Title: Investigation of Possible Pretreatment Effect of Photo Biomodulation through Alterations of MicroRNAs (21 and 124a) Expression in Animal Model of Ischemic Stroke

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Abstract:

Through the careful regulation of neuronal activity in brain circuits damaged by a stroke lesion, the developing and promising optogenetic stimulation method can aid in functional recovery. This investigation assesses the potential pretreatment effects of optogenetic stimulation on an ischemic stroke animal model. Lentiviruses containing pLenti-CaMKIIahChR2 (H134R)-mCherry-WPRE were administered to adult male Wistar rats and injected into the right striatum for this purpose. 26 days following virus injection, the animals were exposed to blue laser light for six days in a row for 30 minutes at a time. 24 hours after the final light stimulation, the transient middle cerebral artery occlusion (tMCAO) was produced. One day after reperfusion, the neurological processes and the sizes of the brain infarcts in ischemic rats were evaluated. In the striatum and hippocampus, the transcript levels of microRNAs 21 and 124a—epigenetic indicators for neuroprotection and neurogenesis—were also assessed. Our findings suggested that pretreatment with glutamatergic striatum optogenetic stimulation could reduce neurological impairments in rats and boost neuronal survival in both striatum and hippocampal regions. Also, the expression of microRNA-21 (miR-21) in the striatum was significantly increased in rats that had been optogenetically stimulated. Additionally, miR-124a expression was elevated in both regions in rats given tMCAO, and pretreatment with optical stimulations may considerably lower its expression in the hippocampus. According to our findings, optogenetic stimulation pretreatment of the striatum's positive effects on stroke recovery are at least partially mediated by altering miRNAs involved in neurogenesis and subsequently activating its downstream signaling cascade.

Key words: Ischemic stroke; Optogenetic stimulation; Ischemic tolerance; MicroRNA; Neuroprotection; Neurogenesis.

Introduction

One of the leading causes of adult long-term disability, stroke has a significant negative economic and social impact on human society Ischemic stroke is a noncommunicable disease that is on the rise in both developed and developing nations. It happens when a blood vessel supplying the brain is blocked (Martinez & Peplow, 2017). By 2030, it is anticipated that many nations would join aging societies (1 in 5 people will be 65 or older), and regrettably, it is predicted that the number of people who suffer from stroke problems will rise every year (Sakai & Shichita, 2019). Most stroke survivors experience a variety of neurological problems and disabilities that affect their long-term everyday activities. However, there is currently no specific treatment for ischemic stroke, necessitating a new viewpoint on the pathophysiological and molecular conditions (Panagal et al., 2019).

The primary neurogenic niches that contain neural progenitor cells and neuroblasts in the adult brain are the subventricular zone (SVZ) in the lateral ventricles and the subgranular zone (SGZ) in the hippocampus. According to Ohab and Carmichael (2008), the neurogenesis in the SVZ reacts to brain injuries including stroke and traumatic brain injury. The striatum is anatomically close to the SVZ region and is known to send axons and dendrites there. Therefore, the neurogenesis process in the SVZ can be started by stimulating glutamatergic receptors in the striatum, resulting in the survival of neurons in the striatum. In order to influence cellular activity, the striatum is therefore likely in the proper location next to the SVZ (Morimoto et al., 2011).

Today, optogenetic technique, as a revolutionary method for stimulating specific brain areas, scrutinizing the role of specific neurons activity in the brain function (Sayed Javad Javaheri, Bigdeli, Zibaii, Dargahi, & Pouretemad, 2019). Some studies highlighted that stimulating direct flow of striatum cells in the chronic phase of the cerebral ischemia leads to an amelioration pattern (Morimoto et al., 2011; Song et al., 2017). Besides, it has been reported that selective activation of glutamatergic neurons in the striatum area using optogenetic technique causes cascading SVZ cellular responses by increasing regenerative activity and eventually, triggers ischemic brain function (Song et al., 2017). Given the above finding, it can be assumed that, the optical stimulating effect of striatum cells may operate by activating glutamatergic neurons in the striatum (C. Lu et al., 2019). Although, glutamate is an excitatory neurotransmitter in the central nervous system, its over-accumulation in the extracellular matrix may shows cytotoxic effect, and finally, lead to activate of cell death processes such as apoptosis. Apoptosis caused by focal ischemic stroke in a short period (30 to 60 minutes) is an outstanding feature of penumbra in the ischemic stroke (Sun et al., 2017).

