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Optogenetic Approaches to Study Stroke Recovery

Michelle Y. Cheng,* Eric H. Wang, and Gary K. Steinberg*

Department of Neurosurgery and Stanford Stroke Center, Stanford University School of Medicine, Stanford, California 94305, United States

ABSTRACT: Treatment for stroke is very limited, and potential new therapies are focusing on promoting brain repair and plasticity, as they offer a longer therapeutic time window than the current U.S. Food and Drug Administration approved drug. Functional recovery can occur after stroke, and strategies such as direct brain stimulations that promote recovery are promising. Here we review how selective stimulation of neurons in the motor cortex using optogenetics enhances plasticity mechanisms and promotes functional recovery after stroke.

KEYWORDS: *Optogenetics, stroke recovery, channelrhodopsin, plasticity, neurotrophin*

Stroke is a devastating neurological insult and a leading cause of adult disability worldwide.[1] There is only one approved drug in the United States, tissue plasminogen activator (tPA), and not many patients can benefit from it due to its narrow treatment window and some contraindications.[1] Functional recovery can occur in both humans and animals, and increasing attention has shifted toward promoting endogenous repair and plasticity mechanisms, thereby enhancing recovery after stroke.[2] After injury, structural and functional plasticity occurs in areas adjacent to or remotely connected to the infarct. Electrical activity in these surviving neurons can release activity-dependent factors such as neurotrophins that can rewire neural connections and enhance recovery. Modulating the excitability in these neurons is a promising strategy to promote stroke recovery.

Brain stimulation allows direct activation of a target area. However, current stimulation techniques, like electrical and transcranial magnetic stimulation, indiscriminately target diverse cell types, which makes it difficult to identify which cell types and mechanisms underlie recovery. Optogenetics circumvents these issues because it activates or inhibits specific cell types with high precision and spatial-temporal resolution.[3] Optogenetics works by expressing a light sensitive protein, such as channelrhodopsin (ChR2), in specific cell populations, and when illuminated with blue light, these cells are depolarized and activated with precise control.[3] To study stroke recovery, we used optogenetics to selectively increase neuronal activity in the ipsilesional primary motor cortex (iM1) poststroke, in transgenic mice expressing ChR2 under a neuronal promoter (Thy1), and examined its effects on functional recovery and plasticity mechanisms.

Typical rodent models of stroke affect the circuitry underlying sensory-motor behavior. By inserting a suture through the common carotid artery to transiently occlude the middle cerebral artery, we are able to produce a large infarct in the striatum and sensory cortex that significantly impedes performance on a rotating beam test—a measure of how fast and how far a mouse can run across a rotating 120 cm beam. We aimed to restore motor function in stroke mice by activating the undamaged circuits in layer V of the iM1. We hypothesized that repeated neuronal stimulations in iM1 poststroke can improve behavior performance. In our experiments, we demonstrated that stimulated stroke mice performed better on the rotating beam test in both distance and speed traveled by day 14[4] (Figure 1). Furthermore, stimulated mice exhibited faster weight gain and improved stimulus-induced cerebral blood flow,[4] which also reflects improved recovery.

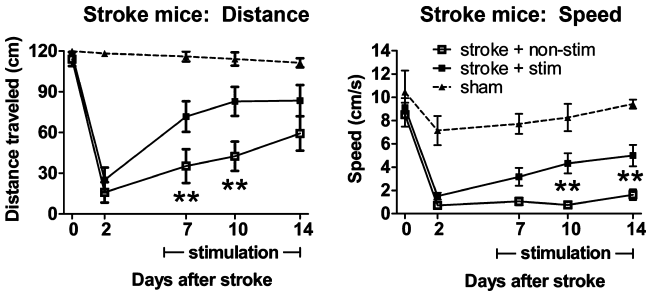

Figure 1. Optogenetic neuronal stimulations improved functional recovery after stroke. Stimulated stroke mice performed significantly better in the rotating beam test, with a longer distance traveled and a faster speed. *$P < 0.05$, **$P < 0.01$, significant difference between stim and non-stim group, Two-way RM ANOVA with Fisher's LSD. Sham: $n = 8$, non-stim: $n = 16$, stim: $n = 21$. This figure was retrieved from our original study published in 2014 in the journal PNAS (Cheng et al., 2014).

