

Optogenetics

(Ernst Bamberg)

Contents

1. At a glance
2. Definition of topic
3. Status of the field
4. Research needs, opportunities and challenges
5. Expected outcome and benefits
6. Figures
7. References

1. At a glance

The use of light in a non-invasive manner to remotely control neuronal cells opens completely new opportunities in neurophysiology (in cultured tissues cells as well as in living animals) with up until now unknown improvements in temporal and even more importantly spatial resolution. This was possible with the discovery of the light activated cation channel Channelrhodopsin 2 together with the light activated chloride pump Halorhodopsin (NphR), i.e. "multimodal control of neurons by light". The new and very fast growing field of 'Optogenetics' was established with the perspective of new approaches on basic science and on the gene therapy of neural diseases such as Parkinson disease, neuropsychiatric diseases and malfunctions in vision.

2. Definition of topic

Optical stimulation of electrically excitable cells is superior to classical activation by microelectrodes. The reason for this is due to its high temporal and spatial resolution. Optical stimulation can be achieved by using caged compounds, e.g. caged ATP, caged Glutamate, whereby the substrates for depolarizing ion channels are delivered to membranes and activated by pulses of UV-light to the chemical photolabile cage in the micro and millisecond time scale. An improved approach is the application of chemical photo-switches attached to ion channels, which allows to depolarize cells in a reversible manner with high temporal and spatial resolution. These elegant techniques, named chemical genetics, are applicable for neural cells in culture as well as in small animals like drosophila and zebrafish.

The discovery of channelrhodopsin2 (ChR2) from the unicellular alga *Chlamydomonas reinhardtii* was the starting point for the optogenetic approach. When transfected into mammalian cells and activated by blue light (λ_{\max} 470nm) ChR2 acts as an inwardly rectifying cation channel, thus depolarizing the cells. Together with the light-activated inwardly directed, hyperpolarizing Cl-pump Halorhodopsin (NphR, λ_{\max} 580nm) from the archaea *Natronomonas pharaonis*, the two form an ideal pair for the activation and inactivation of neural cells (at two different wavelengths). ChR2 activates the cells with blue light by depolarization, whereas NphR inactivates the cells with yellow light by hyperpolarization of the cells (fig1.). In contrast to the chemical approach the microbial rhodopsins do not need any extra addition of chemicals, because the light sensing cofactor retinal is produced by the host cells and binds correctly to the opsins, forming the functional rhodopsins. ChR2 and NphR can be expressed in a more selective manner in certain cell types of the brain by use of finer tuned promoters and molecular biology methods. Because ChR2 and NphR and their variants can be easily expressed in neural cells or used to form transgenic animals, these microbial rhodopsins are long sought tools for neurobiological research.

3. Status of the field

After the first application of ChR2 to neural cells the research field on optogenetics developed extremely fast. Major progress has been made on the transfection methods for targeting different cell types in living animals, which allowed the manipulation of ensembles of cells by light. Genetically adapted to be expressed in the mammalian brain by viral vectors, the microbial rhodopsins do the work with high precision in their host cells. Alternatively, transfection has been achieved by electroporation. However the virus approach appears to be more effective and has a biomedical perspective.

Recent work on cultured cells and on living mammals deals with mapping of the brain and cellular mapping of neurons. These studies allow a better insight into the communication of neurons and demonstrate the high potential for the analysis of neural networks.

Promising research is going on for the recovery of vision by transfection of different cell types such as ganglion and bipolar cells with ChR2 in the retina of photoreceptor deficient rodents. These results represent an alternative approach when compared to those obtained by implantation of photosensitive chips. Interesting progress has been made on rodents to investigate areas in the brain which presumably are associated with Parkinson disease or epilepsy with the optogenetic approach. The results on the above mentioned topics prove that the optogenetic approach is superior compared to the conventional electrophysiology.

For intracellular applications preliminary results exist from use of a light activated Adenylate-cyclase for the production of cAMP and by use of a chimera from Rhodopsin and another G-protein coupled receptor for studies of intracellular signalling.

4. Research needs, opportunities and challenges

Although in the young field of optogenetics spectacular results have been obtained, many improvements are necessary with respect to 1.) transfection methods, 2.) optogenetic tools, and 3.) development of appropriate light sources for studies in the brain or the retina of mammals.

1.) Transfection methods.

Transfection methods are focussed on the construction of vectors and promoters for cell specific expression of the microbial rhodopsins. The molecular biology aspect is a challenge, because of the enormous variety of constructs, which have to be tested by time consuming screening. As carriers for gene transfer different viruses have been used, preferably Lentiviruses and Adeno Associated Viruses (AAV's). The virus approach is quick and efficient and has a biomedical implication, whereas the construction of transgenic animals (rodents, fruitflies, zebrafish and *C. elegans*) are time consuming but have been proven to be ideal for a variety of different experiments in basic research.

2.) Improvement of the optogenetic tools.