It has been revealed that MicroRNAs (miRs), as novel biomarkers, could be targeted in diagnosing and treating ischemic stroke. According to some studies, more than 20% of miRs dysregulate in ischemic stroke and it can presume that miRs may act as mediators in the pathogenesis of stroke. These small non-coding RNAs that are considered as an essential biological modulator that regulate the substantial signaling pathways in stroke pathology (Han et al., 2014; W. Liu, Chen, & Zhang, 2016). Among

the reported miRs, miR-21 is an important one that is often elevated in a variety of diseases and has been shown to play an important role in cell proliferation and apoptosis (Sekar, Venugopal, Sekar, & Ramalingam, 2016). It has been reported that, the level of miR-21 in rodent model of ischemic stroke is relatively high and it is a potent anti-apoptotic in some biological systems (F. J. Liu et al., 2013). The expression of miR-21 protected neurons from cell death caused by hypoxia-activated microglia (Zhang, Dong, Li, Hong, & Wei, 2012). Under ischemic and reperfusion conditions, increased miR-21 expression via suppression of phosphatase and tensinhomolog (PTEN) expression cause upregulation the Akt signaling, consequently it inhibited apoptosis by suppress pro-apoptotic factors such as caspase-3 and increasing ratio of Bcl-2/Bax (Yang, Yang, & Li, 2014).

Another small non-coding RNA that dysregulates during stroke is miR-124a. Under physiological and pathophysiological conditions, the Notch signaling pathway regulates neurogenesis by controlling stem cell survival and determining cellular fate in the brain (Zhu, Liu, & He, 2019). According to previous studies, during ischemic stroke, the Notch signaling pathway is activated in neural progenitor cells (NPCs) located in the SVZ region in the lateral ventricles, leading to the proliferation of NPCs (Kageyama, Ohtsuka, Shimojo, & Imayoshi, 2009; Zhu et al., 2019). It has been reported that miR-124a is involved in the regulating of neurogenesis in the SVZ region of the adult brain through suppressing SOX9 (X. S. Liu et al., 2011). The presence of miR-124a in NPCs significantly reduces JAG1 transcription and protein levels, leading to inactivation of the Notch signaling pathway. it has been shown that in adult rats with

stroke, reduced expression of miR-124a in NPCs of SVZ, inversely related to the activation of the Notch signaling pathway (X. S. Liu et al., 2011; J. Wang, Huang, Ding, & Wang, 2019).

Thus, we propose that specific signatures of reducing of infarct volume with optogenetic stimulation could be obtained from brain tissues and behavioral tests and can be used in the identification of biomarkers (miRNA 21, 124a) for diagnosis, prognosis or even etiology of ischemic stroke. Regarding to these finding showing that increased expression of miR-21 protects against neuronal death in ischemia and decreased expression of miR-124a leads to induction of neurogenesis in neurogenic niches, miR-21 and miR-124a may be important molecules for ischemic tolerance. Therefore, we aimed to answer whether pretreatment with optogenetic stimulation through modulating of expression levels of miR-21 and 124a can restore neurological deficits as well as tissues injury in a rat model of transient middle cerebral artery occlusion (tMCAO).

Materials and methods

Animals and experimental design

The Institutional Animal Care and Use Committee of Shahid Beheshti University, Tehran, Iran, approved all animal procedures and ensured that they adhered to NIH standards. Wistar adult male rats weighing 230–300 g was housed at 21±3ºC on a 12-hour light/dark cycle with free access to enough food and drink. Figure 1 depicts the experimental layout for this study.

