

Optogenetics for visual restoration: From proof of principle to translational challenges

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ARTICLE INFO

Keywords:

Optogenetics

Gene therapy

Retina

Melanopsin

Channelrhodopsin

Remodelling

ABSTRACT

Degenerative retinal disorders are a diverse family of diseases commonly leading to irreversible photoreceptor death, while leaving the inner retina relatively intact. Over recent years, innovative gene replacement therapies aiming to halt the progression of certain inherited retinal disorders have made their way into clinics. By rendering surviving retinal neurons light sensitive optogenetic gene therapy now offers a feasible treatment option that can restore lost vision, even in late disease stages and widely independent of the underlying cause of degeneration.

Since proof-of-concept almost fifteen years ago, this field has rapidly evolved and a detailed first report on a treated patient has recently been published. In this article, we provide a review of optogenetic approaches for vision restoration. We discuss the currently available optogenetic tools and their relative advantages and disadvantages. Possible cellular targets will be discussed and we will address the question how retinal remodelling may affect the choice of the target and to what extent it may limit the outcomes of optogenetic vision restoration. Finally, we will analyse the evidence for and against optogenetic tool mediated toxicity and will discuss the challenges associated with clinical translation of this promising therapeutic concept.

1. Introduction

1.1. Retinal degenerative disorders are a common cause of blindness

Degenerative retinal disorders are among the commonest cause of vision loss in industrial countries and represent a major socioeconomic burden (Chavarayan et al., 2020; Galvin et al., 2020; Resnikoff et al., 2004). These include the rather common multi-factorial Age-related Macular Degeneration (AMD) and the Inherited Retinal Degenerations (IRDs) like retinitis pigmentosa. While AMD leads to a predominantly central vision loss in late stages, usually after retirement age (Klein et al., 1993; Lim et al., 2012; Lindner et al., 2018), IRDs may lead to full peripheral and central vision loss in working age (Galvin et al., 2020; Hartong et al., 2006). Current estimates suggest that IRDs affect 1:3000 people worldwide (Hartong et al., 2006), and to date disease causing mutations have been identified within >100 genes and loci (Perea-Romero et al., 2021; Pontikos et al., 2020; Stone et al., 2017), comprising

>1000 individual variants (Perea-Romero et al., 2021). Thus, degenerative retinal disorders are highly heterogeneous in terms of their underlying genetics, pathophysiology and their clinical course (Bax et al., 2019; Cundy et al., 2020; Fritsche et al., 2016), they typically share a final common pathology and ultimately lead to irreversible photoreceptor death and loss of vision (Fig. 1).

1.2. Gene therapy has become a clinical reality

The emergence of gene replacement therapies has begun to offer therapeutic options for some inherited retinal disease, providing a mechanism to correct underlying genetic defects that ultimately lead to photoreceptor loss. Clinical trials have begun for a number of conditions (Thompson et al., 2020) and have now received regulatory approval in some jurisdictions. The first retinal gene therapy to be approved was Voretigene neparvovec (Luxturna®), an Adeno-Associated Virus serotype 2 (AAV2) vector containing the human RPE65 gene designed for

Abbreviations: Melanopsin, Opn4.

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<https://doi.org/10.1016/j.preteyeres.2022.101089>

Received 18 February 2022; Received in revised form 17 May 2022; Accepted 19 May 2022

Available online 9 June 2022

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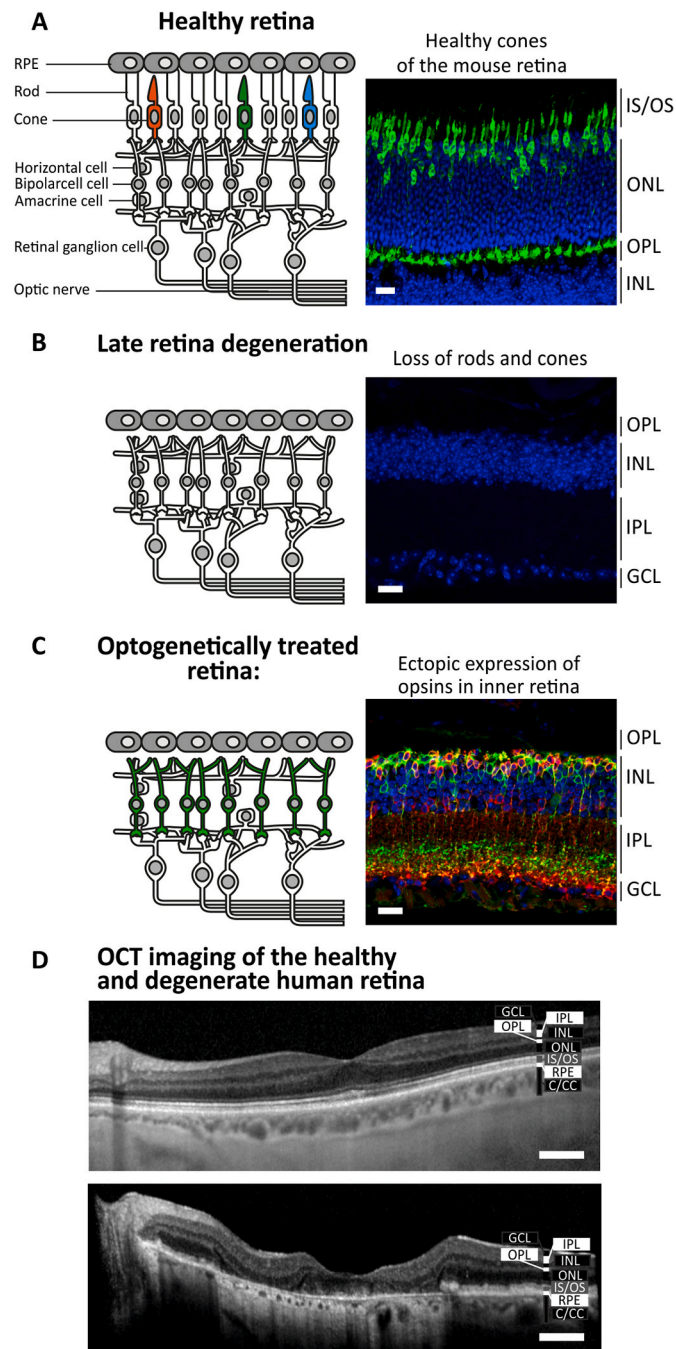


Fig. 1. Retina degeneration and the concept of optogenetic vision restoration.

(A-C – Left column) Cartoon schematics depicting the concept of optogenetic vision restoration, showing a healthy retina with normal rods and cones (A), a degenerate retina (B) and (C) an optogenetically treated retina where cells of the inner retina (here: bipolar cells) have been converted into artificial photoreceptors. Right column shows corresponding confocal microscope images of mouse retina, with (A) showing cone morphology (green) and cell nuclei (blue) in normal wildtype retina, and (b) in rd1 retina at postnatal day P150 (Phase III degeneration). Note the complete loss of the outer nuclear layer and cone staining at P150. (D) Optical coherence tomography (OCT) images from human patients with healthy retina (upper image) and retina degeneration (lower image, here: age-related macular degeneration). Note the thinning and disruption in particular of the outer retina layers following retina disease compared to healthy retina. Schematics and confocal microscope images are presented photoreceptor-side up while the OCT images are presented photoreceptor-side down as per convention. IS/OS: Photoreceptor inner and outer segments, ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: inner plexiform layer, GCL: Ganglion cell layer, RPE: Retinal pigment epithelium, C/CC: Choroid and choriocapillaris. In D label colours are chose according to the OCT band colour.

the treatment of recessive RPE65-linked Leber's congenital amaurosis (Maguire et al., 2019; Russell et al., 2017). Luxturna®, like other gene replacement therapies under development, are primarily intended to be administered early during retinal disease to correct the genetic cause of on-going pathology and arrest further visual loss. Recent years have seen an explosion in the molecular diagnosis of retinal disease – which enables a genetic diagnosis in up to three quarters of cases (Farrar et al., 2017). Hence it would appear that gene replacement therapies may in future offer viable treatments for IRDs. However, the large number of disease-causing mutations identified to date (in >100 genes), the relatively large number of cases for which a genetic cause is yet unknown (around 30%; Birtel et al., 2018; Birtel et al., 2019; Perea-Romero et al., 2021; Stone et al., 2017), and the high cost of developing such tailored treatments (costs for Luxturna® are \$400,000 per eye, with some differences between markets/jurisdictions) potentially limits this approach for widespread treatment of IRDs. Furthermore, and potentially most importantly, it is clear that such gene replacement approaches are not an effective treatment for end stage retinal degeneration, where a near complete loss of post-mitotic photoreceptors has already occurred. There is therefore a clinical need to identify regenerative medicine approaches to restore visual function for the large number of patients who are already in the later stages of retinal degeneration, lacking any significant number of photoreceptors but retaining the remainder of their visual pathways.

To this end, one approach being investigated is the use of prosthetic 'bionic eye' type devices. These devices comprise an external camera which records and then processes and transmits visual signals to a small electrode array-based device surgically implanted onto or beneath the retina (Yue et al., 2016). Some of these devices are FDA approved and have shown early promise, restoring basic visual perception and improving performance in a number of visual tasks (Finn et al., 2018; Ostad-Ahmadi et al., 2021; White et al., 2021). At present, technical limitations regarding the total number of electrodes, and the density of electrodes that can be incorporated into such devices, the inability to cover large areas of the retina, and issues of long-term biocompatibility remain the major hurdles for these devices. In 2019 both SecondSight Medical Products who manufacture the Argus II device, and Retina Implant AG who manufacture the Alpha IMS device ceased manufacturing and implantation of these artificial retina devices (Ayton et al., 2020a; Strickland, 2022). In addition to prosthetic devices, various types of cell transplantation therapy are also currently under investigation: Pre-clinical studies aimed at directly replacing lost photoreceptors using either immature photoreceptor precursor cells, human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) (Singh et al., 2020; Zuzic et al., 2022). Although clinical trials have begun in a number of cases, in general there has been only limited success with photoreceptor stem cell therapy (Singh et al., 2020), not least due to problems of donor cell survival and functional integration, but also disease related changes in the retinal pigment epithelium that is essential for normal photoreceptor function. The current state of cell transplantation therapies for visual restoration have been recently covered in a number of excellent reviews (Caras et al., 2021; Gasparini et al., 2019; Jin et al., 2019; Singh et al., 2020; Zuzic et al., 2022) and will not be covered in further detail here.

1.3. Ectopic expression of opsins renders inner retinal neurons light sensitive

A novel strategy, termed *therapeutic optogenetics* now offers a viable treatment for late-stage retinal degeneration. Optogenetics involves the expression of transgenes encoding light sensitive proteins in surviving retinal cells to make them directly light sensitive (Berry et al., 2019; Bi et al., 2006; Cehajic-Kapetanovic et al., 2015; Gaub et al., 2015; Lagali et al., 2008; Lindner et al., 2021; Sengupta et al., 2016; van Wyk et al., 2015). Such an optogenetic gene therapy could work independent of the underlying genetic cause of degeneration and does not depend on the

integrity of the retinal pigment epithelium-photoreceptor complex. Thereby, it offers the potential to restore vision even in late-stage retinal degenerations, potentially using a universal approach for the treatment of many different degenerative retinal disorders. Work by several groups has shown that light responses can be restored in rodents with degenerate retina using this method (Bi et al., 2006; Busskamp et al., 2010). There is moreover data indicating that restored light responses can drive visually guided behaviours (functional vision) in treated animals. Consequently, early-stage clinical trials of optogenetic vision restoration have been initiated (for examples see the Phase I/II trials RST-001 and PIONEER including patients with advanced retinitis pigmentosa, NCT02556736 and NCT03326336 on clinicaltrials.gov, respectively).

2. Different classes of opsins are being studied as tools for vision restoration

To-date a broad spectrum of light sensitive proteins (opsins) have been studied for the purpose of vision restoration, but most commonly include microbial light gated ion channels, or mammalian light sensitive G-protein coupled receptors. There are several review papers describing their features in much detail (e.g.: Klapper et al., 2016; Simon et al., 2020; Tomita and Sugano, 2021). Most of these reviews put a focus on one particular class of opsins. Here we will briefly introduce each of the relevant classes, highlighting their key features as well as the potential advantage and disadvantages of each in the context of vision restoration. A summary of preclinical studies in optogenetic vision restoration is given in Table 1.

2.1. Microbial opsins are fast but insensitive

Depolarizing opsins. The first opsin to be used in optogenetic vision restoration was Channelrhodopsin-2 (ChR2), a microbial ion-channel type opsin (Bi et al., 2006) (Fig. 2 A). Upon light stimulation the chromophore bond in ChR2 photoisomerases resulting in the opening of an ion permeable pore. Under physiological conditions this leads to a depolarizing influx of cations. After a short time, the chromophore relaxes back into its resting state resulting in pore closure. The chromophore is not released from its pocket and hence Channelrhodopsins can be readily re-activated by the next incident photon (Klapper et al., 2016; Zhang et al., 2011). As chromophore is recycled, the presence of any intact retinal pigment epithelium/Müller cell chromophore recycling machinery is not a necessity for using ChR2-type optogenetic tools. Moreover, Channelrhodopsins operate with high temporal fidelity, showing rapid onset and offset kinetics – a feature that is critical for delivering useful visual information. Both these aspects make ChR2-type opsins highly attractive as tools for vision restoration. However, these advantages come at a price, which is a low level of light sensitivity (in terms of macroscopic conductance), the absence of any intracellular signal amplification cascade, and levels of desensitization (Lin, 2011). Channelrhodopsins have received major attention as tools in research as well as therapeutics (Yawo et al., 2021), leading to the discovery or engineering of several variants with favorable properties. In particular, variants with a long wavelength-shifted excitation spectrum and larger macroscopic currents (at the cost of switching kinetics) like ReaChR or ChrimsonR have become available and have been employed in optogenetic vision restoration studies (Klapoetke et al., 2014; Lin et al., 2013; Lindner et al., 2021; Sahel et al., 2021a; Sengupta et al., 2016). These modifications have enabled stimulation of retinal neurones in vivo using light intensities below the safety thresholds as defined by regulatory authorities (Sengupta et al., 2016), and in the case of ChrimsonR their successful translation into clinical trials (Sahel et al., 2021a). Light intensities required to stimulate either of these Channelrhodopsins remain significantly above ambient light levels, and current clinical studies typically include the use of electronic 'signal amplifying' headwear (i.e. glasses, see also Section 9) to first detect, process and then amplify the intensity of light signals before they are

Table 1
Classes of optogenetic tools and their use in vision restoration studies.

Opsin class	Key features	Studies using these tools
<i>Microbial Opsins</i>		
Channel Opsins	Fast poorly light sensitive Independent of external retinal recycling Concerns about immunogenicity No amplification/second messenger coupling	(Bi et al., 2006; Busskamp et al., 2010; Chaffiol et al., 2017; Cronin et al., 2014; Doroudchi et al., 2011; Ganjawala et al., 2019; Garita-Hernandez et al., 2018; Greenberg et al., 2011; Ivanova et al., 2010; Ivanova and Pan, 2009; Lagali et al., 2008; Lu et al., 2020; Mace et al., 2015; Pan et al., 2014; Sato et al., 2017; Sengupta et al., 2016; Tomita et al., 2007, 2010, 2014; Watanabe et al., 2021)
Light-driven pumps	Hyperpolarizing Independent of external retinal recycling No amplification/second messenger coupling	(Busskamp et al., 2010; Chuong et al., 2014; Garita-Hernandez et al., 2018; Greenberg et al., 2011; Khabou et al., 2018; Zhang et al., 2009)
<i>Mammalian Opsins</i>		
Melanopsin	Native human Independent of external retinal recycling Couples to ubiquitous amplifying second-messenger cascade Works at ambient light conditions	(De Silva et al., 2017; Lin et al., 2008; Liu et al., 2016, 2019)
Rhodopsin	Native human Couples to inhibitory amplifying cascades Works at ambient light conditions	(Cehajic-Kapetanovic et al., 2015; Eleftheriou et al., 2017; Gaub et al., 2015)
Cone Opsin	Native human Couples to inhibitory amplifying cascades Works at ambient light conditions	Berry et al. (2019)
Engineered mammalian opsins	Currently tested based on melanopsin Improved kinetics	(Kralik and Kleinlogel, 2021; van Wyk et al., 2015)
<i>Other</i>		
Vertebrate switchable opsins	Relatively fast On and off switchable	Rodgers et al. (2021)
Chemically sensitized mammalian ion-channels	Independent of external retinal recycling No amplification/second messenger coupling	(Caporale et al., 2011; Gaub et al., 2014)
Chemically sensitized mammalian receptors	Independent of external retinal recycling	Berry et al. (2017)
Multi-characteristic opsins	Broad spectrum Reported to work at ambient light conditions	Batabyal et al. (2021)

transmitted to the retina (Sahel et al., 2021a). Ultimately, there has been one report detailing the generation of a ChR2 type variant that operates at ambient light levels and can be used to restore some degree of visual function (Watanabe et al., 2021).

Hyperpolarizing opsins. The other important family of microbial opsins in the context of vision restoration are the light-activated ion pumps, such as halorhodopsin. Upon light stimulation these pumps typically transport either chloride into or protons out of the cell (Klapper et al., 2016) (Fig. 2 B). Like channel opsins, these light gated pumps are capable of internal chromophore re-isomerisation making them attractive tools for visual restoration. Furthermore, the hyperpolarising effects of these pumps can be an attractive feature when targeting surviving/dormant cone photoreceptors or “OFF-pathway” cells, that

naturally hyperpolarise in response to light (thus allowing for a conservation of signal polarity). However, light activated pumps show temporal kinetics that are about tenfold slower than channel opsins (Feldbauer et al., 2009; Klapper et al., 2016). Still, this has been shown to be fast enough to recover key features of retinal signal processing when expressed in residual cone photoreceptors of blind, retinally degenerate, mice (Busskamp et al., 2010).

2.2. Mammalian opsins show higher sensitivity and a broader dynamic range

In contrast to microbial opsins, mammalian opsins (including rhodopsin, cone opsin and melanopsin) are type A G-protein coupled receptors (Fig. 2 C&D). Upon stimulation with light, they initiate an intracellular second-messenger cascade, which has a signal amplifying effect and thereby allows these opsins to evoke cellular responses following exposure to light stimuli that are considerably dimmer than those required for microbial opsins (Gaub et al., 2015; Gilhooley et al., 2022). This apparent sensitivity is one of the key arguments for the use of mammalian opsins for vision restoration. Given that ambient light levels are sufficient to activate these tools, when expressed *in vitro* and *in vivo* (Cehajic-Kapetanovic et al., 2015; De Silva et al., 2017), it is expected that normal daytime vision and perhaps even some night vision can be restored without the need for signal amplifying goggles and light safety thresholds would not be of a concern.

While the overall light-sensitivity of optogenetic tools is of clear relevance in the context of vision restoration, at least as important is the dynamic range of signal encoding that can be achieved. In their native cellular environment (i.e. rods, cones and intrinsically photosensitive retinal ganglion cells; ipRGCs) mammalian opsins enable encoding of a broad range of signal intensities, spanning at least 5 log-units of intensity (Dacey et al., 2005), while microbial opsins operate over a range of only about 2 log-units. It is understood that at least in rods and cones intracellular modulatory mechanisms contribute to this property; still, a similarly broader dynamic range has been observed when comparing rhodopsin to ChR2 (Gaub et al., 2015) and also when comparing melanopsin to ReaChR (Gilhooley et al., 2022) outside their native environments as tools for vision restoration.

Melanopsin. The first mammalian opsin tested in the context of visual restoration was melanopsin (Lin et al., 2008; Melyan et al., 2005), the photopigment of the intrinsically photosensitive retinal ganglion cells (Hughes et al., 2016; Kinder et al., 2021). This choice had been straight-forward as melanopsin had already been shown to be functional when expressed outside its native environment and confer photosensitivity to a number of cell types (Lin et al., 2008; Melyan et al., 2005). Melanopsin can initiate both, ubiquitous $G_{q/11}$ - and G_i -protein pathways (Jiang et al., 2017; Sonoda et al., 2018; Spoida et al., 2016) making it likely that melanopsin could also evoke cellular responses when expressed in other types of retinal neurons. Moreover, melanopsin putatively uses an intrinsic chromophore recycling mechanism and hence, similar to channelrhodopsins, does not depend on external retinal pigment epithelium/Muller cell mediated retinal supply (De Silva et al., 2017; Koyanagi et al., 2005). We have previously used this approach to restore vision in the rd1 mouse model, leading to restoration of retinal and cortical light responsiveness that persisted for at least one year after treatment (De Silva et al., 2017) (Fig. 6 C).

Rhodopsin and iodopsins. The main downside of melanopsin are its physiologically slow signalling kinetics, which has motivated researchers to test alternative opsins, like rhodopsin and cone-opsins (Berry et al., 2019; Cehajic-Kapetanovic et al., 2015; Gaub et al., 2015) (Fig. 2 D). Both these photopigments physiologically activate G_t (Transducin, member of the $G_{i/o}$ family) to initiate a cGMP dependent signal cascade specific for rods and cones (Fain, 2019). They are also capable of activating ubiquitous G_i (Bailes and Lucas, 2013; Ballister et al., 2018; Li et al., 2005), though in case of the cone-opsins they do so with low efficiency (Hickey et al., 2021). With regard to signalling speed

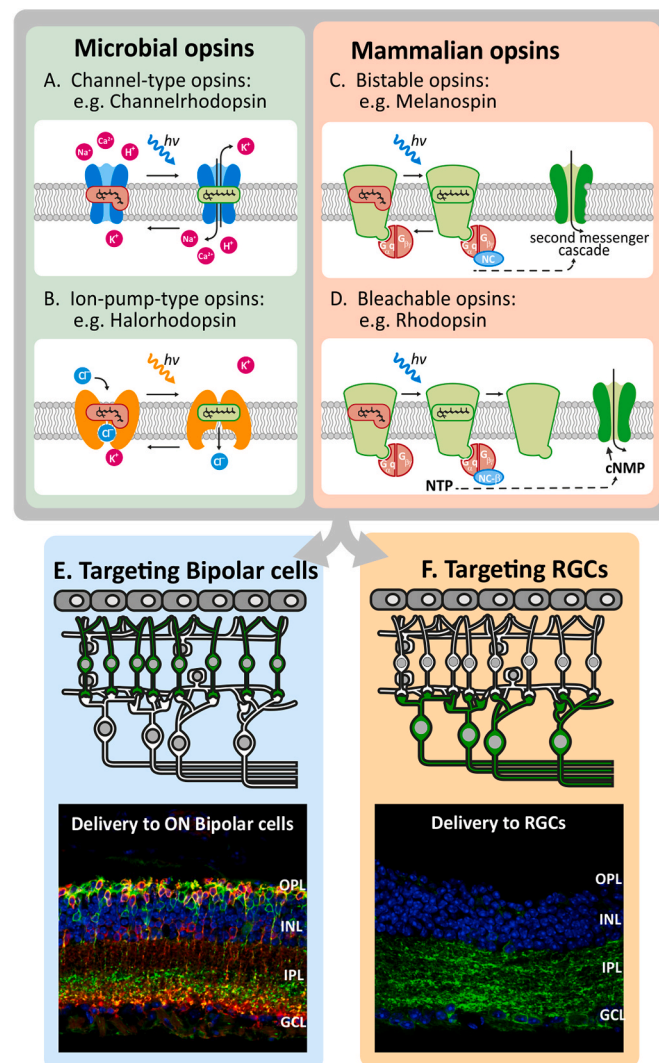


Fig. 2. Classes of tools and targeting strategies in optogenetic vision restoration.

