A Review: Combination of Photon Emission & Genome Engineering in Brain Stimulation

By

Atiya Sunjida Audithi
19146017

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
BRAC University
February 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing 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 which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Atiya Sunjida Audithi

19146017

Approval

The thesis titled “A Review: Combination of Photon Emission & Genome Engineering in Brain Stimulation” submitted by Atiya Sunjida Audithi (19146017) of Spring 19 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

Dr. Md. Aminul Haque
Associate Professor
School of Pharmacy
BRAC University

Approved By:

Program Director:

Professor Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
BRAC University

Dean:

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
BRAC University

Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

Genome engineering and photon emission enables optogenetic brain stimulation. Neurological disorders and brain function research require brain stimulation. This review discusses optogenetics, a new technology that controls genetically defined neurons using light. Genetically engineered cells express light-sensitive opsin first. When engineered cells are illuminated, opsin-bound retinal conformationally shifts to open channels or pumps, depolarize or hyperpolarize cells, and activate or mute neurons. After the invention optogenetics, many opsin variants have been discovered or produced, allowing different wavelengths of light to activate or inhibit neural activity. Optogenetics has helped scientists comprehend brain circuit dysfunction that causes mental illness, stroke, heart disease, etc. Genome engineering and photon emission also enabled brain mapping. Optogenetics has transformed neuroscience and enabled new research to investigate casual roles of any particular brain circuit.

Keywords: Optogenetics; Brain stimulation; Opsin; Genome engineering; Photon emission; Light.

Dedication

Dedicated to my father, who has given me wings without considering the constraints of our society. And to Awal sir, my late primary school teacher, without whose supervision I would not be where I am today.

Acknowledgement

I am grateful to almighty Allah for providing me the opportunity to work with such wonderful people from the school of pharmacy who have always been idealistic and encouraging throughout my journey.

First and foremost, I am indebted to my supervisor Dr. Md. Aminul Haque (Associate Professor, School of Pharmacy, Brac University) for giving me the privilege to work as one of his thesis students. In addition, his support, guidance, dedication, enthusiasm, and expertise in this arena have driven me more interested in thesis work and helped me to complete the research properly.

Secondly, I would like to thank Professor Dr. Hasina Yasmin (Program Director and Assistant Dean, School of Pharmacy, BRAC University) for providing me with huge knowledge to make my journey easy and convenient. Most significantly, I would love to thank Professor Dr. Eva Rahman Kabir (Dean, School of Pharmacy, BRAC University) for her support, motivation, and kind words. Furthermore, I am grateful to all the faculty members of School of Pharmacy, BRAC University for their enormous efforts for the accomplishment of my graduation. Finally, I want to express my eternal gratitude to my friends and seniors for their guidance and my family members for supporting me all the way.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ChR2	Channelrhod-Opsin-2
DBS	Deep brain stimulation
ZFNs	Zinc Finger Nucleuses
YFP	Yellow Fluorescent Protein
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats

Chapter 1

Introduction

1.1 Background

Brain stimulation is a crucial technique which is useful while treating critical neurological diseases as well as to investigate brain function. Although the first real brain stimulation therapy was not discovered until the 20th century, humans have long been interested in how it might be used to cure illness. The conventional brain stimulation therapies included giving electricity given directly to the brain by electrodes or invasively by inserting electrodes into the scalp (Wang & Guo, 2016).

Some of the therapies are –

- VNS- Vagus nerve stimulation
- rTMS- Repetitive transcranial magnetic stimulation
- MST- Magnetic seizure therapy
- DBS- Deep brain stimulation
- ECT- Electroconvulsive therapy (*NIMH » Brain Stimulation Therapies*, 2016)

The brain stimulation therapy with the longest history of use and finest research is electroconvulsive therapy. These are some recent, and in a few cases still experimental, stimulation therapies. But recently optogenetic stimulation of brain has shown more benefits by bringing longer stimuli without undesirable side effects (Williams & Entcheva, 2015).

1.2 Brain Simulation

Neurological disorders can be treated, and the workings of the brain can be studied using brain stimulation. In conventional electrical stimulation, electrodes are used to directly produce intervening electric fields close to neural tissues (Wang & Guo, 2016). Therapies for brain stimulation involve electrically stimulating or inhibiting the brain. Electrodes can be inserted in the brain to deliver electricity directly, or electrodes can be painlessly applied to the scalp. Magnetic fields that are provided to the head can also induce electricity. These therapies show promise for treating some mental diseases that are unable to response to other treatments, while being less commonly utilized than medicine and psychotherapies. The brain stimulation therapy with the longest history of use and finest research is electroconvulsive therapy. These are some recent, and in certain cases still experimental, stimulation therapies (*NIMH » Brain Stimulation Therapies*, 2016).

The target areas of the brain could not be localized in large part by the first brain stimulation therapies mostly in 1930s. With the aid of X-ray pneumoencephalography, which involved replacing the cerebrospinal fluid with air, oxygen, or helium to enable X-ray imaging of brain, doctors were able to identify and focus brain lesions with a great deal more precision, though at the patient's expense of excruciating pain and adverse effects. A collection of knowledge about the results of activating different parts of the brain was amassed by doctors.

Brain stimulation has been most frequently utilized to treat Parkinson's disease throughout the 1950s. In the 1960s, when the novel drug levodopa began to show promise in the treatment of Parkinson's disease, doctors mostly stopped using brain stimulation because they believed it to be an unsafe and invasive alternative to levodopa. Additionally, as news of controversial techniques including lobotomies and electroshock treatment spread, the public's

opinion of neurotherapies and therapies deteriorated (*Brain Stimulation Therapy: The Past, Present, and Future of Treatment through Brain Stimulation | IEEE Brain*, n.d.).

Neuronal depolarization is altered by brain stimulation, which activates activity-related pathways of neuronal plasticity. In turn, activity-associated mechanisms have a significant impact on the post-mitotic organization and functionality of adult neurons (Hogan et al., 2020).

It is essential to distinguish between changes in brain activity produced along by behavioral activity as well as endogenous stimuli vs those caused by exogenous stimulation via electrodes, magnetic coil, or another device (*NIMH » Brain Stimulation Therapies*, 2016).

Through physiological alterations in local ion gradients brought about by synaptic transmission or environmental sensing receptors, endogenous stimulation brought on by physical exercise or sensory input starts brain activity. When administered magnetically or electrically, exogenous stimulation results in changes in ion gradient in a non-physiological and wider scale way. The natural depolarization of such a neuron is undoubtedly started by this kind of stimulation, but it can also have an impact on other systems and result in non-physiologic reactions. Exogenous stimulation methods have the potential to indirectly modify local ion gradients and start other changes that are not evident via natural stimulation of neural pathways, so even though the result may be similar, the question of whether external stimulation is genuinely comparable with exogenously or natural stimulation of neurons remains. Nevertheless, studies on rats and non-human primates have demonstrated that exogenous stimulation can result in spike-timing-dependent plasticity via closed loop stimulation timed with intentional activity or endogenous stimulation (Rebesco, 2010).

1.3 Photon Emission

A photon emission is a probabilistic event, meaning that a probability per unit of time may be used to quantify the chances of its occurrence. The typical amount of time before a photon spontaneously emits from multiple excited forms of atoms is between 109 and 108 seconds. There are numerous natural processes that emit photons. For instance, whenever a charge is accelerated, synchrotron radiation is produced. Photons of different energies, ranging from radio waves to gamma rays, are released during a molecular, atomic, or nuclear transition to a lower level of energy. The procedure through which an arriving photon of a certain frequency interacts with just an excited atom (or other excited chemical state) and causes it to fall to a lower level of energy is known as stimulated emission. In the electromagnetic field, the released energy produces a new photon that shares the same frequencies, polarization, & direction of travel as the photons from the incident wave. In contrast, spontaneous emission happens regardless of the outside electromagnetic field at a predictable rate for every atom and oscillator in the higher energy state (Glenn, 2022).