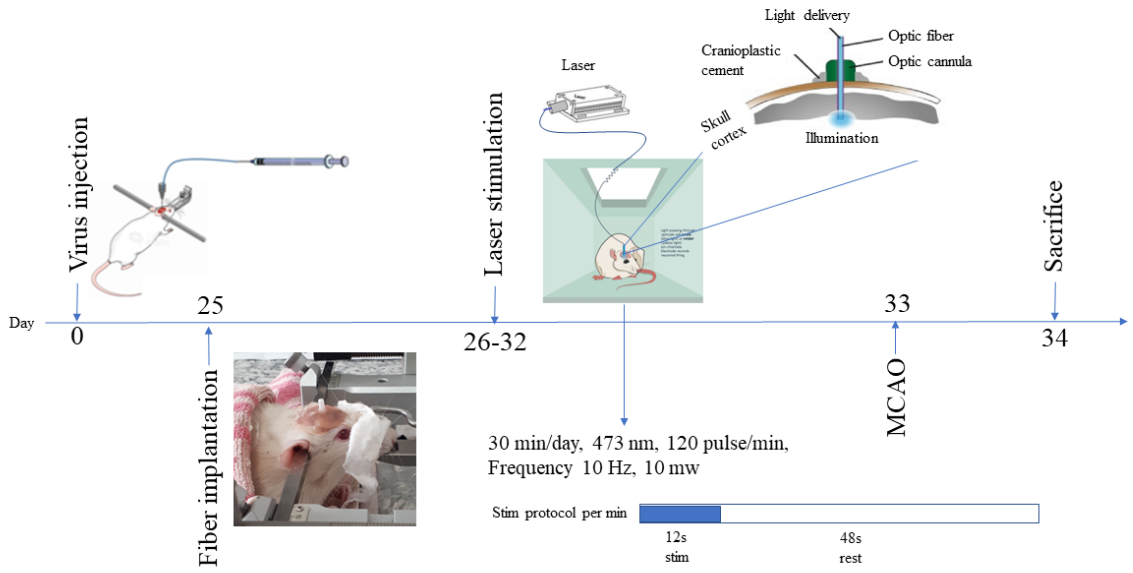

Fig 1. Schematic illustration of the experimental protocol.

In this study, twenty-four rats were randomly divided into three groups 1) sham group (stereotaxic surgery with the injection of virus carrying empty vector, optical cannula implantation, and MCAO surgery without suture thread insertion); 2) stroke group (stereotaxic surgery with the injection of virus carrying empty vector, optical cannula

implantation and tMCAO induction); 3) pretreatment group (stereotaxic surgery with the injection of virus carrying opsin, optical cannula implantation, optogenetic stimulation for six days, and tMCAO induction).

Briefly, lentivirus suspension was injected to striatum. On day 25 post-injection, optical fiber was implanted and laser stimulation was carried out from day 26 to day 32, for 30 minutes daily. Finally, tMCAO surgery was performed on day 33. Evaluation of neurological functions and infarct volume were performed one day following stroke.

Lentivirus injection

The lentiviral particles were prepared according to our previous work with Chavoshinezhad et al. Rats were anesthetized with intraperitoneal ketamine/xylazine (80 mg/20 mg per kg) injection. Then 2.5 microliter lentivirus carrying hChR2 (H134R)-mCherry or the empty vector were injected into the right striatum (AP = 0.36mm, ML= 3mm, DV= -4.8mm relative to the bregma) according to rat brain atlas of Paxinos and Watson (Paxinos & Watson, 2007). It was performed using Hamilton syringe (0.05µl/min) under stereotaxic surgery (Hamilton, Reno, Nevada). At last, after finishing injection, the needle was maintained in the brain for additional 10 minutes before it was withdrawn. Then animals were placed in a recovery chamber(Chavoshinezhad et al., 2021).

