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Optogenetics: lights, camera, action! A ray of light, a shadow unmasked

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DOI: 10.1533/9781908818782.185

Abstract: The field of optogenetics has been progressing at a rapid pace, serving both as an investigative tool and a therapeutic solution for biomedical applications, especially in the field of neuroscience. With the recent BRAIN Initiative launched by the National Institutes of Health, and the breakthrough technology (called CLARITY) to render whole mouse brains transparent, the concept of using light to probe and control cellular functions is fast becoming a clinical reality. Given the fact that light-sensitive (microbial) ion channels are required to be integrated into animal cells, it is envisaged that the application of optogenetics would have a far-reaching impact on biotechnology, nanoscience, and medicine. In this chapter, we seek to provide an in-depth account of the current landscape of optogenetics, its potential clinical applications, and implications for the future.

Key words: optogenetics, photomedicine, channelrhodopsin, halorhodopsins, nanoscience.

10.1 Introduction

The discovery of optogenetics has played an instrumental role in enabling the pursuit of new frontiers in neuroscience. This neuromodulation

technology unites gene therapy with fiber-optic technology to enable precise control and visualization of light-responsive neurons in living tissue [1]. Using the analogy of controlling a microscope, the coarse control is derived from delivering light to specific tissues while fine control is achieved by genetically introducing light-gated channel proteins to certain cells. Apart from being cell-type specific, optogenetic control possesses the advantages of speed, being minimally invasive, and temporal resolution [2]. Coined as "Method of the Year" by *Nature* in 2010 and dubbed as the "holy grail" of neuroscience, this photostimulation technology can be summed up by a director's favourite catchphrase, "Lights, camera, action!" To begin this optogenetic movie, the gene of a photo-responsive channel has to be introduced into genetically targeted cells. Once the cellular stage is set, all that is needed is a light beam. When the ray of light is shone, specific cells lying along the light path activate in concert. The illumination causes the channel (*the camera*) to open, permitting the movement of ions. This movement causes an action potential to be generated or inhibited to either depolarize or hyperpolarize the cell. This ability to drive or silence cellular activity by turning the light switch on or off, has opened new doors to the field of neuroscience, with applications ranging from mapping neuronal circuits to clinical implications such as restoring vision and ameliorating Parkinsonian deficits [3] (Figure 10.1).

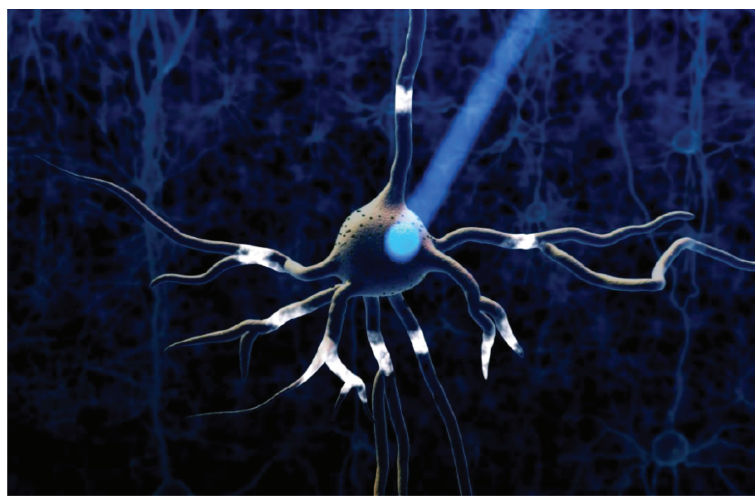

Figure 10.1 Neuron firing an action potential upon illumination with a beam of blue light [3]. Image reproduced with permissions from Boyden et al., 2011. Copyright © 2011 Sputnik Animation

