McPherson Eye Research Institute
UNIVERSITY OF WISCONSIN–MADISON

InSights

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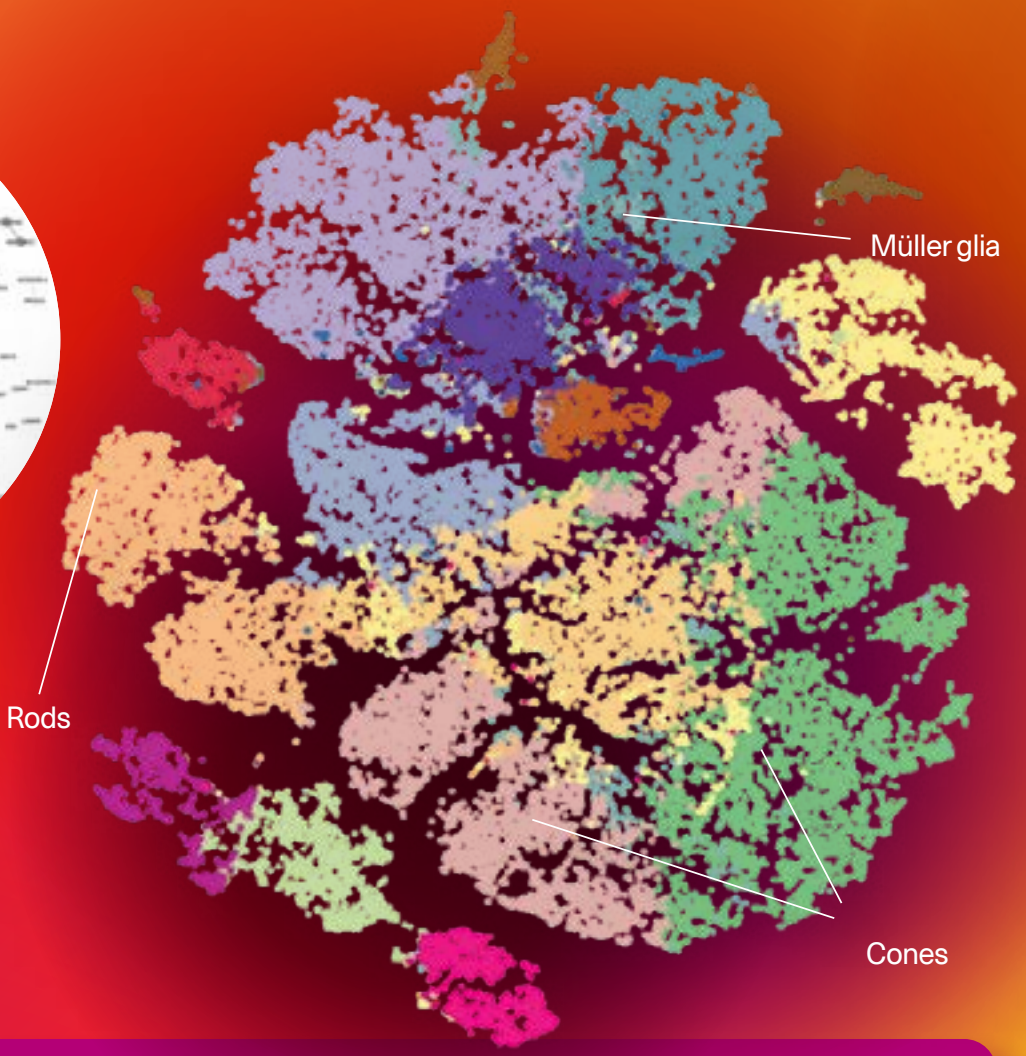
Regulating the Retina

WITH SUSHMITA ROY, PHD

The human retina is composed of a large number of cell types. Characterizing the cellular diversity of adult and developing retinal tissue is important for understanding how the eye functions and for identifying molecular differences underlying normal and disease states. A major determinant of cell type identity is the set of genes a cell can express. Each cell's genes express proteins that control the development and function of the cell. Gene regulatory networks (GRNs) specify how and what genes must be expressed when and where. These networks in turn play a major role in determining the type or identity of a cell. GRNs "direct traffic" within the cell, so to speak, defining connections between specific regulatory proteins (e.g., transcription factors and signaling proteins) and genes whose expression is under the control of these regulatory proteins. However, identifying these GRNs for individual cell populations in a scalable and cost-effective manner is difficult.

