaneous recording and stimulation experiments in Aim 3.

Professional Development
I have begun to participate in several virtual meetings at my new institution to learn about the landscape of grant administration, mentoring, and teaching environment. Once I re-locate to Atlanta, I plan to participate in more opportunities offered by both Georgia Tech and Emory, as well as my department. I have researched outlined these opportunities in the new Resources statement.

Student Mentoring
I have begun to interview prospective graduate students in the Georgia Tech & Emory Biomedical Engineering PhD Program who have expressed interest in joining my lab. I have also submitted paperwork to be able to recruit from the Neuroscience PhD Program at Emory. My goal is to have 1-2 students join my lab during the upcoming year, who I can mentor to participate in the R00 research.

Grants Writing
I have begun the process of mapping deadlines for grant opportunities at the NIH, NSF, and private foundations; as well as early career awards. I have a meeting with mentors scheduled for the end of September to make a grants submission plan for 2022. Currently, my goal for NIH grants is to submit an NIH DP-2 in 2022, and an NIH R01 in 2023. I also plan to be a co-investigator on an SBIR grant that will enable me early access to equipment useful for Aim 3 of the R00 proposal.

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Publications and presentations

- *Peer-Reviewed Journal Articles*
    - Unpublished
    - **Fong M-f**, Finnie PSB, Kim T, Thomazeau A, Kaplan ES, Cooke SF, and Bear MF (2020). Distinct laminar requirements for NMDA receptors in visual cortical plasticity. *Cerebral Cortex* 30 (4), 2555–2572.
    - Hanes HL, Koesters AG, **Fong M-f**, Altimimi HF, Stellwagen D, Wenner P, Engisch KL (2020). Divergent synaptic scaling of miniature excitatory synaptic currents following activity blockade in dissociated neuronal cultures. *Journal of Neuroscience* 40 (21) 4090-4102.
- *Manuscripts Under Review*
    - Unpublished
    -
- *Manuscripts in Preparation*
    - Unpublished
- *Presentations:*
    - **Fong M-f**, Duffy KR, Leet MP, Candler CT, Deere JU, Martin L, Yao JS, Liang NT, Liu K, and Bear MF (2021). Correction of amblyopia in cats and mice after the critical period. Society for Neuroscience Annual Meeting. Virtual & in Chicago, IL.

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Specific Aims

Disrupted sensory experience during early postnatal life can lead to improper development of the nervous system. Later in life, brain injury or disease can alter neural circuits that process sensory information. These common adversities can result in profound disabilities in sensory processing which affect quality of life. A central goal of neuroscience research is to leverage the brain's inherent plasticity to treat these developmental or injury-induced neurological disorders and restore normal processing of afferent sensory information. I recently discovered a strategy for restoring cortical responses and acuity to visually-impaired mice and cats. Following temporary silencing of spiking activity in the retinas, I observed a remarkable recovery from the otherwise stable loss of vision that follows early life monocular deprivation. Now, an urgent question is how retinal inactivation alters synapses, cells, and circuits within the visual cortex to allow for subsequent visual enhancement. In this proposal, I will test the hypothesis that bilateral silencing of retinal activity leads to homeostatic plasticity and increased correlated activity in the mouse primary visual cortex (V1). Further, I will test whether reproducing the cortical activity conditions associated with retinal inactivation can directly drive visual enhancement. I will address these overarching hypotheses in the following specific aims:

Aim 1: Identify homeostatic plasticity mechanisms in V1 engaged by retinal inactivation. Temporary inactivation of the retinas promotes recovery from the consequences of monocular deprivation, but how retinal silencing rejuvenates synapses in V1 to facilitate recovery remains unclear. To address this gap in knowledge, it will be necessary to identify the V1 plasticity mechanisms driven by retinal inactivation. I ***hypothesize*** that retinal inactivation drives both homeostatic synaptic and cellular plasticity in V1, as well as an increase in the propensity for synapses to undergo long-term potentiation. To test this hypothesis, I will measure synaptic strength, excitatory-inhibitory ratio, and intrinsic membrane properties, as well as capacity for potentiation or depression, in acute slices of mouse V1 at multiple time points following onset of retinal inactivation.

Aim 2: Dissect spatial and temporal changes in circuit activity in V1 and the visual thalamus driven by retinal inactivation. Preliminary data reveal that binocular inactivation temporarily suppresses visually-driven V1 activity, but subsequently leads to a striking enhancement of spontaneous and evoked activity just 24 hours later as retinal activity and visual experience are gradually restored. To understand the nature of this rapid potentiation, we must identify when and where neural activity changes are occurring. This aim tracks the time course of changes in cellular and circuit excitability within the visual thalamus and cortex following retinal inactivation. I ***hypothesize*** that retinal inactivation will drive a homeostatic increase in circuit excitability within the visual thalamus and cortex, with the timing of the shifts in excitability correlating with to their proximity to the retina. I will test these hypotheses in mice using a combination of multichannel electrophysiology and calcium imaging *in vivo* to monitor changes in spiking activity before, during, and after binocular inactivation.

