Chapter

Recent Advances in Optogenetic Retinal Prostheses

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Abstract

Optogenetics has emerged as a revolutionary technology that enables circuit-specific restoration of neuronal function with millisecond temporal resolution. Restoring vision is one of the most promising and forefront applications of optogenetics. This chapter discusses essential components, mechanisms, present challenges, and future prospects of optogenetic retinal prostheses. The theoretical framework and analysis of optogenetic excitation of retinal ganglion neurons are also presented, which are useful in developing a better understanding and guidance for future experiments. It shows that the newly discovered ChRmine opsin provides control at light powers that are two orders of magnitude smaller than that required with experimentally studied opsins that include ChR2, ReaChR, and ChrimsonR, while maintaining single-spike temporal resolution, in retinal ganglion neurons.

Keywords: optogenetics, computational optogenetics, retinal prostheses, channelrhodopsin, vision restoration

1. Introduction

Loss of vision due to retinal degenerative diseases, including retinitis pigmentosa (RP) and macular degeneration affects millions of people worldwide [1–3]. The degeneration of light-sensitive rod and cone photoreceptors in the retina breaks the process of converting the light signal into an electrical signal. It thus results in partial vision loss or complete blindness. Earlier studies have confirmed that the remaining retinal tissue retains functionality and connections to the brain without these photoreceptors [4, 5]. Direct electrical stimulation of the remaining retinal neurons enables light sensation and visual perception, which confirms that the remaining retinal neural circuits can transmit information to the brain even in the late stage of retinal degenerative diseases [6–8]. However, applications of electrical prosthetic devices are limited due to their poor spatial resolution and highly invasive nature [4, 9, 10]. Although there are alternative methods for treating blindness, that includes pharmaceuticals, gene therapy, and stem cells, they are limited in certain conditions [11–14].

The emergence of optogenetics has revolutionized neuroscience by providing unprecedented spatiotemporal resolution in bidirectionally controlling neural activity with relatively lower invasiveness [15, 16]. In optogenetics, the gene of a light-sensitive protein is introduced into light-insensitive cells. In response to light,

the expressed protein generates either inward or outward current and thus depolarizes or hyperpolarizes the cell [17]. It has contributed to our knowledge of how neural circuits operate and holds tremendous prospects in neural prostheses, particularly in vision restoration [18–23]. As one of the most promising technologies, optogenetics has applications in and beyond neuroscience, including cardiac optogenetics, sensory restoration, all-optical manipulation, and imaging of neural circuits in living animals [24]. More recently, a pioneering human clinical study on optogenetic retinal prostheses has reported successful partial vision recovery in a blind patient [25].

2. Optogenetic strategies for retinal prostheses

The method of optogenetic retinal prostheses is fundamentally different from electrical prostheses [26]. In optogenetics, injection of genetically encoded opsin viral vector into the surviving retinal neurons imparts light sensitivity to the light-insensitive retinal neurons. Thus, vision can be restored with the help of an optoelectronic device that converts visual scene pixels into appropriate light pulses and delivers them to the targeted cells. The implantation of optoelectronic headsets does not require the same level of invasiveness as their electronic counterpart [18, 26].

As the retina is a layered structure, the signal from photoreceptor cells in the foremost layer undergoes various processing before reaching the inner most layer *i.e.,* retinal ganglion neurons (RGNs). After that, the processed signal is transmitted to higher visual areas in the brain. As optogenetics allows cell-specific opsin expression, in principle, it would be best to transfect as early as possible in the visual pathway taking advantage of internal signal processing in the retinal circuits [26]. Once transfected, the retinal neurons require spatiotemporal patterns of high-intensity light depending on the dynamic range and spatial configuration of the targeted neurons and the kinetics and photosensitivity of light-sensitive proteins [27–29]. The selection of targeted cell type and opsin construct decides the quality of restored vision. Furthermore, the safety of retinal tissue after the expression of opsin into the desired neural population must be ensured [30].

In patients with RP, cone cells are not necessarily completely destroyed. These cells lose their outer segment and survive until very late stages of the condition [31]. Typically, the cone photoreceptor cells hyperpolarize in the presence of light and get depolarized in the dark. Thus, it would be better to use light-driven chloride pumps to perfectly mimic the signal in response to light [32–34]. Optogenetic excitation of the surviving cone cells through genetic expression of these cells with halorhodopsin has restored visual responses in the mouse with RP [35]. However, a major drawback of targeting cone cells is their very few surviving populations. Furthermore, there might be no chance of survival of these cells in late stages of other retinal dystrophies. Thus, the restored vision will be restricted to tunnel vision similar to mid-stage RP [8, 26]. The next best layer to target is the bipolar cells. There are two types of these bipolar cells, *i.e.,* ON and OFF cells, which depolarize and hyperpolarize in response to light. Thus, these cells need to be expressed with excitatory and inhibitory opsins to avoid conflicting responses to visual stimuli, respectively [28, 34].

Alternatively, direct optogenetic excitation of RGNs is possible. RGNs also come in ON and OFF varieties. A key issue with the direct excitation of inner retinal neurons is the absence of normal retinal processing. A possible solution to overcome the issue is to process the visual scene by the computational circuit of retinal encoding before delivering it to inner retinal neurons [36]. In the case of direct excitation of RGNs,

optoelectronic goggles have been used to mimic retinal processing and to modify visual scene intensity and color compatible with the light-sensitive protein expressed in RGNs [26].

To accurately restore vision by direct photostimulation of inner retinal neurons, these neurons are to be driven at their natural frequencies, evoked in response to dynamic visual stimuli [18]. There are at least 16 different types of RGNs having different temporal properties [37]. Recently, monitoring 342 RGNs in the human retina revealed that the maximum neural population fires at ~50 Hz, in response to full-field contrast steps, but it could reach up to 180 Hz in a few cells [37]. Although opsins with fast turn-off kinetics would allow precise spiking in those neurons, they invariably require high irradiances, as light sensitivity and kinetics are inversely correlated. Furthermore, the intensity threshold to evoke spikes in opsin-expressing retinal neurons should be below a safety threshold to reduce the possibility of photochemical damage in the retina [38]. Also, the opsins should respond to large intensity variations [26].