1.4 Genome Engineering

Genome engineering, which is also called genome editing is a process of doing alteration in any organism's genetic code. It allows to change physical traits such as eye color, hair color, disease risk etc. It basically customizes the genetic makeup utilizing some enzymes. The basic is to engineer the nucleus to target any specific DNA sequence by cutting DNA strands, then removing existing DNA & inserting replacement DNA (*The Human Genome Project*, 2022). It all started when in 1953 double helix was invented and described by Watson, Crick, and Franklin. After that in 1972 invention of recombinant DNA, which showed result in

1981. First transgenic animal was created my Thomas Wagner and his team. Followed by in 1982, first genetically engineered drug- insulin was created, which got FDA approval later. Invention of PCR was done in 1983 and after that Human Genome Project was initiated and completed within 1990s to 2000s. Genome editing/ engineering started to develop more when in 2012, CRISPR was discovered as gene editing tool with the ability to work on nucleotides to participate in scientific discoveries. Engineered genome editing makes it possible to conduct knockout and knock-in experiments in any species where the collection and subsequent transfer of one-cell embryos is feasible, negating the need for the use of embryonic stem cell intermediaries. We provide a list of tools and vendors to aid in the planning, development, and execution of genome engineering research as well as an introduction to the ZFN, TALEN, homing endonuclease, and Cas/CRISPR approaches (Dunn & Pinkert, 2014).

1.4.1 Techniques & Methods Used in Genome Engineering

Genome engineering is a worldwide activity that makes use of a variety of tools and methods. However, the various tools use the same fundamental method: either a guide sequence or specific DNA binding domains inside the nuclease itself lead the enzyme to a specific target region in the genome. The nuclease can be utilized for editing DNA in several ways once it has recognized and bound to the target DNA. Newer methods of gene editing do not create DSBs (double stranded breaks). A single strand of DNA can be cut using designed or inactivated nucleases, or the target sequence can be identified and bound to, which will then guide a separate enzyme to modify the DNA. Scientist have been researching on DNA modification for years (Mah & Roberts, 2022). Here some of the strategies of modifying or editing DNA is mentioned-

Restriction Enzyme: As restriction enzymes are limited in the nucleotide sequences that they can recognize, they are not as commonly employed for editing genes as they once were. Despite this, they are nevertheless routinely utilized in the process of molecular cloning. In addition, particular kinds of restriction enzymes are required to construct DNA libraries, map the epigenome, and map DNA. These enzymes can break certain DNA sequences because they recognize those sequences as being unique. In addition, bacteria and archaea can employ these enzymes to recognize and eliminate foreign DNA in their environment. The DNA was cut in certain locations, and then researchers used restriction enzymes and other tools to introduce new genes at the locations where the DNA was cut (Mah & Roberts, 2022).

Zinc Finger Nucleuses (ZFNs): Zinc-finger nucleases (ZFNs), which can target DNA, have gained popularity as gene-targeting substances. Targeted gene replacement and targeted mutagenesis are typically produced by cellular DNA repair systems that are activated by ZFN-induced double strand breaks (Carroll, 2011).

CRISPR-Cas9 Genome Editing: Gene therapy has gotten a terrible label in the past, but the discovery and development of the CRISPR/Cas9 system has given it a second chance to prove that it is a successful therapeutic strategy and shake off its negative image. Because of their exceptionally high rate of delivery effectiveness, AAV vectors continue to be an essential component of CRISPR gene therapy and are used extensively in the field. The conventional gene therapy approach, which makes use of viruses, has been analyzed for its potential to cause immunotoxicity as well as insertional oncogenesis (Uddin et al., 2020).

Base Editing- Single-base Substitutions: The CRISPR-Cas9 genome editing tool has potential applications in a variety of industries. The CRISPR-Cas9 system provides the foundation for another relatively recent technique for modifying genomes known as base editing. Single-base substitutions via single-stranded oligonucleotide-directed nucleases

(ssODNs) can be employed to add and/or correct disease-associated mutations in order to construct human sickness models that can be used for medication development and disease elucidation. We have explored the best configurations for 'single-stranded oligo DNA nucleotides' (ssODNs) in aspects of blocking mutation, orientation, size, and length of homology arms in order to determine the most effective ssODN design parameters using reporter systems for the identification of single-base substitutions. This was done by looking at the best configurations for single-stranded oligo DNA nucleotides in terms of blocking mutation. Because of this, the efficiency of knock-in using ssODNs has been significantly increased (Okamoto et al., 2019).

Prime Editing: Because it can create all possible transition mutations, insertions, and deletions without producing double-strand breaks (DSBs), prime editing is safer to employ than other methods of gene editing. This makes it extremely helpful for therapeutic applications. The primary editing process adds the desired genetic construct to the gRNA, which is then converted to DNA by the RT enzyme. This allows the desired genetic construct to be delivered (Matsoukas, 2020).

1.5 Optogenetics

The use of light and genome engineering to regulate a neuron's activity is known as optogenetics. Scientists can alter a living thing's genetic code through a method called genome engineering. Due of its widespread application in researching the neural circuitry of the brain, it is currently regarded as a subfield of biotechnology. Without the evolutionary perspective of optogenetics, it would have been impossible to understand the brain (Singh, 2022).

When conducting experiments using optogenetics, researchers insert a whole new section of genetic code into the DNA of the neurons they are interested in studying. Because of this new coding, these neurons now have the ability to make opsins, which are singular proteins that have a specific response to a certain wavelength of light (for example, ChR2 only responds to blue light). The field of neurology uses these proteins to control the activity of neurons, which are triggered by the presence of light (Lim & LeDue, 2017).

Utilizing a range of light-sensitive proteins originating from microbes or plants, recent developments in "optogenetics" have made it feasible to precisely alter biochemical as well as electrical signal pathways in cells (Pastrana, 2013).

This started when in 1979 Francis Crick had an idea that light might be used to quickly control spatiotemporal targeting of specific neurons. Which gave rise to the idea of optogenetics for neuroscience, but at the time, neuroscientists lacked the knowledge necessary to use photosensitive proteins in this way (Joshi et al., 2020).

Recent developments in optogenetics have completely changed the discipline of neuroscience by making it possible to analyze the causal link between neural activity and behavior. Powerful tools for comprehending the operation of neural circuits are made available by the increase of the list of optogenetic actuators and sensors, as well as transgenic lines expressing these actuators. These technological developments also make it possible to apply optogenetics to other behavioral situations, such as zebrafish that can move freely and adults that use transparent zebrafish lines (Tsuda, 2020).

Optogenetic modules can be modularly engineered into host cells to regulate physiological processes by light-switchable allosteric regulation, oligomeric transitions, protein-protein heterodimerization, and self-cleavage. To control transcription of genes, DNA & RNA modifications, DNA recombination, and genome engineering, a variety of genetically

encoded non-opsin photosensory modules have been utilized. These modules use photons emitting in the broad range of 200–1000 nm when engineered into protein machineries that control the cellular information flow as shown in the central dogma (Lan et al., 2022).

1.5.1 Key Tools in Optogenetics

Opsin: Optogenetics offers methods for genetically modifying the use of light to control the activity of excitable cells. Opsins, which are light-activated proteins (channels or pumps) that allow ion transmembrane transport, are microbial proteins that are used in the technique. Opsins come in two different varieties: Type I, which is present in prokaryotes, algae, and fungus, and Type II (found in animals) (Ferenczi et al., 2019).

Suitable Vector (Plasmid/viral): To distribute vectors containing genetically modified or desired genes, researchers create direct and indirect delivery techniques. Viral vectors including adenoviruses, lentiviruses, and adeno-associated viruses are utilized in most gene delivery techniques because they are more efficient than plasmid vectors at delivering genes (Singh, 2022).

Light Source: The needed light wavelength and intensity are the two most important parameters to take into account when choosing an optogenetic light source. The opsin you choose has a certain activation spectrum, meaning that it can only be activated by a specified range of light wavelengths. To achieve optimal activation, it is therefore better to pick a light source that is closer to the peak wavelength. For instance, many studies will employ wavelengths about 470 nm to activate ChR2 optogenetically. Second, the field of view and the opsin you choose will determine how much light is needed for your experiment (Cloke, 2021).

