A Review on Optogenetics and its Potential in Neurological Disorder Management

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis titled "A Review on Optogenetics and its Potential in Neurological Disorder Management" submitted by Fariza Armin, ID: 18346052 of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on October, 2022.

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Ethics Statement

This study does not involve any human or animal trials.

Abstract

Neuroscientists have a remarkable capacity to influence neurons because of the latest research technology known as optogenetics. Optogenetics offers significant possibilities as a treatment option for neurological disorders as well as has been acknowledged for a wide range of applications including in cancer treatment, management of cardiovascular disease, treatment of diabetes, gene editing, and regulation of epigenomes. Optogenetics has made significant progress in recent years, supplying us with opsins for possible uses in the management of the neurological disorder. In this technique, the ion channels which are sensitive to light are expressed in neurons, allowing them to be regulated particularly by light. This paper aims to provide an overview of optogenetics, its mechanism, applications, and its advances in the management of neurological disorders.

Keywords: Optogenetics; Neurological disorders; Parkinson; Alzheimer; Epilepsy; Schizophrenia.

Dedication

This project work is dedicated to my respectable supervisor Tanisha Tabassum Sayka Khan ma’am and my parents.

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List of Acronyms

ChR	Channelrhodopsin
NpHR	Halorhodopsin
Arch	Archaerhodopsin
TBI	Traumatic brain injury
NSCs	Neural stem cells
C1V1	Chimeric ChR composed of CrChR1 and VcChR1
BLINK1	Blue-light induced K+ channel 1
VSFP	Voltage-sensitive fluorescent protein
LED	Light-emitting diode
ChETAH	Characterization of cell types aided by hierarchical classification
VTA	Ventral tegmental area
TH	Tyrosine hydroxylase
ACT	Adoptive cell transfer
CXCR-4	Chemokine receptor type 4
NIR	Near-infrared
IECDs	Implantable electronic cardiovascular devices
AAV	Adeno-associated virus
Gαq	G protein alpha q subunit

PLC	Phospholipase C
PKC	Phosphokinase C
TRPC	Transient receptor potential canonical
hESCs	Human embryonic stem cells
IP_3	Inositol trisphosphate
T1D	Insulin-deficient type 1 diabetics
GLP1	Glucagon-like peptide-1
HEK	Human Embryonic Kidney
NFATs	Nuclear factor of activated T-cells
LOV	Light oxygen voltage
VVD	Vivid
UASG	Upstream activating sequences for galactose
LoxP	locus of X-over P1
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CIB1	Calcium and integrin-binding protein 1
CRY2	Cryptochrome-2
Cre	Cyclization recombinase
nMag	Negative magnet
pMag	Positive magnet

PA	Photoactivatable
SOD	Superoxide dismutase
EPS	Extrapyramidal symptoms
AD	Alzheimer’s disease
ChEIs	Cholinesterase inhibitors
PD	Parkinson's disease
COMT	Catechol-O-methyl-transferase
DA	Dopamine agonists
SCZ	Schizophrenia
HPC	Hippocampus
CA1	Cornu ammonis 1
PNs	Peripheral nervous system
PCP	Phenylcyclohexyl piperidine
ENS	Enteric nervous system
mPEC	Medial prefrontal cortex
STN	Subthalamic nucleus
DBS	Deep brain stimulation

Chapter 1

Introduction

1.1 Background

Optogenetics is a method that uses light to monitor or control the activity of the neurons. It does this by genetically introducing proteins that are sensitive to light into the neuronal cells. Neurons are regulated by optogenetic activators such as ChR (Channelrhodopsin), NpHR (Halorhodopsin), and Arch (Archaerhodopsin). In this technique, light is the effector, which has some benefit of operating with great temporal and spatial precision at different locations and wavelengths (Duebel et al., 2015).

Neurological conditions like Parkinson's disease and Alzheimer's disease, as well as acute brain injury from stroke and TBI (Traumatic brain injury), is followed by neuron or assisting glial cell loss, which has serious consequences on the patient's standard of living. Human brain's endogenous regeneration capacity is much lower compared to many animal models. Therefore, cell or tissue replacement procedures are being thoroughly examined as a therapeutic option to compensate for injured brain tissue (Habibey et al., 2020).

Optogenetic methods now make it possible to efficiently enhance neuronal function. It is a minimally invasive method as the gene delivery into the cells is non-invasive (intravenously), however, require implantation of optical fibers for light delivery. Optogenetics refers to the use of light stimulation to regulate the actions of cells, such as activity in the brain, in genetically engineered cells expressing actuators with optical sensitivities. At millisecond timescales, optogenetic technique provides high temporal accuracy, making it an excellent method for studying brain circuit activity in vivo or in vitro. Viral tools or can be used to convey and express the genetic data of optogenetic instruments in a "cell-type-specific" way. Optogenetic

actuators are expressed in neural stem cells (NSCs) and transplanted as a consequence. Therefore, it is possible to identify brain stem cells that express optogenetic actuators following transplanting and excite certain neurons selectively using implanted or transcranial optical light (Habibey et al., 2020).

1.2 Objectives

The primary goal of this study is to -

- Provide an overview of the concept of optogenetics.
- Summarize the role of optogenetics in the management of neurological disorders.

1.3 Rationale

Studying optogenetics is essential in identifying how and when neurons communicate with one another. Moreover, we can explore the mechanism by which neuron functions via optogenetics by turning certain neurons on and recording the reaction of the other neurons. Also, by using optogenetics, we can understand what happens when the brain is damaged in a specific location. Furthermore, we can comprehend the prospects of optogenetics in controlling neurons and treating neurological disorders. However, there are some limitations of optogenetics which will be also addressed in this study.

Chapter 2

Methodology

This study reviews the innovative optogenetic technology and its role in the management of neurological disorders. All the data and information for this comprehensive study were gathered from authentic primary and secondary research articles indexed in Pubmed, Scopus, Science Direct, and Springer, as well as from renowned websites. Firstly, an outline was constructed in order to carry out the review in a systematic manner. The articles for this review were searched by using keywords like optogenetics, neurological disorders, Parkinson, Alzheimer, Epilepsy, and Schizophrenia. After reviewing a significant number of research articles, around 50 relevant articles were chosen to collect information for the study.

Chapter 3

Optogenetics

3.1 Development of Optogenetics

The discovery of bacteriorhodopsin (which is a rhodopsin-like protein and it is from the purple membrane of *Halobacterium halobium* that in the presence of light pumps protons), by Stoeckenius and Oesterhelt in 1971 was among the early stages in the evolution of optogenetic system. Later, in the years 1984 and 2002, halorhodopsin (NpHR) and channelrhodopsin (ChR) were considered to be members of the opsin family. Other initial strategies were established and used (in New York city, at Sloan-Kettering Cancer Center) by the teams of Gero Miesenböck and Boris Zemelman, as well as at the University of California (in Berkely) by Dirk Trauner, Ehud Isacoff, and Richard Kramer. A crucial discovery was indeed the observation that neurons responded to light once a “microbial opsin gene” was inserted alone (Duebel et al., 2015).

3.2 Mechanism of Optogenetics

When nerve cells were made to produce opsins in order to enable light-regulated neural functions in cell cultures, the concept "optogenetics" was invented. Originally, the term was used as a collective noun to describe strategies that combined "genetic targeting of particular neurons or proteins with optical technology enabling imaging or regulation of the targets within intact, live neural circuits". Since optogenetic actuators have proven so successful, some writers have recommended a more limited application of optogenetics, concentrating on "the combination of genetic and optical approaches to accomplish gain or loss of function of well-defined events in specific cells of living tissue" (Song & Knöpfel, 2016).

Using microbial opsins, light-sensitive proteins produced by a variety of microbes, it is possible to influence neuronal firing by optogenetic means. Channelrhodopsin-2 (ChR2) and halorhodopsin (NpHR) are two of the opsins that are most frequently employed in optogenetic studies. Channelrhodopsin-2, produced by the green alga *Chlamydomonas reinhardtii*, develops a cation channel which opens in response to blue light (Figure 2). As shown in the figure, a large influx of positively charged sodium (Na+) ions takes place when a ChR2 channel in a nerve cell membrane is activated, (however, because the channel is nonselective, it can also pass hydrogen, calcium, and ions). ChR2 activation rapidly depolarizes the cell membrane and has a significant excitatory effect on the cell, mainly due to this inward Na+ influx. NpHR, on the other hand, is a pump produced by the bacteria *Natromonas pharaonis* that, when triggered by yellow light, moves chloride (Cl-) ions across the cell membrane (Figure 2). As would be expected, the migration of these negatively charged ions results in a significant degree of membrane hyperpolarization and, as a result, an inhibition of cell firing (Meyer et al., 2013).

Actuators and indicators are two categories of optogenetic instruments (they are proteins or the cDNA that codes for those proteins) that has been developed for controlling and observing neural circuits. The “actuators” are proteins that convert light into neural signals to be controlled, and the “indicators” are the proteins which convert the neuronal signals to optical signals to be observed (Figure 1). The prototypical actuators include- NpHR (halorhodopsin), that produces an “inhibitory current” after being triggered by yellow light, and ChR2 (channelrhodopsin 2), that produces an “excitatory cation current” after being triggered by blue light. Although ChR2 (channelrhodopsin 2) and NpHR (halorhodopsin) are still frequently applied, more recently invented optogenetic actuators including Arch, Jaws, Chronos, C1V1, BLINK1, and anion channel rhodopsin (ACR) will likely take their place in the future. Genetically encoded voltage and calcium indicators can be found in the indicator segment of the optogenetic toolkit (Figure 1) (Song & Knöpfel, 2016).

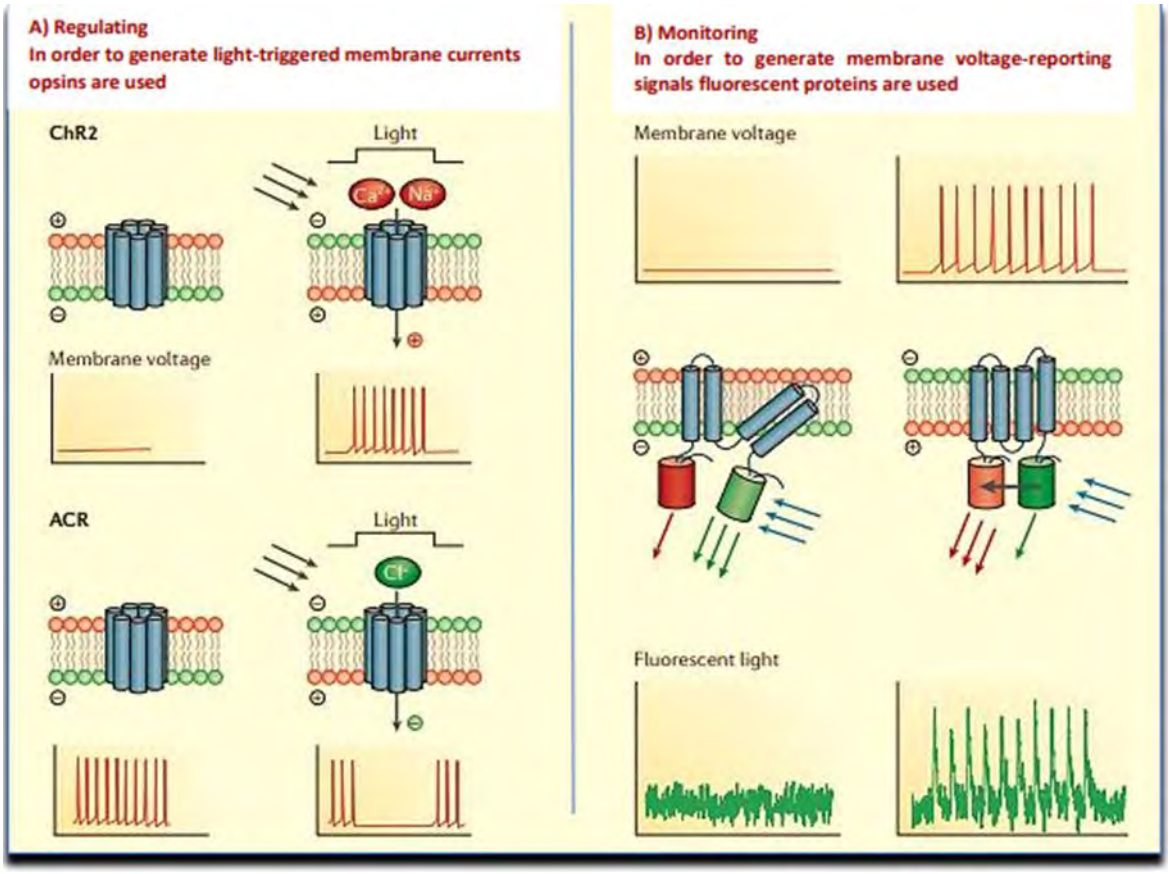

Figure 1: The reporter proteins and actuator that can be targeted are genetically encoded and allow the use of light to regulate or report the electrical activity of nerve cells. A) In order to produce "membrane currents" that are activated by light opsins can be utilized. Light-gated ion channels which either stimulate (ChR2) or inhibit (ACR) neuronal activity are examples of optical control. Membrane potential actuators with a wide range of structural and functional variations have been created and effectively applied to optogenetically regulate neuronal activity. Action potential generation is induced (ChR2) or inhibited (ACR) by light (black oblique arrows). B) To produce membrane voltage-reporting signals, fluorescent proteins can be utilized. Calcium and voltage markers that are genetically encoded can be used to optically read out neural activities. A chimeric butterfly made of the voltage-sensitive fluorescent protein (VSFP) is illustrated. Because calcium indicators, despite being widely and successfully employed, only partially and indirectly report neural activities, a voltage indicator is chosen as an example for simplicity. Numerous genetically encoded markers of neural activity have been created, each with a different structural and functional makeup. Diagrammatically, the red highlighted one's are the "membrane potential traces". When the voltage-sensitive fluorescent protein is illuminated by blue light (illustrated in blue angled arrow), two different colors of fluorescent light are emitted (illustrated in red and green oblique arrows) which indicate action potential firing (green trace) (adapted from Song & Knöpfel, 2016).

