ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA INFORMÁTICA

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OPTOGENÉTICA: El uso de la luz para revertir la parálisis por accidentes cerebrovasculares

OPTOGENETICS: using light to reverse stroke-induced paralysis

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Resumen: Los ictus son lesiones cerebrales en las que el riego sanguíneo se ve drásticamente reducido y se forman coágulos de sangre que causan muerte celular en el tejido cerebral. Es la tercera causa de muerte en todo el mundo y supone la primera causa de deficiencia motora en las personas de la tercera edad. En este estudio se presentan las diferentes maneras en las que es tratada actualmente y se explora una nueva técnica que está siendo estudiada actualmente para la rehabilitación de pacientes: la optogenética. Se exponen los principios básicos de la técnica, las diferentes opsinas que se pueden usar para la activación y silenciación de neuronas y los métodos en los que se introducen en el cerebro. También se presenta el uso de la optogenética para la mejora de los métodos de obtención de imagenes biomédicas y para la promoción de la plasticidad neuronal para la rehabilitación de pacientes con parálisis por ictus. Asimismo, se compara la optogenética con las Interfaces Cerebro Computador para la restauración motora, ya que esta última es una de las técnicas más seguras usadas actualmente para este fin. La comparación está respaldada por un estudio sobre Interfaces Cerebro Computador para determinar la posibilidad de analizar correctamente los impulsos nerviosos para detectar intención de movimiento y su restauración mediante miembros robóticos o mediante la inervación de músculos usando microelectrodos.

Palabras claves: Optogenética, Interfaz Cerebro Computador, ictus, parálisis

Abstract: A stroke is a cerebral lesion in which the blood flow to the brain is drastically reduced and a blood clot is formed, causing cell death in brain tissues. It is the third cause of death worldwide and it accounts for the first cause of motor impairment in elders. In this study we present the different ways in which it is currently being treated and explore a new technique which is being studied for rehabilitation of patients: optogenetics. We explore the basis of the technique, different opsins for activation and silencing of neurons and the method in which they are introduced into the brain. Its uses for improving optical imaging and induce plasticity in order to drive rehabilitation of patients with paralysis due to stroke are discussed. We also compare optogenetics to Brain Computer Interfaces for restoration of motor function, as the latter is one of the safest techniques being used currently for this purpose. This comparison is supported by a study on Brain Computer Interfaces to determine the possibility of correctly analyzing brain impulses to detect intention of movement for restoring motor function through robotic limbs or through innervation of muscles using microelectrodes.

Keywords: Optogenetics, Brain Computer Interface, stroke, paralysis

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Acronyms

BCI Brain Computer Interface

CCD Charged-couple device

ChR2 Channelrhodopsin-2

cLCN Contralesional lateral cerebellar nucleus

cM1 Contralesional primary motor cortex

cPPT central polypurine tract

CTX Motor/Premotor Cortex

DPSSL Diode-pumped solid state lasers

EEG Electroencephalography

FEI Federación Española de Ictus (Spanish Stroke Federation)

fMRI Functional magnetic resonance imaging

HIV-1 Human Immunodeficiency virus-1

iLCN Ipsilesional lateral cerebellar nucleus

iM1 ipsilesional primary motor cortex

LCN Lateral cerebellar nucleus

LED Light-Emitting diode

LSL loxP-flanked STOP cassette

NPCs Neural Progenitor Cells

NpHR Halorhodopsin

ofMRI Optogenetic functional magnetic resonance imaging

SFO Step function opsin

SSFO Stabilized step function opsin

tDCS transcranial direct current stimulation

Th Thalamus

TMS Transcranial magnetic stimulation

tPA Tissue plasminogen

VSD Voltage sensitive dye

WHO World Health Organization

WHP Woodchuck Hepatitis Virus

WPRE Woodchuck Hepatitis Posttranscriptional regulatory element

XFP Yellow Fluorescent Protein

Definitions

Angiogenesis: Formation of new blood vessels.

Cations: Positive ions.

Contralesional hemisphere: Hemisphere of the brain that has not been affected by the stroke, healthy hemisphere.

Cortical reorganization: Migration of lost functions to the uninjured side of the brain.

Depolarization: Movement of a cell's membrane potential to a more positive charge.

Electrolyte gel: Gel used for transformation of ionic changes into electric currents.

Hyperpolarization: Movement of a cell's membrane potential to a more negative value.

Ipsilesional/**Ipsilateral hemisphere:**Hemisphere of the brain that has been affected by a stroke.

Neural plasticity: Neurological phenomenon in which new cells and neuronal connections are created.

Opsin: Group of proteins that is made light-sensitive via the chromophore *retinal* (molecule related to Vitamin A).

Periinfarct region/**Ischemic penumbra:** Part of the brain surrounding the stroke.

Plastic window: Period in which rehabilitation is the most effective.

Promoter: Region of DNA that initiates transcription.

Rhodopsin: Light-sensitive protein.

Striatal neurons: Neurons, from the deep nuclei of the brain, whose role is to facilitate voluntary movement.

Transcranial: Going through the skull.

Tumorigenesis: Formation of tumours.

1. Introduction

1.1 Motivation

A *stroke* is a cerebral lesion that occurs when the blood flow to an area of the brain is interrupted or drastically reduced; this leads to an oxygen and nutrient deprivation of brain tissue and, consequently, cell death (*Stroke - Symptoms and Causes*, n.d.). There are different types of strokes; it can be due to a rupture in an artery (15% of cases), the so-called *hemorrhagic stroke*; it can be due to a blood clot, in which case it is called an *ischemic stroke* (80% of cases); or it can be a *transient ischemic attack*, in which case the blood flow decreases temporarily but it does not lead to permanent damage.

Strokes are a common occurrence: it can be estimated that a stroke happens every 40 seconds, being one of the main causes of death and disability worldwide. According to the World Health Organization (WHO), strokes are the first cause of motor impairment in elders, the third cause of death and, for instance, it accounts for the highest mortality rates for specific diseases among women in Spain (Sociedad Española de Neurología, 2006; *What is a stroke?*, n.d.). Aside from motor disability, an additional possible after-effect of ischemia is memory loss.

In Spain, the annual incidence of all cerebrovascular events is of 187 per 100,000 people (with a 95% confidence interval). It has been studied that rates increase with age, being frequent after 55 years old, peaking at 85 years old or elder. The in-hospital mortality rate is 14%, peaking in the northern regions of Spain as opposed to the southern and Mediterranean regions. In Spain, around a 21% of the population older than 60 is at a high risk of suffering a stroke. Taking into account the rate at which the population is aging, the Spanish Stroke Federation (FEI) stipulates that by 2050, 46% of the population will be older than 65 years old and the statistics will increase to half of this sector of the population being at risk of suffering a stroke (Díaz-Guzmán et al., 2012; *Ictus: un problema-socio sanitario*, n.d.).

The way in which an ischemic cerebral insult affects a person depends on where it occurs and the intensity in which the brain is damaged. The effects could be reversed naturally or with physical or stimulating therapy after the stroke or they could be permanent, which is the most common case. Two thirds of the survivors from stroke suffer from some type of disability (*What is a stroke?*, n.d.), 30% have some kind of paralysis, balance problems or cognitive deficits, and the survivors are at a high risk of suffering another stroke within the first three months (*Ictus: un problema-socio sanitario*, n.d.). This implies that this neurological condition leads to a large health care cost. In 2015, in Europe, an approximate 45.000 million euros were destined for stroke, either direct health care costs (in-hospital care, drugs) or informal health care costs (cost of unpaid care, loss of productivity because of death or disability) (King's College London, n.d.).

When someone suffers a stroke, the blood flow going to one particular area is cut off and neurons surrounding this area can die. Subsequently, the functions from these neurons are either lost or taken on by neurons on the contralesional hemisphere of the brain (non-damaged hemisphere) (Wahl et al., 2017). Right after an ischemic stroke, there is a "cascade of genetic, molecular cellular and electrophysiological events" that will aid in the plastic remodeling process (Coleman et al., 2017). After an initial short period, in which the contralesional hemisphere controls the motor functions that were controlled by the dead neurons, the functionality goes back to the ipsilateral hemisphere (damaged hemisphere), where the neurons in the perilesional area take on these functions. This reorganization of functions is called *neuroplasticity* (Coleman et al., 2017).

Currently, there are some methods being used for avoiding and for rehabilitating motor impairments due to ischemic cerebral insults. Specifically, the methods being used in the acute phase (first 24 hours after stroke) for avoiding the aftermath of cerebral insults are:

- Tissue plasminogen activator (tPA): it is a protein used for treating acute ischemia in pharmacological thrombolysis, which is the break down of blood clots through medication. It has to be administered via intravenous within a very short period of time after the stroke. This leads to a very few amount of patients benefiting from the treatment (Azad et al., 2016).
- Endovascular therapies: It is based on pharmacological or mechanical thrombolysis by introducing a microcatheter using a microguide in the artery where the blood clot has formed. (Ciccone et al., 2013)

If the artery occlusion cannot be reversed, we would, ideally, have efficient neuroprotective techniques:

- Neuroprotection: it aims to protect the ischemic penumbra, which is the region of the brain that is closest to where the insult has occurred and the one that shows the biggest ability for recovery. It can be done through mild neurological hypothermia (33ºC) and postsynaptic density-95 protein, which has an improved outcome. Another way in which we can attempt neuroprotection is through the inhibition of Na^{+} and Ca^{2+} receptors (Azad et al., 2016).

On the other hand, if in the end, the cerebral damage is permanent, there are rehabilitation treatments that include:

- Cellular transplantation: stem cell therapies are used as a neurorestorative treatment. They can be endogenous or exogenous strategies.
  - Endogenous strategies are based on neural progenitor cells (NPCs) differentiating into the predominant type of cell that has been damaged. These NPCs are stem cells that are naturally in the brain. This suggests a form of neurogenesis, however, due to the fact that glial cells scar faster than NPCs differentiate into the needed cells, the creation of neural connections can be prevented. This strategy is also a dangerous strategy as it carries risks of tumorigenesis (Azad et al., 2016).
  - Exogenous strategies are based on transplantation of cells from another origin. These cells include cells from bone marrow, umbilical cord and adipose tissue. This type of therapy uses immortalised cell lines such as NT2N, which are obtained from tetracarcinoma and have been genetically manipulated to resemble the cells needed with specific acids and inhibitors and to minimize the risk of tumorigenesis. Studies showed it

was safe for cellular transplantation, however, motor function hypotheses were not met (Azad et al., 2016).

- Neuromodulation: it is used as a neurorestorative therapy. It can be used for inhibiting inhibitors, i.e. there are inhibitors in the contralesional hemisphere that are impeding the connection attempts from the ipsilateral neurons that can still communicate. If we can silence the inhibitors, the remaining neurons located near the space in which the insult occurred and which are not damaged but were being inhibited, will be able to carry out their functions. This kind of therapy can be invasive, like epidural stimulation, or non-invasive, like transcranial magnetic stimulation (TMS). Studies show promise for non-invasive methods, like transcranial direct current stimulation (tDCS). However, these methods cannot discriminate the type of cell they stimulate, therefore, when stimulating one specific area, they can activate or inhibit all cell types. This can lead to psychiatric, motor or speech problems (Coleman et al., 2017; *Transcranial Magnetic Stimulation*, n.d.; Azad et al., 2016; Cheng et al., 2014). Invasive cortical stimulation allows a bigger stimulus at a more precise location and have been proven to be safe in preclinical and pilot human studies but remain unstudied because of a discontinuity in the sponsorship of the group studying this (Azad et al., 2016). Another type of neuromodulation that has been studied is cerebellar stimulation, which leads to clinical improvements in motor recovery and plastic reorganization (Azad et al., 2016). Vagal stimulation has also been stipulated to be efficient and has been shown to be safe, however, further studying is needed.(Azad et al., 2016)
- Physical therapy: it can be paired with neuromodulation or without it and should be done within the plastic window and after the first 24h within a stroke (Coleman et al., 2017). It has been proven to be useful in rehabilitation, helping in the reshaping of motor-function circuitry. A delay in the mobilization might lead to an ineffective rehabilitation (Wahl et al., 2017).
- Brain Computer Interface: it is designed to be able to make paralyzed patients regain their mobility. It is based on the fact that neural networks not affected by the stroke are still functioning and can still drive a stimulus. Using microelectrodes, a computer can interpret the movement that the subject wishes to make and then perform it with a robotic limb or by innervating the needed muscles through microelectrodes. The limitation with this technique is the long-term interface between the brain tissue and the microelectrodes. The algorithm is also a challenge especially when it comes to obtaining information about cognitive functionality (Azad et al., 2016).