1.6 Purpose of the Study

The purpose of this review paper is to summarize and to highlight the utilization of light/photon emission in genome engineering, which is also called optogenetics. The present methods for stimulating the brain, such as deep brain stimulation (DBS), transcranial direct current stimulation, and transcranial magnetic stimulation, are unable to target a particular cell type or neuronal circuit. This issue has been solved by optogenetics, which allows for precise temporal alterations to investigate the mechanisms behind neurological illnesses. By using optogenetics instead of electrical stimulation, excitable cells can have their action potentials triggered or altered. Also, optogenetics is used in other fields of medical for the betterment of the industry. In this review paper, it has been tried to present all the possible techniques used to utilize light/ emitting photons in genome engineering which are carried out by different researchers.

Chapter 2

Methodology

This review study is conducted using current and pertinent research papers and articles from high-impact journals. Peer-reviewed journals, official papers, and articles were all thoroughly searched. To enhance the review article, basic and supplementary information was gathered from numerous sources. Some search engines have been used to gather data for this review paper- which are Scopus, Science- Direct, Springer & PubMed. The major publication which was focused on to retain needed data are- Nature, Frontiers, Meghtex etc. A thorough screening of journals was done before focusing on the most recent and pertinent ones to create an ideal quality review on the "Combination of Photon Emission and Genome Engineering in Brain Stimulation."

Chapter 3

Result& Discussion

3.1 Optogenetic Actuators and Components for Stimulation of Brain

When a cell is exposed to light, optogenetic actuators change the activity of the cells in within which they are expressed. With millisecond control over event timing, these actuators can create a single or also can create multiple nerve impulses, reduce brain activity, or alter biochemical signaling pathways (Guru et al., 2015). Opsins are the most powerful and often employed actuators. Opsins can either be utilized in the same way they are found in nature, or they can be modified to function more efficiently. Vertebrate opsins, also known as Type II opsins, and microbial opsins, also known as Type I opsins, are the two most common types of opsins found in nature (Type II). There are prokaryotic and eukaryotic species of microbiological organisms that have type I opsins. Type I opsins may be found in bacteria, archaea, and algae. They are made up of a single protein component that is linked to the membrane and functions as either a pump or a channel (Nagel et al., 2002).

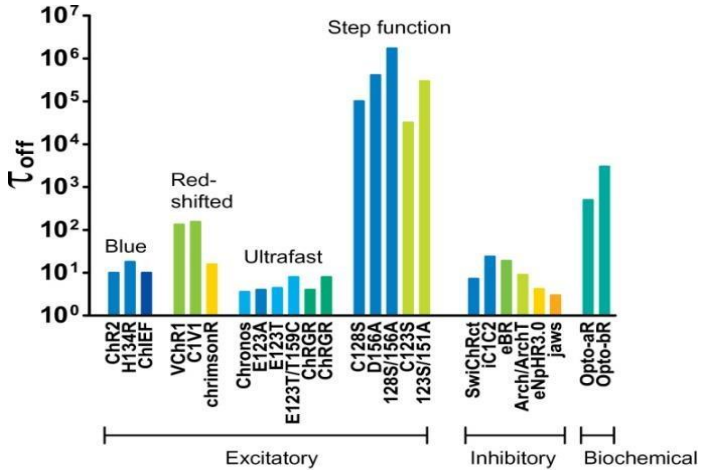

Figure 1: Illustration of some of the currently available optogenetic actuators (Fenno et al.,2011).

3.1.1 Opsins

With the invention of opsins like ChR2, optogenetics had its start. Opsins are basically light-sensitive channels that generate depolarization as well as hyperpolarization of neurons by ion influx or protein signaling cascades, among other methods. Microbial opsins (type I) & animal opsins are the two separate superfamilies that make up the opsin gene family (type II). Both kinds of opsin proteins are in need of retinal, an organic cofactor linked the vitamin A that acts as a photon antenna; when retinal is attached, the functioning opsin proteins are known as rhodopsins. Through the formation of a protonated retinal Schiff base (RSBH+), retina forms a covalent bond with a conserved lysine residue of helix 7. Even though both opsin groups encode seven-transmembrane structures, there is very little sequence homology in between two families. On the other hand, there is a lot of homologies within families (between 25% and 80% residue similarity) (Man, 2003). Type I opsins, on the other hand, frequently encode proteins that use retinal in an all-trans configuration, that photo isomerizes on photon absorption towards the 13-cis configuration. In contrast to type II rhodopsins, type I rhodopsins thermally revert to their all-trans state while preserving a covalent link with their protein partner, preventing an activated retinal molecule from dissociating from its opsin protein. Opsins can either activate or inhibit brain activity because they are sensitive to certain light wavelengths. As an illustration, blue light stimulates ChR2, which results in an influx on Na+ ions & depolarizes the cell (Boyden et al., 2005).

Opsins have been found to control the on/off neurons at various speeds and with various light wavelengths. Table 1 shows the difference between these-

Opsin Proteins	Wavelength for excitation	Functionality
ChR2	470nm	Activating
GtACR2	470nm	Inhibiting
Arch T	540nm	Inhibiting
C1v1	560nm	Activating
NpHr	590nm	Inhibiting
bReaChES	590nm	Activating
Chrimson	590nm	Activating
ReaChR	620nm	Activating
JAWS	620nm	Inhibiting

Table 1: Different opsins having different wavelength and functionality (Cloke, 2021a).

3.1.2 Biological Components Needed for Optogenetics.

Understanding the biological components is the first step to performing optogenetics successfully. This entails picking the ideal optogenetic probe, inserting an optical cannula, and producing the optogenetic probe inside the targeted brain area and neurons. Choosing the right optogenetic probe is an essential initial step in optogenetic experiments. The optogenetic probe must be expressed by the brain after being chosen. Viral expression &

transgenic mice models are two strategies used by neuroscientists to produce constructs in the brain. Injecting a virus with an opsin expressing gene into the brain is known as viral expression (Cloke, 2021b).

To control opsin expression or to confine it to a specific area of the brain, neuroscientists use viral expression. Because expression can differ based on the brain area, cell type, or virus, this is helpful. Additionally, to map neuronal circuits across different brain areas, neuroscientists can utilize viral expression to generate opsin into brain projections. Transgenic mouse models are created to express opsins all through the entire brain, as opposed to viral injections. Based upon that transgenic model, the expression of the opsin can differ between regions, with one region occasionally expressing the opsin at a higher level than the other. To create more reliably replicating mice lines for optogenetic production in particular cell-types, transgenic mouse models could be used. Or, since they call for considerably more extensive expression than just a particular region of interest, these models are used by neuroscientists studying huge cortical areas (Mei & Zhang, 2012).

3.1.3 Equipment Components

Optogenetic investigations are conducted using the microscope and freely behaving experimental setups. There are distinctive elements in each of these two methods for optogenetic activation (Cloke, 2021b). A light source as well as a filter set are the two major elements needed to include optogenetics in these microscopy setups. It is crucial to choose the right source of light for the optogenetic probe because it will be utilized to brighten the sample and operate the probe. Every back epi-fluorescence port of the majority of microscopy setups will be wired with a collimated light source to illuminate the sample. The light source may be coupled directly to the backport or via a lightguide or fiber to an epi-

fluorescence port, depending on the application. The epi-fluorescence port directs light to the filtering turret, where it is focused by the objective onto your sample. The field of vision and intensity of the light source used for optogenetic stimulation will be determined by the objective. As previously discussed, before reflecting onto the sample, the light will be sent to the filter turret. Therefore, it is essential to choose the correct filter set to send the appropriate wavelength towards the sample and, depending on the experiment, to prevent this from reaching the camera (van Haren et al., 2018). An approach to evaluate the causal relationship between brain circuits and behavior is offered by optogenetics. As a result, unlike microscopy investigations, this procedure must be carried out by scientists on a freely moving animal. For freely behaving tests, optical cannula, fiber-optic cable, as well as a light source are the three essential parts. As was already explained, to shine light into the brain, the optical cannula gets inserted into the area of interest and expresses the optogenetic probe. The optical fiber has been normally linked to a fibres light source and coupled to an optical cannula. This makes it possible for light to move from light source to a brain of the animal that is acting freely. The animal may behave naturally while the brain is illuminated for optogenetic stimulation thanks to a flexible optical wire (Cloke, 2021b).