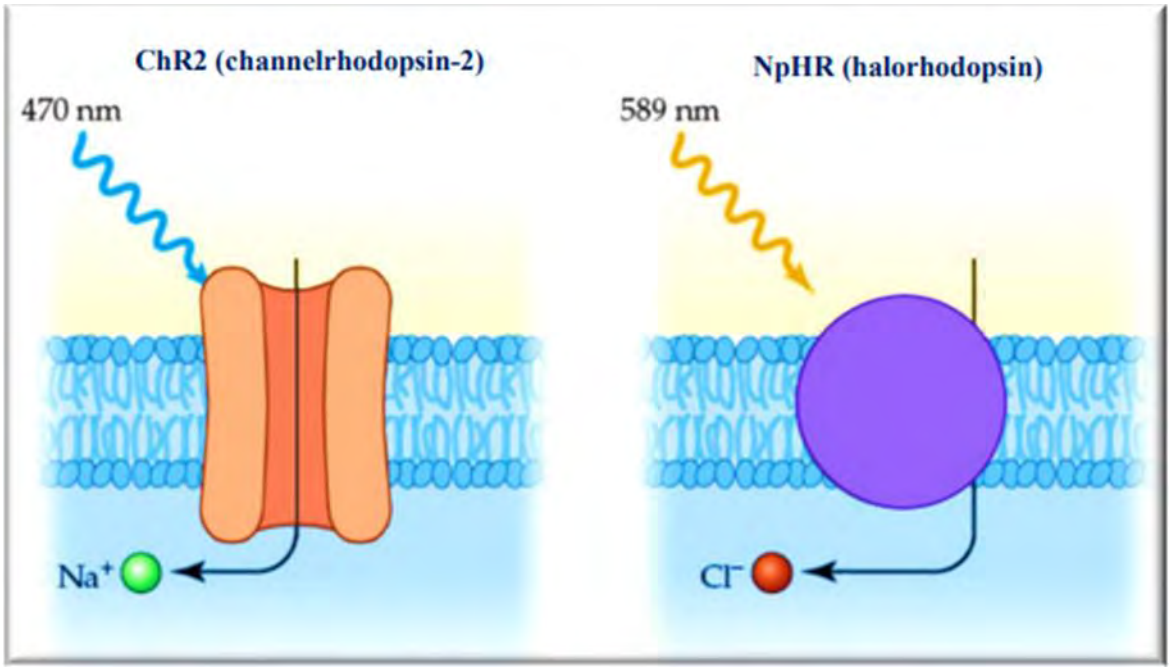

Figure 2: Optogenetic techniques utilizing the light-sensitive proteins ChR2 and NpHR to regulate neuronal activity. When neurons which express channelrhodopsin2 are illuminated by blue-colored light (wavelength- 470 nm), enables the entry of Na^+ ions into the cell resulting in depolarization. When nerve cells which express NpHR are exposed to yellow-colored light (wavelength- 589 nm), and the Cl^- ions are released into the cell, causing hyperpolarization (adapted from Vlasov et al., 2018).

Figure 2 shows that in the optogenetic technique, opsins that stimulate like ChR2 are generally utilized in order to depolarize nerve cells, in contrast, opsins which are inhibitory like NpHR and Jaws can be utilized in order to mute nerve cells. A fiber-optic device must be placed over the selected area in the brain for illuminating opsin-containing neurons. The implantation is normally carried out at a single surgical procedure right after the viral injection, though it may also happen during a different procedure many weeks later when transfection and protein expression have already occurred. To allow for enough recuperation and healing before experimentation, it is advised that patients recuperate after neurosurgery for at least one week (Vlasov et al., 2018).

Light-emitting diode (LED) or a laser as a light origin and a technique for manipulating the light pulse time (such as a stimulus generator) are required in optogenetics investigations in

order to accurately time the inhibition or activation of nerve cells. It is important to make sure that the right wavelength is utilized to modify the chosen opsin because each opsin is stimulated by certain light wavelengths. In order to link the implanted fiber optic to the light source, a patch cord is also required. An alternative is to utilize a fiber optic with an LED put on it (Vlasov et al., 2018).

For the light pulses, three primary considerations should be given which are duration (5-15 ms), intensity (1-10 mW), and frequency (1-50 Hz). As preliminary setups, it might be useful to take into account the typical firing rates of firing and the neuronal patterns that have been aimed since it is frequently desirable for neural activation to match "normal" neurophysiology. The most widely utilized opsin, ChR2, reliably triggers neuronal firing at a frequency of 20–40 Hz. New ChR2 mutants have been introduced, like ChETAH (Characterization of cell types aided by hierarchical classification), that enable nerve cells to fire at a frequency of up to 200 Hz for faster firing rates. A 473 mm blue-colored light laser having a light pulse of about 5 ms at a frequency of 5 Hz and 5mW of light intensity of 5 mW is a common set of parameters for ChR2 (Vlasov et al., 2018).

There are a number of inhibitory opsins for neural inhibition, including NpHR (halorhodopsin), which is a pump for chloride and it causes hyperpolarization of nerve cells in response to green or yellow colored light. Since red light has a greater ability compared to blue light to enter the tissue of the brain, Jaws (cruxhalorhodopsin) with a red shift which has higher photocurrents compared to NpHR makes it possible for noninvasive photoinhibition (Vlasov et al., 2018).

In order to inhibit or activate each of the neurons which are targeted in a particular area of the brain (such as the dopaminergic neurons in the ventral tegmental area), cell bodies may be lit. However, lighting of the terminal area is indeed applicable for examining the results of altering a neural circuit which extends to a particular area of the brain. For instance, VTA (ventral

tegmental area) dopamine neurons containing tyrosine hydroxylase will produce channelrhodopsin-2 in both the axons that extend to different parts of the brain as well as the cell bodies found inside the ventral tegmental area when a channelrhodopsin-2 is encoded via a viral structure is injected into the ventral tegmental area of TH (tyrosine hydroxylase)- Cre mice. It is possible to selectively activate dopamine neurons that project from the ventral tegmental area to a particular area of brain by illumination of the light on the terminals in the selected area instead of the cell bodies in the ventral tegmental area. This method was recently used to show that dopamine neurons projecting from the ventral tegmental area to the nucleus accumbens are stimulated by light to induce alertness (Vlasov et al., 2018).

3.3 Applications of Optogenetics

3.3.1 Optogenetic Tools for Cancer Therapy

Immunotherapy has gained popularity as a powerful cancer treatment in recent years. In patient with metastatic cancer, adoptive cell transfer (ACT) immunotherapy has the potential to mediate tumor regression (Ye & Fussenegger, 2019). These therapies, however, have limitations due to the inadequate transport of transplanted cells to desired tissue locations ((Kalos & June, 2013; Vivier et al., 2012; Restifo et al., 2012). Optogenetic methods have been created to get around this restriction and allow immune cells to identify and destroy tumor-specific antigens.

One such is “an optical oncotherapy system" that couples the CXCR-4 (chemokine receptor-4) subunit and rhodopsin subunit that when illuminated with light at a wavelength of 505 nm, can cause intracellular chemokine signals and T-cell movement towards a selected tumor. This technique enables the transmission of cytotoxic T lymphocytes (CD8+ T cells) in an adoptive way to locate tumors in the body and target them, which inhibits tumor growth in response to light stimulation. Through the optical modulation of T cell trafficking to specific target tissues,

this type of chemokine receptor technique that is photoactivatable can initiate new possibilities for cancer immunotherapy. This is done by employing light of a particular wavelength (Figure 3) (Xu et al. 2014).

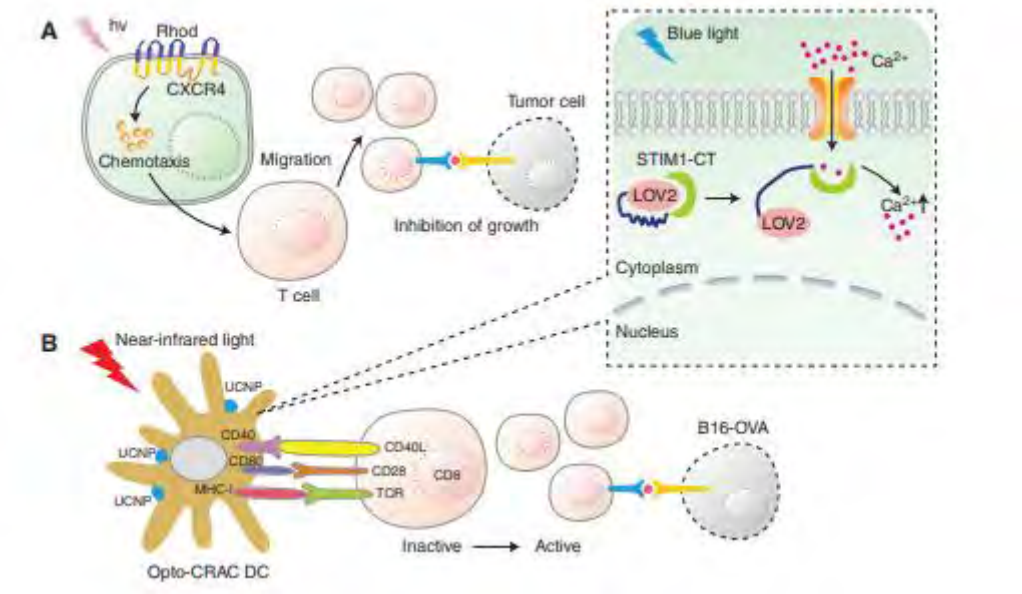

Figure 3: Oncotherapy using synthetic optogenetic devices. A) Controlling a chemokine receptor with light for the treatment of murine melanoma. The chimeric photoactivatable chemokine receptor (CXCR4), made up of the chemokine receptor-4 and rhodopsin α subunit, is activated under 505-nm light illumination to draw transmitted cells to the selected tumor, increasing regional effector actions and inhibiting the development of tumor. B) Opto-CRAC is a "NIR (near-infrared)-stimulatable" optogenetic system which is constructed in order to utilize in cell-based immunotherapy (Ye & Fussenegger, 2019).

3.3.2 Optogenetic System for the Management of Cardiovascular Diseases

Neurobiologists are the ones who initially used this technology to modulate the activity of neurons, being influenced by the neurobiologists' "cardiac optogenetics" evolved succeeding thirty years of study utilizing the optical instruments for tracking cardiac arrhythmias, originating with "optical mapping in the 1970s". The potential of optical mapping has been adopted by cardiologists to test hypotheses concerning arrhythmia progress and regression, and also in order to notify the advancement of the latest therapeutic approaches and innovations. In

addition to overcoming the commonly abrasive characteristics of conventional optical mapping investigations using “small-molecule dyes”, the genetic component of such optical analyses enabled “cell-specific probing in the heart” (Entcheva & Kay, 2021).

Throughout the world, the principal reason of death and morbidity as well as disability is cardiovascular disease (Covas et al., 2009). Despite advances in pharmacology and surgical techniques that may decrease the rate of deaths, numerous individuals have a significant possibility of additional cardiovascular incidents, such as myocardial ischemia and death. Therefore, there is still a dire need for a novel therapeutic strategy to treat myocardial damage. A novel biological system developed by Joseph Woo's team at Stanford University used the cyanobacterium “*Synechococcus elongatus”* photosynthesis to protect the myocardium from acute ischemia. An acute myocardial infarction mouse model was given a direct injection of the cyanobacterium *S. elongatus* into the ischemic region. After being exposed to light, *S. elongatus* created oxygen by photosynthesis, which improved heart function, metabolic activity, and tissue oxygenation. Additionally, this photosynthetic system does not cause any harmful side effects or major pathogenic immunological reactions. This approach, which uses photosynthetic bacteria to deliver vital oxygen to the patient who has myocardial ischemia, and it is a viable approach with excellent possibilities for treating microvascular disease and ischemic disease (Figure 4A) (Ye & Fussenegger, 2019).