Optical fiber implantation and laser stimulation

On day 25 after virus injection, animals underwent optical fiber cannula implantation. the ceramic LC fiber optic cannula was implanted into the right striatum, the same coordination of virus injection through a small burr hole generated with a drill. The implanted optical fiber was immobilized on the surface of skull via dental cement. One day later, laser stimulation was performed once daily for six consecutive days, from 26 to 32 days after virus injection. Each stimulation session lasted for 30 minutes. Every session had 30 explosions and each containing 120 pulses. The parameters of lasers were controlled by LabVIEW software. Each 60-second stimulation cycle composed of 12-seconds stimulation phase and 48seconds rest phase. In the stimulation phase, 473nm solid state laser (CNI laser) pulses were administrated at 10 Hz. The laser powers were ~10-11mW output at the tip of a 200μm fiber. Laser waves are transmitted through optical fiber attached to a rotary joint patch cable (Thorlabs) so that the rat is enable to rotate freely in the chamber(Sayed Javad Javaheri et al., 2019).

Transient Middle Cerebral Artery Occlusion Surgery

Twenty-four hours after the last stimulation, rats were anesthetized with intraperitoneal injection of chloral hydrate, (385 mg/kg, Merck, Germany). The protocol of MCAO surgery was described by Longa et al (Longa, Weinstein, Carlson, & Cummins, 1989). The right middle cerebral artery was occluded by insertion a 3–0 nylon suture. After 60 minutes, the suture was withdrawn to establish reperfusion of the ischemic areas.

Neurological and infarct volumes assessments

To evaluate neurological function, neurological severity score (NSS) was conducted 24 hours after the reperfusion. It was used for the estimation of injury caused by ischemic stroke and the assessing recovery with laser stimulation. NSS scaled from 0 to 18 (normal score 0; maximal deficit score 18). Category of the neurological finding was included raise the tail, motor function, sensory function, beam test and reflex activity (Long et al., 2013).

For infarct volume assessment, triphenyl tetrazolium chloride (TTC) staining, as a reliable histochemical indicator, was carried out on one day after MCAO. Animals were sacrificed and brains were quickly removed and cut into coronal sections with 2mm thickness. The sections were stained with 2% TTC solution. Then, they were incubated at 37˚C for 15 minutes. The stained brain sections were photographed and evaluated via Image J software and total, striatal, and hippocampal infarct volumes were calculated separately (Khaksar & Bigdeli, 2017). The volume was assessed as the following formula:

The corrected volume of damaged area=left hemisphere volume - (left hemisphere volume - damaged area volume).

RNA extraction and quantitative polymerase chain reaction (qPCR)

One day following stroke, 3 rats from sham, stroke and pretreatment groups were sacrificed. Right striatum and hippocampus tissues were immediately dissected on ice, snap-frozen in liquid nitrogen, and kept at −80 °C until qPCR analysis. Total RNA from

striatum and hippocampus were extracted via Trizol solution (Favorgen, Taiwan). NanoDrop 2000c UV-Vis spectrophotometer (Thermos Scientific, USA) was used to detect RNA quality. Reverse transcriptions of RNA to specific cDNA were performed via cDNA synthesis kit (Smobio, Taiwan). The amplification was performed by a real-time PCR system (ABI, USA) using a SYBR Green master mix (Ampliqon, Denmark). The relative expression level of miR-21 and 124a was normalized to the U6 control and fold change was calculated through 2-ΔΔCt formula.

microRNA	Primer sequence
miR-21-5p	uagcuuaucagacugauguuga
miR-124a-3p	uaaggcacgcggugaaugcc

Table1. microRNA sequence

Statistical Analysis

All statistical analyses were performed with GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). The volume of infarction was determined by the ImageJ software version 1.50 and findings were analyzed by t-test. The findings of NSS were analyzed by the nonparametric test (mann-whitney test). One-way ANOVA followed by post hoc Tukey's test was applied to compare molecular assessments between groups. All data are presented mean ± SEM and statistically significant result were considered as $p < 0.05$.