Sushmita Roy's lab focuses on the development and application of computational methods to define gene regulatory networks. Roy Lab scientists use high-throughput gene expression measurements and examine how they change across different spatial contexts, such as cell types and tissues, and temporal contexts, such as during development and disease progression. Such measurements are becoming increasingly available through pioneering technologies, such as single-cell RNA sequencing (scRNA-seq), which, as the name implies, rapidly sequences the genetic makeup of individual cells. These measurements offer unique opportunities to define GRNs for known and new cell types.



Right, Two-dimensional plot of cells of a retinal population. Each color represents a different cell population and each dot is a single cell. **Left,** a gene regulatory network for photoreceptors. Circles denote genes and lines denote interactions. The NRL gene is shown as a major hub. Image courtesy of Sushmita Roy.

Effective mining of these huge masses of data requires advanced computational approaches to identify statistically significant patterns, especially as these data tend to be "noisy." The Roy Lab develops methods based on machine learning that can analyze large and complementary genomic datasets and extract meaningful statistical patterns from these data that can indicate regulatory relationships. The lab works together with developmental and molecular biologists to define and validate these GRNs in different biological systems, including the retina and brain.

Together with McPherson Eye Research Institute researchers David Gamm and Kris Saha, Roy and her colleagues are collecting and analyzing single-cell RNA sequenced datasets to define gene regulatory networks in the developing and mature retina. In particular, they are working to identify key GRN components that control a major decision point in retinal cell development: how a cell "chooses" to turn into a rod versus a cone, the two major photoreceptor cell types that enable vision. Understanding this process is important for the development of gene editing and gene therapy approaches for diseases resulting in photoreceptor degeneration, such as retinitis pigmentosa and macular degeneration. The lab is also developing tools to make sure these therapies are safe and effective when implemented. In these and many other ways, the Roy lab is using cutting-edge statistical analyses to better understand how our retina develops, how it works, and how it may be repaired in the future.

Dear Friends of the McPherson ERI,

Over the past year, we have been bombarded with scientific and health-related data—both discouraging and, more recently, quite hopeful. But data are only as good as the methods used to acquire, analyze, and validate them. Indeed, with advances in computer science and technology, our ability to acquire information can easily outpace our ability to understand it. Biostatisticians and medical informaticists are on the frontline of this battle, and the McPherson ERI is fortunate to have one of the best. In this issue, we feature the work of Dr. Sushmita Roy, who develops cutting-edge analytical tools to mine the mountains of genetic and other data that are routinely generated from a single experiment. She and her laboratory apply their knowledge and tools to better understand all aspects of photoreceptors and other retinal cells.

If the food of biostatisticians is data, then the farmers are the researchers who design and carry out experiments in the hope of advancing knowledge and developing effective treatments. The McPherson ERI is proud to operate as a scientific "co-op", building teams to tackle the toughest problems. The success of this collaborative approach is recognized nationally and internationally through grant support and publications. As a prominent example, we are pleased to highlight a group of McPherson ERI researchers, led by Dr. Krishanu Saha (and including Dr. Roy), that comprises an integral part of the National Institute of Health's Somatic Cell Genome Editing (SCGE) Consortium. This nationwide program is tasked with safely and effectively moving gene editing technology toward patient clinical trials, and McPherson ERI researchers are the only ones dedicated to the eye and retina. The SCGE Consortium is one of many high-profile efforts that we are engaged in for the benefit of Wisconsin and the world, and we are fortunate and proud to do so here at UW–Madison.

