

Applications of Optogenetics as Alternative Treatment for Neurological Diseases

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Abstract

Neurological diseases are some of the most devastating illnesses due to the limited understanding of the complex nervous system network and its intricate functions. Moreover, lack of basic understanding in pathophysiology of disease hinders the development of new treatment. Optogenetics is a biological technique involving light modulated cells in living tissue. Successful application of this technique to control function of genetically modified neurons galvanizes efforts to utilize optogenetics in neurological disorders, either as treatment option or investigating tool of disease pathophysiology. This review seeks to summarize optogenetic history and highlight its application in neurological diseases, specifically: Stroke, Alzheimer's disease, Parkinson's disease, and Epilepsy. Current treatments in these diseases are summarized with discussion on how optogenetics may overcome some of the pitfalls in current treatment options. In conclusion, optogenetics is an exciting and promising tool in neurological diseases that may provide therapeutic benefit and elucidate disease mechanism.

Keywords: optogenetics; neuroscience; neurological disorder, stroke, Alzheimer's

1.0 Introduction

Optogenetics is a technique that uses optics and genetics to deliver a light sensitive tool to a living cell or tissue to gain or lose a function (Yizhar, Fenno, Davidson, Mogri, & Deisseroth). This requires engineering of a gene, which not only must be delivered in a cell specific manner but also be expressed at adequate levels. In addition, a mode of light delivery and a method for detecting the effect of optogenetics is needed (i.e. Electric current, fluorescently labelled protein, behavior in animal models) (Yizhar et al.). The concept of optogenetics was first introduced in 1979 (Crick, 1979) when Francis Crick discussed the complexity of the human brain and challenges in the field of neuroscience. The major challenge in neuroscience at the time was to develop a technique that achieved both temporal and cellular precision, since neuronal networks function with these features. While electrical manipulation with deep brain stimulation (DBS) does give temporal control of neurons, it lacks cell type precision (Li et al., 2012). Even though pharmacologic therapy can allow precise targeting, it lacks temporal control of cellular processes. Therefore, there was pressure in neuroscience to create a technique that will allow temporal and cellular precision. Crick postulated light may be the means by which this can be achieved. Years before Francis Crick discussed the challenges in neuroscience, a microbial light activated single-component ion pump, bacteriorhodopsin, was identified in an unrelated field of research (D & W., 1971; Oesterhelt & Stoerkenius, 1973). Decades of research led to thousands of papers expanding the knowledge of bacteriorhodopsin and discovery of new opsin genes encoding 7-transmembrane domain proteins functioning in response to light. The newly discovered genes were halorhodops in (Matsuno-Yagi & Mukohata, 1977) and channel rhodops in (Nagel et al., 2002). These three opsins are ion channels and pumps functioning in response to light and modulating concentration and electrical gradients in cells (Yizhar et al.). Halorhodopsin (NpHR) pumps chloride ions into the cells, and therefore hyperpolarizes the cell membrane upon light activation, resulting in spiking and neurotransmission inhibition (Matsuno-Yagi & Mukohata, 1977). Channelrhodops in (ChR) is a cation channel, allowing inward flux of positive charge (Nagel et al., 2002; Nagel et al., 2003), which can be used to induce action potentials in neurons. Although opsins were extensively understood, it took decades for optogenetics to be used by neuroscientists (Boyden, Zhang, Bamberg, Nagel, & Deisseroth, 2005) since there certain doubts about its application.

