# The Bionic Retina: A Small Molecule with Big Potential for Visual Restoration

Theodore G. Drivas[1,*] and Jean Bennett[1,*]
[1]F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: tdrivas@mail.med.upenn.edu (T.G.D.), jebennet@mail.med.upenn.edu (J.B.)
http://dx.doi.org/10.1016/j.neuron.2012.06.010

In this issue of *Neuron*, Polosukhina et al. (2012) intravitreally deliver the light-activatable molecule acrylamide-azobenzene-quaternary ammonium (AAQ) to the eyes of mice with end-stage retinal degeneration. Results show that, with the appropriate illumination, AAQ restores light sensitivity and visual behavior.

More than three million Americans currently suffer from devastating inherited and acquired forms of blindness. This includes Americans with age-related macular degeneration (AMD), retinitis pigmentosa (RP), complications of diabetic retinopathy, retinopathy of prematurity, and individuals unable to take advantage of timely treatments for traumatic retinal detachment. Patients with retinal blinding disorders have no or limited treatment options, and the societal and public health burdens of these diseases are substantial. Despite distinct etiologies, each of the above diseases is characterized by a pathologic degeneration of the light-sensitive rod and cone photoreceptor cells of the retina, eventually resulting in blindness, with persistence of inner retinal neural circuitry (Figure 1A).

This common pathology has led many to ask whether the remaining retinal wiring can be harnessed in advanced retinal degeneration in an attempt to develop a generic therapy. That is exactly the question that Polosukhina et al. (2012) sought to address in their paper in this issue of *Neuron*. The approach they took utilized the light-activatable AAQ (acrylamide-azobenzene-quaternary ammonium) molecule as an agent for imparting light sensitivity to remaining inner retinal neurons, even in the complete absence of photoreceptors (Figure 1B).

AAQ is a small molecule K+ channel photoswitch that can exist in both a *cis* and *trans* form. The *trans* form of AAQ binds to cellular K+ channels, blocking the flow of K+ ions, thereby increasing neuronal excitability. In the presence of short wavelength (380 nm) light, AAQ is photoisomerized to the *cis* form, abrogating its inhibitory effects and decreasing neuronal excitability. The relaxation from *cis* back to *trans* is characterized by relatively slow kinetics but occurs much more rapidly upon exposure to longer wavelength (500 nm; green) light. Thus, upon incubation with AAQ, individual neurons can be specifically and rapidly activated and inactivated by exposure to 500 nm and 380 nm light, respectively (Figure 1B).

Polosukhina et al. (2012) first incubated retinal explants from rd1 mice (characterized by a near-complete degeneration of photoreceptors) with AAQ and tested the ability of the explants to respond to light. Polosukhina et al. (2012) measured the electrical output of the retinal ganglion cells (RGCs), the sole output cells of the retina, and found a dose-dependent increase in light sensitization in the explants in response to AAQ. However, Polosukhina et al. (2012) noted that, paradoxically, the RGCs exhibited an increase in firing in response to 380 nm light, opposite to the effect of AAQ on cultured neurons.

As the normal role of the amacrine cells of the retina is to provide a strong inhibitory input on the RGCs, Polosukhina et al. (2012) reasoned that the paradoxical response of RGCs to AAQ treatment might be due to AAQ-mediated inhibition of amacrine cells. Thus, by reducing the inhibitory amacrine input on RGCs, AAQ might appear to have a paradoxical effect on RGC firing. Through a series of elegant experiments, Polosukhina et al. (2012) dissected out the contribution of each retinal cell type to the final RGC output and showed their hypothesis to be correct—AAQ inhibits firing of amacrine, bipolar, and RGCs upon exposure to 380 nm (UV) light, with the final integrated effect of increasing RGC output.

Having shown a robust effect on retinal explants, Polosukhina et al. (2012) went on to show that AAQ treatment could also confer in vivo light responsiveness. First, the pupillary light reflex (PLR), the constriction of the pupil in response to light, was measured. No PLR could be elicited in sham-injected animals, while a subset of animals that had received an intravitreal injection of AAQ was found to have an improved PLR, approaching the wild-type response. Polosukhina et al. (2012) attributed the lack of response in some of the treated animals to the technical difficulties relating to drug delivery to the very small volume of vitreous in the mouse eye.

