Optogenetics Will Make Electrical Stimulation Irrelevant

BSE656 – MIDTERM DEBATE REPORT – FOR THE MOTION – TEAM 3

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INTRODUCTION

In this debate, we are going to give arguments on why Electrical stimulation, a technique widely used in neurobiology to stimulate a neuron or neural network in the brain through the direct or indirect excitation of its cell membrane with the help of electric current, **stand no chance against,** a relatively new technique that uses light to control neurons that have been genetically modified to express light-sensitive ion channels known as **Optogenetics**.

At the beginning of the 1980s, Nobel laureate Francis Crick came up with a new idea to overcome the challenge of "Controlling all cells from one type in the brain while leaving the others more or less unaltered" with the help of light as it does not affect signalling while scattering. To implement this idea to make revolutionary advancements in terms of stimulating the brain, the field of Optogenetics came into the picture, which paved the way for replacing extensively used Electrical Stimulation with a new and significantly better domain of Optogenetics. The temporal resolution of Optogenetics has the order of milliseconds that will help us keep track of fast-changing biological data.

In 2010, the interdisciplinary research journal chose Optogenetics as the "Method of the Year" across all fields of Science and engineering. At the same time, Optogenetics was highlighted in the article "Breakthroughs of the Decade" in the academic research journal *Science*.

However, this only doesn't make Optogenetics better than electrical stimulation. Arguments **given ahead support why Optogenetics Will Make Electrical Stimulation Irrelevant**.

PRINCIPAL ARGUMENTS

Better Temporal/Spatial Control

Since optogenetics involves expression of light sensitive proteins called opsins into the body, it can provide spatial and temporal control: the expression of genes can be controlled spatially by incorporating enhancers specific to a type of cell, and temporal control can be provided by shining light at the right moments. The best part is that once the required gene is inserted into the organism, the technique is totally uninvasive, unlike most electrical stimulation methods.

Opsins can be many types of molecules

The development of Light-Inducible Transcriptional Effectors (LITEs) has allowed opsins to be one of many types of proteins-channels, pumps, G-protein coupled receptors, or transcription factors. When light is shone on these specific types of proteins, experimenters can associate a particular phenotype to a type of protein in an area, rather than just the area in the brain. Thus, LITEs provide much more specific modification capability to optogenetics. On the other hand, electrical stimulation has no such option in which only one type of protein is affected. At its very best, it can affect a particular area at a particular time, without any distinction in the type of proteins affected. [1]

Type of Modulation can be controlled in Optogenetics

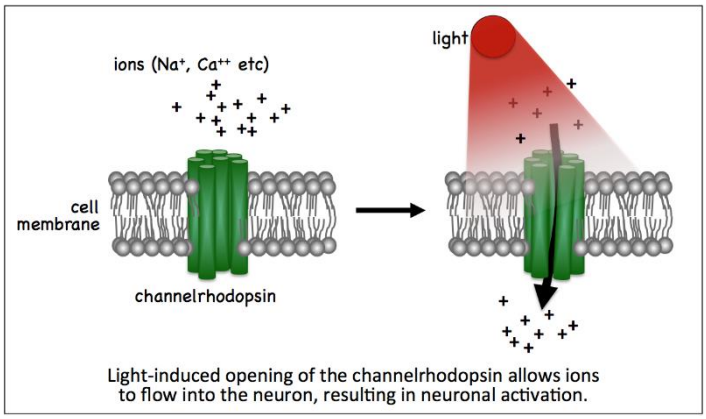

Fig 1: Channelrhodopsin-2 functioning
(Source: bloomington drosophila stock center, indiana university)

Type of modulation (excitation/inhibition) can be controlled by optogenetics, unlike electrical stimulation. Channelrhodopsin-2 (ChR2) is a naturally occurring ion channel, found in the algae *Clamydomonas reinhardtii.* When stimulated by blue light, this channel opens and allows the flow of K+, Na+, H+ and Ca+. Thus, when opened, it allows these ions to flow along their concentration gradient, depolarising the cell. [2]

Halorhodopsin (NpHR) is a chloride ion channel expressed by the bacterium Natromonas *Pharaonis*. When stimulated by orange light, this channel opens and allows chloride ions to flow along their concentration gradient, thus hyperpolarizing the cell.

Constraints of Electrical Stimulation

Despite the extensive use of Electrical Cell Stimulation (ECS) methods in the current time, various constraints in the technique limit the utilization of this technology to its full extent. Adoption of Optical Cell Stimulation (OCS) methods can resolve multiple constraints which arose in the ECS technique. [3]

Specificity:

We have already discussed the lack of precise stimulation in ECS methods which can be resolved by OCS-based approaches to achieve better precision. Developing Opsins to express in specific cells results in increased temporal accuracy, and at the same time, high frequencies achieved by OCS increase the accuracy of neuron excitation (Fig 2).