Those concerns included: photocurrents (current induced by photo activation) would be too weak and slow in neurons to control them efficiently and precisely, opsins may be poorly expressed or toxic to mammalian cells, and cofactors such as all trans-retinal (the light absorbing chromophore used by microbial opsins) would also need to be delivered to cells. However, contrary to these concern, Boyden et al 2005 reported the delivery of opsins into mammalian neurons allowed control of action potentials with millisecond precision (Boyden et al., 2005). In their work, channelrhodopsin was introduced into hippocampal neurons and conferred precise temporal control of neuronal spiking and inhibitory and excitatory synaptic transmission. Furthermore, retinoids were found present in sufficient amounts in mature mammalian brains for opsins to function (Deisseroth et al., 2006; Zhang, Wang, Boyden, & Deisseroth, 2006). This conferred optogenetics as a single-component strategy in modulating neuronal activity, since the co-delivery with retinoids is not necessary. Because of this technology, neurons can be precisely controlled for quick, specific excitation or inhibition. Since the original discovery of optogenetics in neurons, this field has vastly expanded. Constructs have been engineered to optimize gene expression and trafficking to the membrane in mammalian cells (Gradinaru, Thompson, & Deisseroth, 2008; Zhang et al., 2006). There are now dozens of single-component optogenetic tools with different spectral and kinetic properties to allow experimental manipulation (Yizhar et al.). Optogenetics has shown to be an invaluable tool in the field of neuroscience. Since the introduction of optogenetics in neurons, this technique has been a useful to study circuitry, pathophysiology, and mechanisms of current treatments in neurological diseases. Moreover, this tool has also been studied as a treatment possibility. In this review, current treatments and applications of optogenetics in stroke, Alzheimer's disease, Parkinson's Disease, and epilepsy will be discussed.

2. Applications

2.1 Stroke

Great efforts are being focused on improving stroke recovery including: rehabilitation, stem cell transplantation, brain stimulation and pharmacological treatment (Floel & Cohen, 2010; Murphy & Corbett, 2009). Brain stimulation is a capable tool, since it employs direct activation and manipulation of target area. Though studies have shown electrical and transcranial magnetic stimulation improve recovery following stroke (Bashir, Mizrahi, Weaver, Fregni, & Pascual-Leone, 2010; Fregni & Pascual-Leone, 2007; Murphy & Corbett, 2009), these techniques have their limitation. These techniques lack precision and discrimination between excitation or inhibition of all cells at the site of focus. This may lead to adverse effects such as mania, depression or confusion (Mandat, Hurwitz, & Honey, 2006). Likewise, the other alternative treatment, pharmacotherapy also lacks precision and causes many unpleasant side effects (Floel & Cohen, 2010). In addition, it has been difficult to elucidate the mechanism of stroke recovery from the previously mentioned treatments, because they non-specifically activate or inhibit a target area. The use of optogenetics in stroke models has overcome these limitations.

In a study done by Cheng et al (Cheng et al., 2014), transgenic mice expressing Channel rhodopsin 2 in layer 5 of the primary cortex were used to study the functional recovery of mice following stroke. Following stimulation to the ipsilateral primary motor cortex (iPMC) using an implanted optical fiber, mice showed significant improvement in movement of previously affected limb. Furthermore, there was an increase in cerebral blood flow in affected area and significant increase in neuroplasticity markers, which suggests functional improvement, may be mediated by neuroplasticity. In this study, stimulated mice also had a faster weight gain and performed better in sensory-motor behavior tests. These results showed the ability of optogenetics, specifically in targeting the ipsilateral motor cortex, in improving stroke outcomes. Implantation of neural stem cells (NSC) has shown to be safe and effective for stroke recovery in mice models and patients (Bhasin et al., 2013; Bliss, Guzman, Daadi, & Steinberg, 2007; Marcel M. Daadi, Maag, & Steinberg, 2008; Hao et al., 2014; Honmou et al., 2011; Jiang et al., 2013). Though it had been widely studied transplantation of neural stem cells improves sensorimotor function following stroke, it was not known whether the electrophysiological activity of NSC progeny participate in recovery of function or if progeny with excitatory function are beneficial or deleterious to functional improvement. In a study by Daadi et al (M. M. Daadi et al., 2015), NSC were genetically engineered to express ChR2, an excitatory opsin, and implanted in mice stroke models. Following optogenetic stimulation, mice showed significant improvement in sensorimotor function, suggesting excitatory function of implanted cells aids functional recovery. In addition, there was a significant increase expression of genes involved in neurotransmission, neuronal differentiation, axonal guidance, neuroplasticity, and regeneration.

Interestingly, inflammatory mediators were decreased. These results suggest the potential benefit of using optogenetics tool to further improve outcomes in NSC implantation following stroke.

2.2 Memory and Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, or cognitive dysfunction, causing up to 75% of cases (Qiu, Kivipelto, & von Strauss, 2009). Two pathological markers have been widely studied in Alzheimer's: extracellular senile plaques made of beta amyloid protein and intracellular neurofibrillary tangles made of hyperphosphorylated tau protein aggregates. The relationship between these pathologies and memory impairments in late stages of AD has been the focus of most research (Selkoe, 2001, 2002; West & Bhugra, 2015). However, early memory impairments in AD have received less focus. Nonetheless, studies in mouse models and patients with AD have shown neuronal dysfunction before the accumulation of amyloid plaques and neurodegeneration. Specifically, synaptic phenotypes correlating with cognitive impairment have been discovered (Jacobsen et al., 2006; Terry et al., 1991) Furthermore, a study by Jacobsen et al (Jacobsen et al., 2006) reported: decrease in dendritic spine density in the dentate gyrus, impairment in long-term potentiation (LTP, persistent strengthening of synapses based on patterns of activity) and behavior deficits occur prior to formation of senile plaques. This study also showed perforate pathway input from entorhinal cortex to dentate gyrus is dysfunctional and structurally compromised in mice with memory deficits prior to amyloid plaque deposition, supporting early neuronal dysfunction in AD. This finding has led to increased interest in studying Alzheimer's disease in earlier stages.

Previous research studies showed memory deficits in AD patients is the result of aberrant memory encoding (Granholm & Butters, 1988; Hodges, Salmon, & Butters, 1990). However, the methods used in these studies relied on memory retrieval and could not distinguish between a disruption in memory encoding and recall. In a study done by Roy et al (Roy et al., 2016), mice models were used to elucidate whether amnesia in early stages of AD is caused by a disruption of consolidation and storage or problem in retrieval of a memory. Early AD was induced by overexpression of delta exon 9 variant of Presenilin along with Swedish mutation of beta amyloid precursor protein (APP). To determine if mice showed early AD, 7-month-old mice were compared to 9-month-old mice. The younger mice did not show amyloid plaque deposition in the entorhinal cortex and dentate gyrus and did not show short-term memory (STM) loss but did show long-term memory (LTM) loss whereas 9 month mice showed amyloid plaque deposition and STM and LTM loss. Therefore, 7-month-old mice were chosen as a model of early AD. Adeno-associated virus with ChR2 conjugated to enhanced yellow fluorescent protein (eYFP) reporter protein was injected into dentate gyrus. Mice which were amnesic to a long-term memory test, showed memory retrieval after optogenetic activation of hippocampal engram cells (neurons holding traces of specific memories) The study also found amnesia in these mice is age-dependent, and progressive amnesia correlated with reduction in dendritic spine density of dentate gyrus engram cells.

Furthermore, optogenetic induction of LTP of dentate gyrus engram cells at perforate path connections reestablished spine density and long-term memory (Roy et al., 2016). This study showed memory loss in early AD is due to aberrant retrieval and demonstrated the potential in using optogenetics as a therapeutic method to improve memory in AD patients. Optogenetics has also been used to study the causal relationship between LTP and memory encoding, which was previously difficult to demonstrate (Stevens, 1998). In a study by Nabavi et al (Nabavi et al., 2014), optogenetics was used to overcome this limitation by inducing LTP and long-term depression (LTD). In behavior studies using mice, cued-fear conditioning (CFC) was used by pairing foot shock (unconditioned stimulus; US) and tone (conditioned stimulus; CS). This resulted in mice having a tone-driven conditioned response (CR), indicating memory of the foot shock. To determine whether LTP is the cause of this memory storage, tone was replaced with blue-light activation of ChR2 containing neurons in the medial geniculate nucleus projecting to the lateral amygdala. In mice, which had light stimulus paired with foot shock, light stimulus alone caused the CR, whereas light alone without the initial pairing did not result in CR. LTP caused by pairing of light stimulus with US was found by measuring the ratio of AMPA to NMDA component of optogenetically driven synaptic responses in amygdala slices. Furthermore, if the memory storage was caused by LTP, then memory amnesia should be successfully induced by LTD. They used optogenetics to induce long-term depression (reversal of potentiation) in mice previously showing CR to light stimulus. Following LTD, the CS (light) no longer led to CR. Subsequent light induction of LTP using optogenetics reactivated the CR. Therefore, memory can be inactivated and reactivated using LTD and LTP, respectively, which supports the causal link between these synaptic processes and memory.

2.3 Parkinson's Disease (PD)

PD is a neurodegenerative disease caused by loss of dopaminergic neurons in the substantia nigra. Clinical manifestations of Parkinson disease (PD) usually begin at age 55-65, and it occurs in 1-2% of the population above 60 years old (Tanner & Aston, 2000). Hallmark signs of PD are: bradykinesia, cogwheel rigidity, resting tremor, and shuffling gait (Lang & Lozano, 1998). Other signs are cognitive, sleep, mood, and autonomic dysfunction such as orthostasis (Chaudhuri et al., 2006). There are currently no therapies that may reverse the neurodegenerative process, but pharmacotherapies may significantly improve quality of life (Connolly & Lang, 2014). However, potential side effects of pharmacotherapy include: nausea, psychosis, impulse control disorders, constipation, increased salivation, and orthostasis. Moreover, following years of therapy with the levodopa, which is the most effective treatment for PD, medication-associated resistance and complications occur (Fahn, 2000). These complications include "on-off" phenomena whereby patients have dystonia when levodopa concentration is at its lowest, also known as the "off" state, and dyskinesia when levodopa concentration is at its highest, referred to as "on" state (Aquino & Fox, 2015). This phenomenon develops over time, and most cases occur after 5 years of treatment with levodopa (Okun, 2012). Once it occurs it is difficult to eliminate (Fahn, 2000), hence the need for other forms of therapy. Deep brain stimulation (DBS) is another option for treatment in patients. This is a surgical technique that implants electrodes in specific sites in the basal nuclei, the subthalamic nucleus and internal segment of globus pallidum. These electrodes are connected to an impulse generator which delivers electrical stimuli to modulate neuronal activity (Okun, 2012). However, DBS is not without its own problems. A wide range of neurological and neuropsychiatric effects have been reported such as: suicidal ideation, mania and depression (Temel, 2010). It has also been known DBS lacks spatial specificity. Electrical pulse provokes changes in electric field in all directions causing modulation of neuronal circuits outside the region of interest (Li et al., 2012). Therefore, there is a need for a more specific targeting therapy for PD.

Optogenetics has been used in the field of Parkinson's disease to develop dopaminergic neurons responsive to light and better understand cell transplantation and DBS for the treatment of PD (Gradinaru, Mogri, Thompson, Henderson, & Deisseroth, 2009; Steinbeck et al., 2015). Dopaminergic cells obtained from pluripotent stem cells have been efficiently transplanted into PD mice models (Kriks et al., 2011). However, the mechanism had not been deduced. Steinbeck et al (Steinbeck et al., 2015) used the optogenetic tool eNpHR3.0 (halorhodopsin) to control electrophysiological and neurochemical properties of implanted mesencephalic dopaminergic neurons (mesDA) in real time. Their studies showed strong evidence functional improvement of PD symptoms relies upon neuronal activity and dopaminergic release of implanted cells. Also, dopaminergic grafts modulate glutamatergic synaptic transmission on striatal medium spiny neurons similar to host mesDA (Steinbeck et al., 2015). A major promise in the field of optogenetics in PD is the ability to deduce disease circuitry and mechanisms of current treatment. For example, Gradinaru et al (Gradinaru et al., 2009) studied the mechanism by which DBS has therapeutic benefits. This was done by selectively inhibiting (eNpHR) or activating (ChR2) circuit elements in parkinsonian rodents using optogenetics. Using rodents, they found activation of afferent neurons projecting to the subthalamic nucleus significantly reduces Parkinsonism, whereas activation of efferent neurons from subthalamic nucleus did not result in any improvement. This study showed therapeutic benefits from DBS can be credited to direct stimulation of afferent axons extending to the subthalamic nucleus (Gradinaru et al., 2009). These results presented how optogenetics can be used to dissect neuronal circuitry in disease by controlling individual components.

2.4 Epilepsy

Epilepsy, a disease of recurrent seizures, is characterized by an over activity of various circuits. It is one of the most common neurological disorders affecting about 50 million people worldwide (Banerjee, Filippi, & Hauser, 2009). Pharmacologic treatment focuses on seizure prophylaxis, requiring daily dosing. Furthermore, anti-seizure medications are associated with a wide range of side effects and toxicities (Walia, Khan, Ko, Raza, & Khan, 2004). Hence, the need for better therapeutic intervention. To strengthen this notion, a prevalence study done on a city in France showed 15.6% of patients had pharmaco-resistant epilepsy (Picot, Baldy-Moulinier, Daurès, Dujols, & Crespel, 2008). In a retrospective study, patients with drug-resistant epilepsy had an increased risk of premature death (Mohanraj et al., 2006). This population also had increased risk of injury (Lawn, Bamlet, Radhakrishnan, O'Brien, & So, 2004), reduced quality life and psychosocial dysfunction (McCagh, Fisk, & Baker, 2009).

Another treatment strategy is to use implantable anti-epileptic devices. These include open-loop devices such as vagus nerve stimulator and deep brain stimulation, which have been shown to be effective in refractory epilepsy (Fisher et al., 2010; Hodaie, Wennberg, Dostrovsky, & Lozano, 2002; Kerrigan et al., 2004; Lawn et al., 2004; K. J. Lee, Jang, & Shon, 2006; Lim et al., 2007; Orosz et al., 2014; Osorio, Overman, Giftakis, & Wilkinson, 2007). However, a limitation with this technology is it lacks neuronal feedback to modulate activity with temporal precision i.e. before seizure will occur. Therapy with open-loop devices require repetitive stimulation, which depends on automated settings being programmed by the physician (Stacey & Litt, 2008). However, epileptic seizures occur erratically with possible long interictal intervals (Schiff et al., 1994). This leads to unnecessary brain stimulation in between seizures. Furthermore, repetitive stimulation with open-loop devices has been shown to cause detrimental side effects (Ben-Menachem, Revesz, Simon, & Silberstein, 2015; Hartikainen et al., 2014; Lawn et al., 2004). Closed-loop devices may overcome these limitations by detecting epileptiform patterns and sending an electric current to disrupt this activity on demand. Several closed-loop devices have shown efficacy in treating animal models and patients. (Berényi, Belluscio, Mao, & Buzsáki, 2012; Bergey et al., 2015; Lawn et al., 2004; B. Lee et al., 2015; Liang et al., 2011). NeuroPace RNS System, the first responsive neuro stimulator, has been FDA approved for the use in medication resistant partial onset seizures and has been shown to improve quality of life with no effect on cognition and mood (Morrell, 2011; Morrell & Halpern, 2016). Closed-loop systems may circumvent the drawback of continuous brain stimulation from open-loop devices, which may cause side effects such as: paranoia, visual hallucinations, and impairment in spatial memory (Andrade et al., 2006; Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009; Lawn et al., 2004). Although closed-loop devices stimulate seizure foci with temporal specificity and have shown to be safe, they lack spatial specificity at the neuronal level, which may cause alteration of function in different brain areas (Li et al., 2012). Optogenetics will be a suitable tool to overcome this limitation due to its high spatiotemporal precision.

In vitro optogenetic inhibition of hippocampal principal cells in regions CA1 and CA3 was shown to be sufficient to reduce seizure activity (Tønnesen, Sørensen, Deisseroth, Lundberg, & Kokaia, 2009). In the same study by Tønnesen et al, cells in this region were transduced by injecting lentiviral vector with halorhodopsin (NpHR) under the control of calcium/calmodulin protein kinase II (CaMKII). Transduction efficiency was observed by the co-expression of reporter enhanced yellow fluorescent protein (EYFP). Pharmacoresistant epilepsy model was created by stimulation train-induced bursting (STIB) in tissue slice culture from the hippocampi of mice. Duration of STIB was reduced with optogenetically stimulating hippocampal cells with orange light (573-613 nm) in regions CA1 and CA3 (Tønnesen et al., 2009). This study showed selective inhibition of excitatory principal neurons in the hippocampus by optogenetics reduces epileptiform activity in in vitro model. Furthermore, it showed the potential in using optogenetics in the treatment of temporal lobe epilepsy, since it originates in hippocampus. This study encouraged further studies in in vivo models. In a study by Krook-Magnuson et al, a closed-loop device was developed to detect early seizure activity in mice with temporal lobe seizures and modulate cells in the hippocampus using optogenetics (Krook-Magnuson, Armstrong, Oijala, & Soltesz, 2013). Kainate was injected into the dorsal hippocampus to induce temporal lobe epilepsy in mice.

Detection of early seizure activity was done using custom software tuned to signal power, frequency and spike properties. To study the potential of optogenetics to abolish seizure activity in mice, halorhodopsin (HR) was used to inhibit excitatory principal cells. Mice expressing Cre under the control of Calcium/calmodulin protein kinase II alpha (CaMKII α) promoter were crossed with mice expressing Cre-dependent HR. This resulted in mice with HR gene expressed under the control CaMKII alpha promoter. They observed on-demand optogenetic stimulation using this construct significantly controlled electrographic seizures and reduced behavioural seizures. Furthermore, they also studied the effect of exciting inhibitory parvalbumin (PV) containing GABAergic neurons in hippocampus with channelrhodopsin, since studies have shown these GABAergic neurons are important in synchronizing principal cells during gamma oscillations in seizure activity (Cobb, Buhl, Halasy, Paulsen, & Somogyi, 1995; Lawn et al., 2004; Sohal, Zhang, Yizhar, & Deisseroth, 2009). In order to test whether activation of these neurons could prevent seizure activity, mice containing PV-Cre were crossed with mice expressing channelrhodopsin Cre-dependently in order to obtaining mice with parvalbumin containing GABAergic neurons expressing channelrhodopsin. They found optogenetically stimulating these neurons reduced seizure activity and behaviour as well. However, more recent in vitro studies have shown activation of these parvalbumin containing interneurons may provoke epileptic activity (Ellender, Raimondo, Irkle, Lamsa, & Akerman, 2014; Lawn et al., 2004; Yekhlief, Breschi, Lagostena, Russo, & Taverna, 2015). Nonetheless, this study shows the potential in using optogenetics together with closed-loop seizure detection for patients suffering from temporal lobe epilepsy.