We appreciate your interest. Take care, stay safe, and have an outstanding summer.

Wishing you the best,

David M. Gamm, MD, PhD
RRF Emmett A. Humble Distinguished Director
Sandra Lemke Trout Chair in Eye Research

2021 Kenzi Valentyn and Expanding Our Vision Research Grant Recipients Announced

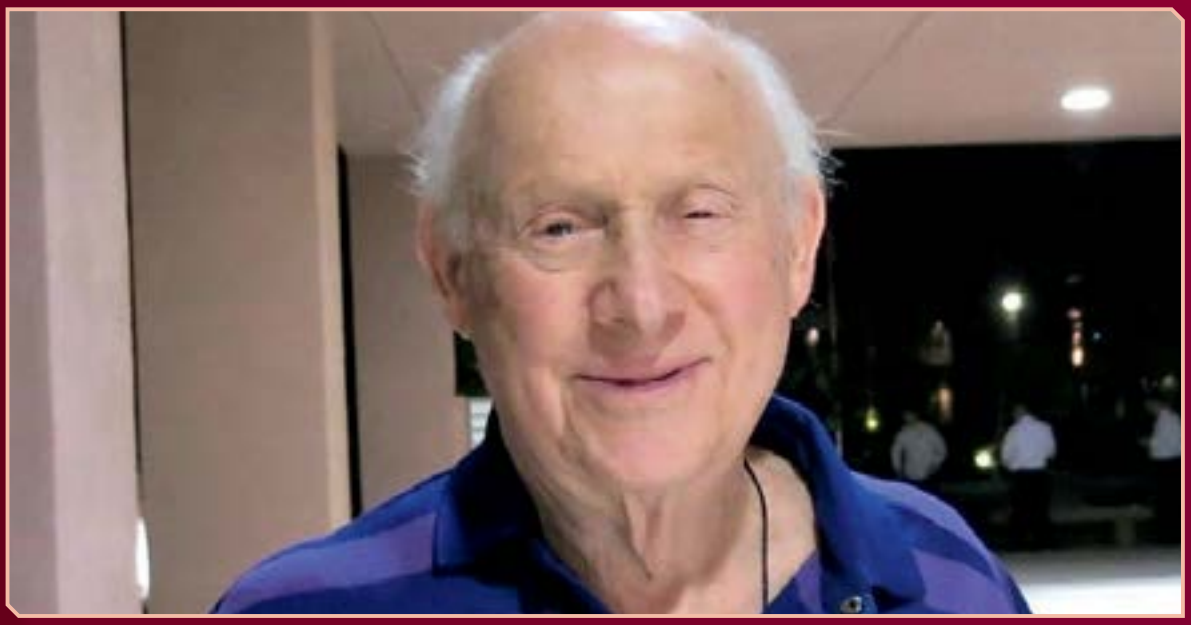
The 2021 recipients of the **Kenzi Valentyn Vision Research Grants**, one-year grant awards of $5000 funded by the Institute's Cycle for Sight event, were announced this spring. This research trainee grant is named in honor of Kenzi Valentyn's courage and positive attitude throughout her battle with Kearns–Sayre syndrome, a degenerative disease with symptoms including vision loss. We are grateful to the Valentyn family and all donors for their support for this grant program. This year's recipients are **Allison Ludwig** (Veterinary Medicine, Gamm Lab), **Kushin Mukherjee** (Psychology, Rogers Lab), **Aindrila Saha** (Neuroscience, Sinha Lab), and **Kara Vogel**, (Ophthalmology & Visual Sciences, McLellan Lab).

Congratulations also to Dr. Yuhang Zhao (Computer Science), recently awarded the 2021 **Expanding Our Vision Award** ($10,000) for ***Designing Augmented Reality Systems to Facilitate Safe Cooking for People with Low Vision***, a collaboration with Bilge Mutlu (Computer Science) and Sanbrita Mondal (Ophthalmology and Visual Sciences).