One strategy that is growing and that can make motor recovery a reality is *optogenetics*. This is a growing field that consists of the use of light to stimulate targeted cells. The cells that are going to be stimulated are genetically altered to express a light-sensitive protein such as Channelrhodopsin-2 (ChR2) or Halorhodopsin (NpHR), which makes the cells sensitive to blue or yellow light respectively, and therefore the cells can be activated or silenced at a given moment. Optogenetics would solve the problem of selecting the specific neurons that we want to stimulate without affecting the nearby healthy neurons (Cheng et al., 2014).

This strategy has been shown to be effective in mitigating seizures (George & Steinberg, 2015), proving neural circuits can be manipulated.This is why using light to activate or silence neurons in order to control their excitability can offer a

solution to motor impairment.

1.2 Aim and structure of this project

In this review, the fundamentals of stroke and optogenetics and its implications in modern medicine will be explored. A study on Brain Computer Interfaces (BCIs) will be carried out in order to map the brain and single out the specific region of the brain which is used to perform a specific movement. This study will aid in determining the use of BCIs for restoring motor functions and how it compares to the expected results from using optogenetics based on the results by other groups.

For achieving this goal, this review will be structured in the following chapters:

- Chapter 1 introduces the project, explaining the motivation for the work and the aim of the project.
- Chapter 2 consists of a review on stroke, how the brain recovers from it and the adequate timeframe for rehabilitation.
- Chapter 3 describes the fundamentals of optogenetics, its current applications in medicine and the benefits and limitations of the technique.
- Chapter 4 explores the ethical implications of using optogenetics.
- Chapter 5 contains a review of the state-of-the-art of Brain-Computer Interface, the experimental setting used for the purposes of this study and the results obtained.
- Chapter 6 draws the conclusions of the review and study.
- Chapter 7 is the translation of chapter 6 to Spanish

2. Fundamentals of stroke

In this chapter the bases of neuroanatomy relating to this study will be briefly laid out. Moreover, the consequences of a stroke will be presented. We will also explore the timeframe for rehabilitation.

2.1 Neuroanatomy

In this study we focus on the recovery of voluntary movement after a stroke, which is controlled by the motor cortex, depicted in Figure 2.1 in yellow as *Motor function*. Another region that will be of importance is the somatosensory cortex, which is used for propioception (sensation from muscles and skin), in light blue in Figure 2.1 as *Sensory Area*.

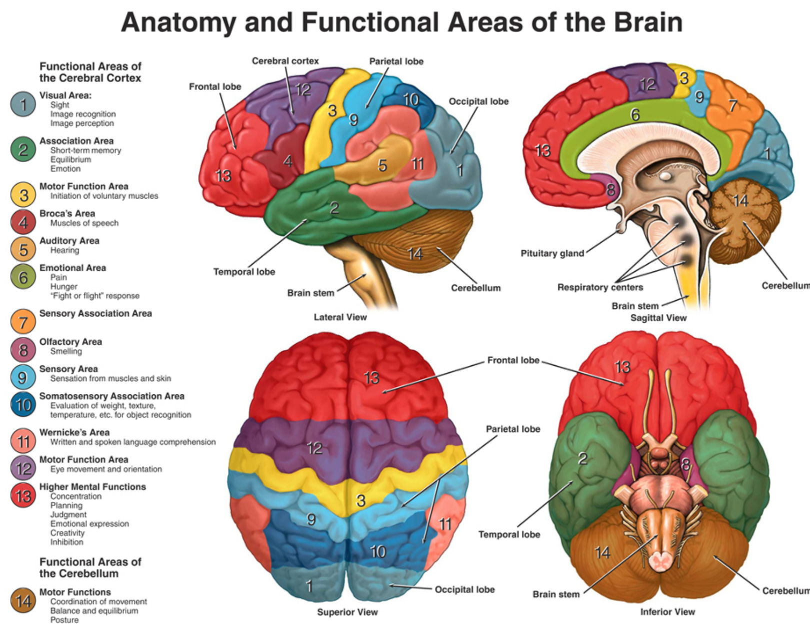

Figure 2.1: Anatomy of the brain and functions

Picture from (*Neuroanatomy-A Primer(1)*, n.d.)

2.1.1 Cortical reorganization

As described in Chapter 1, a stroke is a neural injury in which blood flow is drastically reduced. This leads to neural cell death and, ultimately to cerebral damage. Afterwards, an inflammatory response is triggered. Initially, it leads the cerebral damage, however, later on it promotes recovery by enabling synaptic remodeling / cortical reorganization (George & Steinberg, 2015).

Cortical reorganization is the migration of the lost functions to the uninjured part of the brain. After some time, the functions return to the ipsilateral side and the periinfarct region takes on the responsibility of carrying out the functions of the now dead neurons. However, not all functions can be recovered (Coleman et al., 2017) (see Figures 2.2 and 2.3 for visual representation of ipsilateral, contralateral and periinfarct region/ischemic prenumbra).

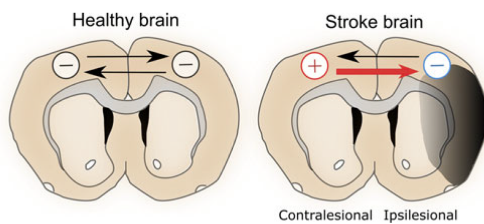

Figure 2.2: Stroke hemispheres in healthy and stroke brains.
Picture from: Cheng et al. (2016)

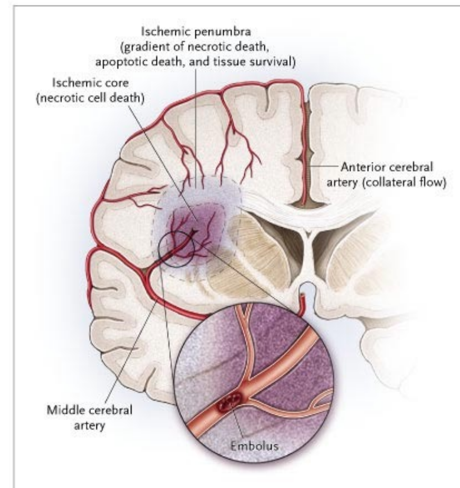

Figure 2.3: Parts of a stroke lesion
Picture from Friedlander (2003)

Strokes can trigger angiogenesis (formation of new blood vessels) and sprouting of new cells. This leads to a functional reorganization called neural plasticity, in which the new cells and connections serve as support for the ischemic penumbra. (Coleman et al., 2017)

Neurological impairments usually improve after the first few weeks/months after stroke, nevertheless, there can still be chronic neurological damage. Brain plasticity encompasses the different ways in which the brain can overcome the loss of neurons for recovering the lost functions through their assumption by neural circuits that are anatomically connected to the damaged ones, whether close or distant to the lesion. This reorganization can be seen through imaging methods and, in monohemispheric strokes, by comparing the contralateral hemisphere to the ipsilesional one (Rossini et al., 2003).

The factors that promote plasticity are many; some examples, as presented by Rossini et al. (2003), are changes in neuronal-membrane excitability, removal of inhibition, and improved synaptic transmission among others.

2.2 Timeframe for rehabilitation

There is a specific window of time after a stroke in which rehabilitation can be more effective than at other times; this is called a plastic window. This period is well defined for rodents, peaking around days 7-14 and ends 30 days later, which is the time at which the functions transfer back from the contralesional hemisphere to the ipsilesional side after a stroke. This period is less defined for humans, as the same process that takes 1-2 weeks for rodents starts around week 2 after stroke for humans and lasts 3 months instead of 1. However, there have been various studies assessing the potential benefits or harms that training at different times can produce, suggesting that training or mobilization of a subject within the first 24 hours after a stroke might lead to harmful events, however, doing this at a later time (e.g.:2 weeks after stroke) leads to beneficial effects (Coleman et al., 2017).

3. Fundamentals of optogenetics

Optogenetics is a biological technique in which light is used as a biomedical instrument. Different wavelengths are used to control neurons that have been genetically modified to be light sensitive through actuators such as Channerlrhodopsin-2 (ChR2) and Halorhodopsin (NpHR). These actuators are light-sensitive proteins coming from algae and, expressed by neurons, they render them light sensitive, therefore, allowing light to *switch* them *on* or *off* selectively. This technique allows the activation and inhibition of neurons within milliseconds, which allows a greater specificity and precision than the aforementioned techniques for brain mapping and rehabilitation (see Section 1.1). This Chapter reviews the basic principles of *optogenetics*, the benefits, side effects and applications being studied at the moment.

3.1 Basic principle

Zhang et al. (2006) postulate that there are three types of photostimulation techniques. Thus, the photostimulation can be through light-mediated uncaging of chemically modified signaling molecules; chemical modification of ion channels and receptors; and introduction of light sensitive proteins inside the cells. An example of light-mediated uncaging of signaling molecules is glutamate uncaging. This method is used for dendritic integration and mapping of the brain, however, it is not useful for specific neurons, as glutamate is expressed largely and is not cell-specific. Capsaicin receptors have been used to overcome this problem, however, millisecond control over spike firing has not been demonstrated (Zhang et al., 2006). Chemically modified ion channels and receptors use photoswitching of an azobenze group which is photoisomerizable. However, it poses a similar problem to uncaging methods (Zhang et al., 2006).

The optogenetics technique is based on the use of naturally occurring photosensitive proteins (light-sensitive rhodopsins) to activate or inhibit a specific set of neurons through their depolarization or hyperpolarization. For achieving this, these neurons need to be able to express sensitivity to light. Therefore, they need to be genetically modified to express this characteristic. For the purpose of this genetic modification, lentiviral vectors containing light-sensitive proteins are used and introduced into the brain (Zhang et al., 2006). After this is done, the neurons must be illuminated in order to be activated or silenced, this is usually done through a fibre optical device.

3.1.1 Light-sensitive proteins

The first light sensitive protein used was ChARGe, a rhodopsin cascade from *Drosophila sp*. It can be used for depolarization using light within the 400 to 600nm wavelengths. However, it takes seconds to minutes to spike an

impulse, rendering it impractical for this task (Zhang et al., 2006).

Hence, it can be considered that the first useful light-sensitive protein to be studied for the purposes of optogenetics was Channelrhodopsin-2 (ChR2); a microbial opsin[1] from *Chlamydomonas reinhardtii*. When shone with blue photons (470nm wavelength), this opsin allows positive ions (cations) to flow into the cell within 50 μs of illumination (Figure 3.1 - right), making the cell depolarize and fire (phase 2, as illustrated in Figure 3.2) (Cheng et al., 2016; Zhang et al., 2006). If this is combined with an ultrafast light switching, neurons can be activated with high and reliable temporal precision. The effect of the expression of this light-sensitive protein on the neurons' health, membrane integrity and electrical properties has been studied by Boyden et al. (2005). Their studies show that neurons expressing ChR2 appear to have similar characteristics as non-ChR2-expressing neurons regarding these parameters. Later on, the light-sensitive protein *Halorhodopsin* (NpHR) was found to be a good neuronal inhibitor, seeing as it is a chloride pump that activates when yellow light (580nm wavelength) is shone on the cell expressing the opsin (Figure 3.1 - left), causing a hyperpolarization (phase 4 in Figure 3.2). This protein is retrieved from the halophilic bacteria *Natronobacterium pharaonis*, it is stable at room temperature and easy to obtain. (Cheng et al., 2016; Hegemann & Nagel, 2013). When both proteins are being expressed in the cells and both blue (470nm) and yellow (580nm) lights are being shone on the cells, the effect of the hyperpolarization overshadows that of the depolarization, which means that the effects of ChR2 are temporarily suppressed while the yellow light is on. This effect can be seen in Figure 3.3.

