

## Review Article

# Optogenetic Stimulation Enhanced Neuronal Plasticities in Motor Recovery after Ischemic Stroke

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Motor capability recovery after ischemic stroke involves dynamic remodeling processes of neural connectomes in the nervous system. Various neuromodulatory strategies combining direct stimulating interventions with behavioral trainings for motor recovery after ischemic stroke have been developed. However, the effectiveness of these interventions varies widely due to unspecific activation or inhibition of undefined neuronal subtypes. Optogenetics is a functional and structural connection-based approach that can selectively activate or inhibit specific subtype neurons with a higher precision, and it has been widely applied to build up neuronal plasticities of the nervous system, which shows a great potential in restoring motor functions in stroke animal models. Here, we reviewed neurobiological mechanisms of enhanced brain plasticities underlying motor recovery through the optogenetic stimulation after ischemic stroke. Several brain sites and neural circuits that have been previously proven effective for motor function rehabilitation were identified, which would be helpful for a more schematic understanding of effective neuronal connectomes in the motor function recovery after ischemic stroke.

## 1. Introduction

Ischemic stroke, a leading cause of severe disabilities in the adult population, has an annual economic cost of approximately \$34 billion all over the world [1]; it also brings about a large number of familial and social burdens in lost productivity. A stroke occurs when a loss of blood flow disables both afferent and efferent connectivities, which finally result in a loss of sensory and motor connections to the world. A number of neurological functions are impaired after a stroke, among which motor disability contralateral to the stroke lesion side accounts for most dysfunctions [2]. Autonomously, the brain functional networks undergo the reorganization and rewiring after a structural lesion from a stroke [3–5]. These remodeling processes are often associated

with the sprouting of spared axons that can innervate denervated target areas and the establishing of new circuits for the recovery of lost functions [6–8]. Such spontaneous reorganizations of connectomes can partially restore motor functions, but the effects are limited and can sometimes be harmful to neurological recovery in large stroke lesions [9]. To maximally regain sensory and motor functions after stroke, surviving neural circuits should be regularly reorganized to make new connections [3, 10, 11]. Thus, multiple strategies have been introduced to promote better neural rewiring and ultimately achieve better recovery of the motor function [11]. These strategies include pharmacological treatment, rehabilitation, cell transplantation and brain stimulation [12–16]. Among them, brain stimulation is considered as a direct and promising neurorestorative strategy. For one

thing, neuronal activity is a major factor that influences neural plasticity and the recovery from ischemic stroke [3, 17]. For another reason, the brain stimulation allows direct manipulation of the activities of a specific brain area, as evoked cortical activities in the damaged hemisphere are decreased after focal stroke [5, 7, 8, 18]. Traditional brain stimulation strategies include microelectrode stimulation, deep brain stimulation, and noninvasive stimulation methods, such as transcranial magnetic stimulation and transcranial direct current stimulation (tDCS). All these stimulations have been shown to promote motor function recovery [19–21]. However, an intrinsic limitation of these approaches is that the cellular effects of TMS or tDCS are much less focal: both of them activate mixed populations of neurons (regardless of neurons or astrocytes), thus blurring the underlying mechanisms of stimulation and producing a range of effects [22–24]. Consequently, which brain areas or circuits are activated or inhibited, and whether stimulations of these targets are efficacious in terms of the motor functional recovery, are still poorly known so far. With the emergence of the optogenetic approach, specific cell types and neural circuits could be well activated or inhibited with a high precision both temporally and spatially [25–27]. For instance, one study comparing optogenetic versus electrical stimulation of dopamine terminals in the nucleus accumbens showed that electrical stimulation induces both local multisynaptic and indirect modulation of DA release, which are absent in optogenetically targeted stimulation [28]. Here, we reviewed recent studies on optogenetic techniques aiming at rewiring or reestablishing brain circuits for motor function regain in stroke rodents. We have summarized several effective target circuits that have the potential for neuromodulatory intervention in the brain after stroke. These potential targets include the ipsilesional motor cortex (iM1), the afferent circuits to the motor area, the efferent corticospinal circuits, and the neurogenic niche subventricular zone (SVZ) (Table 1). Notably, the viral vectors with promoters such as CaMKII to drive the expression of ChR2 in neurons of target regions will allow for selective stimulation of certain subtype neurons, which is critical in understanding the ultimate neuronal activity in functional restoration after stroke. More importantly, the underlying neurobiological mechanisms on mesoscopic (regional interactions) and microscopic (synapses) levels are also elucidated, which might provide an advantage for other stimulation methods, possibly by offering similar or greater therapeutic efficacy in clinical works.

