Optogenetic modulation in stroke recovery

*Arjun V. Pendharkar, MD, Sabrina L. Levy, BS, Allen L. Ho, MD, Eric S. Sussman, MD, Michelle Y. Cheng, PhD, and Gary K. Steinberg, MD, PhD

Department of Neurosurgery, Stanford University School of Medicine, Stanford, California

Stroke is one of the leading contributors to morbidity, mortality, and health care costs in the United States. Although several preclinical strategies have shown promise in the laboratory, few have succeeded in the clinical setting. Optogenetics represents a promising molecular tool, which enables highly specific circuit-level neuromodulation. Here, the conceptual background and preclinical body of evidence for optogenetics are reviewed, and translational considerations in stroke recovery are discussed.

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STROKE is the leading cause of long-term disability in the United States,⁶⁷ with a projected prevalence of 3.4 million by 2030⁶⁷ and an annual cost of approximately \$34 billion.⁶⁷ Treatment options include intravenous tissue plasminogen activator and endovascular thrombolysis but are limited to ischemic stroke and a narrow administration window. From a basic science perspective, numerous experimental therapies have shown success in the laboratory, yet none have successfully reached the clinic.¹⁰⁷

Neuroprotective strategies, including glutamate receptor antagonists, ion channel regulators, free radical scavengers, and antiinflammatory compounds, have shown promise in preclinical models but have failed in human studies.^{18,63} Stem cell-based strategies represent another promising therapeutic avenue, but are pending FDA approval. Transcranial stimulations such as transcranial direct current stimulation and transcranial magnetic stimulation have also produced some preliminary success.^{7,45,72,96}

Optogenetics is an emerging method for studying central nervous system function. Genetically encoded channels and receptors that are responsive to single-wavelength light sources are used to allow optical control of the neural circuits. This technology can be used to target specific cell

populations and restore central nervous system function. Here, we review the conceptual background and preclinical body of evidence for optogenetics and discuss translational considerations in stroke recovery.

Optogenetics

Optogenetics uses light-responsive cellular elements to produce stimulation, inhibition, and silencing of neurons.^{26,29,64,66,98} One major advantage of this tool is the rapid kinetics that lead to precise temporal and geographic targeting.^{31,102} When a photon hits naturally occurring opsins, the molecules undergo a conformational change. Channelrhodopsin, the first photo-responsive channel to be widely implemented, is a light-responsive, cation-selective ion channel found in *Chlamydomonas reinhardtii*.^{9,69,70} In the case of channelrhodopsin-2, a blue photon induces a conformational change from all-trans to the 13-*cis*-retinal conformer, which opens a cation pore in the ionotropic ion channel that directly leads to depolarization and an ensuing action potential.^{9,103}

Several additional optogenetic channels have been developed to allow further modulation (Table 1). Halorhodopsin, which is found in the bacterium *Natronobacterium*

ABBREVIATIONS ArchT = archaerhodopsin; BDNF = brain-derived neurotrophic factor; CAP-23 = cytoskeleton-associated protein 23; CNO = clozapine-N-oxide; DREADD = designer receptor exclusively activated by designer drugs; EGF = epidermal growth factor; FGF-2 = basic fibroblast growth factor 2; GABA = γ -aminobutyric acid; GAP-43 = growth-associated protein 43; GDF10 = growth differentiation factor 10; GDNF = glial cell line-derived neurotrophic factor; IGF1 = insulin-like growth factor 1; iM1 = ipsilesional primary motor cortex; MARCKS = myristoylated alanine-rich protein kinase C substrate; NGF = nerve growth factor; NTF3 = neurotrophin-3; WGA = wheat germ agglutinin.

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* Dr. Pendharkar and Ms. Levy contributed equally to this work.

