TOWARD TRANSLATIONAL OPTOGENETICS: A SURVEY OF THE LIMITATIONS AND ADVANCEMENTS IN BRINGING LIGHT-INDUCED NEUROMODULATION TO THE CLINIC

FINAL PROJECT REPORT

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Abstract

Optogenetics, the use of optical energy to target genetically modified cells, stands among the most critical advancements in modern neuroscience. By offering cell-specificity, fast temporal dynamics, and reduced invasiveness from traditional modalities, optogenetics has equipped the scientific world with a tool for deliberately investigating neural circuits, deepening our understanding of cognition, behavior, and disease. Though its roots lie in a half-century of biological sciences, the seminal optogenetics findings relevant to neuroscience were established less than two decades ago. Limitations have since existed in the development of optical techniques for neural interfacing and addressing these challenges has been the focus of many academic pursuits, with a vision for translational optogenetics serving as a significant motivation for the field. Much work remains in closing the gap between optogenetics at the benchtop and a viable method for that in a clinical setting but promise and efforts remain in evolving the field from academic investigation to patient therapy.

Introduction

The human central nervous system, capable of complex intellectual learning, precise motor control, deep emotional connection, autonomic regulation, and much more, remains one of the most prominent and exciting frontiers in science. To study the brain's form and function is not just an exercise in existentialism, but rather an opportunity to understand the mechanisms which underlie a healthy brain and as such better address disease and dysfunction as they arise. By nature, the hierarchical and complicated subsystems within the brain, from its gross anatomical features to its molecular signaling paradigm, are difficult to dissect. For as long as the field has existed, neuroscience has pushed toward techniques and technologies which could enable specific and controlled investigation at the spatiotemporal scales relevant to the brain. While implantable electrodes and pharmacological approaches remained a research focus for decades, each fell short in producing such a tool.

In 1971, biologists described a light-sensitive protein within the cell membrane of a bacteria known as *Halobacterium halobium*, providing the first known recognition of a naturally occurring mechanism for light to modulate ionic flow in living cells [1]. In the following decades, various opsins serving similar functions were also discovered, including halorhodopsin and channelrhodopsin. However, it wasn't until 2002 that living cells were first engineered to express rhodopsins, and not until 2005 that Boyden *et al.* pioneered the official field of optogenetics with the fast-acting Channelrhodopsin-2 (ChR2) [2], [3] . By demonstrating cell-type specificity, high temporal precision, function within excitatory and inhibitory signal transmission, and mechanistic reversibility, this work introduced a new generation of neuroscience research. While monumental in academic research, the traditional methods for optogenetic interrogation were limited. Just two opsins, each with a distinct peak optical sensitivity, were used in the seminal work, allowing for minimal simultaneous but exclusive control of different cell types [3]. Subsequent *in vivo* studies required invasive and bulky light delivery methods, and light attenuation in tissue constrained control to superficial regions of the brain [4]. Further, these challenges, along with the genetic nature of the technique, have prevented significant progress toward clinical applications [5], [6]. Here, we discuss two subfields of research dedicated to addressing these limitations and provide an outlook on translational optogenetics.

Advancements

Sensitivity Spectrum

Soon after the first opsin was successfully introduced by Boyden et al. (2005), many publications came to investigate new opsins with different wavelength sensitivity and functionality to enrich the existing neuroscience toolbox. One such advancement came in 2007 by Zhang et. al. exploiting light-activated chloride pump, halorhodopsin, from Natronomonas pharaonis (NpHR) which can be hyperpolarized and inhibited from firing action potentials when exposed to yellow light [7]. This was a major achievement as it allowed researchers to have an on/off switch when expressing both ChR2 and NpHR using distinct wavelengths. After these seminal discoveries, the field slowed down for several years due to the complexity, lack of guidance and available tools in modular genetic methods for specific cell targeting. However, under the right conditions, more discoveries were made for variants exhibiting faster kinetics, differentiated color response, and altered ion conductance [8]. Altogether, scientists now have a plethora of specialized opsins to choose from, enabling them to reach higher complexity and spatiotemporal precision in their experiments. A large color palette is an important steppingstone to reach such desirable complexity, allowing researchers to target multiple cell types simultaneously with a multichromatic approach. While blue, green and yellow light sources have been used first, groups started investigating red light as well as infrared as these frequencies allow for greater penetration through biological tissue like the brain [8]. It is also noteworthy that genetically encoded fluorescent reporters, attached to opsin-derived voltage sensors have been shown in recent years [9]. Though not traditionally used in optogenetic research, these reporters open the door for bright and fast readouts of action potentials.