Aim 3: Drive retinal inactivation-associated changes in excitability and plasticity through closed-loop V1 stimulation. Binocular retinal inactivation dramatically alters V1 activity, but the causal role this altered activity plays in subsequent plasticity remains unclear. I ***hypothesize*** that re-creating the initial V1 activity conditions associated with retinal inactivation will be sufficient to drive changes in circuit excitability and synaptic plasticity, even when retinal activity and visual experience are normal. To test this hypothesis, I will design a closed-loop optogenetic stimulation system to control firing rate and cell-to-cell correlations within the cortical circuit in mice. I will use this system to reproduce the V1 spiking statistics observed in Aim 2 without manipulating retinal activity conditions. I predict that just a few hours of closed-loop V1 stimulation will be sufficient to drive the same synaptic and cellular plasticity (Aim 1) and changes in circuit excitability (Aim 2) observed following binocular retinal inactivation.

Public Health Impact: Understanding which synapses and cell types are altered by retinal inactivation will help in designing targeted treatments for amblyopia and other neurological visual impairments. Further, designing a stimulation paradigm for directly driving neural plasticity and recovery in the visual cortex will inform non-invasive stimulation therapies. Given the common organizational features and plasticity mechanisms observed across mammalian primary sensory cortices, these findings may well translate to other sensory modalities.

Revision Summary: The overall aims of the proposal remain the same. This updated research plan includes slight adjustments to experiments in order to better address scientific questions, incorporate previous feedback from reviewers, optimize use of animal subjects, and adjust to technological advances from the past 3 years. The research strategy includes a description of all specific aims, with key outcomes presented for completed K99 work and experimental design presented for future R00 work.

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Research Strategy

R00 Revisions Summary: The project aims remain the same as the original K99-R00 proposal, but there have been minor changes to sub-aim experiments. The revisions and rationale are summarized below, followed by the updated research strategy. Additional justification is included in the K99 Progress Report.

Aim 1 of the original proposal focused on solely on identifying homeostatic synaptic plasticity mechanisms in at excitatory synapses onto excitatory cells. Two additional components have been added. First, I now measure the contribution of inhibition and assess the excitatory-inhibitory balance (E/I). The rationale for this change is that my calcium imaging experiments (Aim 2.2) suggested shifts in inhibitory cell activity might contribute to a reduced E/I. Second, I now examine homeostatic intrinsic plasticity *ex vivo* (rather than *in vivo* as stated in former Aim 2.3) because I determined that assessment *in vivo* did not merit the significant increase in resources and animal numbers that would be required given the marginal scientific gain. Assessment *ex vivo* allowed me to run protocols on slices already being generated for synaptic plasticity experiments.

Aim 2 of the original proposal included a breadth of metrics for evaluating intrinsic and network plasticity. The aim has been re-focused to solely use chronic electrophysiology and calcium imaging to assess circuit-level plasticity. As stated above, the rational for removing the *in vivo* whole cell experiments is that *ex vivo* methods offered the opportunity to answer the same experimental question about intrinsic plasticity, but with zero additional animals or resources required. In addition, one reviewer noted that the techniques in the aim were overly complicated; therefore, simplifying the approach seeks to address that concern. A second change in this aim is that the electrophysiology portion has been consolidated to use chronic high-density probes alone (previously a combination of acute high-density recordings and chronic recordings with classical silicone probes was proposed). This change is enabled by newer commercially-available high-density silicon probes that allow for chronic implantation with simultaneous behavioral tracking. Further, the newer probes enable simultaneous recording from all V1 layers as well as the lateral geniculate nucleus (LGN), enabling me to expand our scientific question without using additional animals or resources beyond computing power.

Aim 3 has been updated to use more modern recording techniques (high-density probes, rather than classic silicone probes with fewer contacts), but is otherwise unchanged.

Significance

Problem, importance, and barriers. Amblyopia is a widespread form of visual disability that affects 2-4% of the world population[45]. Poor quality vision during early postnatal life alters the developmental trajectory of neural circuits underlying vision, such that one eye becomes weaker (amblyopic) despite normal ocular health. Current clinical interventions for amblyopia occlude or blur vision in the fellow eye to promote recovery of vision through the amblyopic eye. Unfortunately, this intervention loses efficacy with age, and is ineffective beyond pre-adolescent years. Even if initiated early, the treatment can be hindered by poor compliance among children, penalization of the fellow eye, and/or failure to develop stereoscopic vision[23,41,43,47]. Thus, there is a significant and unmet need for improved treatments for amblyopia, particularly in adults.