TABLE 1. Survey of widely used optogenetic tools

Tool	Description	Advantages	Limitations	Select References
Channelrhodopsin-2 (<i>Chlamydomonas reinhardtii</i>)	Excitatory; cation channel responsive to blue light	Millisecond & temporal precision; single-gene basis facilitates use w/ other genetic tools	Light scattering in tissue can lead to imprecise activation	Boyden et al., 2005; Cheng et al., 2015
Halorhodopsin (<i>Natromonas pharaonis</i>)	Inhibitory; chloride channel responsive to yellow light	Millisecond & temporal precision; single-gene basis facilitates use w/ other genetic tools	Chloride channels enter into inactivation phase & constitutive inhibition requires exposing tissue to light for long periods & can lead to heat damage; unsuited for long-scale or high-quantity silencing	Nagel et al., 2003 Kandori et al., 2015
Archaerhodopsin (<i>Halorubrum sodomense</i>)	Inhibitory; proton pump silences neurons in response to yellow light	Spontaneously recovers from light-driven inactivation; good for large-scale silencing	Light scattering in tissue can lead to imprecise activation	Chow et al., 2010
Mac (<i>Leptosphaeria maculans</i>)	Inhibitory; proton pump silences neurons in response to blue-green light	Responds to a different color light; can be used w/ another channel for the dual control of 2 separate populations	Light scattering in tissue can lead to imprecise activation	Waschuk et al., 2005
LiGluR	Light-activated ionotropic glutamate receptor via photoswitchable ligands; agonized & antagonized variants available	Light control of GPCR enables dissection of neurocircuitry; provides more nuanced control	Requires separate addition of MAG photoswitch solution prior to activation	Gorostiza et al., 2007
LimGluR	Light-activated metabotropic glutamate receptor via photoswitchable ligands	Light control of metabotropic GPCR enables dissection of messenger systems; provides more nuanced control	Requires separate addition of MAG photoswitch solution prior to activation	Levitz et al., 2013
DREADDs	Modified muscarinic receptors respond to biologically inert CNO	Circumvents need for a light source	Systemic administration of inert CNO agonist depends on metabolism for "off" switch; less temporally precise than optical controls	Wahl et al., 2014

GPCR = G protein–coupled receptor; LiGluR = light-activated ionotropic glutamate receptor; LimGluR = light-activated metabotropic glutamate receptor.

pharaonis, is a chloride channel that responds to a green/yellow light and enables the inhibition of neurons.^{48,70,105} Archaerhodopsin (ArchT) is another inhibitory bacteriorhodopsin and functions as an outward proton pump.²¹ The differences in kinetics between halorhodopsin and ArchT provide an example of the complexity of the optogenetics platform: the former recovers slowly, while ArchT recovers spontaneously after light-induced inactivation. Additional light-responsive glutamate receptors have been engineered using photosensitive ligands, enabling investigation of synaptic transmission mechanisms as well as an emerging treatment strategy for retinitis pigmentosa.^{32,58,80}

From an experimental perspective, our group has recently reviewed the technical considerations of using optogenetic technology in the laboratory. Briefly, imaging and stimulation can be achieved using 2-photon microscopy via cranial windows. Rodents can be awake or anesthetized, and several groups have used integrated microscopy and fiberoptics to measure and record circuit responses in experimental models. Thermal tissue stimulation must be considered when stimulating with light and may confound results.¹⁷

The strength of the optogenetic platform lies in the temporal and geographic precision of the system. Millisecond-scale responses have been demonstrated using photon induction,^{9,39,104} and the effect begins and ends immediately. This is in stark comparison with traditional electrical stimulation, which produces artifactual or unwanted effects in surrounding tissues. Delivery platforms include viral injection that utilizes a host of vectors and constructs, as well as transgenic animals.^{31,102} Viral injections have the benefit of limiting optogenetic expression to a specific geographic region, depending on the amount of virus injected and promoter specificity (Table 2).

Optogenetics in combination with targeted genetic strategies creates the possibility of the highly specialized targeting of the cells that are linked functionally, structurally, by identity, or even by seemingly idiosyncratic synchronized activity. A proposed therapeutic modality would benefit from geographic and temporal specificity.

Proposed Targets for Stroke Recovery

Optogenetics may be applied toward recovery after

TABLE 2. Cell type-specific promoters for genetic targeting

Cell Population	Promoter	Selected References
Pan-neuronal	Synapsin, hThy1	Kügler et al., 2003; Diester et al., 2011
Glutamatergic (excitatory)	CaMKII	Rongo et al., 1999
GABAergic (inhibitory)	VGAT	Zhao et al., 2011
Dopaminergic	THp	Oh et al., 2009
Serotonergic	TPH	Zhao et al., 2011
Cholinergic	ChAT	Zhao et al., 2011
Purkinje cells	L7	Tsubota et al., 2011
Oligodendrocytes	MBP	Lawlor et al., 2009
Astrocytes	gfaABC1D	Lee et al., 2008
Activity-dependent promoters	Fos, Arc	Guenthner et al., 2013

CaMKII = calcium- and calmodulin-dependent protein kinase type II; ChAT = choline acetyltransferase; hThy1 = human thymocyte-1; MBP = myelin basic protein; THp = tyrosine hydroxylase promoter; TPH = tryptophan hydroxylase promoter; VGAT = vesicular GABA transporter.

stroke at the hemispheric level, within specific circuits, or targeted to cells promoting plasticity.