10.2 Overview – from birth to cradle

In 1979, Francis Crick voiced that the neuroscience community faced the challenge of creating a technique to control a single cell type without affecting other cells in the vicinity. Traditional approaches such as electrical stimulation have failed to selectively target electrically excitable cells, which are usually found embedded and densely packed in different tissues [2]. Pharmacological intervention, on the other hand, allows for such discrimination among cells but lacks temporal precision [4]. Crick pointed out that light might be the solution though it was not till 2005 that the idea of using light as a tool for selective neuromodulation translated into reality with the advent of optogenetics, a term coined by Deisseroth et al. in 2006 [5]. This versatile technology possesses the twin advantages of being both temporally precise and fast (within milliseconds), enabling control on the millisecond time scale. Since then, there has been a proliferation (if not explosion) of publications dedicated to the study of optogenetics, both as an investigational tool, and describing its potential for regenerative medicine.

10.3 Optogenetics

The field of optogenetics can be broadly categorized into effectors or actuators, and sensors. Optogenetic effectors or actuators are tools that can be used to control neural circuits while optogenetic sensors are used as probes to monitor neural circuits (Figure 10.2).

10.3.1 Optogenetic effectors/actuators

Conventional optogenetic effector or actuator tools can be classified into two superfamilies – microbial (type I) opsins and animal (type II) opsins. These proteins can be genetically introduced into cells where they alter the movement of specific ions and hence neuronal firing, in response to light. There are two main similarities between these two families.

First, both require a compound that is structurally related to vitamin A, retinal, which serves as an organic cofactor [6]. Retinal is a bound chromophore, which possesses the ability to absorb photons and hence enable the photocycle to ensue [7]. An opsin protein with bound retinal is termed rhodopsin [6]. When retinal covalently binds to a conserved

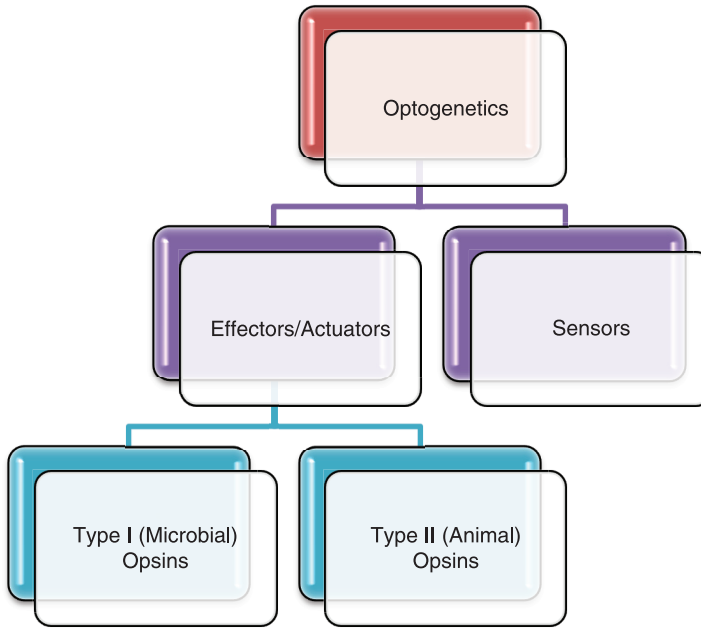

Figure 10.2 Optogenetic classification chart

lysine residue of helix 7, a protonated retinal Schiff base ($RSBH^+$) is formed. The residues that make up the binding pocket define the ionic concentration of RSB, which in turn defines the spectral properties and specific kinetics of each protein [6]. When light of a specific wavelength is present, the isomerization of retinal triggers a cascade of conformational changes [6].

The second similarity is that the genes from both opsin families encode seven-membrane structures. However, there is very low sequence homology between the two, although intra-family homology is high with a 25–80% residue similarity [8] (Table 10.1).