## 2. Mesoscopic Plasticity of Brain Sites and Circuits in the Motor Restoration after Ischemic Stroke

**2.1. Enhanced Neuronal Activities in Ischemic Infarcted iM1 and Peri-Infarcted Areas.** In normal conditions, each cortical hemisphere inhibits the other through transcallosal fibers [29]. Stroke disrupts the interinhibition mechanism and results in excessive inhibition from the contralesional cortex, where evoked cortical activity decreases [30]. Within the

ipsilesional cortex, a significant increase in tonic neuronal inhibition in the peri-infarct zone has been detected after stroke [5]. Based on this interhemispheric competition model and tonic inhibition mechanisms in the peri-infarct zone, the iM1 of the damaged hemisphere became one of the target cortices for the stimulation. A preclinical study revealed that invasive cortical electrical stimulations in the ipsilesional M1 enhanced dendritic plasticities in both the peri-infarct areas and contralateral cortex after stroke [21]. However, limited by indiscriminate activation of all cell types near the stimulated site, the actual effects and mechanisms driving motor recovery remained uncertain. The first attempt using optogenetics to manipulate specific cell types to promote stroke recovery was studied [31]. Optogenetics was used to excite neuronal activities selectively in the ipsilesional primary motor cortex (iM1) poststroke in transgenic mice expressing ChR2. During stimulation, the laser was set to 10 Hz, 20 ms light pulses with a power range of 0.4–0.8 mW. Recordings in the ipsilesional striatum (iStr) and ipsilesional somatosensory cortex (iS1) revealed that iM1 neuronal stimulation could activate peri-infarct regions (Str and S1) as well as cM1. Moreover, repeated neuronal stimulations within the iM1 significantly promoted corresponding motor function recovery after stroke. Although no direct observation on the structural remodeling of axons was made, mechanically, the stimulation promoted the expression of activity-dependent neurotrophins and GAP43, a growth-associated protein critical for axonal sprouting. GAP43 expression was mainly upregulated after stroke in the peri-infarct areas and the contralesional cortex, suggesting that new connections formed from the motor cortex to the premotor cortex and primary and secondary somatosensory cortices [32, 33]. Moreover, these patterns of axonal sprouting overlapped with changes in human functional maps of motor control in peri-infarct motor, premotor, and somatosensory areas [34].

As tonic neuronal inhibition increased in the peri-infarct zone after stroke, to help regain the excitatory-inhibitory balance of the ischemic region within the infarct hemisphere, perhaps direct manufacturing of GABAergic neurons in the peri-infarct zone can become a candidate target for optogenetic stimulation in the future [35, 36].

**2.2. Reorganization of Afferent Circuits Restored the Motor Function.** Although iM1 stimulation has demonstrated significant effects in promoting motor function recovery, the overall motor performance of the mice only recovered to about 50% of the prestroke baseline [31]. To optimize the effect of stimulation-promoted recovery, we focused on the contralesional lateral cerebellar nucleus (cLCN), a deep cerebellar nucleus that sends major excitatory output to multiple motor and sensory areas. Electrical stimulation studies have demonstrated that chronic electrical stimulation of the cLCN could enhance contralateral cortical excitability, which in the end enhances motor function recovery in rats after stroke [37, 38]. With the method of optogenetics, researchers demonstrated that selective stimulation of neurons in the cLCN produced more robust and persistent motor function restoration than the iM1 stimulation. The stimulation laser

TABLE 1: Summary of targeted brain circuits or sites in motor recovery after ischemic stroke using the optogenetic stimulation.