Results

Effect of optogenetic stimulation on neurological deficits

The statistical analysis revealed the significant difference in NSS scores between groups. As shown in Fig. 2, optogenetic stimulation pretreatment could improve neurological deficits one day after ischemia in comparison to stroke group ($p < .001$, Fig. 2a).

Also, in the Fig. 2b–f, the mann-whitney analysis indicated that the raising the tail, sensory, moving and balance tests, were significantly reduced in the pretreatment of optically stimulated group versus stroke group (Fig. 2b–e, $p < .05$).

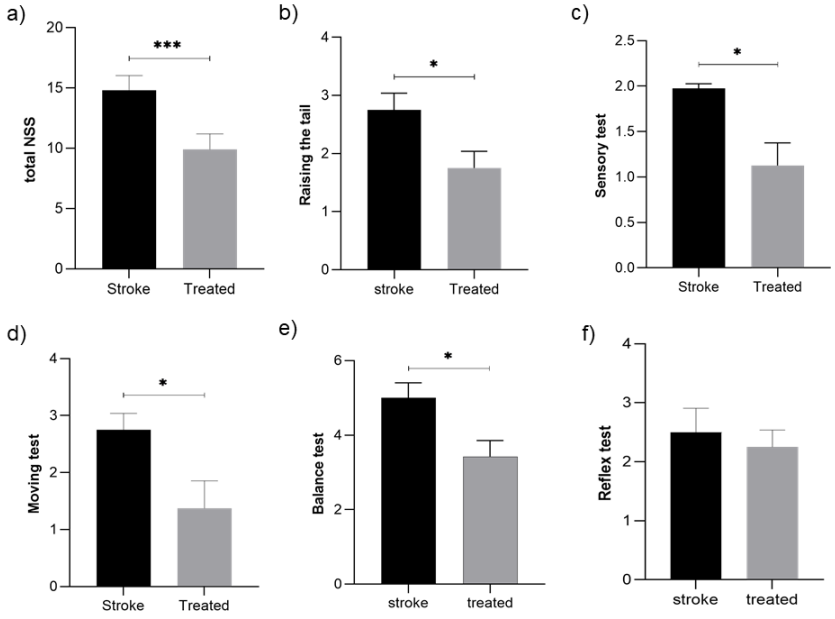

Fig 2. The effect of optogenetic stimulation pretreatment on NSS (n=4/group). optogenetic stimulation pretreatment could reduce NSS one day after ischemia Data are presented as mean ± SEM (n=4/groups). a) Total NSS b) Raising the tail c) Sensory test d) Moving test e) Balance test f) Reflex test. * p<0.05, *** p<0.001 versus stroke group.

Effect of optogenetic stimulation on infarct volumes

The results showed that in the group with light stimulation of glutamatergic neurons, total infarct volume was decreased compared to the stroke group ($p<0.01$, Fig. 3A). Furthermore, statistical analysis demonstrated that infarct volume significantly reduced in the ipsilateral hippocampus and striatum of the pretreated rats compared to stroke a group (Fig. 3B and 3C, $p <0.001$ and $p <0.01$, respectively).