3. The first human clinical trial of retinal prosthesis

The first human clinical study has recently shown partial recovery of visual function in a blind patient with RP using optogenetics [25]. In the study, a serotype AAV encoding ChrimsonR fused to the red fluorescent protein tdTomoto was administered in one eye of the patient to target mainly foveal RGNs. After injecting the opsin into the ganglion cells, the retina was stimulated by an optoelectronic goggle after 7 months, as the expression takes two to six months to get stabilized in the cells [25, 39].

Recovery of visual function was tested under three conditions with three psychophysical tests. The conditions were as follows:

I. Both eyes were open in the absence of the goggles (Natural binoculars).

II. Untreated eye covered and treated eye opened in the absence of the goggles (Natural monocular).

III. Untreated eye covered and treated eye opened in the presence of the goggles (Stimulated monocular).

The conducted psychophysical tests were as follows:

I. Perceiving, locating, and touching a single object on a white table (Test-I)

II. Perceiving, locating, and counting more than one object (two or three tumblers) (Test-II)

III. Combined the assessment of vision with extracranial multichannel electroencephalography (Test-III)

The average response of the patient under the above conditions and psychological tests are summarized in **Table 1**.

The study has shown that wearing the goggle, which transforms the changes in intensity, pixel by pixel, into a monochromatic image and projects them in real time onto the retina, the patient could perceive, locate, count, and touch different objects (**Table 1**) [25].

Tests/Conditions	Success rate		
	Natural binocular	Natural monocular	Stimulated monocular
Test-I	No detection	No detection	92%
Test-II	No detection	No detection	63%
Test-III	5.8%	6%	41%

Table 1.
The success rate of visual perception under different conditions and psychological tests [25].

4. Challenges and limitations in optogenetic retinal prostheses

Channelrhodopsin-2 (ChR2) from *Chlamydomonas reinhardtii* was the first light-sensitive opsin used to restore light responses in blind mice [40]. ChR2 was injected in thalamic projecting RGNs via adeno-associated virus (AAV) serotype 2 [40]. However, the required light intensity to evoke spiking with ChR2, having its maximum absorption at 460 nm, exceeds the standard safety threshold and might cause photochemical damage in the human retina and retinal pigment epithelium [41–43].

To overcome the limitation, new opsins with increased light sensitivity were developed through mutations and discovery that increased the dynamic range of neural activity and, thus, image contrast within regulatory limits. Since the safety limits of light intensity have a strong wavelength dependence, opsins with red-shifted wavelength activation peaks safely allow the use of higher irradiance than their blue-shifted counterparts. Shifting the wavelength from blue o red allows for safely increasing the photon flux by three orders of magnitude. Deeper penetration of longer wavelengths is an additional advantage with the red-shifted opsins [41].

To reduce irradiance requirement, opsin with Ca^{2+} selectivity named CatCh was expressed into RGNs [22, 44, 45]. Although the activation threshold of CatCh-expressing RGNs is below the damage threshold and allows normal displays to evoke responses, CatCh suffers from inherent toxicity associated with Ca^{2+} influx [9]. Engineered mutants of CoChR, developed by optimizing the off-rate through site-directed mutagenesis, have also been used to restore functional vision under normal light conditions. Although the mutants functions at safe irradiances, they do not provide sufficient temporal resolution [46].

At present, red-shifted opsins are being intensively investigated as red-shifted photons can reach deeper areas in the tissue, which is important for noninvasive light delivery in *in vivo* experiments [47–49]. Three orders of magnitude higher irradiance can be used at 590 nm in contrast to light at 470 nm for safe illumination of the retina [41]. The safety threshold shifts on changing wavelengths of light as 7.52×10^{13} photons mm^{-2} s^{-1} at 470 nm to 5.94×10^{15} photons mm^{-2} s^{-1} at 590 nm [41, 50]. Recently, a red-activable variant of ChR2, named ReaChR, has restored light sensitivity in blind rd1 mice, primate retina, and post-mortem human retinae when targeted to RGNs [41, 47]. However, ReaChR could not drive RGNs beyond 30 Hz [41]. Although ReaChR requires similar irradiance as ChR2, it is safe at the red-shifted wavelength [4, 41]. To get high-frequency along with red-shifted activation wavelength, ChrimsonR was used for optogenetic vision restoration [25, 39, 51–53]. Even though ChrimsonR uses a safe wavelength of light, it still requires extremely bright light [4]. ChrimsonR has been used for the first clinical study to restore vision in a human patient [25]. **Table 2** summarizes different opsins, their characteristic features, and their response in retinal neurons [9, 25, 39–41, 47, 54–59].

Opsin	λ_{max} (nm)	τ_{off} (ms)	EPD50 (mW/mm^2)	Peak current (pA)	Target cell type	Animal Model	Operating photon flux in retinal neurons (Photons mm^{-2} s^{-1})	Ref.
ChR2	460	11.6 ± 0.4	1.3 ± 0.2	~200	RGNs	Wide-type, *rd1* mice, RCS rats	>1e+17	[40, 54, 55]
					ON Biopolar Cells	*rd1, rd10, rd16* mice	>1e+16	[56, 57]
ChR2 (H134R)	460	20.9 ± 2.2	0.43 ± 0.04	~1200	ON Bipolar Cells	*rd1* mice	>1e+16	[58]
ReaChR	590	137.2 ± 7	0.8	349 ± 192	RGNs	Mice, macaque and human retina	>1.25e+15	[41]
CoChR(H94E/L112C/K246T)	480	750	0.05	~ 1500	RGNs	Transgenic mice	>1e+14	[46]
CatCh	460	16 ± 3	0.4 ± 0.1	~ 300	RGNs	*rd1* mice, macaque eyes	>1e+15	[9]
*Ps*CatCh2.0	450	—	—	~ 400	RGNs	*rd1* mice	>4e+16	[59]
ChrimsonR	590	16 ± 2.1	~ 1	~ 400	RGNs	Macaque, Human	>7e+14	[25, 39]

Table 2.
Microbial rhodopsins in optogenetic retinal prostheses.