Experiments in mice and rats have shown the efficacy of introducing ChR2 and NpHR in the body for using optogenetics (Cheng et al., 2014; Wahl et al., 2017; Shah et al., 2017). Additionally, Nagel et al. (2003) introduced ChR2 in the human kidney (among other types of mammalian cells), concluding that the technique could be used in other cells to depolarize them, suggesting the use of optogenetics in humans is possible and would lead to the expected depolarizations. However, Hegemann & Nagel (2013) believe that there are some limitations when using ChR2 because of the small cation conductance. It is suggested that this problem could be overcome by widening the ion pores in the protein, but this would potentially lead to a destabilization and thermal activation in the darkness. Newer versions of ChR would probably lead to better results, seeing as ChR2 can lead to intracellular acidification due to the high conductance of protons as compared to conductance of Na^+ when the depolarization takes place (Hegemann & Nagel, 2013; Schneider et al., 2013).

[1] An opsin is a group of proteins that is made light-sensitive via the chromophore *retinal* (molecule related to Vitamin A).

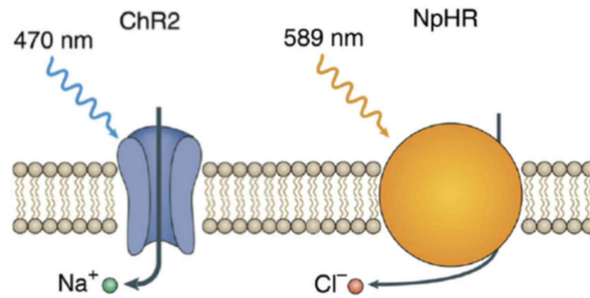

Figure 3.1: Working of ChR2 (left) and NpHR (right) expressing cells.

Picture from: Farrar et al. (2014)

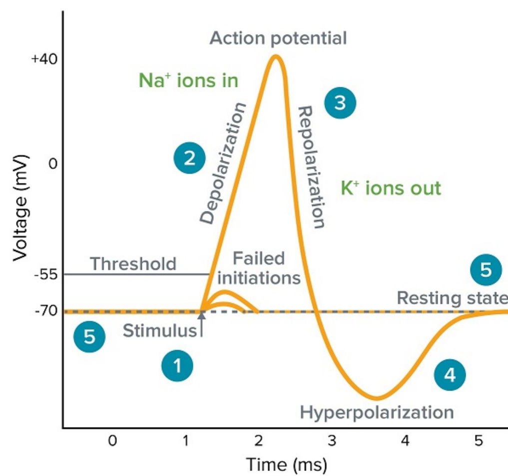

Figure 3.2: Stages of an action potential.

Picture from: *What is an action potential?* (n.d.)

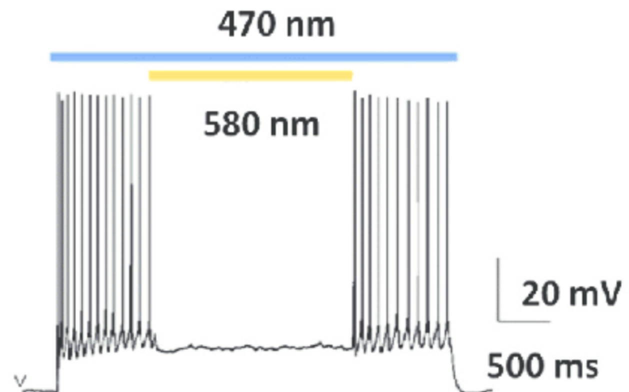

Figure 3.3: Spiking suppression when blue and yellow lights shine on a neuron expressing ChR2 and NpHR.

Picture from: Bregestovski & Mukhtarov (2016)

There have been many developments in the area of light-sensitive proteins afterwards. One of the most used ones is ChR2-H134R, which is a humanized variant of ChR2 that results in higher expression levels and larger photocurrents. Other variants that have been developed are ChETA (490nm wavelength), VChR1 (545nm wavelength), ReaChR (590-630nm wavelengths) and Chrimson (625nm wavelength). All of which are ultrafast variants of yellow/red-shifted ChR, which have proven to be more consistent (Cheng et al., 2016).

Alternatives to NpHR have also been developed for inhibition of neural activity. A chloride-conducting ChR (iC1C2) has been developed and causes hyperpolarization when blue light (470nm) shines on the cell that expresses it. It is highly light-sensitive and prolongs the hyperpolarization stage, making it possible to have a lower laser exposure. Additionally, the hyperpolarization stage can be ended by shining red light on the cell. Another proton pump that drives the hyperpolarization of the cell when blue light shines on it is Mac (from the fungus *Leptosphaeria maculans*). Other (superior) alternatives have also been discovered: Archaerhodopsin-3 (Arch) and ArchT are light-sensitive proton pumps derived from *Halorubrum sodomense*, which lead to a better and more efficient inhibition with less laser exposure

(Cheng et al., 2016). Another advantage that Arch poses over Halorhodopsin is the short recovery time after illumination, which resembles the recovery time of ChR2 (Chow et al., 2010). The safety of using proton pumps for optogenetics was also assessed by Chow et al. (2010), concluding that they were safer than pumps that drove an excessive silencing of the neurons: electrical properties were not affected, cell death remained at the same levels as if the protein was not expressed and cells recovered instantly after illumination ceased. There are also many different proton pumps that can be activated in a variety of wavelengths, therefore, silencing could be done with different wavelengths in different places.

3.1.2 Introduction of photosensitive proteins into the brain

Proteins can be introduced into the brain in different ways. Among others, it can be done through viral delivery, electroporation, DNA microinjection, or creation of transgenic lines. In this study we will review the viral delivery technique, as it can be produced fast (within days) and it can be observed on the subject as early as 8 days after delivery (Zhang et al., 2006).

In order for the delivery to be efficient, the central polypurine tract (cPPT) from the Human Immunodeficiency Virus-1 (HIV-1) or the Woodchuck hepatitis (WHP) B virus post-transcriptional regulatory element (WPRE) are usually included in the lentiviral vector. The vector can also have a fluorescent protein for tracking and it can have a promoter to make the vector cell specific. General promoters are usually preferred as they drive a stronger expression and cell-type specific promoters are weaker.It is however possible to target some types of cells without a promoter (e.g.: targeting dividing cells with a vector based on the Moloney murine leukemia virus) (Zhang et al., 2006).

An example of a possible lentiviral vector that can be used for the genetic modification of neurons with ChR2 is proposed by Zhang et al. (2006) (see Figure 3.4). In this model, WPRE and the HIV-1 virus's cPPT are used for long-term and efficient expression; the protein ChR2 is the protein that we want to express in the neurons and a yellow fluorescent protein (XFP) is introduced for tracking the viral vector. A promoter is also used for cell-specific targeting.

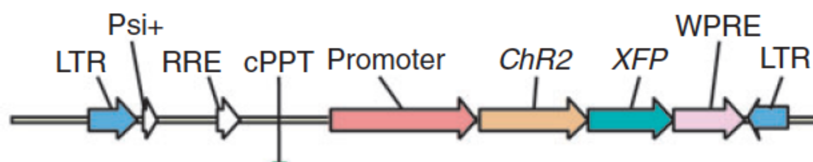

Figure 3.4: Lentiviral vector used for delivery of ChR2.

Picture from: Zhang et al. (2006)

Cheng et al. (2016) present some of the most common promoters for applying optogenetics in the dorsal dendate (motor and premotor cortices). They are (a) CamKII-ChR2, which targets excitatory neurons and their illumination will drive a response in the connected areas of the Thalamus (Th) like the motor/premotor cortex (CTX); (b)Syn-ChR2 can be used for excitation of well-defined neural circuits; (c) LSL-ChR2, used for unknown projections from deep nuclei,

Cre-dependent tracers are injected into both ends of the unknown projection (e.g.: CTX and Th); and (d) for multisynaptic neural circuits, viral tracers from the rabies virus expressing ChR2 can be injected into the beginning of the circuit (e.g.: injection in the dendate nucleus for the dendate-thalamo-cortical pathway). These methods are shown in Figure 3.5.

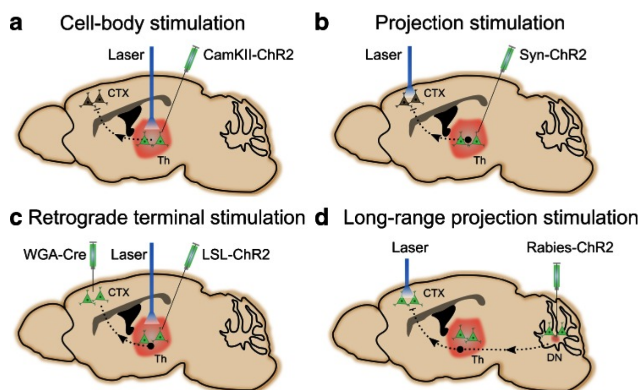

Figure 3.5: Promoters for delivery of ChR2 for targeting (a) excitatory neurons in CTX, (b) well defined neural circuits, (c) unknown projections from deep nuclei (retrograde terminal stimulation) and (d) multisynaptic neural circuits.

Picture from Cheng et al. (2016)

3.1.3 Photostimulation of neurons

There are two possible options for illuminating light-sensitive neurons: Light-Emitting diodes (LEDs) and diode-pumped solid state lasers (DPSSLs). LEDs are smaller, less expensive and have a great variety of colours, however the light is less intense. In order to use LEDs in *in-vivo* applications, they must be introduced into an optical fibre, which is difficult and not practical at the moment. DPSS lasers are easy to use, powerful and can be driven by digital and analog signals. However, the wavelength is non-adaptable, therefore, it must be appropriate for the opsin that is going to be activated from the beginning (e.g.: ChR2 is maximally activated at 473nm, NpHR is maximally activated at 593nm but can be activated with less intensity at 532nm). Driving lasers with TTL pulses (digital signals) allows the intensity to be adjusted and driving them with analog signals allows for delivery of more complex waveforms. (Britt et al., 2012)

The powering of the light source must also be specific for each case, being 5-30mW lasers sufficient for an *in-vitro* experiment and 50-200mW for *in-vivo* applications due to the fact that maximal activation is needed and power can be reduced when it goes through tissue (Britt et al., 2012).

Currently, an optical fibre device must be introduced in the brain. This device must be adapted to the anatomy and size of the subject, therefore, the size of the light source, the fiber tip and the intensity are different for each subject. For the purposes of optogenetics, the type of optical fibre that is used is a multi-mode optical fibre. This type of fibre has a diameter bigger than 10μm and is easy to handle, as opposed to single mode, which is smaller and very delicate. The size of the device has a direct relation to tissue damage; however, smaller devices tend to break more easily,

concluding that the size of the device should be determined by the site that needs to be illuminated. The numerical aperture should also be determined by the site, causing a bigger beam divergence with a large numerical aperture. (Britt et al., 2012)

Tissue heating must remain bellow $1^{o}C$, therefore, direct illumination might be dangerous for the subject. In order to overcome this problem, Cheng et al. (2016) suggest targeting a healthy area that projects onto the injured one. The same group reported beneficial effects on recovery with this approach.

The frequency, duration, intervals and power of the illumination should be dependent on the area that is being stimulated. Cheng et al. (2016) report that brief, high powered laser pulses trigger single action potentials in inhibitory neurons on the primary somatosensory cortex and longer, and low-powered ones are suitable for inducing spiking in striatal neurons.

There are two ways in which an optical fibre can be introduced into the brain. The standard used for *in vivo* rat experiments is with a ferrule. This consists of a fibre implanted in the brain and connected to a ferrule placed on the skull. The other option is implanting a guide cannula with a cable attached to the light source; this method's biggest disadvantage is the possible damage to the optical fibre when introducing or extracting it from the cannula. (Britt et al., 2012)

3.2 Implications of optogenetics

3.2.1 Non-invasiveness

One concern with the optogenetics technique is the invasiveness of the procedure. A fibre optic implant needs to be placed in the brain, therefore, the question of a non-invasive alternative should be taken into consideration. Higher wavelengths penetrate brain tissue better than lower ones due to less absorption and scattering. Lin et al. (2013) put forth the possibility of a non-invasive targeting of the photosensitive neurons based on the fact that red light scatters less and is less absorbed by blood than other lights in the blue-green spectra.

