

# UC Berkeley

## UC Berkeley Previously Published Works

**Title**

Optopharmacological tools for restoring visual function in degenerative retinal diseases

**Permalink**

<https://escholarship.org/uc/item/61z0r4n8>

**Journal**

Current Opinion in Neurobiology, 34

**ISSN**

0959-4388

**Authors**

Tochitsky, Ivan

Kramer, Richard H

**Publication Date**

2015-10-01

**DOI**

10.1016/j.conb.2015.01.018

Peer reviewed



Published in final edited form as:

*Curr Opin Neurobiol.* 2015 October ; 34: 74–78. doi:10.1016/j.conb.2015.01.018.

## Optopharmacological tools for restoring visual function in degenerative retinal diseases

Ivan Tochitsky<sup>1</sup> and Richard H Kramer

Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

### Abstract

Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are progressive retinal diseases that result from the death of rod and cone photoreceptors, ultimately leading to blindness. The only currently approved vision restoration treatment employs an implanted retinal ‘chip’ as a prosthetic device to electrically stimulate retinal neurons that survive after the photoreceptors are gone, thereby restoring light-driven neural signaling to the brain. An alternative strategy has been proposed, which would utilize optogenetic or opto-pharmacological tools to enable direct optical stimulation of surviving retinal neurons. Here, we review the latest studies evaluating the feasibility of these molecular tools as potential therapeutics for restoring visual function in human blinding disease.

Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are degenerative retinal disorders that lead to the progressive loss of rod and cone photoreceptors from the retina, leading to varying degrees of vision loss. Unfortunately, rods and cones do not regenerate, so the two million people around the world suffering from RP face the prospect of irreversible visual decline [1]. To address this unmet clinical need, several methods for reanimating the blind retina have been advanced in recent years [2–4]. Broadly speaking, the proposed strategies for restoring visual function focus either on replacing the photoreceptor cells lost due to degeneration (e.g. with stem cell progenitors) or electrically or chemically manipulating the surviving non-photoreceptive neurons to restore light-driven signaling to the brain. This review will focus on the latter strategy, with a special focus on recent developments in the field.

The light response of rods and cones is transmitted through the neural circuitry of the retina, culminating in the retinal ganglion cells (RGCs), through which all visual information is funneled to the brain. Synaptic connections in the retina undergo profound remodeling as photoreceptor degeneration progresses [5–7], with morphological changes happening quickly in the outer retina and extending to the inner retina in late stages of disease [8].

Corresponding author: Kramer, Richard H (rhkramer@berkeley.edu).

<sup>1</sup>Current address: F.M. Kirby Neurobiology Center, Boston Children’s Hospital and Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA.

### Conflict of interest statement

Richard H. Kramer has a financial interest and is on the Board of Directors of Photoswitch Biosciences, Inc., a company interested in commercializing technologies employing photoswitch compounds.

Edited by **David Julius** and **John Carlson**

However, the number of RGCs somata and the number of their axons in the optic nerve stays constant, implying that RGCs retain their normal connections to the brain. Since visual information is transmitted to the brain via a spatially and temporally encoded pattern of RGC action potentials, vision might, in principle, be restored by artificially stimulating RGCs to reproduce their normal output.

The first method for stimulating surviving retinal neurons involves the use of a surgically implanted electronic prosthetic — an array of stimulating electrodes that triggers the firing of nearby neurons. These stimulating electrodes can be electronically controlled by an external camera or contain a photovoltaic diode [9] to convert optical stimuli into electrical currents. Current electronic prosthetics can be implanted either subretinally or epiretinally and typically consist of a few dozen to a few hundred electrodes [10] although newer devices with higher electrode density are under development [9]. The 60 electrode ARGUS II epiretinal implant has restored simple shape discrimination to blind patients [11] indicating that artificial stimulation of RGCs *in vivo* can generate a useful visual experience. Recently, this implant was approved for clinical use by the FDA — making electronic prosthetics the only currently available treatment for blind RP patients.

