Audacious Goals Initiative (AGI) for Regenerative Medicine in Vision

Status Update 2019

NIH National Eye Institute

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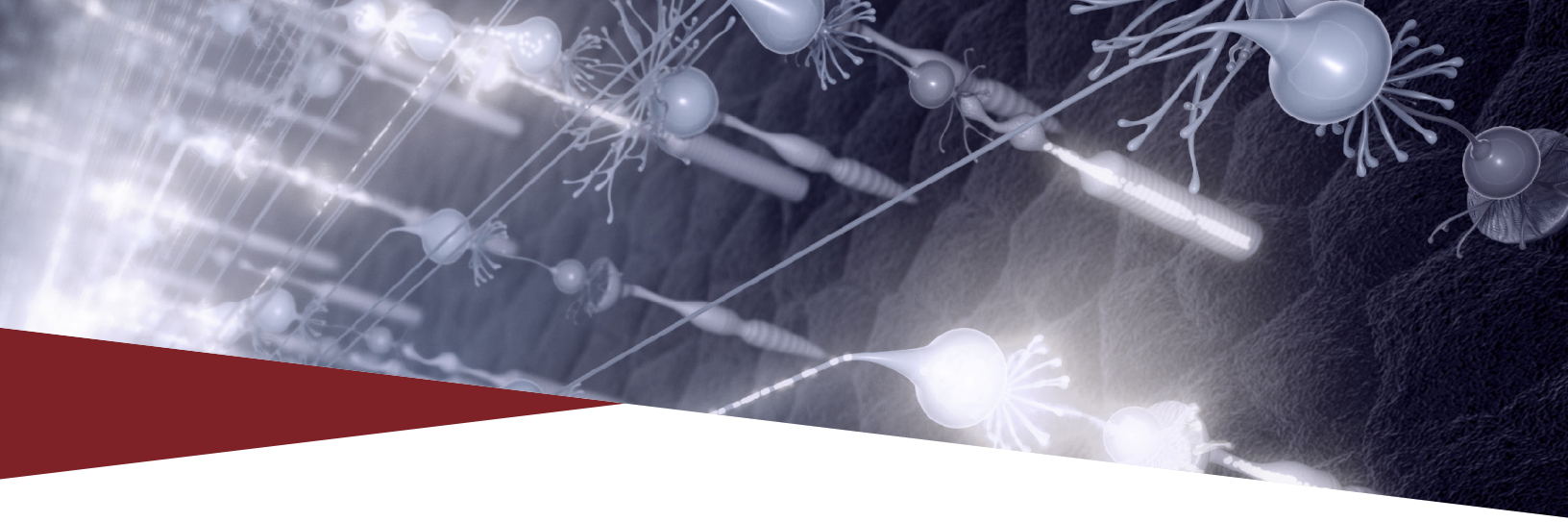


Executive Summary

The Audacious Goals Initiative (AGI) for Regenerative Medicine in Vision is a strategic endeavor by the National Eye Institute (NEI) to expand support aimed at new treatments for vision disorders. By facilitating cross-disciplinary research, the AGI is tackling devastating and difficult-to-treat eye diseases.

Despite recent advances in understanding vision disorders, effective therapies for many conditions are still lacking. The three most common causes of blindness in the United States are age-related macular degeneration, diabetic retinopathy, and glaucoma. All are projected to increase during the next decade[1]. These diseases attack and destroy cells of the retina, the light-sensitive tissue in the back of the eye. Though some animals can regenerate lost retinal neurons, humans cannot. The purpose of the AGI is to develop therapies that replace lost or damaged cells of the retina and thereby preserve or even restore vision.

The AGI has launched three key research consortia, representing 16 projects and $62 million. The AGI Functional Imaging Consortium is addressing the technical needs and opportunities for imaging cells of the visual system as they respond to light. The AGI Regenerative Factor Discovery Consortium is identifying factors that control cell regeneration in the visual system. The AGI Translation-Enabling Models Consortium is developing animal models that have fidelity to human eye disease, a crucial step toward testing regenerative therapies in clinical trials. Beyond direct funding, the AGI has generated interest from the vision research community, helping to expand the NEI regenerative medicine portfolio.

This report presents the current status of the AGI, its activities, and its accomplishments.

Vision and the AGI are at the forefront of regenerative medicine, leveraging new trans-National Institutes of Health (NIH) activities such as the BRAIN Initiative and the Regenerative Medicine Innovation Project, while contributing to the collective effort. The latest AGI workshop, "Pathways for Retinal Cell Replacement Therapies," held in September 2018, marked NEI's most recent effort to translate preclinical advances to clinical testing. NEI anticipates that clinical trials of new regenerative strategies for eye disease will begin in the next few years, as AGI-funded consortia converge. AGI-sponsored activities — including workshops, town halls, and symposia — are informing plans to build capacity for human studies.

NEI is catalyzing a new cross-disciplinary research approach whereby teams collaborate and complement each other, sharing their unique expertise and creativity. Bringing in experts who can cultivate this new scientific approach has been crucial and is embodied by members of the AGI external scientific oversight committees (ESOCs), which oversee the various consortia. Furthermore, with the guidance of a distinguished external steering committee, the AGI is facilitating new ways of advancing science across the NEI research portfolio.

The AGI is making progress. For example, Jessica Morgan, Ph.D., of the University of Pennsylvania, is using AGI imaging technologies to noninvasively assess photoreceptor function in patients. AGI support has also led to a new regenerative factors database that will soon be open to the research community. Importantly, NEI-funded disease models are setting the stage for preclinical studies of cell replacement therapies. Although the AGI is aimed at eye disease, its work is having an impact across regenerative medicine.

Background

Genesis of AGI for Regenerative Medicine in Vision

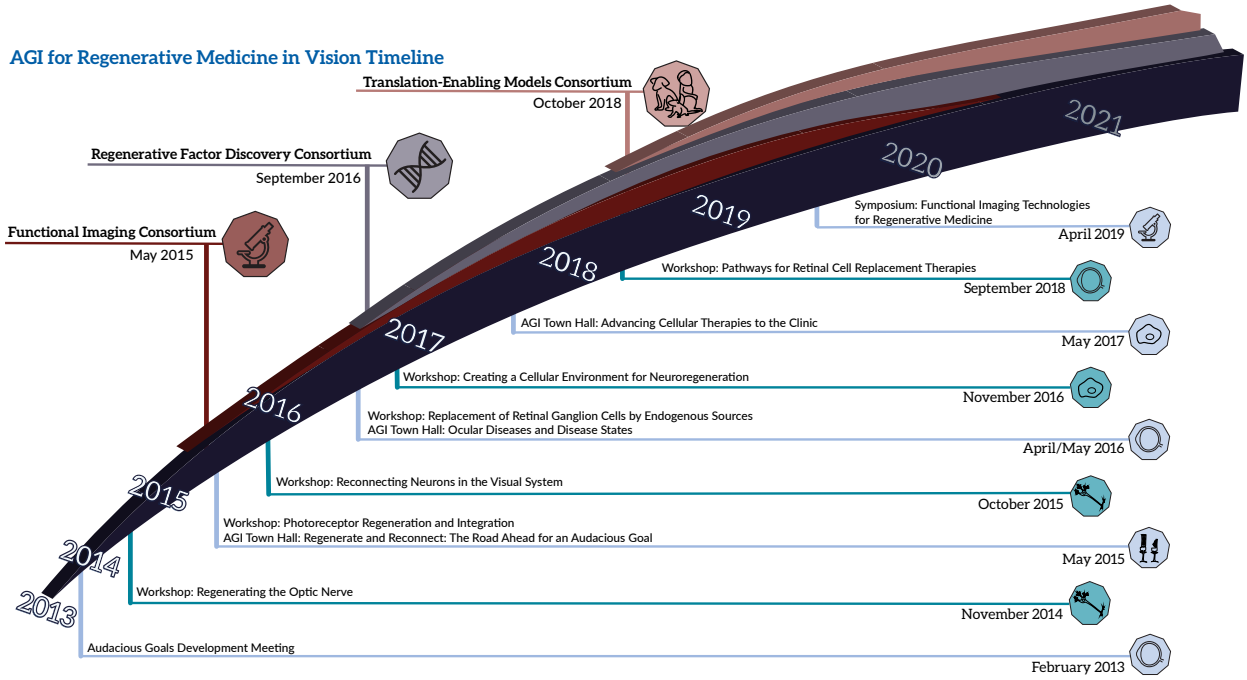
The AGI is catalyzing fundamental research that will enable the restoration of vision through regeneration of the retina. The central goal is to replace cells of the retina that have been damaged by disease or injury and to restore their connections to the visual centers of the brain. Advances from the AGI will stimulate efforts across the research spectrum, within and beyond vision. Through strategic research funding, NEI is enlisting dynamic scientists and teams to develop the knowledge and technology to make this audacious goal a reality.

NEI Director Paul Sieving addresses participants at the 2012 Audacious Goals Development Meeting.

In February 2012, former NEI Director Paul A. Sieving, M.D, Ph.D., addressed the National Advisory Eye Council (NAEC) and proposed that NEI organize a concerted research effort lasting 10 to 15 years to identify and address a major goal that was not yet feasible. The NAEC is a 12-member public panel comprising members of the vision community who are charged by law to help guide NEI activities. The NAEC responded enthusiastically to Sieving's proposal, and NEI proceeded to develop plans for the AGI.

The AGI began in 2012 with the Audacious Goals Challenge, a prize competition that challenged the vision community to imagine the greatest need and achievement for vision research during the next 10 to 15 years. The challenge attracted 548 innovative responses from around the world. From this pool of ideas, NEI awarded prizes to 10 scientists (see appendix) for their concepts, which it consolidated into six themes. In February 2013, NEI invited 200 scientists, engineers, and other stakeholders to the three-day Audacious Goals Development Meeting to explore ideas from the challenge.

In May 2013, with input from the vision research community, NEI set the goal of "restoring vision through the regeneration of neurons and neural connections in the eye and visual system," and specified photoreceptors and retinal ganglion cells (RGCs) as research targets. NEI based its choice to pursue cell replacement therapies on preclinical advances in induced pluripotent stem cell technology and proof-of-principle studies in animal models showing that transplantation of stem cell-derived precursor cells can restore vision. Studies of fish and amphibians showed that some vertebrates utilize endogenous regenerative factors to rebuild the retina after injury. Such studies gave NEI hope that a similar regenerative strategy could be recapitulated in humans.



Workshops, Town Hall Forums, and Symposia

The AGI has coordinated events and working groups to solicit input and guidance from the research community. These include meetings, workshops, town hall forums, symposia, hands-on training courses, and seminars. Some events have resulted in white papers or journal publications. Importantly, they have informed AGI strategic planning and have fostered collaborations among researchers.

Participants in the AGI hands-on training workshop "Stem Cells: Regeneration Methods for the Visual System" on the NIH campus in August 2017.