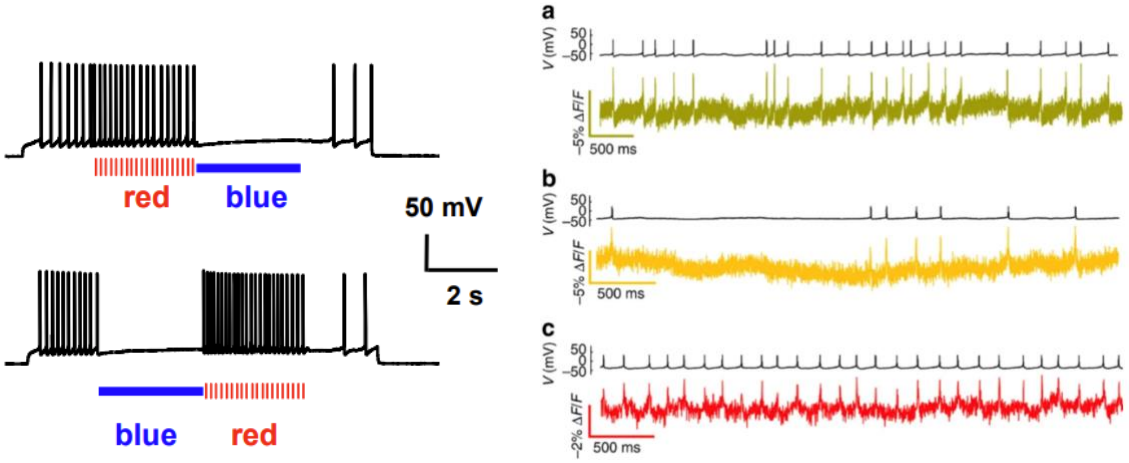

Figure 1. On the left: Spike traces recorded with patch electrodes showing bidirectional activation of the same neuron using a multi-chromatic approach. Adapted from [8]. On the right: patch clamp recordings of APs and corresponding multichromatic optical recording traces [9].

Light Delivery

Fundamentally, optogenetics requires techniques for delivering light efficiently to opsin-expressing neurons to elicit a response. While specific sensitivity varies by opsin, challenges arise particularly *in vivo* as tissue limits the penetration depth of light. Moreover, the traditional approaches utilize light in the visible spectrum, whose high energy nature only exacerbates the attenuation through the skull and neural tissue. Through surgical implantation to bypass the skull and fiber optic methods to deliver more directed light, rodent research has side-stepped these limitations. However, these solutions are largely non-translational as the tethered external devices and surgical procedures are highly invasive and the required penetration depths in human patients are on a much larger scale. Much effort has gone into developing less invasive approaches which can achieve signal transmission into deeper regions of neurological tissue.

A natural progression toward decreased invasiveness in optogenetic research has been in the development of wireless approaches for power and communication. By utilizing radio-frequency (RF) inductive coupling, paired with techniques to fabricate microscale light-emitting diodes (μLED), groups have invoked localized optogenetic neural response in various animal studies and throughout the nervous system [10]–[12]. Advanced photovoltaic systems have improved RF harvesting capabilities and enabled high-efficiency power transmission to drive the LEDs, while alternative circuit configurations have introduced smart-phone control of optogenetic studies. The use of flexible materials in both cortical and spinal cord studies have enhanced the mechanical properties of these wireless approaches to encourage compliance within neural tissue, thus further decreasing their invasiveness. However, these studies have required head stages for housing the necessary harvesting circuitry, as well as long ‘wired’ leads for delivering power to the respective LEDs. More recent work has involved implantation subdurally and on the cortical surface to reduce the physical burden of these devices, minimize their effect on social behavior in rodent experiments, and prevent infection, but has failed to fully address the remaining issues that electronic leads pose in deeper regions of neural tissue.