As it has been previously stated, there is a large variety of proton pumps each activated through a different wavelength, therefore, finding an inhibitor that can be activated by red light can be done relatively easy. This, coupled with the findings by Lin et al. (2013) lead us to believe that a non-invasive optogenetics technique is possible.

3.2.2 Genetic implications and stimulation of healthy patients

Optogenetics also involves the genetic modification of the subjects. This is usually done through viral delivery and the subject's genetic changes to produce a light-sensitive protein that is not present in the rest of the population. This can

be done with a cell-specific promoter or with general promoters as previously stated (Section 3.1.2). In both cases, if the subject had offspring, they would have cell-type specific ChR2 (Cheng et al., 2016), this would not affect the offspring as it has been reported by Cheng et al. (2014) that stimulation in normal, non-stroke mice does not affect the mice, it does not alter neurotrophin expression or motor behaviour, nor does it have an effect on body weight (parameter important for stroke patients, discussed in Section 3.3.2), which suggests that, in order for the optogenetic treatment to work, there must be a neurological damage. The stimulation promotes plasticity by increasing the expression of plasticity marker growth-associated proteins (GAP); if there is no need for plasticity, the stimulation appears to not cause any effect on the subject.

3.3 Applications

Optogenetics is the use of light to manipulate light-sensitive cells for driving depolarization or hyperpolarization in them (activating and silencing respectively). This approach has been used in studies regarding neurological and neurodegenerative diseases such as Parkinson's disease, schizophrenia, epilepsy, Alzheimer's disease and it has also been used for mapping functional organization after stroke. These applications hint at the possibility of using the technique known as optogenetics for the purposes of reversing stroke induced paralysis by studying plasticity through a mapping of brain functions and using this knowledge to induce plasticity.

After a person suffers a stroke, a neurological plasticity windows open in which there is a functional reorganization of the brain. During this process, the contralateral part (non-damaged side) or parts adjacent to the infarct take on the actions of the damaged neurons in the ipsilateral side (damaged side). This knowledge is founded by *in-vivo* imaging, which is usually done with electrodes. However, the combination of optical imaging techniques and photostimulation can lead to a more temporally and spatially precise technique.

3.3.1 Optical imaging in-vivo

Optical imaging of neural activity is an important part of understanding recovery. Fast *in-vivo* imaging can be done through different methods: functional magnetic resonance imaging (fMRI), 2-photon microscopy, voltage-sensitive-dye imaging (VSD)... These methods can be paired with optogenetic stimulation of photosensitive neurons to track functional remapping of the brain after a stroke (Cheng et al., 2016).

In a study by Lim et al. (2014), optogenetics was paired with VSD imaging for recording cortex functionality after stroke. This method proved to have high temporal and spatial resolution and provided a large-scale recording of neuronal activity (both subthreshold and suprathreshold). It is based on the use of voltage sensitive dyes, applied on the exposed cortex and the use of photostimulation to innervate ChR2-expressing neurons. This stimulation was done with a solid state laser at 1ms intervals with 5mW and a 473nm wavelength. When using this technique, changes in

membrane potentials are seen because the dye molecules transform these changes into fluorescent optical signals. The signals are then recorded with a charged-couple device (CCD) camera which allows for a submillisecond temporal resolution (Chemla & Chavane, 2010).

Other works introducing optogenetics in functional brain imaging include optogenetic fMRI (ofMRI), which is the acquisition of a whole-brain fMRI using light to activate or silence specific neurons in order to map functional connections between different regions. Choe et al. (2018) used Arch for silencing of Purkinje neurons, a cannula for introducing the optical fibre for delivering photostimulation and a 532nm DPSS laser at 5-20Hz in 10 cycles of 30 second intervals. The laser was driven by TTL signals and different frequencies were used (5, 10, 20Hz) at 20mW intensity for inducing cerebral output of the forebrain. The optimal parameters were 5Hz at 143mW/mm^2 intensity. The effectiveness of this technique for brain mapping was previously reported by Lee et al. (2010); who concluded that ofMRI extended the possibilities of current imaging methods, including casual connectivities, circuit topology and the conformation of cells' connections *in-vivo*.

3.3.2 Inducing plasticity through optogenetics

As it has been previously presented, there are different approaches for overcoming motor function shortcomings after stroke, however, rehabilitation entails the complete recovery of these functions. This can be done through promoting plasticity. In this section, we will review the results of some of the groups that have studied the use of optogenetics for this purpose.

"Optogenetically stimulating intact rat corticospinal tract post-stroke restores motor control through regionalized functional circuit formation" by Wahl et al. (2017)

In this study, Wahl et al. (2017) used the optogenetics technique to activate corticospinal circuitry in stroke rats paired with intense rehabilitation to assess its effect on restoration of motor functions. The study revealed restoration of these functions as opposed to compensatory actions by promoting axonal sprouting.

The rats received a stroke on the premotor and sensorimotor cortex linked to the preferred paw and were then divided into four rehabilitation groups: two of them ("Optostim" and "Optostim/training") received optogenetic stimulation 3 times/day for 2 weeks starting 3 days after stroke (ChR2 expression, 473nm wavelength, 3 consecutive 1 minute stimulations with 3 minute intervals in between). One of these optogenetically stimulated groups also received training on the impaired paw between weeks 2 and 4 ("Optostim/training"). Another group ("Delayed training") received training from weeks 2 to 4 and the last group ("Spontaneous recovery") did not receive any treatment.

The group reported improvements in forelimb function in both optogenetically stimulated groups as soon as 7 days after stroke. The "Optostim/training" group obtained results similar to the baseline 5 weeks after stroke in non-specific tasks.

The growth promoting Anti-Nogo-A immunotherapy treatment was also paired with training for comparison with the optogenetics technique, finding full recovery of prelesion levels of forelimb function at 3 weeks after stroke (one week previous to ”Optostim/training”). After 4 weeks, ”Optostim/training”, ”Anti-Nogo/Training” and ”Optostim” groups all achieved restoration of function in grasping tasks, obtaining similar results to baseline.

Enhanced corticospinal rewiring was observed in the ”OptoStim/Training” and ”Anti-Nogo/Training” groups, suggesting optogenetic techniques paired with training would have a beneficial effect added to that of the stimulation alone.

Optogenetic silencing was also carried out with Arch in order to assess the effect of silencing corticospinal tract fibres on the performed tasks (grasping tasks). The result of silencing these fibres was a decrease in successful performances.

”Optogenetic neuronal stimulation promotes functional recovery after stroke” by Cheng et al. (2014)

In this experiment, Cheng et al. (2014) use optogenetics to stimulate the ipsilesional primary motor cortex (iM1) to promote functional recovery. They found stimulated mice gained weight faster (weight loss is a problem in stroke subjects) and, while performing sensory-motor behaviour tests they found the stimulated mice to perform significantly better. They also assess the safety of using optostimulation on non-stroke mice.

The experiment consisted of three successive 1 minute laser stimulations with 3 minute intervals in between. Cheng et al. (2014) found that stimulation of iM1 could lead to a reliable activation of the peri-infarct regions and the contralesional primary motor cortex (cM1).

Cerebral blood flow was also examined in order to assess the ability of the technique to activate plasticity mechanisms. For this purpose, a repeated neuronal stimulation paradigm was implemented at days 5-14 post-stroke. The mice were divided into 5 groups (sham, normal without stimulation, normal with stimulation, stroke without stimulation and stroke with stimulation). At the beginning, the results were consistent with depressed blood flow and excitability, however, at day 15 after stroke, the ChR2-expressing stroke mice showed improved blood flow after stimulation, while non-ChR2-expressing stroke mice remained unresponsive to laser stimulation. The stimulated stroke mice also exhibited increased neurotrophin expression, which is tied to recovery post-stroke. They also found that stimulation increased growth-associated protein 43 (GAP43), which suggests that stimulation could promote synaptic plasticity.

Finally, Cheng et al. (2014) found stimulated mice could travel longer distances in less time than non-stimulated mice, that repeated stimulation to the iM1 did not alter infarct size, nor did stimulation affect, in any way, non-ChR2-expressing mice and that the recovery effects were region dependent, not because of general stimulation.

"Optogenetic neuronal stimulation of the lateral cerebellar nucleus promotes persistent functional recovery after stroke" by Shah et al. (2017)

In this study, optogenetics is used to stimulate neurons in the lateral cerebellar nucleus (LCN) to assess recovery after stroke, obtaining results consistent with a persistent recovery and increased GAP43.

Shah et al. (2017) study the LCN because it elicits major excitatory outputs to the cerebral cortex and that its stimulation enhances stroke recovery. In this study, the contralesional LCN (cLCN) is stimulated in order to discriminate whether the recovery that can be seen with excitation of LCN can only be achieved through direct neuronal activation or if activating cLCN can promote functional recovery.

It was found that stimulating the cLCN evoked reliable movements in the damaged limbs, however, stimulating off-target sites did not elicit any limb movement, although some whisker movements were recorded. There was some damage in the cLCN due to stimulation of lateral off-target sites that could be attributed to higher laser power (medial off-target activation did not affect cLCN). However, stimulation of the cLCN did not evoke response in the ipsilesional LCN (iLCN).

The stimulation paradigm consists of three 1 minute stimulations at days 5-14. Tests were conducted on days 4, 7, 10 and 14 after stroke and the results showed that stimulated mice traveled longer distances in shorter periods of time (day 7) and by day 14, the stimulated mice performed similarly to pre-stroke baseline. Levels of GAP43 were also recorded, finding an increase in the ipsilesional hemisphere which correlates with improved recovery.

The results also pointed towards a persistent recovery, seeing as the experiment was performed on a non-stimulated group, a short-stimulated group (days 5-14) and a long-stimulated group (days 5-28), finding that the short-stimulated group exhibited similar results to the long-stimulated groups even after day 14, suggesting the recovery is not transient but persistent.

3.4 Benefits of optogenetics

The main benefit from optogenetics in comparison to other stimulation techniques is its ability to control the sets of neurons that will be activated or silenced with high temporal precision. It also allows the enhancement of plastic reorganization, making it beneficial for the purpose of regaining mobility after a stroke.

Regarding its specific application in optical imaging; functional mapping of the brain through different methods is greatly improved by using optogenetics in combination with imaging techniques due to the fact that specific neural circuits can be activated/silenced and neural connections can be seen.

As opposed to other brain stimulation techniques, optogenetics has a higher spatial and temporal resolution,

overcoming the problems of imprecise and indiscriminate stimulation and, even though invasive at the moment, the technique shows promise as a noninvasive technique as well.

The technique is also patient specific since the promoter in the vector being used to introduce the proteins to the subject is different for each one because of the variability between strokes; the promoters may vary from one case to another, rendering it more precise than other non-specific techniques.

3.5 Limitations

Even though there are many benefits to using optogenetics as a rehabilitation tool, there are also cautionary steps that should be taken when using this technique, for example, the time of activation needs to be taken into account: prolonged activation times can lead to epileptic seizures. This effect can be present when using a step-function opsin (SFO); however, stabilized step function opsins (SSFO) can also be used for the same purposes and it accounts for less laser exposure. Cheng et al. (2016)

Other parameters that should be taken into account are the light scattering for lower laser exposure and, therefore, heat damage, the toxicity problems that the opsin might induce in the subject and the possible long term effects of expressing said opsin in the brain. Cheng et al. (2016)

Another limitation relating to the technique is the ethical implications, which will be explored in the next chapter (Chapter 4).

4. Ethical implications

Optogenetics is an invasive technique which entails the irreversible and permanent modification of a subjects nervous system through genetic modification and device implantation, therefore, ethical questions arise as to immune responses, psychological damage and potential manipulation by a third party. This last concern is unlikely to occur and does not constitute for immediate ethical examination due to the fact that it is part of speculative ethics. In this chapter the most immediate questions about ethicality in optogenetics will be assessed.

The optogenetics technique is based on genetically modifying and implanting a device inside a subject's brain, leading to the possibility of permanent damage to the subject undergoing the treatment should there be any physical or psychological trauma during the process. Genetic modification arises the concerns of mutagenesis and immune responses among others. Regarding the implant and illumination of the opsins, depending on location and design, this phase can bring up concerns with immune responses, implant rejection, device failure and psychological risks to the subject (Gilbert et al., 2014).