3.2 Channel Rhodopsin Working on CNS Neuron Activation

Recent advances in neuroscience research have led to the development of optogenetics, which uses optically stimulated proteins to regulate neural function. With a level of geographical, temporal, and neurochemical accuracy never achieved, optogenetic techniques enable the activation or inhibition of specific groups of neurons. A light-activated cation channel called channelrhodopsin-2 (ChR2) is an algal protein of Chlamydomonas reinhardtii that can cause depolarization as well as action potentials in neurons (Britt et al., 2012).

When triggered, channelrhodopsin-1 (ChR1), which is energized by blue light, allows selective cation influx through into cell. The first commonly used optogenetic instrument, channelrhodopsin-2 (ChR2), is also a cation channel that is activated by blue light. Because ChR2 has a greater permeability at physiological pH that traffics better to the membrane, it is favored to ChR1 in most situations. We discovered that ChR2 could trigger neuronal depolarization and could be produced safely and reliably in mammalian neurons. ChR2 was reliably activated by a series of short light pulses and mediate predetermined spike trains or synaptic events having temporal resolution on the millisecond timescale. As a result, the basic units of brain computation can now be controlled optically in the same temporal domain. It is done to generate lentiviruses bearing a ChR2-yellow fluorescent protein (YFP) fused protein for genetic alteration of neurons to obtain consistent and dependable ChR2 expression for connecting light to neuronal depolarization. ChR2 was expressed for weeks after infection in membrane-localized form in cultured rat CA3/CA1 neurons. Despite ChR2 being inactive after prolonged exposure to light we saw that peak ChR2 photocurrents quickly returned in neurons. This quick recovery is in line with retinal's ability to re-isomerize to the completely trans initial state in a dark reaction without the need for additional enzymes, in addition to the well-known consistency of the Schiff base in microbial-type rhodopsins. After 1 hour of pulsed light exposure, patch-clamped neurons' light-evoked current amplitudes were unaffected (data not shown). As a result, ChR2 was able to mediate rapid activation kinetics, large-amplitude photocurrents, and sustained (Boyden et al., 2005).

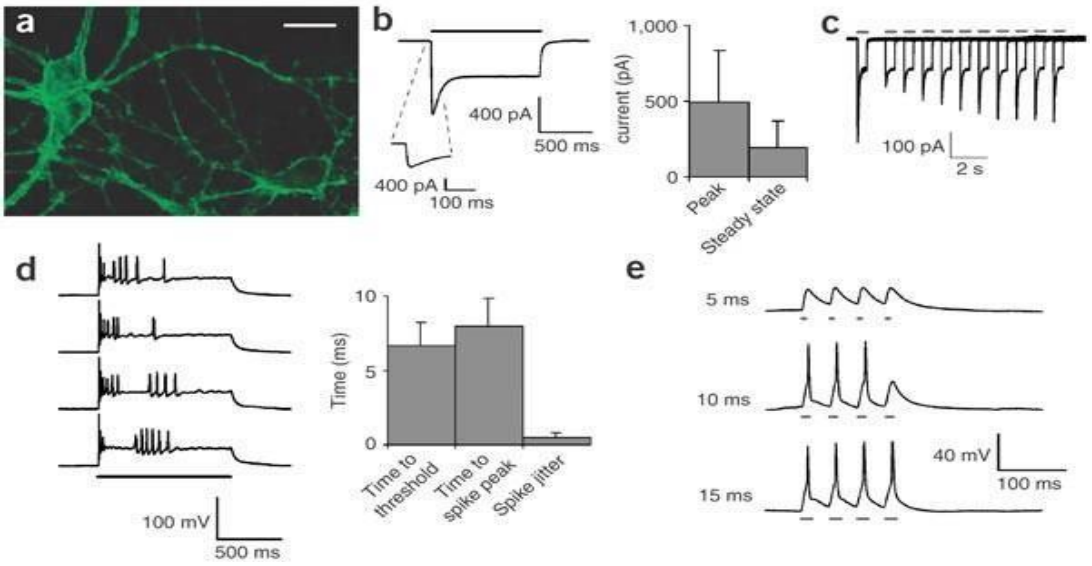

Figure 2: ChR2 enables light driven neural spiking (Boyden et al., 2005).

It was noticed that the single spike reliably generated by continuous illumination had extraordinarily low temporal jitter from trial to trial, as evidenced by the modest standard deviation of the spike durations across trials, while looking for a method to trigger precisely timed series of spikes with ChR2. The methodology described here exhibits voltage control much more quickly than other genetically encoded photo stimulation techniques by combining the best features of earlier technologies that employ light to drive neural circuits. Notably, neither the ChR2 technique nor the genetic orthogonality of a transgene as well as the host organism depend on artificial chemical substrates. All-trans retinal is a necessary cofactor for the ChR2 molecule to transmit light, but none of the experiments discussed here introduced any to the culture media or recording solution (Lima & Miesenböck, 2005).

ChR2 can be expressed in specific subgroups of neurons throughout the nervous system using genetic techniques such as lentiviral vectors and in transgenic mice because it is only encoded by just a single open-reading frame of 315 amino acids. This makes it possible to study the function of different kinds of cells throughout intact neural circuits as well as in vivo. ChR2 may be directed to a variety of clearly defined neuronal subtypes with the use of cell-specific

promoters, allowing for further investigation of their causal role in regulating animal behavior and downstream neural activity. In response to realistic spike trains/ rhythmic activity, ChR2 may also be employed to resolve the functional connections of neurons in intact circuits (Boyden et al., 2005).

3.3 Post-Stroke Functional Recovery is Aided by Optogenetic Neural Activation

When anything prevents blood flow to a brain area or whenever a blood artery in the brain bursts, a stroke, also known as a brain attack, happens. The brain either ages or suffers harm in both scenarios. A stroke may result in permanent brain damage, chronic disability, or even fatality. The brain is the organ that manages our bodily activities, retains our memories, and generates our ideas, feelings, and verbal expression. In addition, the brain regulates a variety of bodily processes, including respiration and digestion. You need oxygen for your brain to function correctly. All of the areas of your brain receive oxygen-rich blood from your arteries. Brain cells begin to die in minutes of a blockage in blood flow because they are unable to receive oxygen. The result is a stroke.

A stroke is a serious acute neurological injury that destroys neurons and impairs brain function. After a stroke, functional recovery has been seen, and is now ascribed towards both brain reorganization and plasticity. There are extremely few treatment options for stroke, though it is the chief reason for disability in the USA. Research into brain stimulation methods that aid stroke recovery is promising, however the target area is often unintentionally activated or inhibited by existing stimulation methods, making it challenging to identify the types of cells and mechanisms that underlie recovery.

Optogenetics is utilized to selectively target neurons that produce channelrhodopsin 2 to get over these obstacles, and it has been shown that such stimulations can help with functional recovery mostly in ipsilesional primary motor cortex (iM1). Repeated neuronal stimulations after stroke significantly improved cerebral blood flow as well as the neurovascular coupling response, and increased the expression of activity-dependent neurotrophins, such as neurologically neurotrophic component, nerve growth factor, and neurotrophin 3, in the contralesional cortex (Cheng et al., 2014).

In rats, optogenetic methods have been utilized to examine neural circuits in the context of Parkinson disease and epilepsy, among other neurological/neurodegenerative illnesses. Optogenetics has also been employed in recent studies to assess functional organization following stroke. Optogenetics' effectiveness and safety in nonhuman primates have also been studied (Gradinaru et al., 2009).

3.3.1 Neural/ Brain Stimulation Activating Peri-infract Areas and Contractional M1 & Repeated Stimulation Enhancing Cerebral Blood Flow in Stroked Mice.