Workshop: Regenerating the Optic Nerve

November 19, 2014
Washington, D.C.
Co-chairs: Jeffrey Goldberg, M.D, Ph.D., Stanford University, and William Guido, Ph.D., University of Louisville
Output: Report on the National Eye Institute Audacious Goals Initiative: Regenerating the Optic Nerve[2]
https://www.ncbi.nlm.nih.gov/pubmed/26990163

Workshop: Photoreceptor Regeneration and Integration

May 2, 2015
Association for Research in Vision and Ophthalmology (ARVO) annual meeting
Denver, Colorado
Co-chairs: David Gamm, M.D., Ph.D., University of Wisconsin, and Rachel Wong, Ph.D., University of Washington
Output: Report on the National Eye Institute Audacious Goals Initiative: Photoreceptor Regeneration and Integration Workshop[3]
https://www.ncbi.nlm.nih.gov/pubmed/26629398

Town hall forum: Regenerate and Reconnect: The Road Ahead for an Audacious Goal

May 3, 2015
ARVO annual meeting
Denver, Colorado
Moderators: Paul Sieving, M.D., PhD., NEI, and John Dowling, Ph.D., Harvard University

Panel discussion: Reconnecting Neurons in the Visual System

October 16, 2015
Society for Neuroscience annual meeting
Chicago, Illinois
Co-chairs: Michael Crair, Ph.D., Yale University, and Carol Mason, Ph.D., Columbia University
Output: Reconnecting Eye to Brain[4]
https://www.ncbi.nlm.nih.gov/pubmed/27798125

Workshop: Replacement of Retinal Ganglion Cells by Endogenous Sources

April 30, 2016
ARVO annual meeting
Seattle, Washington
Co-chairs: Peter Hitchcock, Ph.D., University of Michigan, and Monica Vetter, Ph.D., University of Utah
Output: Report on the National Eye Institute Audacious Goals Initiative: Replacement of Retinal Ganglion Cells from Endogenous Cell Sources[5]
https://www.ncbi.nlm.nih.gov/pubmed/?term=PMC5354473

Town hall: Regenerative Medicine Targets—Ocular Diseases and Disease States

May 1, 2016
ARVO annual meeting
Seattle, Washington
Moderator: Leonard Levin, M.D., Ph.D., McGill University and the University of Wisconsin-Madison
Output: Special Commentary: Early Clinical Development of Cell Replacement Therapy: Considerations for the National Eye Institute Audacious Goals Initiative[6]
https://www.ncbi.nlm.nih.gov/pubmed/28365209

Workshop: Creating a Cellular Environment for Neuroregeneration

November 11, 2016
Society for Neuroscience annual meeting
San Diego, California
Co-chairs: Marie Burns, Ph.D., University of California Davis, and Beth Stevens, Ph.D., Harvard Medical School
Output: Report on the National Eye Institute's Audacious Goals Initiative: Creating a Cellular Environment for Neuroregeneration[7]
https://www.ncbi.nlm.nih.gov/pubmed/29766041

Town hall: Advancing Cellular Therapies to the Clinic

May 7, 2017
ARVO annual meeting
Baltimore, Maryland
Moderator: Mark Blumenkranz, M.D., Stanford University
Presenters: Peter Coffey, DPhil, University of California Santa Barbara; David Gamm, M.D., Ph.D, University of Wisconsin; Jeffrey Goldberg, M.D., Ph.D., Stanford University; and Margaret Sutherland, Ph.D., National Institute of Neurological Disorders and Stroke

Panel discussion: ARVO evening session: Bringing Regenerative Medicine Therapies to the Clinic

May 1, 2018
ARVO annual meeting
Honolulu, Hawaii
Panelists: Ula Jurkunas, M.D., Massachusetts Eye and Ear Infirmary; Susanna Park, M.D., Ph.D., University of California Davis; Kapil Bharti, Ph.D., NEI; and Steven Becker, Ph.D., NEI

Workshop: Pathways for Retinal Cell Replacement Therapies

September 24 to 25, 2018
NIH campus
Bethesda, Maryland
Moderators: Kapil Bharti, Ph.D., NEI; Brian Brooks, M.D., Ph.D., NEI; Mark Blumenkranz, M.D., Stanford University; Russell Van Gelder, M.D., Ph.D., University of Washington; Steven Becker, Ph.D., NEI; Shekhar Jha, Ph.D., NEI; and Ilyas Singeç, M.D., Ph.D., National Center for Advancing Translational Sciences

Symposium: Functional Imaging Technologies for Regenerative Medicine

April 28, 2019
ARVO annual meeting
Vancouver, British Columbia
Moderator: Leonard Levin, M.D., Ph.D., McGill University
Presenters: Sheng-Kwei (Victor) Song, Ph.D., Washington University; Jennifer Hunter, Ph.D., University of Rochester; Krzysztof Palczewski, Ph.D., University of California Irvine; Jessica Morgan, Ph.D., University of Pennsylvania; and Austin Roorda, Ph.D., University of California Berkeley

Training

Hands-on training workshop: Stem Cells: Regeneration Methods for the Visual System

August 28 to September 1, 2017
NIH campus
Bethesda, Maryland

Instructors:

- Anand Swaroop, Ph.D., NEI
- Douglas Dean, Ph.D., University of Louisville
- Gilbert Bernier, Ph.D., University of Montreal
- Haohua Qian, Ph.D., NEI
- Jason Meyer, Ph.D., Indiana University
- Kapil Bharti, Ph.D., NEI
- Valerie Wallace, Ph.D., University of Toronto
- Wei Li, Ph.D., NEI

Seminar Series in Neuroregeneration at the NIH Campus

The AGI Seminar Series in Neuroregeneration explores topics relevant to regenerative neuroscience and medicine, with emphasis on the visual system. Speakers are invited to the NIH campus in Bethesda, Maryland.

Past lectures:

Joshua Sanes, Ph.D., Harvard University
Assembly of Feature-Detecting Circuits in the Retina
November 2, 2015

Dennis Clegg, Ph.D., University of California Santa Barbara
Vision for the Future: Cell Therapy for Ocular Disease
February 11, 2016

John Flanagan, Ph.D., Harvard University
Molecular Cues for Axon Guidance and Regeneration
April 6, 2016

Carla Shatz, Ph.D., Stanford University
Saving the Synapse: Developmental Critical Periods and Amblyopia
October 25, 2016

Andrew Huberman, Ph.D., Stanford University
Visual Restoration: Bridges and Gaps to Curing Blindness in Humans
January 24, 2017

David Gamm, M.D., Ph.D., University of Wisconsin
Production, Characterization, and Application of Stem Cell-Derived Photoreceptors
June 6, 2017

Jeffrey Goldberg, M.D., Ph.D., Stanford University
Optic Nerve Regeneration: Uncovering Molecular Pathways
September 25, 2018

Michael Dyer, Ph.D., St. Jude Children's Research Hospital
Cellular Pliancy in Retinal Development and Disease
December 13, 2018

AGI Charter, Leadership, and Administration

In July 2014, NEI adopted a charter for the AGI, establishing the AGI Office, the AGI Working Group, and the AGI Steering Committee. The charter outlines these parties' roles and responsibilities.

Briefly, the AGI governance structure is as follows: The external AGI Steering Committee helps assess the state of the science, proposes scientific priorities, and assists with evaluation metrics. Planning and implementation are conducted by the AGI Working Group and coordinated by the AGI Office. Working Group staff and the NEI director provide the National Advisory Eye Council (NAEC) updates as necessary. Input from council members is welcome and encouraged. At least one council member attends AGI-sponsored activities. Going forward, the NAEC will propose future AGI Steering Committee members. The personnel and roles of the various groups are detailed in the organizational chart.

AGI Steering Committee

The AGI steering committee comprises three to five members from the NEI extramural community to help guide the AGI.

Current AGI Steering Committee Members:

Name	Affiliation	Term
Mark S. Blumenkranz, M.D.	Stanford University	August 2014 -
Joshua Sanes, Ph.D.	Harvard University	August 2014 -
Clive Svendsen, Ph.D.	Cedars-Sinai	May 2018 -
Russ Van Gelder, M.D., Ph.D.	University of Washington	May 2018 -
Rachel Wong, Ph.D.	University of Washington	May 2018 -

Past AGI Steering Committee Members:

Name	Affiliation	Term
John Dowling, Ph.D.	Harvard University	August 2014 - 2017
Pamela Raymond, Ph.D.	University of Michigan	August 2014 – February 2018

AGI Office

Name	Affiliation	Term
Steven Becker, Ph.D.	NEI Office of the Director	September 2014 -

AGI Working Group

Name	Affiliation	Term
Lesley Earl, Ph.D.	NEI Office of Science Communications, Public Liaison, and Education	January 2019-
Donald Everett, M.A.	NEI Division of Extramural Science Programs	August 2014 -
Thomas Greenwell, Ph.D.	NEI Division of Extramural Science Programs	August 2014 -
Dustin Hays	NEI Office of Science Communications, Public Liaison, and Education	August 2014 -
Paul Sieving, M.D., Ph.D.	NEI Office of the Director	August 2014-2019
Michael Steinmetz, Ph.D.	NEI Division of Extramural Science Programs	August 2014 -
Santa Tumminia, Ph.D.	NEI Office of the Director	January 2019 -
Cheri Wiggs, Ph.D.	NEI Division of Extramural Science Programs	August 2014 -
Maria Zacharias	NEI Office of Science Communications, Public Liaison, and Education	September 2015 -

Projects

The AGI is target-driven, a feature that distinguishes it from investigator-initiated (R01) funding. To ensure that AGI-funded projects meet their milestones, NEI uses the cooperative agreements as funding mechanisms, which encourage collaboration and allow for substantial NEI programmatic involvement. This mechanism also allows for external scientific oversight committees to assist NEI in monitoring progress toward project milestones. NEI is calling on a diverse, multidisciplinary cadre of researchers to achieve AGI's scientific objectives.

Functional Imaging Consortium

The first AGI funding opportunity was announced in April 2014. "Addressing Technical Needs and Opportunities for Imaging the Visual System" (RFA-EY-14-001) called for the development of new methods for imaging cells and tissues of the visual system. The announcement solicited new imaging tools to noninvasively evaluate future regenerative therapies.

NEI funded five projects totaling almost $4 million in 2015 and up to $17.9 million during the next 5 years. Projects are testing technologies in a variety of models, including cell culture, rodents, nonhuman primates, and humans. The end goal of these projects is integration with systems for noninvasive monitoring of retinal neurons in patients. Some projects are developing eye tracking systems to compensate for eye movement, enabling high-resolution optical coherence tomography (OCT) and adaptive optics scanning laser ophthalmoscopy (AOSLO). One project is developing magnetic resonance imaging (MRI) techniques to noninvasively assess the structure and function of regenerating axons in the optic nerve.

The AGI imaging projects are increasing sensitivity and resolution of imaging modalities. Improvements are enabling researchers to study retinal cell death and observe the physiology of replacement cells. The dissemination of improved eye tracking and adaptive optics will better enable vision researchers and clinicians to monitor disease progression and the effects of treatment. Future challenges include adoption by other research groups and adaptation to clinical settings.

Table 1. Goals of Functional Imaging Projects

Funding Objectives	Projects				
	Interferometric optophysiology of the human retina (Roorda)	Accelerating vision restoration with in- vivo cellular imaging of retinal function (Williams)	A two-photon ophthalmoscope for human retinal imaging and functional testing (Palczewski)	Imaging optic nerve function and pathology (Song)	Platform technologies for microscopic retinal imaging (Dubra)
New methods for imaging retinal cells in vivo	x	x	x	x	x
Noninvasive assessment of function/ integration of retinal cells or monitoring of axon regeneration	x	x			x
Imaging methods to measure retinal ganglion cell (RGC) viability and/or regeneration	x	x Rossi EA, et al. [8]			x
Increased resolution and sensitivity to imaging of individual retinal neurons	x	x Walters S, et al. [9]	x Chen Y, et al. [10]		x

Funding Objectives	Projects				
	Interferometric optophysiology of the human retina (Roorda)	Accelerating vision restoration with in- vivo cellular imaging of retinal function (Williams)	A two-photon ophthalmoscope for human retinal imaging and functional testing (Palczewski)	Imaging optic nerve function and pathology (Song)	Platform technologies for microscopic retinal imaging (Dubra)
Biomarkers and imaging agents to monitor specific cell types	x	x	x		x Cooper RF, et al. [11]
Development of safe quantitative longitudinal imaging techniques	x	x	x Palczewska G, et al. [12]	x	x
New 3D image analysis or informatics tools	x Ling T, et al. [13]	x	x Palczewska G, et al. [14] Alexander NS, et al. [15]	x	x

Table 2. Functional imaging projects at a glance

Lead Principal Investigator (PI)	Techniques/Methods	Testing Paradigm	Anticipated Outcomes
Austin Roorda, Ph.D., U. California Berkeley	Wide-field interferometry; phase-resolved OCT; AOSLO and image-based eye tracking.	Ex vivo and in vivo primate and human retina imaging.	Techniques will measure neural activity noninvasively throughout the retinal layers at cellular resolution. Adaptive optics and eye tracking will overcome optical aberrations and eye jitter. The technology will allow activation of photoreceptors with visible light while imaging the resulting electrical activity of individual downstream cells in vivo.