Coding of Spectral Information:

Secondly, ECS performance is also limited by spectral data's inaccuracy, resulting from wide current distribution from each electrode contact. Research involving cochlear implants shows the vulnerability arising due to this constraint as it was observed that the ECS method could only capture up to 8 electrodes as functioning when tested for speech precision in noisy environments, whereas at least 20 are needed. [4][5] Due to higher attenuation achieved in OCS methods, spatial resolution is improved by it compared to ECS, hence removing this constraint

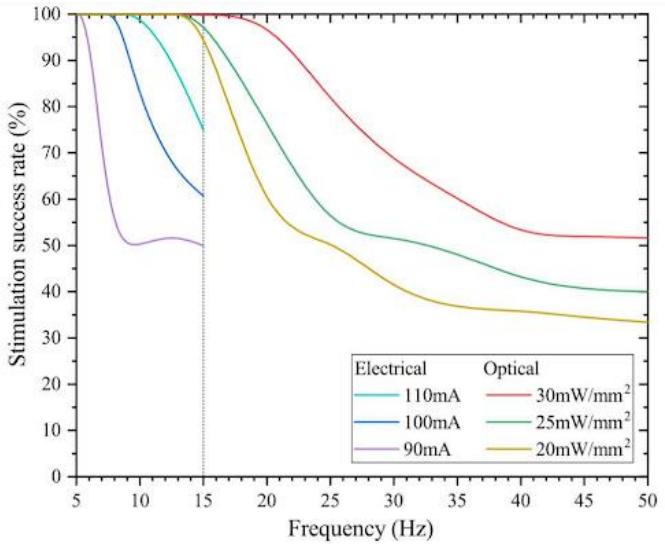

Fig 2: Average Stimulation Success Rate[3]

Waveform of Action Potential:

The superimposition of applied current stimulus and existing membrane potential in ECS methods hinders the ability to acquire feedback and result in the waveform of the action potential caused by current stimulation being indistinguishable from membrane potential. [6] This problem is solved by generating optical action potential using OCS methods that vary in length and strength of the optical stimulation. It produces ion flux through a cell membrane that responds rapidly to any changes in stimulus.

Optogenetics in DBS and Neuromodulation

DBS is a method in which brain regions are stimulated by implanting electrodes, generating electrical impulses. This method is a possible treatment option for many diseases such as Parkinson's and epilepsy. Using electrical stimulation, only a coarse level of neuromodulation can be achieved. But if light-sensitive cells are used, optogenetics can provide a much more selective alternative and a possible therapy in neurological conditions. Recent advances in optogenetics have significantly reduced vector-associated cytotoxicity and immune responses using viral vector technologies and have shown considerable pre-clinical promise. [7] Optogenetics approaches have been shown to reduce epileptiform activity in rodent models of epilepsy. Optogenetics approaches are highly specific due to the use of cell-specific promoters which express opsin in the selected cell where the gene is transcribed. Like electrical stimulation, we can also inhibit action potential in selected cells using infrared pulses. [8] Modern optogenetics promises selectivity and parallelism at par or even better than electrical stimulation [9][10] using custom opsin design. Optogenetics approaches have overwhelming prospects, thanks to modern research in the field. By combining different opsins such as channelrhodopsin 2 (ChR2), Archaerhodopsin-3 (Arch), and Halorhodopsin (NpHR) enables us to control cellular effects sensitive to a different wavelength of lights. Several chemicals such as ions, enzymes, neurotransmitters can be expresses in the brain using light by modifying the genome of the cells or using drug encapsulating Liposomes. [11] Recently, a new study highlighted the possibility to control and modify synapses by changing the composition of excitatory synapses using light by using an optical dimerization. [12] The expression of postsynaptic proteins can specifically and reversibly be controlled with demonstrated utility in research. Possibilities of controlling mechanical interaction between cells are also hypothesized [13], which were not considered possible earlier.

Possible Clinical Applications:

Optogenetics may provide alternative and better treatment strategies for neurodegenerative diseases than conventional electrical methods. Neuromodulation is currently one of the therapy methods for epilepsy, but the pathophysiology of this condition remains unknown. Recently, seizure-like activity in mice was suppressed by optogenetic stimulation of excitatory neurons in the mouse entorhinal cortex. High selectivity of optogenetics can allow us to form a more precise pathophysiology of conditions and suggest efficient treatments [14][15] Optogenetics has a vast potential in prosthetic devices and may prove to have

even better qualities than electrical stimulation. [16][17] New retinal prosthesis approaches involving sensitization of neurons using light-sensitive pumps and ion channels have been proposed that may improve sensation significantly.

The above points strongly support that optogenetic approaches are at par or sometimes better in procedures such as Deep Brain Stimulation and Neuromodulation thanks to their high selectivity and Customizability. As more progress is being made in this area, optogenetics is turning out to be an ideal candidate to replace electrical stimulation. Its results in treating neurodegenerative diseases in model organisms also seem promising. Optogenetics could soon become an alternative to DBS [18] in the treatment of Parkinson's disease [19] and epilepsy. Being a non-invasive approach, it does not require any implantation. As the processing powers of GPU increase, we can provide more performance of optogenetic devices at a much lower cost.[19]

Longer Stimulations Using Optogenetics

Electrical stimulations cannot be applied for longer durations of time. Harmful chemical products are accumulated at the electrodes and their quantity is proportional to the electrical charge delivered. These are called the Faradaic processes. These chemicals can also diffuse into the tissue and affect it, therefore persistent electrical stimulations are not possible electrochemically. The only way to reduce the damage from irreversible Faradaic processes is the shortening of the electrical pulse.