Although recently discovered opsins could allow control at improved light levels, they have not yet been studied for that purpose. To drive opsin-expressing neurons at their firing frequencies, optogenetic approaches are combined with an extraocular device or optoelectronic goggles [26]. These devices consist of a camera and an image processing unit. These devices are crucial for optogenetic retinal prostheses for the following reasons [18]:

i. The light-sensitive opsins used in optogenetics are sensitive to only 3–4 log units of irradiance, which is a very shorter range than at which human vision operates (10 log units).

ii. Microbial opsins have maximal responses at a single wavelength and thus need a monochromatic light source to maximize their response.

iii. The light-sensitive proteins are not sufficiently sensitive to respond to normal daylight.

iv. The optoelectronic goggle converts natural scenes having different wavelengths into monochromatic light pulses.

v. In the case of direct excitation of inner retinal neurons, there is a need for a system that can compensate for the visual processing pathways.

Optogenetic-based retinal prostheses involve the genetic expression of opsin-construct into the retinal neurons. Neurotoxicity and immunogenicity are the key concerns for clinical trials. Attempting functional-level response in the system by enhancing opsin expression is problematic due to the increased risk of cell toxicity and immune response [27, 60]. Although AAVs have a favorable safety profile in gene therapy, their safety is dose-dependent [61]. This limits the expression of microbial opsin with a safe viral dose [9]. Another critical task in clinical trials is to achieve stable opsin expression in the retina. Earlier studies have shown that the virus-mediated expression of ChR2 remained stable beyond 3 months in nonhuman primates [62]. Similar safety and stability checks must be conducted while selecting new opsins for retinal prostheses. Therefore, long-term stability and neurotoxicity with new microbial rhodopsins will need to be carefully evaluated before they can be considered for clinical applications [18].

5. New opsins in optogenetics

In recent years, intense research efforts have been directed to design more efficient opsins with tailored properties, including unitary spectral conductance, retinal binding affinity, faster temporal kinetics, light actuation sensitivity, spectral tuning, and protein stability. Being a primary goal of optogenetics, new opsins are first investigated in neurons in the brain (**Table 3**) [29, 32, 63–68].

CsChrimson is a CsChR-Chrimson chimera, produced by replacing the Chrimson N terminus with the CsChR N terminus. It exhibits more sensitivity than Chrimson, while the spectral and kinetic properties are the same [51]. bReaChES was generated by introducing a Glu123Ser mutation and replacing the first 51 amino-terminal residues with the first 11 amino-terminal residues in the ReaChR construct [63]. It

Opsin	λ_{max} (nm)	τ_{off} (ms)	EPD50 (mW/mm^2)	Peak photocurrent (pA)	Spiking Frequency in Neurons (Hz)	Ref.
CsChrimson	585	30	0.7	~700	20	[49, 51]
bReaChES	585	39 ± 3.6	0.2	~2000	10	[49, 63]
ChRmine	585	60 ± 20	0.03 ± 0.01	~4000	40	[49]
rsChRmine	530	175	0.22	1.5 ± 0.3 nA	40	[64]
hsChRmine	530	35 ± 15	—	2.5 ± 0.4 nA	40	[64]
Chronos	470	3.5	0.3	1100	60	[51]
ChroME	510	5.5 ± 0.2	0.075	1.5 ± 0.1 nA	40	[65, 66]
ChroME2s	510	13 ± 1	0.06	2.5 ± 0.2 nA	40	[66]
ChroME2f	510	9.6 ± 0.6	0.085	2.3 ± 0.2 nA	40	[66]

Table 3.
Newly discovered or engineered microbial rhodopsins in optogenetics.

has allowed simultaneous stimulation and recording using Ca^{2+} indicators [63]. Most recently, screening of residues forming the cation-conducting ChR pore guided by crystal structure-derived knowledge has resulted in ~1000 suitable new opsin sequences. A sequence of a marine opsin gene optimized for mammalian expression, named ChRmine from *Tiarina fusus*, exhibits a very large inward photocurrent ~4 nA with red-shifted excitable wavelength along with an order of magnitude faster recovery than for other red-shifted opsins [49, 68].

More recent efforts to understand the mechanism of ion transport by ChRmine has been enabled by its cryo-electron microscopy structure at 2.0 A° resolution. The structural knowledge has been used to design new variants with faster speed (hsChRmine) and greater red shift (rsChRmine), while retaining high current and light sensitivity [64]. Along with the development of red-shifted opsins, new efficient blue-activated opsins have also been discovered and engineered. *de novo* sequencing of opsins from over 100 algal species has resulted in Chronos, a blue light-sensitive opsin exhibiting high light sensitivity and unprecedented kinetics, with a turn-off of 3.6 ± 0.2 ms. It is the fastest blue-shifted opsin reported to date [51]. Recent efforts to engineer Chronos have resulted in a mutant, ST-Chronos-M140E, or ChroME. It exhibits rapid decay kinetics while exhibiting photocurrents more than 3-5 times larger than Chronos [65]. More recently, two new mutants have been engineered, namely ChroME2f and ChroME2s, with enhanced properties that can support large-scale, temporally precise multiphoton excitation [66].

Although the newly discovered opsins can significantly improve optogenetic retinal prostheses, developing strategies for safe and sufficient expression of new opsins in retinal neurons and experimentation of each opsin pair with different targeted retinal neurons is a lengthy process and needs repetitive investigation and safety checks before clinical studies.