Lead Principal Investigator (PI)	Techniques/Methods	Testing Paradigm	Anticipated Outcomes
David Williams, Ph.D., U. of Rochester	OCT and AOSLO (reflectance, offset aperture, and two-photon) with genetically encoded calcium indicators; fluorescence lifetime imaging of glucose and NADH sensors.	Ex vivo and in vivo mouse and primate imaging with optogenetic tools (ChrimsonR) and labeled human photoreceptor precursor cells into lesioned primate retina.	To develop advanced imaging technology and tools for use in the tracking and assessment of vision restoration.
Krzysztof Palczewski, Ph.D., U. California Irvine	Two-photon ophthalmoscope optimization.	Ex vivo and in vivo mouse and human two-photon imaging of endogenous retinal fluorophores.	To develop a new two-photon ophthalmoscope for safe imaging of cells and tissues in the human retina. Together with novel measurements of retinal function, this tool will permit the early detection, monitoring, and evaluation of treatments for human retinal diseases.
Sheng-Kwei Song, Ph.D., Washington U.	Diffusion basis spectrum imaging (DBSI) and diffusion functional magnetic resonance imaging (fMRI).	Optic nerve crush model in frog and mouse to correlate DBSI with histological counts of axons; improved modeling algorithms and protocols to image optic nerve function and pathology in glaucoma and optic neuritis patients.	DBSI and fMRI will be combined to deliver an improved imaging method to simultaneously assess optic nerve anatomy, function, and pathology, allowing a detailed pathophysiological investigation of optic neuropathies.
Alfredo Dubra, Ph.D., Stanford U.	Real-time eye motion compensation; optics improvements, super-resolution line scanning ophthalmoscopy; opto-physiological method for assessing photoreceptor function.	High-speed retinal and pupil motion compensation; novel adjustable lens for adaptive longitudinal chromatic aberration correction; improved wavefront sensing optimization to remove spherical aberration; eliciting and measuring stimulus-evoked intrinsic signals from individual cone photoreceptors in humans.	Technologies are generalizable to preclinical and clinical imaging systems and applicable to all retinal cell types, retinal diseases, and therapeutic strategies; will enable diagnosis of retinal disease and monitoring of retinal structure and function with unprecedented sensitivity and resolution.

Functional Imaging Projects

Interferometric optophysiology of the human retina (EY025501)

Principal investigator: Austin Roorda, Ph.D., University of California Berkeley
NEI program official: Thomas Greenwell, Ph.D.

Roorda and colleagues are designing a system to map the interaction of cells in the retina. The system will enable scientists to stimulate individual neurons and observe nearby cells as they become active in response. Mapping these intricate signaling patterns will help explain how the retina processes visual information before it sends it to the brain, an important tool for monitoring function in regenerated cells. The system will incorporate eye tracking components and adaptive optics, a technology that corrects for distortion imposed by the cornea and lens.

Progress

Roorda and his team have developed all-optical methods that measure neuronal activity. In cultured cells, they have recorded cellular changes resulting from single action potentials. Their new technique detects cellular shape changes of less than 1 nanometer that last about 1 millisecond. Their experiments provide a template for future human studies.

In collaboration with Ramkumar Sabesan, Ph.D., and colleagues at the University of Washington, Roorda's team is translating their all-optical methods to human eyes. In partnership, they have built a line-scanning optical coherent tomography system with adaptive optics. They recently measured optical changes in photoreceptors that lasted 10 milliseconds, moving between 5 to 30 nanometers. Importantly, these changes exhibited robust and predictable responses to the level of light stimulation.

In collaboration with Hyle Park, Ph.D., of the University of California Riverside team, Roorda and colleagues completed instrumentation that will offer uniquely sensitive and high-speed optical recordings from the human retina, and they have begun initial tests.

Publications

1. Privitera CM, *et al*. "Eye-tracking technology for real-time monitoring of transverse chromatic aberration." *Optics Letters*, 2016;41(8):1728-1731.
2. Winter S, *et al*. "Transverse chromatic aberration across the visual field of the human eye." *J Vis*. 2016;16(14):9.
3. Tong MQ, *et al*. "OCT intensity and phase fluctuations correlated with activity-dependent neuronal calcium dynamics in the Drosophila CNS [Invited]." *Biomedical Optics Express*, 2017;8(2):726-735.

4. Cordeiro C, *et al.* "Optophysiology of cardiomyocytes: characterizing cellular motion with quantitative phase imaging." *Biomedical Optics Express*, 2017;8(10):4652-4662.

5. Domdei N, *et al.* "Ultra-high contrast retinal display system for single photoreceptor psychophysics." *Biomedical Optics Express*, 2018;9(1):157-172.

6. Goetz G, *et al.* "Interferometric mapping of material properties using thermal perturbation." *PNAS*, 2018;115(11):E2499-E2508.

7. Mozaffari S, *et al.* "Versatile multi-detector scheme for adaptive optics scanning laser ophthalmoscopy." *Biomedical Optics Express*, 2018;9(11):5477-5488.

8. Ling T, *et al.* "Full-field interferometric imaging of propagating action potentials." *Light: Science & Applications*, 2018;7:107.

Accelerating vision restoration with in vivo cellular imaging of retinal function (EY025497)

Principal investigator: David Williams, Ph.D., University of Rochester Center for Visual Science
NEI Program official: Thomas Greenwell, Ph.D.

Williams and colleagues are designing an optical system to image responses to light of large numbers of individual cells in the retina. The system uses two main components: 1) a fluorescent marker that can detect cellular calcium fluxes indicative of nerve cell firing, and 2) a two-photon microscopy, which uses infrared light to detect fluorescent signals without damaging living tissue. The team plans to test their system in collaboration with investigators who are exploring three different approaches to vision restoration: 1) genetically re-engineering cells other than photoreceptors to respond to light, 2) replacing lost photoreceptors using stem cells, and 3) preserving photoreceptors with gene therapy.

Progress

Williams' team has developed a high-resolution ophthalmoscope with adaptive optics that resolves individual retinal cells and uses a fluorescence marker called CGaMP6 that, when expressed in cells, measures electrical activity. Using this system, they have obtained stable optical recordings from macaque eyes for a month or longer in a photoreceptor-specific cell-damage model that they developed. The model delivers femtosecond pulses guided with adaptive optics to ablate photoreceptors in small patches without damaging other retinal cells.

This model system, combined with their newly designed ophthalmoscope, has enabled the team to test two approaches to vision restoration:

1. **Optogenetics.** With help from the University of Rochester's William Merrigan, Williams and colleagues used gene therapy to produce light sensitivity in macaque RGCs. The RGCs normally respond to signals from photoreceptors, providing the conduit to the brain. They do not respond directly to light. But using an approach called optogenetics, in which light-sensitive ChrimsonR was incorporated into cells, the team restored vision.

2. **Stem cell therapy.** In collaboration with David Gamm, M.D., Ph.D., and his colleagues at the University of Wisconsin, Williams' team developed a fluorescence adaptive optics imaging system for tracking the fate of photoreceptor precursor cells that were injected subretinally into monkey eyes. With this system, they tracked single cells for many months and witnessed what could have been the first stages of neural integration into the retina, marked by the sprouting of neuronal processes.

Finally, in collaboration with Connie Cepko, Ph.D., and her colleagues at Harvard University, Williams' team has developed a way to use novel intracellular sensors to measure fluorescence lifetimes. These sensors, delivered with viral vectors, can characterize the metabolic pathways inside single cells in the living eye. The researchers have used the technique to monitor NADH metabolism with a sensor called Peredox, which can provide important information about the general metabolic state of different types of cells. They are working on a similar approach with a sensor called Sweetie, which is designed to monitor glucose. Use of Sweetie could help test the glucose hypothesis, which proposes that retinal degeneration of cone photoreceptors results from cones losing their ability to uptake adequate amounts of glucose following the death of adjacent rods.

Publications

1. Walters S, *et al.* "Cellular-scale evaluation of induced photoreceptor degeneration in the living primate eye." *Biomedical Optics Express*, 2019;10(1):66-82.

2. Feeks JA, Hunter JJ. "Adaptive optics two-photon excited fluorescence lifetime imaging ophthalmoscopy of exogenous fluorophores in mice." *Biomedical Optics Express*, 2017;8(5):2483-2495.

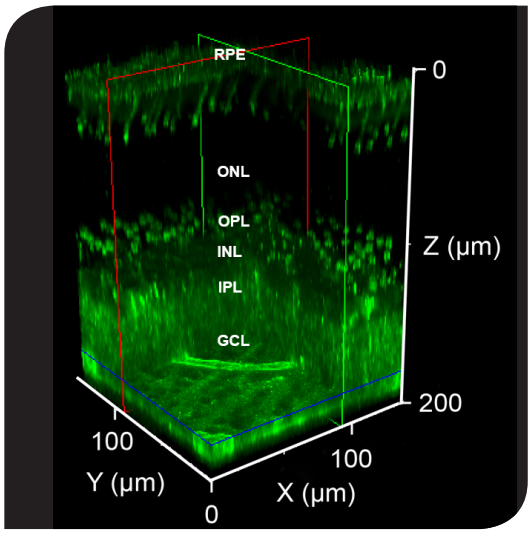
3. Schwarz C, *et al.* "Selective S cone damage and retinal remodeling following intense ultrashort pulse laser exposures in the near-infrared." *Investigative Ophthalmology & Visual Science*, 2018;59(15):5973-5984.

A two-photon ophthalmoscope for human retinal imaging and functional testing (EY025451)

Principal investigator: Krzysztof Palczewski, Ph.D., University of California Irvine
NEI program official: Lisa Neuhold, Ph.D.

Palczewski and colleagues are pursuing tools to visually monitor vitamin A derivatives in the retina. Vitamin A derivatives help power the light-sensitive machinery inside photoreceptors. Many inherited diseases of the retina involve mutations that affect the retina's ability to utilize or recycle vitamin A.

Palczewski's team is developing a two-photon microscope capable of measuring the metabolism and distribution of vitamin A derivatives within photoreceptors. Two-photon microscopy is a technique that enables imaging of living tissue as thick as one millimeter. The principal application of the technology will be establishing baseline disease characteristics and monitoring disease progression and response to future regenerative therapies



3D reconstruction of mouse retina from two-photon excitation fluorescence imaging. Labeled retinal structures are the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and retinal pigment epithelium (RPE).

Progress

Thus far, Palczewski and colleagues have developed a technique to noninvasively visualize metabolic activity in retinal cells using a novel two-photon microscopy technique that excites retinal fluorophores, which are substances that fluoresce when stimulated. They have also developed methods for measuring retinal cell responses to two-photon stimulation using transretinal electroretinography, a technique that measures the electrical responses of cells. They have established that macaques and mice that are modified to lack the GNAT1 gene—the loss of which has been linked to congenital stationary night blindness in humans—have photoreceptors that respond to pulsing infrared light and initiate perception of infrared as visible light. The team has optimized a system for image-

guided direct tests in humans and has demonstrated that two-photon stimulation improves the reproducibility of functional tests with pulsed infrared light, as compared with visible light. Infrared visual sensitivity thresholds also were less influenced by lens opacities, as compared with visible light.

Finally, the team has improved noninvasive imaging of the retina by introducing a laser that delivers 20-femtosecond pulses. The team devised a system that modulates the phases of the individual spectral components of infrared light to compress pulses, which are strongly influenced by optical elements and tissue. The modification resulted in a greater than three-fold increase in two-photon fluorescence of endogenous retinal fluorophores, as compared with a state-of-the art 75-femtosecond pulsing laser at the same average power and wavelength, with no detectable impact on retinal structure.

The team expects further improvements by reducing pulse repetition frequency. In parallel, team members are working on high-sensitivity/low-noise detectors with active cooling, delineation of the subcellular distribution of retinal fluorophores via hyperspectral imaging and fluorescence lifetime imaging, and software algorithms for feature segmentation, image registration, and alignment. These advances will enable examination of biochemical processes in the RPE, photoreceptors, and other retinal neurons. The shorter pulse duration and lower pulse repetition frequency will help to image vitamin A metabolites including fluorescent condensation products such as A2E that form from vitamin A derivatives during normal aging. Such condensation products are biomarkers for potentially toxic compounds that can form during macular degeneration.