Scientific premise. I discovered that temporarily blocking action potential firing in both retinas promotes visual recovery from experimental amblyopia in mice and cats[18]. Proprietary Info Proprietary Info An urgent question is now how this brief period of retinal inactivation creates the conditions that foster visual recovery. Data from my K99 project shows that bilateral retinal inactivation in mice leads to a striking enhancement of visual cortical responsiveness as retinal activity is gradually restored as assessed through longitudinal measurements of visual evoked potentials (VEPs) in cortical layer 4 (L4) of the mouse primary visual cortex (V1). Further, I found that these changes are accompanied by synaptic and intrinsic changes in the mouse primary visual cortex (V1) (**Aim 1**). The updated R00 proposal expands on these findings, using a variety of measurement techniques to carefully dissect synaptic, cellular, and circuit-level changes in V1 that subserve retinal inactivation-driven visual enhancement.

Enhancing scientific knowledge. Augmenting sensory responsiveness is of broad interest in rehabilitation and enhancement. By identifying the synaptic and circuit mechanisms that drive visual augmentation, the knowledge gained by carrying out this project will be of broad relevance for neurologically-based forms of sensory impairment. Moreover, these studies may reveal how neural circuits can "re-booted" to promote plasticity beyond classic developmental critical periods.

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Potential to change concepts, technologies, and treatments. Monocular deprivation is a classic paradigm used in animals to study experience-dependent visual cortical plasticity and the synaptic pathophysiology underlying amblyopia. It is of significant clinical relevance to identify how these deprivation-driven cortical changes can be reversed to restore visual ability. Brief retinal inactivation can enhance visual responses without the requirement of training exercises or sustained patient compliance. The proposed aims will identify synaptic and intrinsic mechanisms that precede and accompany this visual enhancement, advancing current conceptual understanding about how visual deprivation (via retinal inactivation) modifies cortical circuits. Moreover, the final aim will provide a novel stimulation technology for reproducing visual potentiation in the absence of retinal manipulations. These pre-clinical studies may be of great utility in advancing treatments for amblyopia and other neurological visual impairments.

Innovation

Challenging current paradigms. The current standard of care for treating amblyopia is to promote vision through the weaker eye by limiting vision through the stronger eye. This clinical practice is supported by principles of ocular dominance plasticity, wherein synaptic competition in V1 induces use-dependent strengthening or disuse-dependent weakening of synapses downstream of the two eyes. *I propose that engaging homeostatic plasticity in V1 through binocular manipulations to visual experience may provide a more efficacious alternative to competition-based therapies.* Indeed, binocular suppression of vision using dark exposure[16,22,38] or retinal inactivation[18] has been shown to promote functional, behavioral, and anatomical visual recovery in amblyopic rodents and felines, possibly through homeostatic modifications in V1 that render synapses "experience-expectant"[9]. Since the period of dark exposure required for therapeutic effects appears to be at least 7-10 days[17,37], this proposal focuses on the more rapidly-acting retinal inactivation, which requires ≤ 3d to promote subsequent recovery[18] and potentiates V1 responses within 24-48h. I will directly probe for compensatory changes in synaptic strength, cellular excitability, and circuit activity to test the hypothesis that homeostatic plasticity can be leveraged to enhance cortical responses to sensory input.

Novel concepts, approaches, and interventions. Metaplasticity and synaptic scaling represent novel theoretical concepts proposed for stabilizing neural activity during chronic gain or loss of sensory input[4,36,40]. **Aim 1** directly tests these theoretical models of homeostatic synaptic modification following binocular inactivation. I also use and develop several novel technologies in this proposal, including optical stimulation of specific sets of presynaptic inputs to evoke miniature release events onto patched neurons. These innovative methodologies are employed to measure strength of specific synapses from genetically-defined cell types. **Aim 2** employs several advanced technologies for measuring neural activity *in vivo*, including ultra-dense silicon probes with hundreds of recording sites and chronic 2-photon calcium imaging in awake animals. **Aim 3** develops a novel intervention for enhancing cortical responses by stimulating V1 to engage plasticity. My findings may aid in the design of non-invasive stimulation strategies for patients with amblyopia[48] and other neurological visual impairments[28]. Moreover, the results will inform new technologies for sensory augmentation[3,12] and closed-loop systems for controlling neural activity[8,20].