Epilepsy is a potential sequela of stroke (Kelly, 2002; Kotila & Waltimo, 1992; Lawn et al., 2004; J. C. Lee et al., 2009). However, before reported study by Paz and his colleagues (Paz et al., 2013), the pathophysiology of this disease had been unknown. In the study by Paz et al, Rose Bengal dye was injected into the tail vein of rats, and light from a fiber optic cable was focused to induce photothrombotic lesion in the right somatosensory cortex. Adeno-associated virus with construct containing eNpHR3.0 under control of CaMKII, which allowed expression specifically in excitatory neurons, was injected into the right somatosensory ventrobasal nucleus. Using a closed-loop optogenetic control, selective illumination of thalamocortical neurons expressing eNpHR3.0 with 594nm light interrupted cortical and thalamic epileptic activity while stopping seizure behaviour. In this study, inhibition of thalamocortical neurons by eNpHR3.0 was shown to abort seizures from stroke induced epilepsy in rats, hence, suggesting these neurons are important in the development of seizures in stroke induced epilepsy (Paz et al., 2013). This study introduces the potential to treat stroke induced seizures by selectively inhibiting excitatory thalamocortical neurons. Application of optogenetics in epilepsy research has led to discovery of pathways central to its pathophysiology as well as introduced a promising new treatment modality.

3. Future Direction

Introduction of optogenetics to patients is a major obstacle in this field, one that must not be overlooked. It is an invasive treatment, requiring implantation of diodes into brain tissue to supply light to activate opsins. To date, opsins have been delivered to rodent models by transfection or developing transgenic mice. These methods are impractical and the latter raises ethical issues. However, not to be discouraging, we are still at the early stages of optogenetics. Since the introduction into the field of neuroscience by Diesseroth in 2005, it has vastly expanded. Remarkably, the first patient dosed with optogenetics for retinitis pigmentosa (RP) in a clinical trial conducted by Retro Sense Therapeutics was reported earlier this year of 2016. Similar to RP, there are many other diseases which do not have effective treatments or have treatments that are not tolerable. Optogenetics has not only helped us discover pathophysiology of neurological diseases, but has also opened the door to use this as a treatment option for many diseases.

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Figure 1: Summary of Optogenetic Applications in Neurological Diseases

Application	Opsin/Gene	Stimulation	Finding	Reference
Stroke	ChR2	Primary Somatosensory Cortex	increased neuroplasticity markers, improved sensorimotor behavior test, and increased cerebral blood flow	Cheng et al., 2014
Stroke	ChR2	Implanted Neural Stem Cells	Downregulation of inflammatory markers, upregulation of neuroplasticity, differentiation, neurotransmission and regeneration markers, and enhanced forelimb use	M.M. Daadi et al., 2015
Alzheimer's Disease	ChR2	Dentate Gyrus	Early memory loss in AD due to memory retrieval not consolidation	Roy et al., 2016
Memory encoding	ChR2	Medial Geniculate Nucleus	LTP and LTD lead to memory storage and inactivation	Nabavi et al., 2014
Parkinson's Disease	eNpHR3.0	Implanted mesencephalic dopaminergic neurons	mesDA mediated improvement in PD patients by dopamine release and modulation of glutamatergic synaptic transmission	Steinbeck et al., 2015
Parkinson's Disease	eNpHR and ChR2	Afferent neurons to Subthalamic nucleus Efferent neurons from Subthalamic nucleus	DBS mediates therapeutic effects by stimulation of afferent neurons to subthalamic nucleus	Gradinaru et al., 2009
Temporal lobe epilepsy	NpHR and ChR2	Principal cells of hippocampus regions CA1 and CA3	Inhibition of excitatory principle cells in hippocampus reduces epileptiform activity	Tønnesen et al., 2009
Temporal lobe epilepsy	ChR2 and HR	ChR2 in principal cells HR in PV+ GABAergic neurons	Closed-loop device inhibits epileptiform activity and behavior by inhibiting principal cells and exciting PV+ cells	Krook-Magnuson et al., 2013
Post-stroke epilepsy	eNpHR3.0	Excitatory cells of right somatosensory ventrobasal nucleus	Inhibiting thalamocortical neurons using closed-loop device prevents post-stroke seizures	Paz et al., 2013