FROM LEFT: Allison Ludwig, Kushin Mukherjee, Aindrila Saha, Kara Vogel, Dr. Yuhang Zhao

Marv Conney, 1927–2021

McPherson ERI Advisory Board member Marv Conney passed away on March 25th, 2021, at 94 years of age. The McPherson ERI was one of many research, educational, and cultural nonprofits that was fortunate to draw the interest and support of Marv and his late wife, Mildred "Babe" Conney (also an Advisory Board member before her death in late 2016). After his time in the US Navy and at UW–Madison, Marv had a long career as the founder and owner of Conney Safety Products of Madison. Both before and after their 1998 retirement, the Conneys had a "second career" in philanthropy and were known as one of the most insightful, intelligent, and generous couples in Madison. They supported a number of outstanding scientists, including McPherson ERI member and pioneering stem cell researcher James Thomson. Marv and Babe were a delight to know and to talk with, often over Marv's favorite Chinese food lunches, and we will miss them.



Teaming up for gene therapy

Millions of Americans currently battle inherited visual disorders, armed with very few therapeutic options. Recent advances in genome editing, which many believe is the life science breakthrough of our era (and which was awarded a Nobel Prize in 2020), now provide new hope for a life-long durable therapy from a "single shot" by editing the DNA sequence within the eye. These advances have inspired a new $190M federal effort, the Somatic Cell Genome Editing (SCGE) Consortium. The SCGE is supported by the National Institutes for Health, with the aim of accelerating the development of safer and more effective methods to edit the genomes of diseased cells and tissues in patients. The initiative assembles a collection of multidisciplinary teams working on individual projects designed to develop new genome editors, delivery systems, and biological systems to measure the safety and efficacy of various genome-editing strategies. The end goal is a range of new therapies for inherited disorders.

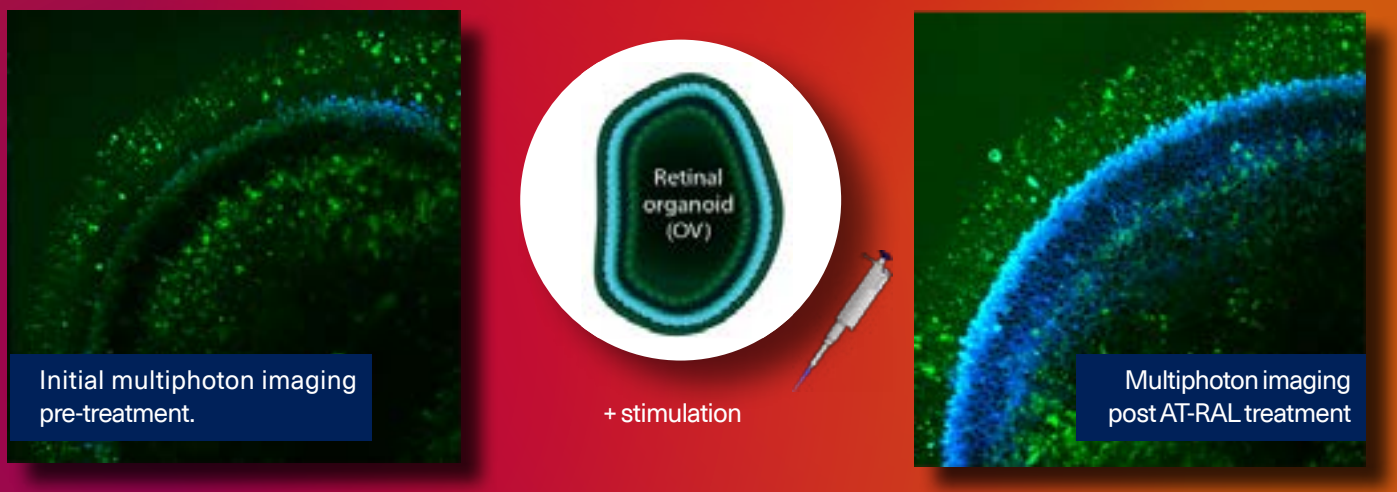
McPherson ERI scientists are heavily involved in this project; in fact, the only team within the SCGE focused on the eye is entirely composed of McPherson ERI investigators. Two of these teams are from UW–Madison, and participants include McPherson ERI researchers **Kris Saha** (1, above), **Sushmita Roy** (2), **Melissa Skala** (3), **Sarah Gong** (4), **Bikash Pattnaik** (5), and **Dave Gamm** (6, opposite page).