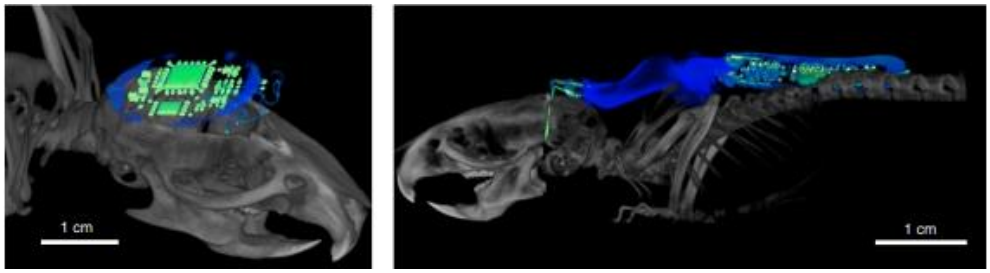

Figure 2. A CT image of a subdermal battery-free wireless microscale device in a mouse model. The device is capable of emitting light via ILEDS and RF power delivery. Adapted from [10].

Another alternative approach for achieving less invasive optogenetic capabilities deeper in the brain is using upconverting injectable nanoparticles. As a technique, upconversion involves the translation of lower energy incident photons to higher energy visible light. While the wireless techniques technically achieve this by inductive coupling, converting RF energy to visible light, nanoparticles can achieve this more efficiently by tuning the nanoparticles to a desired wavelength conversion using a designated doping concentration. This ability, combined with a tunable depth reach via the manipulation of power irradiation of the incident light offers great flexibility. It is also noteworthy to take into consideration that due to limited absorption profile of lower frequencies, the incident light will result in minimal temperature increase and undesired thermal and photochemical damage. Chen et al. (2018), describe a way of using near-infrared light, in concert with highly specialized $NaYF_4$:Yb/Tm@SiO_2 nanoparticles to achieve such local wavelength upconversion [11]. Their approach utilizes near infrared incident photons which are then up-converted into blue light that is optimal for channelrhodopsin-2 activation. In a similar fashion, other groups show similar results, using slightly different tools. For example, $Gd_2(WO_4)_3$:Eu nanoscintillators were used alongside X-rays to mediate energy absorbed into optical photons [12]. Although upconversion of low energy photons allows researchers to control deeper brain regions, with a reach of a few millimeters [11], it is only sufficient for animal models with relatively smaller brain volumes. For example, to reach the limbic network in a human, which has clinical relevance, a few centimeters of tissue are needed to pass through. That depth is further than currently possible with the current conversion efficiencies. While further improvements are necessary, these two methods could pave the way for further advancements in the field of mediating light conversion using injectable agents.

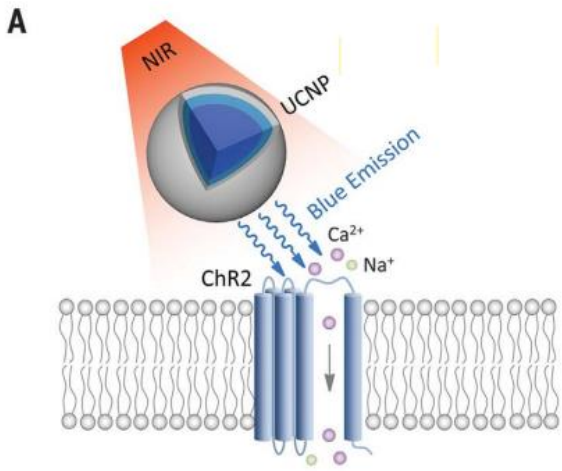

Figure 3. A schematic illustrating the principle using external low energy incident light while nanoparticles locally mediate upconversion into visible light to target respective opsins. Adapted from [11].