Approach

The overall goal of the proposed work is to identify how retinal inactivation creates the conditions that promote visual cortical enhancement, and to reproduce these conditions using direct V1 stimulation.

Animals: Male and female wildtype or transgenic mice on the C57BL/6 background will be used for all experiments. I have found no sex-related differences on how retinal inactivation affects visual cortical activity in juvenile or adult animals so far. If any differences emerge during the course of this study, I am prepared to treat sex as a relevant biological variable and quantify differences between groups.

Aim 1: Identify homeostatic plasticity mechanisms in V1 engaged by retinal inactivation.

Aim 1.1: Probing metaplasticity following retinal inactivation. Metaplasticity is a form of homeostatic plasticity wherein the quality of synaptic plasticity depends on the recent history of activity[1]. For example, a period of heightened neural activity might increase the likelihood of subsequent synaptic strengthening, while a period of inactivity might increase the likelihood of subsequent synaptic weakening. Since retinal inactivation initially reduces overall activity in V1, it is possible that the brief period of cortical inactivity renders synapses more readily potentiated once retinal activity returns[9]. Pioneering work in the Bear lab showed that rearing animals in complete darkness shifts the synaptic modification threshold in V1 neurons to favor long-term potentiation

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(LTP) over long-term depression (LTD)[30]. Here we asked whether similar experience-dependent metaplasticity in V1 also occurs following retinal inactivation.

Key Outcomes: Contrary to my prediction, experiments in L4 revealed no difference in the capacity for LTP or LTD following retinal inactivation, albeit it was not possible to induce LTP in either group[11,25,29]. Experiments for L2/3 experiments are nearing completion, though interim results are unknown due to treatment blinding during experimentation and analysis.

Aim 1.2: Probing for homeostatic synaptic plasticity in V1 excitatory neurons. Synaptic scaling is a widely studied form of homeostatic plasticity wherein all synapses onto a neuron strengthen (or weaken) by a common multiplicative factor to compensate for lack (or excess) of activity[49]. Previous work has shown that monocular retinal inactivation or brief dark exposure can drive upward synaptic scaling in L2/3 pyramidal cells at P21-23[13,19]. In older animals, deprivation-induced increases in synaptic strength still occur, but are no longer multiplicative[19]. In this sub-aim, I test the hypothesis that binocular retinal inactivation drives homeostatic increases in evoked and spontaneous excitatory synaptic strength, which will be most pronounced following recovery of visual activity at 48h.

Key Outcomes: Consistent with my hypothesis, binocular inactivation drove synaptic scaling in L2/3 neurons 48 hours after the onset of retinal inactivation. Interestingly, it produced a broadening of the synaptic strength distribution in L4 neurons at the same time point. Shifts in excitatory synaptic strength were not observed in either population during the early hours following inactivation.

Revision: There is evidence that excitatory-inhibitory balance (E-I) can change with monocular inactivation[35] and brief dark exposure[5]. Our calcium imaging results (Aim 2.2) suggest potential reductions in inhibition, which could shift E-I to compensate for loss of retinal activity [2,31]. I hypothesize that a homeostatic shift in E-I occurs to favor excitation, and have added a set of experiments test this in L4 and L2/3.

Aim 1.3: Probing for homeostatic intrinsic plasticity in V1 excitatory neurons. Increases in firing rate, reductions in spike threshold, and redistribution of membrane conductances have been observed following activity blockade in cultured visual cortical neurons[14]. Further investigations have revealed altered excitability in L2/3 and L4 principal cells of V1 slices following various forms of *in vivo* visual deprivation[33,34]. In this sub-aim, I test the hypothesis that homeostatic shifts in cellular excitability properties are altered by binocular inactivation.

Key Outcomes: Surprisingly, I found that membrane potential in L4 cells is hyperpolarized during the immediate period following retinal inactivation. This shift represents a decrease in excitability, possibly due to Hebbian plasticity. Nonetheless, the shift is no longer apparent by 48h, suggesting that homeostatic mechanisms help restore normal excitability. No changes in membrane properties were detected in L2/3.

Revision: This sub-aim was originally part of *in vivo* experiments Aim 2, but moved to *ex vivo* experiments in Aim 1 for reasons related to ease of addressing the hypothesis using fewer animals and simpler methods.

Aim 2: Dissect spatial and temporal changes in circuit activity in V1 and the visual thalamus driven by retinal inactivation.