Safety is usually assessed during phase one of clinical trials and therapeutic results are assessed in phase two, however, the difficulties that subjects that have already received an implant might phase should they want to withdraw from the experiment might make phase one ethically questionable. This leads Gilbert et al. (2014) to present the possibility of assessing benefits from the treatment at the same time that safety is, in phase one, therefore reducing the number of subjects and research costs.

Gilbert et al. (2014) also discussed that trials regarding terminal diseases might also be more ethically acceptable due to the fact that the subjects suffering from these illnesses have more to gain than to lose, as compared to other subjects with non life-threatening illnesses.

When using this technique for reversing paralysis through inducing plasticity, the subjects are not in this position, however, the safety and efficacy shown in animal studies and the ”unethicality” of only assessing safety in phase one trials suggests that this phase should focus on effectiveness as well as safety. Should the therapy turn out to be ineffective, the possibility of seeking another type of therapy should be assessed in the informed consent document.

5. Activity mapping using EEG-based BCI

5.1 Introduction to BCI

One of the main techniques being used currently for restoring mobility in stroke patients is Brain Computer Interface. In this chapter we evaluate the use of Brain-Computer Interface for addressing this task. The typical application of a BCI system is to convert neural activity into a signal for controlling an external device in real time. In order to achieve this, the BCI collects the signals and the software used for the processing of these signals must be able to correctly identify the specific patterns that the user performs in order to give her/him an effective control of the device. Given this brief background, the main applications of BCI have usually been in the domain of rehabilitation, or control of prosthetics. In order to achieve these goals, BCIs need to be able to process large amounts of data in real time. This suggests that implementation of a BCI that processes data in real time is currently possible and suitable for application in the neurorestorative field.

This chapter provides a brief summary of the fundamentals of Brain-Computer Interface, and describes a preliminary experience with a Electroencephalography-based BCI, for mapping the neural patterns associated to specific motor activities. Our preliminary results are positive and in-line with other similar studies. For a more detailed mapping, the non-invasive EEG-based BCI should be replaced by an invasive BCI.

5.1.1 The motor cortex

The motor cortex is the part of the brain that starts the voluntary movement of each part of the body. A visual representation of the motor cortex can be seen in the Figure 5.1. It can be noted how actuation and sensing are distributed along the brain, involving more than 1×10^9 neurons.

In general, each hemisphere of the brain manages information coming from the opposite side of the body.

The places of the motor and sensory areas within the brain are shown in the figure 5.1. These different areas have a specific organization each, which can be seen in a topographic map with the corresponding placement of the neurons responsible for the sensing (in the sensory area) and movement (in the motor area) of the muscles shown.

When a movement is planned to be done, neurons fire electrical impulses. These impulses, although very small in amplitude, can be detected through various methods:

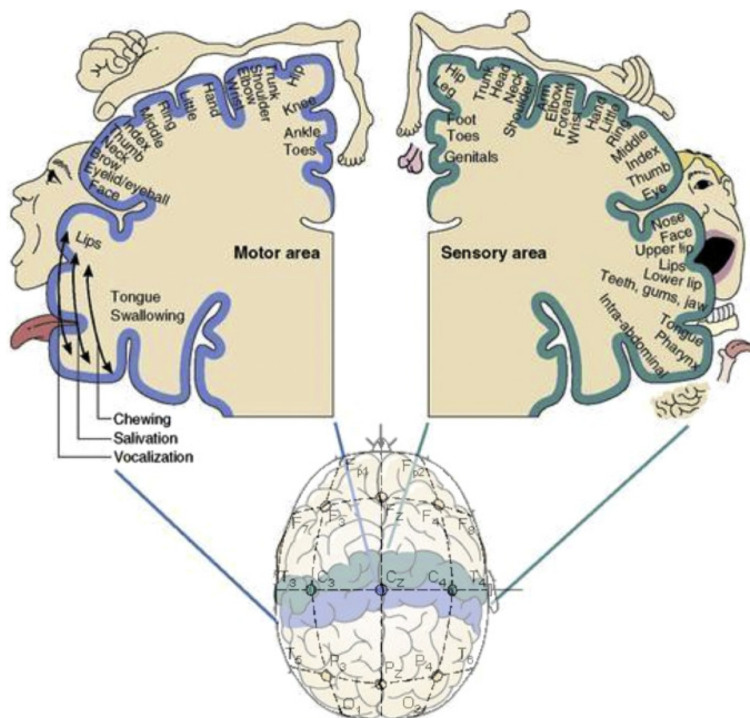

Figure 5.1: Penfield Homunculus. Motor cortex functions (left), sensory area (right) and location in the brain (down). Picture from: *The international 10–20 system of EEG electrode placeme | Open-i* (n.d.)

- Invasive: the goal is to position the electrodes inside the brain. This technique is more reliable than the non-invasive techniques described below due to the fact that noise is reduced. However, it is not the preferred method because of the necessity of a surgical intervention and the possibility of rejection to the implant by the body. Electrical activity can be captured through Electrocorticogram (ECoG) or through micro-electrodes. (Francisco Javier Velasco Álvarez, 2012)
- Non-invasive: this technique does not require surgery and the activity is monitored from outside the skull. The major disadvantages are bigger noise and smaller amplitude on the signals. However, it is the most common technique because of the comfort of the patient. Furthermore, a useful signal can be obtained after filtering the captured values. This method is called Electroencephalography (EEG). (Francisco Javier Velasco Álvarez, 2012)
- Non-invasive methods based on magnetic fields: Another non-invasive option is to capture magnetic signals. There are several options: Magnetoencephalography (MEG), functional Magnetic Resonance Imaging (fMRI) or Near Infrared Spectroscopy (NIRS). (Francisco Javier Velasco Álvarez, 2012)

For the purposes of this study, non-invasive EEG-based Brain-Computer Interfaces (BCIs) will be studied. As aforementioned, BCIs are real-time computer-based systems that acquire, analyze, and translate brain signals into useful output commands. Many BCIs employ EEG signals, although others use alternative modalities such as ECoG or fMRI (see Figure 5.2).

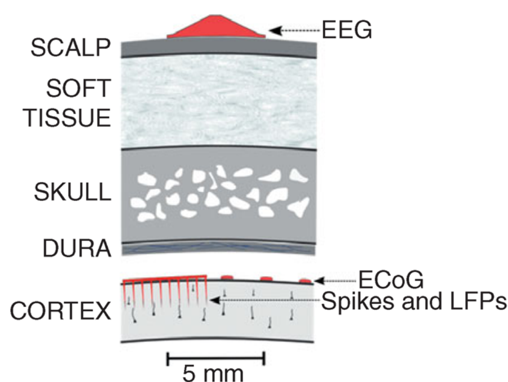

Figure 5.2: Recordings of electrophysiological signals used by BCI systems (EEG and ECoG).
Picture from: Wolpaw & Wolpaw (2012)

5.1.2 Detection of impulses using EEG-based BCI

Electroencephalography (EEG) captures the neuronal activity without positioning electrodes inside the brain. Although isolated neural activity does not create electric currents on the scalp that can be captured by the BCI headset, ionic currents do appear due to the electrical activity of the brain. In order to record these signals, the electrodes of the EEG-based BCI can be used. These electrodes can be active, passive or dry electrodes (Francisco Javier Velasco Álvarez, 2012). In our study, active electrodes were used. Active electrodes include a pre-amplifier and an electrolytic gel, which is used for transforming the ionic changes on the scalp into electrical currents through oxidation-reduction chemical reactions. These currents can then be amplified and filtered (Francisco Javier Velasco Álvarez, 2012).

There are several alternatives for detecting such impulses: (a) they can be detected through one electrode positioned on the part we are interested in and a reference (monopolar); (b) through two electrodes on the part we are interested in and obtaining the signal with the difference between them (bipolar); (c) with a Common Average Reference (CAR), taking into account the signals from all the electrodes connected to the brain; (d) with Common Spatial Patterns (CSP), taking into account all the electrodes connected and their position; and (e) with a Laplacian-based scheme, where there is an electrode on the site that is interesting for the study and a few electrodes near it whose aim it is to obtain an average value of the reference signal. The Laplacian can be either a small Laplacian or a Large Laplacian, depending on the distance between the electrodes surrounding the main one and this main electrode (Francisco Javier Velasco Álvarez, 2012).

5.1.3 Basic Principles of EEG-based BCI Systems

Electroencephalography BCIs create the possibility of analyzing intention of movement without actually performing it. For achieving this, we can study of the different rhythms which are present on the EEG signal and are linked to different actions (Francisco Javier Velasco Álvarez, 2012):

- μ-rhythm: it appears around 8-13 Hz and it is related to motor movement. Intention of movement spikes the same

reaction in this rhythm.

- α-rhythm: this rhythm is related to the subjects having their eyes closed and staying in a relaxed state. It appears around the same bandwidth as the μ-rhythm.
- β-rhythm: this rhythm appears around the 14 to 30 Hz bandwidth and is also affected by movement and intention of movement.

In this study, we focus on the μ-rhythm as a measure to determine the intention of movement. It has been studied that there is a synchronization in the brain activity's μ-rhythm when there is no motor activity. And, on the contrary, the activity dies down and there is a desynchronization when an movement is performed or intended to be performed (Francisco Javier Velasco Álvarez, 2012). Figure 5.3 shows the mentioned synchronization in the resting state in red. The μ-rhythm is located in the peak between the 8 and 13 Hz. This peak is considerably smaller when the state is 'moving the right hand' (Right signal, in blue). The presence of the peak can be studied with the r^2 norm, appearing as the highest value (see Figure 5.3(right)).

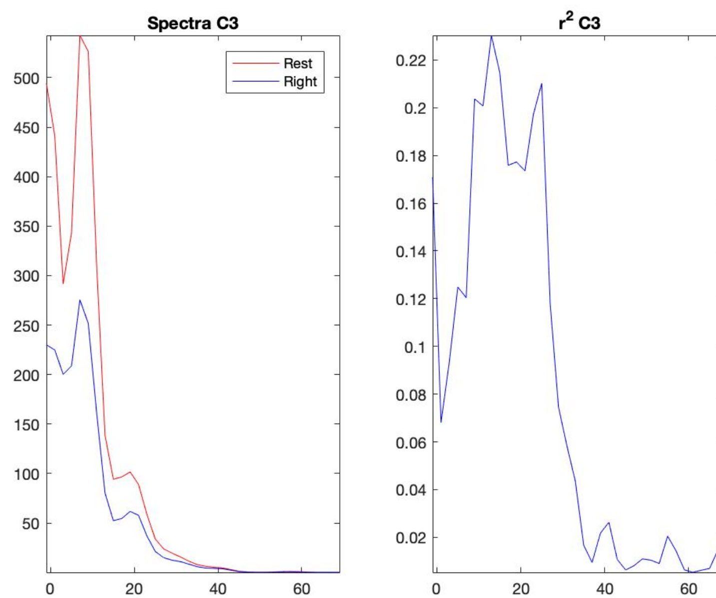

Figure 5.3: Synchronization in the resting state and desynchronization when the subject attempts movement of the right hand: (Left) Spectra C3; and (Right) r^2 C3

5.2 Experimental setting

This Section describes our experimental evaluation using a BCI system in the research Laboratories of the DIANA group at the Department of Electronic Technology of the University of Malaga (*Grupo DIANA*, n.d.).

5.2.1 Overview of our BCI system

Essentially, a BCI system consists of a headset which is used with electrodes and an amplifier. If the electrodes are active electrodes, an electrolyte gel is also required. The headset is placed according to the international system 10-20. The electrolyte gel is needed for transforming the ionic changes on the scalp into an electric current.

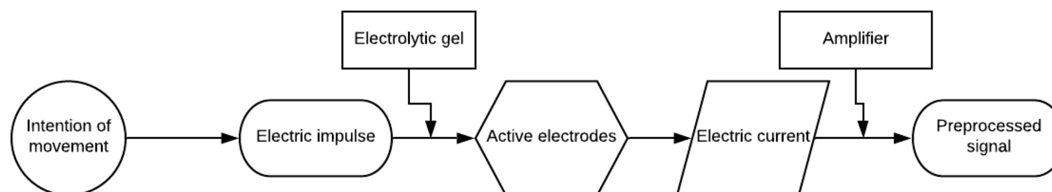

Figure 5.4: BCI system flowchart from electrical impulses to acquired signal (before processing)

The next sections describe the elements in our experimental setting.

