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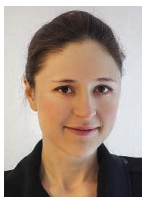

CATEGORY WINNER: MOLECULAR MEDICINE

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MOLECULAR MEDICINE

Engineering near-infrared vision

An optogenetic technology inspired by snakes could aid those with incomplete blindness

By **Dasha Nelidova**[1,2]

Photoreceptor degeneration, including age-related macular degeneration and retinitis pigmentosa, is a leading cause of blindness worldwide. Repair of retinal neurons by optogenetics—a technology that sensitizes neurons to light through the transfer of genes for light-sensitive proteins of microbial origin (*1*, *2*)—has entered clinical trials (*3*, *4*). Trials began in 2018 in patients with advanced retinitis pigmentosa and minimal remaining vision (*4*).

Optogenetic proteins are sensitive only to the brightest visible light, at intensities that overwhelm surviving functional photoreceptors. Yet, in a number of blinding diseases, light-sensitive and light-insensitive photoreceptor zones coexist within the same retina. In macular degeneration, for example, cone photoreceptors of the central retina lose their light sensitivity. Surrounding photoreceptors remain viable, and peripheral vision is largely unaffected. A key challenge for new translational technologies that aim to restore image-acquiring properties of the retina is the compatibility of such technologies with remaining vision.

We reasoned that sensitizing the retina to wavelengths that functional photoreceptors are unable to detect (>900 nm) could supplement deteriorating natural vision, without interfering with the ability to see the visible spectrum. Inspired by infrared vision in snakes, we developed nanogenetic molecular tools that allowed blind mice and ex vivo human retinas to detect near-infrared (NIR) light (*5*).

SNAKE VISION: TWO IMAGES SUPERIMPOSED

Snakes can see the world in two different ways. Like humans, they make use of their eyes to detect wavelengths of the visible spectrum (400 to 700 nm). In addition, several species can also generate thermal images (*6*). Snakes detect infrared light (1 to 30 μm) using temperature-sensitive transient receptor potential (TRP) cation channels expressed in a specialized "pit" organ (*6*). Infrared and visible spectrum images superimpose within the brain (*7*), presumably enabling the animals to react to the environment with greater precision than what is possible by using only a single image. Snakes can switch back and forth between the two imaging systems or use both simultaneously (*7*, *8*).

TRP channels could potentially be targeted to mammalian retinal cell types to make them sensitive to infrared radiation. However, infrared light would raise vibrational energies of water molecules throughout the eye. Shorter wavelength NIR light would be preferable because NIR has lower water absorption, although this same feature also makes direct NIR illumination an inefficient activator of TRP channels.

ENGINEERING RETINAL CELLS TO DETECT NIR

To develop a more efficient NIR light detector for retinal cell types, we engineered a dual system that consists of a genetic and a nanomaterial component (see the figure). The genetic half of the sensor consists of TRP channels, engineered to incorporate an extracellular protein epitope tag recognizable by a specific antibody (*9*). The nanomaterial half of the sensor consists of gold nanorods conjugated to an antibody against the epitope (*10*). Gold nanorods serve as antennas for NIR light and convert light into local heat through surface plasmon resonance (*11*), driving photocurrents through antibody-bound TRP channels. Subretinal microinjection of virally packaged TRP and nanorods delivered the sensor components to cones.

Our initial system was based on TRP vanilloid 1 (TRPV1) channels and gold nanorods with absorption maxima at 915 nm. We began by inserting a 6x-His epitope tag into the middle of the first TRPV1 extracellular loop, measuring sizes of evoked currents before and after the modification, and confirmed that channels remained functional. Next, we used adeno-associated virus (AAV)–mediated gene transfer to transduce cone photoreceptors of blind mice with the nanogenetic sensor. To measure neural ac-