The eye is the frontline for gene therapeutic development for many reasons, including ease of accessibility. The most common use of genome editors is to change the DNA base sequence directly within the genes in our cells. Genome editors could correct small "misspellings" in the genes that cause diseases such as retinitis pigmentosa, sickle cell disease, and others. Alternatively, genome editors might insert new synthetic genes to add functionality to a cell or tissue. Genome editing broadly encompasses diverse technologies that can make many different genomic alterations in different contexts, depending on the part of the gene or cell that needs to be fixed. In the eye, genome editors can target many cell types, including rod and cone photoreceptors, retinal pigmented epithelium (RPE) cells, and ganglion cells (whose axons comprise the optic nerve).

While a single shot cure holds much promise, new studies and tools are required to make this vision a reality. One McPherson ERI team in the SCGE, led by Kris Saha, is using mini retinal tissues in a dish grown from stem cells. This effort builds on pioneering work by the Gamm Lab on differentiating these retinal cells and tissues from human stem cells. New bioengineering techniques are being applied to monitor their health after gene editing, including a multiphotonic imaging process (image below) pioneered by Melissa Skala to measure the light sensitivity of the photoreceptors, and sequencing technology to measure all molecular perturbations in these cells. Machine learning, using novel methods developed by Sushmita Roy, is being applied to these datasets to identify signs of dysfunctional gene editing, which is essential for establishing safe dosing and formulation of a single shot therapeutic.

The second UW–Madison SCGE team, led by Sarah Gong, is developing new non-viral materials to deliver these genome editors safely into the eye. These materials can be thought of as "shrink wrap"—they consist of a thin polymeric shell around the CRISPR-Cas9 protein complex (the actual gene editor). The chemistry of this shell has been tuned so that it protects the genome-editing machinery from degradation before it enters the target cell. Once this configuration, or nanoparticle, is engulfed within the targeted cells, the shell degrades to allow the CRISPR-Cas9 protein complex to edit the DNA. The same materials have already shown promise in delivering genome editors into the eye in collaborative projects with Bikash Pattnaik. It is anticipated that the technologies, tools, and knowledge developed in one tissue system by SCGE investigators will be informative for studies in other tissues. The entire SCGE project, with key contributions from McPherson ERI investigators, will share this information broadly to spur new therapies for the eye and other areas of the body.

For more information, go to:
www.nature.com/articles/s41586-021-03191-1



In the Skala Lab, photoreceptors of a stem-cell derived retinal organoid are shown before (left) and after (right) stimulation with all-trans retinaldehyde (AT-RAL). Image courtesy of Kayvan Samimi and the Skala Lab.

Cycle for Sight Plus 2021, Safe and Successful, Raises $55,000 for Vision Research

Cycle for Sight Plus 2021, held in late April, changed its format from previous years, with teams cycling, walking, or running on their own. Instituted in response to COVID-19, the change was a big success with many teams, allowing outdoor walks and rides, not only in Madison, but from Baraboo and Eau Claire to the Colorado Rockies and Alaska. Teams raised approximately $55,000 for a variety of vision research grant programs. Kenzi's Team, which hosted a walk around the Wisconsin State Capitol, once again took the lead. We're grateful for all of our teams, and for our terrific sponsors, Opsis Therapeutics, the Princeton Club, and (new this year) the Chippewa Valley Eye Clinic.

CYCLE FOR SIGHT WILL RETURN IN 2022 WITH A HYBRID FORMAT, INDOORS AND OUTSIDE.

THANK YOU TO ALL WHO DONATED!