Microbial (Type I) opsins

Type I opsin genes are present in prokaryotic algae and fungi [9]. Despite the lack of complex eye system, microbes are dependent on light for various purposes, including homeostasis. This is possible due to the presence of light-activated proteins [6]. These molecular factories function by coupling light sensation to influence ionic fluxes. Microbial

Table 10.1 **Summary of similarities and differences between microbial (Type I) and animal (Type II) opsins [6]**

Characteristic	Microbial (Type I) opsins	Animal (Type II) opsins
Organic co-factor	Require retinal	Require retinal
Structure	Genes of both opsin families encode seven-membrane structures	
Type of organism	Prokaryotes, algae, fungi	Eukaryotes
Main role	Homeostasis	Vision
Inactive retinal	All-*trans* retinal	11-*cis* configuration
Closing of opsin channel	No dissociation; thermal relaxation closes the channel	Dissociation of activated retinal from opsin protein
Covalent retinal–protein linkage	Linkage maintained	Hydrolysis of linkage
Active retinal	13-*cis* configuration	All-*trans* retinal

(Type I) opsins comrpise many subfamilies, including bacteriorhodopsin (BR), channelrhodopsins (ChR) and halorhodopsins (NpHR).

Type I rhodopsins typically have retinal in the all-*trans* configuration bound to them. When a photon of light is absorbed, photoisomerization to the 13-*cis* configuration occurs. The retinal molecule is thermally inactivated to the all-*trans* state while still maintaining the covalent bond to the opsin [6,10].

Channelrhodopsins

Channelrhodopsins are nonspecific cation channels that depolarize upon blue light illumination. These light-gated ion channels were isolated from green microalgae of the genus, *Chlamydomonas*. Structurally, they have a seven-transmembrane region and a C-terminal extension.

Channelrhodopsin-1 (ChR1) and channelrhodopsin-2 (ChR2) are light-sensitive proteins discovered in the algae species, *Chlamydomonas reinhardtii* [11]. Compared with proton-selective ion channel ChR1, non-specific cations may permeate ChR2 [12]. Additionally, ChR2 triggers larger photocurrents and possesses better host cell expression than ChR1 [11,12]. In 2005, ChR2 was successfully used as the first optogenetic tool, and has since been established as the paradigm in this field [13].

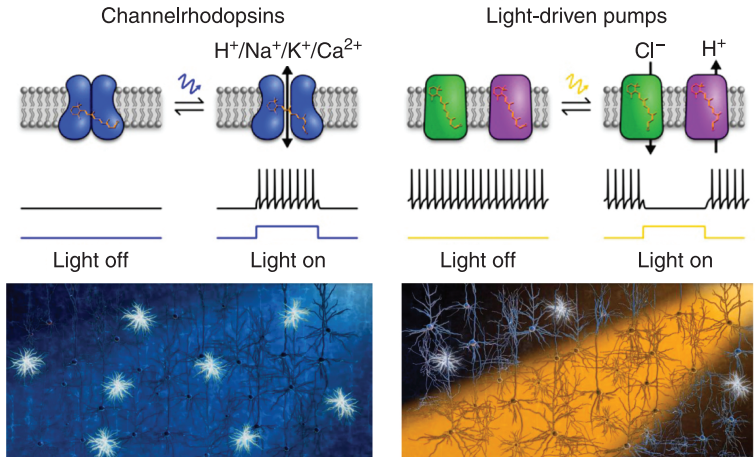

Figure 10.3 **Microbial (Type I) opsins – Channelrhodopsins (blue, on the left) depolarize cells and stimulate neurons upon illumination with blue light. On the other hand, light-driven pumps (on the *right*), including halorhodopsins (green) and bacteriorhodopsin (pink), generate hyperpolarizing currents, silencing neurons in response to yellow light illumination. Halorhodopsins translocate negative chloride ions in to the cell while bacteriorhodopsins pump protons out of the cell [3,47]. Image reproduced with permissions from Boyden et al., 2011 [3]. Copyright © 2011 Sputnik Animation. Image reproduced with permissions from Dugue et al., 2012 [47]. Copyright © 2012 Elsevier B.V.**

A second cation channel, Volvox-Channelrhodopsin-1 (VChR1) is a red-shifted ChR variant isolated from spheroidal alga *Volvox carteri* [14]. Owing to differences in the RSB binding pocket, VChR1 has different spectral properties compared with ChR1 and ChR2, allowing action potentials to be generated at 589 nm [14]. However, neuronal expression of VChR1 is about three times lower than ChR2 [14]. Nevertheless, its ability to be activated upon yellow-light illumination is a useful property that contributes to the diversity in the optogenetic toolbox (Figure 10.3).