Moreover, several cardiac diseases are linked to a reduction of excitation of tissues, which causes problems in the rhythm of the heart. IECDs (termed as “Implantable electronic cardiovascular devices”) such as defibrillators, cardioverters, and pacemakers are frequently applied to treat those cardiac disorders. Furthermore, biological approaches to restoring pacemaking capabilities by providing excitatory ion channels have recently been developed. An optogenetic method provides a tool for selecting and interrogating the cells that are transduced in order to generate an ion which is excitatory. Channelrhodopsin-2, which is a non-

specific cation channel triggered by light, was initially utilized in order to excite the muscle of the heart in vivo as well as in vitro in 2010. With the help of this technique, the illuminated area could be precisely stimulated, and the cardiomyocytes could be depolarized over an extended period of time, results in changes to the heart's pacemaking and causing arrhythmogenic spontaneous additional beats. In 2015, Nussinovitch and Gepstein stated that it is also possible to regulate cardiac excitability in vivo using an optogenetic approach. In this analysis, an adeno-associated virus (AAV) 9 vector containing a ChR2 transgene was injected into one or more myocardial regions. For the purpose of cardiac pacing, blue light flashes were delivered to the location of the ChR2 transgene using a monochromatic LED with optical fiber coupling (wavelength of 450 nm). Dual- and multi-site blue-light-enabled optogenetic pacing synchronized the activation of ventricle and reduced times which is required for the activation of ventricle. The special significance of optogenetic technique for cardiovascular pacing and cardiac resynchronization therapy is highlighted by these findings (Figure 4) (Ye & Fussenegger, 2019).

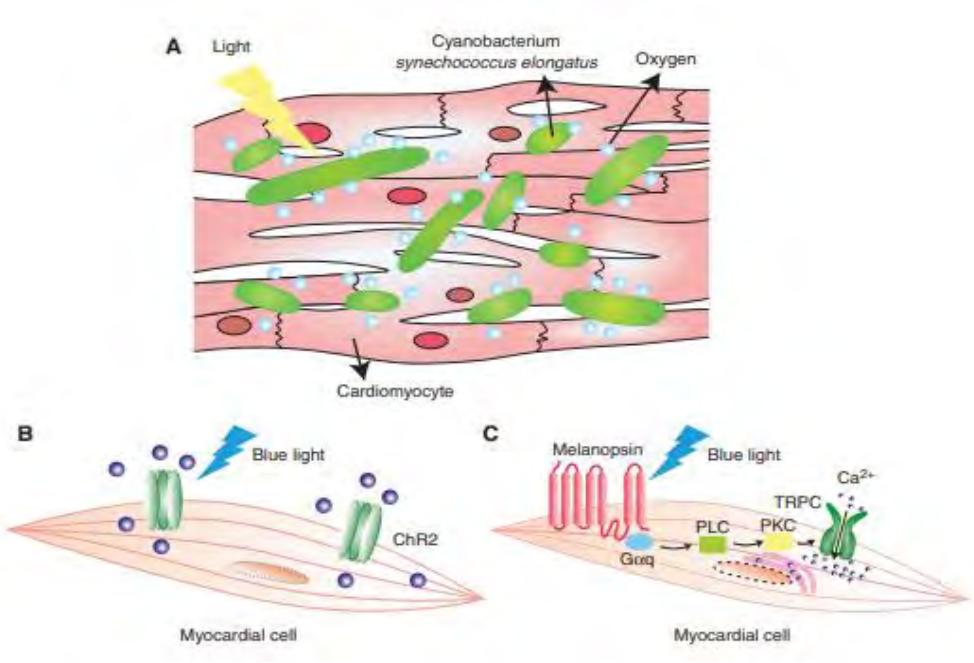

Figure 4: Illustration of optogenetic devices used in the treatment of cardiovascular disorders. A) Cyanobacterium photosynthetic method to treat myocardial ischemia. Rats used as a model for acute myocardial infarction get direct injections of the cyanobacterium *Synechococcus elongatus* into their hearts. The cyanobacterium *S. elongatus* may produce oxygen during photosynthesis under light illumination, which boosts the process of metabolism and enhances the function of the ventricle while reducing severe ischemia of the tissues. B) "Optogenetic pacemaker" for possible cardiovascular pacing and cardiac resynchronization treatments. Here, the nonselective cationic channel ChR2 and other light-sensitive proteins are used by the optogenetic pacemaker to regulate cardiac activity. AAV (termed as Adeno-associated virus) 9 is used for introducing the channelrhodopsin-2 transgene into rats' ventricular locations. The optical fibre is then placed in the selected regions, optogenetically pacing the treated heart while it is illuminated with light which is blue-colored. C) "Optogenetic pacemaker" to control the functions of cardiomyocytes. Blue light (470 nm) stimulates the "GQ-protein-coupled receptor" melanopsin that is triggered by light, which then uses the G-protein-signaling route to speed up heartbeat. Here, PLC stands for phospholipase C; PKC for phosphokinase C (Ye & Fussenegger, 2019).

Utilizing a mixed experimental/computational approach is another illustration of optogenetic control of the heart system. By using video microscopy, the patch clamp technique, and MEA (multi-electrode array) documentation, it was determined that hESCs (human embryonic stem cells) that are stably transgenic and express channelrhodopsin-2 were capable of differentiating

into cardiomyocytes. The channel opens in response to visual stimulation, allowing the entry of Na^+ (sodium ions) into the cell and as a result, an action potential developed. To replicate this effect, the development of a computerized simulation model of the lightly beating heart was developed. By predicting the activation patterns in distinct human heart pacing sites using a computational model, we might potentially treat a variety of cardiac illnesses linked to schizophrenia, melancholy, pain disorders, and irregular heartbeat. Also, in 2014 an optogenetic system was created that uses only the channelrhodopsin-2 which is triggered by light or in conjunction with the Arch-T (archaerhodopsin-T), which is a proton pump that is sensitive to light and hyperpolarizing. This method allowed for the pacing of heart tissue through optogenetic means, coordination of electrical activity in heart-tissue, reduction of the time which is required for electrical activation, and suppression of electrical activity in the cultures when exposed to continuous monochromatic redlight. This proof-of-concept study suggests that optogenetics is appropriate for applications in pacemaking, resynchronization therapy, and the creation of fresh antiarrhythmic approaches (Ye & Fussenegger, 2019).

In addition, cardiac pacing and arrhythmia treatments employed melanopsin, which is a G protein-coupled receptor and is also triggered by light. In 2014, an up-to-date optogenetic device was created on the basis of melanopsin in order to study, both in vitro and in vivo, the impact of the "Gq-signaling cascade" on cardiomyocyte pacemaking. Melanopsin photostimulation caused PLC (termed as phospholipase C) functioning and inositol trisphosphate (IP_3) production when illuminated with blue-colored light (at a wavelength of 470nm), which caused Ca2+ release and an increase in impromptu pacemaking action (Figure 4C). Those results also demonstrated the possible benefit of optogenetic technique in the treatment of cardiac pacing and arrhythmias (Ye & Fussenegger, 2019).

Therefore, it can be said that,

- Cardiac optogenetics uses optical sensors and actuators that are genetically encoded to support fundamental and clinical studies, as shown by the tremendous increase in publications that have been published in the last ten years.
- Heart-safe opsin transport, cell type-specific appearance, and using light to engage the opsins deeply inside the tissues are the key barriers to bringing cardiac optogenetics to the clinic (Entcheva & Kay, 2021).

3.3.3 Diabetes Therapy with Optogenetic Devices

With reference to pancreatic-cell function in homeostasis and pathological states like diabetes mellitus, optogenetics approaches can help us understand how insulin secretion is regulated (Kushibiki et al., 2015).

At least 415 million individuals worldwide suffer from the complex and progressive condition known as diabetes mellitus, which is defined by persistently elevated blood glucose levels. For insulin-deficient type 1 diabetics (T1D) or insulin-resistant type 2 diabetics (T2D), rigorous dietary restrictions and long-term doses of GLP-1 (glucagon-like peptide-1) analogs at daily to weekly intervals are usually used to regulate blood glucose homeostasis in diabetic patients. These tactics need high expense with minimal patient convenience (Ye & Fussenegger, 2019).

Moreover, the endocrine pancreas can secrete hormones, particularly insulin, when the vagus nerve is electrically stimulated. However, blood glucose reductions are not typically reached, a study revealed. Electrical stimulation that is relatively indiscriminately applied may activate the motor and sensory axons that innervate different organs as well as unintended pathways. Scientists used an optogenetic method in the current work that allowed them to optically stimulate exclusively cholinergic parasympathetic (and thus largely efferent) axons. Using this

optogenetic method, insulin secretion increased in response to stimulation of the cervical vagus nerve or the pancreas. Notably, blood glucose was promptly lowered during pancreatic optical stimulation under both basal glucose and glucose-elevated circumstances, in contrast to electrical stimulation approaches. Under pancreatic optical stimulation, blood flow was also enhanced in the pancreatic vasculature (Fontaine et al., 2021).

There are now two effective optogenetics-based therapy approaches for the management of diabetes. The first is a clever strategy developed by Ye et al. (2011) who created an optogenetic device that is controlled by blue light to maintain blood glucose homeostasis. Melanopsin, which is a "GQ coupled receptor" that responds to blue-colored light, was erratically produced in the human embryonic kidney (HEK) 293 cell. While activated by light which is blue in color, it phosphorylates the NFATs (nuclear factor of activated T-cells), activating it through a signaling cascade within the cell. To activate the expression of GLP-1, rewiring was done on this signal cascade to an artificial nuclear factor of activated T-cell-regulated activator. After being microencapsulated, these optogenetically modified cells were transplanted into a type 2 diabetic rat model. The blood glucose homeostasis of the mice who were exposed to blue light was enhanced (Figure 5A) (Ye & Fussenegger, 2019).

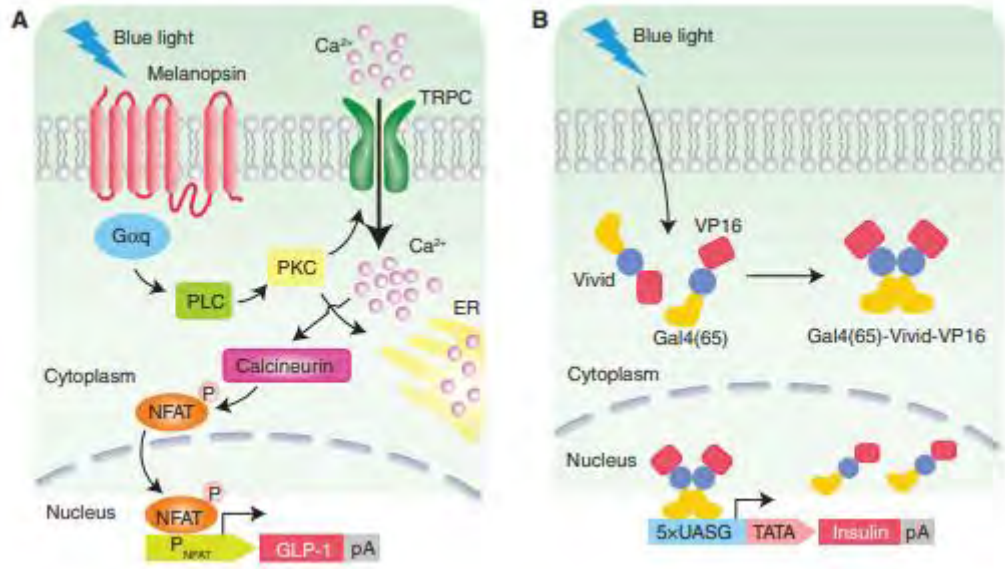

Figure 5: Artificial optogenetic instruments for the management of diabetes. A) Diabetic treatment using blue-light-regulated optogenetic designer cells. PKC (phosphokinase-C) and PLC (phospholipase-C) are activated by blue-light exposure to start the downstream signaling cascade, that as a result via TRPCs (transient receptor potential channels), promotes calcium (Ca^{2+}) influx. Here, melanopsin is a G-q-type G-protein-coupled receptor (GPCRs). A NFAT-responsive promoter induces transgene expression by activating the transcription factor NFAT (nuclear factor of activated T cells) in response to an increase in intracellular Ca2+ (P_{NFAT}). B) For the treatment of diabetes, a LOV (light oxygen voltage) domain-based blue light activated transcription method is used. The fusion protein Gal4 (65)-VVD-VP16 dimerizes when triggered by blue light, attaching to the UASG (upstream activating sequences for galactose) sequence and triggering the transcription of the selected gene. Vivid (VVD) undergoes this structural change. Here, ER stands for Endoplasmic Reticulum (Ye & Fussenegger, 2019).

