Near-IR nanosensors help blind mice see

When blindness sets in gradually, the patient's remaining vision can hinder prospective treatments. In a new experimental strategy, researchers turn to a different wavelength.

Even in the dark, rattlesnakes and their fellow pit vipers can strike accurately at small warm-blooded prey from a meter away. Those snakes, and a few others, can see in the IR—but not with their eyes. Rather, they have a pair of specialized sensory organs, called pit organs, located between their eyes and their nostrils and lined with nerve cells rich in temperature-sensitive proteins that cause the neurons to fire when heated.[1] The pits work like pinhole cameras to focus incoming thermal radiation onto their heat-sensitive back walls; the thermal images are then superimposed with visual images in the snake's brain.

Heat-responsive neurons are not unique to snakes. We have them over every inch of our skin, to feel objects warm to the touch, and on our tongues, to taste spicy food. But the snakes' ability to resolve the source of radiated heat at a distance is unusual.

Inspired by the snakes, Dasha Nelidova and her colleagues at the Institute of Molecular and Clinical Ophthalmology in Basel, Switzerland, are developing a new treatment for forms of blindness caused by the degeneration of retinal photoreceptors.[2] Using gene therapy, they endow remaining retinal cells with thermoresponsive proteins, thereby compensating for their lost light sensitivity with heat sensitivity. The proteins by themselves aren't sensitive enough to rival normal vision, so the researchers tether them to gold nanorods, as shown in figure 1. The 80-nm-long nanorods strongly absorb near-IR light at 915 nm and convey the concentrated heat to the attached proteins.

So far, the researchers have tested the protein–nanoparticle combination on blind mice and on donated postmortem human retinas. The results are promising: The mice could learn a behavioral response to a flash of near-IR light, and the human retinas, like the one shown in figure 2, produced a detectable electrical signal. But it will take years of additional work to turn the procedure into a safe and effective treatment for live humans.

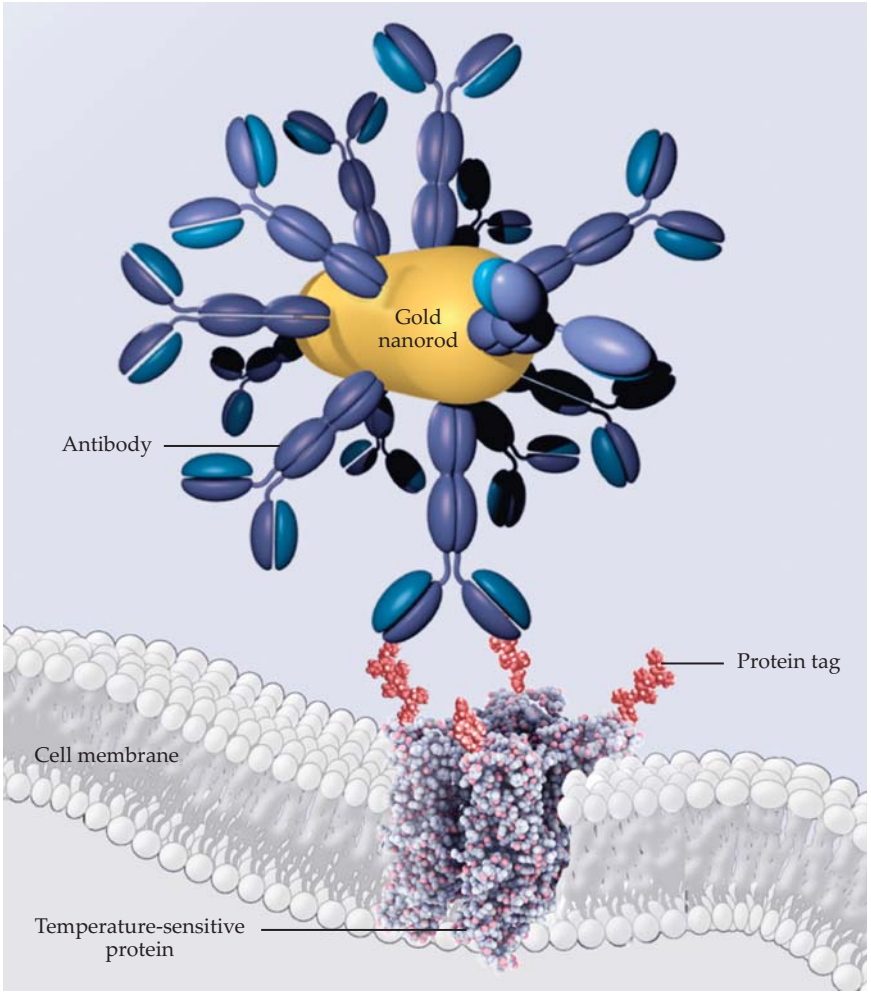

FIGURE 1. HOW TO SEE IN THE NEAR-IR. A gold nanorod strongly absorbs 915 nm light to produce heat, and a temperature-sensitive protein generates an electrical signal when heated. When the protein is embedded in a retinal cell membrane and linked to the nanoparticle via a protein tag and corresponding antibody, the retina becomes sensitive to near-IR light that most animals can't normally see. (Image by Veronique Juvin, SciArtWork.)