5.2.2 actiCAP and actiCHamp by Brain Products GmbH

The actiCAP (Brain Products GmbH, n.d.-a) is a BCI headset developed by Brain Products GmbH for EEG recordings that uses active electrodes and electrolyte-gel 5.2.3.

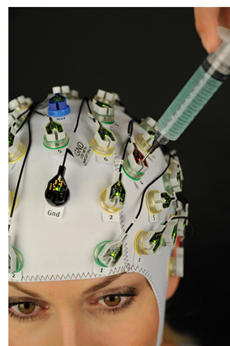

Figure 5.5: actiCAP

Picture from: Brain Products GmbH (n.d.-a)

Figure 5.6 shows the actiCHamp (Brain Products GmbH, n.d.-b), a 24-bit amplifier used for amplifying electric signals from the brain. These signals will be collected and transformed by the active electrodes present on the actiCAP headset. The sampling frequency is 250Hz, the band-pass filter is done between 0.1Hz and 30Hz and a notch filter is also used at 50Hz.

Figure 5.6: actiCHamp

Picture from: Brain Products GmbH (n.d.-b)

The aim of the experiment is to record intention of movement of the left and right hand, therefore, the electrodes will be placed on the actiCAP where those signals are known to spike impulses. In order to obtain the signals around C3 and C4 (known locations for hand movement spiking), a Laplacian channel is used. There are eight channels that will be combined to obtain the signal, taking away the undesirable noise. These channels are F3, F4, T7, T8, C3, C4 and Cz according to the international system 10/20. There is also a ground electrode (GND – AFz) and a reference electrode, which is located in Tp10 for the first two subjects and located near GND (Fz) for the other three subjects.

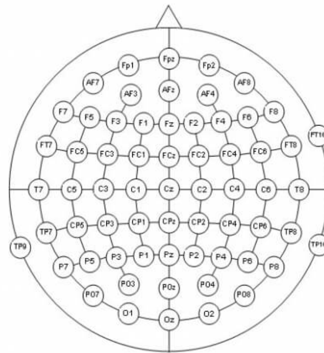

Figure 5.7: Electrode location.

Picture from: *EXTENDED 10/20-SYSTEM WITH 30 CHANNELS - Brain Latam* (n.d.)

5.2.3 SuperVisc by EASYCAP GmbH and active electrodes

The SuperVisc is a high viscosity Electrolyte-Gel for Active Electrodes developed by EASYCAP GmbH. It is used for EEG Recording Caps and related products.

Active electrodes are positioned on the cap making contact with the electrolyte-gel to convert the ionic changes on the surface of the scalp into an electric current. They have a small pre-amplifier which makes the signal ”cleaner” for the amplifier (5.2.2).

5.2.4 BCI2000 software

BCI2000 (Schalk et al., 2004) is a software used for recording data and for monitoring brain impulses. It is used for acquiring the EEG of the patients for the selected channels (Figure 5.7) during the different tasks that will be described in Section 5.2.9.

BCI2000 offers an offline analysis which runs on MATLAB and is used for obtaining a set of images depicting the frequencies and intensity at which the electrodes receive the signals. For different placements (channels), Figure 5.8 shows a frequency map. The y-axis shows 8 channels and the x-axis the frequencies (as a set of bins). The colour of each bin provides an estimation of the intensity measure (see the value scale on the right of the figure). Measures relate one condition with respect to the resting state. Thus, a map of the brain showing the part that is activated during a movement of the dominant hand (right in this case) is illustrated at Figure 5.9. Finally, Figure 5.10 provides a plot showing the frequencies at which the neurons synchronize/desynchronize.

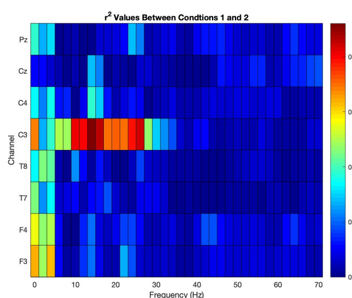

Figure 5.8: Frequency map

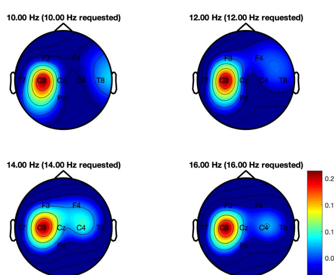

Figure 5.9: Map comparing the resting state to the right hand movement

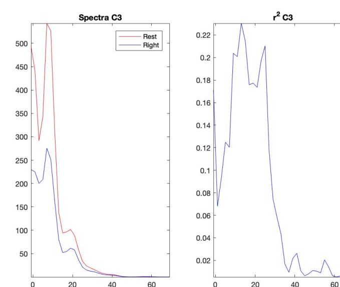

Figure 5.10: Spectra

5.2.5 Additional software tools

Apart of the BCI2000 software, we used in our study the MATLAB and Satistics Toolbox Release 2017b . Specifically, MATLAB by The MathWorks, Inc., version 2017b (with a license from the University of Málaga), is used for running the BCI2000 Offline Analysis software (The MathWorks, Inc., 2019).

5.2.6 Informed consent

An informed consent document is signed by all the subjects used in the study. A copy can be found in the Appendices Section (at the end of this document).

5.2.7 Selection of subjects

The subjects are volunteers between the ages of 19 and 24.

5.2.8 Software Configuration

As Figure 5.11 shows, the BCI2000 Offline Analysis consists of two conditions. In our case, the resting state and the hand with the least amount of noise will be compared as conditions 1 and 2 respectively. The Spectra channel has been set to the one that allows the hand to show the least amount of noise (C3 or C4). On the other hand, the Topo Frequencies have been set to the frequencies in which the impulses are highest (these frequencies should be around 12 Hz).

The data files added to the analysis will be four and will correspond to those of the first type of data, which is that of the training phase.

The Analysis Domain will be *Frequency*, and as Acquisition Type we must choose *EEG*. The trial change condition is *auto* and the spatial filter is *Common Average Reference (CAR)* (see Figure 5.11 for details).

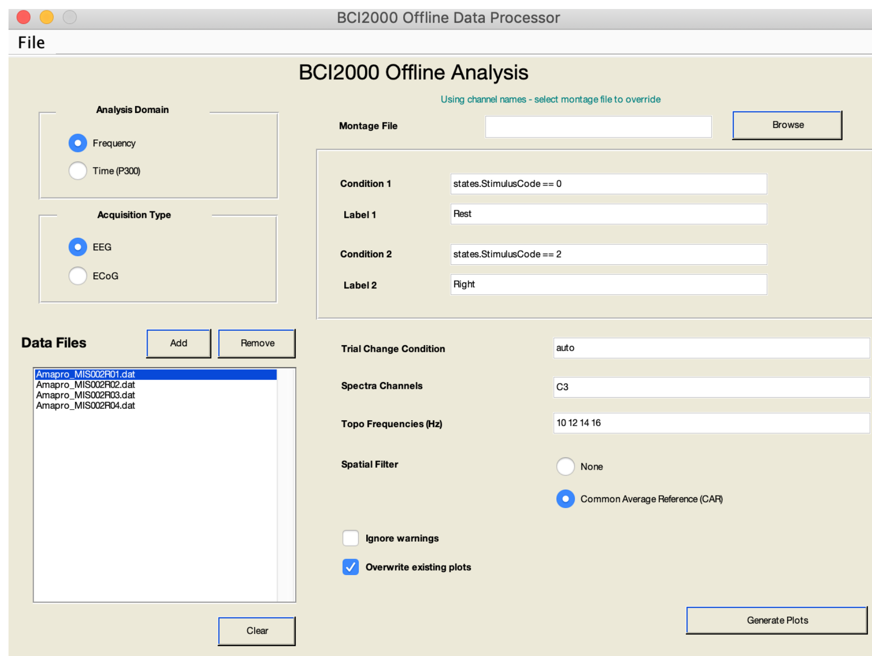

Figure 5.11: Offline Analysis configuration

5.2.9 Recollection of data

The electrodes are placed around the part of the brain known to be the part that controls the right hand (C3). There are also electrodes placed on the opposite side, the side controlling the left hand (C4).

In this study, ”intention of movement” is determined by acquiring signals from 3 different states: the first one is that of the left hand, the second one is the one of the right hand, and the third one that of the resting state.

The subject is asked to imagine the movement in a kinesthetic way. That is, they imagine the movement that they want

to perform as if they were going to carry it out. But they stop themselves right before moving. The subjects are also asked to avoid blinking, swallowing or pressing their teeth during the length of each attempt in order to avoid the α signal to cross-over to the μ bandwidth (8-13Hz), which is the one being studied.

The study consist of two parts:

- Training: during this part the subject sees one of three different options:
  - Right green arrow: the subject has to imagine movement of the right hand.
  - Left red arrow: the subject has to imagine movement of the left hand.
  - Nothing: the subject has to avoid thinking of movement of the hands. In this phase it is suggested that the subject does a cognitive task, for example, think of a number and subtract three until the time passes.

  There are 4 sessions where the subjects sees each option 15 times. During each session the arrows are shown for 8 seconds and the resting state lasts 4 seconds. The appearances are randomized by the program.
- Feedback: in this part, there is a display in which there is a ball and a wall. The objective is to move the ball so it touches the wall in each attempt. The ball moves upwards if the subject's intent is to move the hand that has been established as the selected one and it moves downwards if the subject's intent is to not move the hands (cognitive tasks are suggested for this). The attempts last 8 seconds and there are 100 attempts in total, taking a break after 50 attempts and continuing at wish.

As previously mentioned, the subjects are asked to avoid blinking, swallowing etc. during the task due to the fact that this creates artifacts in the signal. These artifacts are usually larger than the brain signals and can eclipse the data which is being studied (Francisco Javier Velasco Álvarez, 2012).

5.2.10 Data analysis

The analysis of the data is done with BCI2000 (Section 5.2.4). First, the frequencies at which the electrodes capture the signals are plotted (Figure 5.8) comparing the resting state to the dominant hand. Taking this frequency map into account, the channel that collects the data from the dominant hand is studied in the frequency at which r2 values are the highest (where the desynchronization happens) and a map and spectra depicting the right hand in comparison to the resting state is formed (Figures 5.9 and 5.10).

The data from the frequencies is used for the second part of the study, which, using feedback, creates a linear classifier that distinguishes the intent of movement from the resting state at the frequencies where the μ signal is acquired.

5.2.11 Statistical analysis

Due to external factors, the study was cut short to a group of 5 subjects instead of 25, therefore the statistical analysis that is carried out is the percentage of subjects who are responsive and whose signals can be captured. No other analyses are carried out because of the reduced number of subjects, however, in a larger study, sex, age and the ability to play an instrument or practice a specific sport are possible independent variables that can influence the result and should be studied as such.

5.3 Results

The study was performed on 5 subjects and it was structured in two parts. The offline and the online part. After presenting the main characteristics of the subjects involved in our tests, we will present both parts in Sections 5.3.2 and 5.3.3, respectively.

5.3.1 Subject selection

The subjects were volunteers of 21.8 ± 1.92 years ($mean \pm SD$). Four were female and 1 male; all five subjects were right-handed.

Table 5.1: Subjects' characteristics

ID	Sex	Age	Dominant hand
1	Female	23	Right
2	Male	24	Right
3	Female	19	Right
4	Female	22	Right
5	Female	21	Right

5.3.2 Offline Experiment

During the offline experiment, three different results were obtained for each individual.