Experimental ischemic stroke model	Target method	Stimulation protocol	Stimulation time course	Recovery onset	Ref.
Mice, transient middle cerebral artery occlusion	Thy-1-ChR2-YFP line-18 transgenic mice which exhibited high levels of ChR2 in layer V of primary motor cortex pyramidal neurons	Activated pyramidal neurons in iM1 with 473 nm blue laser	Poststroke days 5-10, 10 Hz	Significant improvement from day 14	[31]
Mice, transient middle cerebral artery occlusion	Thy-1-ChR2-YFP line-18 transgenic mice	Activated neurons (mainly excitatory neurons) in cLCN with 473 nm blue laser	Poststroke days 5-14, 10 Hz	Significant improvement from day 14.	[39]
Rat, middle cerebral artery occlusion	Injection of a Cre-recombinase-dependent ChR2 vector achieved the specific expression of ChR in corticospinal-projecting neurons	Activated corticospinal projecting neurons specifically expressing ChR2 with 473 nm blue LEDs	Poststroke days 3-14, 10 Hz	Significant improvement from day 21	[9]
Mice, ligations of distal branches of the middle cerebral artery	ChR2 transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) and ChR2-YFP protein were selectively expressed in the excitatory glutamatergic neurons	Activated glutamatergic neuron in the striatum of the ChR2 transgenic mouse with 473 nm blue laser	Poststroke days 5-10, 10 Hz	Significant improvement from day 3	[58]
Mice, middle cerebral artery occlusion	Gad2-Arch-GFP transgenic mice and Arch-GFP were selectively expressed in GABAergic neurons expressed	Inhibited GABAergic neurons in striatum with 530 nm green laser	Poststroke days 5-14, 10 Hz	Significant improvement from day 14	[54]
Mice, middle cerebral artery occlusion	Injection of AAV2.CaMKIIhChR2(E123A) which drove ChR2 expression in thalamocortical projection neurons	Activated thalamocortical projecting neurons with 475 nm blue light	Poststroke days 3-42, 5 Hz	Significant improvement from day 14	[46].

was set to 10 Hz, 20 msec light pulses with a power range of 0.2–0.4 mW, which mainly stimulate neurons in the LCN with the Thy1 promoter. In addition, repeated cLCN stimulations enhanced the structural plasticity for GAP43 in the ipsilesional somatosensory cortex, suggesting that new connections formed in the joints between afferents from cLCN and the ipsilesional somatosensory cortex [39]. However, as LCN contains three neuronal subtypes including glutamatergic, GABAergic, and glycinergic neurons [40, 41], it calls for more studies to interrogate the effects of stimulating selective neuronal subtypes in the LCN during post-stroke recovery.

Apart from afferent cLCN, sensory-evoked cortical activity also depends on the integrity of afferent inputs from the thalamus. It has been shown that focal ischemia leads to a loss of thalamic axonal synaptic contacts with the peri-infarct cortex, resulting in a reduction in the excitability of surviving thalamocortical circuits [42–44], which are proven to be partly responsible for the well-known deficits in superficial (layers 1–3) cortical responses to sensory stimuli after stroke [17, 45]. During the stimulation, the light was set to a 5 Hz, 5 ms light pulse at  $10 \text{ mW mm}^{-2}$  that reliably activates cortical neurons. As expected, optogenetic stimulation targeting this circuit resulted in a persistent improvement in sensory cortex and paw function. Furthermore, chronic optogenetic stimulation also promotes rewiring of thalamocortical boutons as well as growth and retraction of thalamocortical axon branches [46]. It is worth mentioning that the stimulation focused mainly on thalamocortical sensory circuits rather than those responsible for motor output, providing new cues for mechanisms underlying optogenetic stimulation in improving motor recovery. As the study did not assess the expression of trophic factors in the contralateral hemisphere, the mechanisms underlying the function recovery remain undetermined.