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Imaging optic nerve function and pathology (EY025500)

Principal investigator: Sheng-Kwei Song, Ph.D, Washington University
NEI program official: Cheri Wiggs, Ph.D.

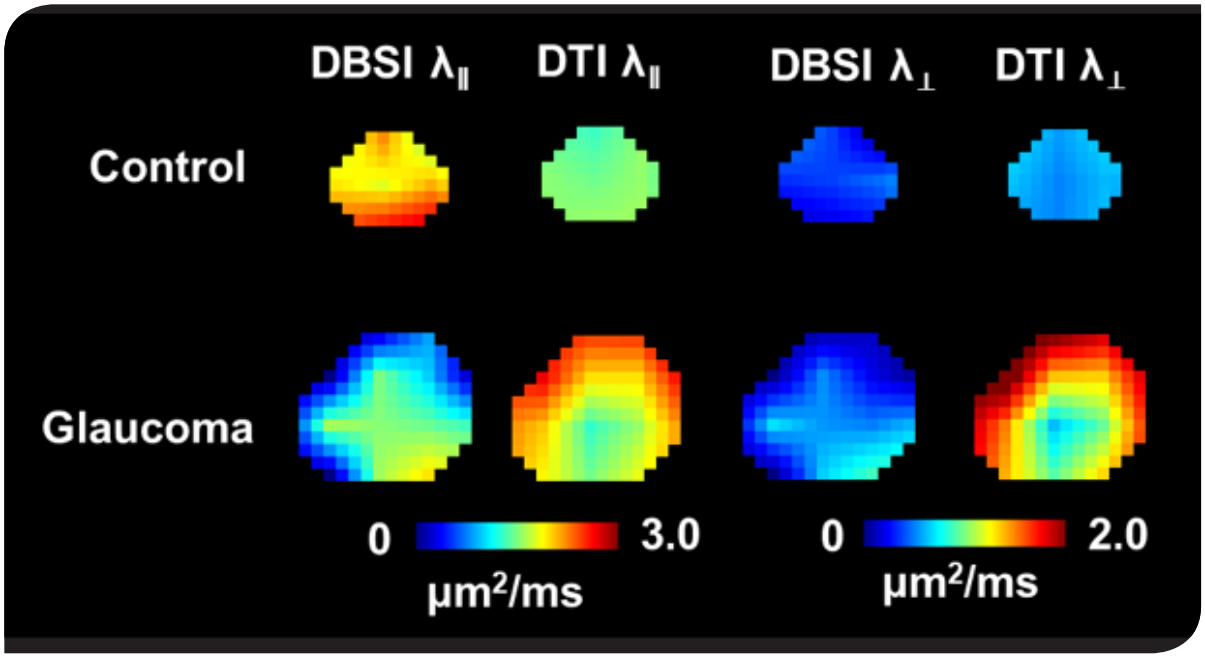
Although the optic nerve originates in the retina, most of it resides within the brain, out of reach of most devices used to see into the eye. Song is adapting two technologies to noninvasively visualize optic nerve anatomy, function, and pathology: diffusion basis spectrum imaging (DBSI) and diffusion functional magnetic resonance imaging

(diffusion fMRI). DBSI is a data-driven multi-tensor model for analyzing diffusion-weighted MRI signals. It detects, distinguishes, and quantifies coexisting axonal injury, demyelination, and inflammation of the optic nerve. Diffusion fMRI images optic nerve responses to visual stimulation through the detection of changes in radial diffusivity (apparent diffusion coefficient perpendicular to axonal tracts). Optic nerve damage, a consequence of glaucoma and other optic neuropathies, is currently irreversible. A system that incorporates DBSI and diffusion fMRI could be used to noninvasively monitor the optic nerve's response to new therapies aimed at regeneration.

Progress

As proof of principle, Song's team has characterized the mechanisms responsible for MRI-based signal changes in myelinated axonal fibers of perfused bullfrog sciatic nerves. Their research has revealed that diffusion fMRI responses are linearly proportional to electrical impulse number, based on simultaneous recordings of compound action potentials (CAPs). Microstructural changes and osmotically driven redistribution of tissue water play a crucial role in the observed diffusion fMRI response in myelinated fibers. The team has demonstrated that DBSI-derived axonal volume can be used to assess loss of axons resulting from optic nerve swelling. In a recent modification of DBSI modeling to separate water signals between inter- and intra-axonal compartments, Song demonstrated a 10% intra-axonal volume increase during electrical stimulation in perfused bullfrog nerves, suggesting multiple DBSI metrics can be used to interrogate optic nerve changes in response to functional activations.

Song and colleagues have developed a preliminary DBSI protocol to image optic nerve function and pathology in patients with glaucoma and optic neuritis. In a small cohort of four patients with glaucoma and seven control volunteers, the team observed a decrease in optic nerve DBSI-derived axial diffusivity (suggesting the presence of axonal injury), less significant increase in DBSI-derived radial diffusivity (mild myelin damage), increased nonrestricted diffusion fraction (reflecting edema), and increased nerve volume (optic nerve swelling) in optic nerves from patients with glaucoma. Among the patients, DBSI detected axonal loss in the presence of optic nerve swelling, consistent with detection of axonal loss with optic nerve swelling in a mouse model of optic neuritis. DBSI-detected axonal injury linearly correlated with OCT-assessed retinal nerve fiber layer thickness. In two preliminary functional DBSI measurements in patients with multiple sclerosis and unilateral optic neuritis, Song detected increased optic nerve response (the extent of decreased DBSI-derived radial diffusivity) in the affected eye. These results support DBSI as suitable for assessing optic nerve function and pathology in patients with glaucoma and optic neuritis.



Imaging of human optic nerve, with and without glaucoma, using various MRI techniques under development by Sheng-Kwei Song and colleagues.

Publications

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Platform technologies for microscopic retinal imaging: development and translation (EY025477)

Principal investigator: Alfredo Dubra, Ph.D., Stanford University
NEI program official: Grace Shen, Ph.D.

With collaborators at several research institutions, Dubra is developing a suite of core technologies to advance and increase the usability of next-generation retinal cameras. The suite will include real-time eye motion stabilization, image resolution doubling, a tunable lens to improve the focusing of all colors of light, and high-throughput methods for testing the function of individual cells. The technology will be applied to noninvasive imaging techniques to compensate for involuntary eye movements, both subtle and extreme cases involving nystagmus.

Progress

Dubra has demonstrated that pupil and retinal position can be calculated simultaneously. Key to this advance is faster processing of images as they are downloaded from the camera. The team's eye tracking system works in a broad spectrum of human eye types, even when the pupil is partially obstructed by the eyelid or eyelashes. The system will function as an add-on to any ophthalmoscope and is designed to work with current and future ophthalmoscopes with or without adaptive optics.

The team has designed two add-on reflective devices for rotating the imaging raster in scanning ophthalmoscopes. Image pairs captured at different orientations can then be combined to remove residual distortion due to eye motion, enabling precise monitoring of structural changes occurring in the retina.

In addition, the team has developed a new adaptive optics scanning light ophthalmoscope specifically designed for rodent imaging. This system enables visualization of the photoreceptor mosaic in near-infrared reflectance confocal imaging with unprecedented detail.

Lastly, the team has developed a novel technique to monitor the physiology of individual cones. The technique measures changes in photoreceptor infrared reflectance in response to visible light stimulation, which they have demonstrated to be a biomarker of cone function.

Publications

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Functional Imaging External Scientific Oversight Committee

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Regenerative Factor Discovery Consortium

NEI issued the second AGI funding opportunity, "Discovery-based science to identify factors influencing neural regeneration in the visual system," (RFA-EY-15-002) in August 2015. The announcement solicited proposals for projects to pursue biological factors that affect the restoration of functional connections within the retina and between the eye and brain. The first two AGI workshops identified the need for regenerative factors as a research priority. This funding opportunity encouraged high-throughput screening, comparative biology, and the development of new animal models that more closely resemble the human visual system. The AGI Regenerative Factor Discovery Consortium comprises six projects, initially funded for $12.4 million for three years.

The funded teams employed a variety of approaches to screen, identify, and validate targets that may prove useful in efforts to restore function to the visual system. All of these projects will eventually contribute to a publicly available data repository that will enable additional researchers to explore related factors and pathways in other animal and disease models.

Table 3. Goals of Regenerative Factor Discovery Projects

Funding Objectives	Projects					
	Molecular Discovery for Optic Nerve Regeneration (Goldberg)	Screening for Molecules that Promote Photoreceptor Synaptogenesis (Zack)	Evaluation of Novel Targets for Retinal Ganglion Cell Axon Regeneration (Strittmatter)	Novel Activators of Regeneration in Muller glia (Levine)	Comparative Transcriptomic and Epigenomic Analyses of Muller Glia Reprogramming (Hyde)	Novel Targets to Promote RGC Axon Regeneration: Insights from Unique RGC Cohorts (Park)
Identify regenerative factors	x	x	x	x	x	x
Identify axon guidance cues	x	x	x			x
Employ high-content/ high-throughput screening	x	x			x	x
Compare factors across species				x	x	
Test factors in human-like models						

Regenerative Factor Discovery Projects

Three groups are identifying molecules associated with optic nerve regeneration. Their collective efforts are providing better understanding of how proteins, genes, and lipids respond after optic nerve damage. Two other teams are researching how cells called Müller glia regenerate retinal neurons in zebrafish and other species, with the hope of unlocking the same regenerative capacity in humans. The sixth team is exploring strategies to integrate transplanted photoreceptors. All projects aim to provide knowledge in support of efforts toward new regenerative therapies.

Table 4. Regenerative factor discovery projects at a glance

Lead PI	Species	Subject	Method	Omic datasets
Jeffrey Goldberg, Stanford U.	Mouse	Optic-nerve-crush-RGCs that regenerate vs. RGCs that don't regenerate vs. uninjured RGCs	Bulk and single cell transcriptome Proteomics, mass spectrometry, newly synthesized proteins	Genes expressed in RGCs associated with regeneration Transportome – proteins connecting RGCs to LGN, SC*; proteins upregulated upon injury
Donald Zack, Johns Hopkins U.	Human	Pluripotent retinal organoids; photoreceptor progenitor reporter	Small molecule screen >5,000	Molecules that stimulate photoreceptors to create neurites
Stephen Strittmatter, Yale U.	Cedars-Sinai	Optic nerve crush	shRNA screen AAV2 delivery into eye; CRISPR screens in mouse to test combinations	shRNAs/genes that increase or decrease optic nerve regeneration
Edward Levine, Vanderbilt U.	Zebrafish Mouse	Uninjured eyes; genetic models of glial activation	Inject extracellular vesicles (EVs) from different cell sources (e.g., C6 glioma)	Identification of EVs that stimulate regeneration; EV proteomics; EV small RNA-seq

Lead PI	Species	Subject	Method	Omic datasets
David Hyde, U. of Notre Dame	Mouse Chick Zebrafish	Light damage (photoreceptor damage), NMDA (inner retina damage), growth factors (no retina damage, but Müller glia activation/ proliferation)	Assess gene expression (bulk RNA-seq, single cell RNA-seq [scRNA], and ATAC-seq*) and biological changes (proliferation) in Müller glia at stages when regeneration can/cannot occur	Bulk RNA-seq, scRNA-seq, and ATAC-seq of Müller glia either changing into neural progenitor cells to regenerate retinal neurons or undergoing gliosis
Kevin Park, University of Miami	Mouse	Optic nerve crush; OPN+RGCs* can regenerate, HB9+RGCs can't regenerate	In vitro screen for neurite-promoting factors; transcriptome of the separate labeled RGC populations; lipids that change after injury	Transcriptome associated with RGC regeneration; lipidomic changes associated with injury

* LGN=lateral geniculate nucleus. SC= superior colliculus. ATAC=assay for transposase-accessible chromatin. OPN= osteopontin. RGC= retina ganglion cell. HB9= homeobox 9. N-Methyl-D-aspartic acid. scRNA-Seq= single cell RNA Sequencing.

Molecular discovery for optic nerve regeneration (EY027261)

Principal investigators: Jeffrey Goldberg, M.D., Ph.D., Andrew Huberman, Ph.D., Stanford University; Larry Benowitz, Ph.D., Harvard University; Hollis Cline, Ph.D., Scripps Research Institute
NEI program official: Neeraj Agarwal, Ph.D.