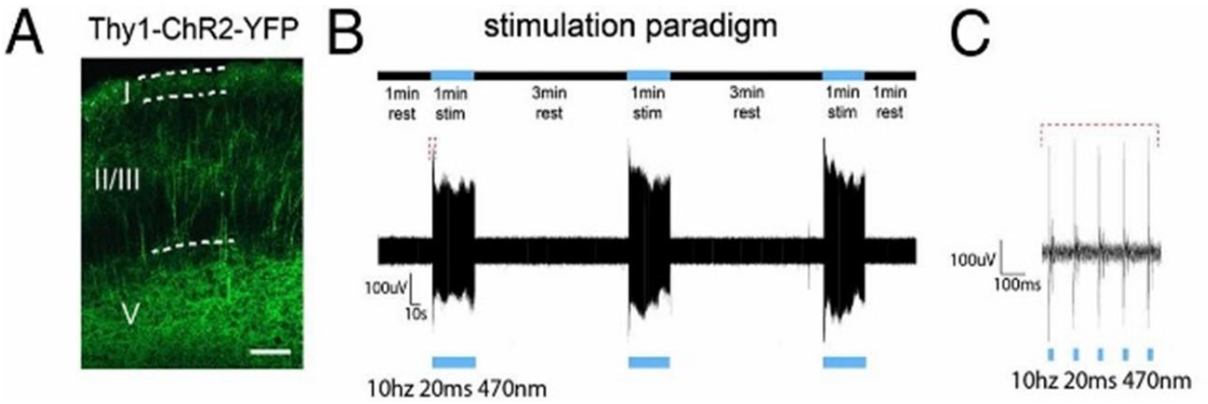

Figure 3a: Neural/ brain stimulation activating peri-infract areas and contractional cortex(Cheng et al., 2014)

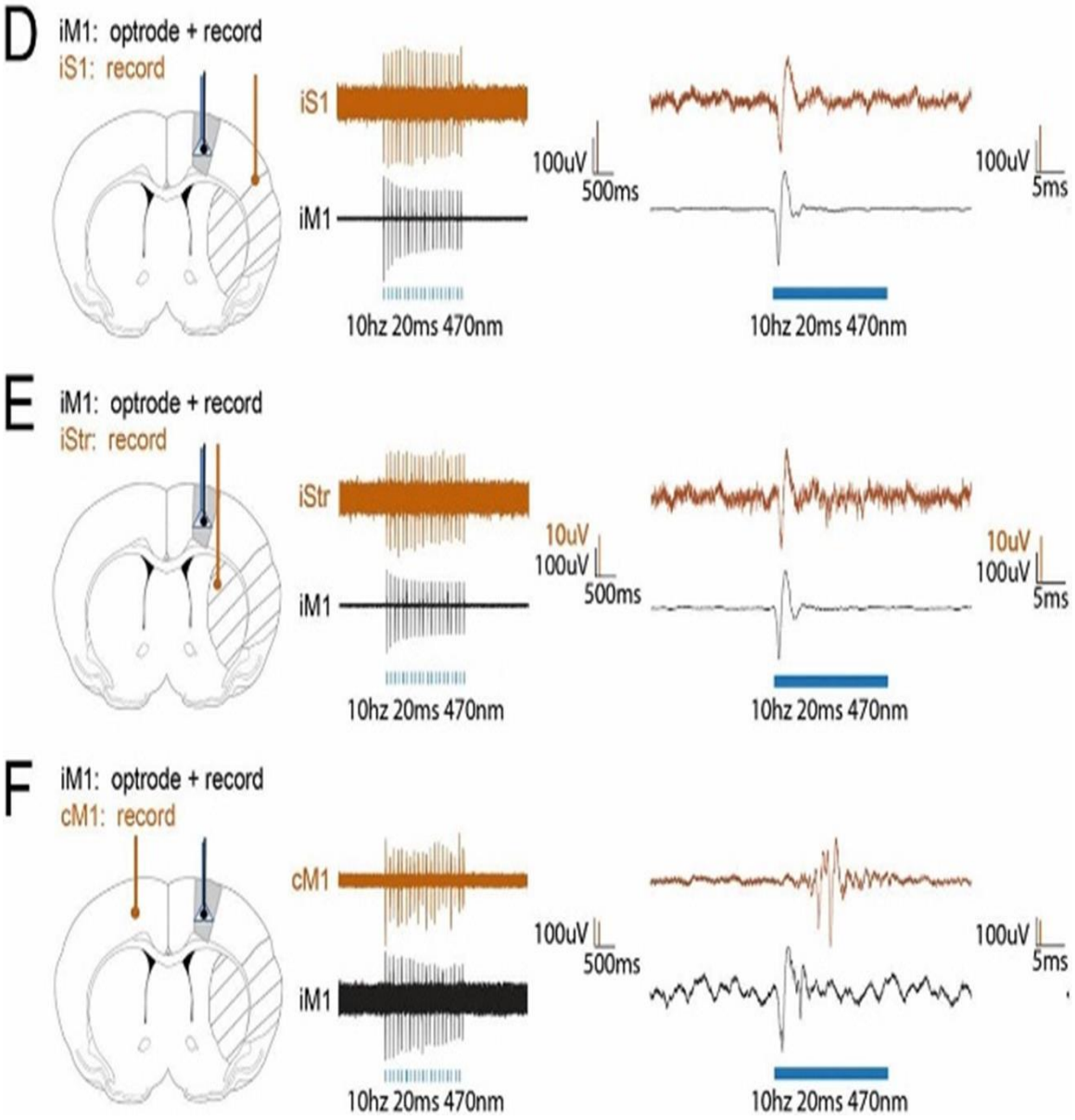

Figure 3b: Neural/ brain stimulation activating peri-infract areas and contractional cortex(Cheng et al., 2014)

In Fig. 1A, transgenic Thy-1-ChR2-YFP line-18 mice were used. ChR2 is highly expressed on layer V primary motor cortex neurons in these mice. The striatum and somatosensory cortex were infarcted using the temporary posterior cerebral artery blockage concept shortly after an optical fiber was stereotaxically placed above layer V of the iM1 brain (S1). It was initially tested to see if the iM1 neural stimulation paradigm—which involves three

sequential brief initial laser stimulations separated by three-minute rest intervals—could consistently activate neurons. The figures in Figures 1B and 1C show this paradigm. In Fig. 1C, individual spiking is shown. Electrical optrode recordings made in vivo in the iM1 brain region showed that this stimulation technique might result in reliable and consistent firing rhythms throughout all three stimulations. The dual recording method was used to demonstrate that iM1 stimulation may activate peri-infarct regions and contralesional M1 (cM1). It involved inserting an optrode into iM1 as well as recording an electrode in the ipsilesional striatum, ipsilesional somatosensory cortex, or contralesional M1 (cM1). Figures 1D, 1E, and 1F demonstrate how iM1 neural stimulation may activate peri-infarct regions (Str and S1) together with the cM1 and indicate that dependable firing in iS1, iStr, and cM1 may be produced by iM1 stimulation (Cheng et al., 2014).

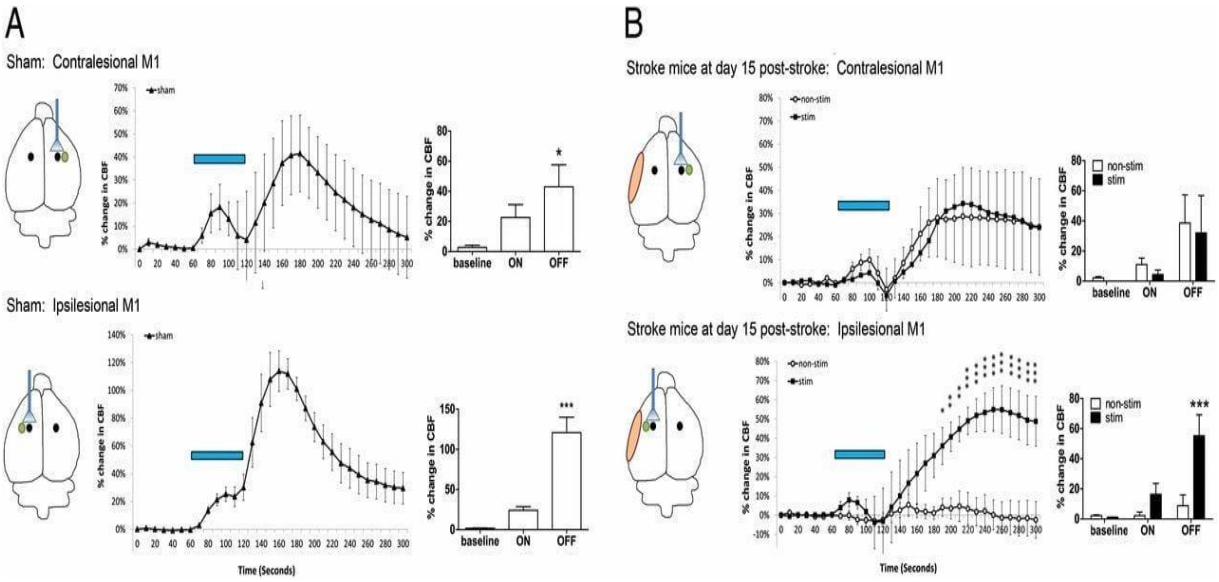

Figure 4: Repeated M1 neural stimulation improving CBF after stroke (Cheng et al., 2014)