Future Technological Direction

Beyond RF coupling techniques and upconversion via nanoparticles, various other methods have attempted to introduce wireless solutions to deep brain optogenetic stimulation, including semiconductor stacks for infrared (IR) upconversion and mechanical devices such as ultrasonic transducers and piezoelectric harvesters [13]. In other subfields, methodologies and protocols have improved concentrated delivery of RF energy in biological tissue, including the brain, at much greater depths without surpassing clinical margins of heat dissipation [14]. Further, channel sensitivity to other forms of energy, such as thermal stimulation, may offer alternatives which mitigate or supplement the optical constraints in optogenetics [15]. With each advancement comes tradeoffs, such as the chronic implications of sustained heat or RF energy to neural tissue, and these must be considered moving forward.

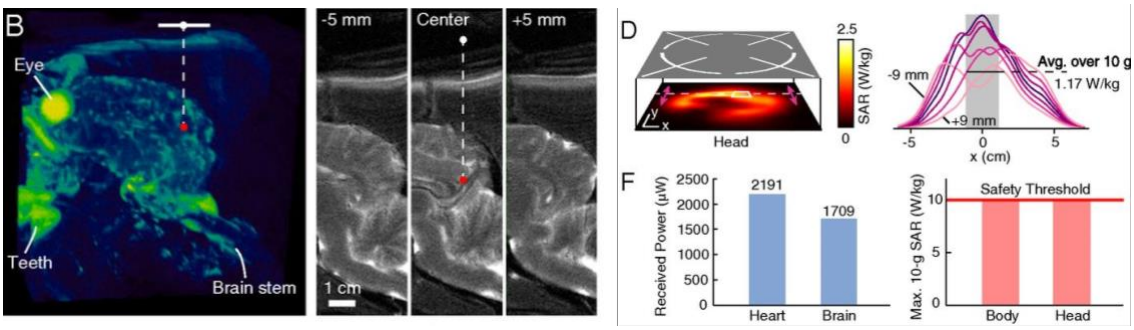

Figure 4. Improvements in delivery of directed RF energy particularly suited for biological tissue adds promise to academic optogenetics while maintaining subthreshold safety values for future translational opportunity. Adapted from [14].

As technology in fields apart from neuroscience continue to progress, the opportunity for integration into optogenetics grows. One integral leap which has not yet been made in the field of optogenetics is the use of magnetic field gradients, such as those in an MRI setting, to simultaneously actuate and track magnetoelectric and magnetothermal nanoparticles through the bloodstream and the blood brain barrier into the brain [16]. By combining the magnetic sensitivity with the ability to upconvert light locally, nanoparticle research has the potential to further shift optogenetics toward a less invasive procedure, at least from a physical and immune perspective. Tangentially, far-field inductive coupling has been used for wireless transceiving in the context of fish, but for the same fundamental reasons as addressed by upconversion discussed here [17]. Micro and nanofabrication techniques enable the development of these devices at a scale that may soon be injectable in the brain. While significant progress has been made, optogenetic techniques remain infeasible for clinical applications, but not without promise.

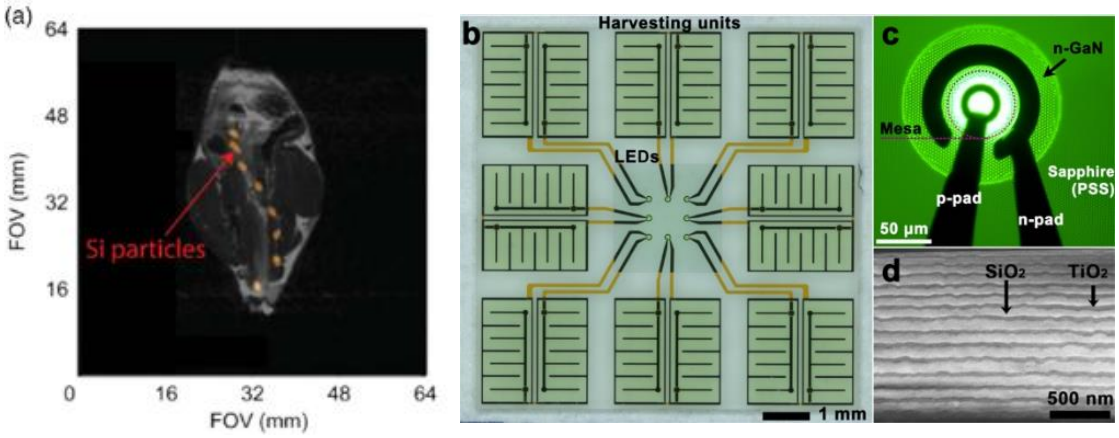

Figure 5. Examples of technological advancements adjacent to optogenetics, including wireless microscale LEDs and MRI-guided nanoparticles, which may be of use in future generations of optogenetic techniques. Adapted from [16] and [17], respectively.