Optogenetic tools currently employed in vision restoration studies are either microbial (A, B) or mammalian opsins (C, D). Microbial opsins are either light-gated ion-channels (A), usually allowing entry of depolarizing currents, or light-driven ion pumps (B) actively transporting charge across the cell membrane commonly resulting in cell hyperpolarization. Mammalian opsins are G-protein coupled receptors that initialize an intracellular signal amplifying cascade. Relevant representatives of mammalian opsins include bistable melanopsin (C) capable of internal chromophore recycling and rhodopsin (D), which is natively faster, but is not capable of internal chromophore recycling. These tools have been tested in different types of surviving neurons of the inner retina (E, F) and it is postulated that delivery to Bipolar cells allows for best possible utilization of the intraretinal signal processing while delivery to Retinal ganglion cells (RGCs) is most resilient against rewiring and remodelling processes in the degenerating retina. Further detail is given in the main text. $h\nu$: photons, $G\alpha$, $G\beta\gamma$: alpha and beta-gamma subunits of heterotrimeric G-proteins, NC: Nucleoside cyclase. Chemical formula encircled in red and green represents respective *cis* and *trans* isoforms of retinal. OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: inner plexiform layer, GCL: Ganglion cell layer.

achieved by the different optogenetic tools it is important to remember that for mammalian opsins, not just the opsin activation and inactivation kinetics are rate limiting. Rather, the availability of downstream effectors and their kinetic properties are important and can be equally rate limiting. Thus, the kinetic behaviour of mammalian opsins will differ depending on the cell type they are expressed in and the signalling cascade they couple to in those cells, and they can't be extrapolated immediately from their kinetic behaviour in rods, cones and intrinsically photosensitive retinal ganglion cells, respectively. Interestingly, in heterologous expression systems, rhodopsin or cone opsin induced signalling show similar temporal kinetics (if not slower) as melanopsin

signalling (Ballister et al., 2018; Hickey et al., 2015). In any case, detailed data on response kinetics of rhodopsin and middle-wave sensitive (MWS) cone opsin treated retinal neurons are available and show times to half-activation in the range of few 100 ms (Berry et al., 2019) and all three opsins seem to be able to mediate some level of image-vision guided behaviour (Berry et al., 2019; Cehajic-Kapetanovic et al., 2015; De Silva et al., 2017; Gaub et al., 2015).

Opsin Chimeras. Given the above-mentioned limitations of mammalian opsins, attempts have been made to identify or engineer opsins with properties that more optimally suit the requirements of vision restoration. One such attempt is the melanopsin – glutamate

receptor chimera Opto-mGluR6 (van Wyk et al., 2015). Herein, the light sensitivity conferring transmembrane and extracellular segments of class A G-protein coupled receptor melanopsin were fused with the intracellular segments of class C G-protein coupled receptor mGluR6, in essence to produce a light sensitive mGluR6 receptor (Tichy et al., 2019; van Wyk et al., 2015). Opto-mGluR6 is one of very few examples of a functional inter-class G-protein coupled receptor-chimera and was designed to utilize the native signal transduction cascade of ON-bipolar cells – that is initiated by changes in mGluR6 activation and ultimately results in TRPM-1 channel gating (Schneider et al., 2015). Indeed, when delivered into ON-bipolar cells, Opto-mGluR6 was able to drive retinal light responses with high temporal precision as well as image-vision guided behaviour (Kralik and Kleinlogel, 2021; van Wyk et al., 2015). Such tools optimized to interact with native ON-bipolar signalling pathways are attractive in the long term as they could enable high-speed, high sensitivity light perception. Other reports of chimeric opsins include the generation of chimeras containing the intracellular domains of melanopsin and extracellular and transmembrane domains of rhodopsin or long-wave sensitive (LWS) cone opsin, designed to confer activation of the ubiquitous G_q signalling pathway natively observed with melanopsin, to the faster switching and more sensitive rhodopsin and LWS-cone opsins, respectively (Hickey et al., 2021). Using this approach, G_q -activity was successfully conferred to rhodopsin (but not LWS-cone opsin) chimeras, albeit with a lower efficiency and slower rate of activation compared to wild-type melanopsin (Hickey et al., 2021) (Fig. 3).

2.3. Further approaches

Multi-characteristic opsins. While microbial and mammalian opsins are receiving most attention, a number of further innovative strategies are also being pursued. One example is the so-called multi-characteristic opsin (MCO, Patent No PCT/US2017/059922), developed by Nanoscope Therapeutics and which has now progressed into clinical trials (NCT04919473, NCT04945772). Delivery of MCO into ON-bipolar cells of the rd10 degenerate mouse retina has been shown to restore

visually guided behaviors in mice at ambient light levels (Batabyal et al., 2021), yet there is little information published on the nature of the MCO construct (Wright et al., 2017) making it difficult to fully interpret these results in the broader context of therapeutic visual restoration.

Light-gated mammalian ion-channels. An alternative to a purely optogenetic approach is to photosensitize normally non-light sensitive mammalian ion channel proteins using synthetic compounds. Such an approach could provide faster signalling kinetics compared to mammalian opsins without necessarily coming with the disadvantages of microbial opsins. However, the significant disadvantage of this approach is that the chemical photosensitising agents need to be regularly supplied to the site of the ion channel. In this regard, maleimide-azobenzene-glutamate tethered mutant GluK2 ionotropic glutamate receptors are most advanced, and have been shown to accurately restore time-resolved electrophysiological and behavioral responses when expressed in either ON-bipolar cells or RGCs (Caporale et al., 2011; Gaub et al., 2014). Perceptively, the hurdle of constant/repeated photosensitizer supply could be overcome by deployment of drug delivery systems similar to those employed in the treatment of neovascular retinal diseases (Rittiphaibroj et al., 2020).

Switchable opsins. Most recently, the spectrum of candidate tools for vision restoration has been expanded to include the class vertebrate opsins from non-mammalian origin. While also being G-protein coupled receptors, certain non-mammalian opsins may possess desirable features not found in their mammalian counterparts. Lamprey paravinopsin, in particular, is a fish opsin coupling to G_i that can be switched on and off by different wavelengths of light with sub-second precision (Eickelbeck et al., 2020) and has been shown to restore retinal light responsiveness when expressed ON-bipolar cells of degenerate mouse retina (Rodgers et al., 2021).

3. Restoring cellular responsiveness is not restoring functional vision

For most of the opsins proposed for vision restoration it has been clearly shown that when ectopically expressed in retinal neurons they

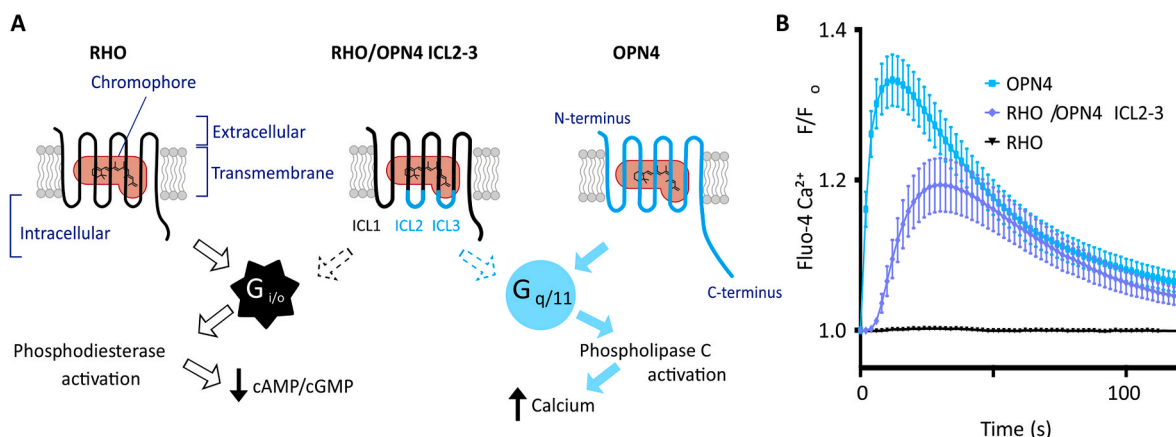


Fig. 3. Chimeric opsins as tools for vision restoration

Opsin chimeras with improved signalling properties: (A) Human rhodopsin (RHO) natively activates G protein partners of the $G_{i/o/t}$ family, which activate phosphodiesterase enzymes, resulting in a reduction in cyclic nucleotide second messenger concentration. Melanopsin (OPN4) also activates the $G_{q/11}$ G protein signalling cascade, leading to phospholipase C activation and intracellular Ca^{2+} release. Activation of practically ubiquitous, $G_{q/11}$ can be advantageous in particular when targeting retinal ganglion cells, where the availability of $G_{i/o}$ has not been confirmed in every subtype. Moreover stimulation of G_q that may elicit excitatory cellular responses seems desirable. Intracellular loops (ICL2 and ICL3) or G-protein coupled receptors govern G-protein specificity. Chimeric opsins consisting of a backbone of e.g. rhodopsin and melanopsin ICL2 and ICL3 may therefore combine kinetic properties of rhodopsin with the G-protein specificity of melanopsin. (B) In vitro $G_{q/11}$ and G_i function of rhodopsin/melanopsin chimeric opsins and wild-type rhodopsin and melanopsin. (A) In a plate reader-based assay of $G_{q/11}$ activity, transfected HEK293T cells were loaded with the Ca^{2+} -sensor Fluo-4 before being illuminated with 485 nm light for opsin activation (arrow). The relative fluorescence unit value (F) was normalized to the value of the first reading (F_0) ($n = 11$). (Hickey et al., 2021).

render these cells light responsive. Moreover, it has been studied how transduced cells integrate with their neural environment inside the retina and to what extent physiological response patterns of healthy retina can be replicated (e.g. (Lindner et al., 2021), Fig. 4). These are essential prerequisites that critically determine the upper limits of any restored functional vision, but high-fidelity responses on retinal level alone are not necessarily sufficient to restore high fidelity functional vision. Signals leaving the retina need to encounter a central neural circuitry that can make sense of these signals in order to create a perceived visual scene that can be made sense of. Several techniques have been employed to demonstrate that exposing optogenetically treated eyes of laboratory animals to light stimuli evokes neural responses also in central brain areas. Examples therefore include optical imaging of changes blood flow or oxygenation in the murine visual cortex (De Silva et al., 2017; van Wyk et al., 2015) (Fig. 2A–C), recording of visually evoked potentials (Liu et al., 2019; Sato et al., 2017; Tabata et al., 2021) or microelectrode array recordings from the dorsal lateral geniculate nucleus (dLGN) (Cehajic-Kapetanovic et al., 2015). Collectively, these data show without doubt that visual stimulation of optogenetically treated retinal neurons results in changes in activity within retino-recipient brain areas. Such non-behavioral data are highly important, as much of the early phase vision restoration studies are performed in mice, and mice are not regarded as highly visual animals. They naturally rely more on scent, and indeed visual performance on a

behavioral level is not easily measured. Still, behavioral level tests are necessary to prove that the entire axis from the “photoreceptorised” retinal neuron to functional image vision has been restored.

The probably best-established assay to assess mouse vision at the behavioral level is the optomotor reflex (Cahill and Nathans, 2008). This is attractive as it is comparably robust and widely validated to measure grating acuity and contrast sensitivity with reasonably good resolution. However, the optomotor reflex is mediated at the subcortical level (Cahill and Nathans, 2008) and thereby does not truly assess what is considered as functional vision. Another widely used assay, the light/dark box, on the contrary does require cortical activity, however this behaviour requires only detection of irradiance – as opposed to space-resolved pattern vision – and is in fact a behaviour that can be driven solely by melanopsin expressing intrinsically photosensitive retina ganglion cells (ipRGCs) in the absence of rods and cones (Semo et al., 2010). Several attempts have been made to overcome such limitations. These include a modification of the light/dark box into an open field arena where optomotor-drum type gratings are presented on one side of a dividing wall and equiluminant grey on the other (Cehajic-Kapetanovic et al., 2015). Also modified “hidden” platform (van Wyk et al., 2015), fear avoidance (Berry et al., 2019; Gaub et al., 2015) and observance of behaviour upon exposure to naturalistic scenes (van Wyk et al., 2015) based approaches have been employed. Our group has successfully expanded the novel object recognition paradigm by adding

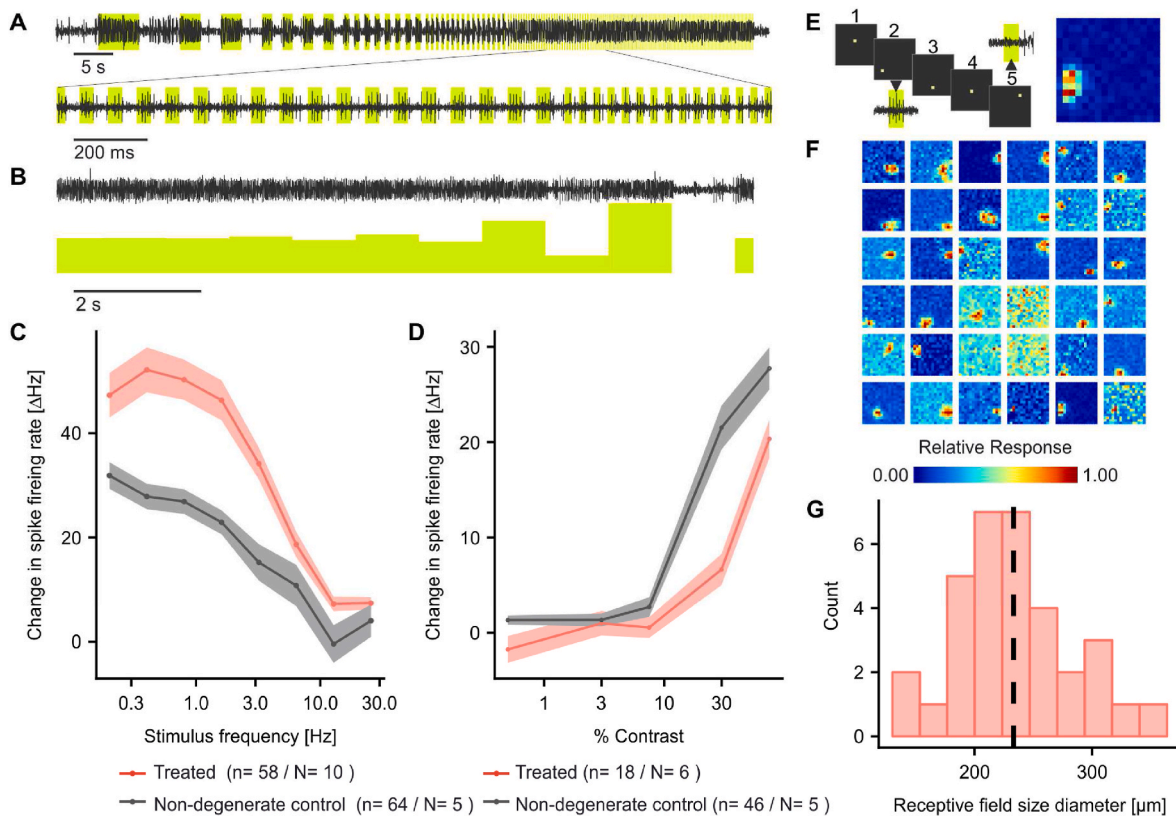


Fig. 4. Features of optogenetically restored light sensitivity on organ level.

Spatiotemporal characteristics of optogenetically restored light responses. Shown are multi-electrode array recordings from retina of blind *rd1* mice. (A, C) Change in spike firing rate in response to a flicker stimulus of increasing frequency as observed after AAV mediated delivery of the microbial opsin ReaChR. A shows a representative recording from a single neuron, with lime areas indicating times where the light was switched on. (C) summarizes responses recorded from treated degenerate (red) and non-degenerate (grey) retinas. (B) Representative recording obtained from a treated degenerate retina in response to a stepwise contrast modulation. Summary curves for treated degenerate (red) and untreated non-degenerate (grey) retinas are shown in D. (E) Receptive field maps (2.38 × 10⁻³ mm²/pixel) obtained in response to a sparse binary noise stimulus. The left panel exemplifies five frames of a stimulus sequence. For frame #2 and #5 the neural responses recorded while the stimulus was projected are also shown. In #2, a sharp increase in firing rate can be observed, indicating that the stimulus was projected into the receptive field of that neuron, while this was not the case for the stimulus shown in #5. The right panel shows the resulting receptive field map. (F) Receptive field mappings from each responding neuron as recorded in response to the stimulus paradigm shown in E (n = 36, N = 8). Responses are normalized for each single neuron. (G) Histogram of corresponding receptive field size diameters. Dashed line indicates mean receptive field size (Lindner et al., 2021).

a modifiable visual context (Fig. 6 D, E) to measure functional vision (De Silva et al., 2017; Tam et al., 2017). All in all, such tests have provided good evidence that all four major candidate mammalian opsins can restore functional vision. However, this approach is limited, and fails to provide a graded measure for restored vision making comparison between different optogenetic tools difficult. Moreover, most of these tests are dependent on some period of learning, which may be impaired in blind mice. And finally, the high light intensities required for some tools – particularly microbial opsins – are likely to induce fear responses in nocturnal, species such as mice via the native light-responsivity of melanopsin-expressing ipRGCs (Semo et al., 2010). Such fear or light aversion responses would potentially mask changes in functional vision.

The discussion in this review thus far has mainly focused on pre-clinical animal models but similar considerations need to be taken into account when it comes to clinical trials, and how best to assess restoration of vision in human patients: It is clear that restoring electroretinogram responses in a patients eye is not predictive of a patients ability to identify optotypes or gratings in a visual acuity test. But even if an optogenetic treatment has enabled a patient to successfully complete a visual acuity test, this does not by itself confirm that they are able to use this visual information to perform tasks (relevant in every day's life). This distinction is made by the terms “visual function” vs. “functional vision” (Ayton et al., 2020b). While the first one, “visual function”, is relatively straight forward to assess and of high importance in the proof-of-principle stage (see also Section 9), “functional vision” is what ultimately matters to the patients. To-date, there is a limited number of validated tools available and usually rather coarsely graded. Examples for such tests are mobility parcourse or standardized street or room-environments (Chung et al., 2018; Sahel et al., 2021b). At the current stage of optogenetic therapy development it is important to include and report validated tests of functional vision to enable inter-trial comparability. Importantly, there is yet no consensus on which assays of functional vision are best suited for this purpose, and it is likely that established assays alone may not be sufficient to ensure signs of therapeutic efficacy are detected.

4. Choice of target cells and subcellular targeting strategies

4.1. By targeting upstream neurons some intraretinal signal processing can be retained

A common hallmark of most degenerative retinal disorders is that early cell loss is largely restricted to the outer retina photoreceptors, with the bipolar cells and retinal ganglion cells, which are involved in the integration and transmission of visual signals and their associated interneurons (horizontal cells and amacrine cells), remaining largely intact. This provides a number of options regarding which cell type to target with optogenetic tools, and two main approaches are being investigated. The first approach is to target RGCs – located in the innermost layers of the retina, and the cells that are ultimately responsible for transmitting visual information to the brain. This approach offers a method to directly control the information transmitted from the retina to central brain regions. It is moreover relatively easy to target RGCs with current viral delivery strategies making this approach relatively easy to translate into clinical settings (Fig. 2 E).

The alternative approach to targeting RGCs is to target the outermost surviving cells of the degenerate retina –degenerate cones or bipolar cells (Fig. 2 F). Restoring photosensitivity at that level has several possible advantages compared to delivery to RGCs, not least the potential to make use of existing retinal circuitry to aid (more natural) processing of visual signals. This could potentially allow a more faithfully recreation of visual code – a feature that will likely be lacking when targeting RGCs, where light detection is simply converted into a widely uniform output to the brain.

Intraretinal signal processing. Visual information is not provided to the brain as simple space and time resolved brightness encoding.

Instead of just relaying visual information to the brain, the retina performs substantial signal processing. In the healthy retina, visual information is processed by as many as 40 parallel pathways and dedicated RGC subtypes (Baden et al., 2016; Tran et al., 2019), each responsible for encoding specific aspects of the visual scene, like contrast or direction of movement (Fig. 7). To date the ability of optogenetics to restore this diversity of functional responses and truly replicate natural visual code has not been examined. The more of these functional channels can be recovered by an optogenetic gene therapy, the more natural will be the information transmitted to the brain, increasing its potential to extract useful information from the received data.