**2.3. Reorganization of Efferent Circuits Restored the Motor Function.** Ischemic stroke disables not only afferent circuits, but also efferent connections. Experimental studies have shown that a stroke denervated corticospinal neurons in the contralateral cortex. Furthermore, after various therapeutic and rehabilitative interventions, the corticospinal tract (CST) on the contralesional side can sprout and terminate in the denervated hemispinal cord [47–49]. Thus, CST has been identified as another critical pathway where rewiring may be associated with the recovery of impaired motor functions after ischemic stroke [50]. Recently, a study by Wahl's group strongly proved the existence of this association. After a large sensorimotor stroke, photoactivation of intact CST neurons in the contralesional motor cortex in combination with motor training can robustly induce CST sprouting of preexisting ipsilaterally projecting axons as well as midline crossing CST fibers in the denervated cervical hemicord [9]. Together with a structural rebuilding of the efferent circuits, light stimulation restored the motor function of the corresponding rats by nearly 100% of their prestroke forelimb movement abilities in the fourth week after the stroke. The stimulation laser was set to a 10 Hz, 20 ms light pulse at  $16.6 \text{ mW/mm}^2$ , which specifically activated corticospinal

projecting neurons. The encouraging effects of this stimulation paradigm were comparable to previously established anti-Nogo-A immunotherapy [51], verifying the growth-promoting effect induced by optogenetic-based stimulations. Interestingly, the initially reestablished motor function can be extinguished by further optical silencing of newly sprouted fibers of intact CST neurons. Such dual effects confirmed that specific axonal sprouting in contralateral corticospinal circuits is causally associated with recovery in these large-volume stroke models. Furthermore, it is worth noting that the above study incorporated rehabilitative training into the recovery protocol and demonstrated that stimulation-promoted intrinsic circuitry might be significantly strengthened by rehabilitative training through the reshaping of specific circuits. Physical therapy is a critical approach in improving motor function after stroke, and robot-based rehabilitation has been shown to promote network plasticity and functional recovery [52]. Indeed, the formation of a new circuit can be enhanced by growth stimulatory therapies, followed by rehabilitative training and the selective shaping of new circuits for motor functions [53]. However, the neural mechanisms underlying this synergistic effect is still unclear. Accompanied by optogenetics, the combined protocol could allow for an enhanced knowledge of the neural processes underlying the synergistic effects and potentially help to improve the effectiveness of rehabilitative therapy.

### 3. Enhancing Neurogenesis Restored the Motor Function after Ischemic Stroke

Brain stimulation strategies based on activity-dependent neuronal plasticity have made great advancements in motor recovery after stroke. Nevertheless, effective treatment of ischemic stroke remains a major challenge mainly because the narrow treatment window of currently available treatment approaches is limited to the acute phase [54]. Recently, strengthening neurogenesis has become a topic of interest in addressing the narrow treatment window of stroke since it was reported that the brain is capable of generating new neurons [55, 56]. In the adult brain, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone in the hippocampus are primary neurogenic niches where neural stem cells and neuroblasts inhabit. The striatum is anatomically adjacent to the SVZ and has been proved to project dendrites and axons into the SVZ. Thus, the striatum is presumably in a suitable location to affect cellular activities in the SVZ. Previous studies reported that direct current stimulation of striatal cells in the chronic phase of stroke promoted recovery [57]. Using optogenetics, a past study demonstrated that selectively activating glutamatergic neurons/axons in the striatum triggered a cascade of SVZ cellular responses with increased regenerative activities and functional recovery in the ischemic brain [58]. Furthermore, laser stimulation-induced increases of cells were mainly concentrated in the peri-infarct cortex. Additional behavior tests of the study revealed that regained motor function paralleled with the migration of newborn DCX+ neuroblasts from the SVZ to the peri-infarct region. Thus, the immediate effect



of the current stimulation of striatal cells might act through activating glutamatergic neurons/axons in the striatum. Inhibiting rather than exciting the activity of striatal neurons after ischemia improved motor function in stroke mice.

Aside from the stimulation of glutamatergic neurons, GABAergic neurons are known to constitute the majority of striatal neurons [59]. Another study on striatum focused on these neurons. In this study, researchers used a stimulation laser with a 20 Hz, 5 ms light pulse at 1 mW/mm<sup>2</sup> and a 473 nm-pulse blue laser with 0.5 mW power to specifically inhibit or activate GABAergic neurons in Gad2-Arch-GFP transgenic mice, respectively. It was demonstrated that optogenetic inhibition rather than activation of striatal GABAergic neurons promoted neurogenesis after cerebral ischemia by projecting dendrites and axons to the SVZ [60], leading to a recovery in corresponding motor function in an experimental ischemic stroke model [54].