6. Computational modeling of optogenetic excitation of retinal neurons

Computational modeling of optogenetic systems has made significant contributions in developing a better understanding of the reaction dynamics behind

photocurrent generation in the opsin molecules and change in membrane potential in opsin-expressing neurons in response to light. It helps in correctly interpreting experiments and optimizing photostimulation conditions within a complex cell/tissue environment, which is often questionable and challenging [69–74]. Due to the naturally occurring structural and functional diversity in the cell types in living animals, a large data set is required for optogenetics to be used in different environments, which is a challenging task. The problem persists while selecting light-sensitive proteins to get desired control over cellular activity. Computational models can help quick (virtual) testing of newly developed light-sensitive proteins in different cell types and within realistic tissue and organ-level settings.

Initial efforts in the field of computational optogenetics include modeling of the photoresponse of ChR2 considering three, four and six-state models of its photocycle, light-mediated spiking in neurons under continuous and pulsed illumination, the effect of illumination of sub-cellular compartments, analysis of activation threshold of opsin-expressing cell within tissue while considering scattering and absorption [69–74]. Although there were many new opsins with improved kinetics, photocurrent, and light sensitivity, theoretical models of these opsins were not formulated until recently.

In the last few years, theoretical models of optogenetic systems consisting of new light-sensitive proteins have been formulated. Accurate theoretical models of optogenetic excitation of neurons with Chronos, CheRiff, ReaChR, Chrimson, ChrimsonR, CsChrimson, f-Chrimson, vf-Chrimson, bReaChES and ChRmine and inhibition with NpHR, eNpHR3.0, Jaws, and GtACR2 have been reported recently. Using these computational models, a detailed theoretical analysis of the effect of photostimulation and physiological parameters was also conducted, which has provided a better understanding of the mechanism, limitations, and advantages of different types of neurons [75, 76]. Further, accurate theoretical models of bidirectional optogenetic control with spectrally separated excitatory-inhibitory opsin pairs, namely ChR2(H134R)-eNpHR3.0, Chrimson-GtACR2, Chronos-eNpHR3.0, Chronos-Jaws, CheRiff-Jaws, and Vf-Chrimson-GtACR2 were also reported. A comprehensive theoretical study on high-frequency low-power bidirectional optogenetic control of neurons with single spike resolution with already tested opsin pairs and with new

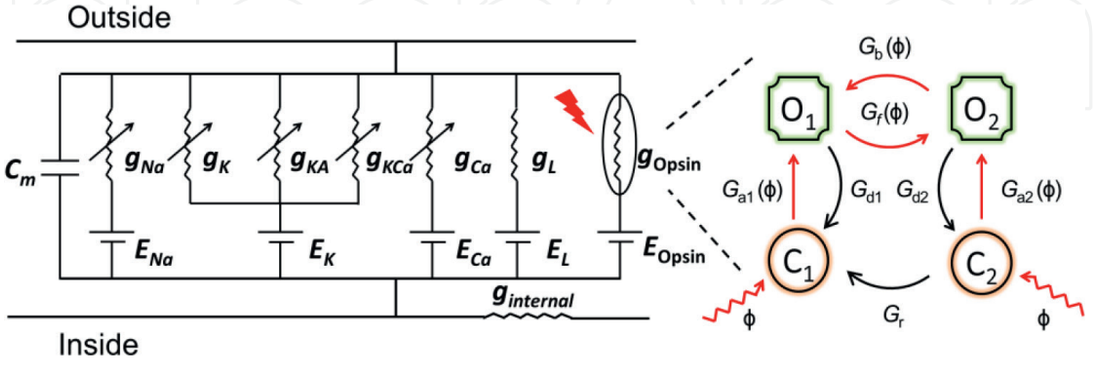

Figure 1.
Equivalent circuit diagram of biophysics model of opsin-expressing retinal ganglion neurons. C_m is the membrane capacitance. g_{Na}, g_K, g_{KA}, g_{KCa}, g_{Ca} and g_L are maximal conductances of naturally occurring sodium, potassium, potassium (A-type), Ca^{2+} activated potassium, calcium, and leak channels. E_{Na}, E_K, E_{Ca}, and E_L are the reversal potential for sodium-, potassium-, calcium-conducting, and leaky channels. g_{Opsin} is the light-dependent conductance through opsin channels having reversal potential E_{Opsin}. C_1 and C_2 are the closed states, and O_1 and O_2 are the open states. G_{a1}, G_{a2}, G_b, and G_f are the light-dependent rate functions, while G_{d1}, G_{d2}, and G_r are the light-independent rate constants. ϕ is the photon flux. (For details, see Ref. [38]).

opsin pairs not experimented till that time for better control was conducted using theoretical models [38]. Recently, computational simulations have proposed a novel method of co-expressing step-function opsins with fast channelrhodopsins to avoid spike failure from desensitization of photocurrent [77]. Integration of theoretical models of photoresponse of ChRmine into biophysical circuit models of cardiac cells has shown that ChRmine could be used for ultra-low power deep sustained optogenetic excitation/suppression of electrical activity in cardiomyocytes [78].

Theoretical simulations of optogenetic visual cortical prosthetic systems have provided a better understanding of the mechanism behind signal encoding by the visual cortex [79, 80]. Theoretical modeling of optogenetic excitation of retinal neurons would be a primary step to design ideal optogenetic prosthetic devices and circuits. Also, the newly discovered light-sensitive proteins have the potential to overcome current challenges. However, designing methods for the efficient and safe delivery of new opsins in the retina is a lengthy process. No mathematical model was reported until recently to study optogenetically evoked spiking in RGNs.

The theoretical framework of the biophysical mechanism of optogenetic excitation of already tested opsins, namely, ChR2, ReaChR, and ChrimsonR, and newly discovered potential opsins, *i.e.,* CsChrimson, bReaChES, and ChRmine has contributed significantly to our knowledge and provided insights into the design of optimized optogenetic retinal prosthetic circuits [38] (**Figure 1**). In the first step, the photocurrent characteristics of different opsins were compared over a wide range of irradiances (**Figure 2**). The variation of photocurrent with time is shown in **Figure 2A**. Peak and plateau photocurrent of these opsins were compared over a wide range of irradiance **Figure 2B** and **C**. Theoretical simulations have helped in determining the minimum pulse width to achieve maximum photocurrent at each irradiance, also called saturating pulse width (SPW), which is important to minimize delivered light power while getting maximum output (**Figure 2D**). SPW decreases on increasing irradiance and saturates at higher irradiances for each opsin [38].