Despite promising results from early clinical trials, retinal implants suffer from a number of limitations. First, implantation of the retinal chip requires invasive surgery. Second, even if the procedure is successful, the restored visual acuity is low. The healthy human retina contains ~1.2 million RGCs, but current retinal chips contain only a few dozen to a few hundred electrodes spaced 100–200  $\mu\text{m}$  apart, up to 10-fold wider than the packing density of RGCs [12]. In the central retina, RGCs are not spread in a monolayer but rather piled several stories high, making selective electrical stimulation of individual cells difficult if not impossible. At present, the resolution provided by retinal prosthetics is several orders of magnitude lower than the theoretical limits imposed by the RGC density in the macula, the retinal region crucial for high-acuity vision. The stimulated area of the retina is likewise limited by the physical size of the chip, which typically only covers the central 20 degrees of vision in the macula [13]. Larger chips with higher electrode densities can be manufactured, but may result in problems with power delivery or crosstalk between neighboring electrodes [14].

Two alternative strategies have been proposed to overcome these limitations. The first involves the expression of light-sensitive microbial opsins, which can generate depolarizing or hyperpolarizing electrical currents in response to light (Figure 1a). Viral delivery of genes encoding optogenetic tools, including light-activated channels [15•,16–18], transporters [19•], or receptors [20–22] can bestow light-sensitivity on retinal neurons that survive after photoreceptor degeneration. Expression of optogenetic tools in RGCs [20], bipolar cells [16,23], or surviving cone remnants [19•] can restore light-elicited behavioral responses in mouse models of RP. In principle, such treatments can confer light-sensitivity to all neurons of a particular cell type, allowing for high visual acuity. However, in practice, the efficiency of viral transduction tends to be low, resulting in the expression in a minority of targeted cells, for example ~5% of mouse bipolar cells [16] or 5–10% of marmoset RGCs [24], although new viral vectors with improved transduction efficiencies are under development [23].

So far, the only type of gene therapy approved for clinical trials involves adding a wild-type human gene to compensate for a dysfunctional or null mutant allele, as has been accomplished successfully in treating Leber's congenital amaurosis [25]. All viral-mediated gene therapies carry the risk of triggering immune responses [26], but there are additional challenges in genetically introducing a microbial opsin for vision restoration. Microbial optogenetics require the robust expression of an alien gene in humans, an unprecedented medical intervention. High level expression of a microbial opsin can alter neuronal morphology and function [27], with uncertain long-term consequences. Light responses can be restored with the gene encoding melanopsin, an opsin from intrinsically photosensitive RGCs [21], but melanopsin-mediated light responses are small, slow and persistent, and they may not be adequate for visual perception. A two-component approach, involving exogenous introduction of a kainate receptor and intraocular drug injection, can also restore light responses [20], but the combined hurdles of gene therapy and repeated intraocular drug administration will be difficult to surmount for clinical applications. In general, the potential permanence of optogenetic interventions might be favorable in the absence of complications but any deleterious side effects of these treatments could be very difficult or even impossible to reverse.

We have developed yet another strategy, which utilizes light-sensitive drugs (opto-pharmacological tools) for conferring light-sensitivity onto endogenous voltage-gated ion channels in retinal neurons. This approach requires no genetic modification. By adding a photoisomerizable small molecule (a 'photoswitch') light can be used to manipulate endogenous channels and thereby control action potential firing in retinal neurons (Figure 1b) [28–30]. The first such photoswitch developed, called AAQ (Figure 2a), is a light sensitive potassium channel blocker. When applied onto the retina of blind *rd1* mice, AAQ enables robust light responses, as observed in extracellular and intracellular electrophysiological recordings [31••]. AAQ photosensitizes multiple types of retinal neurons, primarily amacrine cells, resulting in synaptically amplified responses and center-surround antagonism in RGCs. Beyond electrophysiology, a single intravitreal injection of AAQ is sufficient to restore the pupillary light reflex and simple light avoidance behaviors in blind mice *in vivo*.