Aim 2.1: Characterizing the effect of retinal inactivation across V1 and LGN in the early visual pathway using in-vivo electrophysiology. Altered pyramidal cell spiking and excitability has been observed in V1 slices following retinal inactivation[34]. However, less is known about how activity changes in the awake cortex *in vivo*. I have found that retinal inactivation initially reduces evoked spiking and VEP magnitudes in V1, but dramatically increases both just 24 hours later. In the following experiments, I measure how spontaneous and evoked activity change across the visual cortical microcircuit and the upstream lateral geniculate nucleus (LGN). The results will help us understand how retinal inactivation impacts activity statistics in the early visual pathway to create the conditions that promote response potentiation in V1.

Experimental design: To determine how retinal inactivation alters activity throughout V1, I will use high-density silicon probes that span all layers of V1 and LGN for measuring spiking and LFP activity during retinal inactivation in freely-moving animals[26,27,46]. Implants will occur in P30 mice, and mice will be habituated to tethering and head fixation for 3 days thereafter. Freely-moving recordings will occur in a high-walled behavioral arena under a 3-D video tracking system, and head-fixed recordings will be performed with mice viewing alternating blocks of grey screen and high-contrast sinusoidal phase-reversing grating stimuli in the binocular visual field. Pupillary light reflexes will be assayed before each recording, and pupil diameter will be monitored during head-fixed experiments to assess contributions to enhanced stimulus response[44,50]. Recordings will occur at baseline and at the following time points Proprietary Info 1h, 3h, 6h, 12h,

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24h, 48h, 72h, 168h (7d). A 1h freely-moving recording will precede each head-fixed recording. Raw data will be high-pass filtered, single-units will be extracted[42], and spiking statistics (unit firing rates, within-unit interspike interval, unit-to-unit cross-correlation) will be compared across behavioral states and light/dark periods. Units will also be classified as fast-spiking (FS) or regular-spiking (RS). For LFPs, raw data will be low-pass filtered and VEP and current-source density (CSD) analysis will be performed. For cortical layer identification, the site of the greatest stimulus-evoked current sink will be defined as L4, and electrolytic lesions will be used for post-hoc histological analyses. For LGN field identification, responses to monocular stimulation will be examined to approximate positions in the LGN core versus shell. Data will be compared separately for freely-moving and head-fixed recordings using 1-way repeated-measures ANOVAs, followed by post-hoc tests corrected for multiple comparisons.

Expected outcomes: These experiments will produce a rich dataset that allows us to look at spatial and temporal aspects of V1 and LGN spiking in RS units (presumptive principal cells) and FS units (presumptive parvalbumin-positive interneurons) following retinal inactivation. Based on pilot (unblinded) experiments using classic (lower density) probes, I expect that the initial hours Proprietary Info will be characterized by elevated LGN bursting, reduced spontaneous firing in V1, and elimination of evoked responses in both regions. As LGN bursting gradually ramps up over the first day[32], spiking correlations in L4 and spindle-like oscillatory bouts will increase[21]. This correlated cortical activity will initially be restricted to L4 units as they receive the densest thalamocortical input[6,10]. Overall firing rates will homeostatically increase in both RS and FS cells throughout all V1 layers, and the return of visually-evoked activity (~24h) will re-structure and heighten interlaminar correlations. The laminar dynamics of the CSD will remain generally intact, though the magnitude of the sources/sinks may shift, and temporal dynamics of the VEP may broaden during/after retinal inactivation.

Aim 2.2: Characterizing the effect of retinal inactivation within layers and across cell-types of V1 using in-vivo calcium imaging. While the electrophysiological approaches described in **Aim 2.1** provide fast temporal sampling of cells across cortical layers, calcium imaging can be used to track activity in hundreds of visually-identifiable cells within a layer across many days. These experiments follow calcium transients in L2/3 and L4 of V1, where changes in excitability following visual deprivation have previously been observed[33,34].

Key Outcomes: We used the Emx1-Cre, Som-Cre, and PV-Cre driver lines cross to the Ai95 transgenic line to create mice that expressed the calcium indicator, GCaMP6f, in excitatory principal cells, somatostatin-positive inhibitory cells, and parvalbumin-positive inhibitory cells, respectively. Using 2-photon calcium imaging, we chronically recorded activity in L4 and L2/3 of V1 before, during, and following binocular retinal inactivation. We found that retinal inactivation consistently decreased calcium activity in all three groups of animals, with greater decreases occurring in inhibitory cell populations.

Revision: The original proposal only monitored excitatory cell populations. However, the drop in activity within excitatory populations was a surprise to us. Therefore, we also conducted pilot experiments to evaluate how retinal inactivation impacted inhibitory cells populations (using time made available by eliminating Aim 2.3 as previously discussed). The results from those experiments suggest that we should be looking at the balance between excitation and inhibition as a plasticity feature, which we have now added to Aim 1.2.