Tuning RGCs into photoreceptors. At the current stage, clinical trials employ intravitreal injections of AAV to target optogenetic tools to as many RGCs as possible. This current “non-selective” approaches will usually result in the simultaneous delivery of optogenetic tools to multiple distinct classes of RGCs, regardless of which particular visual feature they naturally deliver to the brain. Such widespread delivery to RGCs is unlikely to replicate normal visual code. In this scenario, instead of encoding specific aspects of visual information (and performing distinct roles in visual processing) different RGC channels will now respond in a uniform manner, collectively now providing the brain with a jumbled or aberrant version of the normal visual code – potentially even sending conflicting signals, via ON and OFF channels for example (now presumably both showing ON responses). This “non-selective” approach also poses the risk of delivering optogenetic tools to RGCs naturally involved in non-visual tasks and may therefore disrupt other biological processes. For example, this may lead to transduction of melanopsin expressing ipRGCs – a class of endogenously photosensitive RGC classically involved in non-image forming pathways such as circadian entrainment (Foster et al., 2020; Hughes et al., 2016; Kinder et al., 2021; Palumaa et al., 2018). Such a situation could conceivably result in the generation of aberrant light responses in ipRGCs, leading to a disruption of circadian pathways and sleep wake cycles (and increased likelihood of conditions associated with circadian disruption) (Abbott et al., 2020; Foster, 2020; Foster et al., 2013).

Notably, to date it is not understood how cortical circuits may handle or adapt to an aberrant visual code received from the retina. While there might be capacity for adaptation at the cortical level, at least efferences from those RGCs projecting into non-visual brain areas (like endogenously photosensitive RGCs) will remain problematic. Speculatively, it may ultimately be preferable to selectively target one or a few major classes of RGCs in order to restore a specific (and useful) feature of vision, rather than target *all* RGCs non-specifically. While more specific targeting of RGCs is possible using cell-type specific promoters (as suggested for bipolar targeting approaches) it is not currently clear which of the 40 plus RGC types (Baden et al., 2016; Tran et al., 2019) should be targeted to encode the most important and most useful features of vision, or indeed which cell type specific promoters can be used to specifically to target such populations.

Reviving intraretinal signal processing by re-sensitizing cones. The most straightforward way to recover as many of these output channels as possible is by targeting optogenetic tools into the lowest order of retinal neurons surviving in the degenerate retina, thereby retaining the natural path the visual signal takes through the retina. The potential thereof has been demonstrated by Busskamp et al. in 2010 who targeted a hyperpolarizing microbial opsin into surviving cone photoreceptors that had no remaining native light responsivity. In fact, by this strategy they were able to restore key features of intraretinal signal processing, including direction sensitivity and center-surround inhibition (Busskamp et al., 2010). These experiments provide the important confirmation that intraretinal signal processing can be revived. Yet, cones are typically only available as target cells for optogenetic vision restoration for a narrow time interval: At the point of intervention, native vision should be mostly absent so that the optogenetic vision would not interfere with it and a sufficient number of cones should still survive. While in humans for the individual cone, this may be a time

span of several years, retinal degeneration progresses over space and time and thus many cones might have already died in one area while they are just beginning to lose light sensitivity in another (Campochiaro and Mir, 2018). This is a valuable proof of principle, though, on the path towards optogenetic vision restoration as a clinical reality it needs to be taken into account that the first patients participating in clinical trials are those with end-stage retinal degeneration and thus likely to have no relevant number of cones remaining. Moreover, cones seem to be unable to survive in a rod-less retina for yet not completely understood reasons (Campochiaro and Mir, 2018) and it is unlikely that optogenetic treatment could halt or slow down cone death. Finally, as retinal degeneration progresses, extensive circuitry remodelling takes place (discussed in further detail in Section 6). Besides the pure availability of cones as a target cell, this remodelling will likely affect the way in which the retina is capable of computing visual features.

Bipolar cells are the lowest order of retinal neurons that are primarily retained throughout the course of retinal degeneration. Targeting these should therefore yield the highest potential to restore lifelike retinal output channels. Indeed, several groups have successfully expressed optogenetic tools in retinal ON bipolar cells of rd1 mice and have assessed features of intraretinal signal processing in some detail (Cehajic-Kapetanovic et al., 2015; Chuong et al., 2014; Cronin et al., 2014; Doroudchi et al., 2011; Gaub et al., 2014, 2015; Lagali et al., 2008; Lindner et al., 2021; Mace et al., 2015; van Wyk et al., 2015, 2017). An overt feature of retinal signal processing is the segregation of stimuli into “ON” and “OFF”-type responses, i.e. into responses that positively correlate with incident light intensity and responses that inversely correlate with incident light intensity. In the absence of native rod and cone input to bipolar cells, this would be mainly a consequence of the synaptic link from ON-bipolar cells via AII amacrine cells to OFF-bipolar cells (Bloomfield and Dacheux, 2001)). Interestingly, while ON responses could be observed in all studies, ON and OFF responses were only reported by some authors (Chuong et al., 2014; Cronin et al., 2014; Gaub et al., 2014, 2015; Mace et al., 2015; van Wyk et al., 2015), while others explicitly report that this feature of intraretinal signal processing had not been observable (Doroudchi et al., 2011; Gaub et al., 2014; Lagali et al., 2008; Lindner et al., 2021). While all these studies were conducted in mice, thereby excluding inter-species effects, one possible explanation for this discrepancy are the strength (and specificity) of the different promoters and/or the transduction efficacy of the different delivery viral vectors used (Kleinlogel et al., 2020). This seems logical but presently alternative explanations cannot be ruled out: It is particularly noteworthy that in studies finding ON and OFF responses, mice were typically younger when they underwent behavioral or electrophysiological testing as compared to those in studies where only ON responses were detected (exception: (Mace et al., 2015)). Therefore, these mice had a shorter course of retinal degeneration and thus probably a lower degree of retinal remodelling. It is therefore possible that active retinal remodelling – while not impeding signal propagation to the brain per se – abolishes the AII-OFF circuitry and with it, possibly other relevant aspects of intraretinal signal processing. This would be consistent with the recently observed formation of novel gap-junctions from ON-bipolar cells to AII cells in the degenerating retina (Pfeiffer et al., 2020a). Finally, it also needs to be considered that obtaining cell type-specific delivery within the retina is challenging (see Section 7), and the different AAV2 serotypes and promoters chosen in the various studies might have had slightly different (off-target) transduction patterns that could potentially explain the OFF-responses observed in some studies.

To date, most studies using specific targeting to bipolar cells have focused on selective transduction of ON bipolar cells. However, a significant advantage of targeting bipolar cells is the ability to also specifically target the OFF pathway via selective delivery to OFF bipolar cells (see also (Suh et al., 2013)). Restoration of the ON pathways is considered to be more functionally important than restoration of the OFF pathway, and thus while cell specific promoters have been

identified for OFF bipolar cells this line of study has received far less attention. Ultimately it may be possible to target both ON and OFF pathways with simultaneous delivery of different optogenetic tools to ON (depolarizing tool) and OFF bipolar cells (hyperpolarising tool) to more fully replicate normal visual processing in the retina.

Evidence for a benefit of targeting upstream neurons is indirect. By simply analyzing the polarity of light-responses in electrophysiological assays, the presence of such ON/OFF circuitry is relatively easy to assess and observations from the aforementioned studies show us that under the right conditions some intraretinal computation can be restored when targeting ON-bipolar cells. But overall, it is unclear whether this is in any way an informative surrogate for the integrity of the retinal circuitry. It is also unclear how it relates to function at a behavioral level. Behavioral assays employed in reported studies are too diverse to allow comparison across study borders and so far, only a few studies employing sensitive behavioral tests directly compare intraretinal computation retaining ON-bipolar cell delivery and non-intraretinal computation retaining RGC delivery: using rhodopsin as an optogenetic tool Cehajic-Kapetanovic and colleagues observed that rd1 mice that expressed rhodopsin in bipolar cells performed better in light/dark box tests as compared to rd1 mice expressing rhodopsin within RGCs. Moreover, they observed that only ON-bipolar cell delivery enabled mice to respond to complex naturalistic scenes (Cehajic-Kapetanovic et al., 2015).

Pertinent in this regard are the findings of Gaub et al. (2014). In their study, a light-activatable ion-channel was delivered into either RGCs or ON-bipolar cells. When targeting RGCs, responses to a single 1s flash stimulus recorded from the retinal output level appeared highly similar, and – consequently – all strongly correlated. In contrast, when delivering the light-activatable ion-channel into ON-bipolar cells, responses to an identical stimulus were much more diverse, and correlated with each other only in clusters, indicating distinct functional RGC groups (Gaub et al., 2014). Indeed, we can confirm this observation re-analyzing our own data (Lindner et al., 2021) in an analogous fashion: When delivering red-shifted Channelrhodopsin (ReaChR) into (predominantly) RGCs, obtained peri-stimulus histograms correlated with 0.58 [IQR: 0.18–0.81] while correlation was significantly weaker, when targeting ON-bipolar cells (0.51 [IQR: 0.14–0.73], data from 76 to 57 responsive units observed on microelectrode array recordings from RGC level, $p < 0.05$).

4.2. Subcellular targeting strategies are explored to mimic ON and OFF responses or improve kinetics

From a translational perspective, delivering optogenetic tools into specific cell types – in particular bipolar cells – bears certain challenges. By contrast, widespread RGC delivery can be obtained relatively easy using intravitreal delivery strategies and ubiquitous promoters (see Section 7). Therefore, efforts are being made to optimize the way optogenetic tools operate inside RGCs.

Physiologically RGCs receive input originating from a number of rod and cone photoreceptors. In many types of RGCs, via differential usage of ON and OFF pathways and lateral connectivity, a center-surround antagonism is obtained which supports contrast and initiates edge detection (Fain, 2019). It has been proposed that by differential subcellular targeting of excitatory and inhibitory optogenetic tools to the soma and dendrites of an RGC, such center-surround inhibition could be re-enacted (Greenberg et al., 2011). Indeed at the level of individual RGCs, this strategy has proven successful to create center-surround inhibition and “edge detection” (Greenberg et al., 2011).

A challenge with this strategy is that until now it is not possible to ensure that both excitatory and inhibitory opsins are expressed in each cell transduced. While smaller targeting motifs could be identified and tested (Wu et al., 2013), the size of two opsins alone exceeds the packaging capacity of a single AAV. Thus, a mix of different AAVs would need to be injected, resulting in the formation of pure ON, OFF RGCs

besides the intended center-surround RGCs. Such functional diversity might seem attractive at first, but as it only depends on the random event of which cell becomes transduced by which virus, it still needs to be determined if and how this would carry additional information to the brain, and how this information might be interpreted. Once the challenge of uneven delivery is solved it will be interesting to see in what way such subcellular targeting strategies are beneficial for system-level responses. In any case, reflecting the size of the dendritic field of an RGC as compared to a rod or cone photoreceptor, sharpening the receptive field of optogenetically treated RGCs by subcellular targeting of a single opsin might be beneficial even without aiming for center-surround inhibition.

4.3. Interaction of optogenetic tool with the target cell environment

Another consideration to make when choosing the optimal target for optogenetic gene therapy is how the selected optogenetic tool operates inside its host cell environment. This is most obvious for mammalian opsins, which need to be able to activate an intracellular signalling cascade which is available in the host cell, as discussed in Section 3. But also, microbial opsins may differentially shape retinal light responsiveness depending on the cell type they are expressed in. For example, in a recent study we observed that when delivered into RGCs, ReaChR-mediated light responses decay relatively quickly upon continuous stimulation. While this is considerably less evident when ReaChR is delivered into (predominantly) ON-bipolar cells (Fig. 5). This observation is not yet well understood but it appears likely that spike-generating RGCs might be less well equipped to handle the large, continuous depolarizing ion-flux mediated by channel opsins. On the other hand, for non-spiking ON-bipolar cells such continuous depolarizing flux might more closely resemble the physiological mode of operation enabling these cells to sustain light responsiveness also upon long-term stimulation.

5. Inner retinal remodelling in retinal degeneration

5.1. The nature of retinal and central remodelling

While it has been known for many years that the post-photoreceptor visual pathway is functional in very late-stage retinal degeneration, it is only more recently that attention has been given to the impact of such

remodelling of visual pathways on the potential success of optogenetic visual restoration. Following the loss of rods and cones, the remaining cells of the retina are initially spared from further widespread degeneration. However, the loss of photoreceptor input is accompanied by extensive retinal network rewiring over time (Fig. 8) (Marc et al., 2003; Pfeiffer et al., 2020b). Significant levels of retinal and central remodelling are apparent in both mouse models (Jones et al., 2003; Strettoi et al., 2003) and human subjects (Jones et al., 2016a, 2016b), and furthermore it appears this remodelling is not confined to the retina, but also occurs in the retino-recipient visual pathways in the brain (Chen et al., 2016; Yoshimine et al., 2018).

Robert Marc's laboratory originally examined the late stages of retinal degenerations in rd1 mice, a widely used model of human retinal degeneration (Carter-Dawson et al., 1978; Farber et al., 1994) containing a mutation in the β subunit of phosphodiesterase 6b (PDE6b) that is also known to cause autosomal recessive retinitis pigmentosa in humans (McLaughlin et al., 1993). Using computational molecular phenotyping Marc et al. revealed a sustained period of remodelling in the degenerate retina (Marc et al., 2003), initially showing relatively subtle changes in neuronal structure in the inner retina, progressing to large-scale reorganization of cell to cell contacts and retinal circuitry, and including cell loss (Jones et al., 2016a; Marc et al., 2003) (Fig. 8). Three distinct phases of degeneration were described by Marc et al. (2003). Phase I and phase II refer to the different stages of rod (phase I) and then cone (phase II) photoreceptor degeneration. Additionally, more subtle changes are also known to occur during these periods including sprouting of neurites from various cell types, changes in bipolar cell glutamate and NMDA receptor expression (Jones et al., 2011; Marc et al., 2007), retraction of bipolar and horizontal cell dendrites, cell movements, and initial glia cell activation (Pfeiffer et al., 2020b). Some particularly interesting changes during these early phases have been described in detail recently: Rod bipolar cells form unphysiological gap junctions with AII-amacrine cells and GABAergic amacrine cells begin to form synapses with horizontal cells (Pfeiffer et al., 2020a). Phase III represents a more sustained period of retinal remodelling, with significant sprouting of neurites, formation of new synaptic sites and re-wiring of cell-to-cell contacts, with increasing levels of cell migration, disruption of inner retinal layers and cell death. Phase III is subdivided into early, mid and late phase III reflecting distinct periods of retina remodelling (Marc et al., 2003). Early phase III involves significant neurite remodelling, with increased sprouting from amacrine and horizontal cells (Fariss

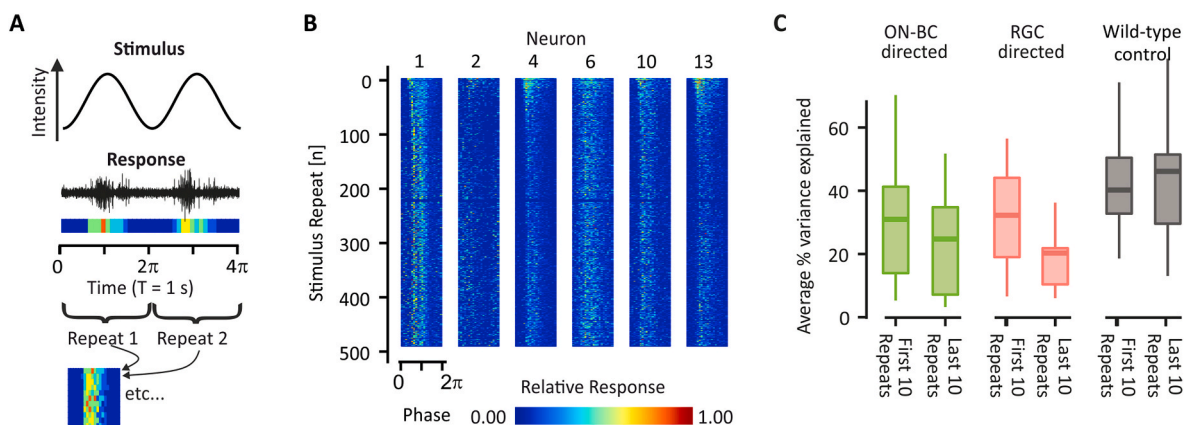


Fig. 5. Long-term stability of ReaChR-Mediated light responses in RGCs vs BCs.

Long-term responsiveness in microbial opsin (here: ReaChR) treated retinas to quasi-realistic light stimuli. (A) Schematic illustrating stimulus shape and processing of the recorded neural responses: A continuous cosine intensity modulated full field stimulus ($T = 1$ s) was displayed over >8 min. Spike firing responses were analysed in a stimulus phase-aligned manner. (B) Raster-plot for exemplary neurons. Spike firing responses in some neurons rapidly wear off (e.g. #2) or become less defined while others appear stable (e.g., #1). (C) Percentage of variance explained (%VE) over time. In degenerate treated retinæ, %VE was significantly lower for the last ten stimulus repeats ($p < 0.05$ each), while in wild-type controls %VE was stable ($p = 1$). While the decay in %VE was observed treated degenerate retinæ regardless of whether the opsin was delivered predominantly into Retinal ganglion cells (RGCs) or into ON-bipolar cells (ON-BCs) this decay was apparently less pronounced upon ON-BC directed opsin delivery ($p = 0.18$) (Lindner et al., 2021).

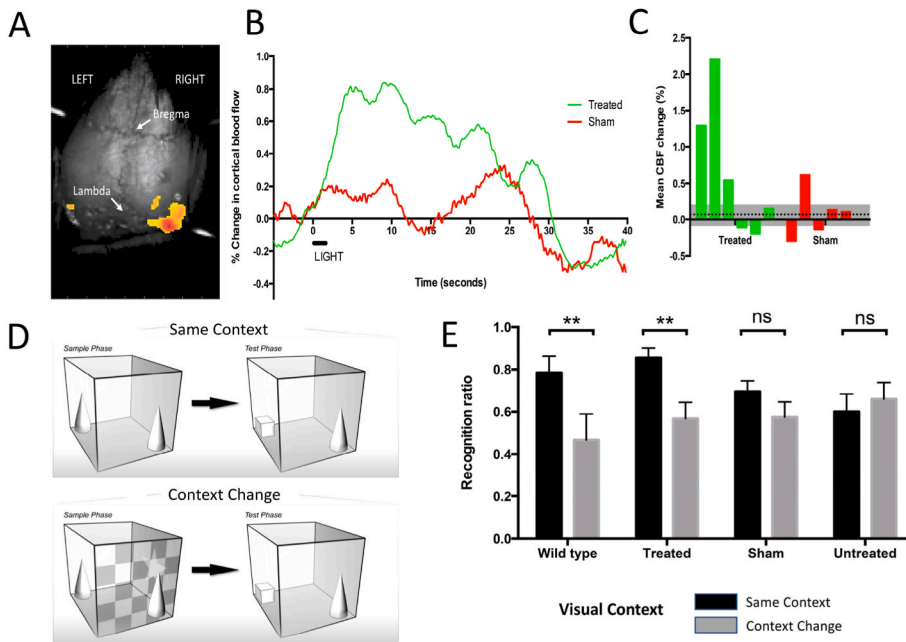


Fig. 6. Measuring optogenetically restored vision on cortical and behavioral level. A-C) Laser speckle contrast imaging can be used to measure changes in visual cortex blood flow (as a surrogate for neural activity) in response to visual stimuli. A) Representative image obtained in response to a 2s, 480-nm light stimulus of 2,000lx intensity. B, C) Blind rd1 mice treated with AAV carrying the Melanopsin gene showed an appropriate light-dependent peak in cortical blood flow, which cannot be observed in untreated controls (treated, $n = 5$; controls, $n = 4$). D, E) A modified novel object recognition test where known or novel objects are presented either in an unchanged, or in a changed visual context can be used to measure functional vision in mice. D illustrates the concept of the test: in the “same context” condition, the visual environment is identical in both phases. In the “Context Change” condition, there is a change in visual environment between phases, but all other factors are constant. E) Recognition ratios for the novel object were significantly different in treated mice (Melanopsin-treated as in A-C) when the visual environment was the same versus when it was changed, indicating a visual environment-dependent change in behaviour (De Silva et al., 2017).

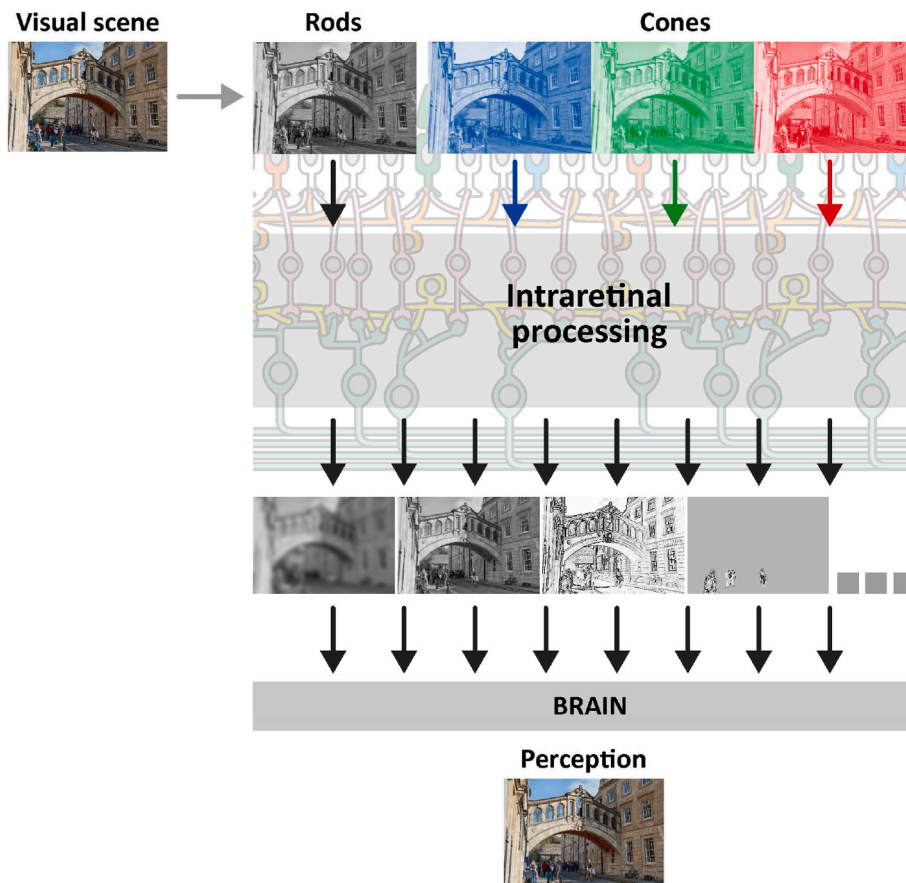


Fig. 7. Image processing in the retina. The retina does not simply relay an unmodified representation of a visual scene to the brain. Rather, rods, cones and intrinsically photosensitive ganglion cells (not show) sense different aspects of a visual scene and provide these to downstream retinal neurons for signal processing. At the output level (ganglion cells) the retina then provides individual features, like irradiance, local brightness, contrasts, or movement, extracted from the visual scene to the brain in the form of multiple parallel outputs.

et al., 2000; Marc et al., 2003), accompanied by Müller cell hypertrophy and initial neuronal cell death. By mid-phase III global remodelling is evident, with progressive cell death, formation of multicellular fascicles, significant microneuroma growth, and migration of amacrine and bipolar cells into the inner plexiform and ganglion cell layers. Late phase III shows a plateau in levels of remodelling with continued cell death

and a regression of microneuromas, increased levels of Müller cell hypertrophy and completion of the glial seal, invasion of blood vessels and migration of retinal pigmented epithelium cells to the inner retina layers and progressive cell death.