Despite its ability to restore visual function to blind mice, several adverse properties of AAQ probably preclude its therapeutic use in humans. Photoswitching of AAQ requires high intensity, UV light and its effect *in vivo* lasts for only one day after an intravitreal injection [31••]. The human lens filters out most UV light [32] and repeated exposure to high intensity light can be damaging [33]. AAQ's short half-life (<one day) would necessitate frequent injections of the compound into the eye, which is unsuitable for long-term treatment in humans. To overcome these shortcomings we tested several candidate photoswitch compounds whose action spectra we found to be red-shifted. The most promising is DENAQ (Figure 2b), which responds to visible light in the blue-green range (450–550 nm) and turns off rapidly in the dark [34].

Like AAQ, DENAQ confers robust light responses onto RGCs from blind *rd1* mice (Figure 2c,d) [35••]. These responses can be elicited with broad spectrum white light of intensity typical of daylight, in the mid-photopic range. The light response terminates within several

hundred milliseconds after light offset [35••], allowing for the detection of moderate frequency visual stimuli. The DENAQ-mediated light response is spatially precise, allowing for pinpoint stimulation of illuminated RGCs and suggesting that high-acuity vision might be possible. Unlike AAQ, DENAQ primarily targets RGCs themselves, since blocking synaptic inputs onto RGCs did not alter the light response.

A single injection of DENAQ *in vivo* photosensitizes blind mouse retinas for up to a week, considerably longer than AAQ, without any evidence of toxicity. Newer photoswitches with more prolonged lifetimes in the eye are under development and encapsulation in slow release biodegradable polymers may enable even longer-term drug delivery to minimize the frequency of intraocular injections [36]. Like AAQ, DENAQ restores light perception in blind mice, allowing treated animals to use light stimuli in a learned behavioral task.

Photoreceptor degeneration in human RP begins in the periphery and progresses centrally over many years, leaving most patients with ‘tunnel vision’, long before complete loss of light perception [1]. DENAQ injected into the eye can diffuse through the vitreous to reach the entire retina, potentially affecting both degenerated and normal regions. This makes it important to evaluate the effects of DENAQ not only on degenerated retinas from mutant mice, but also on normal retinas from wild-type mice. To our great surprise, we discovered that DENAQ has almost no effect on the normal, rod/cone-mediated light response of wild-type retinas, in contrast to its robust effect on retinas from a variety of animal models of RP [35••]. DENAQ affects several types of voltage-gated ion channels in *rd1* RGCs, including potassium channels and hyperpolarization activated, cyclic nucleotide gated (HCN) channels. Nonetheless, we determined that HCN is crucial for the DENAQ-mediated light response. Presumably, rod/cone degeneration leads to changes in HCN channel expression or function in RGCs, and these underlie DENAQ’s disease-specific effects. The nature of the ‘degeneration signal’ that triggers changes in RGCs is still unknown.

Since photoswitches are drug-like small molecules, they have several potential advantages over optogenetic and retinal prosthetic strategies for vision restoration. The effect of the photoswitches is reversible, allowing dosage to be adjusted to maximize efficacy and minimize toxicity. Unlike other vision restoration technologies that commit a patient to one course of treatment, photoswitch treatment can be initiated or terminated as necessary, and improved photoswitch compounds with higher light sensitivity and faster response kinetics can be deployed to patients as they are developed. The disease-selectivity of DENAQ and related compounds could also prove useful in the clinic. These compounds might bypass healthy parts of the retina and target blind regions. If so, this could fill in the gaps in the visual world, which would be especially valuable for patients suffering from localized forms of degeneration, perhaps including AMD. Finally, if they prove safe in preclinical testing on animals, the simplicity and reversibility of the photoswitch approach may streamline regulatory approval by the FDA for clinical trials as an Investigational New Drug (IND).