Aim 3 (R00): Drive retinal inactivation-associated changes in excitability and plasticity through closed-loop cortical stimulation. While retinal inactivation is minimally invasive and uses routine ophthalmic procedures, it is not without risks, such as hazards of inactivating agents and patient safety during the temporary period of visual incapacitation. The previous aims explored how retinal inactivation creates the conditions that promote visual cortical potentiation. The goal of the final aim is to test whether those conditions can be re-created in the absence of retinal inactivation through direct V1 stimulation to produce the circuit-level changes that promote visual potentiation. Indeed, evidence from human rehabilitation studies suggests that brief cortical stimulation can prime neural circuits to undergo subsequent synaptic plasticity and changes in excitability[7,24]. Strategies for non-invasive stimulation of V1 have also been used to alter visual ability in amblyopic patients, with some success[15,48]. In this aim, I create a new stimulation strategy while testing the causal relationship between V1 spiking and subsequent plasticity. Although the proposed methods are invasive, if successful, a future goal will be to translate this to a non-invasive approach.

Aim 3.1: Designing a closed-loop system for controlling V1 activity. I will first design a V1 stimulation strategy that allows me to replicate the effects of retinal inactivation.

Experimental design: I will genetically express the depolarizing opsin, Chronos, and the hyperpolarizing opsin, ArchT, in V1 principal cells by crossing Ai90D (cre/tet-dependent Chronos) and Ai40D (cre-dependent ArchT) mice with Emx1-Cre mice. Mice will be implanted with high-density silicone probes and optogenetic

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Aim 3.2: Open-loop and closed-loop control to drive V1 potentiation. In this sub-aim, I will test whether V1 stimulation can be used to drive visual enhancement in the absence of manipulations to retinal activity.

Experimental design: Using the strategy developed in **Aim 3.1**, I will record activity in freely-moving $Cre^{+/-}$ (opsin expressing) mice for 3 hours and generate single-unit templates from these baseline recordings. I will then begin closed-loop control of firing to reproduce the first and second order spiking statistics in V1 that typically follow retinal inactivation (closed-loop case). The same optical stimuli will be delivered to two yoked littermate controls, with one littermate being $Cre^{+/-}$ (open-loop case) and the other littermate being $Cre^{-/-}$ (non-opsin expressing negative control). I will deliver stimuli for 6h, and then measure freely-moving and head-fixed visual activity as described in **Aim 2.1** at the following time points following the onset of V1 stimulation: 6h, 12h, 24h, 48h, 72h, and 168h (7d). I anticipate using 30 mice for these experiments.

Expected outcomes, potential problems, and alternative approaches: I predict that 6h of closed-loop control will be sufficient to drive a homeostatic rebound in visual responsiveness, significantly heightened over the negative control. The open-loop case will be interesting to monitor, as the success of a pre-determined stimulus would be therapeutically relevant. If closed-loop control is successful, this would establish a causal relationship between changes in V1 activity and subsequent enhancement. On the other hand, it is possible that I observe no change or a reduction in visual responsiveness. Possible interpretations of this outcome include that my stimulus may be driving LTD-like processes or reducing intrinsic excitability, or that other neuron types (e.g. inhibitory cells) may be the drivers of visual potentiation. These possibilities could be tested in follow up experiments. Regardless, the data generated in these experiments could form the basis of an independent grant proposal to investigate the capacity for V1 stimulation to drive recovery from visual impairments, or to reduce cortical hypersensitivity observed in other neurological disorders.

Aim 3.3: Closed-loop control to drive synaptic plasticity. In this sub-aim, I will test whether V1 stimulation can be used to create the activity conditions that promote synaptic plasticity, despite normal retinal activity.

Experimental design: I will use the 6h stimulation strategy as described in the closed-loop and negative control cases in **Aim 3.2**. I will prepare acute slices from these animals and measure changes in synaptic plasticity at 6h (immediately after stimulation ends) and 48h after stimulus onset. I will specifically probe the plasticity mechanisms or connection types found to be significantly altered by retinal inactivation (**Aim 1**). I will use this as a proxy to determine whether 6h stimulation can engage the same plasticity mechanisms driven by retinal inactivation. I anticipate using 30 mice for these experiments.

Expected outcomes, potential problems, and alternative approaches: This experiment is rooted in the hypothesis that using V1 stimulation to reproduce the same activity conditions driven by retinal inactivation will also drive the same plasticity mechanisms. This is admittedly a very V1-centric hypothesis, and it is important to acknowledge the many convergent inputs onto V1 and the roles they play in shaping cortical plasticity. If it is possible for altered V1 activity alone to drive plasticity, I expect that the 6h time point will exhibit plasticity of the same direction and magnitude as identified in **Aim 1**. A more tantalizing question will be whether the 48h time point (well after the end of stimulation) shows any residual effects. If the 48h time point shows the same plasticity as observed 48h Proprietary Info this would bode very well for direct V1 stimulation as a potential therapeutic strategy. Notably, this experiment is not contingent on a particular result in **Aim 3.2**, as it is possible that V1 stimulation will drive visual enhancement but not synaptic plasticity, or vice versa. Dissecting the independent drivers of changes in plasticity and excitability could be the subject of future research.