Disinhibition Hypothesis: Excitation and Inhibition Following Stroke

The disinhibition hypothesis postulates that each cerebral hemisphere inhibits the activity of the other,^{87,101} and mutual inhibition leads to a balanced baseline activity level. In stroke, overactivation of the contralateral hemisphere perpetuates ipsilesional damage, and a positive feedback loop develops that leads to progressive functional deterioration.³⁷ This concept carries significance for timing rehabilitation, and animal studies show that immediate rehabilitation following stroke can prohibit long-term recovery and lead to permanent damage in the penumbra.^{52,53} Early intervention has also been linked to increased cell death in the penumbra.^{30,81} A Phase III trial—A Very Early Rehabilitation Trial (AVERT)—found that rehabilitation beginning 24 hours after stroke leads to a higher likelihood of worse clinical outcomes at the 90-day poststroke time point.⁵

From a mechanistic standpoint, γ -aminobutyric acid (GABA) plays a critical role in poststroke brain repair. There is the downregulation of the GABAergic pathways, leaving the ipsilesional hemisphere^{2,79} and increased GABA receptors in the ischemic core.^{85,86} GABA signaling exists in both tonic (extrasynaptic) and phasic (synaptic) states. The former is increased after stroke in contrast to phasic GABA signaling, which is decreased. Reducing tonic GABAergic inhibitory pathways using a GABA receptor antagonist has produced functional recovery in rodent stroke models, implicating perilesional cortex inhibition as a major mechanism.²² In contrast to tonic signaling, which is largely thought to be detrimental, our group recently demonstrated that increasing phasic GABA signaling after stroke produces a functional benefit in experimental models.⁴³ From these findings, 3 proposed targets have emerged as optogenetic therapies for stroke:

1) perilesional and global hyperexcitability immediately following stroke;^{71,85} 2) decreased ipsilesional inhibitory postsynaptic potentials;⁶² and 3) sustained contralesional hyperexcitability.^{65,85}

Optogenetic Stimulation and Stroke Recovery

Given the body of evidence supporting imbalanced inhibition following ischemia and reperfusion, our group has recently shown that targeted optogenetic stimulation of the ipsilesional primary motor cortex (iM1) produces functional recovery in rodent stroke models.¹⁹ Transgenic Thy1-Channelrhodopsin-2 mice were implanted with an optical fiber over the iM1. In response to targeted iM1 stimulation, the cells in the perilesional and contralesional hemisphere fired reliably and specifically, as verified by electrophysiology recordings. Mice underwent three 1-minute stimulations per day from poststroke Days 5 to 14. Behavior tests showed that iM1-stimulated mice exhibited functional behavioral recovery at Days 10 and 14 following an experimental stroke model. Biochemically, the increased levels of the plasticity markers, including activity-dependent neurotrophins brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NTF3), were observed, as well as higher GAP-43 plasticity marker expression. These data suggest that functional recovery is likely related to plasticity-associated remodeling and provide a proof of concept that optogenetic neuronal stimulation can enhance recovery after stroke.

Poststroke Plasticity State and Growth Factor Release

Another therapeutic strategy involves modulating the endogenous regenerative response after stroke. There are several promising targets. The plasticity markers growth-associated protein 43 (GAP-43), cytoskeleton-associated protein 23 (CAP-23), and myristoylated alanine-rich protein kinase C substrate (MARCKS) are upregulated in the postischemic milieu.^{12,13,68,77} Growth differentiation factor 10 (GDF10), a growth factor, has also been implicated in promoting poststroke plasticity,⁶¹ and upregulation in the perilesional cortex is conserved across species in both in vivo and in vitro studies.⁶⁰ Insulin-like growth factor 1 (IGF1) and Nogo receptor complex-1 are involved in cell adhesion, axonal guidance, and cellular architecture modifications.⁶¹ A neurite inhibitor, Nogo-A, is implicated in inhibiting poststroke axonal sprouting,⁴⁷ and its inhibition mitigates this effect.^{89,90,99} High levels of various growth factors, including BDNF, NGF, NTF3, basic fibroblast growth factor 2 (FGF-2), IGF-1, epidermal growth factor (EGF), and glial cell line-derived neurotrophic factor (GDNF), further characterize the postischemic regenerative environment.^{19,77}