Subject 1

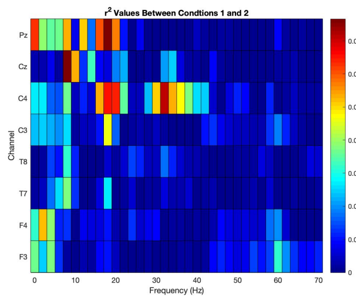

Figure 5.12: Frequency map for S1

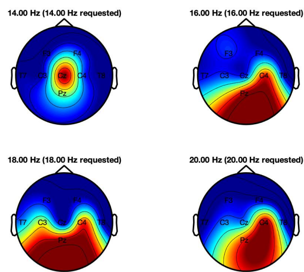

Figure 5.13: Map comparing the resting state to the left hand movement for S1

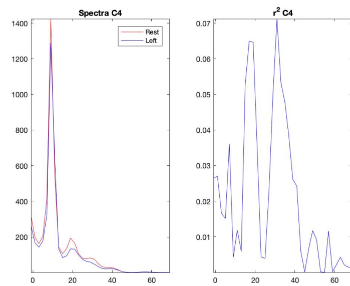

Figure 5.14: Spectra for S1

Subject 2

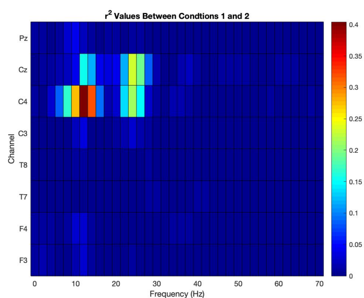

Figure 5.15: Frequency map for S2

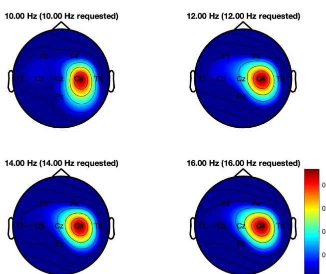

Figure 5.16: Map comparing the resting state to the left hand movement for S2

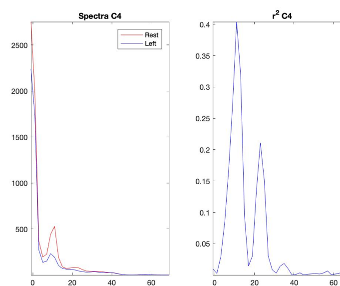

Figure 5.17: Spectra for S2

Subject 3

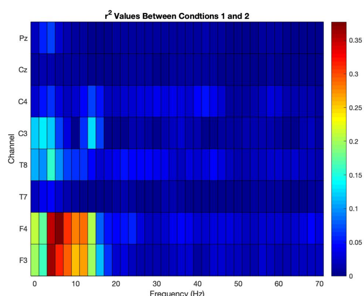

Figure 5.18: Frequency map for S3

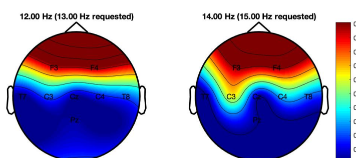

Figure 5.19: Map comparing the resting state to the right hand movement for S3

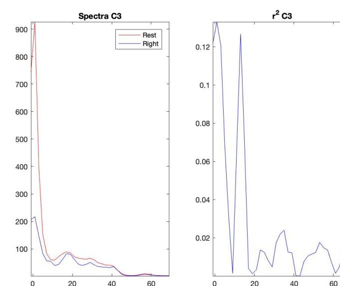

Figure 5.20: Spectra for S3

Subject 4

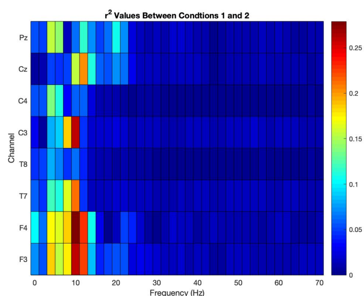

Figure 5.21: Frequency map for S4

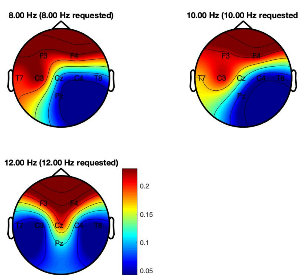

Figure 5.22: Map comparing the resting state to the right hand movement for S4

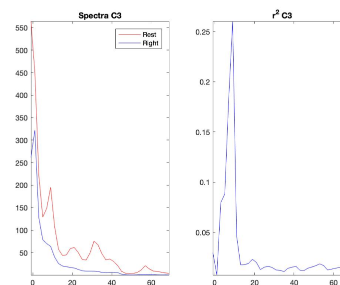

Figure 5.23: Spectra for S4

Subject 5

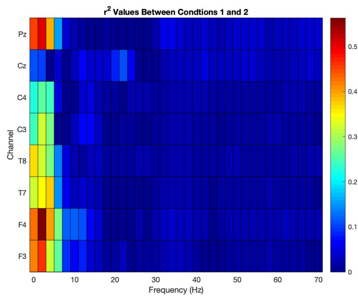
Figure 5.24: Frequency map for S5

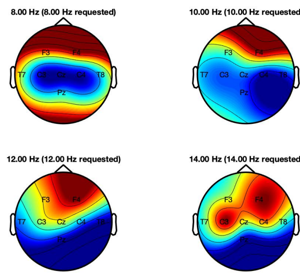
Figure 5.25: Map comparing the resting state to the right hand movement for S5

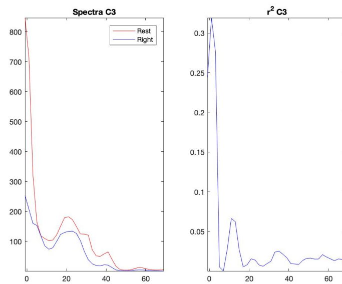
Figure 5.26: Spectra for S5

As we can see from the data, the BCI headset was able to obtain the data from subjects 1, 2 and 4 successfully without much noise, however, in subjects 3 and 5, the majority of the signal is noise that is being caught by the positions F3 and F4 and there is a lack of impulses caught on the μ bandwidth in C3 and in C4 . This means that the online experiment cannot be conducted with these last two subjects as the BCI headset would not be able to catch the signals for the experiment.

It is interesting to acknowledge the fact that all of the subjects are right-handed, however, the first two subjects obtain a stronger signal for the left hand. This could be due to the position of the reference channel, since it was placed on the right side of the head, in Tp10 according to the international system 10/20 (see Figure 5.7). For the following experiments, the reference channel was placed in Fz following the aforementioned international system 10/20 (Figure 5.7)

5.3.3 Online Analysis

The online analysis was carried out by the subjects whose signals were clear in the offline analysis (subjects 1, 2 and 4). The Frequencies in which the subjects were studied were the ones in which they showed the strongest impulses, and the percentage of times the BCI correctly classified the signal is shown in Table 5.5 as correct (%). In order to obtain this classification, BCI2000 uses a linear classifier whose parameters can be seen in Tables 5.2, 5.3 and 5.4. In the linear classifiers, the input channel is the channel that represents the dominant hand, the input element is the frequency where the neurons are desynchronized, output channel is the number assigned for each hand in the experiment, 1 represents right hand and 2 represents left hand and weight is represented by -1 when there is intention of movement and the ball goes upwards and 1 would represent the ball going downwards.

The linear classifiers were configured as seen in Tables 5.2, 5.3 and 5.4 and the results from the online analysis can be seen in Table 5.5.

Table 5.2: Linear Classification filter for Subject 1

id	input channel	input element	output channel	weight
1	C4_OUT	16Hz	2	-1
1	C4_OUT	18Hz	2	-1

Table 5.3: Linear Classification filter for Subject 2

id	input channel	input element	output channel	weight
2	C4_OUT	10Hz	2	-1
2	C4_OUT	12Hz	2	-1

Table 5.4: Linear Classification filter for Subject 4

id	input channel	input element	output channel	weight
4	C3_OUT	8Hz	2	-1
4	C3_OUT	10Hz	2	-1

Table 5.5: Online Analysis Results

ID	Frequencies (Hz)	Correct (%)
1	15-19	62
2	9-13	90
4	7-11	91

As we can see in Table 5.5, the classification for subject 1 is poorer probably due to the fact that there is more noise in the signal, however, subject 4's classification was the best, and it also had some noise. This difference might be because of the placement of the reference channel or how, as we can see in the spectras (Figures 5.14 and 5.23), the desynchronization in subject 1's μ signal is smaller than the desynchronization caught in subject 4's μ signal. But it might also be because of the frustration that a subject can experience the first time s/he operates a BCI system and it does not perform as well as expected, overwhelming the subject to the point of error (Guger et al., 2003).

6. Discussion and conclusions

Optogenetics has been proven to promote plasticity in the brains of mice and rats through the stimulation of light-sensitive neurons through a fibre optical device implanted in the brain. In order to do this, the neurons have to be genetically modified, which is done through the expression of opsins. The most widely used opsin in optogenetics is ChR2, however, there are many more opsins that could be used for inducing neuronal plasticity, being especially interesting those activated by red wavelengths, as they could potentially be used for non-invasive optogenetics techniques as red light produces less scattering and has a higher penetrating power. One of these opsins is ReaChR which has already been proven to drive excitation through intracranial stimulation.

The introduction of the opsins into the brain appears to be optimal using lentiviral vectors with either general or specific promoters to target specific types of cells and introduced in a specific part of the brain depending on the neural circuit that needs to be activated; and the illumination is usually done through a fibre optical device implanted in the brain, ideally in a region that projects onto the injured part so that tissue heating is reduced. However, non-invasive techniques are being studied and could be used for protecting the subject from tissue heating and possible traumas deriving from implants.

This technique has been studied for many different cases such as Alzheimer's disease, epilepsy, Parkinson's disease and motor impairment and has shown promising results. In this study we focused on its use for optical imaging and for inducing plasticity for recovering motor function.

Optical imaging techniques can be done in a variety of ways including fMRI and VSD. These methods have been studied in combination with optogenetics and results show them to provide a higher temporal and spatial precision and the opportunity to image casual connectivities, circuit topology and conformation of connections *in- vivo* when combined with optostimulation.

In regards to inducing plasticity, various groups have studied the application of optogenetics for this purpose. The stimulation paradigms were the same for all of them (3 consecutive 1 minute stimulations with 3 minute intervals in between) and show that optical stimulation benefited the subjects and allowed them to regain motor function to the point of obtaining results similar to baseline. It was also found that the mice/rats that were stimulated showed increased levels of GAP43, increased neurotrophin expression and were able to gain weight and that the combination of optostimulation and training was beneficial for enhancing cortical rewiring and, therefore, for a better recovery. One group also studied the effect of long-term stimulation as compared to short-term stimulation and found that there was no significant difference between stimulating the subjects between days 5-14 after stroke and days 5-28 after stroke,

concluding that the recovery was persistent and not transient.

The technique provides us with high temporal and spatial precision, enhances plasticity and improves imaging methods, however there are also some risks associated with the technique such as laser exposure, heat damage and possible rejection of the implant or the opsin. These risks should be taken into account when using the technique and should be further studied for a safe use.

In order to compare the optogenetic technique to one of the safest techniques currently being used for restoration of movement in patients paralysed from stroke, BCI, we carried out a study on this technique. In this study we use a BCI headset to collect data from μ rhythms from subjects when they intend to perform movements with their dominant hand as compared to these same μ rhythms when the subject is in a resting state.

Results from five subjects were obtained, however, only three subjects had neural impulses caught in the μ rhythm bandwidth. The first result for each subject is the frequency map, we analyzed the frequencies that have the highest intensities within the known μ bandwidth in the desired channel (dominant hand) and then we obtained a map showing the activation within the brain in these frequencies and a spectra showing the synchronization and desynchronization of the neurons at different frequencies. The r^2 peaks from the spectra depict the frequencies at which we can find a desynchronization between the neurons. These peaks were used for obtaining the μ bandwidth of each subject and that information was used for the online analysis.

During the offline analysis, the subjects had to imagine intention of movement with both hands and, initially, the dominant hand was analyzed. However, there were 2 cases (Subject 1 and Subject 2) who were right-handed and obtained higher impulses during intention of movement of the left hand. This could be due to the fact that the reference channel was placed on the right side of the head (hemisphere controlling the left side of the body). For these cases, the online analyses were carried out using intention of movement of the left hand and results are shown for the left hand (dominant hand for these two subjects from now on).

As can be seen in the results from the offline experiment (Section 5.3.2), there was a clear activation of C4 for left hand and C3 for right hand in three out of the five subjects (Subject 1, Subject 2 and Subject 4), combined with some noise or activation from other parts of the brain as well. However, there were no high impulses in the μ bandwidth for the other two subjects (Subject 3 and Subject 5). These results lead us to perform the online analysis only on the three subjects that could drive high enough impulses for the BCI system to recognize and classify correctly.