In the context of optogenetic vision restoration the fate of bipolar cells as one proposed target for optogenetic tools is of particular interest.

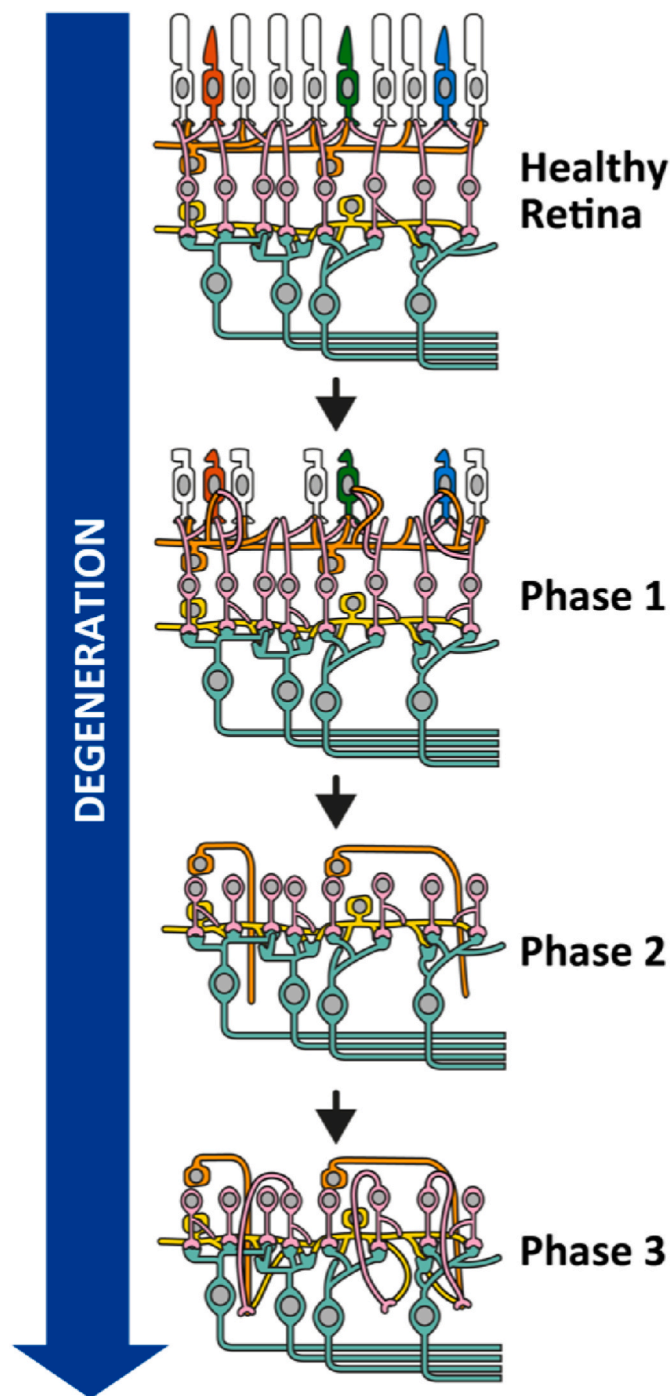


Fig. 8. Phases of retinal remodelling

During degeneration, the retina undergoes significant remodelling that may affect signal processing in the inner retina. (Phase 0) Healthy retina. (Phase 1) Rod and cone photoreceptors lose their outer segments. Subsequently rods and cones die, with rod death usually preceding cone death. Dendrites of deafferented rod bipolar cells make novel chemical synapses with cone presynaptic terminals and gap junctions with amacrine cells. Some data suggests loss of defining components of the rod bipolar-cell signalling machinery (Jones et al., 2011) while other data is not indicative for such changes (Gilhooley et al., 2021a). Rod death is complete at end of Phase 1. (Phase 2) Retraction of bipolar cell and horizontal cell dendrites. Horizontal cells grow neurites into the inner retina. Amacrine cells form novel synapses in the inner plexiform layer. Rod death is complete at end of Phase 2. (Phase 3) Amacrine cells make synapses with horizontal cells in the outer plexiform layer. Microneuromas form and inner retinal neurons die.

Indeed, there is evidence for some bipolar cell death occurring over the late phases (Phase III) of degeneration. A human post-mortem study found that loss of cells in the inner nuclear layer is moderate (20% lower than age matched controls in a population aged 77 years in median), and only evident in eyes from individuals that had a visual acuity of “light perception only” or worse before their death (Milam et al., 1998; Santos et al., 1997). Accordingly, optical coherence tomography (OCT) studies indicate a 12% thinning of the inner nuclear and inner plexiform layer together only in patients with no remaining light perception but not individuals with better visual function (Vámos et al., 2011). OCT data also indicate that this effect might be genotype-dependent, with patients with RPGR mutants even showing an increase in inner nuclear layer thickness (Gargini et al., 2007). In comparison, data for bipolar cell loss reported for mouse models are variable and range from none (Stefanov et al., 2020), over 10% and 20% (Gargini et al., 2007; Strettoi and Pignatelli, 2000) to 60% (Chen et al., 2012). But not only does the survival of bipolar cells matter in the context of using these cells as a target structure for optogenetic gene therapy. Especially when using mammalian GPCR-type opsins it is important to consider to what extent native signal transduction cascades are preserved. Evidence has accumulated over the last fifteen years that bipolar cell glutamate signalling is altered following loss of rod and cone input. In particular, mGluR6 mediated TRPM1 modulation is found to be lost by some authors (Barhoum et al., 2008; Jones et al., 2011; Marc et al., 2007). Notably, this does not essentially mean components of this signalling cascade are no longer expressed. Reduction of mGluR6 mRNA expression is reported to be transient over the course of degeneration (Armata et al., 2006) and we could recently show, that in P90 rd1 mice (resembling stage III remodelling) mRNA expression of both mGluR6 and TRPM1 was back to normal (Gilhooley et al., 2021a). Rather, there is evidence for a mis-localization of its individual components, in particular mGluR6 (Agosto et al., 2021; Dunn, 2015; Puthussery et al., 2009) and TRPM1 (Gayet-Primo and Puthussery, 2015) that likely impedes functional coupling but not necessarily utilization in the context of mammalian opsin-based vision restoration approaches.

5.2. Oscillating retinal networks and their role in vision restoration

It is clear from numerous different animal models of retina degeneration (including rd1 and rd10 mice, and P23H-1 and RCS rats) that loss of photoreceptors leads to the emergence of rhythmic activity in the retina, with the generation of oscillatory local field potentials and spontaneous bouts of RGC spike firing (Borowska et al., 2011; Choi et al., 2014; Goo et al., 2011; Margolis et al., 2008; Menzler and Zeck, 2011; Pu et al., 2006; Stasheff, 2008; Stasheff et al., 2011). Indeed, studies have now shown that two distinct oscillatory networks are evident in the degenerate retina (Euler and Schubert, 2015), including an outer retina network driven by interactions between remaining cones, horizontal cell and rod bipolar cells – where retrograde release of GABA from horizontal cells onto cones produces oscillating local field potentials at a frequency of around 3 Hz (Haq et al., 2014). A second oscillatory network is located in the inner retina, and produces oscillatory field potentials in the range of 8–10 Hz (Borowska et al., 2011; Trenholm et al., 2012). Multiple mechanisms have been proposed to explain this oscillatory activity. It has been proposed that remodelling and rewiring of the retina following degeneration may be responsible (Jones et al., 2012; Marc et al., 2003, 2007; Phillips et al., 2010; Strettoi et al., 2003), yet current evidence suggests that such oscillations are an intrinsic property of electrically-coupled AII amacrine cells and cone ON bipolar cells (Borowska et al., 2011; Choi et al., 2014; Margolis et al., 2014; Trenholm and Awatramani, 2015; Trenholm et al., 2012), and that these events are normally suppressed by photoreceptor input. Support for this theory is supplied by observations that similar ~10 Hz oscillations are induced in healthy wildtype retina following pharmacological blockade of photoreceptor input to bipolar cells (Menzler and Zeck, 2011; Trenholm and Awatramani, 2015; Trenholm et al., 2012), or photoreceptor

bleaching (Menzler et al., 2014). At the molecular level, it has been shown that gap junctions assembled from Connexin36, physiologically functioning to electrically couple AII amacrine cells are critical for the development of retinal oscillations (Ivanova et al., 2016) and that not their expression but rather their phosphorylation state (possibly being the result of an imbalance of dopaminergic and glutamatergic signalling) changes during retinal degeneration (Ivanova et al., 2015). Thus, no major rewiring of the retina seems to be required to generate these rhythmic bouts of oscillatory activity – just a loss of photoreceptor input.

This increase in spontaneous activity, and – at an output level – the increased spontaneous firing of RGCs has implications for visual restoration and may impact the success of optogenetic treatments. Increased spontaneous firing from RGCs likely adds significant noise to transmitted signals, reducing signal to noise ratio, and ultimately reducing the clarity of information sent to brain (Eleftheriou et al., 2017). Furthermore, the efficiency by which external electrical stimulation induces changes in RGC spike firing has been shown to be reduced in multiple mouse models of retina degeneration compared to healthy wildtype retina (Goo et al., 2011; Haselier et al., 2017; Margalit et al., 2011; O'Hearn et al., 2006; Ye and Goo, 2007; Ye et al., 2008), and there appears to be a strong correlation between levels of oscillatory activity and efficiency of RGC stimulation (Haselier et al., 2017). Furthermore, pharmacological blockade of oscillatory activity results in improved RGC responses to electrical stimulation in rd10 mice (Gehlen et al., 2020). Thus it seems possible that such oscillatory signals may directly act in some way to suppress or reduce the efficacy of exogenous stimulation of RGCs. Therefore, combined such oscillatory potentials may act to both reduce the potency of optogenetic based stimulation, whilst also reducing the signal to noise ratio of restored responses. Indeed, several studies have now investigated the potential consequences of these oscillatory signals for optogenetic vision restoration. Pharmacological blockade of oscillations with gap junction blockers have been shown to increase the signal-to-noise ratio of optogenetic restored light responses, following viral delivery of channelrhodopsin to RGCs (Barrett et al., 2015) and also following delivery of human rhodopsin to ON bipolar cells of degenerate retina (Eleftheriou et al., 2017). Interestingly, recent studies have shown that expression of the optogenetic tool Opto-mGluR6 selectively in ON bipolar cells can by itself reduce levels of oscillatory activity and spontaneous RGC firing to levels similar to that seen in wildtype retina (Kralik and Kleinlogel, 2021). Notably, it has been shown that benzodiazepines can be used to abolish retinal oscillations and improve RGC stimulation efficiency (Gehlen et al., 2020), and similar pharmacological approaches may in future be combined with optogenetic therapies to improve functional outcomes.

5.3. How does remodelling impact upon optogenetic restoration therapy?

In the majority of human retinal diseases degeneration is slow and not uniform across the retina. As such, patients can experience prolonged periods of only partial visual loss, where the retina still retains some level of basic physiological function. Current clinical trials for restorative therapies typically only target patients with end stage disease (see Section 9). However, it is currently unknown how Phase III remodelling would affect therapeutic outcome.

What is the impact of remodelling on the functional capacity of surviving visual circuits? The profound nature of retinal remodelling raises some important issues regarding optogenetic therapies for visual restoration. It is strongly predicted that the anatomical changes associated at least with Phase III, but possibly also with earlier phases of degeneration, will lead to changes in retinal pathways and connectivity and potentially impact the quality of restored vision following regenerative therapy. Whether photoreception is restored at the level of bipolar cells or retinal ganglion cells, the visual signal produced must be transferred through remaining circuits in the retina and brain to support vision. In visually intact individuals these circuits efficiently transmit the visual signal and perform appropriate computations. As elements of

this circuitry degenerate and connections between remaining neurones become disordered, it would be most surprising if the functional properties of the circuit were not impacted. In this regard, Pfeiffer et al. recently speculated how pathologic gap junctions formed between rod bipolar cells and AII amacrine cells, as early as in Phase I remodelling could impact basic intercellular interactions such as lateral inhibition, a key prerequisite for complex vision (Pfeiffer et al., 2020a). In fact, while there is a wealth of information on the anatomy of remodelling, the impact on circuit function remains largely untested. There has not been a systematic assessment of the impact that remodelling has on the spatial and temporal resolution of the visual code, or on its ability to perform neuronal computations. This is of fundamental importance for restorative therapies, as circuit performance could realistically place an upper limit on the quality of vision supported, and it is possible that late intervention may limit the efficacy and success of regenerative therapy in the retina.

Is intervention at early stages of degeneration advantageous to functional outcome? While there are obvious advantages to intervening later in disease progression, when the possibility of disrupting residual rod and cone driven vision is avoided, it is possible that remodelling at later stages of disease could potentially degrade the fidelity of retinal circuitry to such an extent as to fundamentally limit the quality of restored vision. While studies in animal models at late stage of degeneration (De Silva et al., 2017; Kralik and Kleinlogel, 2021) and early results from clinical trials (Sahel et al., 2021a) (see Section 9) suggests that optogenetic therapies can function in a highly remodeled state, it does not exclude the possibility that intervening earlier could deliver better therapeutic outcomes. If end stage remodelling does indeed significantly impede higher levels of restored visual function, this could lead to poor results in ongoing late-stage remodelling trials and an overall underestimation of the potential benefits of optogenetics for visual restoration. Intervening in earlier stages of retinal remodelling would be associated with additional risks for the trial participants. Thus, animal studies that are able to evaluate if and what the functional consequences of advanced remodelling for optogenetic vision restoration are, are urgently needed.

Late-stage remodelling may affect the choice of retinal target. The profound changes in connectivity or rewiring associated with late-stage Phase III remodelling may ultimately affect the choice of retinal survivor cells to target with optogenetic treatments. In the later stages of disease, it may be more effective to target the retinal output neurons – the RGCs, rather than bipolar cells. Whilst you may lose benefits associated with increased retinal processing you may retain some fidelity through the preservation of retinotopic mapping. It needs to be mentioned that, to date, this is still a theoretical concept. Worth considering, RGCs are also affected by aberrant connectivity in the late stages of degeneration. Though possibly to a lesser extent than upstream neurons, it has not been investigated what this exactly means in functional terms.

Expression patterns may change during degeneration. Besides circuit rewiring – an additional aspect needs to be taken into account: (how) does the protein machinery in the different retinal neurons change during degeneration and how does this affect the suitability of that cell type as a target for optogenetic gene therapy? This question is particularly pressing in the context of G-protein coupled receptor-type opsins, as these need an intracellular cascade to couple to. In fact, data from our group and others indicate that this seems not to be a major issue: even after long-term degeneration melanopsin is able to induce light-evoked responses following expression in RGCs (De Silva et al., 2016) and similarly Opto-mGluR6 (see Section 3) can do so when expressed in ON bipolar cells (Kralik and Kleinlogel, 2021). There is still relatively little understanding of which intracellular signalling cascades are utilised. With regard to ON bipolar cells we recently showed that key components of the native G_o-signalling pathway remain transcriptomically unchanged over the course of retinal degeneration, suggesting that expression of a compatible opsin will likely simply revive the native

cascade (Gilhooley et al., 2021a).

Retinal remodelling in response to regenerative therapy – potential for neuroprotection and compensatory remodelling of visual circuits? The final critical unknown regarding the significance of remodelling for visual restoration is whether early intervention (prior to large scale remodelling) could either prevent disruptive remodelling or ensure that it progresses in such a way as to maximize the impact of restored photoreception. Either or both outcomes are possible. If delivered during early stages of retina disease optogenetic therapy may by itself impact on the rate of further retinal degeneration, remodelling of visual circuits and pathological cell loss. Delivery of therapy could prove neuroprotective, not just for the visual circuits, but also for the primary photoreceptor degeneration. There are reports that reactivation of rod signalling circuitry can lead to a reversal of retinal remodelling and recovery of visual signalling circuitry (Wang et al., 2019). Secondly, it is possible that therapy may lead to compensatory remodelling. Increased external input could constitute a form of environmental enrichment, which is protective for degenerative conditions in general, and retinal degeneration specifically. Optimistically, this could lead to gradual improvements in therapeutic outcome over time as circuits reorganise to optimally process and interpret the newly restored visual signals.

5.4. Central remodelling

Whilst the implications of photoreceptor loss on retinal anatomy have been studied, significantly less is known about the consequences of photoreceptor loss for central visual circuitry. It has been established that in the early stages of retinal degeneration in a rat model cortical V1 cells exhibited weaker orientation selectivity, lower optimal spatial and temporal frequency values, and smaller receptive fields compared to controls (Chen et al., 2016). It remains unclear to what extent this reflects a central reorganization of visual circuitry. And there are no functional studies of central visual circuit properties when all retinal input is lost. However, from clinical studies on retinitis pigmentosa patients it is understood that post-thalamic pathways remain structurally mostly intact (Ohno et al., 2015; Schoth et al., 2006). Moreover, subjects treated with gene replacement therapy for Leber's congenital amaurosis appear to show normal visual pathway anatomy and cortical responses measured by (functional) magnetic resonance imaging (Begenisic et al., 2020). Using established paradigms to test for cortical plasticity in ex-vivo electrophysiological recordings from rd10 mouse visual cortex a recent study could moreover show that cortical plasticity is retained, even in very late disease stages of retinal degeneration (Begenisic et al., 2020). Certainly, more research is required in this regard, but the current evidence suggests that cortical remodelling secondary to retina degeneration – if any – is not a significant limiting factor in optogenetic vision restoration.

6. Strategies for targeted optogenetic tool delivery

Cell type specific targeting of optogenetic tools has proven feasible in animal models, most notably the targeted expression of optogenetic tools in ON-bipolar cells. While potentially beneficial in terms of final outcomes and the nature of vision restored (see Section 5), there are a number of hurdles to overcome before these approaches can be translated into clinical therapies. In animal research models, many studies have used Cre-lox based approaches, typically employing a genetically engineered mouse in which Cre recombinase is expressed in a specific cell type (under control of a cell type specific promoter), and thus permits expression of AAV constructs containing floxed genes only within cell types of interest.

While successful in animal models it is clear that such Cre-lox based approaches are obviously not translatable into human studies. An alternative approach is the inclusion of cell type specific promoters within the AAV constructs themselves. Again, proof of concept has been

demonstrated using the *Grm6* promoter (and shortened variants) for targeting ON-bipolar cells (Cronin et al., 2014; Hulliger et al., 2020). However, there are some disadvantages of using such promoters. Cell type specific promoter constructs (such as *grm6-sv40* (Cronin et al., 2014)) are often seen to produce lower levels of trans-protein expression compared to non-specific constructs using more ubiquitous promoters (such as CAG or EF1 α) (Cronin et al., 2014). This results in a practical trade-off between the potential advantages of targeted expression described above and achieving sufficient levels of optogenetic protein expression to induce functional light responses – as they might be limiting for some optogenetic tools.

The use of cell type specific promoters may be further compounded in the context of retina degeneration and remodelling, where changes in gene expression may reduce the functional specificity and efficacy of such approaches. However, transcriptomic studies from our own group (Gilhooley et al., 2021b) suggest only relatively minor changes in gene expression within the ON-bipolar cells during the progression of degeneration, and *Grm6* has been specifically demonstrated to persist during degeneration (e.g.:Hulliger et al., 2020). However, studies at the protein level (van Wyk et al., 2017) show a markedly reduced ability of the *grm6-sv40* construct to drive cell specific protein production in degenerate retina compared to wild type.

Equally important for translation to human studies is the specificity and efficacy of vector constructs across species. Evidence is accumulating that cell specific promoter constructs shown to work in mice are likely to have altered efficacy and specificity when expressed in other species (Hickey et al., 2017; Juttner et al., 2019; Lu et al., 2016) and thus careful consideration will be required before adopting this approach for human trials (Gauvain et al., 2021). Ultimately, due to reasons of technical complexity (and issues of remodelling), at the current stage, it may be necessary to settle on the simpler delivery of optogenetic therapies to RGCs, and forgo the potential benefits of specifically targeting bipolar cell populations.

7. Safety and toxicity of optogenetic therapies

In the human data thus far reported, there has been no evidence of severe toxicity or immune reaction following deployment of retinal optogenetic therapies, however, of necessity these examinations have been limited to assessments of gross anatomy on clinical examination (e.g.: Sahel et al., 2021a). Whilst this precludes any cataclysmic systemic rejection of the tools so far tested, it does little to assess toxicity at lower, microscopic levels that nonetheless may have damaging effects, slowly over time, which may limit efficacy of the therapy. Such potential toxic effects can be thought of in four main categories: 1. Phototoxicity, 2. Immunotoxicity, 3. Direct toxic effects of optogenetic protein, or 4. Indirect toxic effects due to the function of the protein. Each of these should be considered and investigated while we are now more and more entering the stage of clinical translation.

7.1. Phototoxicity

Phototoxic effects, defined as effects on the retina related to light incident onto the retina are today well investigated and have been reviewed in detail elsewhere (Hunter et al., 2012; Lawwill et al., 1977; Youssef et al., 2011). In brief, photothermal, photomechanical and photochemical toxicity can be distinguished, with photochemical toxicity (light-induced changes to biological molecules) being the one of main relevance in the context of optogenetic vision restoration. The potential of a certain light stimulus to induce phototoxic damage mainly depends on the energy delivered, being a function of its intensity and wavelength. Consensus agreement has been achieved regarding which light intensities are acceptable for ocular exposure and which must be avoided (ICNIRP, 2013). Such consensus has been widely implicated into national legislations. As discussed in Section 3, some optogenetic tools cannot be activated by ambient light would require stimulation by

light intensifying goggles to function and such goggles must operate within the limits of light safety thresholds. While more recently a number of microbial opsin variants have emerged that can be operated within these safety thresholds, phototoxicity concerns had been a hurdle for opsins operated by (high energy) blue light. This point has been excellently made in (Sengupta et al., 2016), where details on the calculation of light safety thresholds are presented.