Our group has demonstrated that iM1-stimulated mice exhibited an increase in the expression of neurotrophin factors BDNF, NGF and NTF3 in the contralesional motor cortex at poststroke Day 15. Additionally, these changes were absent in stimulated, nonstroke mice. The axonal

growth protein GAP-43 marker was also upregulated in stimulated mice.¹⁹ We hypothesize that optogenetic stimulation during the acute postischemic period will help strengthen and rebuild potentially beneficial connections that are degraded during ischemia. New connections that are activated by a selective optogenetic paradigm can recapitulate and bolster an “adolescent critical period” that can help rebuild necessary inhibitory interhemispheric connections and reestablish interhemispheric balance.

Stem cell therapies have shown promise in preclinical studies, and optogenetic modulation may provide an additional strategy for improving outcome.^{6,24,42} Targeted cell migration and the activation of endogenous neurogenesis pathways may be augmented by photomodulation.^{6,8,24,33,34,40,42,44,50,74,75,84} Each of these well-characterized pathways and mechanisms may be targeted using optogenetic stimulation to enhance endogenous mechanisms or exogenously introduced cells and their contribution to functional recovery.

In a recent example, neural stem cells were optogenetically modified to express channelrhodopsin-2, then engrafted in vivo. Chronic optogenetic stimulation of the transplanted cells led to the genetic expression of the transcripts involved in neuronal differentiation, axonal sprouting, and synaptic plasticity. The overall inflammatory response was also reduced, and stimulated transplanted rodents demonstrated behavioral improvement compared with controls.²³

Combination With Genetic Strategies for Improved Precision and Control

The power of optogenetics lies in the ability to exploit molecular genetic tools to develop cell or circuit specific targets that allow geographic and temporal control. Several well-described cell-specific promoters are outlined in Table 2. For example, driving channelrhodopsin expression under the CaMKII promoter allows for the selective stimulation of excitatory cell circuits. Similarly, expressing halorhodopsin under the somatostatin promoter can induce the specific inhibition of inhibitory circuits. The identification of the promoters associated with elevated activity in neurons, such as c-Fos and p-CREB, are further paving the way for the selective isolation of active circuits.⁴⁹ One recent example of a cell-specific optogenetic strategy involves selective targeting of the excitatory cells in the subthalamic nucleus through lentiviral CaMKII α -driven halorhodopsin expression.³⁶ More recently, 4 cell-specific channelrhodopsin-2 transgenic mouse lines were generated and characterized to employ VGAT, ChAT, Tph2, and Pvalb promoters for GABAergic, cholinergic, serotonergic, and parvalbumin-expressing neuron specificity.¹⁰⁶

Perhaps one of the most useful genetic tools for selective circuit-specific control is the 2-component Cre-inducible genetic system.⁷⁶ The Cre enzyme, which is coded for by the Cre recombinase gene, selectively excises LoxP regions. By flanking a STOP codon with LoxP sites upstream of the gene of interest, the selective expression of a gene of interest occurs after the addition of Cre. Cre is delivered using a separate virus (AAV-Cre), conjugation

to a neurotracer (i.e., wheat germ agglutinin [WGA]-Cre), or by crossing with another transgenic animal line that expresses Cre. Cell-specific promoters can drive both viral and transgenic Cre, as well. Circuit-level targeting can be achieved by administering Cre that has been conjugated to a retrograde, anterograde, or transsynaptic neural tracer such as the ubiquitously used WGA tracer.⁵⁹ Temporal activation is possible using Cre that has been modified with an estrogen receptor that selectively responds to tamoxifen administration.^{27,38} Finally, Cre-mediated gene expression can be targeted to specific regions using a viral Cre injection.

As optogenetic strategies come to fruition, one major shortcoming will be the need for direct photon delivery in vivo, which may be difficult to apply in the clinical setting. Another emerging chemogenetic strategy may obviate this translational hurdle.

Chemogenetic Manipulation: DREADDs

Similar to channelrhodopsin, genetically encoded designer receptors exclusively activated by designer drugs (DREADDs) respond selectively to a systemically administered compound (i.e., the role of light in the optogenetic system), resulting in the excitation or silencing of the receptor. The DREADD system involves a genetically expressed G protein-coupled receptor system that is modified to exclusively respond to a biologically inert compound, clozapine N-oxide (CNO), and has no response to endogenous compounds.^{3,56,92,93,98} CNO is safe and inert to the endogenous system, yet selectively binds to the DREADD receptor to either activate or inhibit the cell. Thus, chemogenetic manipulations are possible with precise selectivity, similar to optogenetics. One recent study used DREADDs to selectively modulate anti-Nogo-A (a growth inhibitory protein implicated in poststroke insult) in the corticospinal tract and elucidate a novel mechanism in postischemic recovery.⁹⁴

Translational Considerations

The temporal, cell-specific, circuit-specific, and rapid kinetics of optogenetics has brought about significant advances in multiple disease models, including Parkinson's, epilepsy, retinitis pigmentosa, and rhythmic heart disorders.^{4,10,11,16} There are, however, many challenges that must be addressed first, ranging from gene therapy, light delivery technology, and the optimization of stimulation paradigms.^{20,100}

Channelrhodopsin-2 has been successfully expressed in human embryonic cells and subsequently differentiated into cardiac cells, which successfully responded to light activation, using mechanical, biochemical, and electrical verification.¹ The optogenetic toolset can also manipulate nonhuman primate models.^{25,41,46} Lentiviral and adeno-associated viral optogenetic delivery platforms are safe and effective for nonhuman primates. Viral transfection localizes to the injection site and leads to successful inhibition and stimulation in response to optogenetic activation.²⁸ ArchT, which is expressed under the CAG promoter, directed neuronal silencing in nonhuman primates on behavioral and histological levels.¹⁴ Transfection of human

embryonic stem cells with channelrhodopsin-2 under the excitatory human synapsin promoter, followed by differentiation into neurons, enabled optical control in vitro.⁹⁷ Additionally, lentiviral expression in humans effectively transduces cells with effects that last 1.5 years.⁸⁸ As gene therapy gains more traction and public acceptance, optogenetics and DREADDs will stand at the forefront of the therapeutic methods to be implemented.

Technical innovations are rapidly solving the challenges of regionally precise light delivery for stimulation. Novel bioelectronics employ biocompatible materials, low power requirements, and microscale components. One such cell-scale device measures 50 × 50 μm and integrates micro-light-emitting diodes, wireless powering, and temperature sensors to detect overheating.⁵¹ Such a system enables implantation at a precise location with minimal light scattering, especially compared with the scattering present in optical fibers, which can be millimeters wide. A similarly pliable wireless optogenetic system delivers light to the cortex for optical neural control.⁷⁸

DREADD technology is an additional strategy for obviating the need for a light source. The oral administration of CNO, a biologically inert ligand, can replace light delivery. CNO benefits from its blood-brain barrier-permeable properties.⁸² One notable risk in humans is the metabolism of CNO to clozapine, a drug used in schizophrenia that stimulates multiple neural targets, and this effect is not seen in mice.¹⁵ DREADD activation relies on the pharmacokinetics of CNO, which limits the temporal precision of neural manipulations. Activation is sustained on the order of hours and reduced to the order of minutes for the localized injection of CNO.⁸² More recently, an alternative to CNO has been engineered that uses the pharmacologically inert Salvinorin B. Salvinorin B in conjunction with the κ-opioid receptor DREADD may provide improved temporal kinetics compared with CNO.⁹²

Conclusions

Stroke remains a major contributor to morbidity, mortality, and cost in the United States. Although preclinical experimental strategies have been promising, there is a dearth of translatable targeted therapies. Optogenetic and DREADD technologies provide an opportunity for circuit- and cell-specific modulation. Further studies are needed to further bring these treatments to fruition.

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Disclosures

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Author Contributions

Conception and design: Steinberg, Pendharkar, Levy, Ho, Sussman. Acquisition of data: Steinberg, Pendharkar, Levy, Cheng.

Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Steinberg. Administrative/technical/material support: all authors. Study supervision: Steinberg, Cheng.

Correspondence

Gary K. Steinberg, Department of Neurosurgery, 300 Pasteur Dr., R281, Stanford, CA 94305-5327. email: gsteinberg@stanford.edu.