Previous research has demonstrated that stroke disrupts the interhemispheric interactions between the ipsilesional and contralesional hemispheres.[5] This results in *overactivation* of the contralesional hemisphere and corresponding *suppression* of the ipsilesional hemisphere. Similarly, our nonstimulated stroke mice also exhibited reduced neurovascular coupling in the iM1.[4] Data from our lab and others have shown that increasing excitability in the iM1 is beneficial and promotes poststroke recovery.[4,5] Specifically we show that repeated neuronal stimulations in iM1 can restore the impaired neurovascular coupling response to patterns comparable to normal nonstroke mice,[4] suggesting that repeated neuronal stimulations of the

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iM1 increases its excitability and potentially re-establishes the balance of interhemispheric interactions. Future studies will determine the relationship between interhemispheric excitability, neurovascular coupling and functional recovery.

Stimulation may promote functional recovery by enhancing structural plasticity, such as axonal sprouting or dendritic branching. This is supported by our observation that repeated neuronal stimulations significantly increased expression of activity-dependent neurotrophins, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin 3 (NTF3).[4] These trophic factors have been shown to enhance regrowth and remapping of neural circuits.[2] Indeed, we also detected a significant increase in GAP43 expression, a growth-associated protein critical for axonal sprouting.[2] However, whether these increased neurotrophins and GAP43 expression enhance structural plasticity to promote recovery needs further investigation. Interestingly, stimulation of the iM1 increased these neurotrophin and GAP43 expressions in the contralesional motor cortex (cM1),[4] highlighting its involvement in stroke recovery. It will be important to determine the role of the contralesional circuits involved in recovery by manipulating specific neuronal projections using optogenetics. Interestingly, the pro-recovery effect of stimulations was dependent on the stroke environment, as stimulations in nonstroke mice did not enhance their behavioral performance or increase neurotrophin expression.[4] This suggests that stroke may alter the circuits and the microenvironment, and this may prime the surviving circuits to be more responsive to stimulations.

Our study provides the first evidence that optogenetics can be used to manipulate specific cell types to promote stroke recovery, and stimulation of neurons in the stroke hemisphere is sufficient to enhance recovery.[4] Unlike current brain stimulation tools that activate/inhibit indiscriminately, optogenetics allows precise control of excitability in specific cell types and circuits, which is critical to clearly address the mechanisms that underlie stroke recovery. With the optogenetic tool kit rapidly expanding, many rhodopsin variants are now available for probing how circuits and specific signaling pathways contribute to biological functions. It is now possible to express channelrhodopsin under specific cell-type promoters (excitatory vs inhibitory), or in specific neuronal projections by using CRE-inducible ChR2 (DIO-ChR2) with retrograde tracers carrying CRE (AAV-WGA-CRE).[3] Furthermore, other rhodopsin variants such as halorhodopsin or archaerhodopsin can be used to hyperpolarize the cells, causing an inhibitory effect.[3] Thus, in addition to activating specific circuits that are beneficial, optogenetics can also be used to inhibit maladaptive circuits.

Optogenetic approaches have been used in rodents to probe neuronal circuits for several neurological/neurodegenerative diseases, including Parkinson's disease and epilepsy.[3] Recent studies have shown that optogenetic stimulation of cortical excitatory neurons increases blood oxygen level dependent (BOLD) signals. Others have used optogenetics to probe functional remapping after stroke. The safety and efficacy of using optogenetics in nonhuman primates has also been characterized.[3] We now demonstrate a proof-of-concept that optogenetics can be used to promote stroke recovery; undoubtedly, our stimulation parameters can be further optimized to maximize the beneficial effects on stroke recovery. Future studies will also determine an optimal stimulation region for promoting recovery which may have relevance for designing electrical stimulation clinical studies. Whether optogenetic stimulations can be applied to humans would heavily depend on overcoming hurdles in gene therapy or the development of pharmacological photoactivatable compounds and infrared optogenetics. Despite this, optogenetics is a powerful tool for understanding the mechanisms of neurological/neurodegenerative diseases.

AUTHOR INFORMATION

Corresponding Authors

*M.Y.C.: Mailing address: Stanford University, Department of Neurosurgery, 1201 Welch Rd. MSLS P352, Palo Alto, CA 94305. Telephone: 650-725-3111. E-mail: mycheng@stanford.edu.

*G.K.S: Mailing address: Stanford University Medical Center, 300 Pasteur Drive, R301A, MC 5325, Stanford, CA 94305. Telephone: 650-725-5562. E-mail: gsteinberg@stanford.edu.

Notes

The authors declare no competing financial interest.

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