Ideally, a vision restoring therapy would be tested in primates with eyes similar to ours, but no good primate model of inherited retinal degeneration is available. Instead of primates, mouse and rat models of RP are the best available options for preclinical testing. In mice and rats, various mutations in the phototransduction machinery can lead to photoreceptor

degeneration that progresses at different rates [37,38]. Large animal models of inherited photoreceptor degeneration such as dogs and pigs also exist [38–40], but are expensive and have long generation times. Despite the well characterized retinal degeneration in animal models, the integrity of the downstream components of the visual system in the brain has received remarkably little attention. It is unknown whether the circuitry of the thalamus or the visual cortex is altered, as has been shown in blind human patients [41]. Moreover, it is possible that gain-adjusting modulatory mechanisms involved in visual attention may be altered in blind animals, perhaps taking visual recognition partly or fully ‘offline’. Our incomplete understanding of the visual system in a blind animal makes it difficult to predict whether visual perception will be regained when retinal light responses are restored. Till date, both optogenetics and opto-pharmacology have been able to restore simple light/dark discrimination behaviors in blind rodents, but no treatment has yet convincingly restored object recognition.

The ultimate test of how well restoration of retinal photosensitivity translates into vision will depend on evaluation in blind humans. The recent technological advances discussed in this article are highly encouraging and the next few years may see one or more clinical trials employing these technologies on RP patients, hopefully with a successful outcome.

## Acknowledgments

This work was supported by grants from the NIH (PN2 EY018241 and P30 EY003176), a research grant from the Beckman Institute for Macular Research, and a Wynn-Gund grant from the Foundation Fighting Blindness.

## References and recommended reading

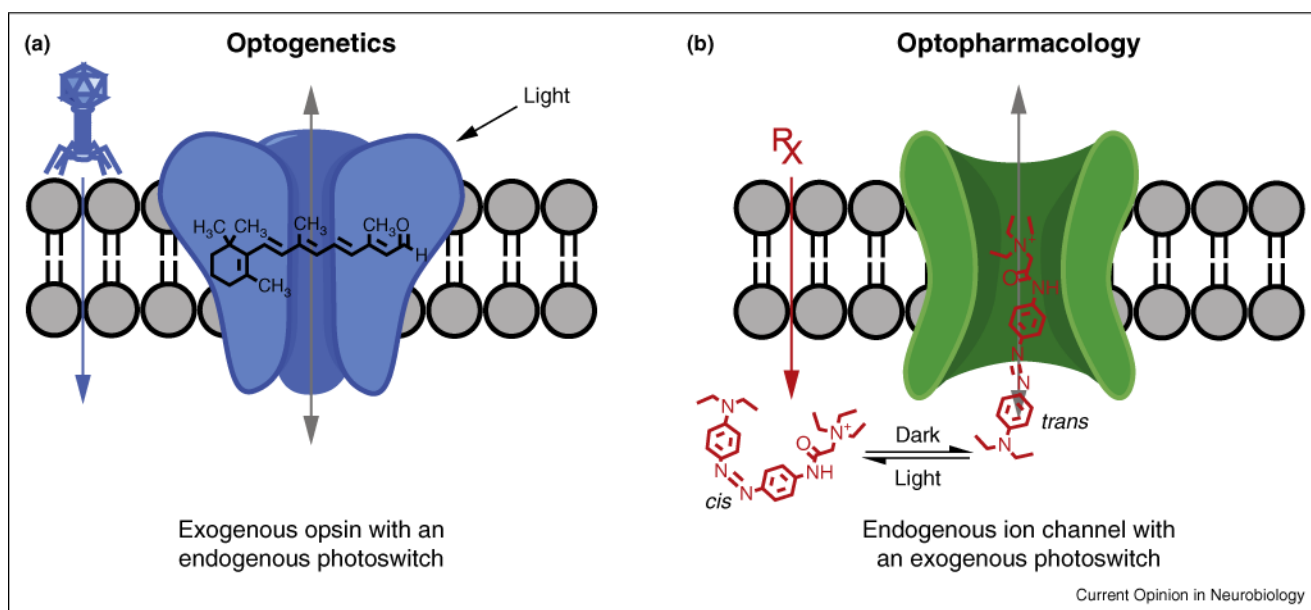
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