Goldberg and colleagues are identifying genes and proteins that help and hinder the ability of RGCs to regenerate axons. RGC axons are fibers that coalesce at the optic nerve and transmit visual information from the eye to the brain. The team's prior work showed that mice with optic nerve injury can successfully regrow axons under specific conditions. In their AGI project, Dr. Goldberg's team aims to grow functional RGC axons toward specific targets. They will investigate promising regenerative candidate molecules in long-term animal studies designed to assess their therapeutic effect on vision.

Progress

Goldberg and colleagues have optimized a method to identify proteins that are transported into the optic nerve before and after optic nerve damage. They have also developed a method to purify RGCs and measure their gene expression in the days following optic nerve damage and/or treatment. Using data from these methods, they have found new genes or proteins associated with degeneration or regeneration. They have validated the importance of three of these genes in RGC death and axon regeneration and have manipulated two with gene therapy to promote RGC axon regeneration.

Publications

None to date.

Screening for molecules that promote photoreceptor synaptogenesis (EY027266)

Principal investigators: Donald Zack, M.D., Ph.D., Johns Hopkins University; David Gamm, M.D., Ph.D., University of Wisconsin, Madison
NEI program official: Thomas Greenwell, Ph.D.

Using induced pluripotent stem cells, Zack, Gamm, and their teams are searching for factors that coax retinal precursor cells into mature and integrated photoreceptors. They are generating a list of small molecules and candidate genes that guide photoreceptor cells to target bipolar cells, which are intermediaries between photoreceptors and RGCs. Re-establishing these connections is essential to restoring vision via photoreceptor transplantation.

Progress

The team has identified molecules that affect neurite outgrowth in photoreceptors. Neurites are cellular appendages that are crucial to the formation of synapses between photoreceptors and other retinal cells. The team worked with cells dissociated from retinal organoids using a technique that Gamm pioneered. Organoids are cultured, organ-like structures made from stem cells. Gamm's technique makes organoids from a human embryonic stem cell (hESC) line that was genetically engineered to differentially express visible compounds when mature. The technique enables the scientists to distinguish rod and cone photoreceptors in real time. The hESC-derived photoreceptors display characteristics of authentic human photoreceptors.

The team established procedures to scale up and ship the organoids from Gamm's lab in Madison, Wisconsin, to Zack's lab in Baltimore, Maryland. Safe and efficient transport of high numbers of human retinal organoids will be useful for future studies worldwide. With photoreceptors from the organoids, Zack's team used a high-throughput assay to evaluate 5,000 compounds for their ability to stimulate neurite outgrowth. They are currently studying these compounds to understand their mode of action and to assess their therapeutic potential.

Gamm's team has shown that photoreceptors derived from organoids elongate their axons in an unconventional way: They appear to fix their axon terminal and then pull the axon via migration of the photoreceptor cell body. The team has also shown that neighboring cells pull photoreceptors, so that neurite elongation is non-cell autonomous, meaning that this process requires interactions among cells. This mechanism of axonogenesis starkly differs from the mechanism used for long projection neurons such as RGCs.

Additionally, Gamm's team is "tracing" connections between neurons to study how stem cell-derived photoreceptors and other cell types communicate with each other after dissociation and re-association.

Publication

1. Phillips MJ, *et al*. "A novel approach to single cell RNA-sequence analysis facilitates *in silico* gene reporting of human pluripotent stem cell-derived retinal cell types." *Stem Cells*, 2018;36(3):313-324.

Evaluation of novel targets for retinal ganglion cell axon regeneration (EY027256)

Principal investigator: Stephen Strittmatter, M.D., Ph.D., Yale University
NEI program official: Neeraj Agarwal, Ph.D.

Strittmatter and his team are searching for genes that contribute to the regeneration of axons from RGCs. Starting with 450 candidate genes culled from more than 17,000, they will test each candidate in a mouse optic nerve injury model to see if any act as mediators of regeneration. Positive genes will then be validated by looking to see if they are also active in the C. elegans worm, an indication that a gene's function is preserved across species. The strongest gene candidates will then be analyzed in greater detail to understand their molecular action better.

Progress

This team is testing each of these 450 candidate genes by genetic suppression in the eye, followed by nerve injury and measurement of regeneration. They have completed in vivo testing of more than 75% of these candidate genes and observed more than 20 genes with previously unknown roles in axon regeneration. They are now completing tests of the entire list and combining gene suppression to assess synergistic action.

Publications

1. Sekine Y, *et al.* "Functional genome-wide screen identifies pathways restricting central nervous system axonal regeneration." *Cell Reports*, 2018;23(2):415-428.
2. Sekine Y, *et al.* "PlexinA2 and CRMP2 signaling complex is activated by Nogo-A-liganded NgR1 to restrict corticospinal axon sprouting after trauma." *Journal of Neuroscience*, 2019.
3. Huebner EA, *et al.* "Diltiazem promotes regenerative axon growth." *Molecular Neurobiology*, 2018.
4. Fink KL, *et al.* "Identification of intrinsic axon growth modulators for intact CNS neurons after injury." *Cell Reports*, 2017;18(11):2687-2701.
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Novel activators of regeneration in Müller glia (EY027265)

Principal investigators: Edward Levine, Ph.D., James Patton, Ph.D., and David Calkins, Ph.D., Vanderbilt University
NEI program official: Thomas Greenwell, Ph.D.

Levine and colleagues are investigating exogenous and endogenous factors—that is, factors with an external or internal origin—that contribute to the reprogramming of support cells in the retina called Müller glia. In zebrafish, Müller glia can give rise to photoreceptor cells after injury to the retina. The investigators are first testing a novel combination of pharmacological agents and genetic manipulation for the ability to reprogram Müller glia in mice. If the therapy is successful, the team will then study conditions that support regeneration by determining which genes are turned on or off in regenerating zebrafish and mouse Müller glia. A second component of their project will look at the regenerative role of extracellular vesicles (EVs), which are tiny cell-secreted blebs commonly found in blood and other bodily fluids.

Progress

The team has screened more than 40 distinct EV preparations in zebrafish and more than 20 in mice. In zebrafish, they found 13 different exosome preparations that stimulate Müller glia proliferation, with exosomes from a rat glioma cell line (C6) being the most consistent. The team has completed proteomic and RNA sequencing analyses for highly purified vesicles from C6 cells. Bioinformatic analysis of the RNA sequencing data sets is underway. For proteomics, the most enriched EV protein is a relatively uncharacterized member of the epidermal growth factor family that is thought to be involved in cell-cell interactions via the extracellular matrix. The team is now in the process of determining the role of this protein during induction of regeneration. Team members are also generating C6 lines expressing known regenerative factors with the goal of generating designer EVs for intravitreal injection and induction of regeneration.

Many of the exosome preparations tested in mice promoted proliferation. Cell type identification is ongoing, and the team has identified effects in limited numbers of Müller glia, but more often the proliferating cells are of non-retinal origin. This is not unexpected since the EV injection itself can produce a local injury, and vesicle uptake is not necessarily cell type-specific. To provide additional context for assessing regeneration, the team is testing EVs in animals with retinal injury caused by laser lesioning and in combination with a genetic model in which Müller glia are induced to proliferate.

In addition to proliferation, the team is examining reactivity, a general response of glia to retinal injury, disease, and degeneration. Their hypothesis, based on research in the retina, brain, and spinal cord, is that reactivity hinders the ability of mammalian Müller glia to take a regenerative path. Thus far, most EV preparations promote reactivity, but one induces minimal reactivity, and recent results suggest it suppresses reactivity. In addition to being relevant for promoting regeneration, a means to suppress reactivity could be beneficial for managing many types of retinal pathologies. Further characterization of both EV-treated retinas and the reactivity-suppressing EV preparation is ongoing with EV transcriptomic and proteomic profiling to be completed in the second half of 2019.

Publications

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2. Webster MK, *et al*. "Stimulation of retinal pigment epithelium with an alpha7 nAChR agonist leads to Müller glia dependent neurogenesis in the adult mammalian retina." *Investigative Ophthalmology & Visual Science*, 2019;60(2): 570-579.

Comparative transcriptomic and epigenomic analyses of Müller glia reprogramming (EY027267)

Principal investigators: David Hyde, Ph.D., University of Notre Dame; John Ash, Ph.D., University of Florida; Andy Fischer, Ph.D., The Ohio State University; Seth Blackshaw, Ph.D., and Jiang Qian, Ph.D., Johns Hopkins University
NEI program official: Thomas Greenwell, Ph.D.

In zebrafish and chicks, retinal damage induces Müller glia to reprogram and re-enter the cell cycle, producing neuronal progenitor cells. These progenitors are capable of moving to damaged retinal tissue and turning into missing neuronal cell types. Though Müller glia can initiate a regenerative response in damaged zebrafish and chick retinas, mammalian Müller glia cannot, thus preventing retinal regeneration and restoration of vision in humans. Hyde and his colleagues are comparing the capacity of Müller glia cells from zebrafish, chicks, and mice to perform this type of reprogramming. From the Müller glia of each animal, they will determine what gene activity is upregulated or downregulated (transcriptomics)—as well as look for modifications to the genomic DNA (epigenomics)—during retinal development and in response to different forms of retinal damage. These types of cross-species comparisons are designed to detect differences in gene expression, as well as to identify potential regulators that control Müller glia reprogramming. The team's work will shed light on why some species possess the ability to regenerate their damaged retinas but humans do not.

Progress

Hyde's team has damaged zebrafish, chick, and mouse retinas with either light or the drug NMDA, waited various lengths of time for Müller glia to respond, and then separately purified Müller glia and remaining retinal neurons with fluorescence-activated cell sorting (FACS). They performed bulk RNA-seq from each sample to identify all the genes that were expressed in the Müller glia and neuronal fractions and assay for transposase-accessible chromatin using sequencing (ATAC-seq) to identify chromosomal regions that were open or closed for gene expression. The team has analyzed 267 samples by bulk RNA-seq and 78 samples by ATAC-seq.

Additionally, the team has explored how individual Müller glia and neurons change gene expression relative to every other cell in a single retina. For this, they used single-cell RNA-seq to analyze 84 samples from developing retinas, undamaged retinas, and damaged retinas. Using this approach, they determined gene expression profiles from more than 316,000 individual retinal cells. They are currently using software to analyze data from 1) different retinal treatments within the same organism to identify

similarities and differences within an organism to different types of damage, 2) the same treatment between different organisms to identify how different organisms respond to the same damage, 3) the relationship between changes in the DNA that correlate to changes in gene expression within and between organisms, and 4) similarities and differences between developing and damaged retinas to determine how similar or different gene expression is between the de-differentiated Müller glia and the progenitor cells that form the retina.

The team is generating data to help explain why the zebrafish retina can spontaneously regenerate and the mammalian retina cannot. They are identifying genes essential for Müller glia-dependent regeneration in zebrafish and chicks that are not expressed in mice, and genes that may block the regenerative ability of Müller glia in mice that are not expressed (or expressed differently) in zebrafish. Team members are currently testing the roles of these candidate regeneration regulatory genes in all three organisms. They are then using small molecules and viruses to modify gene expression and corresponding protein activity. They have identified genes that are essential for regeneration in zebrafish and some that allow the mouse retina to be more "ready" to reenter a regeneration process (although they fail to fully enable the mouse retina to regenerate).

Hyde and colleagues have reported several findings. First, reprogrammed zebrafish Müller glia and developing retinal progenitors are nearly identical at the gene expression level as the retinal progenitor cells that are involved in retinal development. This suggests specific transcriptional regulatory pathways that are employed during retinal regeneration. Second, using both bulk and scRNA-seq, along with ATAC-seq analysis, they have identified both evolutionarily conserved and species-specific transcriptomic and epigenomic events that occur in Müller glia following retinal injury. Integrating these data sets revealed gene regulatory networks that are strong candidates for both promoting and restricting neurogenic and proliferative competence in Müller glia. Third, Müller glia in the damaged zebrafish and chick retinas progress through a transient gliotic state before advancing to the reprogrammed/neurogenic state. This defines gliosis as a critical point in regeneration, where mammalian Müller glia either lack activators or possess repressors that prevent them from progressing through to a regeneration response.