Translational Outlook

Existing Research

While the regulatory limitations of optogenetics currently prevent use in humans, the technique has been used extensively to study disease models in animals and extrapolate the results to better inform physicians and improve clinical outcomes. One study used rodent models to improve the mechanistic mapping of depression in humans, targeting symptoms such as anhedonia, changes in appetite, and social avoidance [18]. In another study, the role of the infralimbic brain region in PTSD was

investigated, in which optogenetic stimulation reversed the characteristic hypoactivity in trauma, demonstrating an improvement in fear related symptoms but not anxiety [19]. In Parkinson's research, optogenetics has been used in various studies to dissect the various motor pathways in the basal ganglia. Aristieta et al. specifically investigated the pathways which invoke the external region of the Globus Pallidus (GPe), known as the indirect and hyperdirect pathways, and better defined their roles in movement regulation [20]. Another study demonstrated the mitigation of Parkinsonian motor symptoms in rodents by optogenetically stimulating the direct pathway [17]. Similarly, another study looking to reverse bradykinesia and hypokinesia in mice treated with 6-OHDA, found that optogenetic stimulation with a 100-130Hz of the afferent pathway from the STN saw significant reduction in symptoms [21].

Although not extensively discussed here, translational research has been conducted beyond the nervous system. For example, extensive work has been produced in identifying uses in the cardiovascular system, including monitoring, conformal changes, and cardiac pacing [18]. Not only for the benefits of the cardiovascular system, but the whole regenerative field in medicine has the potential to benefit from translational optogenetic research. In the review by Spanuolo et al. (2019), applications to bone repairing, tissue engineering and post-stroke recovery seem possible [22]. Though these and countless other studies have helped guide translational neuroscience and neurology, they can neither replace human studies nor provide therapy directly to patients. However, longitudinal progress in the medical field itself may yield progress toward truly translational optogenetics.

Medical Advancements

Just as technology advances to miniaturize light-emitting technology, improve energy transmission and conversion, and introduce new light-responsive proteins, so does medicine shift toward modern approaches. This parallel evolution of fields may prove fruitful for translational optogenetics in various ways. For example, novel surgical techniques have been developed to endoscopically remove tumors in deep regions of the brain with minimal invasiveness [23], a technique that has the potential to be borrowed for implantation and injection of optogenetic stimulation devices. The recent use of mRNA technology to address the Covid pandemic by viral vaccinations, along with the ensuing mainstream breakthrough of the approach, also offers potential solutions for clinical optogenetics. In one study, genetically encoded voltage and calcium indicators were introduced to human stem cell cardiomyocytes by mRNA transfection, enabling reporter capabilities with reduced toxicity and constraints on stability [24]. This research lends to translational optogenetics by reducing the implications of genetic modification, though this sword is two sided as the effects are unlikely to be long lasting and instead require repeated injections. Altogether,

advancements in medical fields beyond neuromodulation may continue to open new doors for clinical optogenetics, though much work is required to bridge these efforts.

Conclusions

Optogenetics has been invaluable in modern neuroscience, acting as a tool for specific interrogation of neural circuits to understand disease, cognition, emotion and much more. As a field, it has grown to vast heights and countless efforts have derived from the goal of improving science's optogenetic capabilities, including bringing it to the clinic. Though human trials may not be seen in the near future, it is important to look ahead and take incremental steps towards it, remembering the power of optogenetics a tool for cell specificity, high temporal resolution and wide range of spectral proteins. It only remains to optimize the delivery method so that humans could benefit from these capabilities, and to address the concerns regarding genetic approaches for opsin expression. We believe that accessible solutions for light delivery and neural sensitivity exist, and that efforts in tangential fields will lend in achieving translational optogenetics. Together, the path toward patient-focused, therapeutic optogenetics, though unlikely trivial, is certainly traversable.

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