Later, research supported the feasibility of using light-controlled insulin expression to treat diabetes. Wang created a different variation of the optogenetic instrument (a technique known as LightOn) according to the LOV domain vivid, that upon blue-light activation forms a quickly exchanging dimer. He put the LightOn system to the test on rats having diabetes and demonstrate that blue-colored light may stimulate the production of insulin in animals, improving homeostasis of blood glucose (Figure 5B). Compared to conventional approaches, both optogenetic technologies offer novel options for the treatment of diabetes. A beam of light

may one day be all that diabetes people need to control their blood sugar levels (Ye & Fussenegger, 2019).

3.3.4 Optogenetic Tools for Editing Genes and Regulation of Epigenomes

In order to alter the transgenes in the chromosomes of certain cells, genome engineering typically makes use of the potent tools known as site-specific Deoxyribonucleic acid (DNA) recombination systems. The most popular site-specific recombinase is called Cre; it facilitates DNA recombination which is directed among two loxP (locus of X-over P1) regions as well as allows for location-specific DNA alterations like the specific gene inversion, addition, deletion, or swap via various loxP areas, opening up a range of options for genome-editing under certain conditions. Cre-LoxP recombination technologies that are chemically inducible were created in order to establish time-based and conditional regulation of "genome engineering" in live organisms. These methods, however, fall short of the requirements for great spatiotemporal resolution. Additionally, due to unintended consequences, the inducers may have adverse impacts on alive things and the adverse impact includes cytotoxicity (being toxic to cells) and disruption of communication within the cells (Ye & Fussenegger, 2019).

Through allosteric regulation that is light-exchangeable, oligomeric transformation, protein-protein heterodimerization, and self-cleavage, optogenetic modules may be built modularly engineered into host cells to regulate biological activities. In addition to the transcriptional apparatus, the RNA-linking proteins, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based genome-editing instruments and DNA recombinases, optogenetic tools may be used to precisely regulate genetic recombination, DNA or RNA (ribonucleic acid) alterations, genetic recombination, epigenome or genome engineering, and transcriptional modification. Optogenetics can be used in conjunction with genome-editing techniques,

biophotonics and synthetic biology to enhance individualized treatments for human diseases and precision medicine (Lan et al., 2022).

With great spatiotemporal accuracy, optogenetics blends biophotonics and genetics in order to provide non-invasive control of physiological mechanisms. Scientists present a set of modular strategies for optogenetic engineering that are broadly applicable. They also highlight recent developments in the widespread use of optogenetic technology without opsin-free to accurately modify the genome of mammalians and program transcriptional outputs. Scientists also discussed about this optogenetic technology without the use of opsin, that is quickly developing to satisfy the expanding demands in genetics studies and synthetic biology, as well as its present problems and potential future directions (Lan et al., 2022).

Recently, two Cre (cyclization recombinase)-loxP systems that are responsive to blue light were created. According to research, A. *thaliana* cryptochrome 2 and CIB1 (Calcium and Integrin Binding 1)-based protein interaction modules with subsecond temporal precision and subcellular spatial resolution dimerize upon exposure to blue light. These modules are genetically encoded. These researchers have created DNA recombination using blue light and Cre recombinase by utilizing "split Cre recombinase and blue-light-dependent dimerization of plant photoreceptor CRY2 (cryptochrome-2) and its connecting domain CIB1" (Figure 6). Nevertheless, the cryptochrome2-calcium and integrin binding1 split Cre device performs poorly, as both in vivo and in vitro recombination efficiency is low. In order to facilitate light-induced DNA recombination in living systems, scientists created an additional Cre-loxP recombination method (termed as PACre) in 2016 that is triggered by blue light. The foundation of this Cre-loxP recombination method is the reconstruction of split Cre domains joined to the magnet process, which is a dimerization method that is dependent on blue light and has nMag (negative magnet) and pMag (positive magnet) "photosensitive domains", which were created from "a flavin-connecting fungal photoreceptor" (Figure 6). Upon illumination,

this PA (photoactivatable)-Cre technology effectively and precisely induced DNA recombination. Although Cre recombinase which is regulated by light for genome editing has been developed, blue light's phototoxicity to the cells of mammals and its inability to permeate cells seriously limit its useability for future scientific study and therapeutic use. So, there is a need for more sophisticated optogenetically controlled Cre recombination systems (Ye & Fussenegger, 2019).

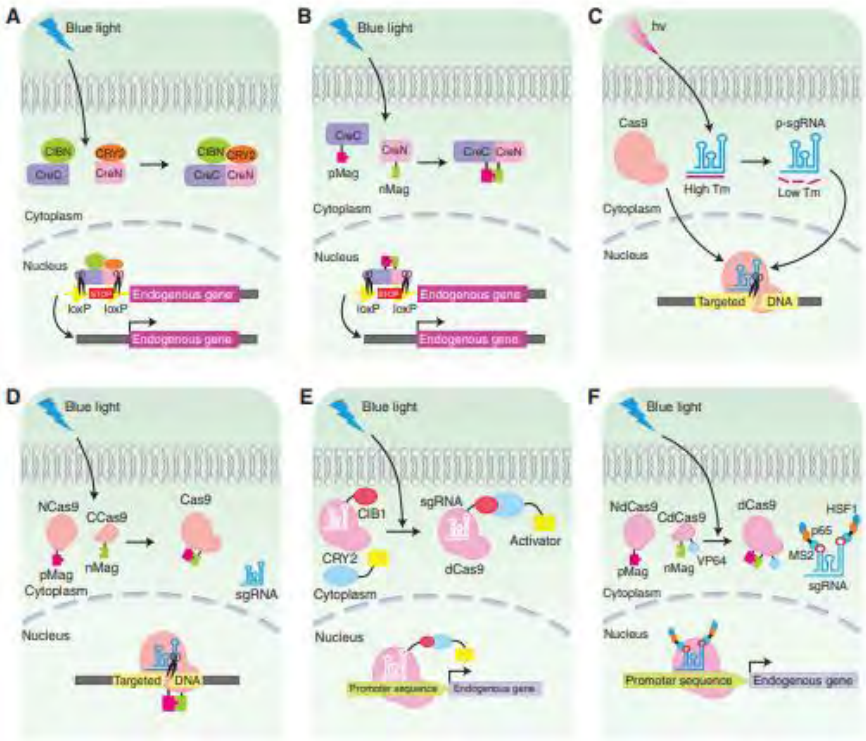

Figure 6: Illustrations of optogenetic instruments that are used in editing genes. A) Gene editing can be controlled spatially and temporally using the enzyme Cre recombinase (Cre recombinase activated by blue light). B) When light is absent, Cre is divided into 2 pieces which are inactive for loxP sites. Cre rapidly regains its enzymatic activity when exposed to blue light. C) In human cells, ultraviolet light activates the CRISPR system, which is combined with the CRISPR-plus system, which uses light to reveal sgRNAs. D) Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein-9 method for gene modification through optogenetics that is stimulated by blue light. In order to create the photoactivatable Cas9 (paCas9), split Cas9 fragments were combined with photo-inducible nMag and pMag known as "dimerization domains". Light-inducible targeted genome editing is made possible by the heterodimerization of nMag and pMag, which reassociates the broken Cas9 pieces and restores RNA-guided nuclease activity. E) CRY2/CIB1-based blue-light-mediated CRISPR-dCas9 transcription method. While illuminated with light that is blue in color, heterodimerization of CRY2PHR and CIB1 initiates downstream gene transcription. F) The split-d-CRISPR-associated protein-9 transcription method in order to photoactivate endogenous genes that is triggered by light which is blue in color. When exposed to bluelight, pMag and nMag heterodimerize, allowing for the reconstruction of the broken Cas9 pieces (Ye & Fussenegger, 2019).

Chapter 4

Neurological Disorders and its Management

4.1 Neurological Disorders

Diseases of the central and peripheral nervous systems are referred to as neurological disorders. The brain, spinal cord, cranial nerves, peripheral nerves, nerve roots, autonomic nervous system, neuromuscular junction, and muscles are all included. Epilepsy, Alzheimer's disease and other dementias, cerebrovascular diseases such as stroke, migraine, and other headache disorders, multiple sclerosis, Parkinson's disease, neuroinfections, brain tumors, traumatic nervous system disorders caused by head trauma, and neurological disorders caused by malnutrition are examples of these disorders (*Mental Health: Neurological Disorders*, n.d.).

4.1.1 Epilepsy

Epilepsy is a relatively common disease characterized by seizures, which can take many different forms and are caused by episodic neuronal discharges, with the kind of seizure varying depending on the portion of the brain affected (Ritter et al., 2018). 10% of people will experience at least one seizure over their lifetime. After Alzheimer's and cerebrovascular disorders, epilepsy is the third most prevalent neurologic condition worldwide. Epilepsy is a collection of distinct seizure types and syndromes caused by a variety of processes that all share the rapid, excessive, and synchronized firing of brain neurons. This aberrant electrical activity can cause a number of symptoms, including loss of consciousness, irregular movements, anomalous or strange behavior, and altered perceptions that last for a short time but reoccur if left untreated. The symptoms are determined by the location of the aberrant neural activity. If the motor cortex is affected, for example, the patient may suffer aberrant motions or a widespread convulsion. Auditory, visual, and olfactory hallucinations may occur in parietal or occipital lobe seizures. Medicines are the most often utilized way of treatment for epileptic

patients. In average, 75% of individuals can have their seizures managed with just one medicine. Patients may take more than one drug to achieve complete seizure control, and some patients may never achieve complete seizure control (Whalen et al., 2015).

Mechanism of epilepsy

In most situations, there is no known cause of epilepsy. Changes in physiologic variables, such as changes in pH, blood gases, electrolytes, and blood glucose, and changes in environmental factors, such as lack of sleep, alcohol use, and stress, can activate functionally aberrant foci. In epilepsy, neuronal discharge is caused by the firing of a limited number of neurons in a particular location of the brain known as the "primary focus". Neuroimaging methods such as positron emission tomography scans, magnetic resonance imaging, and single photon emission coherence tomography may detect regions of concern. Epilepsy can be caused by a structural, genetic, or metabolic defect, or it might be caused by an unknown factor (Whalen et al., 2015).

Moreover, acute epileptic activity can be generated by inhibiting synaptic and voltage-gated inhibitory conductances or stimulating synaptic and voltage-gated excitatory conductances. Seizures are prevented by doing the opposite: enhancing inhibition or reducing excitement. Several decades of pharmacological research have demonstrated that an imbalance in inhibitory and excitatory conductances causes seizures (i.e. is ictogenic) in otherwise normal brain tissue. This imbalance is clinically shown by toxic exposures such as domoic acid, which stimulates excitatory GluK1 glutamate receptors, or through theophylline excesses, which block the inhibitory adenosine A1 receptor. In these circumstances, ordinarily normal patients are subjected to acute, recurring, and medically intractable seizure activity. Thus, an imbalance between inhibition and excitation is a proven ictogenic process. Difficulties occur when this method is extended to epileptogenesis, which is, a mechanism that causes a sustained increase in the likelihood of spontaneous seizures (Staley, 2015).

Prevalence of epilepsy

Epilepsy contributes significantly to the global illness burden, impacting around 50 million individuals globally. At any one time, the estimated proportion of the normal population with active epilepsy (continuous seizures or the need for medication) is between 4 and 10 per 1000 persons (*Epilepsy*, n.d.).

Every year, around 5 million individuals worldwide are diagnosed with epilepsy. Every year, in wealthy countries, 49 people from each 100,000 are diagnosed with epilepsy. In low- and moderate-income countries, this estimation can exceed 139 people from every 100,000 people. And, it is most likely related to an increase in the danger of common illnesses like as neurocysticercosis and malaria; the increased frequency of road accidents; complications that are related to birth; and disparities in healthcare facilities, the availability of preventative health programs, and available medical care. Nearly eighty percent of persons with seizures reside in moderate and less-than-average-income nations (*Epilepsy*, n.d.).

Signs and symptoms of epilepsy

Epilepsy varies in its features based on which area in the brain the disturbance originates and to what extent it progresses. Temporary signs like losing consciousness or awareness, also problems in mobility, sensibility (including sight, listening, and tasting), emotion, and various cognitive aspects, might occur (*Epilepsy*, n.d.).

People who have seizures are more prone to experience physiological difficulties (including broken bones and bruising caused by epilepsy) and greater rates of mental illnesses like depression and anxiety. Likewise, the likelihood of death from seizures is at least three times greater than in the normal nation, with low- and middle-income nations and rural regions having the greatest rates of early mortality (*Epilepsy*, n.d.).

Epilepsy treatment

Epilepsy can be treated. With the accurate use of anti-epileptic drugs, the epileptic patient may reach an epilepsy-free condition in approximately 70% of cases. Considering relevant clinical, societal, and individual aspects, anti-epileptic drugs should be stopped after 24 months with no epilepsy (*Epilepsy*, n.d.).