The eyes have it

From the cornea to the optic nerve, any component of the visual system can malfunction, and correspondingly, there are many types of blindness, some more treatable than others. Cataracts, the clouding of the normally transparent lenses, are routinely treated by surgically replacing the faulty lenses with artificial implants. If the cornea becomes cloudy or opaque, part or all of it can be replaced with a transplanted one. Although organ and tissue transplants are never easy, corneal transplants are among the most straightforward: The eyes are an immune-

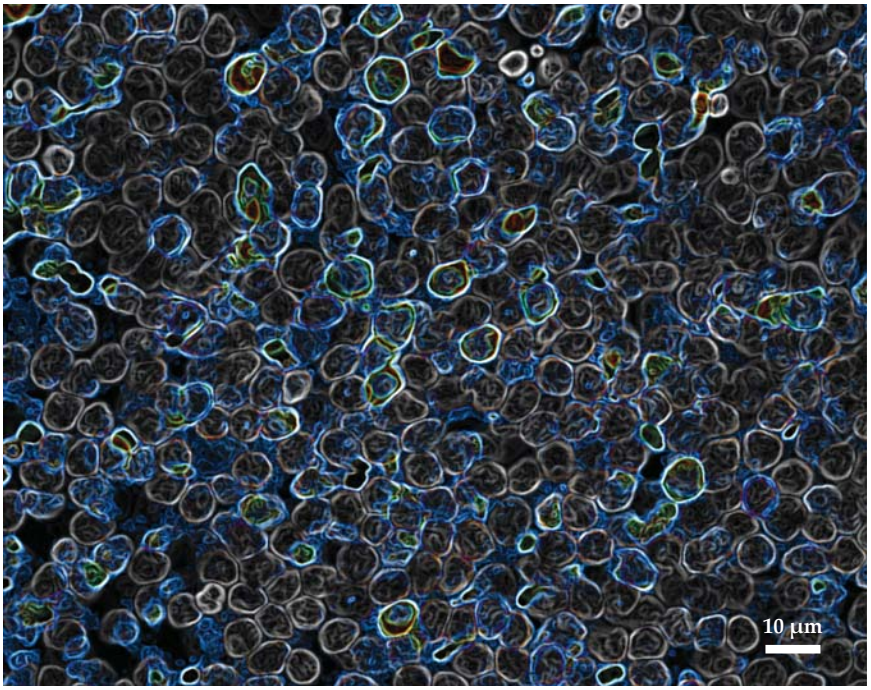

FIGURE 2. DELIVERING A NEW GENE. Retinal cells don't normally manufacture the thermoresponsive protein shown in figure 1, but they can be made to do so through gene therapy. Benign viruses, packed with the DNA that encodes the protein, inject their genetic material into the cells, which take up the gene and start expressing the protein. In this image of a treated postmortem human retina, the thermoresponsive protein is stained with a fluorescent dye. The cells shown in color are all producing the new protein; the cells shown in gray are not. (Courtesy of Dasha Nelidova.)

privileged site, meaning that the body is less likely to attack and reject the foreign tissue.

In the industrialized world, most vision loss is due to photoreceptor degeneration, a category that includes age-related macular degeneration—which affects millions of people in the US alone—and several less common inherited conditions. In each of them, the rods and cones in the retina gradually lose their light-sensitive parts (and may die off altogether) until vision is noticeably impaired and eventually lost. Treatments can slow the progress of macular degeneration, but none yet exist to reverse it.

For several years, Nelidova and her colleagues have been working to combat photoreceptor degeneration with optogenetics, a technique for controlling neurons with light by endowing them with genes for light-sensitive membrane proteins borrowed from bacteria or algae. Optogenetic methods are typically used for basic neuroscience research—optically switching neuronal activity on or off to figure out which neurons do what in the neural circuits in the brain. The researchers hope to restore lost vision by inserting the same light-sensitive proteins into cells in the retina: the photoreceptors themselves if they're still alive, or neurons in the next layer of retinal cells if they're not.

The eye is an advantageous target for gene therapy. It's small and compartmentalized, so the therapeutic gene can be selectively conveyed to the cells that need it. Its immune privilege means that the foreign genetic material is protected from attack and rejection. And researchers have developed a toolkit of gene-delivery vectors, called adeno-associated viruses, for targeting specific types of retinal cells. The viruses don't cause disease, and they reliably insert their genetic payload into the cells' genome in a way that doesn't interfere with normal cellular function. The viruses are introduced to the retina through subretinal injection, a surgical procedure.