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Vertebrate Animals

1. Description of Procedures

All procedures listed in the Research Strategy are described in animal protocols that have been submitted for approval by the Georgia Tech Institutional Animal Care and Use Committee (IACUC), with an expected approval date in October 2021. The procedures include craniotomies, chronic electrode implants, electrode recordings, viral injections, intravitreal injections, eyelid suture, and euthanasia/tissue harvest. More detailed descriptions of each procedure are included below. All animal subjects will be mice (C57BL/6 background), some genetically altered, crossed to produce the described genotypes. Animals of both sexes will be used, with ages ranging from P23-P60. Based on power analysis using estimated effect sizes, I anticipate that 90 mice will be needed to complete this proposal.

2. Justifications

Early studies of visual cortical plasticity were conducted in felines and non-human primates. Previous observations on the effects of retinal inactivation have been performed in both mice and cats, and we have selected mice as the species for follow-up studies as they are ideally suited for mechanistic studies. Mice have a known genetic background and there are numerous tools available for manipulating the mouse genome to test questions about cell-type specificity and for introducing optogenetic constructs. At this time, it is not possible to test the effects of retinal inactivation on specific cell/synapse types in the mammalian visual cortex using computational models, invertebrates, or in vitro models. For our current studies, we do not feel it is appropriate to use higher mammals (e.g. cats, monkeys, humans) both based on ethical use of these model systems and based on experimental tractability.

3. Minimization of Pain and Distress

Every effort is made, in consultation with Redacted by agreement, to minimize discomfort and/or pain. Care is taken to familiarize animal subjects to restraint, head-fixation, weight of any recording devices placed on the head, and different handlers. For all surgical procedures, animals are kept warm with a heating pad and eyes are kept moist with either sterile saline or opthalamic ointment. For anesthesia, isofluorane (0.5-3% vaporized in oxygen) is used for surgical procedures and ocular manipulations, with continuous ventilation provided. For surgical procedures, we have also invested in newly-available low-flow vaporizers designed specifically for small animals like mice. Analgesics are administered pre- and post-operatively, and in any animals as suggested by veterinary consult. At present, analgesics recommended by veterinary staff include sustained-release buprenorphine (1 mg/kg IP, pre-operative) and meloxicam (1 mg/kg subcutaneously, post-operatively every 24 hours). For longer procedures, warm fluids are also administered immediately post-operatively. All experiments are eventually terminated by euthanasia using methods compatible with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

All procedures will be performed at Georgia Tech within Unpublished All personnel will complete required trainings and be added to the IACAC protocol prior to working with vertebrate animals.

Additional details about specific procedures are provided below:

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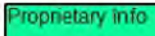
Select Agent Research



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Program Director/Principal Investigator (Last, First, Middle): Fong, Ming-fai

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ RESUBMISSION of application number:
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number:
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number:
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.

Name of former program director/principal investigator:

☐ CHANGE of Grantee Institution. Name of former institution:

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

INVENTIONS AND PATENTS *(Renewal appl. only)* ☐ No ☐ Yes

If "Yes," ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in the NIH Grants Policy Statement, Section 4: Public Policy Requirements, Objectives and Other Appropriation Mandates. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ HHS Agreement dated: ☐ No Facilities And Administrative Costs Requested.

☐ HHS Agreement being negotiated with Regional Office.

☒ No HHS Agreement, but rate established with Office of Nave Research Date July 1, 2021

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

	Amount of base $	x Rate applied	% = F&A costs $
a. Initial budget period:	205221	58.2	249000
b. 02 year	172111	58.2	249000
c. 03 year	161075	58.2	249000
d. 04 year			
e. 05 year			
		TOTAL F&A Costs $	747000

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

MTDC does not include tuition and equipment costs in amount of base amount.

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Wallace H. Coulter Department of
Biomedical Engineering

July 19, 2021

National Eye Institute
6700B Rockledge Drive
Bethesda, MD 20892

RE: Institutional Support for the National Institutes of Health (NIH) K99/R00 Transition Application to NEI proposed by Ming-fai Fong, Ph.D.