Antiepileptic medications include benzodiazepines, carbamazepine, eslicarbazepine, ethosuximide, ezogabine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, perampanel, topiramate, valproic acid, vigabatrin, zonisamide, phenobarbital, phenytoin, pregabalin, rufinamide, tiagabine. Multiorgan hypersensitivity reactions, a rare idiosyncratic response marked by rash, fever, and systemic organ involvement, have been linked to all antiepileptic drugs (Whalen et al., 2015).

Furthermore, surgery may be advantageous to individuals who do not react well to pharmacological therapy (*Epilepsy*, n.d.). Outcomes of successful surgery include a lower chance of injuries or early death, the ability to drive, more independence, and maybe enhanced occupational alternatives. However, surgical therapy is still underutilized, and promising candidates are frequently not referred or are referred late, potentially due to misunderstandings and anxieties (Thijs et al., 2019).

4.1.2. Schizophrenia

Schizophrenia is a kind of prolonged psychosis marked by hallucinations (typically in the form of voices), delusions, and problems with planning or speaking. This condition usually begins in late adolescence or early adulthood. It is a chronic and debilitating condition that affects roughly 1% of the population. Schizophrenia has a significant hereditary component and is most likely the result of a basic metabolic aberration, such as a failure of the mesolimbic or mesocortical dopaminergic neural systems (Whalen et al., 2015).

Mechanism of schizophrenia

According to some research, there is no common cause of schizophrenia. It is hypothesized that schizophrenia is caused by a combination of genetic and environmental causes. Psychosocial variables may also play a role in the onset and progression of schizophrenia. Cannabis usage is linked to an increased risk of illness (*Schizophrenia*, n.d.).

Free radicals are thought to play a role in the pathophysiology of schizophrenia. According to research, superoxide dismutase (SOD) is a critical enzyme engaged in the superoxide radical detoxification and cellular oxidative stress limitation, and its levels were observed to be high in patients with chronic schizophrenia or low in first-episode schizophrenia patients who lack neuroleptic experience. Researchers used a radioimmunometric technique to measure superoxide dismutase levels in the blood. They examined sixty-eight individuals with persistent schizophrenia to fifty healthy regulated people in their research. The scientists discovered that blood SOD levels in schizophrenia patients were considerably higher than in controls. It has been proposed that higher oxidative tone may produce a rise in SOD activity. As a result, the discovery of higher blood superoxide dismutase ranges showed that radicals that are free can be linked to the pathophysiology of schizophrenia. Also, the researchers hypothesized two possible interpretations for this finding. The 1st hypothesis is that neuroleptic therapy may have influenced SOD rates in the blood. Chronically unwell individuals must take neuroleptic medicines for an extended length of time, which prevents red blood cell (RBC) enzymes from returning to normal levels. According to several research, red blood cell superoxide dismutase levels are higher in patients on standard antipsychotics and smaller among individuals with first-episode psychosis who are drug-independent. The 2nd explanation for this condition may be the withdrawal of antipsychotic medication, which can cause symptoms to deteriorate. This phenomenon is thought to be caused by increased dopamine (DA) function, mostly due to the termination of DA receptor blockage (Figure 7) (Bukowska et al., 2006).

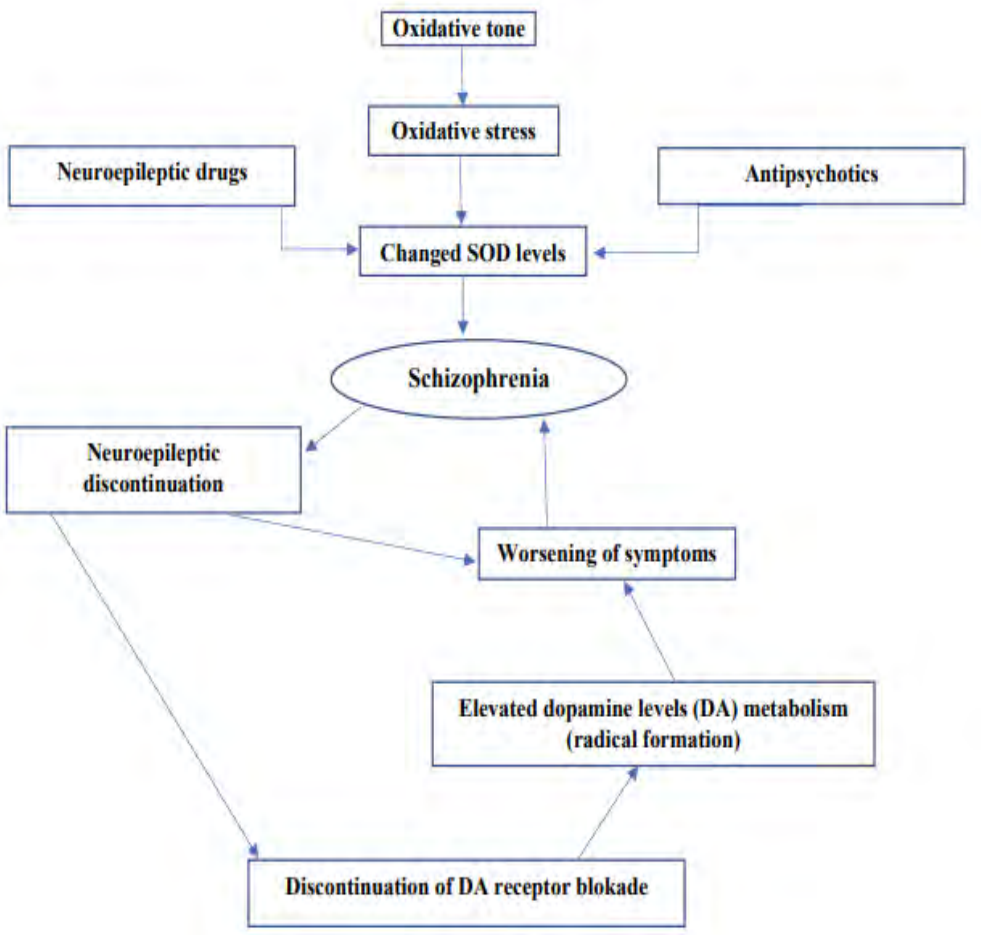

Figure 7: The mechanism of schizophrenia (adapted from Bukowska et al., 2006).

Prevalence of schizophrenia

Schizophrenia affects roughly 24 million individuals globally, or one in every 300 people (0.32%). In adults, this prevalence is about 0.45% (1 in 222). It is not as widespread as most other mental illnesses. Onset occurs most frequently in late adolescence and the twenties, and it occurs earlier in males than in women (*Schizophrenia*, n.d.).

Schizophrenia patients have a two to three times higher risk of dying young than the normal population. Physical issues, including cardiovascular, metabolic, and viral diseases, are frequently to blame (*Schizophrenia*, n.d.).

Signs and Symptoms of schizophrenia

Positive, negative, and cognitive symptoms are the three types of symptoms associated with schizophrenia.

The positive symptoms are,

- Delusions (in nature, people are frequently paranoid.)
- Hallucinations (frequently in the type of voices, which sometimes with encouraging messages)
- Disorder of thought
- Disorganized, abnormal behavior (such as repetitive gestures, confusion, and, in rare occasions, violent behavior)
- Catatonia (might manifest as immobility or aimless motor activity) (Ritter et al., 2018).

The negative symptoms are,

- Withdrawal from social interactions
- Emotional reactions are flattened
- Anhedonia (an incapacity to enjoy pleasure)
- Reluctance to carry out routine chores (Ritter et al., 2018).

The Cognitive symptoms include,

- Deficiencies in cognitive ability (e.g. memory, attention) (Ritter et al., 2018).

Treatment of schizophrenia

Medications, psychoeducation, family interventions, cognitive-behavioral therapy, and psychosocial rehabilitation (training on life skills) are all viable treatment choices for patients

with schizophrenia. Organized assisted living, supported housing, and supported employment are all important care alternatives for persons with schizophrenia. People with schizophrenia, as well as their family and/or caregivers, require a recovery-oriented approach that gives them agency in treatment decisions (*Schizophrenia*, n.d.).

Antipsychotic medications are primarily used to treat schizophrenia. Antipsychotic medications are classified as first-generation and second-generation agents (Whalen et al., 2015).

First-generation antipsychotic drugs

First-generation antipsychotics (also known as conventional, usual, or classic antipsychotic drugs) are competitive blockers of a number of receptors, though their antipsychotic impacts are due to competitive blockage of DRD2 (dopamine D2) receptors. Extrapyramidal symptoms (EPS) have a higher likelihood of being linked with first-generation antipsychotics, particularly medications which firmly bind to DA neuroreceptors, like HAL-oh-PER-i-dol (haloperidol). Medication that binds poorly, such as chlorpromazine [klor-PROE-ma-zeen], reduces the likelihood of movement problems. Clinically, no one medicine is more successful than another (Whalen et al., 2015).

Second-generation antipsychotics

2nd-generation antipsychotic medicines (known as "atypical" antipsychotic drug) have a lower EPS occurrence compared to 1st-generation treatments, though they are linked to a greater possibility of metabolic adverse effects which including diabetes, hypercholesterolemia, and weight gain. The distinctive effect of the second-generation medications appears to be due to the inhibition of both serotonin and dopamine receptors, as well as potentially additional receptors (Whalen et al., 2015).

4.1.3 Alzheimer's Disease

Alois Alzheimer, a German psychiatrist, initially characterized Alzheimer's disease (AD) as "presenile dementia" in 1906 (Šerý et al., 2013). Alzheimer's disorder is a brain disease that gradually damages memory and cognitive skills, as well as the capacity to do the most basic activities (*What Is Alzheimer's Disease? | National Institute on Aging*, n.d.).

Mechanism of Alzheimer's disease

Alzheimer's disease is considered to be caused by an abnormal protein buildup within and around brain cells. Amyloid is one of the proteins involved, and deposits of it create plaques surrounding brain cells. Tau is the other protein, and deposits of it form tangles among brain cells (*Alzheimer's Disease - Causes - NHS*, n.d.). The causes are most likely a mix of age-related brain changes, as well as genetic, environmental, and lifestyle variables. The impact of any of these variables in increasing or reducing the likelihood of Alzheimer's disease varies by individual (*What Causes Alzheimer's Disease? | National Institute on Aging*, n.d.).

The formation of extracellular amyloid-beta (A) plaques and neurofibrillary tangles in the environment of the cell, neuronal death, and synaptic loss are all hallmarks of Alzheimer's disease (AD), all of which lead to gradual cognitive decline (Kocahan & Doğan, 2017).

In healthy aging, the brain shrinks to some extent but, unexpectedly, does not lose a substantial number of neurons. However, with Alzheimer's disease, damage is extensive, with many neurons ceasing to function, losing neural connections, and dying. Alzheimer's disorder impairs critical processes in neurons and their networks, such as communication, metabolism, and repairing (Coman & Nemeş, 2017).

Firstly, Alzheimer's disease often damages neurons and their connections in memory-related areas of the brain, such as the entorhinal cortex and hippocampus. It subsequently affects parts of the cerebral cortex that control language, logic, and social interaction. Many other parts of

the brain are eventually affected. A person with Alzheimer's disease step by step loses his or her capacity to live and operate independently over time. The condition is ultimately lethal (Coman & Nemeș, 2017).

Prevalence of Alzheimer's disease

The most prevalent cause of dementia, Alzheimer's disease (AD), affects more than 10% of individuals over 65 (Šerý et al., 2013). Its frequency climbs rapidly with age, from around 2% in the 65-69 age group to 20% in the 85-89 age group (Ritter et al., 2018).

Symptoms of Alzheimer's disease

Alzheimer's disease symptoms are often minor at first, but when more brain cells are destroyed over time, the symptoms worsen and begin to interact with a person's daily life. This distinguishes them from the changes that many individuals experience as they age, such as becoming slower to consider things through or occasionally forgetting something (*Symptoms of Alzheimer's Disease | Alzheimer's Society*, n.d.).

There are certain common symptoms of Alzheimer's disease, but each one's experience will be identical (*Symptoms of Alzheimer's Disease | Alzheimer's Society*, n.d.).

Most people's initial symptoms of Alzheimer's are memory impairments, namely, trouble recalling recent incidents and acquiring new information. This is because the hippocampus, a region of the brain, is frequently damaged early in Alzheimer's disease. Nevertheless, in the early stages, the person's memory for incidents that took place a long while ago is not normally impacted (*Symptoms of Alzheimer's Disease | Alzheimer's Society*, n.d.)

Memory issues often worsen as Alzheimer's disease advances, and they may include:

- Items (like keys and spectacles) are frequently misplaced around the house.
- Forgetting a friend's name or having difficulty finding the correct word in a discussion

- Forget recent discussions or incidents
- Become disoriented in a known location or route
- Don’t remember about meetings or important dates.