7.2. Immunotoxicity

Investigations of immunotoxicity have so far focused on the immune responses to AAV based vectors themselves (Xiong et al., 2019) and this area has recently been elegantly reviewed in this journal (Bucher et al., 2021). Less has been reported on the immunogenic effects of the delivered tools to the retina independent of this. Khabou et al. (2018) found greater toxic effects on delivery of a non-mammalian protein (GFP) when compared to a native, retinal protein (retinoschisin) using the same AAV vector. Thus, giving some reason concerns regarding the immunogenic potential of exogenous expression of non-mammalian proteins (such as channelrhodopsins) in the retina and highlights an area of unmet need in research.

While the immunotoxic effects of channelrhodopsin expression have been examined at the retinal level in a small number of studies (Batabyal et al., 2021; Doroudchi et al., 2011; Sugano et al., 2011; Wright et al., 2021), this has generally been limited to qualitative or semi-quantitative immunohistochemistry. Examining such local immune responses (e.g. glial activation) is further complicated by the process of retinal degeneration, which can in itself activate similar processes (see Section 6). While these, and other, qualitative studies have been helpful to exclude massive deleterious immune responses to optogenetic tools (Batabyal et al., 2021; Doroudchi et al., 2011; Sugano et al., 2011) they require expansion with a systematic quantitative and likewise sensitive analysis of reactions that may have subtle effects on efficacy or long-term toxic effects.

Indeed, *in vivo* primate data raises concerns that such low-level toxic effects might exist: Gauvain et al. report levels of ocular inflammation assessed by fundoscopy (Gauvain et al., 2021). These data (Gauvain et al., 2021, Suppl Fig 1) shows that after treatment, vitreous cells were present in eight out of twelve examined treated macaque eyes and persisted over several examinations until the end of the study in six eyes. In the wider (non-ocular) literature, more in-depth studies on optogenetic tool immunotoxic have been performed (Shen et al., 2020) including on central (Miyashita et al., 2013) and peripheral (Maimon et al., 2018) neurons, demonstrating the existence of immune responses that limit long-term functionality (Maimon et al., 2018).

Only microbial optogenetic tools have so far moved to clinical trial (see below) and while similarly there is no evidence of massive, deleterious immune reactions in one trial (Martel et al., 2021); another trial reports one patient with vitritis and anterior chamber cells (NCT02556736) – however details have not yet been published. Further observations are therefore eagerly awaited from these early trials to determine the risk of a significant immune-type reaction in human eyes/patients.

7.3. Direct protein toxicity

Further observation both in disease models and humans will also be essential in forms of optogenetic induced toxicity beyond immune considerations. There is a considerable body of work regarding the direct toxic effects of proteins in retinal cells – especially in relation to dominant negative effects leading to autosomal dominant retinitis pigmentosa (Mendes et al., 2005) secondary to mal- and over-expression of rhodopsin. These effects can lead to cell death by various routes (Athanasίου et al., 2018) and are in themselves providing a fruitful target for novel gene therapies (e.g. (Orlans et al., 2021)). While the mechanisms of direct protein toxicity resulting from opsin expression

could be inferred from the example of rhodopsin, as yet *direct* toxic effects of *exogenous* (over)expression of mammalian opsins have not yet been systematically investigated.

While, similar to mammalian opsins, the direct toxic effects of microbial opsins have not been reported for the retina, observations have been made of such proteins when exogenously expressed in both central (Miyashita et al., 2013) and peripheral (Maimon et al., 2018) neurons. Indeed, Miyashita et al. demonstrate morphological changes in cortical neurons following ChR2 expression. They speculate on possible mechanisms for this: either as a direct toxic effect of protein overexpression, or indirect, perhaps via disruption of intracellular calcium signalling due to the non-native conductance introduced by the channelrhodopsin protein (Miyashita et al., 2013). Notably, observations regarding protein toxicity of microbial opsins are yet limited and observations made for one opsin can not necessarily be generalized to all opsins of that class. There are, for instance, engineered microbial opsins that are codon optimized (Gradinaru et al., 2008; Lin et al., 2013) and are designed for improved membrane targeting which may affect toxicity of such tools (Lin, 2011).

7.4. Functional toxicity

Exogenously expressed optogenetic tools could be predicted to have deleterious effects secondary to their functional activation, especially when we consider microbial opsins mediating directly light activated membrane conductances. This direct action is in contrast to the highly regulated receptor cascades activated by mammalian opsins with associated compensatory pathways to efficiently restore homeostasis and protect the cell. Such control is particularly important to bring intracellular calcium concentrations back into the physiological range to avoid excitotoxic processes (Arundine and Tymianski, 2003; Feldbauer et al., 2009; Kleinlogel et al., 2011). While activation of exogenously expressed microbial opsin does not appear to lead to loss of retinal ganglion cells (Tabata et al., 2021; Wright et al., 2021) our own data indicates that it may alter neuronal physiology to the extent that electrophysiological parameters – such as the rate of resting action potential firing could be attenuated (Gilhooley et al., 2022). Therefore, reinforcing that toxicity can have effects short of cell survival and further investigation is necessary to ensure the most efficient deployment of optogenetic approaches to visual restoration.

8. Translation of optogenetic vision restoration into clinics is ongoing

8.1. Optogenetic studies in large animals

Targeted approaches to optogenetic therapies can be well modelled in small animal models – the mouse is particularly attractive both due to its ease of husbandry, the extensive experience in its genetic manipulation and available models of retinal degeneration. Modelling the higher register of visual processing (especially that related to foveal vision) will however be more difficult in mice and along with regulatory requirements for preclinical safety work there is a need for optogenetic approaches to be investigated in larger animal models.

Indeed, there is increasing evidence (Table 2) that some but not all (Simon et al., 2020) optogenetic constructs delivered by AAV can induce appropriate expression also in larger animal models. These can even restore electrophysiological responses to light at the level of the retina and cortex and a case report of optogenetics being used in a patient has been presented (Sahel et al., 2021a). As primate models of retinal degeneration become increasingly available (Moshiri et al., 2019), this will become an increasingly interesting area of investigation, over and above its attraction for gathering regulatory pre-clinical safety data.

Also of note are the development of other large eye animal models of retina degeneration. These include the P23H miniature pig (Ross et al., 2012) and the P347L rabbit (Jones et al., 2011; Ueno et al., 2019)

Table 2

Summary of notable reports of ocular optogenetic therapy in large animals.

Study	Species	Approach	Construct	Results
Ivanova et al. (2010)	Marmoset	In vivo intravitreal injection; ex vivo Immunohistochemistry (IHC) & Multiple Electrode Array Electrophysiology (MEA).	<i>ChR2</i>	Protein expression confirmed by IHC, Light dependent responses on MEA
Sengupta et al. (2016)	Macaque, human	Ex vivo culture; with IHC and MEA	<i>ReaChR</i>	Protein expression & MEA light responses confirmed in cultured retinal explants of both species
Chaffiol et al. (2017)	Macaque	In vivo intravitreal injection, in vivo ocular imaging and cortical electrophysiology, ex vivo IHC and MEA electrophysiology	<i>CatCh</i>	Protein expression & MEA light responses confirmed, visually evoked cortical responses confirmed. In vivo ocular inflammation noted in small number of animals
Ameline et al., (2017)	RPE65-deficient Briard dog	In vivo intravitreal injection & OCT imaging. Ex vivo IHC & MEA	<i>Melanopsin, ChR2</i>	Protein expression confirmed by IHC, no change in retinal thickness over 21 months on OCT; restoration of light dependent responses on MEA.
Khabou et al. (2018)	Macaque, human	Ex vivo human retina explant; in vivo intravitreal and subretinal injection to macaque.	<i>Jaws</i>	Protein expression confirmed by IHC. Patch clamp electrophysiology demonstrates Jaws mediated light response
Liu et al. (2019)	Macaque, Royal College of Surgeons Rat	In vivo intravitreal injection, examination electroretinography & visual evoked potentials. Ex vivo patch clamp electrophysiology	<i>Melanopsin</i>	Protein expression confirmed by IHC. Reconstitution of electrophysiological light responses.
Garita-Hernandez et al. (2019)	Human induced pluripotent stem cells	Ex vivo expression of optogenetic tool in ipRGC derived human cone photoreceptors which were transplanted into transplanted blind mice (Cpfl1/Rho ^{-/-})	<i>Jaws</i>	Restoration of electrophysiological responses at photoreceptor and ganglion cell level
(McGregor et al., 2020, 2021)	Macaque	In vivo expression of optogenetic tool with adaptive optics ophthalmoscopy & in vivo calcium imaging	<i>Chrimson R</i>	Light induced optogenetic responses seen in vivo up to one year post injection.
Sahel et al. (2021a)	Human	In vivo ("PIONEER" clinical trial)	<i>Chrimson R</i>	Light dependent tasks performed by reported patent

models of human RP. These animals have a visual streak which represents a better model of the human fovea than any available mouse model. They are also far more affordable and logistically simpler to work with than primate models, yet to date these models have not been studied in the context of optogenetic vision restoration. Future studies of optogenetic therapies in non-primate large eye animal models are likely an important step in translating optogenetic therapies to clinical use, and will likely be highly beneficial for optimization of AAV delivery to the human retina. Finally, it is worth mentioning that more recently primate models for retinitis pigmentosa ([Ikeda et al., 2018](#)) and a cone dystrophy ([Moshiri et al., 2019](#)) have been identified and may have their place in translational optogenetic vision restoration studies in future.

8.2. Clinical trials: challenges and directions

This increasing body of evidence illustrates the potential of optogenetic techniques to restore, at the very least, sensitivity to light in degenerate retina in multiple animal models as well as behavioral level light responses in some cases. The logical extension of this has been to translate these encouraging results into human patients. However, in common with the panoply of emerging therapies in IRDs, this presents a collection of challenges, not least in constructing a well-controlled clinical trial. The vast heterogeneity of retinal degenerations, especially in their natural history, emphasizes the importance of patient assessment and selection ([Jacobson et al., 2013](#)) to both ensure a fair comparison and to allow an appropriately phrased research question to be answered efficiently. Similarly, adopting useful, comparable, functional endpoints is a challenge in an area where therapeutic trials are still in their infancy. More practical realities of such trials are also important with a tension in trial design between demands for best-possible safety and the necessity to proof efficacy ([Harris and Gilbert, 2021](#); [Lindner, 2022](#)). For instance, while from a safety perspective it seems logical to include only patients with end stage retinal degeneration it could be argued that advanced retinal remodeling may impede optogenetic gene therapy to restore functional vision (see Section 6). Similarly, with several gene therapy vectors now being studied in humans (and some even approved for clinical use) it could be argued that optogenetic trials should only utilize these given their existing safety data. Conversely, with optogenetic approaches to visual restoration, transfecting a sufficient proportion of the target cell is critical and therefore utilizing the most efficient vectors is ever more important and could justify the selection of alternative vectors ([Lindner,](#)

[2022](#)). Many of these challenges are articulated, and associated areas of unmet research need are presented in a recent manifesto by the Monaciano consortium ([Thompson et al., 2020](#)).

8.3. Questions for clinical trials: selecting a tool and target cell population

Selecting the most appropriate optogenetic tool and target population has proven complex in animal models (Section 5), even before the documented interspecies variability discussed above (Section 7) is considered. In such a complex system, animal models are best employed to determine the "most likely" candidates to pursue in humans. While clinical trials may then provide the information to what extent a chosen optogenetic strategy can result in a restored functional visual perception, they will likely not be able to deliver the details necessary to fully understand why a chosen treatment has – or has not – worked and where optimization is needed. Herein, a steady flow of information between clinical trials and more flexible cell or animal models-based research that can resolve modes of operation of a certain treatment approach in organ and even cellular detail will be needed.

Multiple factors may influence candidate selection at different stages of clinical investigation: the selection of CrimsonR in the PIONEER trial provides an apposite example ([Sahel et al., 2021a](#)). The overall light sensitivity of Crimson R is below the level that would allow activation by ambient light ([Klapoetke et al., 2014](#)), and is therefore not immediately an obvious choice for visual restoration. Its selection, and the incorporation of signal amplifying goggles, does however have several advantages. Firstly, in this phase I/II trial, any activity mediated toxicity or adverse effects (e.g. circadian disruption, unpleasant sensation of light, photophobia) could be avoided simply by keeping the patient in general room light. Secondly, the requirement for light amplifying goggles for activation allowed for an additional control: where light responses seen only with the goggles on, but not off, could more confidently be ascribed to the treatment. Secondly, although not activated by low levels of light, channelrhodopsin variants do provide virtually optimal kinetic performance under bright light conditions, with fast onset and offset kinetics and independently of the availability of second-messenger cascades – and therefore offer a near perfect tool to test the potential of optogenetics. Finally, the advantages of including computer assisted glasses are that they allow a level of signal processing and optimization to be performed prior to presentation of the light signal the retina, including increasing levels of contrast and or edge detection, and image stabilisation. Combined, these features potentially offer the best opportunity to

assess the feasibility of optogenetic visual restoration in humans at this stage. What needs to be kept in mind is that, if goggles are required, there is a need for long-term technical support – beyond the duration of the trial – and discontinuation of support of two main retinal implant devices has recently exemplified how this can be problematic (Strickland, 2022). Finally, these goggles may come with a (possibly neglectable) wearing discomfort.

Current clinical trials have made use of high efficiency, non-specific capsid and promoter constructs to maximize the quantity of optogenetic protein expressed (and so the effect size seen). Again, this approach is useful in early phase clinical trials where questions relate to proof of principle, requiring transgene expression at sufficient levels in the maximum number of cells possible. However, there is now evidence that cis-regulatory elements evoking strong ubiquitous transgene expression are somehow linked to toxicity (Xiong et al., 2019) and thus consideration should be given in clinical trials to population specific delivery possibly tolerating more moderate expression levels.

8.4. Efficacy measurement and design considerations in clinical trials

Selecting efficacy endpoints for clinical trials in optogenetic vision restoration gene therapy bears certain challenges that are substantially different from those faced in other gene therapy trials. In many ways, the obstacles are more akin to the situation in retinal implant development (Bloch et al., 2019) and experience gathered in this field can support efficient translation of optogenetic vision restoration into clinics. This is reflected in recent consensus paper on outcome measures for vision restoration approaches (Ayton et al., 2020b).

Controls. Comparison of a functional metric before and after treatment is the classical way of assessing treatment efficacy. Like retinal implants, stimulation of optogenetically treated retinas with tools that require light intensities above the ambient light levels (microbial opsins) requires utilization of signal enhancing goggles. Functional tests can be performed with goggles switched on and off thereby offering a valuable additional instance of control. This approach has been chosen in the PIONEER trial (Sahel et al., 2021a) and is recommended by the HOVER taskforce (Ayton et al., 2020b). Also, in instances where no signal enhancement is required, an additional instance of control can be obtained. For example, by using spectrally defined stimuli that do or do not match the spectral sensitivity of the optogenetic tool.

Measuring light responsiveness. In early optogenetic trials – from a researcher's perspective – it will be critical to provide objective measures that evidence restoration of light responsiveness in a treated retina. This will not answer the question whether a patient is able to make use of this light responsiveness in any meaningful way (functional vision), but it will provide robust biological proof of principle. Electrophysiological approaches like electroretinogram recordings and visually evoked potentials can be employed and the (temporal) properties of the optogenetic tool under study can serve to discriminate tool-restored from residual native responses (Ayton et al., 2020b).

In some cases, it may be possible to identify the area of the retina or even the individual cells that express an optogenetic tool after treatment. This may be the case after subretinal vector delivery or when fluorescent proteins are delivered together with the optogenetic tool (Sahel et al., 2021a). In those cases abovementioned electrophysiological approaches may be paired with focal stimuli to provide a spatial correlation between the treated cells and obtained responses. Similarly, psychophysical techniques either using modified, validated microperimetry devices (Cideciyan et al., 2016) or in a more experimental context single (photoreceptor) stimulating adaptive-optics devices (Reiniger et al., 2021) may be informative.

Particular to the field of optogenetic vision restoration is the great variability amongst the optogenetic tools that could be employed. This includes aspects like light sensitivity, desensitization or “slow” response kinetics. Stimulus paradigms therefore need to be carefully designed to meet the requirements of the optogenetic tool used in a particular trial.

On the other hand, some low-level stimuli (bright, broadband, sustained, long inter-stimulus-intervals) that are compatible across the spectrum of optogenetic tools currently under debate could enable basic inter-study comparability should be included into trial protocols.

Measuring vision in the ultra-low range. The first patients receiving a trial optogenetic therapy are those with little, or no, remaining perception of light. The improvement in visual function from current optogenetic therapies should be expected to bring patients beyond the range of ultra-low vision. Therefore, endpoints are needed that can robustly and sensitively detect functional gain within the range of ultra-low vision – and ideally beyond. As detailed in the HOVER consensus paper the availability of validated tests operating in this range is limited (Ayton et al., 2020b). Nevertheless, adherence to validated tests is highly important, not only to ensure best possible comparability between studies (Ayton et al., 2020b). This situation becomes even more complex when aiming to measure visual ability (i.e. how well a patient is able to complete visual tasks of every day relevance), instead of pure visual function.

What if trials fail to meet their functional endpoint? We currently know hardly anything about how optogenetic “vision” would manifest in a treated individual. Moreover, there is a possible tension between regulators preferring primary functional endpoints that are both, validated and relevant to the patient and researchers prioritizing those best ensuring detection of any biological sign of efficacy. While we are still in the early days of optogenetic vision restoration it is important that functional data from trials failing to meet their predefined functional endpoint is not simply disregarded but equally reported. Where the reported tests or analysis strategies diverge from what was primarily planned, this obviously needs mentioning, but they may still contain valuable information for the design of future optogenetic treatment approaches.

8.5. Current clinical trials

Four groups have currently registered early phase clinical trials of optogenetic therapy in retinal degeneration patients: the ‘PIONEER’ trial (NCT03326336), ‘RESTORE’ (NCT04945772), ‘RST-001 Phase I/II Trial’ (NCT02556736) and the ‘BS01 Phase I/II Trial’ (NCT04278131). All four have involved delivery of microbial type opsins via AAV constructs under high efficiency, non-specific promoters with a primary endpoint of safety and tolerability and improvement in visual function as a secondary endpoint – details of their specific trial protocols are summarised in Table 3.

PIONEER in particular sparked much interest when individual results from a patient were reported in (Sahel et al., 2021a). In this case report a patient with IRD and visual acuity of “light perception only” was seen to recover the ability to perform low-level visually guided tasks and electroencephalographic light responses to return following injection with AAV encoding the microbial opsin ChrimonR. There were no apparent deleterious alterations in anatomy following treatment or evidence of intraocular inflammation. However, full results of this phase I/II dose escalation study yet to be reported.

The Multi-characteristic opsin (MCO) construct used in the RESTORE trial has been demonstrated to be safe and tolerable in a phase I/IIa trial (NCT04919473, press release: (Eramian, 2021)). Little detail has been published with regard to the nature of MCO in the academic literature. It has however allowed a Phase IIb trial to commence with a saline injected control group with the hope of demonstrating efficacy (NCT04945772).

Recently several major pharmaceutical companies have entered the field to successfully translate further optogenetic vision restoration into clinics. Novartis has recently acquired Arcutos Medical (aiming to commercialize Opto-mGluR6 (van Wyk et al., 2015; Wolf, 2021)) as well as Vedere Bio (cone-opsin (Berry et al., 2019; Von Treskow, 2020; Wolf, 2021)) and Acucela has licensed a Rhodopsin based approach (Cehajic-Kapetanovic et al., 2015) and is preparing to enter clinical trials (Arnold, 2018). It is therefore likely that we will see a significant

Table 3
Overview on current clinical trials.

	Phase	Patients (main inclusion criteria)	Intervention (<i>tool, promoter, capsid, injection route</i>)	Outcome/endpoints	Visual assessment (<i>method used</i>)	Inflammation assessment (<i>method used</i>)	Toxicity Assessment (<i>method used</i>)	Any results reported?
PIONEER (NCT03326336)	I/IIa	"Non-syndromic Retinitis Pigmentosa (confirmed on full field ERG)"	Chrimson R; AAV 2.7m8 intravitreal injection to worse seeing eye	Primary: safety & tolerability of escalating doses Secondary: Visual acuity, Visual function, mobility, quality of life measures, immune response	Freiburg Visual Acuity & Contrast Test, full field threshold stimulus test (FST), perimetry, Novel visual search, counting, mobility and detection tasks	Slit lamp biomicroscopy and grading according to Standardization of Uveitis Nomenclature Working Group	Slit lamp biomicroscopy, fundus autofluorescence, OCT	Results from one participant have been published (Sahel et al., 2021a)
RESTORE (NCT04945772, NCT04919473)	I/IIa&IIb	"Advanced Retinitis Pigmentosa based on clinical examination, dilated fundus examination, and genetic testing"	Multi-characteristic opsin (MCO); delivered by AAV2 vector via intravitreal injection	Primary: Effect on ability to perform Y maze task; Adverse reactions Secondary: Effect of therapy on vision, light sensitivity, visual function. Secindary: Pharmacokinetics	Freiberg visual acuity, pupilometry, FST, perimetry, VQ-25, Shape recognition, optical low task	Not reported	Not reported	Press release only https://www.opthalmologytimes.com/view/optogenetic-gene-therapy-restores-vision-in-11-rp-patients
RST-001 Phase I/II Trial' (NCT NCT02556736). Allergan	I/IIa	"Advanced Retinitis Pigmentosa, vision no better than hand motion"	Intravitreal delivery of "RST-001 a gene therapeutic"	Primary: Number of Participants with Any Grade 3 or Greater Adverse Event (AE) considered Related to RST-001 (Grade 3 being: Severe or medically significant but not immediately life-threatening). Secondary: Change in Amulation, Visual Acuity, Quality of life (VQ-25 score)	Not reported	Not reported	Not reported	Primary result summary on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/results/NCT02556736)
NCT04278131	I/II	"Retinitis Pigmentosa, bare light perception in at least one eye"	AAV vector delivering channelrhodopsin-variant ChronosFP	Safety, change in threshold sensitivity	Electrophysiology	Not reported	Not reported	

increase in the number of active clinical trials in the field. From a scientist's perspective, this underscores the importance of efficacy and safety metrics that enable best possible inter-trial comparability.