Although the wild type ChR2 and NphR work quite well, an improved light sensitivity is important for experiments in the mammalian brain because of its low transmittance. All microbial rhodopsins utilize retinal as their chromophore, whose sensitivity for light can be influenced only slightly by changing the surrounding pocket within the protein. Certain ChR2 mutants, however, show an improved light sensitivity by an increased lifetime up to minutes in the open state of the channel. These channels can be switched on and off at variable time intervals by different wavelengths, but the increased life time and light-induced off response makes these mutants too slow for many neurobiological applications. More attractive would be ChR2 mutants with an increased single channel conductance, larger than the 35 fS of the wild type ChR2 with unaltered kinetics. Those will have to be identified in the future. In addition a search for other channelrhodopsins could be helpful. For the Cl⁻ pump

NphR the chances to increase the activity are low, because in this case the photo (reaction) cycle has to be accelerated substantially for a more efficient Cl^- pumping in the cell. To find such mutants is not probable, because in the field of the light-activated ion pumps those have not been identified in the last 25 years of research on bacterial rhodopsins.

3.) Improvement of appropriate light sources.

For high spatial resolution in the micrometer range light emission diodes (LED's) arrays, which are separately addressable are necessary for the optimal mapping of the brain and the retina. An alternate method consists of the design of arrays of micro light pipes. Such devices are sufficient for light stimulation on the surface of the brain or in the environment of the light source within the brain in the submillimeter range. For applications on the dense tissue in the brain, however, the two photon excitation would be desirable to increase the transmittance.

5. Expected outcome and benefits

The optogenetic approach offers for the future an enormous potential for basic research, because nerve excitation and silencing can be performed simply by light with high precision in a reversible manner. As has been already demonstrated, the microbial rhodopsins are tolerated in the brain of living mammals without obvious impairments. Therefore research is focussed on neurological diseases, because all the advantages of the light stimulation contain a promising potential for gene therapy as described below.

Cell culture, Network analysis.

The optogenetic method provides new opportunities to analyse neural networks. This can be achieved by growing cultured nerve cells on micro or nano patterned substrates. Cells can be stimulated or silenced simply by a light-beam with up to now unknown spatial precision. Only for registration of the light evoked signals electrodes devices are necessary. Results from these experiments are expected to be used for theoretical work on neural nets.

Mapping of the brain and behaviour

Immediately after having demonstrated that ChR2 can be used for remote control of neurons, many laboratories started projects for the mapping of the brain in living animals. Excellent work by various groups shows, that the application of the optogenetic methods opens the door in the near future for more detailed studies, which have not been possible with the traditional electrical and optical methods. To name some examples; studies are possible on which certain areas of the brain are stimulated via light pipes. Results are obtained on the movement of whisker of rodents; on the olfactory system where light replaces the ligands, and on the movement of animals after stimulation of the motor cortex.

Gene therapy

In the future gene therapy with the optogenetic tools appears possible. Transduction via AAV's has been performed successfully on the human eye to cure Lebers Congenital Amaurosis, by transduction of cells in the human retina to replace the missing retinal isomerase. In analogy to this AAV's could be loaded with the microbial rhodopsins and could be used for gene therapy on the diseases listed below.

Recovery of vision

Experiments on photoreceptor deficient mice have shown, that light evokes potentials in the visual cortex after the transduction of the ON bipolar cells with ChR2 in the retina. This indicates that the retina of the animals regained photosensitivity, which is transmitted via the optic nerve to the brain. Trajectories of the movement of the animals in the dark and in the light show clearly an increased activity in the light as it is obtained for wild type animals. It is conceivable that such an approach might be possible for blind humans, suffering e.g. the dry or the wet macula degeneration. However, in order to come to this point many biomedical, biophysical and technical hurdles have to be surmounted. This would be an alternative to the technology, which implants photosensitive chips in the human eye, which is far away from a satisfying treatment.

Parkinson disease, Epilepsy

Besides the application of drugs *Parkinson disease (PD)* can be treated by **Deep Brain Stimulation (DBS)**. The method consists of the stereotactic application of a metallic bipolar or quadrupole electrode to the *nucleus subthalamicus* within the brain. With help of the electrodes an oscillating electric field is applied stimulating the neuronal cells. With this approach spectacular results are obtained, which represent a substantial improvement compared to the drug therapy. Because of the geometry of the electrodes a precision of about one millimeter can be achieved. The extracellular stimulation by the electrodes induces not only the required depolarization of cells, but also partially a hyperpolarization, which inactivates cells with unwanted side effects. This means that parts of the target area are not under perfect control.

The optogenetic method is completely different. If successful this approach will lead to an improved treatment of *PD*: Virus induced transduction of cells with ChR2 allows the activation of the target structure in the brain via appropriate light sources without the side effects. As discussed above the advantages are the cell specificity, high temporal and spatial resolution in the micrometer range, which would open ways for the stimulation of substructures of the nucleus subthalamicus. The latter would give the chance to get a deeper understanding of the cause of this disease.