In addition to memory impairments, Alzheimer's disease patients have a greater likelihood to acquire additional complications. The complications are including cognitive, thinking, and speaking abilities, and perceptual problems like:

- Speech - Patients might repeat words frequently or have difficulty following a discussion.
- Viewing objects in three dimensions and evaluating distances (visuospatial abilities)
- Climbing stairs or parking the vehicle might become considerably harder.
- Planning, and focusing- They might struggle to make plans, solve difficulties, or executing a set of actions (like cooking a meal).
- Orientation — they can get lost or forget the time or day.

An individual in the early stages of Alzheimer's will frequently experience mood swings. They may become more worried, unhappy, or easily irritated. Many individuals lose interest in socializing, as well as in hobbies and other activities (*Symptoms of Alzheimer’s Disease | Alzheimer’s Society*, n.d.)

Treatment of Alzheimer’s disease

Current Alzheimer's disease therapy options have included the cholinesterase inhibitors (ChEIs) donepezil, galantamine, and rivastigmine, and also the N-methyl-D-aspartate receptor antagonist rivastigmine (Memantine). Memantine is authorized in the United States, Europe, and Japan for the management of moderate-to-severe Alzheimer's disease. Donepezil is approved in the United States, Japan, and Europe for the medication of all stages of Alzheimer's disease. Galantamine is approved for the management of mild-to-moderate Alzheimer's

dementia in the United States and Japan, and mild-to-moderately severe Alzheimer's dementia in Europe. Rivastigmine is available in the form of a transdermal patch as well as an oral version. Oral rivastigmine is licensed in the United States for the therapy of mild-to-moderate Alzheimer's dementia, and in Europe for the management of mild-to-moderately severe Alzheimer's dementia. The rivastigmine patch is licensed in the United States for all stages of Alzheimer's disease, in Europe for mild-to-moderately severe Alzheimer's disease, and in Japan for mild-to-moderate Alzheimer's disease (Blesa et al., 2018).

4.1.4 Parkinson's Disease

Parkinson's disease (PD) represents one of the most incapacitating central nervous system illnesses (DeMaagd & Philip, 2015).

Mechanism of Parkinson's disease

The brain region known as the substantia nigra loses nerve cells, which leads to Parkinson's disease. These areas of the brain's nerve cells are in charge of creating a neurotransmitter called dopamine. Dopamine communicates between regions of the nervous system and brain, which assist govern and coordinate bodily movements. The quantity of dopamine in the brain gets lowered after these nerve cells died or get damaged. This implies that the region of the brain that controls movement cannot function appropriately, leading motions to become sluggish and irregular (*Parkinson's Disease - Causes - NHS*, n.d.).

The buildup of misfolded protein aggregates, failure of protein clearance routes, mitochondrial injury, oxidative stress, excitotoxicity, neuroinflammation, and genetic alterations are the most prominent factors implicated in the development of Parkinson's disease (Maiti et al., 2017).

Several variables appear to be involved with Parkinson's disease, including:

- **Genes.** Specific genetic alterations which can cause Parkinson's disease have been found by researchers. However, except in rare circumstances where many family members have Parkinson's disease, they are unusual. Certain gene variants, on the other hand, appear to enhance the chance of Parkinson's disease, but with a relatively low likelihood of Parkinson's disease for every one of these genetic markers (*Parkinson's Disease - Symptoms and Causes - Mayo Clinic*, n.d.).
- **Environmental triggers.** Toxins or external conditions may raise the likelihood of developing Parkinson's disease later in life, but the risk is minimal (Parkinson's Disease - Symptoms and Causes - Mayo Clinic, n.d.).

Prevalence of Parkinson's disease

Globally, PD-related disability and mortality are growing faster than any other neurological condition. In the last 25 years, the frequency of Parkinson's disease has more than doubled. According to 2019 estimates, there are approximately 8.5 million people throughout the world who have Parkinson's disease. Based on current statistics, PD caused 5.8 million disability-adjusted years of life in 2019, a rise of 81% since 2000, and 329 000 deaths, an increase of more than 100% since 2000 (Parkinson Disease, n.d.).

Symptoms of Parkinson's disease

Parkinson disease (PD) is a degenerative brain ailment characterized by motor symptoms (slow movement, tremor, stiffness, walking, and imbalance) as well as a wide range of non-motor consequences (cognitive impairment, mental health disesase, sleep disease and pain and other sensory problems). Motor impairments, including dyskinesias (uncontrollable movements) and dystonias (painful involuntary muscle contractions), lead to speech and mobility difficulties, as well as constraints in many areas of life. The development of these symptoms leads to a high

prevalence of impairment and care needs. Many persons with Parkinson's disease acquire dementia as the condition progresses (*Parkinson Disease*, n.d.).

Parkinson's disease treatment

Huge strides have been achieved in Parkinson's disease (PD) treatment. Many prospective medicines for Parkinson's disease are emerging as a consequence of advancements in investigational treatments (Jankovic & Aguilar, 2008).

I. Levodopa: Levodopa is among the most effective medicine for managing Parkinson's disease symptoms, notably bradykinesia. Nevertheless, because levodopa medication is usually linked with motor problems like fluctuations and dyskinesias, there is ongoing discussion about whether in the course of Parkinson's disease levodopa therapy should be initiated (Jankovic & Aguilar, 2008).

II. COMT (Catechol-O-methyl-transferase) inhibitors: Another method for extending DA (Dopamine) response is to inhibit COMT with medicines like entacapone (Comtan®). The short half-life of entacapone necessitates regular administration (Jankovic & Aguilar, 2008).

III. Dopamine agonists: Dopamine agonists (DA) have a pharmacologic effect by directly activating DA receptors, bypassing presynaptic DA production. Activation of the D2 receptors is critical in mediating the favorable anti-parkinsonian effects of DA agonists, according to experimental and clinical research, although concurrent D1 and D2 stimulation is necessary to provide optimal biological and behavioral benefits (Jankovic & Aguilar, 2008).

Aside from traditional pharmaceutical and surgical therapies, numerous new options are now being investigated in the treatment of various PD symptoms (Jankovic & Aguilar, 2008).

Chapter 5

Role of Optogenetics in Neurological Disorders

Nerve cells are electrically excitable and use a number of different of pumps and ion channels to keep a voltage gradient throughout their membranes. While positive ions enter a neuron, depolarization of the membrane potential occurs, and if the voltage shift is significant enough, an action potential is formed. While negative ions enter the neuron, hyperpolarization of membrane occurs, making firing of the action potentials harder. Consequently, approaches that depolarize or hyperpolarize the membrane can directly alter neuronal excitability (Wykes et al., 2016).

The use of optogenetics in neuroscience is enabling scientists to unravel the neural circuits applicable to a broad range of behavioral reactions. Moreover, optogenetic neuromodulation is being researched as a potential treatment for a variety of neurological illnesses (Wykes et al., 2016).

5.1 Optogenetics in Epilepsy

Optogenetics has significant potential as a direct neuromodulatory treatment for preventing seizure activity. The capacity to activate or inhibit certain types of cells has resulted in a variety of ways for preventing seizure activity in mouse epilepsy models (Tung et al., 2016).

A treatment that exclusively targets the brain areas responsible for seizure genesis and has no negative behavioral side-effects would represent a significant leap in optogenetics in the treatment of epilepsy. Opsins can be expressed by targeting neurons inside an epileptic focal. Light-activated gene therapy has been shown to effectively decrease seizures in a variety of mouse models of epilepsy while not interfering with normal behavior. An optogenetic

technique, however still in its early phases of research, may show potential to treat drug-resistant epilepsies (Wykes et al., 2016).

An ideal epilepsy therapy strategy would target specifically the brain areas responsible for seizure development, which is not attainable with systemically administered anti-epileptic medications. This treatment should also not interfere with regular biological activities. Since seizures are intermittent, establishing a technology for the quick and reversible inhibition of activity in a confined part of the brain would be a significant step forward. Photoactivating ion channels and transpoters that are sensitive to light expressed in neurons is one method for suppressing seizure activity 'on demand'. There are two basic optogenetic techniques to seizure prevention. The first would be to produce an inhibitory opsin (like halorhodopsin) in excitatory (primary) neurons to diminish excitability and output. The second strategy would be to produce an adequate opsin in populations of interneurons to modulate their firing in such a way that main neurons are inhibited more. Selecting and expressing an adequate opsin for interneurons is a difficult task that will be discussed in further detail later (Wykes et al., 2016).

Halorhodopsin expression in main neurons has been employed successfully to modulate electrically and chemically generated epileptiform activity in the slice preparations. Several groups have begun to study whether optogenetic modulation of neurons may reduce in vivo seizure activity in several mouse models of epilepsy in recent years. Initially, these researches relied on the expression of halorhodopsin (whether NpHR2.0 or NpHR3.0) in main neurons in mouse models of “neocortical, thalamic, and temporal lobe epilepsy” (Wykes et al., 2016).

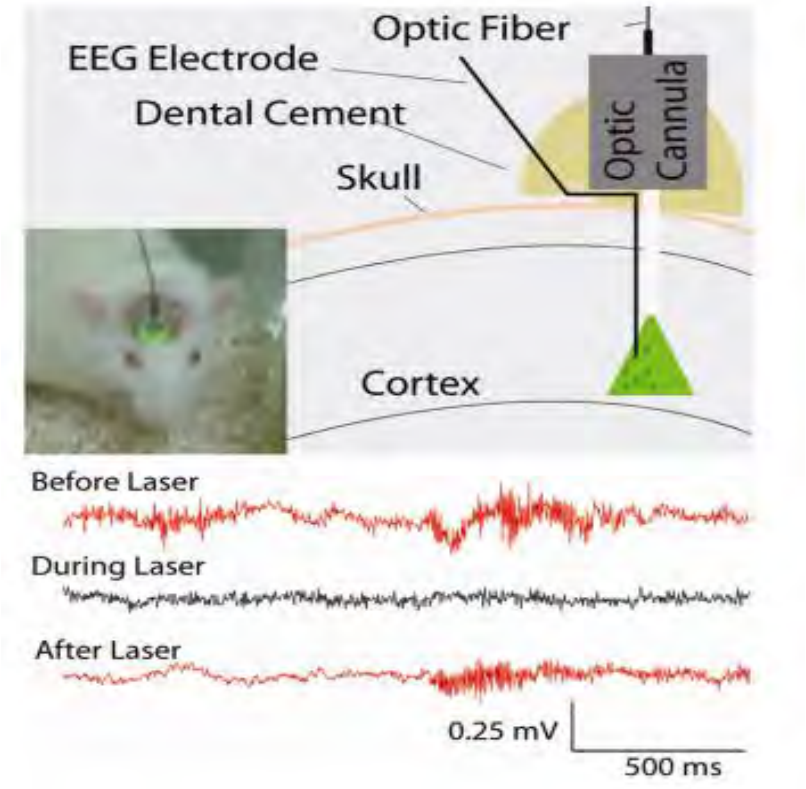

Figure 8: *In vivo* optogenetic inhibition of epileptiform activity. Epileptic activity is acutely attenuated *in vivo* using optogenetics (Wykes et al., 2016).

Figure 8 shows that pyramidal neurons inside the epileptic foci were infected with a virus that expressed halorhodopsin and was able to activate with a 561-nm laser through an attached fiber optic. EEG traces taken before, during, and after laser illumination. During laser stimulation, bursts of abnormal high frequency activity were controlled (Wykes et al., 2016).

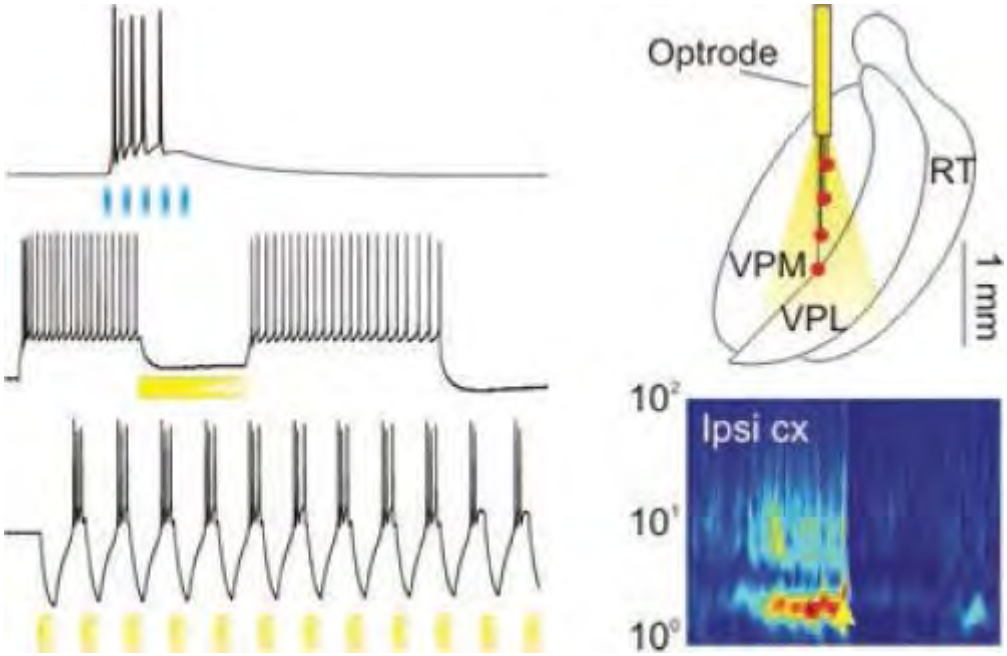

Figure 9: Controlling neuronal activity necessitates careful evaluation of the best opsin to use in conjunction with the best lighting protocol. Left upper trace: To cause excitatory synaptic currents and cause thalamic reticular neuron firing, blue light depolarizes corticothalamic axons that express the ChR2 receptor. Left middle trace: Yellow light causes an eNpHR (enhanced NpHR) -expressing thalamic relay neuron to hyperpolarize and silences continuing activity. Left lower trace: Contrary, repeated yellow light pulses because large, high-frequency rebound bursts of action potentials to occur at the offset of each pulse. Right panel, upper: An illustration of an optrode implanted in the ventroposteromedial thalamus (VPM) that delivers yellow light to thalamic relay neurons that express the eNpHR (Paz & Huguenard, 2015).