The next question was whether the animals enjoyed functional vision. For this, Polosukhina et al. (2012) subjected sham- and AAQ-injected mice to behavioral studies. AAQ-treated animals showed light-induced behavior more similar to wild-type than to sham-injected animals. The responses were sustained for a few hours, but the next day, the performance of the AAQ-treated mice was similar to sham-injected animals, an expected consequence of the dissipation of the drug (Polosukhina et al., 2012).

While these results are encouraging, there are a number of caveats that must be addressed. First, it will be important to test this approach in large animal models. Testing could be carried out on the rcd1 dog, for example, which has a mutation in the same gene (*PDE6B*)

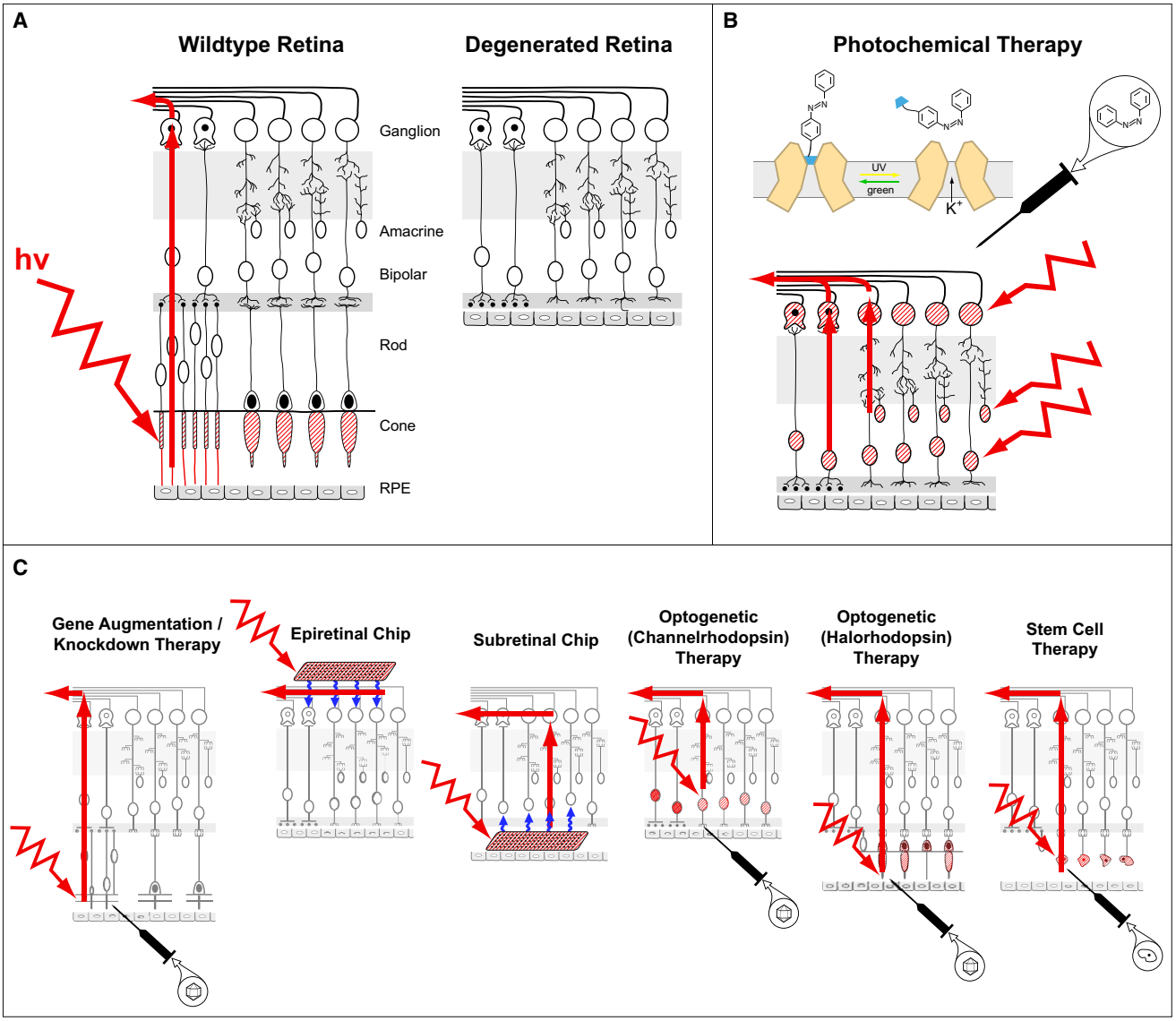


**Figure 1. The Bionic Retina**

(A) Architecture of a normal versus a degenerated retina. The latter lacks both rod and cone photoreceptors. In the wild-type retina, incident light penetrates through the layers of the retina and is absorbed by the photoreceptor outer segments, where it is converted to an electrical signal (straight red arrow). The signal is propagated through and modified by the inner retinal neurons and eventually integrated by the ganglion cells before being passed to the brain via the optic nerve. Since photoreceptors are absent in the degenerated retina, there is no electrical response to illumination.

(B) The mechanism of action of AAQ, a small-molecule photoactivatable K+ channel inhibiter, is represented schematically at the top. Intravitreal injection of AAQ in retinal degeneration mice results in light-dependent activation of the remaining amacrine, bipolar and ganglion cells, and output of electrical signals to the brain.

(C) Some of the many methods currently being developed as therapies for retinal degeneration. Each diagram shows the light-activatable cell type or device (red hatches) and the pathway of the light-generated electrical activity (straight red arrows) from the retina to the brain. More detailed discussion of each of these strategies can be found in the text of this article.

Illustration is by Mary Leonard, Biomedical Art & Design.

as the rd1 mouse. The anatomical and size similarities between the canine and the human eye make this model much more useful in terms of determining doses, treatment protocols, and other parameters that would probably be useful in designing human trials. In addition, it would be easier to test the effects of repeat administrations of AAQ within the same eye in a large, rather than in a small, animal model. Finally, it will be important to evaluate whether AAQ treatment can provide these large animals with the ability to discern shapes and movement.