The online analysis was carried out within the frequency bandwidth where the desynchronization was the highest (wihtin the μ bandwidth) as seen in the spectras for each subject. The BCI2000 collects information from the brain impulses in the frequencies selected and creates a linear classifier based on the results obtained and the tasks that are

supposed to be performed. Two out of the three subjects performed the online experiment with a result of over 90% correctly classified actions and the other subject obtained a result of 62%. This suggests a very high accuracy classification of two of the subjects and it could be further improved in all three cases with more training, however, in this experiment, more training was not possible due to external factors.

These findings are consistent with other studies using BCI systems, with 40% of the population not being able to drive strong enough impulses to be caught by the BCI system (30% in other studies with larger populations) and therefore not being able to use it as a tool for restoring movement in these cases. These results could be improved by using implantable microelectrodes; this would improve the results and make it potentially useful for more subjects, however, it would become a very invasive procedure. Moreover, BCIs do not *rehabilitate* the patient, but help him/her to perform the lost functions by using a robotic limb (or innervating the needed muscles), as compared to optogenetics, which would drive restoration of function by inducing plasticity and, therefore, *rehabilitation* by creating new neural connections.

We conclude that, even though there is more research needed on the topic, optogenetics shows promise for rehabilitating motor functions that have been lost due to stroke. This technique suggests important advantages as compared to the methods that are currently being used in neuro-rehabilitation, which promote restoration of motor function through bypassing the damaged neurons instead of creating new connections (BCIs), or pose a high risk for the patient that can overshadow its benefits (endogenous strategies, tDCS).

7. Discusión y conclusiones

La optogenética ha demostrado ser útil en la promoción de la plasticidad neuronal en ratones y ratas mediante la estimulación de neuronas fotosensibles con un dispositivo de fibra óptica implantado en el cerebro. Para poder llevar esto a cabo, las neuronas tienen que ser modificadas genéticamente, lo que se hace mediante la expresión de opsinas. La opsina más utilizadas en la optogenética es ChR2, sin embargo, hay muchas más opsinas que podrían ser usadas para inducir la plasticidad neuronal, siendo especialmente interesantes aquellas que se activan con luz roja, ya que podrían ser usadas para un tratameinto optogenético no invasivo, ya que la luz roja produce menos dispersión y tiene mejor penetración. Una de estas opsinas es ReaChR, que ya ha sido usada y se ha demostrado que puede excitar neuronas de manera intracraneal.

La introducción de la opsinas en el cerebro parece ser óptima usando un vector lentiviral con promotores generales o específicos, que son introducidos en una parte específica del cerebro dependiendo del circuito neuronal que necesite ser activado. La iluminación se suele hacer mediante un dispositivo de fibra óptica implantado en el cerebro, idealmente en una región que proyecte sobre la dañada para reducir los efectos del calor sobre el tejido. No obstante, los métodos no invasivos se están estudiando actualmente y podrían ser útiles para la protección del sujeto ante calentamiento del tejido y ante posibles traumas creados por los implantes.

Esta técnica ha sido estudiada para diferentes casos como el Alzheimer, la epilepsia, el Parkinson y para deficiencias motoras, y muestra resultados prometedores para su tratamiento. En este estudio nos enfocamos en su uso para la obtención de imágenes biomédicas y para inducir la plasticidad para la recuperación del movimiento.

Las técnicas de obtención de imágenes son varias e incluyen fMRI y VSD. Varios grupos han estudiado estos métodos en combinación con la optogenética y se han obtenido resultados que muestran una mayor precisión temporal y espacial y la oportunidad de ilustrar conexiones casuales, circuitos topográficos y la conformación de conexiones *in-vivo* cuando se combinan con la fotoestimulación.

En cuanto a la promoción de la plasticidad, varios grupos han estudiado la aplicación de la optogenética para este fin. El paradigma de estimulación coincide en los tres (tres series de estimulaciones de un minuto consecutivas con tres minutos de descanso entre ellas) y muestran que la fotoestimulación beneficia a los sujetos y les ayuda a recuperar las funciones motoras hasta el punto de obtener resultados parecidos a los que tenían antes del ictus. También se observó que los ratones y ratas que fueron estimulados obtuvieron niveles mayores de GAP43, mayor expresión de neurotrofinas y fueron capaces de aumentar su peso, y que la combinación de fotoestimulación con entrenamiento era beneficioso para el “recableado” cortical y, por tanto, para una mejor rehabilitación. Un grupo también estudió los

efectos de la estimulación a largo plazo en comparación con la estimulación a corto plazo y no encontraron una diferencia significativa entre estimular a los sujetos entre los días 5 y 14 después del ictus y estimularlos entre los días 5 y 28, concluyendo que la rehabilitación es persistente y no transitoria.

La técnica nos brinda una alta precisión temporal y espacial, promueve la plasticidad y mejora las técnicas de obtención de imágenes. No obstante, también hay riesgos asociados con la técnica, como pueden ser la exposición al laser, el daño por calor y el posible rechazo del implante o de las opsinas. Estos riesgos deberían tenerse en cuenta al usar la técnica y se debería estudiar en profundidad para un uso más seguro.

Con el objetivo de comparar la optogenética con una de las técnicas más seguras usadas actualmente para la restauración de movimiento en pacientes con parálisis por ictus, BCI, realizamos un estudio sobre esta técnica. En este estudio usamos un casco de BCI para recolectar datos de los ritmos μ del cerebro de los sujetos cuando pretenden hacer un movimiento con su mano dominante y se comparan con los mismos ritmos μ que se obtienen cuando el sujeto está en estado de reposo.

Obtuvimos resultados de cinco sujetos, no obstante, solo tres sujetos generaron impulsos neuronales en la banda μ que pudieron ser captados. El primer resultado de cada sujeto fue un mapa de frecuencias, en el que se analizó qué frecuencia dentro de la banda μ obtiene la mayor intensidad en el canal deseado (mano dominante) y entonces obtenemos un mapa que muestra la activación dentro del cerebro para estas frecuencias y un espectro que muestra la sincronización y desincronización de las neuronas a diferentes frecuencias. Los picos de r^2 muestran las frecuencias a las que las neuronas se desincronizan. Estos picos fueron usados para la obtención de las bandas μ para cada sujeto, y esto para el análisis online.

Durante el análisis offline, los sujetos se debían imaginar la intención de movimiento de ambas manos e, inicialmente, la mano dominante fue la analizada. No obstante, hubo dos casos (Sujeto 1 y Sujeto 2) que eran diestros y mostraron mayores impulsos con la mano izquierda. Esto podría ser por la posición del canal de referencia, que se posicionó en el lado derecho de la cabeza (hemisferio que controla el lado izquierdo del cuerpo). Para estos casos, los análisis online se hicieron usando la intención de movimiento de la mano derecha y los resultados se muestran con la mano derecha (mano dominante para estos sujetos de aquí en adelante).

Como podemos observar en los resultados del experimento offline (Sección 5.3.2), obtuvimos una clara activación de C4 para la mano izquierda y de C3 para la mano derecha en tres de los cinco sujetos (Sujeto 1, Sujeto 2 y Sujeto 4), combinadas con ruido o activación de otras partes del cerebro. Sin embargo, no obtuvimos impulsos fuertes en la banda μ para los otros dos sujetos (Sujeto 3 y Sujeto 5). Estos resultados nos llevaron a realizar el análisis online solo con los tres sujetos que podían generar impulsos lo suficientemente altos como para que el sistema BCI los pudiese reconocer y clasificar correctamente.

El análisis online se llevó a cabo en la banda de frecuencia en la que la desincronización era la mayor (dentro de la banda μ) como se muestra en los espectros de cada sujeto. El sistema BCI2000 recoge la información de los impulsos cerebrales en las frecuencias seleccionadas y crea un clasificador lineal basado en los resultados obtenidos y las tareas que se debían realizar. Dos de los tres sujetos obtuvieron un porcentaje de acciones clasificadas correctamente de alrededor de un 90% y el otro sujeto obtuvo un porcentaje de un 62%. Esto sugiere una clasificación de alta precisión para dos de los sujetos y podría mejorar en los tres sujetos con más entrenamiento. No obstante, en este experimento no fue posible más entrenamiento debido a factores externos.

Estos resultados son consistentes con otros estudios de sistemas BCI, obteniendo un porcentaje de la población que no puede generar impulsos lo suficientemente altos como para ser diferenciados por el BCI de un 40% (30% en otros estudios con poblaciones mayores) y que, por tanto, no pueden usar esta técnica como método de restauración de movimiento. Estos resultados podrían ser mejorados mediante el uso de microelectrodos implantables. Esto mejoraría los resultados y podría hacer la técnica útil para más sujetos, no obstante, el procedimiento se volvería muy invasivo. Además, los sistemas BCI no *rehabilitan* al paciente, sino que lo ayudan a realizar las funciones mediante un miembro robótico o mediante la inervación de los músculos necesarios. Sin embargo, la optogenética llevaría a una restauración de las funciones mediante la promoción de la plasticidad, y, por tanto, a la *rehabilitación* mediante la creación de nuevas conexiones neuronales.

Concluimos que, aunque es necesaria más investigación en el campo, la optogenética resulta prometedora para la rehabilitación de las funciones motoras perdidas a causa de accidentes cerebrovasculares. Esta técnica sugiere importantes ventajas en comparación con los métodos usados actualmente en la neuro-rehabilitación, que promueven la restauración de movimiento mediante un bypass de las neuronas dañadas en lugar de crear nuevas conexiones (BCIs) o suponen un gran riesgo para el paciente que puede eclipsar sus posibles beneficios (estrategias endógenas, tDCS).

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Appendices

Informed Consent

INFORMACIÓN SOBRE LA TAREA EXPERIMENTAL

1. IDENTIFICACIÓN Y DESCRIPCIÓN DEL PROCEDIMIENTO

Durante la realización de este estudio, usted participará en una sesión en la que realizará diversas tareas en un ordenador. Tan sólo tendrá que seguir las instrucciones de los investigadores respondiendo de manera adecuada al procedimiento experimental. Al mismo tiempo se realizará un registro de su actividad electroencefalográfica, para lo que se le colocarán una serie de electrodos en diversos puntos de la cabeza.

2. CONSECUENCIAS PREVISIBLES DE SU REALIZACIÓN

El proceso de colocación y ajuste de los electrodos pueden producirse molestias muy menores. Asimismo, durante el experimento tendrá que intentar moverse lo menos posible, y reducir sus parpadeos. Aparte de esto, no produce ninguna consecuencia negativa previsible y no se puede producir ningún tipo de daño a corto ni largo plazo.

3. CONSECUENCIAS PREVISIBLES DE SU NO REALIZACIÓN

Su participación en esta prueba es totalmente voluntaria. Si en cualquier momento, antes o durante su realización, quisiera abandonarla, podrá hacerlo sin ninguna repercusión.

4. RIESGOS FRECUENTES

Esta prueba no entraña ningún riesgo en sí misma.

CONSENTIMIENTO INFORMADO

Yo ____, mayor de edad (___ años) y con DNI ____________________ ACEPTO participar como sujeto de investigación en este estudio realizado en la Universidad de Málaga, por el grupo de investigación UMABCI. Asimismo, declaro no tener ningún historial de enfermedad neurológica o psiquiátrica, así como estar tomando algún tipo de medicación regular (esto, en principio, no invalidaría su participación, pero sería algo a tener en cuenta por el personal investigador).

Con la firma del presente consentimiento manifiesto haber entendido cómo se realizará el presente estudio utilizando una tarea experimental con medidas electroencefalográficas, haber recibido suficiente y completa información sobre su realización, ventajas y posibles molestias, haber realizado cuantas preguntas me han surgido y haber recibido una respuesta satisfactoria a todas ellas.

Con la firma de este consentimiento comprendo y consiento que todos los datos que proporcione o se obtengan sean almacenados en una base de datos informática, manteniéndose la estricta confidencialidad de acuerdo a la legislación nacional vigente de protección de datos (Ley Orgánica 15/1999 y Real Decreto 561/93). Dichos datos serán usados exclusivamente con fines de investigación y respetarán el anonimato.

Finalmente, manifiesto que he leído y comprendido perfectamente lo anterior y que todos los espacios en blanco han sido completados antes de mi firma y que me encuentro en plena capacidad de expresar mi consentimiento.

Fecha y firma participante: Fecha y firma investigador: