The Development of Optogenetics and its Potential to Cure Blindness

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ABSTRACT

In humans, sight resides atop the hierarchy of senses. The eyes allow people to perceive the world around them, interpret information they observe, and make informed decisions when faced with new situations. Without vision, a person can no longer connect with their surroundings, leaving them vulnerable to physical dangers as well as the socioeconomic consequences of blindness (Khanna et al., 2007). Blind individuals have a 44.2% employment rate compared to a 77.2% employment rate for people without disabilities according to the American Community Survey (ACS). Additionally, blind individuals have jobs with lower salaries (McDonnall et al., 2019). A cure for blindness would therefore prove invaluable as over 1.1 million people in the United States are considered legally blind. Despite centuries of research into the prospect of restoring sight, a panacea remains unknown. However, modern advancements in biotechnology and gene therapy offer promising results in helping individuals regain their vision. One such novel technique is optogenetics, an approach that involves genetically modifying cells so that they can sense light, allowing the precise manipulation of neural activity. Researchers hypothesize that through optogenetics, light activated cells could replace damaged sensory cells in the retina, possibly mitigating photoreceptor-related impairments to a person's sight. Here, we delve into new optogenetic therapies being developed to cure blindness. First, we provide a brief summary of the visual system, discuss the advent of optogenetic research in its early stages, and finally analyze how this technology has been implemented as a method to restore sight in animal models and a human case study.

Vision

To approach the mechanisms of blindness, one must first understand the visual system. Light acts as the primary input for the eyes, interacting with the optical organs. The wavelength of light determines perceived color, yet humans can only detect wavelengths between 400-700 nm. Light enters the eye through the pupil, which is controlled by the iris to limit the amount of light allowed in. Next, the lens focuses light onto the retina, a thin layer of tissue that lines the back of the eye (Figure 1). Five types of neurons populate the retina: photoreceptors, horizontal cells, bipolar cells, amacrine cells, and retinal ganglion cells (Figure 2). Light reaches the two types of photoreceptor cells, rods and cones, before traveling to the retinal ganglion cells. Cones contribute to photopic vision in the presence of substantial light, characterized by low sensitivity but high visual acuity (Cao, 2013). On the other hand, rods are highly active in the absence of light, supporting the highly sensitive but less acute scotopic vision. While cones occupy the fovea in the center of the retina, the more numerous rods are spread out along the outer perimeter of the eye.

Photoreceptor cells play a crucial role in visual transduction. In the dark, they release glutamate, an excitatory neurotransmitter that closes cation channels in bipolar cells, keeping them hyperpolarized, preventing a signal from occurring at the ganglion cells. However, this system shifts when pigment molecules found in rods, or rhodopsins, activate (Feher, 2012). These rhodopsins serve as receptors that, when activated, close sodium channels in the rods and cones, resulting in hyperpolarization. As a result, glutamate production is minimized, and the bipolar cells can release neurotransmitters that cause action potentials in the ganglion cells. Action potentials generated in the retina must then travel along the optic nerves and through the lateral geniculate nuclei found in the thalamus before reaching the primary visual cortex (Wang, 2009) (Figure 3). Visual information arrives at the primary visual cortex (V1), where

it is received and then segmented for easier integration. It then proceeds to the secondary visual cortex (V2) and the visual association cortex for further processing. V2 and the visual association cortex are the cortices responsible for higher order visual analysis. Once information has been evaluated, it then advances to the prefrontal cortex through the ventral and dorsal streams (Figure 4). The dorsal stream consists of spatial information such as location while the ventral stream details object characteristics like color (Milner, 2001). This simplified description of the elaborate visual system defines the boundaries of sight and how light entering the eyes can be converted to useful information.

Yet, as a result of its complexity, minor disruptions to the visual pathway can lead to major consequences. The most common types of blindness include cortical visual impairment (CVI), retinitis pigmentosa, macular degeneration, and glaucoma (Jeffery et al., 2021). CVI commonly occurs in children when V1, V2, or the visual association cortex has been damaged. Although there is nothing wrong with the eyes themselves, the analysis pathway of the visual stream is disrupted, so patients with CVI cannot process the images they see. Retinitis pigmentosa is a genetic condition that involves the breaking down of rods in the retina. Without functioning rods, an individual's eyes fail to respond appropriately to light, subsequently impairing one's vision. On the other hand, Macular degeneration, which is common in older people, affects the cones located in the fovea. The macula refers to the area encompassing the fovea in the center of the eye, responsible for one's central and keenest vision. Glaucoma refers to eye diseases stemming from damage to the optic nerve. This deterioration usually occurs because of high eye pressure and halts the transmission of information from the eyes to the visual cortices. Blindness can occur due to disturbances in all aspects of the visual system. However, as of now, retinitis pigmentosa offers the clearest path for recovery using optogenetics, as optogenetic tools can be directly implemented in the retina to circumvent damaged rods.

Basics of Optogenetics

Modern optogenetics is defined by the use of viral vectors to insert genes for light-sensitive proteins into living cells in order to make the cells themselves respond to light. As a result, scientists can manipulate and analyze the functions of these altered cells by activating them with light signals. In the late 1970s, molecular biologist and neuroscientist Francis Crick emphasized the need to reliably analyze individual components of the brain before moving on to the sum of its parts. However, early attempts at neuronal control were widely limited in scope and accuracy (Fenno, 2019). For example, using electricity to stimulate the nervous system lacked the precision required to isolate individual neurons. Additionally, the field of chemogenetics, pharmacological manipulations such as utilizing GABA agonists, failed to meet the temporal requirements associated with stimulating neurons and measuring their immediate responses. However, microbial opsins, which are ion channels or pumps found in all organisms, offer a single component mechanism that allows for manipulating neurons with high specificity and speed. Microbial opsins that react to light are special in that they actually occur naturally in algae and can be differentiated by their response to different colors of light. Any animal cells expressing opsins serve as photoreceptors that signal a physiological response upon light absorption. Generally, they either hyperpolarize or depolarize neurons depending on the specific characteristics of the opsin. Depolarization causes a neuron to fire an action potential, whereas hyperpolarization prevents a neuron from firing. Channelrhodopsins were the first opsins implemented in optogenetic experiments, characterized by their excitatory nature and sensitivity to blue light specifically (Figure 5). Recently, the effects of inhibitory halorhodopsins and archaerhodopsins are being tested in research, activated by yellow and green light respectively.

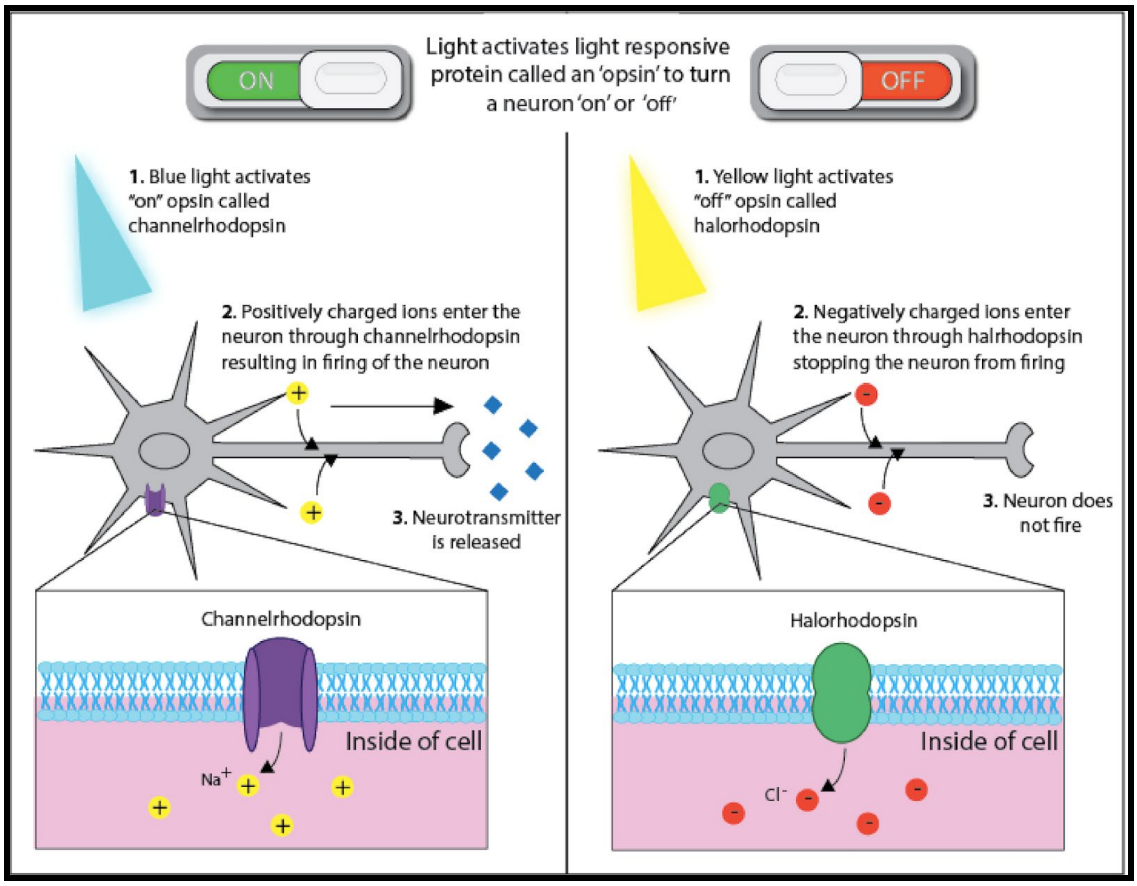

Figure 5: *Left panel: Turning a neuron on. Blue light activates the 'on' opsin resulting in the neuron firing and releasing neurotransmitter. Right panel: Turning a neuron off. Yellow light activates the 'off' opsin preventing the neuron from firing. (Caption taken from Lyon et al., 2016)*

Furthermore, scientists have even created step function opsins by altering channelrhodopsins so that they remain stimulated for longer periods of time (up to thirty minutes compared to a few seconds). This enhanced stimulation period allows researchers to analyze behavioral effects such as learning and motor control over longer periods of time to better quantify their durations.

To create light-activated cells, scientists utilize viruses as gene therapy vectors to insert the DNA for specific microbial opsins into the target cell. Lenti and Adeno-associated (AAV) viruses have proved particularly successful because of their fast and wide spreading infection rates. In addition to targeting regions of the brain, some researchers focus on targeting more precise neural circuits. One method for accomplishing this is monosynaptic rabies tracing, which involves using rabies as the viral vector due to its trans-synaptic properties that allow it to travel from neuron to neuron (Osakada et al., 2013). However, viral expression is limited in that only small specific promoters, the segments of DNA that are relevant to transcription, can be used. This caveat is due to only some promoters allowing for targeting gene expression in certain cell types. Since such specificity is rare, researchers needed animals that could handle larger genetic payloads. Thus, scientists developed transgenic mouse lines, genetically modifying mice that could incorporate a wider range of promoters and directly express opsin genes. Though costly and time consuming, this innovation reduced the downsides of viral vector use. Furthermore, due to the specificity of gene expression in transgenic mouse lines, scientists identified distinct promoters expressed in certain regions of cells, such as individual layers of the prefrontal cortex. With this knowledge, researchers are able to manipulate a particular neocortical layer, allowing them to establish relationships between a singular region of the brain and any resulting behaviors. By

targeting areas of the prefrontal cortex important for behavioral development, scientists implemented optogenetic tools in mice embryos to assess physiological changes at an earlier age (Bitzenhofer 2017). To deliver light to the brain in order to control neurons, researchers utilize a variety of mechanisms such as Mercury Arc lamps, lasers, light-emitting diodes, and LED arrays. For in vivo experiments, laser beams are delivered via optical fibers inserted through chronically implanted cannulas. Activity is measured using silicone multisite electrodes and movable tetrode arrays as well as 2-photon calcium imaging to observe relative neural activity in brain tissue. Through this complex and dynamic intersection of genetics and technology, researchers can control cell function optically, facilitating further investigation of specific cells as well as setting the stage for potential clinical applications.

Versatility of Optogenetics

The earliest applications of optogenetic experiments were aimed at discovering the functions of various types of neurons and their effects on behavior. In one study, researchers found that stimulating hypocretins/orexins, neuropeptides that modulate the sleep cycle, in the hypothalamus of live mice would wake them up, whereas inhibiting those neurons would cause them to fall asleep (Adamantidis et al., 2007). Such results hint at a future for drugs that target these types of neurons in order to combat sleep disorders. Other studies identified neurons contributing to aspects of hunger, thirst, energy balance, respiration, arousal, and more. For example, optogenetic activation of certain neurons produced intense drinking behavior, even in fully water-satiated animals (Oka et al., 2015). Even social behaviors such as aggression and risk taking have been analyzed using optogenetics as researchers stimulate or inhibit certain groups of neurons in live mice and track their subsequent behavior.

As optogenetics advances, one possible application emerged to restore sight. As stated before, retinitis pigmentosa occurs when the photoreceptor rods in the retina become damaged. However, optogenetics approaches have shown that ordinary neurons transform into photoreceptors when injected with rhodopsin DNA through viral vectors (McClements et al., 2020). Thus, researchers hypothesized that other cells in the retina could assume the role of rods if they could sense light. This theory was tested and proved successful when completely blind mice treated with the aforementioned gene therapy vectors showed evidence of recovering vision (van Wyk et al., 2015). Engineered Opto-mGluR6 opsins, which are sensitive to ambient light, were induced in the retinal bipolar cells of the mice, allowing them to regain retinal function and detect changes in motion. Remarkably, the treated mice were even able to complete mazes with their reintroduced sight. No noticeable immunogenic or cytotoxic changes occurred in the retinas of the treated mice, indicating that the procedure was essentially harmless.

The safe implementation of viral vectors paved the way for this technology to be introduced in primates. By using primates, researchers are able to test and perfect the methods they plan to implement in humans. Gauvain (2021) attempted to inject a specific channelrhodopsin into the eyes of blind macaques, then realizing that the blue light required for neural stimulation was potentially dangerous. Instead, they used a red-shifted opsin with a lower chance of causing harmful radiation. The 18 macaques' retinal responses were measured ex vivo, as the cellular activity of transfected retinas were tracked while they were being shown digital moving bars and shapes. Although the animals' behavioral changes couldn't be adequately assessed since the therapy's efficacy was analyzed ex vivo, the specs of the viral preparation and integration proved successful and would later be used in human clinical trials. The implementation of the therapy effectively raised visual acuity to 20/249, measured through response tests performed on the still functioning ex vivo retinas, which is above the threshold for legal blindness (20/400) (Figure 6). This study brought researchers one step closer to being able to implement this treatment in humans.

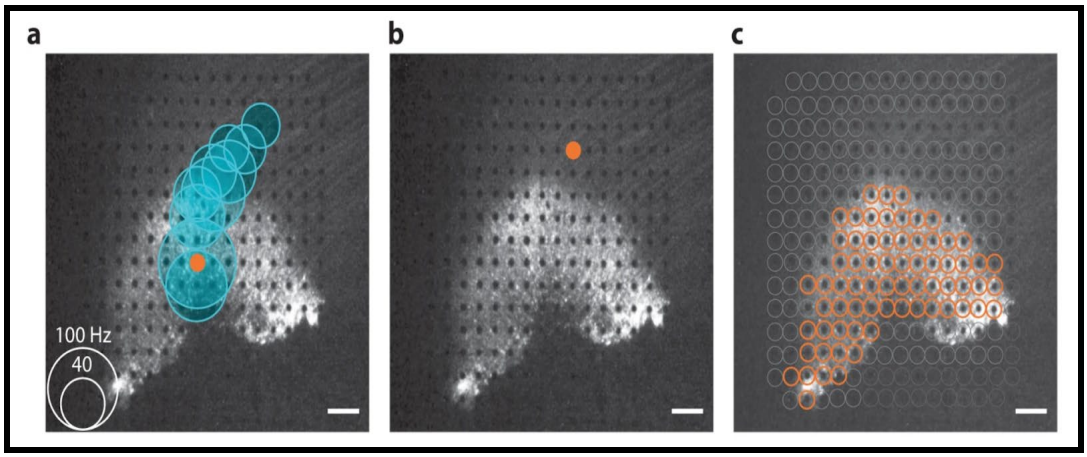

Figure 6. a: *The white area of the image represents the perifovea and the black dots show electrodes to measure responses. The orange dot displays the area of light stimulation, and the blue circles, representing neural spikes, demonstrate a positive reaction of vision in the presence of light.* ***b:*** *When the light stimulation is outside the perifovea, hitting the axons of the affected RGCs instead of their soma, no response is detected.* ***c:*** *An overall stimulation of the area results in activity spikes shown by the orange circles, almost exactly matching the shape of the perifovea (Caption taken from Gauvain et al., 2021).*

These studies reveal the tremendous possibilities optogenetics has to offer, particularly in the medical field where its applications extend beyond the original scope of analyzing neural circuits.

Recovering Sight in Humans: A Case Study

In order to develop optogenetic therapies for human clinical trials, regulations need to be followed to mitigate the risks of the experimental treatments. As of now, implementation of optogenetics in humans' brains has not yet been achieved. Therapies implemented in humans' brains will likely utilize both a viral vector to introduce the opsin and an implantable medical device to deliver light (White et al., 2020). First human drug trials for optogenetic therapies will be implemented in the brains of clinical populations. As such, preclinical testing on a variety of models, from non-human primates to human organotypic tissue slices, is essential to determine possible adverse effects of these therapies. Some risks include tumor formation, negative immune responses to viral vectors, and cellular damage caused by opsin expression (Shen et al., 2020). While implementation of optogenetics in the human brain remains unproven, optogenetic therapies in human eyes are more promising as the eyes already observe natural light: there is no need for an implantable medical device. Therefore, once the safety and reliability of the chosen viral vector has been established, clinical trials can progress.

GenSight Biologics, a biotechnology company based in Paris, sponsored a clinical study to assess the efficacy of GS030-DP, a specific optogenetic vector containing channelrhodopsin ChrimsonR, in patients with retinitis pigmentosa (Sahel et al., 2021). The chief innovative aspect of this study was the incorporation of GS030-MD, visual interface stimulating glasses (Figure 7).

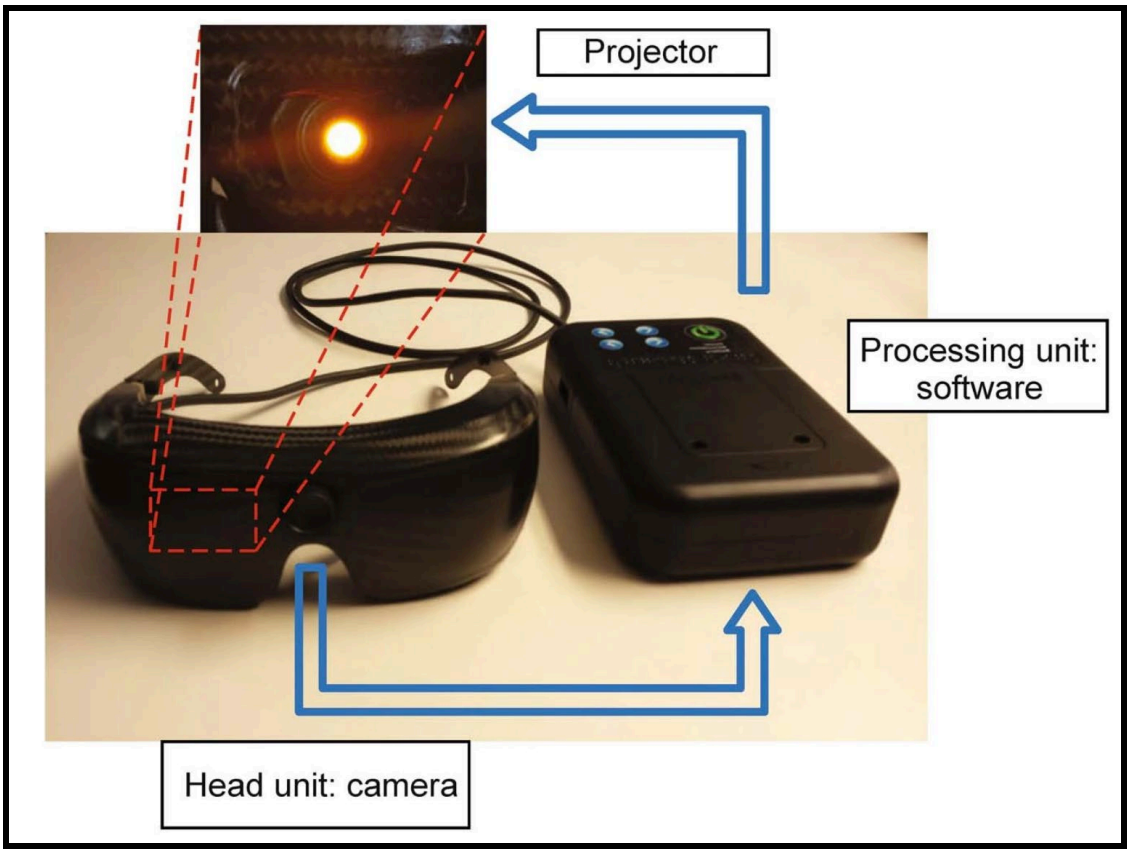

Figure 7. *GS030-MD consists of two units connected by a high-speed link. The head unit hosts the camera which acquires the natural scene in a stream of asynchronous address-events representing pixel coordinates of local relative light intensity changes. The processing unit encodes the visual stream in real time and creates binary images that are sent to the projector in the head unit. (Caption taken from Sahel et al., 2021).*

These glasses employed a neuromorphic camera, which analyzes real world inputs in a way that mimics human physiological processing, to interpret the scene in front of the subject and convert it to monochromatic images. The glasses then project these images onto the retina of the patient as 595-nm amber light pulses. The study entailed three dose escalation cohorts for the vector: a low dose cohort, a medium dose cohort, and a high dose cohort, with each cohort consisting of 3 patients. Additionally, an extended cohort of 6 patients treated with the high dose was included, for a total of 15 patients in the study. While the primary objective of the study was to determine the safety of the therapy, secondary objectives included evaluating the effects on visual function and acuity.

Each patient was injected with the ChrimsonR viral vector combined with tdTomato, a red fluorescent protein that facilitates ChrimsonR expression and makes the viral expression visible, into the worse-seeing eye of the subject. The intravitreal shot targeted foveal retinal ganglion cells in order to circumvent the damaged photoreceptor cells caused by retinitis pigmentosa. The particular viral vector used, AAV2.7m8-CAG, contained specific promoters that optimized inner retinal expression. Because of the red shifted nature of the opsin, the optimal color sensed by the affected eye is amber (around 590 nm). As discovered in the nonhuman primate study, red shifted opsins are safer, because unlike the blue light sensed by ordinary channelrhodopsins, amber light does not constrict the pupils as much and yields safer results.

Full testing and analysis was only completed for one patient, a 58 year old male, and the study is estimated to be completed by 2025. For the 84 weeks that he was under observation, they found no evidence of intraocular inflammation, changes in the anatomy of the retina, or systemic adverse events. Systemic training of the glasses was

administered four and a half months into the experiment, allowing for adequate time for the opsin expression to stabilize. After seven months of visual training, the patient reported improvements in vision when wearing the goggles. The metric for visual function was established using three tests under three conditions: both eyes open without the light-stimulating goggles (natural binocular), only the treated eye open without the goggles (natural monocular), and only the treated eye open with the goggles (enhanced monocular).

The first test involved perceiving, locating, and touching an object 40 cm away from the patient. The object was either a large notebook or a small stapler box, placed in variable grayscale contrasts of 40%, 55%, or 100% (different colored backgrounds). The researchers observed that while the subject could not perceive the objects at all in the natural binocular or monocular conditions (without the goggles), he could perceive, locate, and touch the large notebook 92% of the time in the enhanced monocular condition (with the goggles). Furthermore, the data reveals that while the relative contrast of the objects did not matter, the larger objects were easier to perceive than the smaller ones. Additionally, there was no significant difference between perceiving, locating, or touching the object, indicatin that the patient could coordinate his motor skills upon object perception (Figure 8).

Table 1 First test: finding the notebook or staple box

From: Partial recovery of visual function in a blind patient after optogenetic therapy

Stimulus	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Perceive	Locate	Touch	Perceive	Locate	Touch	Perceive	Locate	Touch
Notebook, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Notebook, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	4/5	4/5	4/5
Notebook, Contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Staple box, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	3/6	3/6	2/6
Staple box, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	2/5	2/5	1/5
Staple box, contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	1/4	1/4	1/4

No test repetition was performed because the patient was unable to complete the task. He could not see anything and did not want to try again.

Figure 8. *The data results from the first test involving perceiving, locating, and touching a singular object*

The second test is built upon the first one by displaying multiple tumblers and having the patient perceive, locate, and count them. Either two or three tumblers were placed in one of six positions along two lines 40 cm and 66 cm away from the subject. The objects were placed in the same three variable contrasts as the first test. Once again, the patient could not perceive the objects without the light-stimulating goggles, and contrast did not affect the success rate of the trials. With the goggles, he successfully perceived the objects 63% of the time, correctly counting and locating them at almost the same rate. From this test, the researchers concluded that the patient was able to perceive multiple objects as opposed to just one (Figure 9).