Halorhodopsins

In contrast to the ChRs, which open up a channel pore to activate neurons in response to illumination, the halorhodopsins (NpHR) are light-driven

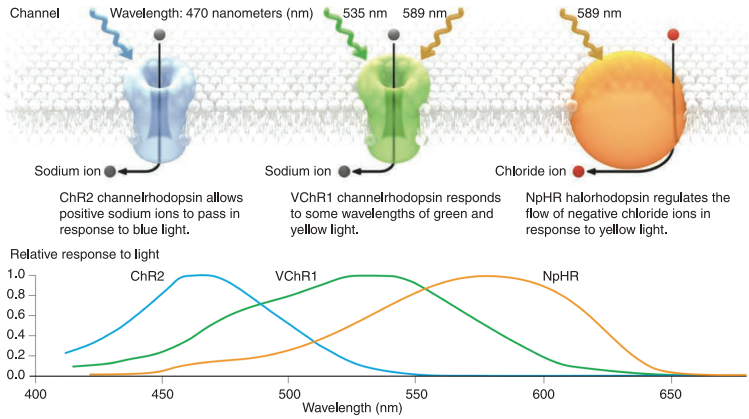

Figure 10.4 **Summary of ChR2, VChR1 and NpHR [1]. Image reproduced with permissions from Deisseroth, 2010 [1]. Copyright © 2010 Redux Pictures**

pumps, translocating one ion per photon absorbed resulting in the optogenetic inhibition of neurons [15].

Halorhodopsin generates this hyperpolarizing current by pumping a chloride ion into the cytoplasm. Other proteins, such as BRs, proteorhodopsins and archaerhodopsins, extrude protons.

Halorhodopsin is the first microbial opsin to be used as an optogenetic silencer in 2007 by Han and Boyden [16]. Identified in the halobacterium *Natronomonas pharaonis*, halorhodopsin is a chloride ion pump that hyperpolarizes the cell upon illumination with yellow light (589 nm) [6]. As a true pump, NpHR can pump ions against a concentration gradient but requires constant light to pass through its photocycle [6]. Nonetheless, optogenetic silencers are important complements to the activators because they are crucial in the understanding of neuronal function of various brain disorders and behaviors (Figure 10.4; Table 10.2).

The Optogenetic toolbox team up

The ChR2/NpHR system provides researchers with a means to bidirectionally control the same cell. By co-expressing ChR2 and NpHR in the same cell, this system of dual control enables independent photostimulation or photoinhibition of neurons by turning on the light switch. Aside from these three major optogenetic tools, there are numerous variants and opsin fusions with enhanced properties that make up the expanding optogenetic toolbox [17]. For instance, step

Table 10.2 **Summary of similarities and differences of the three main microbial opsins, the channelrhodopsin 2, Volvox-channelrhodopsin 1 and halorhodopsin [1]**

Microbial opsin	Channelrhodopsin 2	Volvox-Channelrhodopsin 1	Halorhodosin
Classification	Channelrhodopsins	Channelrhodopsins	Light-driven pumps
Structure	■ 7 transmembrane region ■ Ion Channel	■ 7 transmembrane region ■ Ion channel	■ 7 transmembrane region ■ Inward chloride ion pump
Microbe source	*Chlamydomonas reinhardtii*	*Volvox cateri*	*Natronomonas pharaonis*
Function	■ Allows passage of sodium ions into cell in response to blue light (470 nm)	■ Allows passage sodium ions into cell in response to certain wavelengths on green (535 nm) and yellow light (589 nm)	■ Pumps chloride ions into cell response to yellow light (589 nm) ■ Able to pump ions against a concentration gradient

function opsins or bistable opsins are variants that induce a prolonged sub-threshold depolarization [18] (Figure 10.5).