Altogether, these studies partially illuminate the underlying mechanisms of how altering neuronal activity regulates neurogenesis in a semiphase synaptic way. This photoactivation of neural regeneration may provide a promising direction in extending the treatment window for stroke.

#### 4. Microscopic Mechanisms of Optogenetically Promoted Neuronal Plasticities in the Motor Restoration after Ischemic Stroke

Motor function recovery after stroke depends on the reorganization of neuronal connections throughout the central nervous system (CNS). The contents above have elucidated several potential brain circuits or sites where new connections can grow after optogenetic stimulation. However, the mechanisms of how new functional circuits are formed remain poorly known. We examined three neurobiological mechanisms underlying optogenetic-stimulation-promoted motor function recovery on a synaptic level, including synaptic plasticity, axonal sprouting, and dendritic morphology changes.

Among them, synaptic plasticity refers mainly to the function of synaptic circuits or changes in inhibitory control of circuits. While axonal sprouting and dendritic morphology changes are the structural basis of new connection formations, the axonal sprouting in peri-infarct and connected cortical areas is causally associated with motor recovery and can be triggered by synchronized low frequency neuronal activity [61]. Although different optogenetic stimulation strategies or brain targets lay particular stress on different mechanisms above, neuronal activity is proposed to be the most important driving force in incorporating all these mechanisms of synaptic connections and reorganization of adult cortices [54]. Thus, the internal molecular substrates that underlie these persistent anatomical changes may overlap greatly.

As with activity-dependent synaptic plasticity, both long-term potentiation (LTP) and long-term depression (LTD) have been proposed to be involved in cortical plasticity [62, 63]. It has been proved both *in vitro* and *in vivo* that optogenetics can drive synaptic plasticity. Firstly, synaptic composition and function can be directly tuned with light [64]. Secondly, optogenetics can promote selective

regeneration of refractory axons in living vertebrates [65]. Indeed, almost all processes of the electrical firing of neurons in the brain are complex assortments of neuronal signaling proteins, including myriad ion channels and receptors. It is well known that the cAMP-dependent pathway is one of the neurobiological basis of optogenetics-promoted synaptic recovery [66]. Diverse forms of synaptic plasticity rely on postsynaptic mechanisms that converge in the AMPA-type glutamate receptors on the postsynaptic membrane. The recruitment of AMPA receptors to PSD by photostimulation has been observed. Once these artificially recruited receptors have access to the synapse, they would occupy the same sites occupied by receptors recruited through synaptic activity [64]. Furthermore, rehabilitation training is helpful in optogenetically stimulated motor recovery after stroke. Specifically, the second messenger cyclic adenosine monophosphate (cAMP) acts as a critical factor promoting axonal regrowth [2–5].

As to axonal sprouting, several sprouting transcriptomes and molecules like EphA4, ATRX, Lingo1, and IGF have been evaluated to be responsible for axonal sprouting after stroke [32, 34, 67]. Among them, EphA4 and Lingo1 are growth inhibitors, while IGF is a growth-associated factor. Activity shapes neural connections by inducing both growth-promoting and growth-inhibiting molecules and neutralizing or enhancing either subset to drive structural and anatomical changes [68]. These paradigms are key signaling pathways for activity-dependent plasticity in the adult brain. However, whether the prompting of new connections by optogenetics shares similar mechanisms with these transcriptome and molecular paradigms is still uncertain.

The dendritic morphology of a neuron determines where and how the new connections of that neuron will be made [69]. Although there is only a finite amount of evidence on optogenetic stimulation-related dendritic morphology changes after stroke, it has been shown that optogenetics can regulate the remodeling of dendritic spines in neurons of patients with Alzheimer's disease by modulating the activity of cofilin, a small protrusion on the surface of dendrites that receives inputs from neighboring neurons [70].