9. Conclusion and future directions

Animal models have demonstrated the power and potential of optogenetic vision restoration and proof-of-principle clinical data has just recently been released. The next big challenge will be to move from restoration of a rudimentary form vision to a vision that offers the space and time resolution necessary for (basic) routine daily tasks. Achieving this goal will require a closer synergy between clinical and preclinical efforts: Clinical trials will provide the information to what extent a chosen optogenetic strategy can result in a restored functional visual perception. They may even provide cues on what is missing to deliver a better perception. However, they will be unlikely to deliver the level of detail for a systematic optimization of the approach. Flexible cell or animal models enable resolving modes of operation of a certain treatment approach in organ and even cellular detail. Fed with specific questions from clinical trials, these allow designing next, improved treatment approaches for re-translation.

Decisions are still to be made regarding the optimal tool and target cell type(s) for clinical treatments, ultimately timely progress is more likely to be constrained by our ability to develop protocols allowing safe and reliable delivery of appropriate levels of tools to the target cells of choice – and obviously the translation of these approaches to human subjects. In this respect there is an urgent need to develop reliable short-promoter constructs to allow optogenetic tools to be delivered to individual cell populations in human subjects.

It is expected that future developments in opsin engineering may yield tools with more favorable properties (improved combinations of light sensitivity and temporal kinetics for example). Moreover, the potential of specifically targeting the soma of survivor retinal ganglion cells with an optogenetic tool offers the potential to spatially sharpen light responses at the retinal output level.

Currently from the animal experiments we know that treatment with optogenetic therapy at the very late stage of the disease, when all photoreceptors have been lost and the retinal circuitry is remodeled, is still beneficial – including at behavioral level. However, as these optogenetic approaches translate into treatments, we are yet to really define the optimum time window to result in maximum treatment benefit. This may make the difference between restoring rudimentary vision allowing for coarse orientation and restoring complex functional vision.

Immediate questions are how safe such treatments are in long-term, and if delivery of such tools does not accelerate degeneration or cause damage to recipient cells. Equally, from what we know about retinal remodelling, early intervention could even result in slowing the rate of inner retinal remodelling as a form of neuroprotection. Many of these questions could and should be approached in the current preclinical model systems.

In the immediate future the promise of optogenetic treatments rests on the outcome of ongoing and commencing clinical trials aimed at showing the potential efficacy of such approaches. Though expectations are high, at this stage what is most needed are robust, reproducible signs of efficacy that justify and direct future studies.

Financial support

1. University Hospital Gießen and Marburg Research Funds (15/2020MR to ML)
2. German Research Foundation (LI 2846/6–1 to ML)
3. ProRetina Foundation (Pro-Re/Projekt/Gilhooley-Whitehead-Lindner.04-2021 to ML and MJG)
4. Medical Research Council UK (MR/S026266/1 to MWH)
5. Wellcome Trust (205151/Z/16/Z to MJG)
6. NIHR Clinical Lectureship (CL-2019-18-004 to MJG)

References

- Abbott, S.M., Malkani, R.G., Zee, P.C., 2020. Circadian disruption and human health: a bidirectional relationship. *Eur. J. Neurosci.* 51, 567–583.
- Agosto, M.A., Adeosun, A.A.R., Kumar, N., Wensel, T.G., 2021. The mGluR6 ligand-binding domain, but not the C-terminal domain, is required for synaptic localization in retinal ON-bipolar cells. *J. Biol. Chem.* 297, 101418.
- Armata, I.A., Giompres, P., Smith, A., Stasi, K., Kouvelas, E.D., Mitsacos, A., 2006. Genetically induced retinal degeneration leads to changes in metabotropic glutamate receptor expression. *Neurosci. Lett.* 393, 12–17.
- Arnold, K., 2018. SIRION Biotech and Acucela Set Sights on Gene Delivery Tools for Ocular Gene Therapy.
- Arundine, M., Tymianski, M., 2003. Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium* 34, 325–337.
- Athanasios, D., Aguila, M., Bellingham, J., Li, W., McCulley, C., Reeves, P.J., Cheetham, M.E., 2018. The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy. *Prog. Retin. Eye Res.* 62, 1–23.
- Ayton, L.N., Barnes, N., Dagnelie, G., Fujikado, T., Goetz, G., Hornig, R., Jones, B.W., Muqit, M.M.K., Rathbun, D.L., Stingl, K., Weiland, J.D., Petoe, M.A., 2020a. An update on retinal prostheses. *Clin. Neurophysiol. : Off. J. Int. Fed. Clin. Neurophysiol.* 131, 1383–1398.
- Ayton, L.N., Rizzo 3rd, J.F., Bailey, I.L., Colenbrander, A., Dagnelie, G., Gerasch, D.R., Hessburg, P.C., McCarthy, C.D., Petoe, M.A., Rubin, G.S., Troyk, P.R., Taskforce, H. I., 2020b. Harmonization of outcomes and vision endpoints in vision restoration trials: recommendations from the international HOVER taskforce. *Transl. Vis. Sci. Technol.* 9, 25.
- Baden, T., Berens, P., Franke, K., Roman Roson, M., Bethge, M., Euler, T., 2016. The functional diversity of retinal ganglion cells in the mouse. *Nature* 529, 345–350.
- Bailes, H.J., Lucas, R.J., 2013. Human melanopsin forms a pigment maximally sensitive to blue light (lambda max approximately 479 nm) supporting activation of G(q/11) and G(i/o) signalling cascades. *Proc. Biol. Sci.* 280, 20122987.
- Ballister, E.R., Rodgers, J., Martini, F., Lucas, R.J., 2018. A live cell assay of GPCR coupling allows identification of optogenetic tools for controlling Go and Gi signaling. *BMC Biol.* 16, 10.
- Barhoum, R., Martínez-Navarrete, G., Corrochano, S., Germain, F., Fernandez-Sanchez, L., de la Rosa, E.J., de la Villa, P., Cuenca, N., 2008. Functional and structural modifications during retinal degeneration in the rd10 mouse. *Neuroscience* 155, 698–713.
- Barrett, J.M., Degenar, P., Sernagor, E., 2015. Blockade of pathological retinal ganglion cell hyperactivity improves optogenetically evoked light responses in rd1 mice. *Front. Cell. Neurosci.* 9, 330.
- Batabyal, S., Gajjaraman, S., Pradhan, S., Bhattacharya, S., Wright, W., Mohanty, S., 2021. Sensitization of ON-bipolar cells with ambient light activatable multi-characteristic opsin rescues vision in mice. *Gene Ther.* 28, 162–176.
- Bax, N.M., Valkenburg, D., Lambertus, S., Klevering, B.J., Boon, C.J.F., Holz, F.G., Cremers, F.P.M., Fleckenstein, M., Hoyng, C.B., Lindner, M., Foveal Sparing Atrophy Study, T., 2019. Foveal sparing in central retinal dystrophies. *Invest. Ophthalmol. Vis. Sci.* 60, 3456–3467.
- Begenisic, T., Mazzotti, R., Sagana, G., Lupori, L., Sale, A., Galli, L., Baroncelli, L., 2020. Preservation of visual cortex plasticity in retinitis pigmentosa. *Neuroscience* 424, 205–210.
- Berry, M.H., Holt, A., Levitz, J., Broichhagen, J., Gaub, B.M., Visel, M., Stanley, C., Aghi, K., Kim, Y.J., Cao, K., Kramer, R.H., Trauner, D., Flannery, J., Isacoff, E.Y., 2017. Restoration of patterned vision with an engineered photoactivatable G protein-coupled receptor. *Nat. Commun.* 8, 1862.
- Berry, M.H., Holt, A., Salari, A., Veit, J., Visel, M., Levitz, J., Aghi, K., Gaub, B.M., Sivyer, B., Flannery, J.G., Isacoff, E.Y., 2019. Restoration of high-sensitivity and adapting vision with a cone opsin. *Nat. Commun.* 10, 1221.
- Bi, A., Cui, J., Ma, Y.P., Olshevskaya, E., Pu, M., Dizhoor, A.M., Pan, Z.H., 2006. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* 50, 23–33.
- Birtel, J., Eisenberger, T., Gliem, M., Muller, P.L., Herrmann, P., Betz, C., Zahnleiter, D., Neuhaus, C., Lenzner, S., Holz, F.G., Mangold, E., Bolz, H.J., Charbel Issa, P., 2018. Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci. Rep.* 8, 4824.
- Birtel, J., Gliem, M., Oishi, A., Müller, P.L., Herrmann, P., Holz, F.G., Mangold, E., Knapp, M., Bolz, H.J., Charbel Issa, P., 2019. Genetic testing in patients with retinitis pigmentosa: features of unsolved cases. *Clin. Exp. Ophthalmol.* 47, 779–786.
- Bloch, E., Luo, Y., da Cruz, L., 2019. Advances in retinal prosthesis systems. *Rev. Adv. Ophthalmol.* 11, 2515841418817501.
- Bloomfield, S.A., Dacheux, R.F., 2001. Rod vision: pathways and processing in the mammalian retina. *Prog. Retin. Eye Res.* 20, 351–384.
- Borowska, J., Trenholm, S., Awatramani, G.B., 2011. An intrinsic neural oscillator in the degenerating mouse retina. *J. Neurosci. : Off. J. Soc. Neurosci.* 31, 5000–5012.
- Bucher, K., Rodriguez-Bocanegra, E., Daulebekov, D., Fischer, M.D., 2021. Immune responses to retinal gene therapy using adeno-associated viral vectors - implications for treatment success and safety. *Prog. Retin. Eye Res.* 83, 100915.
- Busskamp, V., Duebel, J., Balya, D., Fradot, M., Viney, T.J., Siegrist, S., Groner, A.C., Cabuy, E., Forster, V., Seeliger, M., Biel, M., Humphries, P., Pagues, M., Mohand-Said, S., Trono, D., Deisseroth, K., Sahel, J.A., Picaud, S., Roska, B., 2010. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* 329, 413–417.
- Cahill, H., Nathans, J., 2008. The optokinetic reflex as a tool for quantitative analyses of nervous system function in mice: application to genetic and drug-induced variation. *PLoS One* 3, e2055.

- Campochiaro, P.A., Mir, T.A., 2018. The mechanism of cone cell death in Retinitis Pigmentosa. *Prog. Retin. Eye Res.* 62, 24–37.
- Caporale, N., Kolstad, K.D., Lee, T., Tochitsky, L., Dalkara, D., Trauner, D., Kramer, R., Dan, Y., Isacoff, E.Y., Flannery, J.G., 2011. LiGluR restores visual responses in rodent models of inherited blindness. *Mol. Ther.* 19, 1212–1219.
- Caras, I.W., Collins, L.R., Creasey, A.A., 2021. A stem cell journey in ophthalmology: from the bench to the clinic. *Stem Cells Transl. Med.* 10, 1581–1587.
- Carter-Dawson, L.D., LaVail, M.M., Sidman, R.L., 1978. Differential effect of the rd mutation on rods and cones in the mouse retina. *Invest. Ophthalmol. Vis. Sci.* 17, 489–498.
- Cehajic-Kapetanovic, J., Eleftheriou, C., Allen, A.E., Milosavljevic, N., Pienaar, A., Bedford, R., Davis, K.E., Bishop, P.N., Lucas, R.J., 2015. Restoration of vision with ectopic expression of human rod opsin. *Curr. Biol.* 25, 2111–2122.
- Chaffiol, A., Caplette, R., Jaillard, C., Brazhnikova, E., Desrosiers, M., Dubus, E., Duhamel, L., Mace, E., Marre, O., Benoit, P., Hantraye, P., Bemelmans, A.P., Bamberg, E., Duebel, J., Sahel, J.A., Picard, S., Dalkara, D., 2017. A new promoter allows optogenetic vision restoration with enhanced sensitivity in macaque retina. *Mol. Ther.* 25, 2546–2560.
- Chen, K., Wang, Y., Liang, X., Zhang, Y., Ng, T.K., Chan, L.L., 2016. Electrophysiology alterations in primary visual cortex neurons of retinal degeneration (S334ter-line-3) rats. *Sci. Rep.* 6, 26793.
- Chen, M., Wang, K., Lin, B., 2012. Development and degeneration of cone bipolar cells are independent of cone photoreceptors in a mouse model of retinitis pigmentosa. *PLoS One* 7, e44036.
- Choi, H., Zhang, L., Cembrowski, M.S., Sabottke, C.F., Markowitz, A.L., Butts, D.A., Kath, W.L., Singer, J.H., Riecke, H., 2014. Intrinsic bursting of All amacrine cells underlies oscillations in the rd1 mouse retina. *J. Neurophysiol.* 112, 1491–1504.
- Chung, D.C., McCague, S., Yu, Z.F., Thill, S., DiStefano-Pappas, J., Bennett, J., Cross, D., Marshall, K., Wellman, J., High, K.A., 2018. Novel mobility test to assess functional vision in patients with inherited retinal dystrophies. *Clin. Exp. Ophthalmol.* 46, 247–259.
- Chuong, A.S., Miri, M.L., Busskamp, V., Matthews, G.A., Acker, L.C., Sorensen, A.T., Young, A., Klapeotke, N.C., Henninger, M.A., Kodandaramaiah, S.B., Ogawa, M., Ramanlal, S.B., Bandler, R.C., Allen, B.D., Forest, C.R., Chow, B.Y., Han, X., Lin, Y., Tye, K.M., Roska, B., Cardin, J.A., Boyden, E.S., 2014. Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat. Neurosci.* 17, 1123–1129.
- Chuvpayan, Y., Finger, R.P., Koberleind-Neu, J., 2020. Economic burden of blindness and visual impairment in Germany from a societal perspective: a cost-of-illness study. *Eur. J. Health Econ.* 21, 115–127.
- Cideciyan, A.V., Roman, A.J., Jacobson, S.G., Yan, B., Pascolini, M., Charnig, J., Pajaro, S., Nirenberg, S., 2016. Developing an outcome measure with high luminance for optogenetics treatment of severe retinal degenerations and for gene therapy of cone diseases. *Invest. Ophthalmol. Vis. Sci.* 57, 3211–3221.
- Cronin, T., Vandenbergh, L.H., Hantz, P., Juttner, J., Reimann, A., Kacso, A.E., Huckfeldt, R.M., Busskamp, V., Kohler, H., Lagali, P.S., Roska, B., Bennett, J., 2014. Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter. *EMBO Mol. Med.* 6, 1175–1190.
- Cundy, O., Broadgate, S., Halford, S., MacLaren, R.E., Shanks, M.E., Clouston, P., Gilhooley, M.J., Downes, S.M., 2020. Genetic and clinical findings in an ethnically diverse retinitis pigmentosa cohort associated with pathogenic variants in EYS. *Eye* 35 (5), 1440–1449.
- Dacey, D.M., Liao, H.W., Peterson, B.B., Robinson, F.R., Smith, V.C., Pokorny, J., Yau, K. W., Gamin, P.D., 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature* 433, 749–754.
- De Silva, S.R., Barnard, A.R., Hughes, S., Tam, S.K.E., Martin, C., Singh, M.S., Barnea-Cramer, A.O., McClements, M.E., During, M.J., Peirson, S.N., Hankins, M.W., MacLaren, R.E., 2017. Long-term restoration of visual function in end-stage retinal degeneration using subretinal human melanopsin gene therapy. *Proc. Natl. Acad. Sci. U. S. A.* 114, 11211–11216.
- De Silva, S.R., Charbel Issa, P., Singh, M.S., Lipinski, D.M., Barnea-Cramer, A.O., Walker, N.J., Barnard, A.R., Hankins, M.W., MacLaren, R.E., 2016. Single residue AAV capsid mutation improves transduction of photoreceptors in the Abca4(-/-) mouse and bipolar cells in the rd1 mouse and human retina ex vivo. *Gene Ther.* 23, 767–774.
- Doroudchi, M.M., Greenberg, K.P., Liu, J., Silka, K.A., Boyden, E.S., Lockridge, J.A., Arman, A.C., Janani, R., Boye, S.E., Boye, S.L., Gordon, G.M., Matteo, B.C., Sampath, A.P., Hauswirth, W.W., Horsager, A., 2011. Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. *Mol. Ther.* 19, 1220–1229.
- Dunn, F.A., 2015. Photoreceptor ablation initiates the immediate loss of glutamate receptors in postsynaptic bipolar cells in retina. *J. Neurosci. : Off. J. Soc. Neurosci.* 35, 2423–2431.
- Eickelbeck, D., Rudack, T., Tennigkeit, S.A., Surdin, T., Karapinar, R., Schwitala, J.C., Mücke, B., Shulman, M., Scherl, M., Althoff, P., Mark, M.D., Gerwert, K., Herlitze, S., 2020. Lamprey parapinopsin ("UVLamp"): a bistable UV-sensitive optogenetic switch for ultrafast control of GPCR pathways. *ChemBiochem* 21, 612–617.
- Eleftheriou, C.G., Cehajic-Kapetanovic, J., Martial, F.P., Milosavljevic, N., Bedford, R.A., Lucas, R.J., 2017. Meclofenamic acid improves the signal to noise ratio for visual responses produced by ectopic expression of human rod opsin. *Mol. Vis.* 23, 334–345.
- Eramian, D., 2021. Positive data from nanoscope's phase 1/2a trial of gene therapy to restore vision in patients blinded by retinitis pigmentosa to be featured at 2021 American society of retina specialists meeting. *Opus Biotech. Commun.*
- Euler, T., Schubert, T., 2015. Multiple independent oscillatory networks in the degenerating retina. *Front. Cell. Neurosci.* 9, 444.
- Fain, G.L., 2019. *Sensory Transduction*, 2 ed. Oxford University Press, Oxford.
- Farber, D.B., Flannery, J.G., Bowes-Rickman, C., 1994. The rd mouse story: Seventy years of research on an animal model of inherited retinal degeneration. *Prog. Retin. Eye Res.* 13, 31–64.
- Farrar, G.J., Carrigan, M., Dockery, A., Millington-Ward, S., Palfi, A., Chadderton, N., Humphries, M., Kiang, A.S., Kenna, P.F., Humphries, P., 2017. Toward an elucidation of the molecular genetics of inherited retinal degenerations. *Hum. Mol. Genet.* 26, R2–R11.
- Feldbauer, K., Zimmermann, D., Pintschovius, V., Spitz, J., Bamann, C., Bamberg, E., 2009. Channelrhodopsin-2 is a leaky proton pump. *Proc. Natl. Acad. Sci. U. S. A.* 106, 12317–12322.
- Finn, A.P., Grewal, D.S., Vajzovic, L., 2018. Argus II retinal prosthesis system: a review of patient selection criteria, surgical considerations, and post-operative outcomes. *Clin. Ophthalmol.* 12, 1089–1097.
- Foster, R.G., 2020. Sleep, circadian rhythms and health. *Interface Focus* 10, 20190098.
- Foster, R.G., Hughes, S., Peirson, S.N., 2020. Circadian photoentrainment in mice and humans. *Biology* 9.
- Foster, R.G., Peirson, S.N., Wulff, K., Winnebeck, E., Vetter, C., Roenneberg, T., 2013. Sleep and circadian rhythm disruption in social jetlag and mental illness. *Prog. Mol. Biol. Transl. Sci.* 119, 325–346.
- Fritsche, L.G., Igl, W., Bailey, J.N., Grassmann, F., Sengupta, S., Bragg-Gresham, J.L., Burdon, K.P., Hebbaring, S.J., Wen, C., Gorski, M., Kim, I.K., Cho, D., Zack, D., Souied, E., Scholl, H.P., Bala, E., Lee, K.E., Hunter, D.J., Sardi, R.J., Mitchell, P., Merriam, J.E., Cipriani, V., Hoffman, J.D., Schick, T., Lechanteur, Y.T., Guymer, R. H., Johnson, M.P., Jiang, Y., Stanton, C.M., Buitendijk, G.H., Zhan, X., Kwong, A.M., Boleda, A., Brooks, M., Giesler, L., Ratnapriya, R., Branham, K.E., Foerster, J.R., Heckenlively, J.R., Othman, M.I., Vote, B.J., Liang, H.H., Souzeau, E., McAllister, I. L., Isaacs, T., Hall, J., Lake, S., Mackey, D.A., Constable, I.J., Craig, J.E., Kitchner, T. E., Yang, Z., Su, Z., Luo, H., Chen, D., Ouyang, H., Flagg, K., Lin, D., Mao, G., Ferreyra, H., Stark, K., von Strachwitz, C.N., Wolf, A., Brandt, C., Rudolph, G., Olden, M., Morrison, M.A., Morgan, D.J., Schu, M., Ahn, J., Silvestri, G., Tsironi, E. E., Park, K.H., Farrer, L.A., Orlin, A., Brucker, A., Li, M., Curcio, C.A., Mohand-Said, S., Sahel, J.A., Audo, I., Benhaboune, M., Cree, A.J., Rennie, C.A., Goverdhan, S.V., Grunin, M., Hagbi-Levi, S., Campochiaro, P., Katsanis, N., Holz, F. G., Blond, F., Blanche, H., Deleuze, J.F., Igo Jr., R.P., Truit, B., Peachey, N.S., Meuer, S.M., Myers, C.E., Moore, E.L., Klein, R., Hauser, M.A., Postel, E.A., Courtenay, M.D., Schwartz, S.G., Kovach, J.L., Scott, W.K., Liew, G., Tan, A.G., Gopinath, B., Merriam, J.C., Smith, R.T., Khan, J.C., Shahid, H., Moore, A.T., McGrath, J.A., Laux, R., Brantley Jr., M.A., Agarwal, A., Ersoy, L., Caramoy, A., Langmann, T., Saksens, N.T., de Jong, E.K., Hoyng, C.B., Cain, M.S., Richmond, A. J., Martin, T.M., Blangero, J., Weeks, D.E., Dhillion, B., van Duijn, C.M., Doheny, K. F., Romm, J., Klaver, C.C., Hayward, C., Gorin, M.B., Klein, M.L., Baird, P.N., den Hollander, A.I., Fauser, S., Yates, J.R., Allikmets, R., Wang, J.J., Schaumberg, D.A., Klein, B.E., Hagstrom, S.A., Chowers, I., Lotery, A.J., Leveillard, T., Zhang, K., Brilliant, M.H., Hewitt, A.W., Swaroop, A., Chew, E.Y., Pericak-Vance, M.A., DeAngelis, M., Stambolian, D., Haines, J.L., Iyengar, S.K., Weber, B.H., Abecasis, G. R., Heid, I.M., 2016. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat. Genet.* 48, 134–143.
- Galvin, O., Chi, G., Brady, L., Hippert, C., Del Valle Rubido, M., Daly, A., Michaelides, M., 2020. The impact of inherited retinal diseases in the republic of Ireland (ROI) and the United Kingdom (UK) from a cost-of-illness perspective. *Clin. Ophthalmol.* 14, 707–719.
- Ganjawala, T.H., Lu, Q., Fenner, M.D., Abrams, G.W., Pan, Z.H., 2019. Improved CoChR variants restore visual acuity and contrast sensitivity in a mouse model of blindness under ambient light conditions. *Mol. Ther.*
- Gargini, C., Terzibas, E., Mazzoni, F., Strettoi, E., 2007. Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: a morphological and ERG study. *J. Comp. Neurol.* 500, 222–238.
- Garita-Hernandez, M., Guibbal, L., Toualbi, L., Routet, F., Chaffiol, A., Winckler, C., Harinquet, M., Robert, C., Fouquet, S., Bellow, S., Sahel, J.A., Goureau, O., Duebel, J., Dalkara, D., 2018. Optogenetic light sensors in human retinal organoids. *Front. Neurosci.* 12, 789.
- Garita-Hernandez, M., Lampic, M., Chaffiol, A., Guibbal, L., Routet, F., Santos-Ferreira, T., Gasparini, S., Borsch, O., Gagliardi, G., Reichman, S., Picard, S., Sahel, J.A., Goureau, O., Ader, M., Dalkara, D., Duebel, J., 2019. Restoration of visual function by transplantation of optogenetically engineered photoreceptors. *Nat. Commun.* 10, 4524.
- Gasparini, S.J., Llonch, S., Borsch, O., Ader, M., 2019. Transplantation of photoreceptors into the degenerative retina: current state and future perspectives. *Prog. Retin. Eye Res.* 69, 1–37.
- Gaub, B.M., Berry, M.H., Holt, A.E., Isacoff, E.Y., Flannery, J.G., 2015. Optogenetic vision restoration using rhodopsin for enhanced sensitivity. *Mol. Ther.* 23, 1562–1571.
- Gaub, B.M., Berry, M.H., Holt, A.E., Reiner, A., Kienzler, M.A., Dolgova, N., Nikonov, S., Aguirre, G.D., Beltran, W.A., Flannery, J.G., Isacoff, E.Y., 2014. 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. U. S. A.* 111, E5574–E5583.
- Gauvain, G., Akolkar, H., Chaffiol, A., Arcizet, F., Khoei, M.A., Desrosiers, M., Jaillard, C., Caplette, R., Marre, O., Bertin, S., Fovet, C.M., Demilly, J., Forster, V., Brazhnikova, E., Hantraye, P., Pouget, P., Douar, A., Pruneau, D., Chavas, J., Sahel, J.A., Dalkara, D., Duebel, J., Benosman, R., Picard, S., 2021. Optogenetic therapy: high spatiotemporal resolution and pattern discrimination compatible with vision restoration in non-human primates. *Commun. Biol.* 4, 125.