With respect to *Epilepsy* similar arguments would hold, because here certain areas in the cortex are affected. One could speculate, that optogenetics would attract focus also to other diseases including neuropsychiatric diseases.

To summarize, optogenetics offers great opportunities to for basic research in the neurosciences, as already has been demonstrated by many laboratories worldwide. The biomedical applications, however, hold unpredictable challenges and risks.

6. Figures

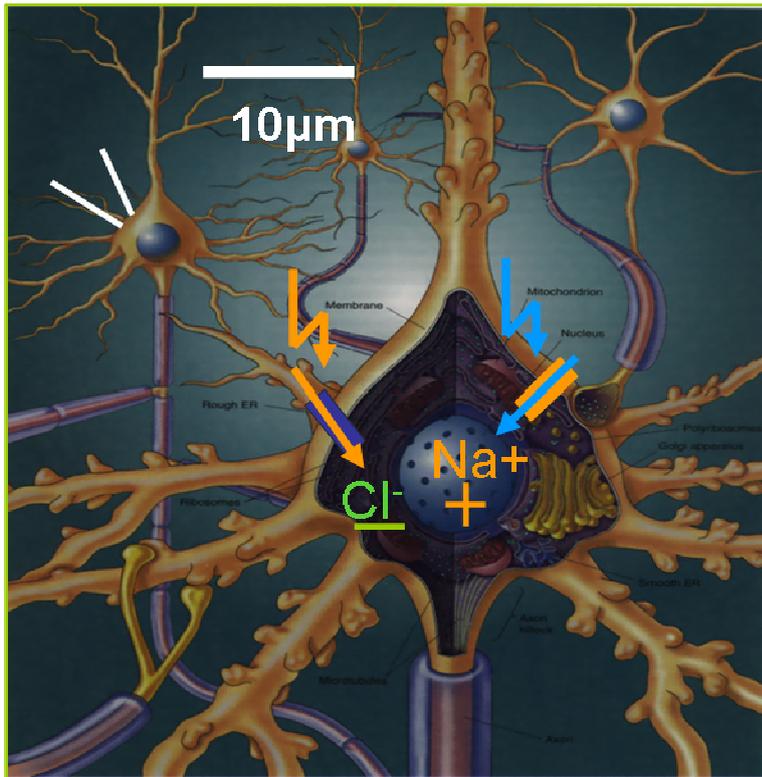


Fig. 1a: Schematic representation of the action of Channelrhodopsin2 and Halorhodopsin on neural cells.

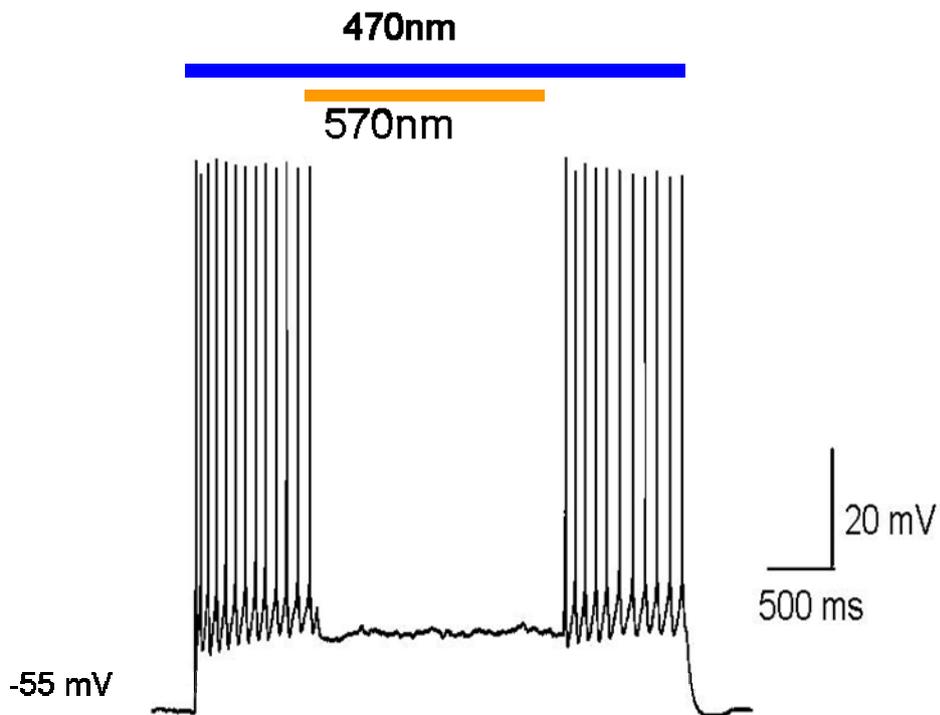


Fig 1b: Activation of action potentials by ChR2 (blue light) and inactivation by NphR (yellow light) of a cultured hippocampal cell

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