Publications

1. Clark BS, *et al.* "Single-cell RNA-Seq analysis of retinal development identifies NFI factors as regulators of mitotic exit and specification of late-born cells." *Neuron*, 2019 Jun 19;102(6):1111-1126.e5.

2. Stein-O'Brien GL, *et al.* "Decomposing cell identity for transfer learning across platforms, tissues and species." *Cell Systems*, 2019 May 22;8(5):395-411.e8.

3. Campbell WA, *et al.* "Matrix-metalloproteinase expression and gelatinase activity in the avian retina and their influence on Müller glia proliferation." *Experimental Neurology*, 2019 Jun 25;320:112984.

4. Todd L, et al. "Reactive microglia and IL1β/IL-1R1-signaling mediate neuroprotection in excitotoxin-damaged mouse retina." *Journal of Neuroinflammation*, 2019 Jun 6;16(1):118.

Novel targets to promote RGC axon regeneration: Insights from unique retinal ganglion cell cohorts (EY027257)

Principal investigators: Kevin Park, Ph.D., Vance Lemmon, Ph.D., Sanjoy Bhattacharya, Ph.D., University of Miami
NEI program official: Neeraj Agarwal, Ph.D.

Dr. Park and Dr. Lemmon are using RNA sequencing in cultured mouse RGCs to identify differences in the expression of genes in regenerative versus nonregenerative RGCs. In parallel, Dr. Park and Dr. Bhattacharya will use mass spectrometry to determine what lipids (or fat molecules) may give subclasses of RGCs more robust regenerative capacities. The researchers will then perform a set of experiments aimed at understanding the function of the genes found to be involved in regeneration. The most promising gene candidates will be used as a therapy aimed at regenerating the optic nerve in a mouse model with optic nerve injury.

Progress

Park and colleagues have identified approximately 20 genes that affect neurite outgrowth of RGCs. They have compared expression of more than 400 genes in regeneration-competent versus incompetent RGCs to identify genes that endow regenerative capacity. They identified genes that alter neurite length by increasing or knocking down expression during embryonic development using DNA plasmid transfection and small-interfering RNAs. Their subsequent analyses found that knock-

down of Thbs1, Cd86, and Nptx2 causes a reduction in total neurite length, and overexpression of different genes promote neurite lengthening. Their more stringent analysis identified 11 genes that upregulate in regeneration-competent RGCs in response to axonal injury.

The team is now assessing the genes in vivo for their ability to regulate RGC axon regeneration. In doing so, they have discovered that manipulation of distinct extracellular matrix (ECM) molecules and ECM-binding proteins that are highly expressed in the regeneration-competent RGCs—including Thbs1—promote axon regeneration. The results of these studies have been submitted for publication.

The team has also identified 65 lipids (sphingolipids + ceramides) from analyses of wild type and Thy1-CFP mouse RGCs, finding lipid signatures in regenerative RGCs. They have also found growth-promoting effects of distinct sphingolipids when injected intravitreally.

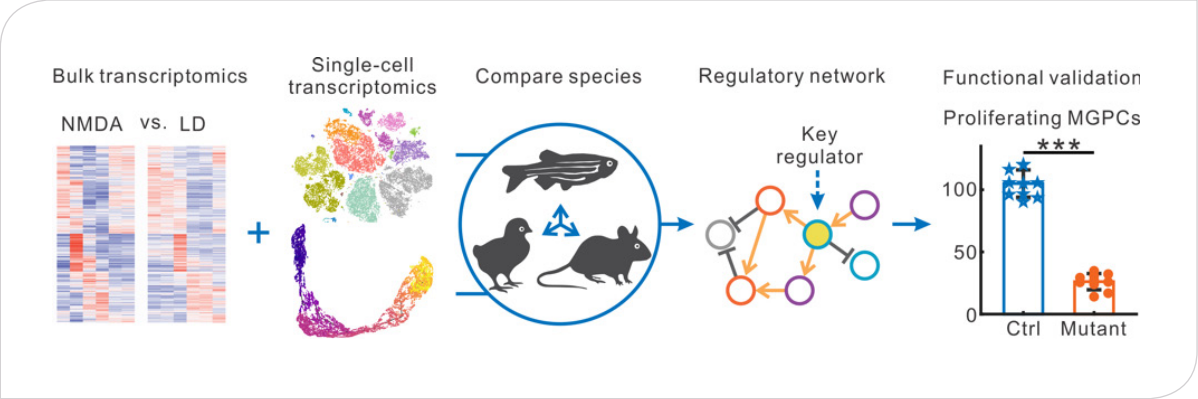


Diagram for the Hyde team's project workflow.

Publications

1. Bray ER, *et al.* "3-D visualization of individual regenerating retinal ganglion cell axons reveals surprisingly complex growth paths." *eNeuro*, 2017;4(4).

2. Luo X, *et al.* "Enhanced transcriptional activity and mitochondrial localization of STAT3 co-induce axon regrowth in the adult central nervous system." *Cell Reports*, 2016;15(2):398-410.

3. Trzeciecka A, *et al.* "Lipid profiling dataset of the Wnt3a-induced optic nerve regeneration." *Data in Brief*, Available online 24 May 2019.

4. Bray ER, *et al.* "Thrombospondin-1 mediates axon regeneration in retinal ganglion cells." *Neuron*, 2019 Jun 18. pii: S0896-6273(19)30497-0.

Regenerative Factor Discovery Consortium External Scientific Oversight Committee

Sally Temple, Ph.D., Neural Stem Cell Institute
Mark Tuszynski, M.D., Ph.D., University of California San Diego
Michael Dyer, Ph.D., St. Jude's Children's Research Hospital
Steve Finkbeiner, M.D., Ph.D., Gladstone Institute of Neurological Disease

Translation-Enabling Models Consortium

The AGI issued its third funding announcement in January 2018: "Translation-enabling models to evaluate survival and integration of regenerated neurons in the visual system" (RFA-EY-17-003).

In October 2018, the AGI funded five projects for a total of $6 million per year for five years to aid development of animal models of human eye disease for testing regenerative therapies.

Scientists use disease models throughout the development of new treatments. From cell or animal models of eye diseases, researchers can learn the root cause of disease, study the changes that occur to eye tissues as disease progresses, and test potential therapies. New and emerging treatments, like gene therapy or stem cell-based tissue replacement, also require novel surgical techniques and methods to measure efficacy, all of which must be tested before trying in humans. Having models that closely match human biology and disease will help vision scientists create and test new methods to preserve and restore sight. Key are models that mimic important aspects of human physiology, including similar light-sensing cells, pathways for connecting the eye to the brain, and brain regions.

Kickoff meeting of the AGI Translation-Enabling Models Consortium, February 2019.

Table 5. Translation-enabling projects at a glance

Funding Objectives	Projects				
	Retinal disease models for translational photoreceptor replacement (Wolfe)	RGC replacement in optic neuropathies (Goldberg)	Novel model systems for the study of cone disorders and other heritable retinal diseases (Rogers)	Developing cone-dominant retinal disease models as a resource for translational vision research (Carroll)	RGC replacement in clinically relevant models of optic neuropathy (Rex)
Develop models emulating critical aspects of human blinding diseases that might be amenable to regenerative therapy	Dog	Squirrel monkey	Rhesus macaque	Cone-dominant models: 13-lined ground squirrel and tree shrew	Tree shrew

Funding Objectives	Projects				
	Retinal disease models for translational photoreceptor replacement (Wolfe)	RGC replacement in optic neuropathies (Goldberg)	Novel model systems for the study of cone disorders and other heritable retinal diseases (Rogers)	Developing cone-dominant retinal disease models as a resource for translational vision research (Carroll)	RGC replacement in clinically relevant models of optic neuropathy (Rex)
Treat vision loss in model by regenerating PRCs* and/or RGCs and their connections	Transplant labeled PRC precursor cells into inherited retinitis pigmentosa models	RGC* transplantation in induced-microbead model of glaucoma	PRC transplantation in animal with cone dystrophies	PRC transplantation in induced or rAAV-generated* human retinal diseases	RGC transplantation in glaucoma and traumatic optic neuropathy models
Use electrophysiology, functional imaging, behavioral measures, or other technology to demonstrate circuit integration and restoration of visual function	ERG,* multi-electrode array analysis, pupillometry, fMRI of visual cortical activity, and visually guided behavioral tests	AO imaging, ERG, and VEP* testing	Histology and immunohistochemistry	Functional imaging (two-photon calcium imaging) and ERG measurement	OCT, retinal imaging, calcium imaging, pERG,* multi-electrode array, histology and immunohistochemistry

* PRC=photoreceptor cell. rAAV= recombinant adeno-associated virus. ERG=electroretinogram. AO=adaptive optics. VEP=visual evoked potential. pERG=pattern electroretinography.

Translation-Enabling Models Projects

Retinal disease models for translational photoreceptor replacement (EY029890)

Principal investigators: John Wolfe, V.M.D., Ph.D., Children's Hospital of Philadelphia; William Beltran, D.V.M, Ph.D., University of Pennsylvania; David Gamm, M.D., Ph.D., University of Wisconsin
Program official: Thomas Greenwell, Ph.D.

Wolfe, Beltran, and Gamm and their colleagues have been using animal models to develop gene therapies for degenerative eye diseases, including retinitis pigmentosa. However, because gene therapies currently can only save regions of the retina that retain living cells, these treatments cannot restore function to damaged areas of the retina. In this new project, the team will be developing models to study how to implant replacement adult stem cell-derived light-sensing photoreceptor cells into these damaged retinal regions. The models will enable them to test surgical techniques, evaluate how well the replacement cells are working, and determine whether the treatment restores vision.

Retinal ganglion cell replacement in optic neuropathies (EY029903)

Principal investigators: Jeffrey Goldberg, M.D., Ph.D., Stanford University; David Calkins, Ph.D., Vanderbilt University; Thomas Reh, Ph.D., University of Washington; Donald Zack, M.D., Ph.D., Johns Hopkins University
Program official: Ellen Liberman, Ph.D.

Goldberg and colleagues study glaucoma, a condition in which progressive degeneration of the optic nerve threatens vision. The optic nerve, made up of RGC nerve fibers, conducts visual information from the retina to the brain. This team is generating a new animal model system to study how to place new RGCs into the eye and guide the cells' nerve fibers to appropriate regions of the brain. Success would constitute overcoming a major hurdle in the development of treatments for vision loss due to glaucoma and other optic neuropathies.

Novel model systems for the study of cone disorders and other heritable retinal diseases (EY029904)

Principal investigators: Jeffrey Rogers, Ph.D., Rui Chen, Ph.D., and John Stout, M.D., Baylor College of Medicine; Sara Thomasy, D.V.M, Ph.D., and Ala Moshiri, M.D., Ph.D., University of California Davis, California National Primate Research Center (CNPRC)
Program official: Lisa Neuhold, Ph.D.

Rogers and colleagues are exploring cases in which animals have naturally occurring ocular diseases. The team will use these animal models to help develop therapies for diseases that cause the loss of cone photoreceptors, which are cells in the retina that detect color. In humans, cones are concentrated in an area of the retina responsible for central vision called the macula. Few models of cone disorders exist because many of the animals most commonly used in research primarily have rod photoreceptors, which cannot detect color, and few cone photoreceptors. The investigators at the CNPRC have discovered several animals with naturally occurring visual impairment and cone dysfunction. The Baylor team has identified specific mutations in those impaired animals in genes like PDE6C, which in humans cause cone photoreceptor degeneration. The project will characterize retinal degeneration in these animals, explore ways to replace cone photoreceptors and restore visual function, and survey additional animals to identify other valuable naturally occurring disease models.

Developing cone-dominant retinal disease models as a resource for translational vision research (EY029891)

Principal investigators: Joseph Carroll, Ph.D., Medical College of Wisconsin; Jacque Duncan, M.D., University of California San Francisco; Deepak Lamba, Ph.D., University of California San Francisco; Dana Merriman, Ph.D., University of Wisconsin, Oshkosh; Jay Neitz, Ph.D., University of Washington
Program official: Lisa Neuhold, Ph.D.