Table 2 Second test: counting and locating tumblers

From: Partial recovery of visual function in a blind patient after optogenetic therapy

Stimulus	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Perceive	Count	Locate	Perceive	Count	Locate	Perceive	Count	Locate
Tumblers, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	4/6	4/6	4/6
Tumblers, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	5/7	5/7	5/7
Tumblers, contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	3/6	3/6	2/6

No test repetition was performed because the patient was unable to complete the task. He could not see anything and did not want to try again.

Figure 9. *The data results from the second test involving perceiving, locating, and touching multiple objects.*

The third test combined the metric of object perception with extracranial multichannel electroencephalography (EEG) to analyze neuronal activity associated with partially recovered sight. EEGs were used because the metallic components of the glasses were incompatible with functional magnetic resonance imaging (fMRI). A tumbler was placed either 66 cm away from the patient or not placed at all, and the subject was asked if an object was present or not (Figure 10).

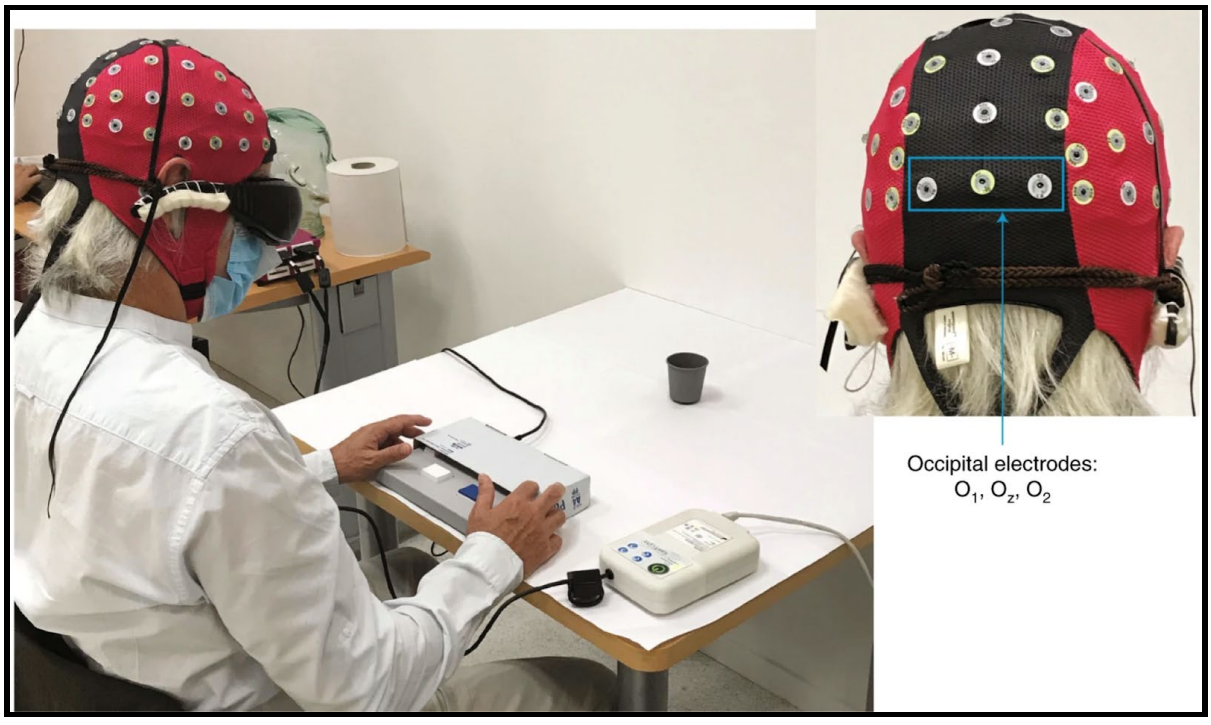

Figure 10. *Experimental setup. Behavioral responses and brain activity were simultaneously recorded during the visual test. EEG data analysis focused on the activity recorded from the occipital channels O1, Oz and O2. (Caption taken from Sahel et al., 2021)*

During the trials, EEG traces were measured for the patient's eyes-open states and eyes-closed states across all three eye conditions. Again, rate of accuracy for object perception was significantly higher when the patient wore

the light-stimulating glasses, with 41% success as opposed to 5.8% for both natural conditions. Analysis of the EEGs revealed that the distinguishing information for the third test resided above the occipital cortex contralateral to monocular stimulation. The researchers trained a decoder that read the amplitudes of occipital channels located in this area to simulate the brain discriminating object versus no-object trials. The decoder yielded a mean success rate of 78%, greater than the rate of random chance. The success of the decoder confirms that the cortical activity detected in the EEGs wasn't simply random and reiterates the extent of the partial recovery of vision in the patient (Figure 11).