In 2017 the FDA approved a gene therapy treatment for Lebers congenital amaurosis, a form of photoreceptor degeneration caused by a specific genetic mutation; the treatment isn't optogenetic

but instead replaces the problematic gene. Optogenetic treatments, which can restore light sensitivity even when the cause of the degeneration is not known, are currently in clinical trials.

But optogenetic therapy suffers from a major unmet challenge. A healthy retina can detect light over eight orders of magnitude in brightness, from a faintly lit room to a sunny summer day. The microbial proteins used in optogenetics are sensitive only to the brightest end of that range. To see under dimmer conditions, an optogenetic patient must wear video goggles that image the visual scene and amplify its brightness. That strategy works well for someone who's totally blind, but photoreceptor degeneration is a progressive condition that's debilitating long before blindness is complete. For a patient with some remaining vision, the healthy photoreceptors would be overwhelmed by the goggles' bright light.

The new work aims to surmount that hurdle by creating vision at a wavelength that healthy rods and cones can't see. Because the world doesn't look the same in the near-IR as it does in the visible regime, patients would still need to wear goggles to convert incoming visible light to 915 nm. The remaining healthy photoreceptors can't help to process that image—but they also won't be overwhelmed.

Engineering nanovision

Gold nanorods, an essential component of Nelidova and colleagues' near-IR sensors, are no newcomers to biomedical applications. Because of their surface plasmon resonances, metal nanoparticles are exceptionally good at absorbing light and converting it into heat (see the article by Mark Stockman in PHYSICS TODAY, February 2011, page 39), and they can be tuned through their size and shape to absorb at a particular desired wavelength. Among their uses is photothermal therapy for cancer, which involves targeting them to a tumor and zapping them with a laser to cook the tumor to death. Although the heat they give off can wreak selective havoc on tissues, the nanoparticles themselves appear to be safe and biocompatible. And they can be injected into the retina in the same surgery as the gene-carrying viruses.

Plasmonic nanorods aren't the only nanotechnological route to seeing in the near-IR. Last year, Tian Xue and colleagues of the University of Science and Technology of China showed that they could endow mice with near-IR vision using so-called upconversion nanoparticles, whose structure of a core wrapped in an outer shell allows them to absorb light at one wavelength and emit it at a shorter one.[3] (For more on upconversion nanoparticles, see the article by Marco Bettinelli, Luís Carlos, and Xiaogang Liu in PHYSICS TODAY, September 2015, page 38.) They chemically anchored their nanoparticles to retinal photoreceptors—the mice they used weren't blind—so that when the particles absorbed in the near-IR and emitted in the visible, the light was visually processed in the normal way.

Because Xue and colleagues' approach relies so heavily on the existing retinal structure, their mice could recognize and distinguish simple near-IR spatial patterns. Nelidova and colleagues, so far, have shown only that their mice can detect an undifferentiated near-IR flash. And in seeking to restore vision to degenerated human retinas, they face an additional challenge. The topology of neural connections in the retina is complicated, and it's not clear whether a lost photoreceptor cell can be adequately replaced by a neuron in the same location. As Nelidova notes, "Restoration of high-resolution vision is still many years away."

Another unknown is how long the treatment will last: Is a single injection of genes and nanoparticles good for a lifetime, or does its effectiveness eventually wane? The researchers are hoping for the former: A subretinal injection is a difficult surgery that's especially challenging to perform more than once in the same eye. Although gene therapy is still a young technology, the coming years will provide a clearer picture of its long-term outcomes in Lebers congenital amaurosis patients and the subjects of optogenetic clinical trials.

As for the nanoparticle half of the sensors, mature retinal cells don't divide, so a single procedure to dose the existing cells with nanoparticles should suffice. But if the particles aren't securely anchored in place, they could be cleared by the body and lost.

To solve those problems, the Basel researchers are working on optimizing their near-IR sensor by tinkering with its parts. Across the animal kingdom, there are many variants of the thermosensitive protein, each of them responsive to a slightly different temperature. Nanoparticles can be tuned in size and shape, and the protein tags and antibodies can also be reengineered. The researchers have tested several of the possible combinations so far, and they plan to explore further. Says Nelidova, "One reassuring thing shown by the experiments is that we can disconnect, reconnect, and exchange sensor components, with predictable final outcomes."

Johanna Miller

References

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2. D. Nelidova et al., *Science* **368**, 1108 (2020).
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Photoelectron spectra explain how ammonia solutions become metallic

The gradual emergence of delocalized electron states in lithium–ammonia solutions underlies their transition.

Dropping a chunk of sodium into water and watching it explode is a classic high-school chemistry demonstration. The violent reaction is caused by the alkali metal's dissociation into Na^+ ions and electrons when it enters H_2O. The electrons react with the water to liberate hydrogen atoms, and those quickly pair to form H_2 gas that is ignited by the exothermic reaction.

The same demonstration becomes less incendiary if H_2O is replaced with liquid ammonia, because NH_3 is harder to break apart. Whereas about 1 in every 10^9