Carroll and colleagues are pursuing new small animal models that will enable translational research in diseases that affect cone photoreceptors. Their team is working with two small mammals with high cone density: the thirteen-lined ground squirrel and the tree shrew. Key aims of the project are to use molecular and chemical tools to generate models of cone diseases that mimic those seen in humans and to evaluate stem cell-based treatments of these disease models. Further, the team seeks to develop imaging and functional assays to assess cone structure and function, both to validate the disease models and assess the treatment efficacy.

Retinal ganglion cell replacement in clinically relevant models of optic neuropathy (EY029893)

Principal investigators: Tonia Rex, Ph.D., Vanderbilt University; Petr Baranov, M.D., Ph.D., Harvard University; Brian Samuels, M.D., Ph.D., University of Alabama at Birmingham; Sunil Chauhan, D.V.M., Ph.D., Harvard University
Program official: Ellen Liberman, Ph.D.

Rex and colleagues are developing optic cup organoids, which are lab-grown tissues with the potential to regrow RGCs. Using tree shrews, the team will first characterize what happens after optic nerve trauma or glaucoma in these animals. They will then test how to transplant RGCs isolated from stem cell-derived organoids. The team hopes the model will yield a better understanding of how transplant-type therapies may help preserve and restore lost vision due to optic nerve damage.

Translation-Enabling Models Consortium External Scientific Oversight Committee

Dennis Clegg, Ph.D., University of California Santa Barbara
Donald Hood, Ph.D., Columbia University
Steven Schwartz, M.D., University of California Los Angeles
Valerie Wallace, Ph.D., University of Toronto

Other Funding

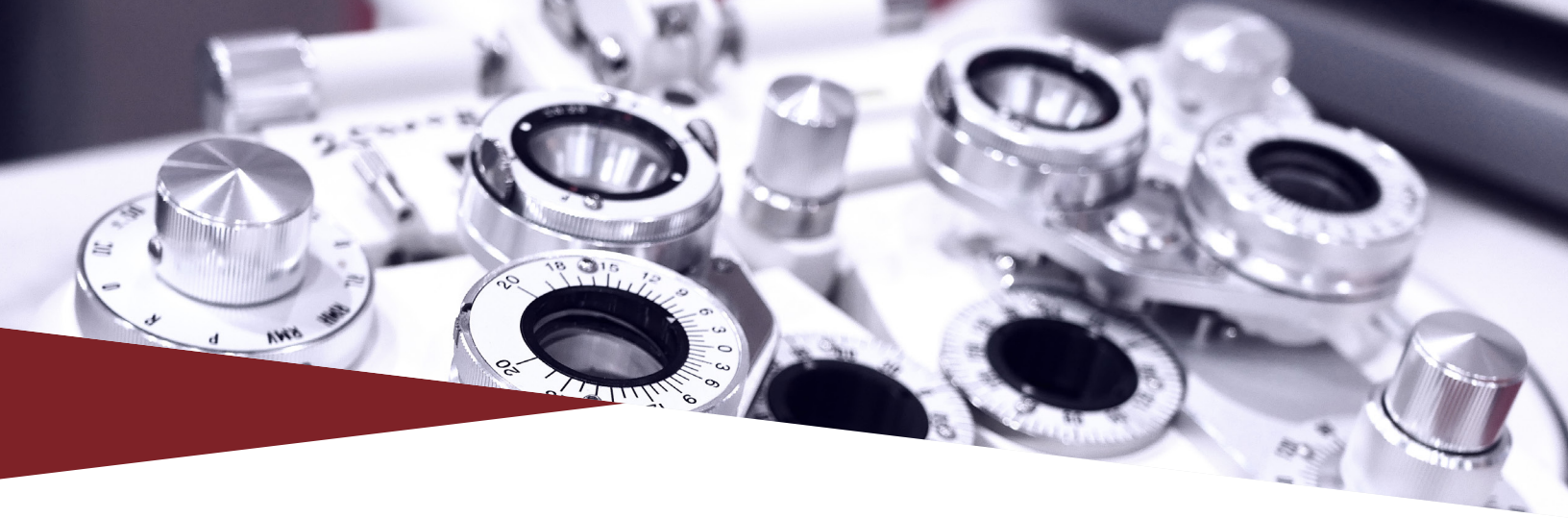
Preliminary Studies for Translation-Enabling Models

In December 2018, the AGI issued its fourth funding announcement: "Preliminary studies for translation-enabling models of the visual system" (RFA-EY-19-001). This funding announcement solicited applications for short-term, proof-of-principle research projects to provide preliminary data for a future funding opportunity aimed at the development of new models that emulate human visual system anatomy, physiology, and disease processes. NEI awarded funds for nine projects under this announcement in August 2019.

Table 6. Preliminary studies for translation-enabling projects at a glance

Lead PI Name	Project	Model
Deniz Dalkara, Fondation Voir et Entendre	Macaque models of photoreceptor degeneration compatible with stem cell transplantation	Cynomolgus monkey
Feng Lin, Cleveland Clinic Lerner COM-CWRU	Development of new models of AMD	Rhesus macaque

Lead PI Name	Project	Model
Glenn Yiu, U of California at Davis	Optogenetic Control of Oxidative Stress as a Model of Geographic Atrophy	Mouse & Rhesus macaque
Huaiya Hu, Upstate Medical U.	Role of Myosin VIIa In Usher Syndrome	Marmoset
Jason Meyer, Indiana U.-Purdue U. at Indianapolis	Targeting the diversity of retinal ganglion cells for replacement therapy	Rhesus macaque
Karl Wahlin, U of California, San Diego	A stem cell based optic nerve model for studies of axon guidance and regeneration	3D retinal organoids
Marina Gorbatyuk, U. of Alabama at Birmingham	Development and Validation of a Tree Shrew Model of Diabetic Retinopathy	Tree shrew
Martha Neuringer, Oregon Health & Science U.	A Novel Model of Photoreceptor Degeneration	Rhesus macaque
Yang Hu, Stanford U.	Development And Characterization Of Silicone Oil-Induced Reversible Ocular Hypertension Glaucoma Model	Rhesus macaque



Appendix

AGI for Regenerative Medicine Funding

NEI AGI Funding Opportunity Announcements	Issue date	Awards	Annual funding	Duration (yrs.)	Total funding
Addressing Technical Needs and Opportunities for Imaging the Visual System	May 2015	5	$4M	5	$20M
Discovery-based Science to Identify Factors Influencing Neural Regeneration in the Visual System	Sept. 2016	6	$4M	3	$12M
Translation-enabling Models to Evaluate Survival and Integration of Regenerated Neurons in the Visual System	Oct. 2018	5	$5M	5	$25M
Preliminary Studies for Translation-Enabling Models of the Visual System	Aug. 2019	9	$275K	1	$2.8M

AGI Organizational Chart

National Advisory Eye Council

NEI Office of the Director

Santa Tumminia, Ph.D.

AGI Steering Committee

Mark Blumenkranz, M.D.

Joshua Sanes, Ph.D.

Clive Svendsen, Ph.D.

Russell Van Gelder, M.D., Ph.D.

Rachel Wong, Ph.D.

Functions

- Assesses the state of the science
- Recommends scientific priorities
- Assists in AGI evaluation metrics/milestones

AGI Office

Steven Becker, Ph.D.

Functions

- Coordinates meetings and workshops
- Communicates AGI progress to stakeholders
- Supports AGI activities

AGI Working Group

Lesley Earl, Ph.D.

Donald Everett, M.A.

Thomas Greenwell, Ph.D.

Dustin Hays

Michael Steinmetz, Ph.D.

Cheri Wiggs, Ph.D.

Maria Zacharias

Functions

- Analyzes grant portfolio
- Develops workshops and distills outputs
- Communicates to research community

AGI Charter

NEI, in conjunction with the National Advisory Eye Council (NAEC) and the advice of a dedicated AGI Steering Committee (AGI-SC), will oversee and implement scientific programs to meet the expectations of the initiative within a 10- to 15-year time frame.

The AGI Steering Committee

1. An AGI-SC composed of 3 to 5 members from the extramural community will be created to provide recommendations on the overall direction of the AGI.
2. Members will be appointed by the NEI Director to serve renewable three-year terms and will report to the NAEC.
3. The AGI-SC, in conjunction with the AGI Working Group, will assist NEI with evaluating the scientific needs and obstacles to progress and prioritizing research opportunities in the context of the emerging science.
4. The AGI-SC will meet as needed, provide reports to the NAEC on the overall progress, and make recommendations on the future direction of the initiative. The committee will assist in the evaluation of the outcomes of conferences and workshops and may solicit expert advice in order to guide future directions of the AGI.
5. The AGI-SC will offer recommendations on the overall integration and coordination of AGI initiatives.

The NAEC

1. The NAEC will provide concept clearance for AGI funding opportunities.
2. Workshops may be held and their reports will be provided to the NAEC, and their concurrence will be deemed as concept clearance.
3. The NAEC will provide the second level of review on proposals to AGI funding opportunities and will advise the NEI Director on the funding plans for the AGI projects.
4. The NAEC will be kept informed of all AGI activities.

AGI Working Group

1. An AGI Working Group (AGI-WG), composed of NEI staff, will provide information to the AGI-SC, the NAEC, and the NEI Director.
2. The AGI-WG will recommend workshops, solicit input from the scientific community, and in conjunction with the AGI-SC will identify gaps in knowledge, obstacles to progress, and potential solutions toward achieving the audacious goal.
3. The AGI-WG will be responsible for preparing Funding Opportunity Announcements (FOAs) and funding plans for the approval of the NEI Director and the NAEC.
4. The AGI-WG will be responsible for monitoring progress of individual research programs and will report progress and make recommendations to the AGI-SC, the NAEC, and the NEI Director.

AGI Office

1. An AGI Office will be established to provide logistical support for the AGI.
2. The AGI Office support function will include organizing workshops, conferences, and other AGI activities.
3. The AGI Office will assemble progress and budget reports for the AGI-SC, the NAEC, and the NEI Director.
4. The AGI Office will coordinate data sharing among AGI investigators and provide other technical support as necessary.
5. The AGI Office will regularly.

Audacious Goals Challenge

NEI launched an open challenge competition in August 2012, advertising in the Federal Register and scientific journals, to its grantee network, at medical conferences, and through social media. The institute reached out to biomedical, bioengineering, and computational science professional societies and to the general public to encourage submissions of one-page audacious concept papers that completed the thought: "It would be fantastic if…" Entries had to identify barriers, feasibility, and impact. NEI received 548 submissions. The success of the Challenge was that NEI received 308 entries (56%) from individuals with no NIH grant experience, indicating that we had reached a wide audience. Of the remaining 240 entries, 198 were from current or former NEI grantees and 42 represented grantees from other NIH institutes.

All submissions were de-identified, reviewed by 81 external scientific experts, and ranked. Finally, a panel of senior NIH leaders judged the entries based on the relevance to the NEI mission, audaciousness, feasibility, scope, and impact. This led to identifying 10 challenge winners who were each awarded $1,500.

Audacious Goals Challenge Winners

Dennis Clegg, Ph.D., University of California, Santa Barbara. *Regenerative therapy for retinal disease*—Treat degenerative retinal disease with an off-the-shelf tissue graft implanted in the back of the eye to replace cells lost to disease.

Robert Duvoisin, Ph.D., Oregon Health and Science University. *Restoration of vision by opto-electronic stimulation*—Restore vision by making nerve cells in the eye light-sensitive so that camera images can be converted to nerve signals and sent to the brain.

Yingbin Fu, Ph.D., University of Utah. *Precise gene editing in vivo*—Permanently correct disease-associated mutations in patients using molecules specially designed to target mutated DNA sequences and to be delivered safely and efficiently into the eye.

Steven Pittler, Ph.D., University of Alabama. *Using molecular scissors genome editing to cure ocular genetic disease*—Permanently correct gene defects in patients at the site of the mutation using scissor-like molecules to precisely replace genome errors with the correct DNA sequence.