Further, the variation of photocurrent with time on illuminating with repetitive light pulses at different light stimulation frequencies was compared (**Figure 2E**). At higher frequencies, the photocurrent in all the opsins does not reach the baseline before the arrival of the subsequent light pulse, which results in a non-zero photocurrent plateau indicated by an arrow in **Figure 2F**. The percentage of return to baseline (RTB %) plays a crucial role in temporal resolution at high frequencies. In all the opsins, RTB % decreases with stimulation frequency. The potential interactions between pulse width, irradiance, and stimulation frequency can have a profound impact on temporal fidelity due to the inverse relationship between kinetics and photosensitivity of opsins. The study also reported that RTB % does not change with irradiance, while it is lower at longer pulse [38].

To determine the irradiance range at which each opsin-expressing RGN exhibit spiking, the variation of membrane potential with time were simulated on illuminating with long (500 ms) and short (10 ms) light pulses at different irradiances (**Figure 3**). The variation of membrane potential with time on illuminating 500 ms light pulses at increasing irradiances has been shown in **Figure 3A**. From the variation of the number of spikes with pulse irradiance, the study has revealed that ChRmine is most sensitive among the studied opsins and the maximum firing frequency within the safety limit of irradiance is two-three orders of magnitude higher than the opsins tested for retinal prostheses (**Figure 3A**). Further, the response of these RGNs was simulated under randomized photon fluxes of increasing contrast (**Figure 4B**). Interestingly, ChRmine can respond to irradiances changes from one order to four orders (**Figure 3B**).

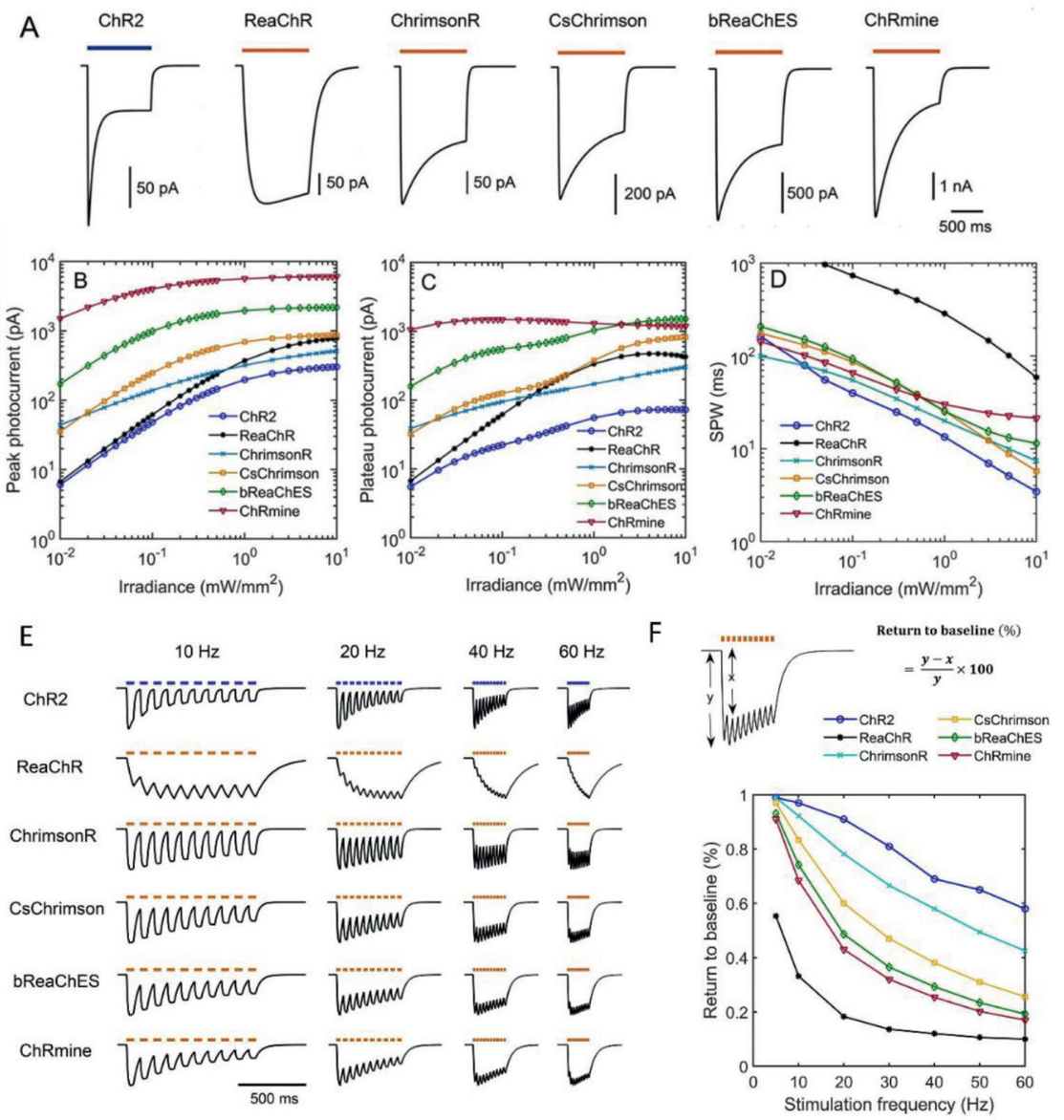

Figure 2.
Photocurrent characteristics of ChR2, ReaChR, ChrimsonR, CsChrimson, bReaChES and ChRmine on illuminating with their peak absorption wavelength i.e., 460 nm for ChR2 and 590 nm for other opsins. (A) Variation of photocurrent with time on illuminating with single light pulse (1 s, 1 mW/mm^2), and (B–D) corresponding variation of (B) peak, (C) plateau photocurrent and (D) saturating pulse width (SPW) with irradiance. (E) Variation of photocurrent with time on illuminating with multiple light pulses at indicated frequencies and different pulse widths (50 ms for 10 Hz, 25 ms for 20 Hz, 12.5 ms for 40 Hz, and 8.5 ms for 60 Hz) at 1 mW/mm^2, and (F) corresponding variation of return to baseline (%) with stimulation frequency, calculated using the formula shown above the box. © IOP Publishing. Reproduced with permission. All rights reserved [38].