First, it was investigated if repeatedly stimulating iM1 neurons would result in an increase in CBF and the neurovascular coupling responses (Fig. 3). he diagrams show the stimulation site, the CBF measurement site, and the stroke site (Fig. 3). CBF increased in the

contralesional as well as ipsilesional hemispheres of the sham group after the 1-min stimulation and then increased noticeably more during the 3-min post-stimulus interval (Fig. 3A). Stroke animals, however, failed to elicit the significant rise of CBF poststimulation in the both contralesional & ipsilesional hemispheres on poststroke day 5 despite relatively high CBF following contralesional stimulation even during 1-min stimulation interval (Fig. S1). This is in line with the current theory that a stroke results in a general decrease in brain blood flow and excitability. At day 15 of poststroke, activated stroke mice demonstrated an enhanced CBF/neurovascular coupling response mostly in ipsilesional hemisphere (Fig. 3B), in contrast to non-stimulated stroke mice who stayed non - responsive to a 1-min laser stimulation and displayed no significant transformation in ipsilesional CBF during or after the 1-min stimulation (Cheng et al., 2014).

The results of the study show that activating recovery-promoting pathways just requires stimulating neurons. Through several repair/plasticity-related mechanisms, such as improved CBF/neurovascular coupling and higher neurotrophin expression, these particular neural stimulations can stimulate movements in the injured forelimb and facilitate functional recovery. Finding prospective pharmacological targets for stroke treatment will be made easier by understanding the mechanisms behind recovery. The work also offers, to our knowledge, the first proof-of-concept application of optogenetics to encourage stroke recovery. The use of gene therapy techniques would be necessary to ascertain if optogenetic stimulation could be used clinically in the future to treat stroke sufferers.

3.4 Brain Mapping Using Optogenetics.

Optogenetic mapping provides several options for understanding how the brain works. With the development of optogenetic techniques and the discovery or production of new opsins, there is potential for more control within brain stimulation studies. Using many opsins, we might be able to govern a variety of different types of neurons at once. Since each opsin responds to a certain form of light, we may employ numerous lights to govern diverse types of neurons. The truth is that certain opsins work to turn neurons off when the right sort of light is present (Lim & LeDue, 2017). By combining light and genetic engineering, optogenetics may manipulate a biological system. For this reason, a wide range of different recombinant can be added into neural systems. The non-selective cation channel Channelrhodopsin-2 (ChR2), which opens in response to blue light, is one of the proteins that has been used most extensively in neuroscience (Nagel et al., 2002).

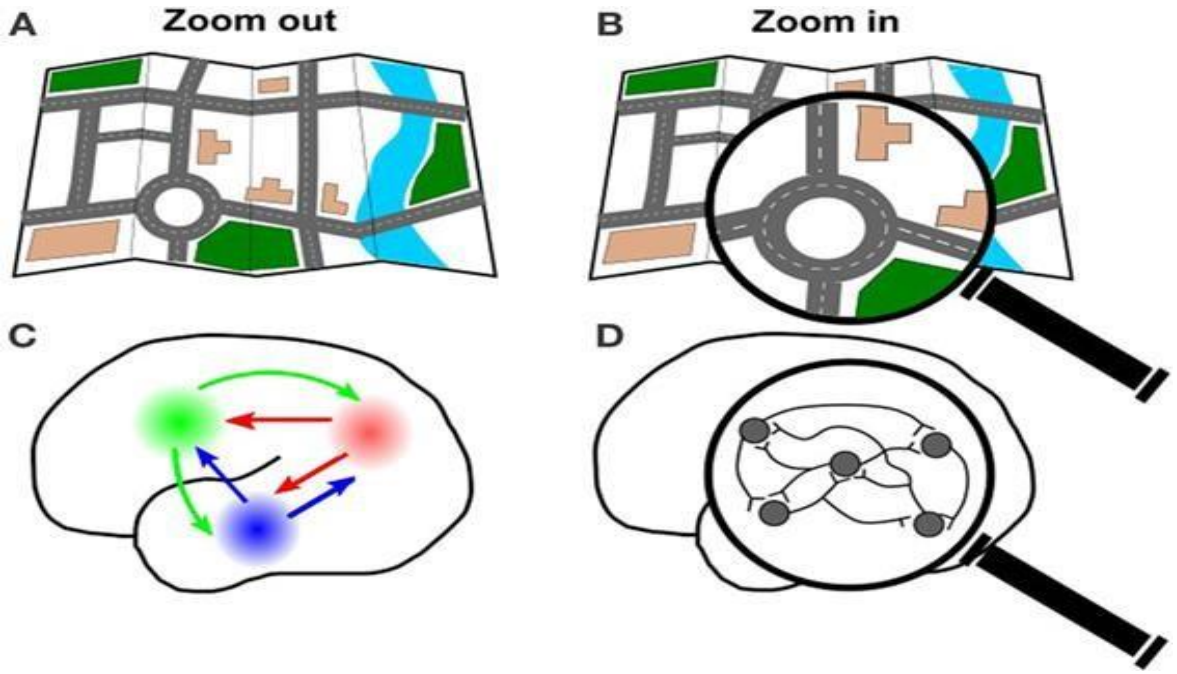

Figure 5: Optogenetic brain mapping (Lim & LeDue, 2017)

We may also enlarge the brain to view the connections between specific neurons (Figure 3D). Using light to turn on some neurons and recording the responses of the other neurons, optogenetics allows us to study how the neurons cooperate. Knowing where and why the neuron communicate with one another is made possible by this in-depth picture. This could be particularly helpful for researching conditions that affect how neurons communicate in a particular region, which is exactly happens when someone suffers from stroke. Blood typically transports oxygen and other critical nutrients into the brain. The brain does not receive the nutrients it needs to operate correctly when the blood flow is compromised or diminished. This is referred to as a stroke, and it may result in malfunction and long-term issues (Lim & LeDue, 2017).

SI No.	**Available Optogenic techniques**	**Advantages**
1	E. phys. single cells with ChR2 activation and CRACM	Excellent temporal resolution able to define connections particular to layers (CRACM)
2	Opto-fMRI	Generally vague BOLD signal
3	Light-based motor mapping via ChR2	Minimally invasive for long-term investigations
4	In vivo ChR2 activation and VSD imaging	Excitation of the VSD does not activate ChR2

Table 2: Optogenetic mapping techniques (Lim et al., 2013)

To examine the functional characteristics of specific individuals and group of neurons throughout response for single-point electrical stimulation, electrophysiological recordings were initially used (Scanziani & Häusser, 2009).

Recently, optogenetics and electrophysiological observations have been used to study cell-type-specific responses in a small network. Through several simultaneous whole-cell recordings, responses of GABAergic fast-spiking (FS) as well as GABAergic non-fast-spiking (NFS) cell were examined after photostimulating Layer 2/3 excitatory neurons that express ChR2. In response to layer 2/3 neuron stimulation, FS GABAergic neurons showed large-amplitude depolarizing postsynaptic potentials, whereas NFS GABAergic neurons showed small-amplitude subthreshold postsynaptic potentials. According to the scientists, the barrel cortex's excitatory neuron inhibition is largely mediated by FS GABAergic neurons. The capability to observe functional synaptic connection and the application of optogenetic techniques within an in vitro brain slice sample are both demonstrated in this paper (Avermann et al., 2012).