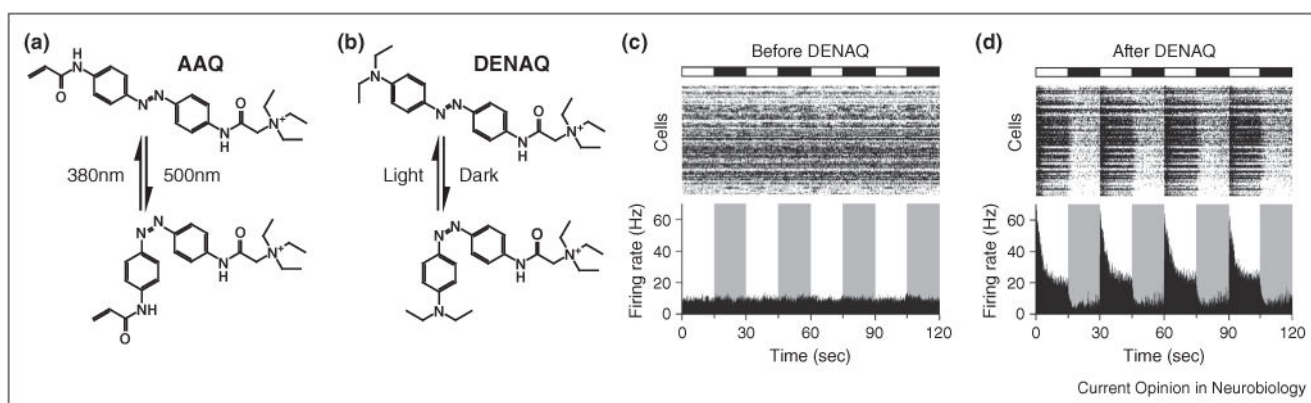
1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006; 368:1795–1809. [PubMed: 17113430]
2. Chuang AT, Margo CE, Greenberg PB. Retinal implants: a systematic review. *Br J Ophthalmol*. 2014; 98:852–856. [PubMed: 24403565]
3. Busskamp V, Roska B. Optogenetic approaches to restoring visual function in retinitis pigmentosa. *Curr Opin Neurobiol*. 2011; 21:942–946. [PubMed: 21708457]
4. Ramsden CM, Powner MB, Carr AJ, Smart MJ, da Cruz L, Coffey PJ. Stem cells in retinal regeneration: past, present and future. *Development*. 2013; 140:2576–2585. [PubMed: 23715550]
5. Jones BW, Kondo M, Terasaki H, Lin Y, McCall M, Marc RE. Retinal remodeling. *Jpn J Ophthalmol*. 2012; 56:289–306. [PubMed: 22644448]
6. Margolis DJ, Newkirk G, Euler T, Detwiler PB. Functional stability of retinal ganglion cells after degeneration-induced changes in synaptic input. *J Neurosci*. 2008; 28:6526–6536. [PubMed: 18562624]
7. Mazzoni F, Novelli E, Strettoi E. Retinal ganglion cells survive and maintain normal dendritic morphology in a mouse model of inherited photoreceptor degeneration. *J Neurosci*. 2008; 28:14282–14292. [PubMed: 19109509]
8. O’Brien EE, Greferath U, Fletcher EL. The effect of photoreceptor degeneration on ganglion cell morphology. *J Comp Neurol*. 2014; 522:1155–1170. [PubMed: 24519018]

