Optogenetics – Overview methods and areas of application

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***Abstract*— Optogenetics and optogenetic systems cover a wide range of applications in the research of neurological pathways. In this overview, relevant aspects regarding the development of optogenetic systems should be shown. These are the optogenetic systems – opsins and optical switches – as well as optoprobes, materials, application areas and limitations of these applications. Additionally, a short outlook should be given. No detailed analysis for specific optogenetic systems or their partial aspects were carried out.**

I. Introduction

Optogenetic methods mean the investigation of excitable tissue through gene expression of light-controlled microbial channels or pumps that enable transmembrane ion movement. The light activation of these proteins achieves cellular excitation with an accuracy in the millisecond range. [1]

Research in the field of optogenetics increased over the past years. The fundamentals for optogenetic research can be seen in Figure 1. The development of an optogenetic system consist not only of the genes but as well of the light sources and recording electrodes. Some aspects of the latter are picked up in the chapters III-B and III-C.

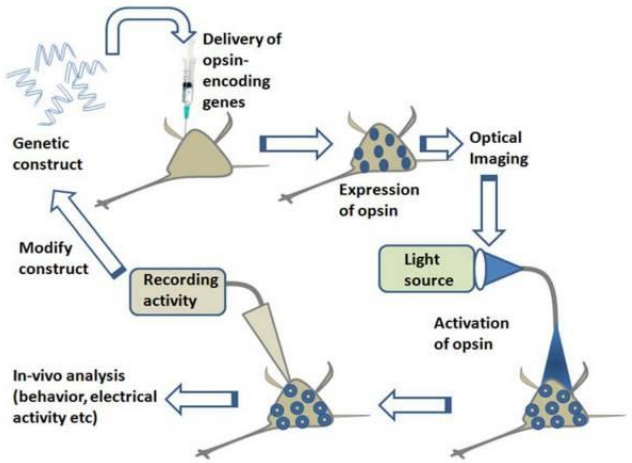

Figure 1: Basic elements of an optogenetic system: opsins, activation by light and analysis shown by Mohanty et al. (2015) [2]

This article should give a short overview of some aspects, which are important regarding the use of optogenetics. These are for example the optogenetic systems (especially the proteins), the optoprobes (combination of optical stimulation and electrical recording) as well as the materials of the implanted parts. Additionally, a short overview of the common areas of application and a selection of limitations should be given.

II. Materials and Methods

A. Research procedure

In the first step a research in the online library PubMed was performed to get an overview of the topic optogenetics. After the selection of the topics of interest – which are the different methods (opsins and optoprobes), the application areas and limitations – the literature [3–9] recommended by Dr. Volker Bucher was analyzed. Then additionally literature was searched online for specific topics and analyzed as well.

B. Fixed requirements

The scope of this meta-analysis was set to a maximum of four pages, therefore a detailed analysis of the different aspects of optogenetic systems cannot be performed. For this reason, a selection of noteworthy aspects was made, leading to incomplete results.

III. Results

A. Optogenetic systems: Opsins and optical switches

As the main part of the development of optogenetic technologies lies in the microbial function of certain cells and especially particular proteins, some considerable aspects regarding optogenetic systems should be shown in this part. The process of gene expression is not considered here.

One of the important aspects are the opsins (see Figure 1). Opsins are light-controlled ion channels or pumps that absorb light at certain wavelengths [10]. Table 1 shows some of the experimentally used opsins, their functions (exhibitor or inhibitor) and the activating wavelengths. These wavelengths are all in the range of the visible light spectrum and therefore could for instance be activated by light emitting diodes (LEDs) or other light sources, which deliver the relevant properties for the certain use. Relevant light source properties include wavelength, frequency, amplitude and light intensity [3].

Table 1: Selection of used microbial opsins for optogenetic systems [3, 10–15]

Opsin	Excitatory or inhibitory	Wavelength: sensitive spectrum (peak activation)
BR (bacteriorhodopsin)	Excitatory	470-650 nm (570 nm)
ChR2 (channelrhodopsin-2)	Excitatory	400-500 nm (470 nm)
Chrimson	Excitatory	(600 nm)
iC1C2	Inhibitory	450-550 nm
NpHR(halorhodopsin)	Inhibitory	550-620 nm (589 nm)
NpHR3	Inhibitory	(590 nm)
ReaChR	Excitatory	590-630 nm
SFO	Excitatory	450-590 nm
VChR1	Excitatory	500-550 nm (540 nm)

Those listed opsins mostly react as cation channels or as chloride channel or pump and are therefore able to activate or deactivate neurons et cetera.