Application of this approach to human disease will also probably require the development of a device to transmit light of the appropriate wavelength and intensity for AAQ activation. Additionally, the wavelength needed for AAQ photoisomerization is outside of the visible spectrum, shifted toward UV, a wavelength nearly completely absorbed by the human lens before ever reaching the retina. Also, the

intensity of light required to affect behavioral changes in mice was log units higher than that encountered in normal working environments. However, much effort is being put into addressing these points—groups are working on modifying AAQ so that photoisomerization occurs at the desired wavelengths and intensities.

The delivery requirements for AAQ treatment in humans also present a challenge. The logistics of delivering AAQ via intravitreal injections, potentially every 12–24 hr, will be difficult for both patients and physicians. It may be possible, however, to deliver AAQ with a slow release device. Such devises can be efficacious at delivering a constant dose of drug in the eye over long periods of time.

While the challenges of developing a photochemical restoration of vision are formidable, there are many significant advantages of a small molecule therapeutic compared to other approaches currently being tested.

Gene replacement approaches hold great promise for the treatment of inherited retinal degenerations (Stieger and Lorenz, 2010). Such approaches are designed to reactivate the remaining (sickly) photoreceptors or retinal pigment epithelium (RPE) cells by delivering the correct form of a defective gene. Gene replacement approaches would be expected to provide the biggest gains in visual function since they harness the intact retinal circuitry in which much of the processing of visual information takes place (Figure 1C). However, since strategies aimed at a specific gene defect require the presence of at least some of the target cells, there can be a limited window of opportunity. Due to cargo constraints of many of the available gene transfer vectors, it is also difficult or impossible to deliver regulatory sequences and cDNAs above a certain size. In addition, the financial burdens of developing a gene therapy drug are significant, and when one considers that over 180 different gene therapy products (each specific for a different retinal gene) would be required to treat all of the inherited forms of retinal degeneration, this approach seems daunting. Direct, light-gating approaches like the AAQ strategy assessed by Polosukhina et al. (2012) provide a potentially more generalizable approach.