Optogenetics on the other hand has been successfully used to apply longer pulses. Long oscillatory optical pulses were given to Purkinje fibre and sino-atrial cells. Pacemaking frequency increased with the irradiance and a very large dynamic range of frequencies could be obtained. All this was done under constant illumination. The experiment was also successfully carried out without any harmful side effects. Suppression of activity of a number of neurons both in vivo and in vitro has also been obtained using hyperpolarizing opsins.

These results give us confidence that whenever longer stimulations are required optogenetics is better than electrical stimulations and is the way forward. [20]

Optogenetics for Enhanced Neurite Growth

Neural tissue growth rate has been studied with respect to optogenetic stimulation and compared with AC electrical stimulation. Neurite growth is influenced by a number of factors including neurotrophic factors, chemical gradients or schwann cells. Electrical stimulation for a few hours resulted in increased expression of neurotrophic factors and receptors which helped in nerve growth while optogenetics increased it by three times. Optogenetics expresses light-gated ion channels like channelrhodopsin 2 (ChR2) and controls neural activity in specific desired areas. Neurons were optically stimulated with different frequencies and on different parameters and neuronal growth was observed using light sensitive dorsal root ganglia (DRG). There was an increase in neurotrophic factors like nerve growth factor (NGF) and brain derived neurotrophic factor(BDNF) which even correlated to and polarized the growth of wild type neurons which were in close vicinity to stimulated neurons. An LED array assembly was used to check for the effects of optogenetics with varying parameters such as time, intensity, wavelength and frequency. It was observed that there was a roughly three-fold increase in the area of expansion in stimulated ChR2 DRGs as compared to wild types and unstimulated ChR2 DRGs. It was better than AC electrical stimulation as it used a significantly greater pulse width (100 microseconds) and provided around 3.2 mm growth with respect to unstimulated DRGs. It was still lower than the results obtained by optogenetically stimulated results at 5 microseconds pulse width at 5 Hz frequency (maximum outgrowth).

Other than enhanced neurite growth and inducing growth in surrounding neurons, other effects were also studied. To observe the changes in expression of soluble neurotrophic factors, ELISA array method was used. It was observed that NGF factor increased consistently with a greater increase in optogenetically stimulated ChR2 DRGs. BDNF factors increased for the first 2 hours and decreased after 5 hours of optogenetic stimulation, however the increase was almost 4 times to that of unstimulated and wild type DRGs. With the increase in NGF factor, increased migration of Schwann cells was also observed, thus, expanding Schwann cell networks.

Optogenetics can be extremely helpful in treating peripheral nervous system injuries or neuronal damages by inducing neurite growth.[21]

Advancements in Electrical Stimulation

Transcranial Direct Current Stimulation (tDCS) is a modern electrical stimulation method, which is capable of non-invasive electrical stimulation. The method also promises the ability of choosing the type of modulation (excitation/inhibition). However, tDCS is in the developmental stage currently. Though it promises a cheap and painless version of electrical stimulation, its effectiveness is not as good. It has been found to work only in a fraction of subjects and in them too, it doesn't necessarily show the desired results. They also have some short-term side effects like itching of the scalp and mental fatigue. [22][23][24]

Provided that optogenetics has already achieved what tDCS vouches for, and shows even more promise for the future, it seems that optogenetics will in fact make electrical stimulation irrelevant in future.

CONCLUSION

Conclusively, a lot of research has been conducted in this field which tells us that optogenetics will indeed make electrical stimulation irrelevant. Various points such as better temporal and spatial resolutions, a wide variety of control options, option for longer stimulation and giving a better option for neurite growth are indicative of the fact that optogenetics can do much of the work that electrical stimulation does and even does better at in many aspects. Further, the coherence of the results of various promising studies suggests that optogenetics will in the future replace electrical stimulation. In this debate report, we have strengthened the claim by citing many important studies and papers which have been conducted for optogenetics so far. But there's still some way to go to replace electrical stimulation completely but future promise shows us that work is being done in this direction. Optogenetics has the potential to evolve the field of neuroscience as neuronal circuits become a new favourite topic among researchers paving the way for the revolution in molecular neuroscience. Further optimizations and properly parametrizing the stimulation, optogenetics can be as efficient as the electrical stimulation in power consumption too. With researchers continuing their effort to develop even more efficient opsins that can be fully customizable for every experimental design and integrating Optogenetics with other technologies to study the brain circuits better, researchers are more confident than ever to achieve the objective of making Optogenetics the universal brain-stimulating method. As we continue to join the gaps in the method and resolving inconsistencies every day, the day optogenetics makes electrical stimulation irrelevant is not very far away.

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