Light-based mapping (LBM), a novel technique for in vivo mapping of the mouse sensorimotor cortex, has been created by researchers. This approach uses optogenetics to capture evoked motor movements and activate the sensorimotor cortex using high spatiotemporal resolution in order to understand cortical function. LBM selectively activates neurons that express ChR2, enabling more precise control over the connections that are activated (Figure 2B). Although ICMS can be utilized to map a sensorimotor cortex reasonably well, elevating and decreasing the activating electrode at each spot requires time, and electrode implantation will damage surrounding tissue and might affect cortical function. LBM, in contrast, allows for recurrent mapping across time without the possibility of tissue damage linked with ICMS, and thousands of cortical locations can be optically activated in a short amount of time (Harrison et al., 2012).

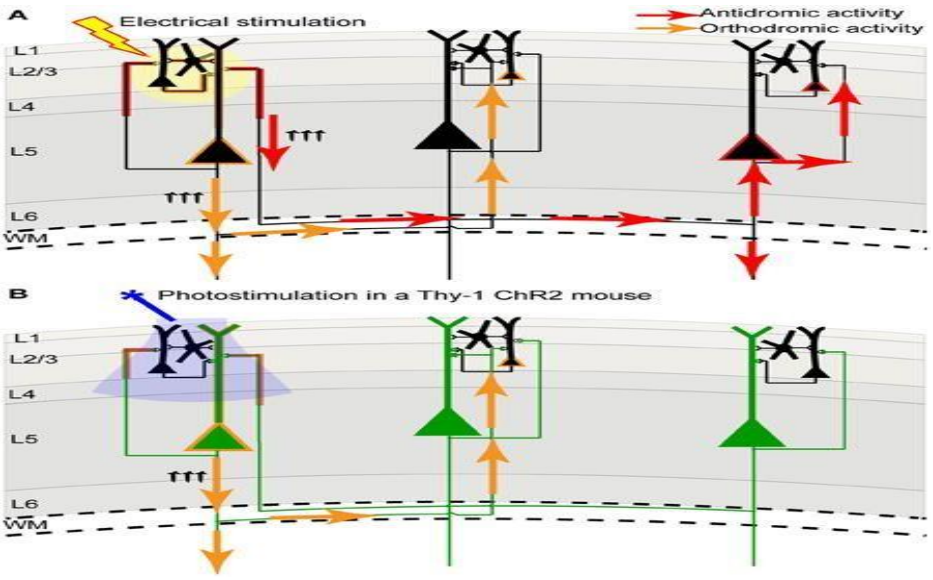

Figure 6: ChR2 stimulation in the THY-1 transgenic mouse may be more specific than directelectrical stimulation (Lim & LeDue, 2017)

To further improve our knowledge of brain function and malfunction, future studies may also take into account the developing brain, a wounded brain, learning, and plasticity models. The methods discussed here could be used to determine abnormal functional relationships between different cortical areas throughout disease models, particularly for conditions like autism in which the underlying mechanism is unclear (Qiu et al., 2011), or they could be used to examine and track recovery following a brain injury like a stroke (Krakauer et al., 2012).It is also conceivable that neural connection maps and maps of a cerebrovasculature will someday be coupled in order to comprehend the impact of disruptions on flow even during relatively minor strokes (Tsai et al., 2009).

3.5 Discussion

The methods of brain stimulation that are now in use, such as deep brain stimulation (DBS), transcranial direct current stimulation (tDCS), and transcranial magnetic stimulation (tMS), do not have the capability of targeting specific cell types or neural circuits. Optogenetics has provided a solution to this issue and enables manipulations with temporal precision, which enables researchers to better understand the underlying mechanisms of neurological illnesses. However, in order for it to be useful for people, a number of other related scientific fields, such as gene therapy, opsin engineering, including optoelectronics, will need to advance as well. Recent advancements in brain interface technologies have produced findings that are adequate for human study (Mahmoudi et al., 2017).

The development of a sufficient quantity of opsins is the primary obstacle for the adoption of optogenetics in people. These opsins need to be triggered for neural stimulation and the extraction of certain behaviors without causing damage from heat. There is a possibility that an infrared opsin or one with a high photocurrent would be appropriate for the job. In addition to this, a light source that generates little heat needs to be devised.Optogenetics, in comparison to other methods of brain stimulation, offers a greater number of benefits and fewer adverse effects, despite the challenges that have been outlined above. Optogenetic stimulation offers a high spatial-time resolution for the stimulation of individual light spots. It is anticipated that such an optically-based biological technology would, in the near future, be implemented in clinical settings for the purpose of providing more precise treatment in brain diseases (Sung et al., 2022).

Chapter 4

Conclusion

Optogenetic is a technique that combines photon emission and genetic engineering to better understand the unique neural function that contributes to an organism's biology and behavior, including such anxiety, fight, desire, and disease conditions. By utilizing a protein called opsin, it aids in mapping the brain circuits and offers deeper understanding into the function of a particular brain region or cell in regulating a certain behavior or set of disease conditions. Numerous investigations of animal behavior, physiology, cardiology, and neurology make use of optogenetic technology. In many clinical settings, involving cardiac and therapeutic disorders, its application is still evolving. As a result, current research focuses on improving optogenetic stimulation's effectiveness in clinics. Many riddles of our brains will be solved by technology with the introduction of opsins with improved light sensitivity, sufficient light devices, and larger models. To conclude, the application of optogenic brain stimulation for a variety of neurological diseases will increase with continued developments in this quickly developing field and promising results from clinical trials.

Future Prospect

The continued development of more effective opsins that can be personalized for every experimental requirement, improved techniques for trying to target one or more opsins on specific cells, better methods of concentrating light upon an individual cells or multiple cells in a particular temporal pattern, everything in freely behaving animals, may facilitate scientific advancement across many fields. Optogenetics could be able to assist paralyzed people regain their ability to move. To do this, modified motor neurons would need to be activated by light, which would then cause muscles to contract. Another option would be to

change the muscle tissue themselves and then utilize light to stimulate contractions by shining it directly on them. With this system, we may detect brain impulses, for example, linked with bending an arm, and then react by lighting the relevant muscles, allowing the arm to stretch. These experiments are currently only carried out on model species like mice and fish. This is because rhodopsins, the proteins at the core of the technique, need to be delivered into cells by viruses. The gene for a certain protein is introduced into the cell by a virus, which causes the cell to begin making the protein. Gene therapy is a treatment that is either heavily regulated or outright prohibited in many nations. The trials that are now being conducted should help to calm the patients' fears around the possibility of receiving foreign genes. It has become a matter of obtaining approval for the broad usage of such protein delivery techniques because the technology is highly safe.

Reference:

Avermann, M., Tomm, C., Mateo, C., Gerstner, W., & Petersen, C. C. H. (2012). Microcircuits of excitatory and inhibitory neurons in layer 2/3 of mouse barrel cortex. *Journal of Neurophysiology*, *107*(11), 3116–3134. https://doi.org/10.1152/jn.00917.2011

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., & Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neuroscience*, *8*(9), 1263–1268. https://doi.org/10.1038/nn1525

Brain Stimulation Therapy: The Past, Present, and Future of Treatment through Brain Stimulation | IEEE Brain. (n.d.). Retrieved February 7, 2023, from https://brain.ieee.org/topics/brain-stimulation-therapy-the-past-present-and-future-of-treatment-through-brain-stimulation/

Britt, J. P., McDevitt, R. A., & Bonci, A. (2012). Use of Channelrhodopsin for Activation of CNS Neurons. *Current Protocols in Neuroscience*, *58*(1). https://doi.org/10.1002/0471142301.ns0216s58

Carroll, D. (2011). Genome Engineering With Zinc-Finger Nucleases. *Genetics*, *188*(4), 773–782. https://doi.org/10.1534/genetics.111.131433

Cheng, M. Y., Wang, E. H., Woodson, W. J., Wang, S., Sun, G., Lee, A. G., Arac, A., Fenno, L. E., Deisseroth, K., & Steinberg, G. K. (2014). Optogenetic neuronal stimulation promotes functional recovery after stroke. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(35), 12913–12918. https://doi.org/10.1073/PNAS.1404109111/SUPPL_FILE/PNAS.1404109111.SM04.WMV