Animal (Type II) opsins

Type II opsin genes are found in eukaryotes and encode G protein-coupled receptors (GPCRs). They play a major role in vision but are also of importance in pigment regulation and maintaining circadian rhythms [6,19,20].

In the dark, the opsin is bound to retinal in the 11-*cis* configuration, which renders them inactive. Illumination causes the isomerization of retinal to an all-*trans* configuration. Consequently, the signal transduction second messenger cascade is triggered. Hydrolysis of the retinal–protein covalent linkage terminates the signal [21]. The free all-*trans* retinal then diffuses away from the opsin, while a fresh 11-*cis* retinal molecule takes its place to allow the cycle to begin again [21].

There are three main superfamilies of animal opsins – vertebrate rhodopsins, ChARGe and OptoXR.

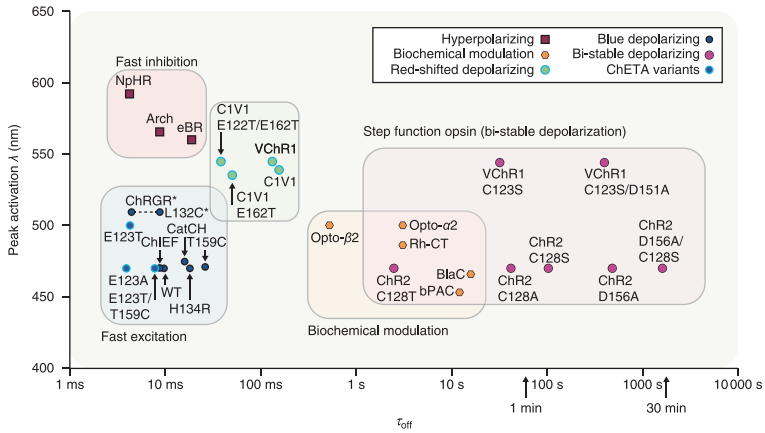

Figure 10.5 **Diagram summarizing kinetics and properties optogenetic tool variants [17]. Image reproduced with permissions from Tye and Deisseroth, 2012 [17]. Copyright © 2012 Macmillan Publishers Limited**

Rhodopsin is a light-sensing protein found in the mammalian eye. It plays a major role in vision, and is both an opsin that is covalently bound to retinal, and a GPCR [6]. With knowledge that there is retinal in adequate amounts found in the mammalian brain tissue by Deisseroth et al. [5], coupled with efforts to understand the structure–function relationship of GPCRs by Khorana and co-workers [22], opsin–receptor chimeras have been developed. These chimeric proteins are known as "OptoXRs", where the X in OptoXR denotes the specific receptor pathway being "hijacked" [23]. The two main OptoXRs are rhodopsin–adrenoreceptor chimeras using the α1 adrenoreceptor and β2 adrenoreceptor – Opto-α1AR and Opto-β2AR [24].

OptoXRs are bovine rhodopsins (green region) but have their intracellular loops replaced with specific adrenergic receptors [23]. OptoXRs "hijack" the downstream intracellular pathways normally recruited by these adrenoreceptors, namely the G_q-IP3 pathway activated by the α1 adrenoreceptor and G_s-cAMP pathways activated by the β2 adrenoreceptor [23].

In 2010, Herlitze et al. successfully produced Rh-CT(5-HT-1A), an opsin chimera between rat rhodopsin and the 5-HT-1A serotonin receptor to allow optical control over the $G_{i/o}$-cAMP pathway [24,25].

The discovery and development of these receptors complement microbial opsin strategies, providing alternative dimension to optical control (Figures 10.6 and 10.7).