Based on evidence from the microscale synaptic level to the mesoscale circuit level, optogenetics can be utilized as a useful tool to sculpt brain connectivity after stroke. However, since the establishment of connections requires guiding axons to correct target areas and forming synapses at particular regions on postsynaptic neurons, there is still no clear mechanistic rationale on molecular details of the process. Consequently, it calls for more mechanistic studies of neuronal plasticities to advance in developing effective treatment strategies.

#### 5. Conclusions and Future Implications

Overall, stroke can be viewed as a disruption of old brain connectomes as well as an origin of new neuronal connection reorganization throughout the central nervous system. Optogenetics is a useful and neuron-specific tool in promoting neural plasticity to benefit motor function rehabilitation after

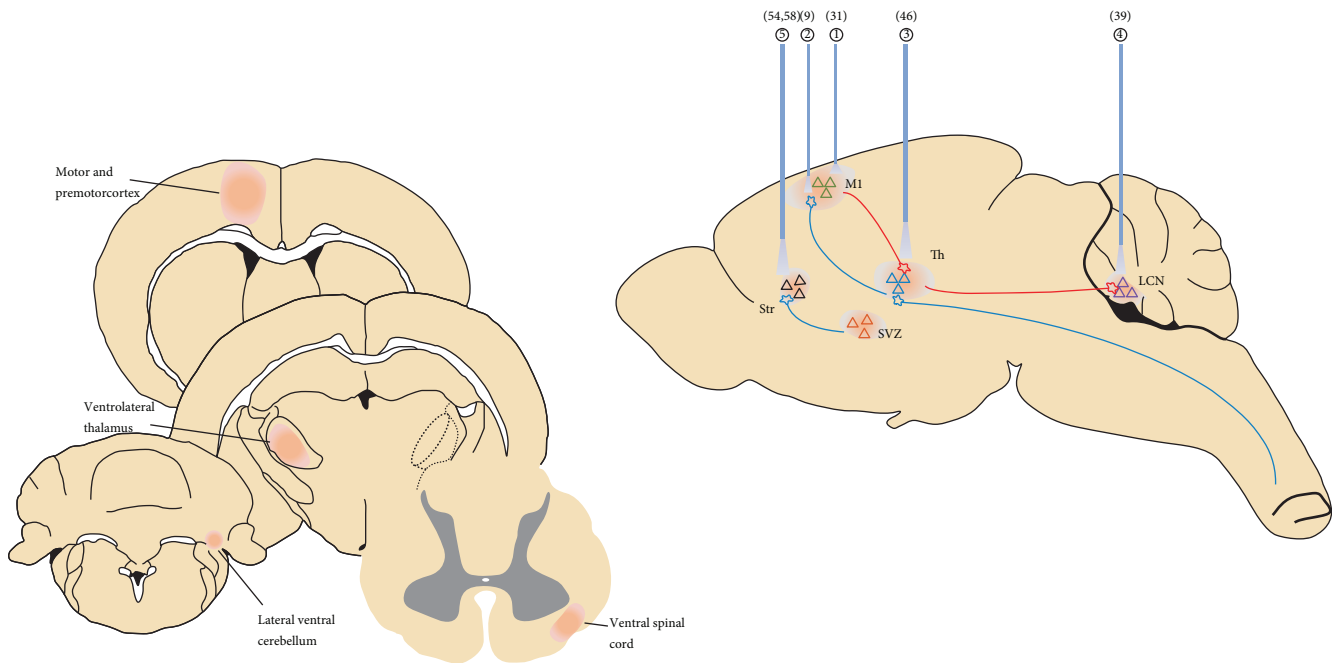


FIGURE 1: Optogenetic targeting of neural circuits in the motor recovery after ischemic stroke in the mouse brain. The illustration depicts several targeting circuits or sites, (1) including stimulation of ischemic infarcted iM1 [31], (2) stimulation of efferent iM1-thalamus-spinal circuits [9], (3) stimulation of afferent thalamocortical circuits [46], (4) stimulation of multisynaptic projections of cLCN-thalamus-M1 [39], and (5) stimulation of striatum-SVZ projection for neurogenesis [54, 58]. Afferent and efferent circuits are distinguished as blue and red color, respectively.