- Gayet-Primo, J., Puthussery, T., 2015. Alterations in Kainate receptor and TRPM1 localization in bipolar cells after retinal photoreceptor degeneration. *Front. Cell. Neurosci.* 9, 486.
- Gehlen, J., Esser, S., Schaffrath, K., Johnen, S., Walter, P., Müller, F., 2020. Blockade of retinal oscillations by benzodiazepines improves efficiency of electrical stimulation in the mouse model of RP, rd10. *Invest. Ophthalmol. Vis. Sci.* 61, 37.
- Gilhooley, M.J., Hickey, D., Lindner, M., Palumaa, T., Hughes, S., Peirson, S.N., MacLaren, R.E., Hankins, M.W., 2021a. ON-bipolar cell gene expression during retinal degeneration: implications for optogenetic visual restoration. *Exp. Eye Res.* 108553.
- Gilhooley, M.J., Lindner, M., Palumaa, T., Hughes, S., Peirson, S.N., Hankins, M.W., 2022. A systematic comparison of optogenetic approaches to visual restoration. *Mol. Ther. Methods Clin. Dev.* 25, 111–123.
- Gilhooley, M.J., Owen, N., Moosajee, M., Yu Wai Man, P., 2021b. From transcriptomics to treatment in inherited optic neuropathies. *Genes* 12.
- Goo, Y.S., Ye, J.H., Lee, S., Nam, Y., Ryu, S.B., Kim, K.H., 2011. Retinal ganglion cell responses to voltage and current stimulation in wild-type and rd1 mouse retinas. *J. Neural. Eng.* 8, 035003.
- Gradinaru, V., Thompson, K.R., Deisseroth, K., 2008. eNpHR: a Natronomonas halorhodopsin enhanced for optogenetic applications. *Brain Cell Biol.* 36, 129–139.
- Greenberg, K.P., Pham, A., Werblin, F.S., 2011. Differential targeting of optical neuromodulators to ganglion cell soma and dendrites allows dynamic control of center-surround antagonism. *Neuron* 69, 713–720.
- Haq, W., Arango-Gonzalez, B., Zrenner, E., Euler, T., Schubert, T., 2014. Synaptic remodeling generates synchronous oscillations in the degenerated outer mouse retina. *Front. Neural Circ.* 8, 108.
- Harris, A.R., Gilbert, F., 2021. Restoring vision using optogenetics without being blind to the risks. *Graefes Arch. Clin. Exp. Ophthalmol.*
- Hartong, D.T., Berson, E.L., Dryja, T.P., 2006. Retinitis pigmentosa. *Lancet* 368, 1795–1809.
- Haselier, C., Biswas, S., Rösch, S., Thumann, G., Müller, F., Walter, P., 2017. Correlations between specific patterns of spontaneous activity and stimulation efficiency in degenerated retina. *PLoS One* 12, e0190048.
- Hickey, D., Hughes, S., Davies, W.I., MacLaren, R.E., Hankins, M., 2015. Human opsin-G-protein fusion proteins as potential light sensitizers for optogenetic gene therapy for retinal degeneration. *Clin. Exp. Ophthalmol.* 43, 16–33.
- Hickey, D.G., Davies, W.I., Hughes, S., Rodgers, J., Thavanesan, N., MacLaren, R.E., Hankins, M.W., 2021. Chimeric human opsins as optogenetic light sensitizers. *J. Exp. Biol.* 224.
- Hickey, D.G., Edwards, T.L., Barnard, A.R., Singh, M.S., de Silva, S.R., McClements, M.E., Flannery, J.G., Hankins, M.W., MacLaren, R.E., 2017. Tropism of engineered and evolved recombinant AAV serotypes in the rd1 mouse and ex vivo primate retina. *Gene Ther.* 24, 787–800.
- Hughes, S., Jagannath, A., Rodgers, J., Hankins, M.W., Peirson, S.N., Foster, R.G., 2016. Signalling by melanopsin (OPN4) expressing photosensitive retinal ganglion cells. *Eye* 30, 247–254.
- Hulliger, E.C., Hostettler, S.M., Kleinlogel, S., 2020. Empowering retinal gene therapy with a specific promoter for human rod and cone ON-bipolar cells. *Mol. Ther. Methods Clin. Dev.* 17, 505–519.
- Hunter, J.J., Morgan, J.L., Merigan, W.H., Sliney, D.H., Sparrow, J.R., Williams, D.R., 2012. The susceptibility of the retina to photochemical damage from visible light. *Prog. Retin. Eye Res.* 31, 28–42.
- ICNIRP, 2013. Guidelines on limits of exposure to laser radiation of wavelengths between 180 nm and 1,000 µm. *Health Phys.* 105, 271–295.
- Ikeda, Y., Nishiguchi, K.M., Miya, F., Shimozaawa, N., Funatsu, J., Nakatake, S., Fujiwara, K., Tachibana, T., Murakami, Y., Hisatomi, T., Yoshida, S., Yasutomi, Y., Tsunoda, T., Nakazawa, T., Ishibashi, T., Sonoda, K.H., 2018. Discovery of a Cynomolgus monkey family with retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 59, 826–830.
- Ivanova, E., Hwang, G.S., Pan, Z.H., Troilo, D., 2010. Evaluation of AAV-mediated expression of Chp2-GFP in the marmoset retina. *Invest. Ophthalmol. Vis. Sci.* 51, 5288–5296.
- Ivanova, E., Pan, Z.H., 2009. Evaluation of the adeno-associated virus mediated long-term expression of channelrhodopsin-2 in the mouse retina. *Mol. Vis.* 15, 1680–1689.
- Ivanova, E., Yee, C.W., Baldoni Jr., R., Sagdullaev, B.T., 2016. Aberrant activity in retinal degeneration impairs central visual processing and relies on Cx36-containing gap junctions. *Exp. Eye Res.* 150, 81–89.
- Ivanova, E., Yee, C.W., Sagdullaev, B.T., 2015. Increased phosphorylation of Cx36 gap junctions in the AII amacrine cells of RD retina. *Front. Cell. Neurosci.* 9, 390.
- Jacobson, S.G., Sumaroka, A., Luo, X., Cideciyan, A.V., 2013. Retinal optogenetic therapies: clinical criteria for candidacy. *Clin. Genet.* 84, 175–182.
- Jiang, Z., Yue, W.W.S., Sheng, Y., Cao, L.-H., Yau, K.-W., 2017. Novel phototransduction pathway in intrinsically photosensitive retinal ganglion cells (ipRGCs). *Invest. Ophthalmol. Vis. Sci.* 58, 4127–4127.
- Jin, Z.B., Gao, M.L., Deng, W.L., Wu, K.C., Sugita, S., Mandai, M., Takahashi, M., 2019. Stemming retinal regeneration with pluripotent stem cells. *Prog. Retin. Eye Res.* 69, 38–56.
- Jones, B.W., Kondo, M., Terasaki, H., Lin, Y., McCall, M., Marc, R.E., 2012. Retinal remodeling. *Jpn. J. Ophthalmol.* 56, 289–306.
- Jones, B.W., Kondo, M., Terasaki, H., Watt, C.B., Rapp, K., Anderson, J., Lin, Y., Shaw, M. V., Yang, J.H., Marc, R.E., 2011. Retinal remodeling in the Tg P347L rabbit, a large-eye model of retinal degeneration. *J. Comp. Neurol.* 519, 2713–2733.
- Jones, B.W., Pfeiffer, R.L., Ferrell, W.D., Watt, C.B., Marmor, M., Marc, R.E., 2016a. Retinal remodeling in human retinitis pigmentosa. *Exp. Eye Res.* 150, 149–165.
- Jones, B.W., Pfeiffer, R.L., Ferrell, W.D., Watt, C.B., Tucker, J., Marc, R.E., 2016b. Retinal remodeling and metabolic alterations in human AMD. *Front. Cell. Neurosci.* 10, 103.
- Jones, B.W., Watt, C.B., Frederick, J.M., Baehr, W., Chen, C.K., Levine, E.M., Milam, A. H., Lavail, M.M., Marc, R.E., 2003. Retinal remodeling triggered by photoreceptor degenerations. *J. Comp. Neurol.* 464, 1–16.
- Juttner, J., Szabo, A., Gross-Scherf, B., Morikawa, R.K., Rompani, S.B., Hantz, P., Szikra, T., Esposti, F., Cowan, C.S., Bharioke, A., Patino-Alvarez, C.P., Keles, O., Kusnyerik, A., Azoulay, T., Hartl, D., Krebs, A.R., Schubeler, D., Hajdu, R.I., Lukats, A., Nemeth, J., Nagy, Z.Z., Wu, K.C., Wu, R.H., Xiang, L., Fang, X.L., Jin, Z.B., Goldblum, D., Hasler, P.W., Scholl, H.P.N., Krol, J., Roska, B., 2019. Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates and humans. *Nat. Neurosci.* 22, 1345–1356.
- Khabou, H., Garita-Hernandez, M., Chaffiol, A., Reichman, S., Jaillard, C., Brazhnikova, E., Bertin, S., Forster, V., Desrosiers, M., Winckler, C., Goureau, O., Picaud, S., Duebel, J., Sahel, J.A., Dalkara, D., 2018. Noninvasive gene delivery to foveal cones for vision restoration. *JCI Insight* 3.
- Kinder, L., Palumaa, T., Lindner, M., 2021. [Intrinsically Photosensitive Retinal Ganglion Cells]. *Der Ophthalmologe : Zeitschrift der Deutschen Ophthalmologischen Gesellschaft.*
- Klapoetke, N.C., Murata, Y., Kim, S.S., Pulver, S.R., Birdsey-Benson, A., Cho, Y.K., Morimoto, T.K., Chuong, A.S., Carpenter, E.J., Tian, Z., Wang, J., Xie, Y., Yan, Z., Zhang, Y., Chow, B.Y., Surek, B., Melkonian, M., Jayaraman, V., Constantine-Paton, M., Wong, G.K., Boyden, E.S., 2014. Independent optical excitation of distinct neural populations. *Nat. Methods* 11, 338–346.
- Klapper, S.D., Swiersy, A., Bamberg, E., Busskamp, V., 2016. Biophysical properties of optogenetic tools and their application for vision restoration approaches. *Front. Syst. Neurosci.* 10, 74.
- Klein, R., Klein, B.E., Franke, T., 1993. The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology* 100, 406–414.
- Kleinlogel, S., Feldbauer, K., Dempo, R.E., Fotis, H., Wood, P.G., Bamann, C., Bamberg, E., 2011. Ultra light-sensitive and fast neuronal activation with the Ca(2) +-permeable channelrhodopsin CatCh. *Nat. Neurosci.* 14, 513–518.
- Kleinlogel, S., Vogl, C., Jeschke, M., Neef, J., Moser, T., 2020. Emerging approaches for restoration of hearing and vision. *Physiol. Rev.* 100, 1467–1525.
- Koyanagi, M., Kubokawa, K., Tsukamoto, H., Shichida, Y., Terakita, A., 2005. Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. *Curr. Biol.* 15, 1065–1069.
- Kralik, J., Kleinlogel, S., 2021. Functional availability of ON-bipolar cells in the degenerated retina: timing and longevity of an optogenetic gene therapy. *Int. J. Mol. Sci.* 22.
- Lagali, P.S., Balya, D., Awatramani, G.B., Munch, T.A., Kim, D.S., Busskamp, V., Cepko, C.L., Roska, B., 2008. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat. Neurosci.* 11, 667–675.
- Lawwitt, T., Crockett, S., Currier, G., 1977. Retinal damage secondary to chronic light exposure, thresholds and mechanisms. *Doc. Ophthalmol.* 44, 379–402.
- Li, X., Gutierrez, D.V., Hanson, M.G., Han, J., Mark, M.D., Chiel, H., Hegemann, P., Landmesser, L.T., Herlitze, S., 2005. Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* 102, 17816–17821.
- Lim, L.S., Mitchell, P., Seddon, J.M., Holz, F.G., Wong, T.Y., 2012. Age-related macular degeneration. *Lancet* 379, 1728–1738.
- Lin, B., Koizumi, A., Tanaka, N., Panda, S., Masland, R.H., 2008. Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16009–16014.
- Lin, J.Y., 2011. A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp. Physiol.* 96, 19–25.
- Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D., Tsien, R.Y., 2013. ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat. Neurosci.* 16, 1499–1508.
- Lindner, M., 2022. The thin line between seeing risks and venturing scientific progress. *Graefes Arch. Clin. Exp. Ophthalmol.*
- Lindner, M., Fleckenstein, M., Schmitz-Valckenberg, S., Holz, F.G., 2018. Atrophy, geographic. In: Schmidt-Erfurth, U., Kohnen, T. (Eds.), *Encyclopedia of Ophthalmology*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 207–209.
- Lindner, M., Gilhooley, M.J., Peirson, S.N., Hughes, S., Hankins, M.W., 2021. The functional characteristics of optogenetic gene therapy for vision restoration. *Cell. Mol. Life Sci.* 78, 1597–1613.
- Liu, M.M., Dai, J.M., Liu, W.Y., Zhao, C.J., Lin, B., Yin, Z.Q., 2016. Human melanopsin-AAV2/8 transfection to retina transiently restores visual function in rd1 mice. *Int. J. Ophthalmol.* 9, 655–661.
- Liu, W., Liu, M., Liu, Y., Li, S., Weng, C., Fu, Y., He, J., Gong, Y., Liu, W., Zhao, C., Yin, Z. Q., 2019. Validation and safety of visual restoration by ectopic expression of human melanopsin in retinal ganglion cells. *Hum. Gene Ther.* 30, 714–726.
- Lu, Q., Ganjawala, T.H., Ivanova, E., Cheng, J.G., Troilo, D., Pan, Z.H., 2016. AAV-mediated transduction and targeting of retinal bipolar cells with improved mGluR6 promoters in rodents and primates. *Gene Ther.*
- Lu, Q., Ganjawala, T.H., Krstevski, A., Abrams, G.W., Pan, Z.H., 2020. Comparison of AAV-mediated optogenetic vision restoration between retinal ganglion cell expression and ON bipolar cell targeting. *Mol. Ther. Methods Clin. Dev.* 18, 15–23.
- Mace, E., Caplette, R., Marre, O., Sengupta, A., Chaffiol, A., Barbe, P., Desrosiers, M., Bamberg, E., Sahel, J.A., Picaud, S., Duebel, J., Dalkara, D., 2015. Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV Restores ON and OFF visual responses in blind mice. *Mol. Ther.* 23, 7–16.
- Maguire, A.M., Russell, S., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A., Wittes, J., Pappas, J., Elci, O., Marshall, K.A., McCague, S., Reichert, H., Davis, M.,