Rajesh Rao, Ph.D., Washington University School of Medicine and The Retina Institute. *An audacious goal: reprogramming the retina*—Directly reprogram easy-to-isolate skin or blood cells to retinal cells using gene therapy and other techniques to enable repair strategies for degenerative retinal diseases.

Tonia Rex, Ph.D., Vanderbilt University. *Functional and structural neuroregeneration*—Restore functional vision in patients who experience loss of axons—the threadlike extensions of a nerve cell that conduct electrical impulses—from the optic nerve as a result of traumatic optic neuropathy or glaucoma by complete axon regeneration.

Julia Richards, Ph.D., University of Michigan. *Fountains of youth for the eye*—Turn back the aging process in the eye so that ocular diseases like age-related macular degeneration or glaucoma start 10, 20, or 30 years later than they do now.

Jeffrey Stern, M.D., Capital Region Retina, PLLC, Albany, New York. *Endogenous retinal repair: releasing our inner salamander*—Repair the retina by activating stem cells residing within the eye, awakening reparative processes that occur naturally in amphibians and other animals but lie dormant in human patients.

Russell Van Gelder, M.D., Ph.D., University of Washington. *Reversing retinal blindness using small molecules*—Restore vision to patients with retinal diseases through the use of a photoswitch, a small molecule that is chemically modified to become active or inactive after exposure to certain wavelengths of light.

Janey Wiggs, M.D., Ph.D., Massachusetts Eye and Ear Infirmary, Harvard Medical School. *Vision BioBank: a network of ocular phenotyping centers using genomic and epidemiologic data to promote personalized ophthalmology*—Create a network of biobanks that collect corresponding phenotype (physical characteristics) and genotype (genetic) data of people with certain eye diseases, and use this to develop sensitive and specific gene tests to accurately determine risk for glaucoma, age-related macular degeneration (AMD), diabetic retinopathy, and other common complex blinding diseases and their response to therapies.

AGI Development Meeting

NEI invited the winners of the Audacious Goals Challenge to discuss their ideas at the AGI Development Meeting, held in February 2013. The meeting brought together nearly 200 "big" thinkers, leading biomedical researchers, and clinicians to transform the winning ideas into a set of targeted audacious goals. Over the course of three days, participants fine-tuned the ideas in breakout groups and arrived at six proposals:

Aging and mechanisms of disease

Audacious goal proposal: Eliminate age-related eye disease

Although eye diseases have different risk factors, mechanisms, and vulnerable populations, and they affect different components of the eye, the effects of aging are a common thread that connects many leading causes of blindness. Age is a significant factor in glaucoma, cataract, diabetic retinopathy, dry eye disease, presbyopia, and, of course, AMD. This audacious goal sought to develop technologies and approaches to identify the very earliest changes and then intervene at a point in the disease process when it may still be possible to prevent or delay progression of the disease.

Molecular therapy at the end gene level

Audacious goal proposal: Preserve and restore vision through the delivery and modification of genetic information

The field of gene therapy celebrated a marquee success by restoring sight in young patients with Leber congenital amaurosis (LCA), an acute, early-onset retinal disease. Building on this success, this audacious goal proposed creating a flexible modular toolkit for manipulating genetic information in different contexts. This included precise and potentially durable gene correction and augmentation, and delivery systems that can handle genetic cargo of variable sizes. Current gene delivery systems cannot handle large-size DNA, limiting their utility for certain genes. Larger DNA cargos would enable packaging protein-coding sequences with contextual genetic information that directs precisely when and how much gene product to synthesize. The toolkit would also require refined methods to target payloads to specific cell types and to provide gene expression controls that could be externally manipulated.

Systems approaches to disease analysis

Audacious goal proposal: Develop a comprehensive systems-based model of vision

Medical research increasingly relies on big data to scan the human genome for risk genes for common complex diseases, assess environmental risk factors, correlate genotype/phenotype relationships, and integrate data from clinical, physiological, and molecular sources. NEI is at the forefront of this effort, having been the first institute to provide a large clinical trial data set—the Age-Related Eye Disease Study (AREDS)—to the database of Genotypes and Phenotypes (dbGaP), a restricted-access NIH database developed to investigate interactions between genotype and phenotype. The landscape of clinical data capture is rapidly changing, with the low cost and high speed of sequencing individual genomes outpacing the capacity to store and analyze the large volumes of data. Health information technology, mandated in a law passed in 2009, led to generation of electronic medical records with phenotypic data. In addition, a growing "quantified self" movement allows users to track their health status, nutrition, activities, and environment using smartphones and wearable devices, creating a trove of user-generated data. Integrating these streams of health data will allow improved prediction, prevention, and treatment of eye disease. The audacious goal would be to create a "crystal ball" comprehensive model so predictive that it could take individual patient parameters and calculate risk factors for various diseases.

Molecular and functional analysis and imaging of ocular tissues

Audacious goal proposal: Create a comprehensive map of visual function in health and disease

The future of imaging is not a static view of structures and how various parts fit together. Rather, it is a dynamic window informing what the individual parts do. Functional imaging incorporates electrical activity, metabolism, blood vessel integrity, molecules, and biomarkers. Expert discussions arrived at two complimentary imaging goals. One goal is to build novel, real-time, 3D, high-resolution, in vivo imaging tools to probe the function of the visual system. This can produce a complete map of the functional circuits for seeing, from the retinal to the visual cortex. A second goal is to image all parts of the human eye in new ways to detect pathological changes, enabling timely diagnosis and appropriate assessment of interventions.

Regenerative medicine for ocular disease

Audacious goal proposal: Improve sight in patients using regenerative therapies

The Audacious Goals Challenge ideas illustrate the promise of regenerative medicine to restore vision lost to degenerative diseases. Some ideas proposed to look for keys in other systems and in embryonic development to unlock the inherent ability to generate tissue. For example, some adult fish and amphibians regenerate naturally, but experiments on birds and mice have suggested that endogenous stem and progenitor cells can also be activated for regeneration. This regeneration might even be coupled with direct reprogramming from one cell type into another. Other AGI ideas focused on exogenous therapies, creating tissues in a dish and transplanting grafts into the eye. Discussions led by Drs. Gamm and Temple at the AGI Development Meeting weaved these ideas together into a formidable goal of restoring function by replacing diseased retinal neurons. Progress in generating various retinal cell types is a first step, but integrating new neurons and inducing them to grow long distances to synapses appropriately is audacious.

Vision restoration by optogenetics, small molecules, and prosthetics

Audacious goal proposal: Restore useful vision to people who are blind due to retinal disease, using prosthetics, optogenetics, and/or small molecules

Many genetic blinding diseases such as retinitis pigmentosa affect specific molecules and cells in the retina that detect light and convey electrical messages to the brain. However, a remarkable discovery is that even in patients who have lost sight for many years, the residual structure in the retina is relatively preserved.

New technologies are attempting to bypass photoreceptor cells by stimulating downstream neurons, thus providing visual information to the brain. This goal explores alternative technologies that can faithfully convert patterns of light into information for the brain, restoring vision to visually impaired individuals.

Glossary

Action potential. The change in electrical potential associated with the passage of an impulse along the membrane of a muscle or nerve cell.

Adaptive optics (AO). A technology that improves the performance of optical systems by reducing light-path distortions.

Adeno-associated virus (AAV). A virus commonly used as a gene therapy vector. Serotype 2 (AAV2) is the variety most extensively used in gene editing.

Assay for transposase-accessible chromatin using sequencing (ATAC-seq). A technique to assess genome regulation by measuring chromatin accessibility.

Axons. Cellular processes that transmit neural signals.

Compound action potentials (CAPs). Signals simultaneously recorded from multiple neurons.

Confocal microscopy. Technique that blocks out-of-focus light using a pinhole, enabling "optical sectioning" in which multiple two-dimensional readings can be assembled to generate three-dimensional reconstructions.

Clusters of regularly interspaced short palindromic repeats (CRISPR). A specialized region of DNA used in the genome editing tool CRISPR-Cas9.

Diffusion basis spectrum imaging (DBSI). A data-driven multi-tensor model for analyzing diffusion-weighted MRI signals.

Diffusion functional magnetic resonance imaging (diffusion fMRI). An MRI-based imaging technique that detects changes in radial diffusivity (apparent diffusion coefficient perpendicular to axonal tracts).

Electroretinogram (ERG). A technique used to assess nerve function in the eye.

Extracellular vesicles. Particles emitted by cells that carry protein, DNA, and lipids.

Fluorescence-activated cell sorting (FACS). A method for sorting a heterogeneous mixture of biological cells based upon the specific light scattering and fluorescent characteristics of each cell.

Fluorophores. Substances that fluoresce when stimulated.

Functional MRI (fMRI). A noninvasive imaging technique that measures brain activity by detecting corresponding changes in blood flow.

Genetically encoded calcium indicator (GECI). Used to monitor calcium transients in living cells for long-term, repeated measurements in vivo.

Glaucoma. A group of diseases that damage the eye's optic nerve and can result in vision loss and blindness.

Glia. Cells in the central nervous system that support neuronal cells, which carry nerve signals.

Human embryonic stem cells (hESCs). Stem cells from human fetal tissue.

In vitro. Latin for "in glass." Term used to describe experiments that take place in a test tube, petri dish, or other vessel.

In vivo. Latin for "within the living." Term used to describe experiments that take place in living organisms.

Immunohistochemistry. Use of antibodies to stain tissue sections.

Induced pluripotent stem cells (iPSCs). Adult cells that are reprogrammed to a naïve, undifferentiated state. Scientists can condition iPSCs to differentiate into specific cell types (e.g., heart cells or retinal cells.)

Lateral geniculate nucleus (LGN). A region of the brain that relays visual information.

Lipidomic. The large-scale study of pathways and networks of cellular lipids in biological systems.

Magnetic resonance imaging (MRI). A noninvasive imaging technique that uses radio waves and magnets to produce detailed pictures of the body's internal organs.

Mass spectrometry. A sensitive technique used to detect, identify, and quantitate molecules based on their mass-to-charge ratio.

Müller glia. Cells that support the structural and functional stability of the retina.

Multi-electrode array. A device that contains multiple microscopic metal electrodes distributed on a small surface through which neural signals are obtained or delivered, essentially serving as neural interfaces that connect neurons to electronic circuitry.

Myelin. A lipid-rich substance that surrounds and insulates nerve cell fibers.

N-Methyl-D-aspartic acid (NMDA). A receptor agonist that mimics the neurotransmitter glutamate.

Nystagmus. Involuntary eye movements.

Optogenetics. Technique used to control or measure the activity of cells by genetically inserting a light-sensitive protein.

Organoid. Lab-grown, organ-like tissues.

Ophthalmoscope. A handheld instrument for examining the eye.

Optical coherence tomography (OCT). A technique that uses light waves to non-invasively image internal structures underneath the skin such as the retina.

Optic neuritis. Inflammation of the optic nerve.

Photoreceptor mosaic. The distribution of rod and cone photoreceptors in the retina.

Photoreceptor cells (PRCs). Light-sensitive neurons in the retina.

Pluripotency. The capability of a cell to turn into all other cell types.

Proteomics. The study of all proteins in a biological system (e.g., cells, tissue, organism) during specific biological events.

Pupillometry. The measurement of pupil size and reactivity.

Retinal ganglion cell (RGC). Type of retinal neuron that transmits visual information from the eye to the brain via axons.

Retinal pigment epithelium. A pigmented monolayer of cells that nourish the neural retina.

RNA-seq. A technique that measures cellular RNA.

Short hairpin RNA (shRNA). An artificial RNA molecule used to silence gene expression.

Scanning laser ophthalmoscopy (SLO). Use of confocal scanning laser microscopy for diagnostic imaging of the eye.

Superior colliculus (SC). An area of the brain important for relaying visual information.

Trans-retinal electroretinography. A technique that measures the electrical activity of retinal cells in response to a light.

Transcriptome. A census of all gene readouts present in a cell.

Transportome. A census of the proteins transported in a cell to another destination (e.g., via an axon of a neuron).

Two-photon microscopy. A technique that enables imaging of living tissue as thick as one millimeter.

Visual evoked potential (VEP). A measurement of the electrical signal recorded in response to a light stimulus. VEP provides important diagnostic information regarding the functional integrity of the entire visual system.

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