Additional to the opsins other elements are investigated which are so called optical switches. These elements also allow protein activation, membrane localization and transcription activation because they are photosensitive. [7, 10]. An overview of four important variants of optical switches is shown in Table 2.

Table 2: Optical switches used for optogenetic systems [7, 10, 16]

	Activation	Reversion
PHYB protein	red light, 650 nm	infrared light, 750 nm, or hours in the dark
CRY2 protein	blue light, 405-488 nm	approx. 5 min in the dark
LOV domains	blue light, 440-473 nm	n.a. - depending (as well as the activation) on the LOV domain
Dronpa protein	390 nm	490 nm

Besides the choice of the optogenetic system (opsin or optical switch) which should be used, it is also important to consider – if necessary – how these proteins can be integrated in the genes, which should be detected. The reason is that only then the optical stimulation of the cells can create reactions.

B. Optoprobes

Optoprobes are technical devices, which link the part for the stimulation (light sources etc.) with the detective part, like electrodes and sensors. The design of an optoprobe needs to be considered carefully to meet the requirements for the specific application. One aspect to differentiate the different types of optoprobes is the approach. With penetrating optoprobes a single cell activation is possible, and the device can be implanted tight to the target cells. On the other hand, surface optoprobes are restricted in their depth and light resolution. As of today, the majority of optogenetic devices were developed to be used in the central nervous system (CNS) and only a few for the peripheral nervous system (PNS). Common designs of optoprobes used are shown in Figure 2. [3]

For detailed information about the different optoprobes, further research is recommended as well as for information about other systems used to stimulate the cells and record signals (especially in other areas of application than the CNS).

C. Material properties

In addition to the structural design of the optoprobes the material of these devices plays a major role in the development process. The material or materials used for the optoprobes, or other implanted devices need to meet the general requirements for medical devices.

The surface biocompatibility, which refers to the biological, chemical, morphological and physical properties of the material, is hereby an important criterion as well as the structural biocompatibility, which refers to the mechanical interaction between the implant (e.g., optoprobe) and the surrounding tissue. [3, 17]

In their researches Ward et al. (2009) [18] found, that the

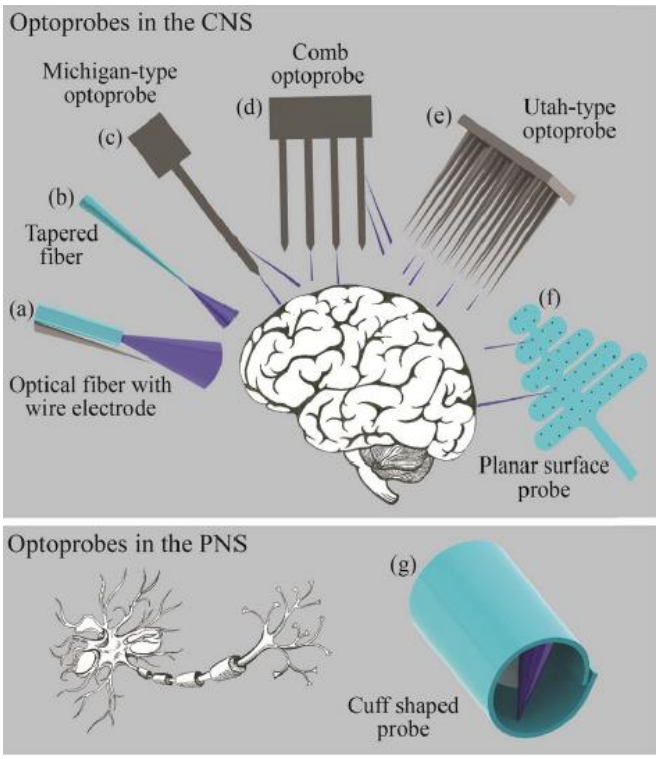

Figure 2: Common designs for optoprobes according to Alt et al. (2017): illustrations (a)-(e) show penetrating optoprobes whereas pictures (f) and (g) show surface optoprobes. The light blue color highlights the light guiding structures and the purple color displays the light rays [3]

material composition and geometry have a strong impact on the tissue as well as the insertion method for microelectrodes. Additionally, for chronic implanted microelectrodes Jorfi et al. (2015) [19] recommend materials where the mechanical properties stiffness and density match the properties of the target tissue.