In moving picture frames, time-dependent change in irradiances of each pixel is the key photostimulation parameter to encode changes in visual scenes. In optogenetics, irradiances below certain threshold results in spike failure, while pulses longer than a certain limit result in spurious spiking patterns. Thus, it would be necessary to determine how larger fluctuations in irradiance and pulse-width can be tolerated by retinal neural circuits. The theoretical study determined a region in the irradiance-pulse-width plot in which each point is a combination of allowed irradiance and pulse-width, while maintaining single-spike resolution (**Figure 4**).

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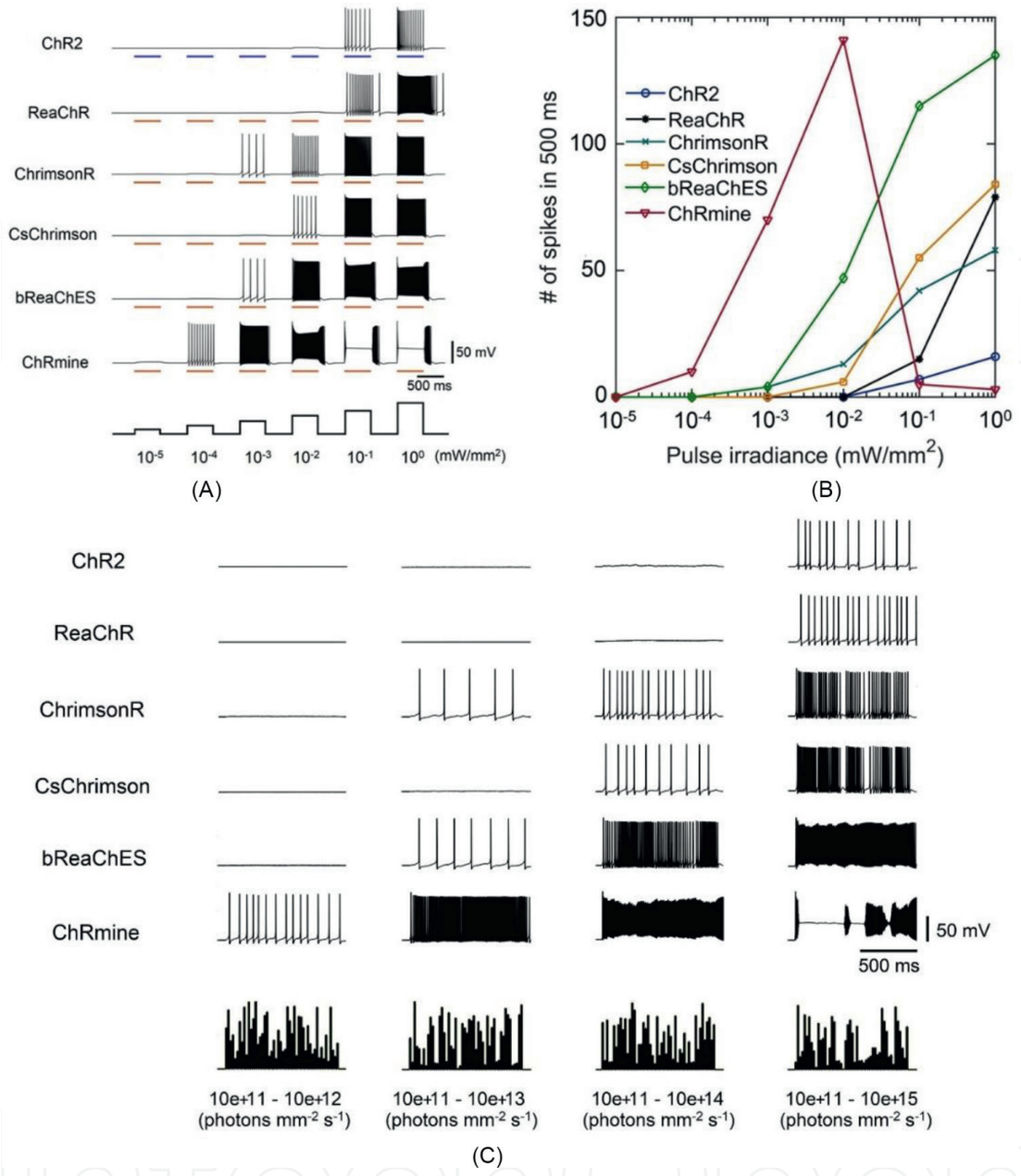

Figure 3.
Frequency response of opsin expressing retinal ganglion neurons in response to long- and short-duration light pulses at different irradiances. (A) Variation of membrane potential with time in response to light pulses (500 ms) at increasing irradiances, and (B) corresponding variation of the number of spikes during illumination at different irradiances. (C) Light-evoked spiking in different opsins-expressing RGNs on illuminating with multiple light pulses each of 10 ms at 50 Hz for 1 s and at randomized photon fluxes of increasing contrast from 10^{11} to 10^{12} photons mm^{-2} s^{-1} to 10^{11} to 10^{15} photons mm^{-2} s^{-1}. © IOP Publishing. Reproduced with permission. All rights reserved [38].

In visual systems, internal delays in signal processing may lead to mislocalization while capturing things changing with time [81]. Thus, the latency of a system is a critical feature for information processing which is determined by the turn-on rate of the opsin photocurrent [48, 81]. Furthermore, due to desensitization of photocurrent in opsins, sustained excitation of opsin-expressing neurons results in spike failure [27]. In the study, they have shown the variation of first spike latency with irradiance

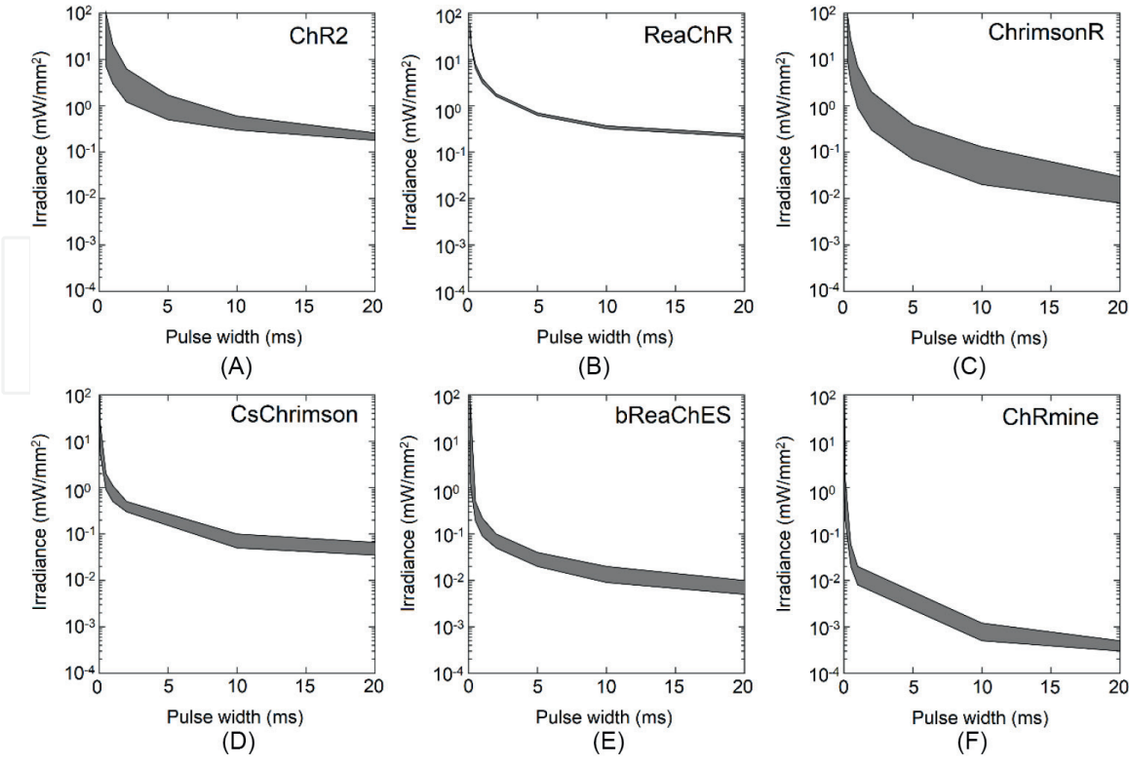

Figure 4.
Tolerance for maintaining single spike resolution by different opsin-expressing neurons against fluctuations in irradiance and pulse width. Variation of minimum (lower boundary of the shaded region) and maximum (lower boundary of the shaded region) irradiance thresholds with pulse-width to evoke 100% spiking at 10 Hz for 2 s in (A) ChR2, (B) ReaChR, (C) ChrimsonR, (D) CsChrimson, (E) bReaChES, and (F) ChRmine-expressing retinal ganglion neurons. © IOP Publishing. Reproduced with permission. All rights reserved [38].

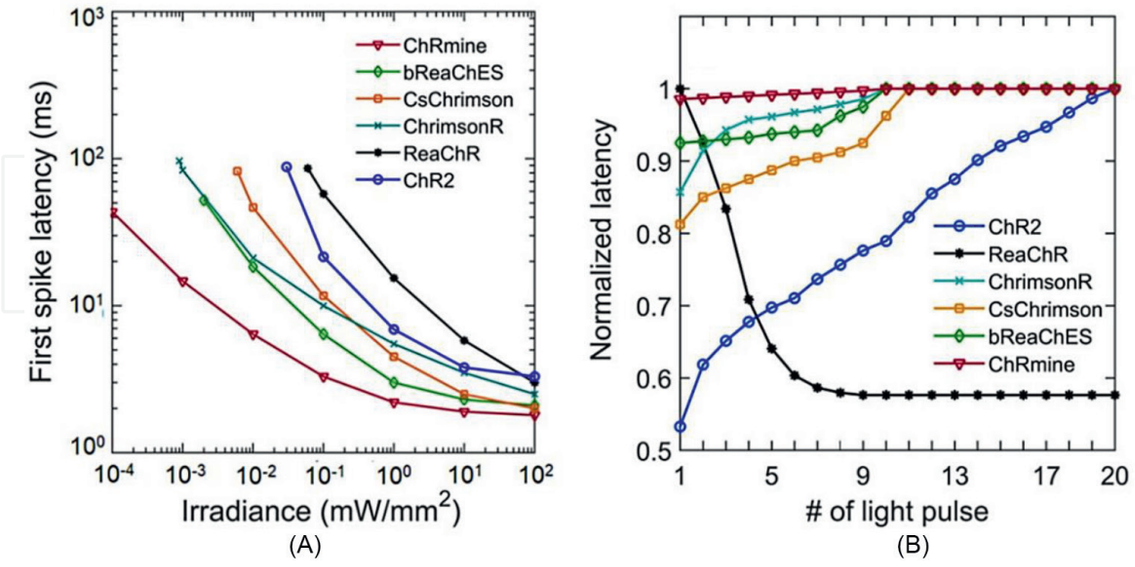

Figure 5.
Latency of spikes in opsin-expressing retinal ganglion neurons in response to light stimulus. (A) Variation of first spike latency with pulse irradiance, and (B) variation of normalized spike latency with sequential position of light pulse each of 5 ms for ChR2, 4 ms for ChrimsonR, 1.8 ms for CsChrimson, 6 ms for ReaChR, 0.4 ms for bReaChES, and 0.13 ms for ChRmine in the stimulus train at 10 Hz and 0.6 mW/mm². © IOP Publishing. Reproduced with permission. All rights reserved [38].

in different opsin-expressing RGNs (**Figure 5A**). Further, latencies of subsequent spikes were also determined to check consistency on illuminating with a light pulse train (**Figure 5B**). Theoretical simulations revealed that under sustained excitation of RGNs, ChRmine exhibits the most stable and shortest latency during the entire stimulus train (**Figure 5B**).