Here, from Figure 9, it is understandable that optogenetic method worked well to stop post-stroke epileptic episodes. Therefore, if optogenetics is used to block excitatory neurons, it can be successful. When it comes to comprehending intricate networks and possible important choke spots, optogenetic methods are pretty advantageous. As a result, these methods are particularly beneficial in the research of epilepsies that signify localized or broad network failure (Paz & Huguenard, 2015).

5.2 Optogenetics in Schizophrenia

Utilizing a mix of light and viral vectors, in vivo optogenetic approaches have identified brain regions and circuits controlling particular characteristics in freely moving rats during the last two decades. The behavioral effects of SCZ (Schizophrenia) were produced or decreased by optogenetic inhibition or activation of PNs (peripheral nervous system) in the HPC (hippocampus), respectively (Patrono et al., 2021).

Scientists used optogenetic over-activation of the ventral hippocampus and peripheral nervous system to test the hypothesis that excitatory over-activity of the HPC-CA1 (hippocampal cornu ammonis) area in humans might be a possible predictor of schizophrenia like psychosis. The results demonstrated that an optogenetic Chronos activator enhanced abnormally PNs activity in mice ventral HPC, causing hyperlocomotion, an SCZ-positive symptom signature, and reduced performance on the spatial novelty preference, an SCZ-cognitive impairment. In contrast, some researchers' work used a methodology in which the previous injection of PCP (phenylcyclohexyl piperidine) generated considerable impairment in the learning of prolonged stimulus-induced delay eyeblink conditioning. After that, optogenetic suppression of PNs of bilateral ventral HPC neurons relieved the reduced acquisition and poor conditioning, suggesting that enhanced activity in the HPC network is important in SCZ (Patrono et al., 2021).

Recent research has revealed whether ChR2-photoactivation or NpHR-photoinhibition can operate on fibers arising from limbic regions, affecting gut colonic sensitivity in freely moving animals. To achieve this purpose, optogenetic applications were paired with behavioral measures of visceral discomfort. First, AAVs encoding ChR2 or NpHR were transfected into the amygdala's central nucleus (CeA), and optic fibers were inserted into the stria terminalis' bed nucleus (BNST). After a few weeks of recuperation and ChR2/NpHR expression, the isobaric colonic distension with and without light treatments was assessed. The researchers

discovered that ChR2-photoactivation caused colonic hypersensitivity, but NpHR-photoinhibition had no impact. This method demonstrates how optogenetic stimulation of limbic brain nuclei can help us comprehend the intricate visceral nociceptive circuitry in independently moving rats (Patrono et al., 2021).

Optogenetic activation of GABAergic transporters (VGATs) of the zona incerta (ZI) that is enclosed among the subthalamic nuclei triggered maladaptive binge-eating in mice in another investigation. This study revealed that ZI GABAergic neurons had an unusually strong orexigenic potential. The researchers wanted to know if the paraventricular thalamic nucleus (PTN)-ZI GABAergic pathways were important for maladaptive food intake control. Axon terminals from ZI GABA (zona incerta gamma-aminobutyric acid) neurons to PTN glutamate neurons were stimulated in vivo to induce an increase in food intake, with high-fat, sweet, and regular meal consumption increasing over the course of a 10-minute stimulation period. Furthermore, the scientists revealed that optogenetic activation of the ZI GABA neurons produced a more robust feeding response than the well-studied lateral hypothalamus stimulation, indicating that the ZI GABA neurons can play a significant role in increasing food consumption (Patrono et al., 2021).

Furthermore, in mouse models of binge eating, targeted uses of light have been utilized to regulate ENS (enteric nervous system) excitability in order to elicit propagating contractions, so boosting colonic transit. Hibberd and colleagues used immunohistochemistry, patch-clamp, and calcium imaging to examine colons from transgenic mice expressing Cre-mediated expression of ChR2 in calretinin neurons. In the meantime, colonic motility was evaluated in vitro utilizing mechanical, electrophysiological, and video recording, as well as fecal output in vivo. When calretinin enteric neurons were stimulated with light, polarized motor reflexes were elicited, followed by premature anterograde propagation contractions. Thus, light stimulation might cause motility from anywhere in the colon. Surprisingly, the researchers implanted a

novel "wireless light-emitting diode" onto the colon wall. A conductive receiving coil, a capacitor, and a rectifier are all part of the about 10 mm diameter machine. A ductile linking trace incorporates metal wires for power transfer from the coil to a 0.06 mm2 lighting area LED on an injectable needle. Magnetic connection to a transmission antenna transfers power to the coil, which is subsequently transmitted by electromagnetic waves at certain frequencies. The device is implanted intragastrically using an insertion among the skin and peritoneum. The needle holding the LED is introduced through a peritoneal incision. Finally, the LED is implanted immediately distal to the cecal-colonic junction, adjacent to the proximal colon. This technological advancement enabled the researchers to validate the results acquired in vitro in vivo. In fact, researchers found that independently roaming mice increased the amount of fecal pellets by administering focused light via the wireless device to the colon, corroborating prior in vitro results. The development of a novel tool that may be useful in understanding how optogenetic regulation can directly alter gastrointestinal motility is one of the study's principal outcomes. Optogenetic interventions of both the CNS and the ENS may thus be useful approaches that should be used (Patrono et al., 2021).

Researchers also discovered that optogenetic stimulation of the ventral hippocampus decreased performance on a hippocampus-dependent short-term memory test, indicating that hippocampal overactivity in mice may result in phenotypes that are possibly related to schizophrenia cognitive symptoms. Furthermore, these SNP differences cannot be explained by changes in anxiety or the possible rewarding effects of ventral hippocampus optogenetic activation. A prior study discovered that blocking hippocampal projections to the medial prefrontal cortex (mPFC) just during the encoding phase of a spatial short-term memory exercise impaired performance. The insufficiency of an interaction between group and stimulation phase in our investigation, on the other hand, showed that activation of the ventral hippocampus is deleterious to spatial short-term memory regardless about whether stimulation

happens during the encoding or retrieval stages of the task. This might be caused by disturbances in state-dependent memory processes, in which performance is degraded as a result of behavioral shifts between encoding and retrieval. However, given that the effects of optogenetic stimulation (and probably the accompanying behavioral state) were shown to outlast the stimulation period on a timeframe of several minutes, the impairment in animals stimulated just during the sampling phase would argue against this hypothesis (Wolff et al., 2018).

This optogenetic over-activation model is a useful tool for investigating which schizophrenia-related impairments and symptom domains may be produced by an overactive hippocampus, to what degree enhanced dopamine signaling or other downstream brain regions are implicated. Manipulation of the dorsal HPC, the thalamic nucleus reuniens, and the mPFC has been demonstrated to impact working memory performance, and hyperactivity of the ventral hippocampus may impact short-term memory by direct projections via these areas, independent of polysynaptic loops through the dopamine system. This would corroborate our contention that indeed dopamine-related phenotypes and memory deficits caused by ventral hippocampus excitation are distinct. In a direct comparison of the effects of dorsal vs. ventral hippocampus pharmacological or electrical stimulation, only the latter resulted in enhanced activity of the mesolimbic dopamine system and phenotypes associated with the positive symptom domain. This structural separation of downstream consequences of ventral hippocampus hyperactivity is consistent with the finding that dopamine-based antipsychotics are mostly not effective against cognitive symptoms of schizophrenia (Wolff et al., 2018).

5.3 Optogenetics in Alzheimer's Disease

In principle, optogenetics might be an alternate therapy option for Alzheimer's disease. The primary benefit of optogenetics over traditional electrical or pharmacological approaches may

be more accurate targeting of specific neuronal parts, higher cellular and temporal specificity, and decreased off-target effects (Mirzayi et al., 2022).

Researchers explored the influence of optogenetic methods on neurotransmitter signaling and discovered that optogenetically stimulating glutamatergic neurons in the hippocampus might aid learning and memory by generating theta waves. Optogenetic stimulation of glutamatergic neurons in the contralateral DG in Alzheimer's disease has been demonstrated to enhance working and short-term memory but not long-term memory, and is related with increased glutamate receptor expression in the hippocampus. It was also shown that glutamate receptor upregulation differs in different hippocampal areas, indicating that a single-target optogenetics technique has geographical constraints and that a multiple-target optogenetics approach to AD treatment should be investigated (Mirzayi et al., 2022).

GABA levels in the brains of wild and AD (Alzheimer's disease) model mice vary. Because GABA inhibits Aβ absorption in neurons, considerably large levels of GABA reduce Aβ-induced cytotoxicity. GABA therapy reduces baseline levels of cell death as well. GABA use during early life, but not later in life, can dramatically increase cognitive performance. Optogenetic approaches for activating or repressing GABA(A) receptors demonstrated that GABA activation at a young age ameliorated Aβ pathology, implying that early life GABA might be used to treat AD. Researchers discovered that optogenetic activation of GABAergic neurons in the hippocampus of APP/PS1 mice activated autophagy, decreased neuroinflammation, reduced A fragments, and totally corrected the learning deficit (Mirzayi et al., 2022).

Scientists proposed that cholinergic projection neurons, despite the physical depth of their cell bodies, are a good target for systems-level optogenetic regulation in AD therapy than

cholinergic interneurons present in multiple brain areas, including the cortex and the striatum, which are stimulated in standard deep brain stimulation (Mirzayi et al., 2022).

Therefore, while optogenetics has the potential to be a successful therapy for Alzheimer's disease, a single-target technique has geographical limits. Alzheimer's disease causes a wide range of damage, and a multi-targeted optogenetics strategy may be a more successful treatment (Cui et al., 2020).

Optogenetic activation of glutamatergic neurons in the bilateral dentate gyrus of an injected animal model of AD increased functioning memory and short-term memory, as well as downregulated neuroinflammation biomarkers in the core and peripheral areas of CHR2 expression and elevated neuroprotection biomarkers in the core region of CHR2 expression (Cui et al., 2020).

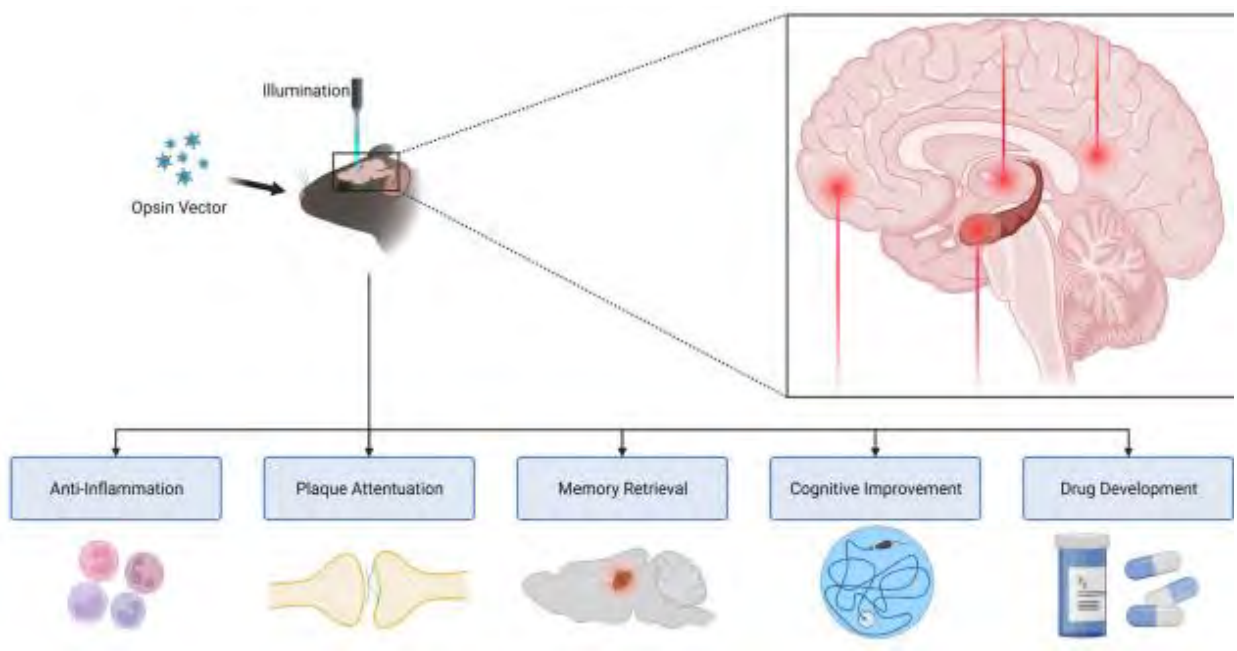

Figure 10: Optogenetics improves AD pathogenesis, supplements pharmaceutical research, and identifies promising areas for future therapies (Mirzayi et al., 2022).