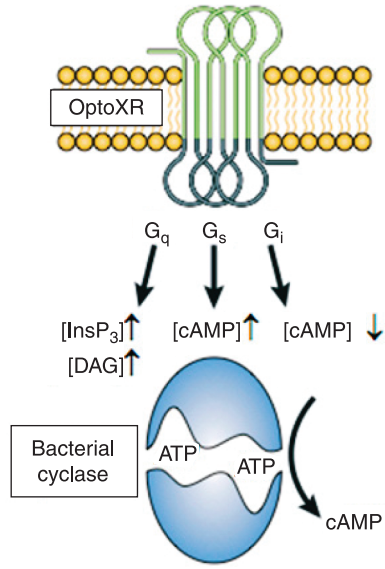

Figure 10.6 Diagram illustrating OptoXR [17]. Image reproduced with permissions from Tye & Deisseroth, 2012 [17]. Copyright © 2012 Macmillan Publishers Limited

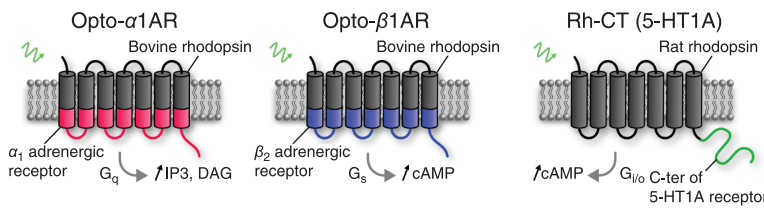

Figure 10.7 Diagram of Opto-α1AR and Opto-β2AR and Rh-CT(5-HT-1A) [24]. Image courtesy Dugue, 2011 [24]. Copyright © 2011 Dugue GP

10.3.2 Optogenetic sensors

Optogenetic sensors include a range of proteins that possess the ability to monitor various stages of synaptic transmission. These include voltage-sensitive fluorescent proteins, genetically encoded calcium indicators, chloride sensors, pH sensors and neurotransmitter release sensors.

The voltage-sensitive fluorescent proteins enable action potentials to be visualized by monitoring trans-membrane voltage differences.

Aside from voltage changes, an action potential also involves an influx of Ca^{2+} into a cell and loss of protons resulting from neurotransmitter release. Synaptic inhibition on the other hand, results from a Cl^- influx. Using this knowledge of ionic movements, genetically encoded calcium indicators, chloride sensors and pH sensors have been used to monitor neural activity by detecting the cellular concentrations of Ca^{2+}, Cl^- and protons, respectively [26,27].

Apart from measuring the concentration of ions, another method used to monitor neural activity is detecting neurotransmitter release. Neurotransmitter release sensors are optogenetic sensors that fluoresce in response to central modulators such as glutamate and acetylcholine [28].

Optogenetic sensors have been useful in enabling researchers to observe neural circuits while optogenetic effectors allow us to manipulate these circuits. These tools have enabled us to reach new frontiers in neuroscience and with further fine-tuning, they could hold key to many unanswered questions in the field of neuroscience.

10.4 Light delivery

Since the advent of optogenetics, light delivery technology has been a key area of constant improvement, enhancing precision and control, both *in vitro* and *in vivo*. There are three main light sources *in vitro* – mercury arc lamps, lasers, and light-emitting diodes (LEDs). *In vivo*, an optic fiber is coupled to either a laser or LED light source, enabling light to be delivered to the transduced tissue [6].

Arc lamps, although easily available, are less suitable for *in vivo* preparations because the efficiency in coupling the light into optic fiber is poor [29]. Laser-based systems can be coupled efficiently and achieve a high intensity per area of illumination, which is a major advantage over other light sources, albeit at a higher cost [29]. LEDs have been a promising technology that has shown rapid advancement. Aside from cost, another advantage is that LEDs can be easily mounted on a rodent, granting the rodent mobility. However, two important disadvantages are their susceptibility to overheating and the relatively weaker light intensity compared with the other light sources when collimated [29].

As each light source has its own pros and cons, the light delivery system chosen would depend on the type of experiment and organism.