Dear Madam or Sir,

As Chair of the Wallace H. Coulter Department of Biomedical Engineering at Georgia Institute of Technology and Emory University School of Medicine, it gives me great pleasure to provide this Statement of Institutional Support for Dr. Ming-fai Fong's NIH K99/R00 Transition Application to NEI proposal entitled "Synaptic and intrinsic mechanisms underlying visual cortical enhancement following retinal inactivation" Dr. Fong will start her tenure-track faculty position in the Coulter Department of Biomedical Engineering on October 16, 2021. Prior to her tenure-track appointment, Dr. Fong will hold an interim position as Transitional Faculty until October 15, 2021. I am pleased to confirm the institution's commitment to Dr. Fong's career development and her success as an independent investigator.

Research Environment
Georgia Tech is committed to the success of Dr. Fong as an independent faculty and we will support her career advancement and progression through tenure process. The College of Engineering at Georgia Tech has an excellent record for progression and retention of junior faculty, and we will ensure that Dr. Fong receives the appropriate resources and support to successfully be promoted while at Georgia Tech. The Wallace H. Coulter Department of Biomedical Engineering has been immensely successful and is currently ranked #2 in both graduate and undergraduate programs, *U.S. News and World Reports*, 2021. I have no doubt that Dr. Fong will thrive in this innovative and collaborative environment. Our department has a strong reputation in neuroengineering and neural plasticity for therapeutic interventions of sensory and motor disabilities, the primary areas of interest to the Fong lab. Furthermore, Dr. Fong's research program lends itself perfectly to the environment at Georgia Tech, and her expertise enhances the research being conducted by other well-established faculty in the U.A. Whitaker building where the Fong Lab will be located.

Dr. Fong will have full access to the equipment and materials needed to fulfill the goals of the proposed research including access to all core facilities in the department of biomedical engineering and across campus.

Redacted by agreement

Wallace H. Coulter Department of Biomedical Engineering is a unique partnership established over 20 years ago between Georgia Institute of Technology and Emory University School of Medicine which will allow her to easily interact with the neuroengineering and vision research communities. Through this partnership, Dr. Fong will have access to many seminars and interactions with the rich and diverse faculty community at Coulter BME.

Susan S. Margulies, Ph.D. • **Georgia Research Alliance Eminent Scholar in Injury Biomechanics**
Wallace H. Coulter Chair • **Wallace H. Coulter Department of Biomedical Engineering**
susan.margulies@gatech.edu • susan.margulies@emory.edu
Georgia Tech The U.A. Whitaker Biomedical Engineering Building • 313 Ferst Drive • Atlanta, Georgia 30332-0535 • www.bme.gatech.edu
Emory University Health Sci[illegible]2-4600 • www.bme.emory.edu
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Appointment
Dr. Fong's position in the Biomedical Engineering Department is a full-time tenure-track position, which will allow her to devote EFFORT of her effort to research. Her academic year salary will be covered by Georgia Tech. She will have a reduced teaching her load her first year, and then will teach two lecture-based courses per year. This teaching commitment amounts to less than EFFO effort reserving at least EFFORT effort for her research

Private Source

Lab Space

Redacted by agreement

Mentorship/Career Development
Coulter BME has a faculty mentoring program that includes a mentoring committee for each Assistant Professor until their promotion to Associate Professor. Dr. Fong will be assigned 3-4 senior faculty mentors, with a range of complementary experiences, to guide her as she sets up her lab and progresses through the tenure process. Additionally, committee members provide strategic input on grant and manuscript submissions; offer counsel on the realities of building an academic career and balancing opportunities as they present themselves; and facilitate networking connections on the local and national science communities. Within Georgia Tech, there are also opportunities for career enrichment, such as the Petite Institute Bioengineering Seminars, Distinguished Lecture Series, Suddath Symposiums, and numerous faculty development workshops through the College of Engineering. The Petit Entrepreneurship Academy provides opportunities to help our Georgia Tech bio-community entrepreneurs learn how to fund and commercialize their technology. Georgia Tech and Emory University routinely provide regular training programs for faculty, post docs and students covering responsible conduct of research, grant writing and laboratory/safety training related topics.

In summary, Dr. Fong is proposing a vibrant integrative research program and a strong commitment to education and training. She is a very promising faculty member for our department. It gives me great pleasure to give Dr. Fong my strongest support in her (NIH) K99/R00 Transition application.

Sincerely,

SSM/clk

Georgia Tech The U.A. Whitaker Biomedical Engineering Building • 313 Ferst Drive • Atlanta, Georgia 30332-0535 • www.bme.gatech.edu
Emory University Health Sciences Research Building • 1760 Haygood Drive, Suite W 200 • Atlanta, Georgia 30322-4600 • www.bme.emory.edu
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