Another point for which reason the structural properties of the material are important is that the implant needs to follow micromovements. Therefore, the materials for the optoprobes or likewise implanted devices for the detection of the signals stimulated with optogenetic principles should have mechanical properties like the tissue (e.g., brain). Care must be taken that this increased structural biocompatibility is not destroyed by the more complex implantation procedures [3, 19]. The choice of the material is thereby dependent on the flexibility of the implant and the insertion as well as the requirements for the signal delivery.

D. Areas of application

As most of the optogenetic systems were developed in terms of understanding neuronal pathways the broadest field of application are neurological applications. As of today, optogenetics are not approved to investigate in humans; therefore many researchers study rats, primates and other animals. Neurological issues are examined as well as the behavior under certain stimulations (behavioral studies). Applications are for example optogenetic microelectrocorticography (micro-ECoG), the identification of connections between different neuronal cell types. [3, 14, 20–22]

Other areas in which optogenetic systems are under investigation are the cardiological area [1, 23–25] and the treatment of retinal diseases [26–28]. Also investigated were optogenetic stimulations to restore hearing [3, 29–31].

The field of applications is wide. Therefore, the listed areas only represent a quantity of the whole number of areas of application. In case of achieving knowledge about the development in specific areas, publications that have a focus on this area are recommended for further information.

E. Limitations

Due to the fact of various aspects which define the optogenetic systems there are a multitude of possible limitations which can occur for specific chosen parameters as light sources, stimulation and detection methods, implant design, operation techniques and many more. This section therefore cannot include all the limitations that occur in optogenetic applications. A few limitations were chosen to be noticeable.

In the area of stimulation and signal recording, the optoprobes have some limitations. To be mentioned here are especially the surface optoprobes which lack in depth and resolution of light [3]. Also, Ronzitti et al. (2017) [32] discovered a limited temporal resolution for single cell or multiple cell approaches via scan-based targeting due to the time it takes to scan the laser sequentially through multiple positions. [32, 33]

Because of the genetic modification needed to achieve light-sensitive cells, optogenetics are not yet approved for humans. Due to this, most researchers work with animals (mice, rats, primates, etc.) or with computational models [1]. Thus, the processes in humans can only be assumed and not definitely be confirmed.

IV. Discussion

This paper can only give a short overview of some aspects regarding optogenetic systems. With a sum of over 8100 publications (8,145 results for the term “optogenetics” in the online library PubMed as of 07/23/21), the analyzed number of publications is not representative to portray the full extent of optogenetics on optogenetic systems.

The parameters for the specific use need to be defined to choose the possible optogenetic parameters which are highly defined by the cells which should be stimulated and the methods with that this can be achieved (for example a specific opsin which ensures a release of ions in neurons). Likewise, the method for detecting the changes needs to be considered to select an appropriate electrode or optoprobe as well as further aspects for the implant.

It was not expected to provide detailed information on the design of optogenetic systems with the fixed requirements for this paper. Therefore, detailed further reading is required when studies in the content of optogenetics want to be carried out. Noticeable is in this occasion, that optogenetics as of today are not approved for humans because of the genetic modification to achieve light-sensitive cells.

V. Conclusion & Outlook

The field of optogenetics includes many different aspects like the different functionalities of proteins, the design and materials of optoprobes and other implanted devices. It is expected that the areas of application increase and that the investigation of certain areas like the treatment of retinal diseases get a new focus.

Concerning the optoprobes, new devices for simultaneous optical neuromodulation and electrophysiological recording must be developed in order to achieve high spatial and temporal resolution [9]. The growth in the area of signal recording already started and is far from over [4].

Further opportunities will arise when the phototransduction mechanisms of the optical switches are further investigated and discoveries are made in this area [7].

As the optogenetics today are only examined in computational human studies like the one from Frenczi et al. (2019) [1] or in animals (especially in rats) future applications in humans are only to be expected after further understanding of the optogenetic systems and pathways and additional improvement of the optoprobes.

VI. Acknowledgment

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