10.5 Applications

Its remarkable precision has resulted in neuroscientists adopting optogenetics as a research tool in uncovering the neural circuitry of targeted cell populations. Dissecting these complex neural circuits is crucial in understanding how their function in a healthy brain might differ from that in a diseased brain. The ability to control and manipulate neuronal firing has enabled researchers to mimic behavioral deficiencies and modulate these symptoms using optogenetic tools [17].

10.5.1 On a micro-level – in vitro applications

Delivering optogenetic tools into neuronal systems can be achieved by three methods: viral vectors, transgenic animals, and lastly, the Cre-dependent expression system, which is a combination of both viral and transgenic methods.

Viral vectors

At present, viral vectors are the standard method used in optogenetic research. Lentiviruses and adeno-associated viruses have been used successfully as vectors to deliver the opsin gene into the host cell genome. This is achieved by fusing the opsin gene with a cell-specific promoter to create the recombinant virus. Thereafter, the virus vector delivers the transgene to the host cell, which incorporates into the host cell genome. This system possesses the twin advantages of being fast to implement (4–5 weeks to achieve expression) and possessing high expression levels over long periods with no reported adverse effects [6]. However, one significant disadvantage is that viruses have a limited genetic payload. This in turn lowers specificity as it limits the length of the promoter sequence to around 4 kilobases [6].

Transgenic animals

The first step in creating a transgenic line involves creating a transgene cassette, which consists of the promoter and the transgene. This cassette, carrying either recombinant promoters or bacterial artificial chromosomes, is subsequently injected into zygotic eggs or mice or rats and integrates randomly into the host cell genome to achieve promoter-specific transgene expression. The strain is then bred until a stable transgenic line is created.

The main advantage of using transgenic animals is that it overcomes viral payload limitations and allows the use of larger promoters to confer greater specificity. Additionally, there is uniformity in the level of expression in transgenic animals. Currently, two lines have been successfully produced under the Thy-1 promoter, one expressing ChR2 [30] and one expressing NpHR [31].

However, the time, effort and cost associated with producing and maintaining these transgenic lines make it a less popular choice. Furthermore, the levels of expression of the transgene are not as high as those obtained by viral methods.

Cre-dependent expression system

The Cre-dependent expression system is a combination of both viral and transgenic methods by producing a transgenic line that expresses Cre-recombinase in the target cells. This involves using a Cre-dependent virus that has been designed to deliver opsins when activated by Cre in specific cells. This virus is designed to carry a doubly floxed inverted opsin fusion gene [32]. When transduced into cells expressing Cre-recombinase, the fusion gene is irreversibly inverted to allow for a cell-specific gene expression of the opsin [32]. Gene expression is dependent on the co-expression of Cre-recombinase. Hence, although cells may be infected with the virus, without Cre-recombinase, expression of the opsin gene will not occur. This approach has been applied in numerous systems and has opened new doors to uncovering relations between specific neuronal populations and animal behavior, which is crucial in understanding clinical conditions such as schizophrenia and depression [6,33].

10.5.2 On a macro-level – in vivo applications: a regenerative approach

In terms of using optogenetics as a powerful biomedical tool, a plethora of *in vivo* applications has emerged in recent years. It mainly revolves around the burgeoning realm of regenerative medicine to control or restore function.

Controlling cardiac and skeletal muscles

It has been recently shown that, as an alternative to electrical stimulation, cardiac muscles could be controlled using light [34]. Classical electrophysiology, with particular reference to probing the mechanisms