For accurate information processing to higher visual centers of the brain, it is essential to drive RGNs up to their natural firing rates. Thus, it was important to determine high-frequency limits of each opsin-expressing RGNs. From MIT-irradiance graph, it is clear that there is a trade-off between pulse-width and required irradiances to evoke spike. However, the light pulse cannot be increased beyond a certain limit at a particular frequency (*e.g.,* 5 ms for 100 Hz). On the other hand, higher irradiance above its saturating value is totally waste of energy. Thus, a systematic study of the effect of photostimulation conditions was carried out to determine the high-frequency limit with each opsin under pulsed stimulation.

Further, the high-frequency limit was determined with each opsin. The variation of spiking frequency with stimulation frequency at optimized photostimulation conditions is shown in **Figure 6A**. Each opsin maintains 100% spike fidelity up to a certain frequency limit. The spiking patterns at the high-frequency limit are shown in **Figure 6B**. Among these opsins, ChrimsonR is the only opsin that allows 100% spiking fidelity up to 100 Hz. However, the required photon flux density with ChrimsonR ~8.3 × 10^{15} photons. mm^{-2} s^{-1} at 590 nm is beyond the safety threshold for the human retina [38, 41]. On the other hand, ChRmine maintains single-spike resolution up to 40 Hz, which is sufficient for RGNs, it needs pulses of the lowest light power (**Figure 6**) [38].

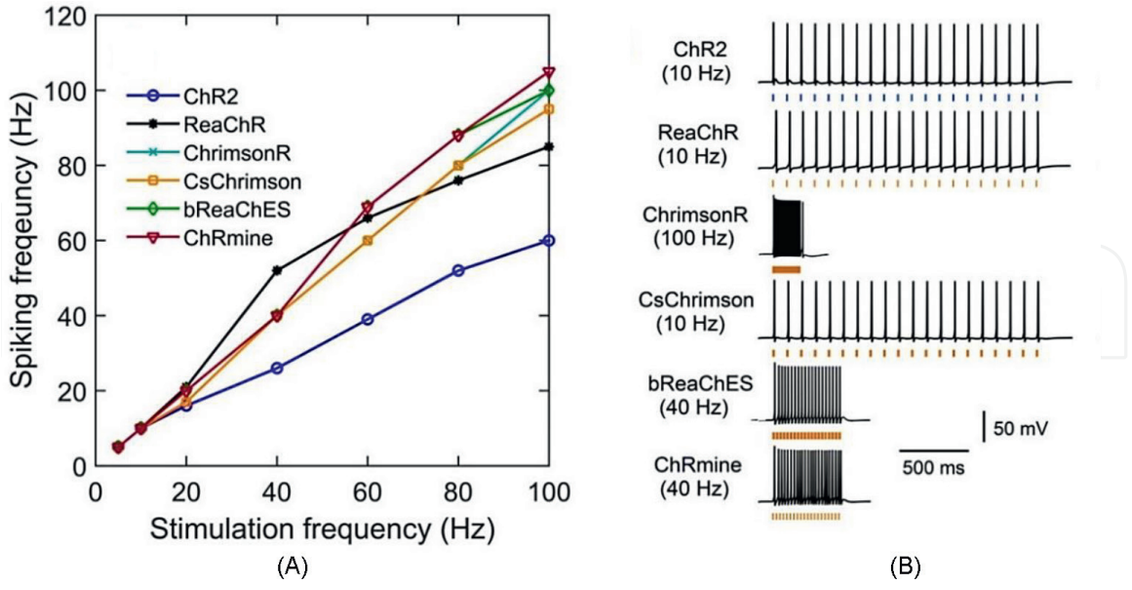

Figure 6.
Frequency response of different opsin-expressing retinal ganglion neurons. (A) Variation of spiking frequency with stimulation frequency on illuminating with 20 light pulses at different frequencies and at different irradiances and pulse widths as follows: 5 mW/mm² and 2.5 ms for ChR2, 2.8 mW/mm² and 1.2 ms for ChrimsonR, 0.13 mW/mm² and 5 ms for CsChrimson, 5 mW/mm² and 1 ms for ReaChR, 0.02 mW/mm² and 6 ms for bReaChES, and 0.013 mW/mm² and 0.93 ms for ChRmine, and (B) corresponding spiking patterns at the high-frequency limit with 100% spike fidelity. ©IOP Publishing. Reproduced with permission. All rights reserved [38].

7. Conclusion

Optogenetic retinal prostheses fundamentally has the potential to restore quality vision in the human retina. Although there are certain limitations and challenges associated with opsin characteristics and delivery methods, in the coming years, repetitive clinical trials with new opsins and safe delivery methods would allow the efficient design of optogenetic retinal prosthetic devices and circuits. Theoretical studies in optogenetics have the potential to guide future experiments with reduced irradiance and enhanced visual contrast. Future experiments by co-expressing excitatory and inhibitory or spectrally orthogonal opsins into different retinal neurons *e. g.,* ON and OFF type of RGNs, would significantly improve the quality of restored vision.

Conflict of interest

The authors declare no conflict of interest.

Nomenclature

AAV	Adeno-associated virus
ChR2	Channelrhodopsins
NpHR	*Natronomonas pharaonis*
RGN	Retinal ganglion neuron
RP	Retinitis pigmentosa
RTB	Return to baseline

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