9. Mathieson K, Loudin J, Goetz G, Huie P, Wang L, Kamins TJ, Galambos L, Smith R, Harris JS, Sher A, et al. Photovoltaic retinal prosthesis with high pixel density. *Nat Photon*. 2012; 6:391–397.
10. Weiland JD, Cho AK, Humayun MS. Retinal prostheses: current clinical results and future needs. *Ophthalmology*. 2011; 118:2227–2237. [PubMed: 22047893]
11. Humayun MS, Dorn JD, da Cruz L, Dagnelie G, Sahel JA, Stanga PE, Cideciyan AV, Duncan JL, Elliott D, Filley E, et al. Interim results from the international trial of second sight's visual prosthesis. *Ophthalmology*. 2012; 119:779–788. [PubMed: 22244176]
12. Winter JO, Cogan SF, Rizzo JF 3rd. Retinal prostheses: current challenges and future outlook. *J Biomater Sci Polym Ed*. 2007; 18:1031–1055. [PubMed: 17705997]
13. Chader GJ, Weiland J, Humayun MS. Artificial vision: needs, functioning, and testing of a retinal electronic prosthesis. *Prog Brain Res*. 2009; 175:317–332. [PubMed: 19660665]
14. Wilke RG, Moghadam GK, Lovell NH, Suaning GJ, Dokos S. Electric crosstalk impairs spatial resolution of multi-electrode arrays in retinal implants. *J Neural Eng*. 2011; 8:046016. [PubMed: 21673395]
15. Bi A, Cui J, Ma YP, Olshevskaya E, Pu M, Dizhoor AM, Pan ZH. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron*. 2006; 50:23–33. First demonstration of optogenetic vision restoration in rd1 mice. [PubMed: 16600853]
16. Lagali PS, Balya D, Awatramani GB, Munch TA, Kim DS, Busskamp V, Cepko CL, Roska B. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci*. 2008; 11:667–675. [PubMed: 18432197]
17. Chuong AS, Miri ML, Busskamp V, Matthews GA, Acker LC, Sorensen AT, Young A, Klapoetke NC, Henninger MA, Kodandaramaiah SB, et al. Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat Neurosci*. 2014; 17:1123–1129. [PubMed: 24997763]
18. Tomita H, Sugano E, Isago H, Hiroi T, Wang Z, Ohta E, Tamai M. Channelrhodopsin-2 gene transduced into retinal ganglion cells restores functional vision in genetically blind rats. *Exp Eye Res*. 2010; 90:429–436. [PubMed: 20036655]
19. Busskamp V, Duebel J, Balya D, Fradot M, Viney TJ, Siebert S, Groner AC, Cabuy E, Forster V, Seeliger M, et al. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science*. 2010; 329:413–417. Optogenetic reactivation of cone photoreceptors in mouse models of RP. [PubMed: 20576849]
20. Caporale N, Kolstad KD, Lee T, Tochitsky I, Dalkara D, Trauner D, Kramer R, Dan Y, Isacoff EY, Flannery JG. LiGluR restores visual responses in rodent models of inherited blindness. *Mol Ther*. 2011; 19:1212–1219. [PubMed: 21610698]
21. Lin B, Koizumi A, Tanaka N, Panda S, Masland RH. Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc Natl Acad Sci USA*. 2008; 105:16009–16014. [PubMed: 18836071]
22. Gaub BM, Berry MH, Holt AE, Reiner A, Kienzler MA, Dolgova N, Nikonov S, Aguirre GD, Beltran WA, Flannery JG, et al. Restoration of visual function by expression of a light-gated mammalian ion channel in retinal ganglion cells or ON-bipolar cells. *Proc Natl Acad Sci USA*. 2014; 111:E5574–E5583. [PubMed: 25489083]
23. Cronin T, Vandenberghe LH, Hantz P, Juttner J, Reimann A, Kacso AE, Huckfeldt RM, Busskamp V, Kohler H, Lagali PS, et al. Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter. *EMBO Mol Med*. 2014; 6:1175–1190. [PubMed: 25092770]
24. Ivanova E, Hwang GS, Pan ZH, Troilo D. Evaluation of AAV-mediated expression of Chop2-GFP in the marmoset retina. *Invest Ophthalmol Vis Sci*. 2010; 51:5288–5296. [PubMed: 20484599]
25. Bainbridge JWB, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008; 358:2231–2239. [PubMed: 18441371]
26. Beltran WA, Boye SL, Boye SE, Chiodo VA, Lewin AS, Hauswirth WW, Aguirre GD. rAAV2/5 gene-targeting to rods: dose-dependent efficiency and complications associated with different promoters. *Gene Therapy*. 2010; 17:1162–1174. [PubMed: 20428215]