of cardiomyocytes, involves the use of electrical stimulation. However, this method could result in the production of toxic gases such as chlorine, oxygen, and hydrogen, which leads to irreversible Faradaic reactions. Furthermore, electrical stimulation can only be used to evoke short depolarizations, and not long-lasting ones. Electrical stimulations would also result in areas of depolarization and hyperpolarization that are not homogeneous. Bruegmann et al. produced a transgenic murine embryonic stem cell line that expressed ChR2 (H134R), in fusion with enhanced yellow fluorescent protein from chicken β-actin promoter (*CAG*) [35]. Electrocardiogram (ECG) readings were then taken from these transgenic mice when their beating hearts were exposed to blue light (475 nm). Characteristic P waves and QRS complexes could be detected on ECG, indicating that light stimulation could evoke depolarization and subsequent action potentials in an *in vivo* murine model. In a similar line, another group also managed to optically control pacemaker cells in zebrafish cardiomyocytes [36]. In this case, NpHRs and ChR2 were genetically encoded in zebrafish cardiomyocytes, and initiation of heartbeat was observed upon light stimulation. This proof-of-principle has far reaching effects in terms of using optogenetics as a powerful tool in cardiology. For example, it is perhaps possible for the next-generation pacemakers to incorporate photostimulation technology as an alternative to electrostimulation [37]. Indeed, there are several drawbacks for the current use of electrostimulation in pacemakers, including mechanical failure of pacing leads as they are in constant contact with contracting heart muscle. Hence, using photostimulation might prove to be a less invasive and durable avenue for pacemakers.

Restoring breathing in a paralysis model

Spinal cord injury at the cervical level can result in paralysis and respiratory insufficiency. Difficulties in breathing can occur due to the interruption of descending presynaptic inputs to respiratory motor neurons. Expression of ChR2 would allow neuronal excitability and evoking action potentials, without the need for presynaptic input. Given this fact, it has been postulated that the artificial expression of ChR2 within the phrenic motor neurons would reinstate respiratory function *in vivo*. Indeed, Alilain et al. showed that photostimulation of transgenic rats expressing ChR2 with cervical spinal cord injury was able to restore respiratory function and persisted even after cessation of photostimulation [38]. This method could pave the way for the eventual development of a minimally invasive technique of restoring breathing after spinal cord injury.

Ameliorating Parkinsonian motor deficits

In a landmark study, Kravitz et al. demonstrated that Parkinsonian motor behavior could be optogenetically controlled in the basal ganglia [39]. The study illustrated that, in a mouse model of Parkinson's disease, direct pathway activation could be elicited via striatal illumination, which ameliorated Parkinsonian motor deficits. Another study further underscored the salutary effects of applying optogentics in a Parkinsonian model *in vitro*. In this case, Tonnesen et al. used optogenetics, together with patch-clamp recordings, to assess graft-to-host synaptic connectivity of stem cell-derived dopamine neurons in a Parkinsonian model [40].

Controlling neural circuitry

An interesting study by Aravanis et al. demonstrated that an optical neural interface implanted in the brain of a mouse model could evoke whisker deflection upon photostimulation [41].

Visual regeneration

The genetic heterogeneity in retinal disorders such as retinitis pigmentosa and age-related macular degeneration has made them a challenge to treat. Optogenetics however, could be the panacea to these retinal disorders because it is an intervention that is independent of the etiology of visual degeneration [42].

By expressing ChR2 on either ON bipolar cells or retinal ganglion cells via a plasmid vector, adeno-associated virus vector, or using transgenic animal lines, the visual pathway can be activated upon blue light illumination [43–46]. This strategy has been successful in restoring vision in multiple mouse models with photoreceptor degeneration.

10.6 Challenges

Although there have been tremendous improvements in optogenetics, this field is still in its infancy but holds great potential. There are a few key areas that need further development – light delivery techniques and molecular methods for transgenic and viral expression. There is a constant need to expand the optogenetic toolbox to create techniques that can target subcellular compartments such as axons; produce possibilities for two-photon activation, and optimize current methods by

improving their conductance, ion selectivity, and kinetics, without unduly compromising light sensitivity [4,6].

10.7 Conclusion

The development of optogenetic technology represents a collaborative success between multidisciplinary fields, including gene delivery, optical technology, and clinical neuroscience. As a key emerging technology showing exponential acceleration in growth, optogenetics is a dream translated into reality with the potential to shed light on the secrets of the brain. Nevertheless, there is still room for this technology to mature and grow, and light can be seen at the end of the tunnel for optogenetics.

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