Cloke, J. (2021a). *Optogenetic Stimulation: The Ultimate Guide | Mightex*. https://www.mightexbio.com/optogenetic-stimulation/

Cloke, J. (2021b). *What Equipment Do You Need to Perform Optogenetics?* https://www.mightexbio.com/optogenetics-equipment/

Dunn, D. A., & Pinkert, C. A. (2014). Gene Editing. In *Transgenic Animal Technology* (pp. 229–248). Elsevier. https://doi.org/10.1016/B978-0-12-410490-7.00008-6

Fenno, L., Yizhar, O., & Deisseroth, K. (2011). The Development and Application of Optogenetics. *Annual Review of Neuroscience*, *34*(1), 389–412. https://doi.org/10.1146/annurev-neuro-061010-113817

Ferenczi, E. A., Tan, X., & Huang, C. L.-H. (2019). Principles of Optogenetic Methods and Their Application to Cardiac Experimental Systems. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01096

Glenn, S. (2022). *Light | Definition, Properties, Physics, Characteristics, Types, & Facts | Britannica*. https://www.britannica.com/science/light

Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M., & Deisseroth, K. (2009). Optical Deconstruction of Parkinsonian Neural Circuitry. *Science*, *324*(5925), 354–359. https://doi.org/10.1126/science.1167093

Guru, A., Post, R. J., Ho, Y.-Y., & Warden, M. R. (2015). Making Sense of Optogenetics. *International Journal of Neuropsychopharmacology*, *18*(11), pyv079. https://doi.org/10.1093/ijnp/pyv079

Harrison, T. C., Ayling, O. G. S., & Murphy, T. H. (2012). Distinct Cortical Circuit Mechanisms for Complex Forelimb Movement and Motor Map Topography. *Neuron*, *74*(2), 397–409. https://doi.org/10.1016/j.neuron.2012.02.028

Hogan, M. K., Hamilton, G. F., & Horner, P. J. (2020). Neural Stimulation and Molecular Mechanisms of Plasticity and Regeneration: A Review. *Frontiers in Cellular Neuroscience*, *14*. https://doi.org/10.3389/fncel.2020.00271

Joshi, J., Rubart, M., & Zhu, W. (2020). Optogenetics: Background, Methodological Advances and Potential Applications for Cardiovascular Research and Medicine. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00466

Krakauer, J. W., Carmichael, S. T., Corbett, D., & Wittenberg, G. F. (2012). Getting Neurorehabilitation Right. *Neurorehabilitation and Neural Repair*, *26*(8), 923–931. https://doi.org/10.1177/1545968312440745

Lan, T.-H., He, L., Huang, Y., & Zhou, Y. (2022). Optogenetics for transcriptional programming and genetic engineering. *Trends in Genetics*, *38*(12), 1253–1270. https://doi.org/10.1016/j.tig.2022.05.014

Lim, D. H., & LeDue, J. (2017). What Is Optogenetics and How Can We Use It to Discover More About the Brain? *Frontiers for Young Minds*, *5*. https://doi.org/10.3389/frym.2017.00051

Lim, D. H., LeDue, J., Mohajerani, M. H., Vanni, M. P., & Murphy, T. H. (2013). Optogenetic approaches for functional mouse brain mapping. *Frontiers in Neuroscience*, *7*(7 APR), 54. https://doi.org/10.3389/FNINS.2013.00054/BIBTEX

Lima, S. Q., & Miesenböck, G. (2005). Remote Control of Behavior through Genetically Targeted Photostimulation of Neurons. *Cell*, *121*(1), 141–152. https://doi.org/10.1016/j.cell.2005.02.004

Mah, A., & Roberts, R. (2022). *Genome Editing Techniques: The Tools That Enable Scientists to Alter the Genetic Code*. https://www.synthego.com/blog/genome-editing-techniques#7-gene-editing-techniques-tools-to-change-the-genome

Mahmoudi, P., Veladi, H., & Pakdel, F. G. (2017). Optogenetics, Tools and Applications in Neurobiology. *Journal of Medical Signals and Sensors*, *7*(2), 71. https://doi.org/10.4103/2228-7477.205506

Man, D. (2003). Diversification and spectral tuning in marine proteorhodopsins. *The EMBO Journal*, *22*(8), 1725–1731. https://doi.org/10.1093/emboj/cdg183

Matsoukas, I. G. (2020). Prime Editing: Genome Editing for Rare Genetic Diseases Without Double-Strand Breaks or Donor DNA. *Frontiers in Genetics*, *11*. https://doi.org/10.3389/fgene.2020.00528

Mei, Y., & Zhang, F. (2012). Molecular tools and approaches for optogenetics. *Biological Psychiatry*, *71*(12), 1033–1038. https://doi.org/10.1016/j.biopsych.2012.02.019

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A. M., Bamberg, E., & Hegemann, P. (2002). Channelrhodopsin-1: A Light-Gated Proton Channel in Green Algae. *Science*, *296*(5577), 2395–2398. https://doi.org/10.1126/science.1072068

NIMH » Brain Stimulation Therapies. (2016). https://www.nimh.nih.gov/health/topics/brain-stimulation-therapies/brain-stimulation-therapies

Okamoto, S., Amaishi, Y., Maki, I., Enoki, T., & Mineno, J. (2019). Highly efficient genome editing for single-base substitutions using optimized ssODNs with Cas9-RNPs. *Scientific Reports*, *9*(1), 4811. https://doi.org/10.1038/s41598-019-41121-4

Pastrana, E. (2013). Light on genome function. *Nature Methods*, *10*(9), 817–817. https://doi.org/10.1038/nmeth.2622

Qiu, S., Anderson, C. T., Levitt, P., & Shepherd, G. M. G. (2011). Circuit-Specific Intracortical Hyperconnectivity in Mice with Deletion of the Autism-Associated Met Receptor Tyrosine Kinase. *Journal of Neuroscience*, *31*(15), 5855–5864. https://doi.org/10.1523/JNEUROSCI.6569-10.2011

Rebesco, J. M. (2010). Rewiring neural interactions by micro-stimulation. *Frontiers in Systems Neuroscience*, *4*. https://doi.org/10.3389/fnsys.2010.00039

Scanziani, M., & Häusser, M. (2009). Electrophysiology in the age of light. *Nature*, *461*(7266), 930–939. https://doi.org/10.1038/nature08540

Singh, A. (2022). *What Is Optogenetics and How Does It Work?* https://conductscience.com/what-is-optogenetics/

Sung, YL. , Wang, TW. , & Lin, TT. et al. (2022). *Optogenetics in cardiology: methodology and future applications*.

The Human Genome Project. (2022). https://www.genome.gov/human-genome-project

Tsai, P. S., Kaufhold, J. P., Blinder, P., Friedman, B., Drew, P. J., Karten, H. J., Lyden, P. D., & Kleinfeld, D. (2009). Correlations of Neuronal and Microvascular Densities in Murine Cortex Revealed by Direct Counting and Colocalization of Nuclei and Vessels. *Journal of Neuroscience*, *29*(46), 14553–14570. https://doi.org/10.1523/JNEUROSCI.3287-09.2009

Tsuda, S. (2020). Optogenetics. In *Behavioral and Neural Genetics of Zebrafish* (pp. 279–292). Elsevier. https://doi.org/10.1016/B978-0-12-817528-6.00017-6

Uddin, F., Rudin, C. M., & Sen, T. (2020). CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/fonc.2020.01387

van Haren, J., Charafeddine, R. A., Ettinger, A., Wang, H., Hahn, K. M., & Wittmann, T. (2018). Local control of intracellular microtubule dynamics by EB1 photodissociation. *Nature Cell Biology 2017 20:3*, *20*(3), 252–261. https://doi.org/10.1038/s41556-017-0028-5

Wang, Y., & Guo, L. (2016). Nanomaterial-Enabled Neural Stimulation. *Frontiers in Neuroscience*, *10*. https://doi.org/10.3389/fnins.2016.00069

Williams, J. C., & Entcheva, E. (2015). Optogenetic versus Electrical Stimulation of Human Cardiomyocytes: Modeling Insights. *Biophysical Journal*, *108*(8), 1934–1945. https://doi.org/10.1016/j.bpj.2015.03.032