Other light-gating therapies are also being explored. Optogenetic gene therapy using ChR or NpHR also shows therapeutic promise for retinal degenerative disease (Busskamp et al., 2012). The delivered genes were originally identified in single cell organisms that exhibit phototaxis. Unlike mammalian opsins, these light-activated proteins directly polarize (NpHR) or depolarize (ChRd) the cell upon photostimulation, without the requirement for additional proteins and enzymes. Delivery of these genes to the appropriate retinal cells using viral vectors (NpHR to degenerating cone photoreceptors and ChRd to the remaining bipolar or ganglion cells; Figure 1C) can restore visual responses in mice. As with AAQ therapy, it will be important to test safety and efficacy of this approach in large animal models. Also, like AAQ therapy, activation of the natural NpHR and ChRd molecules will probably require use of a device able to transmit the appropriate wavelength and intensity of light.

Electronic chips have been placed both epiretinally and subretinally in retinal degeneration patients (Figure 1C). Because they take advantage of the additional processing and cellular connections of the inner retinal neurons, subretinal application of the electronic chips would be expected to provide even more visual detail than epiretinal placement (Figure 1C). Two subretinally applied chips, the ARGUS II (Second Sight Medical Products) and the Alpha IMS (Retina Implant AG), have a fair amount of human patient experience and are in clinical trial. Steps continue to be taken to improve these devices (for example, to develop wireless power transmission and to develop higher resolution chips).

Finally, there is a great deal of interest in stem cell approaches (Ong and da Cruz, 2012). Human stem cells have the potential to develop into a variety of different cell types including photoreceptors or RPE cells. A phase 1 clinical trial is in the process of evaluating effects of transplantation of human embryonic stem cells into the subretinal space (Figure 1C). Additional preclinical studies aim to evaluate the potential of induced pluripotent stem cells to engraft, differentiate, and restore function in the diseased retina. Like the other approaches, there are considerable challenges with stem cell delivery. Will the properties of the cells change over time, will they remain localized or spread to undesirable locations, will there be a harmful immune response, and, in the case of cells destined to become photoreceptors, will they synapse appropriately with target cells?

The passive delivery method for the AAQ compound provides many potential benefits over alternative methods, though there are benefits and drawbacks to all of the above strategies. The utility of the AAQ approach may, however, also extend to organ systems outside of the eye. The transparency of the cornea, media, and retina make it possible to move quickly with the development of retinal therapeutics that harness light-activatable drugs or components, but the AAQ-mediated light activation of other neurons may soon be within our reach. Particular wavelengths of light can penetrate millimeters of skin, for example, and it may be possible to target neurons that control response to touch, temperature, itch, or pain through photochemical therapy. Other organs readily accessible to light through fiber optic technology may be targets as well. With more invasive procedures, it may ultimately be possible to alter broader neurologic functions, such as circadian rhythm and behavior, through photochemical approaches. This is an exciting time for retinal degeneration research given the many promising approaches for visual restoration on the horizon, but the real excitement may come from the revolution in science and medicine already underway from the broader application of these light-activatable technologies.

## ACKNOWLEDGMENTS

J.B. is a cofounder of GenSight.

## REFERENCES

Busskamp, V., Picaud, S., Sahel, J.A., and Roska, B. (2012). Gene Ther. *19*, 169–175.

Ong, J.M., and da Cruz, L. (2012). Br. Med. Bull. *102*, 133–146.

Polosukhina, A., Lin, J., Tochitsky, I., Nemargut, J., Sychev, Y., De, C., Kouchkovsky, E., Huang, T., Borges, K., Trauner, D., et al. (2012). Neuron *75*, this issue, 271–282.

Stieger, K., and Lorenz, B. (2010). Discov. Med. *10*, 425–433.