Table 3 Third test: visual detection task (coupled with EEG recordings)

From: Partial recovery of visual function in a blind patient after optogenetic therapy

Trial	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Answer: yes object	Answer: no object	No answer	Answer: yes object	Answer: no object	No answer	Answer: yes object	Answer: no object	No answer
Object trial	3/30	0/30	27/30	2/30	2/30	26/30	21/32	2/32	9/32
No-object trial	3/30	1/30	26/30	2/30	1/30	27/30	3/31	5/31	23/31

Figure 11. *The data results from the third test involving determining if an object is present or not while the patient's EEG traces are being measured.*

This study not only shows that optogenetic technologies can be safely implemented in humans, but also demonstrates a momentous feat with tremendous implications: the recovery of sight. Even though the regained vision is only a partial recreation of the true nature of human sight, it represents the first step in the long journey ahead towards fully curing blindness. As the rest of the 14 patients continue to undergo treatment, more data regarding the safety and efficacy of the optogenetic treatments and light sensing goggles will emerge. With a greater sample size, researchers can determine an accurate and more detailed scope for beneficial effects of the novel technology. As of now, the combination of the goggles and the altered retinal cells show promising results for the ability to perceive, locate, and touch local objects with success in most cases. In the future, researchers will look to improve the success of object perception, expand the range of the vision, and reintroduce color into patients' lives.

Looking Forward

The clinical applications achieved by integrating opsins in the human eye mark the beginning of a new generation of biomedical technologies based on the findings of optogenetic research. Optogenetics revolves around the principle of turning cells on and off with light. While retinal cells offer the most straightforward approach as they exist naturally in a light-oriented organ, theoretically any cell can become genetically engineered to become responsive to light. Under this assumption, the rationale follows that the cells in any organ, when enhanced optogenetically, can be turned on and off in the presence of light. Therefore, under the right conditions, any organ's function can be artificially manipulated. Although this concept may seem grandiose, this paper has already provided evidence for how optogenetics can drastically manipulate function in the eyes, serving as a means to restore sight.

Other experiments have demonstrated the potential of optogenetic therapies in treating seizures and even chronic pain. In 2013, experiments performed on live mice proved that optogenetic control of thalamocortical neurons connected to the regions experiencing seizures could interrupt the abnormal electrical and behavior activity affecting the brain (Paz et. al 2013). Thus, the technique shows promise as a stop gap measure for spontaneous seizures as well as for improving conditions such as epilepsy. Another study in 2017 found that it was possible to reduce both sudden and ongoing bladder pain by inhibiting pain sensing neurons in the bladders of mice with implanted wireless LED

devices (Samineni 2017). Not only do these results, albeit in mice, display the versatility of optogenetic applications, they also provide an alternative to medical treatments involving potent drugs that carry risks of addiction and side effects.

The target cells are not the only variables in this field subject to change. What if the sensory input was variable? What if cells could sense magnetic fields or heat? Perhaps even sound waves? The transition from light to other means of stimulation emerged as a new goal in the field of genetics. Since light is limited in its ability to penetrate tissue, alternative inputs provide less invasive options for modulating cell function. Recent developments in magnetogenetics have shown that activation of magnetoreceptors can produce movement in transgenic worms (Long et al., 2015). The thermogenetic toolbox also expanded this year, with researchers having discovered new thermosensors for use in temperature-oriented experiments (Chee et al., 2022). Furthermore, the Salk Institute published a paper on sonogenetics this February, describing their successes in manipulating mammalian cells with sound (Duque et al., 2022). However, the regular implementation of genetic therapies to control neuronal activity in humans is still far off. With the variety of sensory mediums that can serve as channels for stimulation, the possibilities for this avenue of biotechnology are practically limitless. As creative and brilliant researchers from across the globe approach the human body with the intent to precisely manipulate its biology through any means, it truly is inspiring to imagine the potential of this intersection of gene therapy and technology to benefit humankind and save lives.

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