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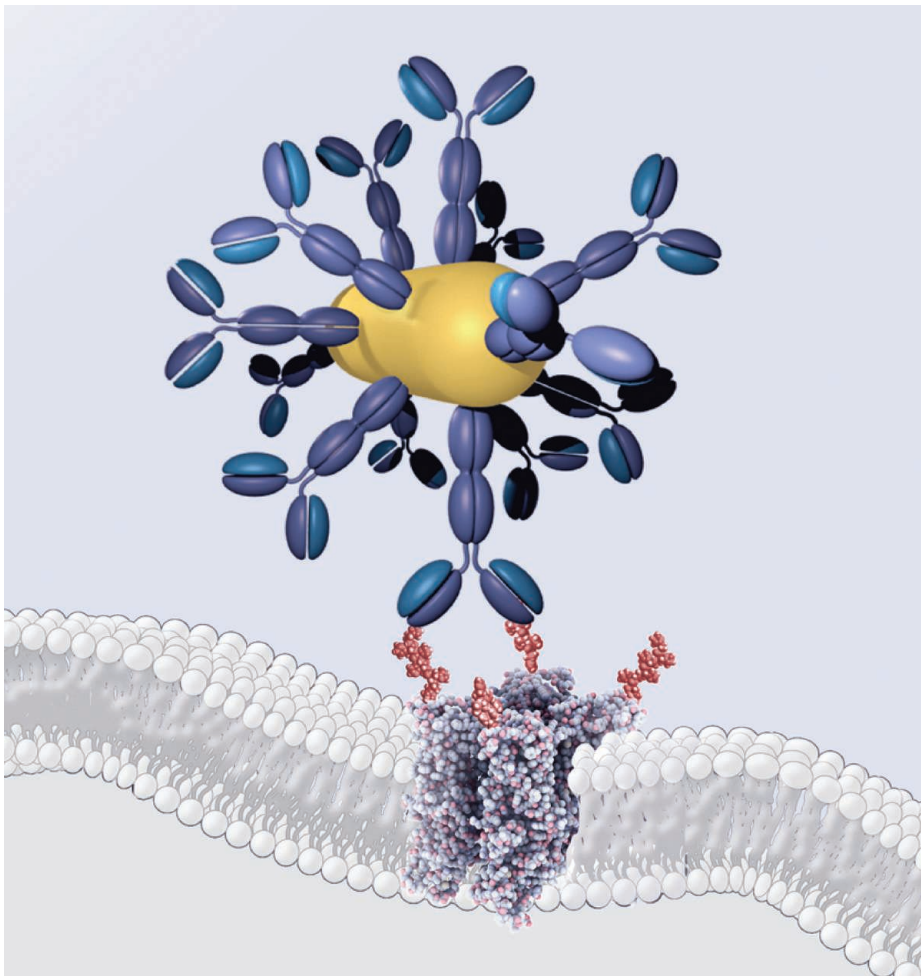

Components of the near-infrared light sensor. Engineered transient-receptor-potential channels (lilac) express protein epitope tags (red) in extracellular domains and bind extracellular antibody-conjugated gold nanorods.

IMAGE: VERONIQUE JUVIN/SCIARTWORK

tivity, we performed two-photon calcium imaging of individual neurons within the retina and primary visual cortex.

Expression of the nanogenetic sensor in cones rendered blind retinas to be sensitive to NIR light. Cone photoreceptors (retinal input) and retinal ganglion cells (retinal output) responded vigorously to 915-nm light, and NIR-evoked retinal activity propagated to the brain. This allowed treated mice to use their newly acquired NIR vision to perform behavioral tasks.

In complementary experiments, we confirmed that NIR light was unable to activate wild-type cones and did not affect their visible light responses. Similarly, awake, wild-type mice failed to exploit NIR light cues during behavioral training.

TUNABLE LIGHT SENSORS

Nanorod properties depend on size and shape (*11*). By changing the length of the gold nanorods from ~80 nm to ~120 nm, we tuned NIR vision to a different NIR wavelength (980 nm). Wavelength tuning is important for several reasons. Certain NIR wavelengths might be better tolerated by patients than others. Also, maximum permissible light doses for the human eye depend on the wavelength. Additionally, NIR vision requires eye goggles that project images composed of specific NIR wavelengths onto the retina. Compatibility with current and future NIR projectors requires tunable NIR detectors.

GAINING FUNCTION BY TWEAKING THERMOSENSITIVE PROTEINS

Across the animal kingdom, multiple variants of thermosensitive proteins can be found, and more can be created through mutagenesis. Channels, tags, and antibodies can be modified to gain additional desirable properties. We selected TRP ankyrin 1 (TRPA1) channels from the Texas rat snake because of their lower thermal thresholds and inserted the newer epitope tag OLLAS (*Escherichia coli* OmpF Linker and mouse Langerin fusion sequence) (*12*) into the first extracellular loop. Mice transfected with engineered TRPA1 channels were better able to anticipate water rewards when lights were dimmed as compared with mice transfected with TRPV1, indicating an improvement in the sensitivity of the sensor. (Both TRPA1- and TRPV1-transduced animals performed behavioral tasks as well as wild-type animals that were trained by using visible light.)

TOWARD HUMAN NIR VISION

The next step was to validate findings in blind human retinas. To do this, we targeted TRPV1 and gold nanorods to light-insensitive photoreceptors of adult human ex vivo retinal explants. (We had previously developed a cocktail of molecules to keep human retinas alive for 8 weeks post mortem, giving gene expression time to take hold.) We then recorded NIR light–evoked calcium activity and saw fast, strong activation of human photoreceptors and downstream retinal neurons, including ganglion cells.

Taken together, these experiments provide proof of principle for the potential therapeutic translation of this technology. Light intensities required to drive genetically encoded NIR sensors met existing safety standards that specify exposure limits for the human eye, and we further demonstrated that components of the sensor may be exchanged, with predictable final outcomes. In the future, targeted central repair would allow an island of NIR sensitivity to be built in a sea of natural vision. Parallel developments in surgery (*13*) and NIR projectors with eye-tracking capabilities (*4*) make targeted central repair feasible. Ultimately, the user may be able to self-select the region of the electromagnetic spectrum most useful to view the external world, a decision guided by the state of their retina and ambient light conditions. ■

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