Figure 10 shows the applications of the optogenetic technique in the management of Alzheimer's disease. Optogenetic methods have also been utilized to evaluate therapeutic efficacy. Memantine has been proven to enhance cognitive abilities in Alzheimer's disease models. Memantine increased entorhinal cortex to CA1 (hippocampal cornu ammonis) synaptic neurotransmission and facilitated dendritic spine regeneration in EC (entorhinal cortex) neurons that projected to CA1 using optogenetics. Caffeine consumption reduces memory impairments in aging and Alzheimer's disease by antagonizing adenosine receptors, which optogenetic stimulation in the hippocampus affects spatial memory function (Mirzayi et al., 2022).

5.4 Optogenetics in Parkinson's Disease

Optogenetics is gaining popularity as a method for understanding the operation of circuits that influence brain illnesses. This method employs proteins that react to certain wavelengths of light. These proteins can be generated using gene therapy techniques to activate or inactivate when exposed to specific wavelengths of light. If this can be adapted for human usage, it has the potential to significantly improve brain function in Parkinson's disease patients (PD). The accuracy of optogenetics has the potential to enhance normalization of malfunctioning circuits in Parkinson's disease, whereas the use of light rather than electricity (as in deep brain stimulation) may lessen the danger of deleterious effects induced by non-specific effects on unintended targets (*Optogenetic Restoration of Basal Ganglia Function to Treat Parkinson's Disease | Parkinson's Disease*, n.d.).

Enhancing the efficacy and result of DBS (deep brain stimulation) necessitates a multi-tiered strategy; optogenetics research gives the precision and practicality to find the processes that will assist in realizing these benefits in patient care (Gittis & Yttri, 2018).

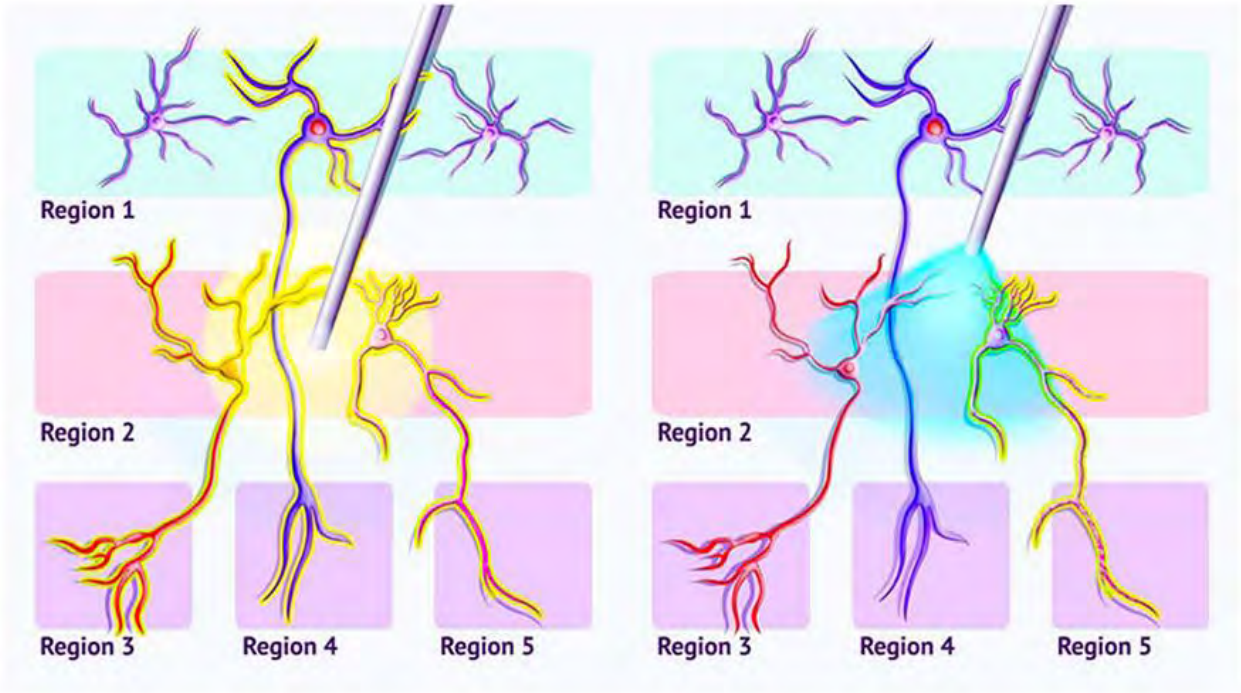

Figure 11: Electrical and optogenetic stimulation's ability to specific targets (Gittis & Yttri, 2018).

In Figure 11, it can be seen that two groups of neurons are represented, each with a distinct downstream target location. Left) Electrical stimulation, like the one used in DBS, will activate all cells in a specific location, even those whose axons just travel through the area, even if their cell bodies are located in a different part of the brain. These complex effects can make it difficult to understand stimulation effects and may result in off-target, negative effects. Right) Optogenetic stimulation targets a single cell type (the neuron expressing ChR2, seen as light-green spots on the neuron's surface), sparing surrounding tissue, including passing fibers (Gittis & Yttri, 2018).

Scientists investigated numerous known "opsins" and discovered that a few of the newest genes did not work as expected. Scientists discovered, however, that at least two additional known medicines were particularly efficient at rectifying several problems in pre-clinical models of experimental Parkinsonism. They discovered that introducing two of the known "opsin" genes into STN (subthalamic nucleus) neurons restored aberrant electrical firing of the (STN), a key target of DBS in current clinical practice. This similar technique resulted in significant

improvements in aberrant spontaneous rotational behaviors, significantly improved levels of activity in an open cage setting, and higher use of the afflicted paw compared to mice receiving genes that did not produce cells sensitive to light. Analyzers findings give critical evidence for the possible continued development of this innovative technology as an unique therapy that combines the benefits of deep brain stimulation with gene therapy (*Optogenetic Restoration of Basal Ganglia Function to Treat Parkinson's Disease | Parkinson's Disease*, n.d.).

In a mouse model of Parkinson's disease, researchers employ optogenetics to investigate the behavior of transplanted dopamine neurons. For the first time, this study shows how optogenetics may be used to investigate how cell treatment enhances rehabilitation in animal models of neurological illness (Chen et al., 2015).

Optogenetics is the use of a light-sensitive molecule, for example the ion channel channelrhodopsin-2, to regulate a biological process with great temporal accuracy by using light. The method is particularly effective for revealing the activity of neurons, which interact on a millisecond time frame. Human stem cell-derived neurons are frequently immature and diverse, and so only mature neurons expressing the optogenetic protein can be accurately controlled. When these latter are included into a neural circuitry in vivo or in vitro, their electrochemical activity may be turned on and off at whim, altering the behavior of downstream neurons. Optogenetics is used by scientists to control neural activity, include dopamine release, enabling this function of the cells to be examined independently of their other probable activities (Chen et al., 2015).

Optogenetic regulation of transplanted human neurons could have therapeutic implications. For example, in Parkinson's disease treatment, one may imagine a switch that either reduces or increases dopamine neuron function to prevent graft-induced dyskinesias (Chen et al., 2015).

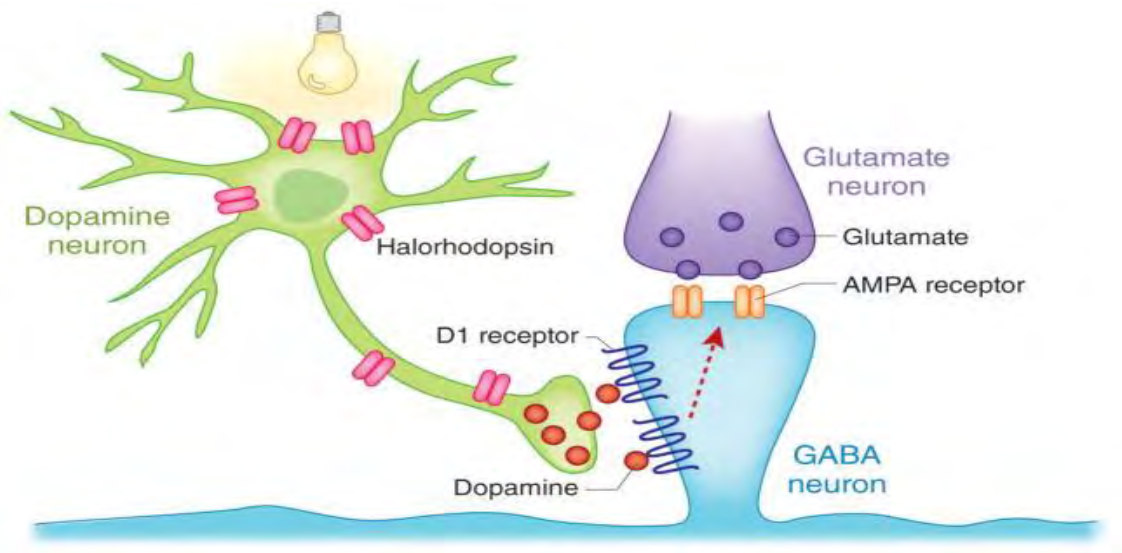

Figure 12: Dopamine neurons modified to express halorhodopsin. Dopamine produce, which binds to dopamine receptors and modulates glutamatergic inputs to GABA neurons, restoring grafted mice's motor function. Light activation of HALO (halorhodopsin) reduces dopamine release, allowing motor impairments to resurface (Chen et al., 2015).

Optogenetic silencing causes a large reversible decrease in evoked excitatory postsynaptic potentials amplitudes, signaling that the transplanted neurons augment excitatory postsynaptic potentials on host striatal GABA neurons via D1 (dopamine) receptor activation (Figure 12). This discovery is intriguing because it shows that transplanted dopamine neurons influence synaptic transmission from cortical and thalamic glutamatergic neurons to striatal GABA neurons, much like endogenous dopamine neurons. This study uses optogenetics to show how transplanting of dopamine neurons helps to restore motor capabilities (Chen et al., 2015).

Chapter 6

6.1 Challenges

Although optogenetics must overcome light supply issues, the increased spatial regulation and time resolution are essential components of this breakthrough technique. In addition, extensive experiments and validation in non-human species will be a critical step in bringing optogenetic technologies to the clinic (Shen et al., 2020).

6.2 Future Directions

These researches serve as the foundation for our understanding of how to employ light-activated channels as a treatment approach for the management of neurological illnesses. Much needs to be learned from the application of opsins and light delivery, and several technical elements must be perfected before they can be used by people, some of which may prove to be significant obstacles. The examination of the human immune system to these foreign proteins, the durability of viral vectors in brain tissue, and the adjustment of light delivery with chronically implanted devices are among these aspects. So far, the use of this potent tool has been effective in revealing normal and abnormal pathways, and some research into therapeutic applications has been conducted. One recent accomplishment that may improve knowledge of how optogenetics might be applied to people is the establishment of a nonhuman animal model (Bentley et al., 2013).

Optogenetics has already advanced significantly in the last decade, with the incorporation of various light-sensitive opsins into cells and the development of unique opsins with specialized features required to address specific research problems (*The Future of Optogenetics...*, n.d.).

However, since the technology is still being developed, there are several possible applications for optogenetics that have yet to be explored. The optogenetics toolset will increase to allow

for wider usage in research as attempts to generate novel opsins and improved light delivery choices continue (*The Future of Optogenetics...*, n.d.).

Chapter 7

Conclusion

Neurological disorders are common diseases that impose a substantial burden on those who suffer from them. Optogenetics has appeared as a potential approach, with numerous recent research investigating its use in animal models. As a result, optogenetics is a crucial research tool for directing translational and clinical research toward remedies that will recover, rather than simply mask, injured circuits, allowing for long-lasting, successful therapy in a broad range of illnesses.

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