27. Miyashita T, Shao YR, Chung J, Pourzia O, Feldman DE. Long-term channelrhodopsin-2 (ChR2) expression can induce abnormal axonal morphology and targeting in cerebral cortex. *Front Neural Circuits*. 2013; 7:8. [PubMed: 23386813]
28. Fortin DL, Banghart MR, Dunn TW, Borges K, Wagenaar DA, Gaudry Q, Karakossian MH, Otis TS, Kristan WB, Trauner D, et al. Photochemical control of endogenous ion channels and cellular excitability. *Nat Methods*. 2008; 5:331–338. [PubMed: 18311146]
29. Mourot A, Tochitsky I, Kramer RH. Light at the end of the channel: optical manipulation of intrinsic neuronal excitability with chemical photoswitches. *Front Mol Neurosci*. 2013; 6:5. [PubMed: 23518818]
30. Kramer RH, Mourot A, Adesnik H. Optogenetic pharmacology for control of native neuronal signaling proteins. *Nat Neurosci*. 2013; 16:816–823. [PubMed: 23799474]
- 31••. Polosukhina A, Litt J, Tochitsky I, Nemargut J, Sychev Y, De Kouchkovsky I, Huang T, Borges K, Trauner D, Van Gelder RN, et al. Photochemical restoration of visual responses in blind mice. *Neuron*. 2012; 75:271–282. First demonstration of optopharmacological vision restoration in rd1 mice with AAQ. [PubMed: 22841312]
32. Artigas JM, Felipe A, Navea A, Fandino A, Artigas C. Spectral transmission of the human crystalline lens in adult and elderly persons: color and total transmission of visible light. *Invest Ophthalmol Vis Sci*. 2012; 53:4076–4084. [PubMed: 22491402]
33. Noell WK, Walker VS, Kang BS, Berman S. Retinal damage by light in rats. *Invest Ophthalmol*. 1966; 5:450–473. [PubMed: 5929286]
34. Mourot A, Kienzler MA, Banghart MR, Fehrentz T, Huber FM, Stein M, Kramer RH, Trauner D. Tuning photochromic ion channel blockers. *ACS Chem Neurosci*. 2011; 2:536–543. [PubMed: 22860175]
- 35••. Tochitsky I, Polosukhina A, Degtyar VE, Gallerani N, Smith CM, Friedman A, Van Gelder RN, Trauner D, Kaufer D, Kramer RH. Restoring visual function to blind mice with a photoswitch that exploits electrophysiological remodeling of retinal ganglion cells. *Neuron*. 2014; 81:800–813. Characterization of DENAQ, an improved and disease selective photoswitch for vision restoration. [PubMed: 24559673]
36. Thrimawithana TR, Young S, Bunt CR, Green C, Alany RG. Drug delivery to the posterior segment of the eye. *Drug Discov Today*. 2011; 16:270–277. [PubMed: 21167306]
37. Chang B, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR. Retinal degeneration mutants in the mouse. *Vision Res*. 2002; 42:517–525. [PubMed: 11853768]
38. Fletcher EL, Jobling AI, Vessey KA, Luu C, Guymer RH, Baird PN. Animal models of retinal disease. *Prog Mol Biol Transl Sci*. 2011; 100:211–286. [PubMed: 21377628]
39. Petersen-Jones S. Advances in the molecular understanding of canine retinal diseases. *J Small Anim Pract*. 2005; 46:371–380. [PubMed: 16119056]
40. Ross JW, de Castro JPF, Zhao J, Samuel M, Walters E, Rios C, Bray-Ward P, Jones BW, Marc RE, Wang W, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. *Investig Ophthalmol Visual Sci*. 2012
41. Merabet LB, Pascual-Leone A. Neural reorganization following sensory loss: the opportunity of change. *Nat Rev Neurosci*. 2010; 11:44–52. [PubMed: 19935836]



**Figure 1.**  
Optogenetics and Optopharmacology: two strategies for restoring visual responsiveness to degenerated retina.



**Figure 2.** Optopharmacological tools restore light responses to the retinas of blind mice. **(a, b)** Photoisomerization of AAQ and DENAQ, two ion channel photoswitches. **(c)** Multi-electrode array recordings from retinal ganglion cells (RGCs) in the retina of blind rd1 mice. Before DENAQ treatment light has no effect on firing, after DENAQ treatment light increases RGC firing. Top shows raster plots of individual RGC activities, bottom shown average of all RGCs.