- Simonelli, F., Leroy, B.P., Wright, J.F., High, K.A., Bennett, J., 2019. Efficacy, safety, and durability of voretigene neparvovec-rzyl in RPE65 mutation-associated inherited retinal dystrophy: results of phase 1 and 3 trials. *Ophthalmology* 126, 1273–1285.
- Maimon, B.E., Diaz, M., Revol, E.C.M., Schneider, A.M., Leaker, B., Varela, C.E., Srinivasan, S., Weber, M.B., Herr, H.M., 2018. Optogenetic peripheral nerve immunogenicity. *Sci. Rep.* 8, 14076.
- Marc, R.E., Jones, B.W., Watt, C.B., Strettoi, E., Kinard, K., Marshak, D.W., Wilson, J.H., Wensel, T., Lucas, R.J., 2007. Neural reprogramming in retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* 48, 3364–3371.
- Marc, R.E., Jones, B.W., Watt, C.B., Strettoi, E., 2003. Neural remodeling in retinal degeneration. *Prog. Retin. Eye Res.* 22, 607–655.
- Margalit, E., Babai, N., Luo, J., Thoreson, W.B., 2011. Inner and outer retinal mechanisms engaged by epiretinal stimulation in normal and rd mice. *Vis. Neurosci.* 28, 145–154.
- Margolis, D.J., Gartland, A.J., Singer, J.H., Detwiler, P.B., 2014. Network oscillations drive correlated spiking of ON and OFF ganglion cells in the rd1 mouse model of retinal degeneration. *PLoS One* 9, e86253.
- Margolis, D.J., Newkirk, G., Euler, T., Detwiler, P.B., 2008. Functional stability of retinal ganglion cells after degeneration-induced changes in synaptic input. *J. Neurosci. : Off. J. Soc. Neurosci.* 28, 6526–6536.
- Martel, J.N., Boulanger-Scemama, E., Degli Esposti, S., Galluppi, F., Blouin, L., Taiel, M., Sahel, J.A., 2021. Optogenetic GS030 therapy in subjects with retinitis pigmentosa: safety and tolerability up to two years after treatment administration in the phase 1/2a PIONEER study. *Invest. Ophthalmol. Vis. Sci.* 62, 3236–3236.
- McGregor, J.E., Godat, T., Dhakal, K.R., Parkins, K., Strazzeri, J.M., Bateman, B.A., Fischer, W.S., Williams, D.R., Merigan, W.H., 2020. Optogenetic restoration of retinal ganglion cell activity in the living primate. *Nat. Commun.* 11, 1703.
- McGregor, J.E., Kunal, K., Xu, Z., Murphy, P.J., Godat, T., Strazzeri, J.M., Bateman, B.A., Fischer, W.S., Parkins, K., Chu, C.J., Puthussery, T., Williams, D.R., Merigan, W.H., 2021. Optogenetic therapy restores retinal activity in primate for at least a year following photoreceptor ablation. *Mol. Ther.*
- Melyan, Z., Tattelin, E.E., Bellingham, J., Lucas, R.J., Hankins, M.W., 2005. Addition of human melanopsin renders mammalian cells photoreceptive. *Nature* 433, 741–745.
- Mendes, H.F., van der Spuy, J., Chapple, J.P., Cheetham, M.E., 2005. Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol. Med.* 11, 177–185.
- Menzler, J., Channappa, L., Zeck, G., 2014. Rhythmic ganglion cell activity in bleached and blind adult mouse retinas. *PLoS One* 9, e106047.
- Menzler, J., Zeck, G., 2011. Network oscillations in rod-degenerated mouse retinas. *J. Neurosci. : Off. J. Soc. Neurosci.* 31, 2280–2291.
- Milam, A.H., Li, Z.Y., Fariss, R.N., 1998. Histopathology of the human retina in retinitis pigmentosa. *Prog. Retin. Eye Res.* 17, 175–205.
- Miyagishi, T., Shao, Y.R., Chung, J., Pourzia, O., Feldman, D.E., 2013. Long-term channelrhodopsin-2 (ChR2) expression can induce abnormal axonal morphology and targeting in cerebral cortex. *Front. Neural Circ.* 7, 8.
- Moshiri, A., Chen, R., Kim, S., Harris, R.A., Li, Y., Raveendran, M., Davis, S., Liang, Q., Pomerantz, O., Wang, J., Garzel, L., Cameron, A., Yiu, G., Stout, J.T., Huang, Y., Murphy, C.J., Roberts, J., Gopalakrishna, K.N., Boyd, K., Artemyev, N.O., Rogers, J., Thomas, S.M., 2019. A nonhuman primate model of inherited retinal disease. *J. Clin. Invest.* 129, 863–874.
- O'Hearn, T.M., Sada, S.R., Weiland, J.D., Maia, M., Margalit, E., Humayun, M.S., 2006. Electrical stimulation in normal and retinal degeneration (rd1) isolated mouse retina. *Vis. Res.* 46, 3198–3204.
- Ohno, N., Murai, H., Suzuki, Y., Kiyosawa, M., Tokumaru, A.M., Ishii, K., Ohno-Matsui, K., 2015. Alteration of the optic radiations using diffusion-tensor MRI in patients with retinitis pigmentosa. *Br. J. Ophthalmol.* 99, 1051–1054.
- Orlans, H.O., McClements, M.E., Barnard, A.R., Martinez-Fernandez de la Camara, C., MacLaren, R.E., 2021. Mitron-mediated RNA knockdown/replacement therapy for the treatment of dominant retinitis pigmentosa. *Nat. Commun.* 12, 4934.
- Ostad-Ahmad, Z., Daemi, A., Modabberi, M.R., Mostafaie, A., 2021. Safety, effectiveness, and cost-effectiveness of Argus II in patients with retinitis pigmentosa: a systematic review. *Int. J. Ophthalmol.* 14, 310–316.
- Palumaa, T., Gilhooley, M.J., Jagannath, A., Hankins, M.W., Hughes, S., Peirson, S.N., 2018. Melanopsin: photoreceptors, physiology and potential. *Curr. Opin. Physiol.* 5, 68–74.
- Pan, Z.H., Ganjawala, T.H., Lu, Q., Ivanova, E., Zhang, Z., 2014. ChR2 mutants at L132 and T159 with improved operational light sensitivity for vision restoration. *PLoS One* 9, e98924.
- Perea-Romero, I., Gordo, G., Iancu, I.F., Del Pozo-Valero, M., Almodovar, B., Blanco-Kelly, F., Carreño, E., Jimenez-Rolando, B., Lopez-Rodriguez, R., Lorda-Sanchez, I., Martin-Merida, I., Pérez de Ayala, L., Riveiro-Alvarez, R., Rodriguez-Pinilla, E., Tahsin-Swafiri, S., Trujillo-Tiebas, M.J., Garcia-Sandoval, B., Minguez, P., Avila-Fernandez, A., Corton, M., Ayuso, C., 2021. Genetic landscape of 6089 inherited retinal dystrophies affected cases in Spain and their therapeutic and extended epidemiological implications. *Sci. Rep.* 11, 1526.
- Pfeiffer, R.L., Anderson, J.R., Dahal, J., Garcia, J.C., Yang, J.H., Sigulinsky, C.L., Rapp, K., Emrich, D.P., Watt, C.B., Johnston, H.A., Houser, A.R., Marc, R.E., Jones, B.W., 2020a. A pathoconnectome of early neurodegeneration: network changes in retinal degeneration. *Exp. Eye Res.* 199, 108196.
- Pfeiffer, R.L., Marc, R.E., Jones, B.W., 2020b. Persistent remodeling and neurodegeneration in late-stage retinal degeneration. *Prog. Retin. Eye Res.* 74, 100771.
- Phillips, M.J., Ottosen, D.C., Sherry, D.M., 2010. Progression of neuronal and synaptic remodeling in the rd10 mouse model of retinitis pigmentosa. *J. Comp. Neurol.* 518, 2071–2089.
- Pontikos, N., Arno, G., Jurkute, N., Schiff, E., Ba-Abbad, R., Malka, S., Gimenez, A., Georgiou, M., Wright, G., Armengol, M., Knight, H., Katz, M., Moosajee, M., Yu-Wai-Man, P., Moore, A.T., Michaelides, M., Webster, A.R., Mahroo, O.A., 2020. Genetic basis of inherited retinal disease in a molecularly characterized cohort of more than 3000 families from the United Kingdom. *Ophthalmology* 127, 1384–1394.
- Pu, M., Xu, L., Zhang, H., 2006. Visual response properties of retinal ganglion cells in the royal college of surgeons dystrophic rat. *Invest. Ophthalmol. Vis. Sci.* 47, 3579–3585.
- Puthussery, T., Gayet-Primo, J., Pandey, S., Duvoisin, R.M., Taylor, W.R., 2009. Differential loss and preservation of glutamate receptor function in bipolar cells in the rd10 mouse model of retinitis pigmentosa. *Eur. J. Neurosci.* 29, 1533–1542.
- Reiniger, J.L., Domdei, N., Holz, F.G., Harmening, W.M., 2021. Human gaze is systematically offset from the center of cone topography. *Curr. Biol.* 31, 4188–4193 e4183.
- Resnikoff, S., Pascolini, D., Etya'ale, D., Kocur, I., Pararajasegaram, R., Pokharel, G.P., Mariotti, S.P., 2004. Global data on visual impairment in the year 2002. *Bull. World Health Organ.* 82, 844–851.
- Rittipahiroj, T., Mir, T.A., Li, T., Virgili, G., 2020. Intravitreal steroids for macular edema in diabetes. *Cochrane Database Syst. Rev.* 11, CD005656.
- Rodgers, J., Bano-Otolora, B., Belle, M.D.C., Paul, S., Hughes, R., Wright, P., McDowell, R., Milosavljevic, N., Orlowska-Feuer, P., Martial, F.P., Wynne, J., Ballister, E.R., Storch, R., Allen, A.E., Brown, T., Lucas, R.P., 2021. Using a bistable animal opsin for switchable and scalable optogenetic inhibition of neurons. *EMBO Rep.* 22, e51866.
- Ross, J.W., Fernandez de Castro, J.P., Zhao, J., Samuel, M., Walters, E., Rios, C., Bray-Ward, P., Jones, B.W., Marc, R.E., Wang, W., Zhou, L., Noel, J.M., McCall, M.A., DeMarco, P.J., Rafter, R.S., Kaplan, H.J., 2012. Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 53, 501–507.
- Russell, S., Bennett, J., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A., Wittes, J., Pappas, J., Elci, O., McCague, S., Cross, D., Marshall, K.A., Walshire, J., Kehoe, T.L., Reichert, H., Davis, M., Raffini, L., George, L.A., Hudson, F.P., Dingfield, L., Zhu, X., Haller, J.A., Sohn, E.H., Mahajan, V.B., Pfeifer, W., Weckmann, M., Johnson, C., Gewaily, D., Drack, A., Stone, E., Wachtel, K., Simonelli, F., Leroy, B.P., Wright, J.F., High, K.A., Maguire, A.M., 2017. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet* 390, 849–860.
- Sahel, J.A., Boulanger-Scemama, E., Pagot, C., Arleo, A., Galluppi, F., Martel, J.N., Esposti, S.D., Delaux, A., de Saint Aubert, J.B., de Montleau, C., Gutman, E., Audou, I., Duebel, J., Picaud, S., Dalkara, D., Blouin, L., Taiel, M., Roska, B., 2021a. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nat. Med.* 27, 1223–1229.
- Sahel, J.A., Grieve, K., Pagot, C., Authié, C., Mohand-Said, S., Paques, M., Audou, I., Becker, K., Chaumet-Riffaud, A.E., Azoulay, L., Gutman, E., Lévêillard, T., Zeitze, C., Picaud, S., Dalkara, D., Marazova, K., 2021b. Assessing photoreceptor status in retinal dystrophies: from high-resolution imaging to functional vision. *Am. J. Ophthalmol.* 230, 12–47.
- Santos, A., Humayun, M.S., de Juan Jr., E., Greenburg, R.J., Marsh, M.J., Klock, I.B., Milam, A.H., 1997. Preservation of the inner retina in retinitis pigmentosa. A morphometric analysis. *Arch. Ophthalmol.* 115, 511–515.
- Sato, M., Sugano, E., Tabata, K., Sannohe, K., Watanabe, Y., Ozaki, T., Tamai, M., Tomita, H., 2017. Visual responses of photoreceptor-degenerated rats expressing two different types of channelrhodopsin genes. *Sci. Rep.* 7, 41210.
- Schneider, F.M., Mohr, F., Behrendt, M., Oberwinkler, J., 2015. Properties and functions of TRPM1 channels in the dendritic tips of retinal ON-bipolar cells. *Eur. J. Cell Biol.* 94, 420–427.
- Schoth, F., Burgel, U., Dorsch, R., Reinges, M.H., Krings, T., 2006. Diffusion tensor imaging in acquired blind humans. *Neurosci. Lett.* 398, 178–182.
- Semo, M., Gias, C., Ahmado, A., Sugano, E., Allen, A.E., Lawrence, J.M., Tomita, H., Coffey, P.J., Vugler, A.A., 2010. Dissecting a role for melanopsin in behavioural light aversion reveals a response independent of conventional photoreception. *PLoS One* 5, e15009.
- Sengupta, A., Chaffiol, A., Mace, E., Caplette, R., Desrosiers, M., Lampic, M., Forster, V., Marre, O., Lin, J.Y., Sahel, J.A., Picaud, S., Dalkara, D., Duebel, J., 2016. Red-shifted channelrhodopsin stimulation restores light responses in blind mice, macaque retina, and human retina. *EMBO Mol. Med.*
- Shen, Y., Campbell, R.E., Cote, D.C., Paquet, M.E., 2020. Challenges for therapeutic applications of opsin-based optogenetic tools in humans. *Front. Neural Circ.* 14, 41.
- Simon, C.J., Sahel, J.A., Duebel, J., Herlitz, S., Dalkara, D., 2020. Opsins for vision restoration. *Biochem. Biophys. Res. Commun.* 527, 325–330.
- Singh, M.S., Park, S.S., Albini, T.A., Canto-Soler, M.V., Klassen, H., MacLaren, R.E., Takahashi, M., Nagel, A., Schwartz, S.D., Bharti, K., Kato, R., 2020. Retinal stem cell transplantation: balancing safety and potential. *Prog. Retin. Eye Res.* 75, 100779.
- Sonoda, T., Lee, S.K., Birnbaumer, L., Schmidt, T.M., 2018. Melanopsin phototransduction is repurposed by ipRGC subtypes to shape the function of distinct visual circuits. *Neuron* 99, 754–767 e754.
- Spoida, K., Eickelbeck, D., Karapinar, R., Eckhardt, T., Mark, M.D., Jancke, D., Ehinger, B.V., König, P., Dalkara, D., Herlitz, S., Masack, O.A., 2016. Melanopsin variants as intrinsic optogenetic on and off switches for transient versus sustained activation of G protein pathways. *Curr. Biol.*
- Stasheff, S.F., 2008. Emergence of sustained spontaneous hyperactivity and temporary preservation of OFF responses in ganglion cells of the retinal degeneration (rd1) mouse. *J. Neurophysiol.* 99, 1408–1421.
- Stasheff, S.F., Shankar, M., Andrews, M.P., 2011. Developmental time course distinguishes changes in spontaneous and light-evoked retinal ganglion cell activity in rd1 and rd10 mice. *J. Neurophysiol.* 105, 3002–3009.

- Stefanov, A., Novelli, E., Strettoi, E., 2020. Inner retinal preservation in the photoinducible 1307N rhodopsin mutant mouse, a model of autosomal dominant retinitis pigmentosa. *J. Comp. Neurol.* 528, 1502–1522.
- Stone, E.M., Andorf, J.L., Whitmore, S.S., DeLuca, A.P., Giacalone, J.C., Streb, L.M., Braun, T.A., Mullins, R.F., Scheetz, T.E., Sheffield, V.C., Tucker, B.A., 2017. Clinically focused molecular investigation of 1000 consecutive families with inherited retinal disease. *Ophthalmology* 124, 1314–1331.
- Strettoi, E., Pignatelli, V., 2000. Modifications of retinal neurons in a mouse model of retinitis pigmentosa. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11020–11025.
- Strettoi, E., Pignatelli, V., Rossi, C., Porciatti, V., Falsini, B., 2003. Remodeling of second-order neurons in the retina of rd/rd mutant mice. *Vis. Res.* 43, 867–877.
- Strickland, E.H.M., 2022. Their bionic eyes are now obsolete and unsupported. *IEEE, IEEE Spectr.*
- Sugano, E., Isago, H., Wang, Z., Murayama, N., Tamai, M., Tomita, H., 2011. Immune responses to adeno-associated virus type 2 encoding channelrhodopsin-2 in a genetically blind rat model for gene therapy. *Gene Ther.* 18, 266–274.
- Suh, J.-K., Kim, J.M., Moon, H.-J., Shin, H.-J., Hwang, E., Kang, D.H., Lee, C., 2013. Optogenetic Targeting of ON and OFF bipolar cells for vision restoration. *Invest. Ophthalmol. Vis. Sci.* 54, 2678–2678.
- Tabata, K., Sugano, E., Hatakeyama, A., Watanabe, Y., Suzuki, T., Ozaki, T., Fukuda, T., Tomita, H., 2021. Phototoxicities caused by continuous light exposure were not induced in retinal ganglion cells transduced by an optogenetic gene. *Int. J. Mol. Sci.* 22.
- Tam, S.K., Hasan, S., Choi, H.M., Brown, L.A., Jagannath, A., Hughes, S., Hankins, M.W., Foster, R.G., Vyazovskiy, V.V., Bannerman, D.M., Peirson, S.N., 2017. Constant light desynchronizes olfactory versus object and visuospatial recognition memory performance. *J. Neurosci. : Off. J. Soc. Neurosci.* 37, 3555–3567.
- Thompson, D.A., Iannaccone, A., Ali, R.R., Arshavsky, V.Y., Audo, I., Bainbridge, J.W.B., Besirli, C.G., Birch, D.G., Branham, K.E., Cideciyan, A.V., Daiger, S.P., Dalkara, D., Duncan, J.L., Fahim, A.T., Flannery, J.G., Gattegna, R., Heckenlively, J.R., Heon, E., Jayasundera, K.T., Khan, N.W., Klassen, H., Leroy, B.P., Molday, R.S., Musch, D.C., Pennesi, M.E., Petersen-Jones, S.M., Pierce, E.A., Rao, R.C., Reh, T.A., Sahel, J.A., Sharon, D., Sieving, P.A., Strettoi, E., Yang, P., Zacks, D.N., Monaciano, C., 2020. Advancing clinical trials for inherited retinal diseases: recommendations from the second Monaciano symposium. *Transl. Vis. Sci. Technol.* 9, 2.
- Tichy, A.M., Gerrard, E.J., Sexton, P.M., Janovjak, H., 2019. Light-activated chimeric GPCRs: limitations and opportunities. *Curr. Opin. Struct. Biol.* 57, 196–203.
- Tomita, H., Sugano, E., 2021. Optogenetics-mediated gene therapy for retinal diseases. *Adv. Exp. Med. Biol.* 1293, 535–543.
- Tomita, H., Sugano, E., Isago, H., Hiroi, T., Wang, Z., Ohta, E., Tamai, M., 2010. Channelrhodopsin-2 gene transduced into retinal ganglion cells restores functional vision in genetically blind rats. *Exp. Eye Res.* 90, 429–436.
- Tomita, H., Sugano, E., Murayama, N., Ozaki, T., Nishiyama, F., Tabata, K., Takahashi, M., Saito, T., Tamai, M., 2014. Restoration of the majority of the visual spectrum by using modified Volvox channelrhodopsin-1. *Mol. Ther.* 22, 1434–1440.
- Tomita, H., Sugano, E., Yawo, H., Ishizuka, T., Isago, H., Narikawa, S., Kugler, S., Tamai, M., 2007. Restoration of visual response in aged dystrophic RCS rats using AAV-mediated channelrhodopsin-2 gene transfer. *Invest. Ophthalmol. Vis. Sci.* 48, 3821–3826.
- Tran, N.M., Shekhar, K., Whitney, I.E., Jacobi, A., Benhar, I., Hong, G., Yan, W., Adiconis, X., Arnold, M.E., Lee, J.M., Levin, J.Z., Lin, D., Wang, C., Lieber, C.M., Regev, A., He, Z., Sanes, J.R., 2019. Single-cell profiles of retinal ganglion cells differing in resilience to injury reveal neuroprotective genes. *Neuron* 104, 1039–1055 e1012.
- Trenholm, S., Awatramani, G.B., 2015. Origins of spontaneous activity in the degenerating retina. *Front. Cell. Neurosci.* 9, 277.
- Trenholm, S., Borowska, J., Zhang, J., Hoggarth, A., Johnson, K., Barnes, S., Lewis, T.J., Awatramani, G.B., 2012. Intrinsic oscillatory activity arising within the electrically coupled AII amacrine-ON cone bipolar cell network is driven by voltage-gated Na⁺ channels. *J. Physiol.* 590, 2501–2517.
- Ueno, S., Kominami, T., Okado, S., Inooka, D., Kondo, M., Terasaki, H., 2019. Course of loss of photoreceptor function and progressive Müller cell gliosis in rhodopsin P347L transgenic rabbits. *Exp. Eye Res.* 184, 192–200.
- Vámos, R., Tátrai, E., Németh, J., Holder, G.E., DeBuc, D.C., Somfai, G.M., 2011. The structure and function of the macula in patients with advanced retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 52, 8425–8432.
- van Wyk, M., Hulliger, E.C., Girod, L., Ebner, A., Kleinlogel, S., 2017. Present molecular limitations of ON-bipolar cell targeted gene therapy. *Front. Neurosci.* 11, 161.
- van Wyk, M., Pielecka-Fortuna, J., Lowel, S., Kleinlogel, S., 2015. Restoring the ON switch in blind retinas: opto-mGluR6, a next-generation, cell-tailored optogenetic tool. *PLoS Biol.* 13, e1002143.
- Von Treskow, A., 2020. Novartis Acquires Vedere Bio, Adding Novel Optogenetic Gene Therapy Technology for Treating Blindness.
- Wang, T., Pahlberg, J., Cafaro, J., Frederiksen, R., Cooper, A.J., Sampath, A.P., Field, G. D., Chen, J., 2019. Activation of rod input in a model of retinal degeneration reverses retinal remodeling and induces formation of functional synapses and recovery of visual signaling in the adult retina. *J. Neurosci. : Off. J. Soc. Neurosci.* 39, 6798–6810.
- Watanabe, Y., Sugano, E., Tabata, K., Hatakeyama, A., Sakajiri, T., Fukuda, T., Ozaki, T., Suzuki, T., Sayama, T., Tomita, H., 2021. Development of an optogenetic gene sensitive to daylight and its implications in vision restoration. *NPJ Regen. Med.* 6, 64.
- White, J., Knight, L., da Cruz, L., Stanga, P.E., Patrick, H., Powell, H., Berry, L., Withers, K., Carolan-Rees, G., Jackson, T.L., 2021. Effects of the Argus II retinal prosthesis system on the quality of life of patients with ultra-low vision due to retinitis pigmentosa: protocol for a single-arm, mixed methods study. *JMIR Res. Protoc.* 10, e17436.
- Wolf, A., 2021. Novartis Acquires Arctos Medical, Expanding Optogenetics Portfolio to Bring Gene Therapies to Patients with Severe Vision Loss.
- Wright, P., Rodgers, J., Wynne, J., Bishop, P.N., Lucas, R.J., Milosavljevic, N., 2021. Viral transduction of human rod opsin or channelrhodopsin variants to mouse ON bipolar cells does not impact retinal anatomy or cause measurable death in the targeted cells. *Int. J. Mol. Sci.* 22.
- Wright, W., Gajjaraman, S., Batabyal, S., Pradhan, S., Bhattacharya, S., Mahapatra, V., Tripathy, A., Mohanty, S., 2017. Restoring vision in mice with retinal degeneration using multicharacteristic opsin. *Neurophotonics* 4, 041505.
- Wu, C., Ivanova, E., Zhang, Y., Pan, Z.H., 2013. rAAV-mediated subcellular targeting of optogenetic tools in retinal ganglion cells in vivo. *PLoS One* 8, e66332.
- Xiong, W., Wu, D.M., Xue, Y., Wang, S.K., Chung, M.J., Ji, X., Rana, P., Zhao, S.R., Mai, S., Cepko, C.L., 2019. AAV cis-regulatory sequences are correlated with ocular toxicity. *Proc. Natl. Acad. Sci. U. S. A.* 116, 5785–5794.
- Yawo, H., Kandori, H., Koizumi, A., Kageyama, R., 2021. Optogenetics : light-sensing proteins and their applications in neuroscience and beyond. In: *Advances in Experimental Medicine and Biology*, second ed. Springer, Singapore, p. 1. online resource (xii, 663 pp).
- Ye, J.H., Goo, Y.S., 2007. The slow wave component of retinal activity in rd/rd mice recorded with a multi-electrode array. *Physiol. Meas.* 28, 1079–1088.
- Ye, J.H., Kim, K.H., Goo, Y.S., 2008. Comparison of electrically-evoked ganglion cell responses in normal and degenerate retina. In: *Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society*, pp. 2465–2468. Annual International Conference 2008.
- Yoshimine, S., Ogawa, S., Horiguchi, H., Terao, M., Miyazaki, A., Matsumoto, K., Tsuneoka, H., Nakano, T., Masuda, Y., Pestilli, F., 2018. Age-related macular degeneration affects the optic radiation white matter projecting to locations of retinal damage. *Brain Struct. Funct.* 223, 3889–3900.
- Youssef, P.N., Sheibani, N., Albert, D.M., 2011. Retinal light toxicity. *Eye* 25, 1–14.
- Yue, L., Weiland, J.D., Roska, B., Humayun, M.S., 2016. Retinal stimulation strategies to restore vision: fundamentals and systems. *Prog. Retin. Eye Res.* 53, 21–47.
- Zhang, F., Vierock, J., Yizhar, O., Fenno, L.E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., Magnuson, J., Hegemann, P., Deisseroth, K., 2011. The microbial opsin family of optogenetic tools. *Cell* 147, 1446–1457.
- Zhang, Y., Ivanova, E., Bi, A., Pan, Z.H., 2009. Ectopic expression of multiple microbial rhodopsins restores ON and OFF light responses in retinas with photoreceptor degeneration. *J. Neurosci. : Off. J. Soc. Neurosci.* 29, 9186–9196.
- Zuzic, M., Striebel, J., Pawlick, J.S., Sharma, K., Holz, F.G., Busskamp, V., 2022. Gene-independent therapeutic interventions to maintain and restore light sensitivity in degenerating photoreceptors. *Prog. Retin. Eye Res.*, 101065.