ischemic stroke. Several proved-to-be effective neural circuits have been identified by optogenetics. Among these stimulation targets, recovery through the stimulation of the primary site of ischemia iM1 is mainly involved in potentiating synaptic plasticity within the core of the infarcted area and promoting new connections within peri-infarct areas by enhancing axonal sprouting. Stimulation of afferent and efferent circuits are mainly involved in axonal sprouting within the ipsilesional somatosensory cortex, the thalamocortical circuit, and CST fibers in the denervated cervical hemisphered. Optogenetic stimulation of SVZ, which is a niche for the genesis of neuroblasts, involves promoting neuroblasts to migrate to the boundaries of the infarct zone, to differentiate into mature neurons, and to integrate into neuronal networks [71].

Optogenetics-enhanced neural plasticities include both long-term potentiation (LTP) and long-term depression (LTD) [72]. At the synaptic level, optogenetics-enhanced neural plasticity involves recruiting AMPA-type glutamate receptors and increasing the efficiency of synaptic transmission after stroke [64]. Furthermore, optogenetics stimulation can promote axonal sprouting. Although a great many molecules like GDF10, Lingo1, and EphA4 are proven to be critical in the formation of new axonal connections [34], these identified molecules are pleiotropic cytokines or growth factors with effects in the noncentral nervous system. Future therapeutics aiming at enhancing axonal sprouting in stroke models might tap into molecules that are both neural activity-dependent and critical to axonal sprouting in the central nervous system [73]. Nevertheless, all the stimulation targets corresponding to poststroke events are mainly due to the

removal of inhibition, activity-dependent synaptic changes, growth of new connections, or unmasking of preexisting connections [74].

Apart from these brain targets, it is worth noting that the contralateral hemisphere has always been of great interest as a target of neuromodulatory interventions [75]. In animal models and human stroke patients, it has been found that increased inhibition in the ipsilesional hemisphere from the hyperexcitable contralesional hemisphere may limit functional recovery after stroke [76, 77]. As traditional rTMS protocols enhancing excitability in the peri-infarct tissue of the ipsilesional hemisphere or downregulating the excitability in the unaffected hemisphere can only grossly rebalance hemispheric excitability, many inconsistencies are generated between studies investigating the effects of rTMS on motor function [78–80]. For these inconsistencies, optogenetic modulation of specific interhemispheric circuits might be a novel approach to specifically modulate the neurons underlying abnormal interhemispheric inhibition after stroke. Due to the imprecise laser power for the stimulation, stimulation parameters like the frequency, duration, and intervals of stimulations should be determined empirically depending on the brain region and cell type of interest [81]. Some stimulation protocols are intended to activate certain neurons, while others are aimed to inhibit target neurons. Depending on the type of opsin used, the stimulation frequency and interval should match the intrinsic photocycle for high stimulation efficiency and low opsin desensitization [24, 82].

Although optogenetics itself could not be applied directly to clinical patients, these studies can be instructive in identifying specific excitatory or inhibitory circuitry that

may work in human patients, thus allowing for more precise and effective outcomes for noninvasive and mature stimulation toolboxes like transcranial magnetic stimulation (TMS) or tDCS.

In conclusion, optogenetics is a useful tool in extending the boundaries of poststroke rehabilitation of motor function by providing a conceptual framework to improve established clinical stimulation techniques (Figure 1). The development of optogenetics has made it evident that brain plasticity is fundamentally a synaptic phenomenon that is largely stimulus dependent, and that brain repair must incorporate biological interventions like brain stimulations and behavioral interventions that are carefully tailored for the reorganization of specific brain circuits [83]. However, more efforts are needed in exploring the basic mechanisms of poststroke paresis as well as the rehabilitation process to guide the tendency in neural repair. Future preclinical trials can mainly focus on two potential directions: firstly, interrogate the effects of stimulating selective neuronal subtypes during post-stroke recovery, and secondly, explore how this approach could be integrated with rehabilitation training and biological approaches to take maximum advantage of the brain's capacity to restore its neural networks after stroke.

### Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

### Authors' Contributions

Changbo Lu and Xianglong Wu contributed equally to the paper.

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