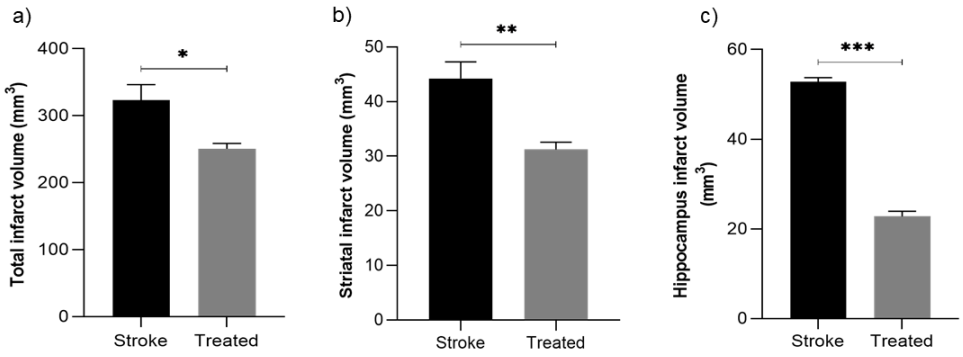

Fig 3. The effect of optogenetic stimulation pretreatment on infarct volume. The data analysis of the IV a) Total infarct volume, b) striatal infarct volume, c) hippocampus infarct volume in the stroke and optically stimulated groups. Data are reported as the mean ± SEM (n=3/group). The volume of infarction was determined by the ImageJ and t-test was used for statistical analysis. *p<0.05, **p<0.01 and ***p<0.001 vs corresponding stroke group.

Effect of optogenetic stimulation on miR-21 expression

One days after stroke induction, the miRNA levels of miR-21 in striatum and hippocampus were evaluated by the qPCR analysis. One-way ANOVA followed by Tukey's test revealed that miR-21 transcripts in both regions of the MCAO group was not altered compared with the sham rats. Furthermore, the pretreatment with optogenetic stimulation increased the expression level of miR-21 in striatum compared to the stroke (Fig 4A. $p<0.001$), while no significant change was found in mRNA level of miR-21 in the hippocampus of the pretreated group compared to the stroke (Fig. 4B, $p>0.05$).

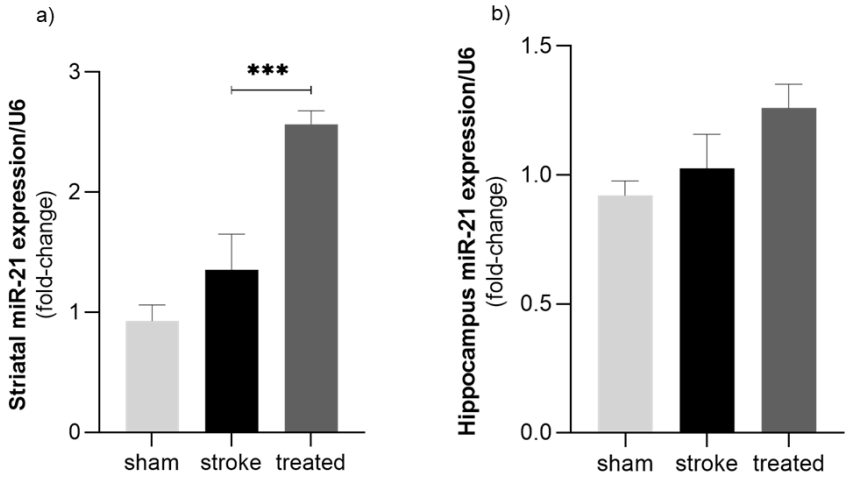

Fig 4: Effect of optogenetic stimulation on miR-21 expression (n=4/group). miR-21 expression by quantitative polymerase chain reaction (qPCR). a) In the striatum area expression level of miR-21 in the treated group increased significantly compared to the stroke group and sham group. b) In the hippocampus expression level of miR-21 in the treated group increased compared to the stroke group and sham group, but this increase was not significant. All data are presented as mean ± SEM (***$p<0.001$).

Effect of optogenetic stimulation on miR-124a expression changes

The real-time PCR technique which was used for evaluation of miR-124a after optogenetic stimulation showed that, in the striatum expression level of miR-124a in the pretreatment group was decreased compared to the stroke group. Anyway, this difference between groups was not significant. There also was a significant increase ($p<0.05$) in the stroke group compared to the sham group. In the hippocampus expression level of miR124a in the pretreatment group was significantly reduced ($p<0.05$) compared to the stroke group. Also, in the stroke group compared to the sham group, we see a significant increase ($p<0.01$) in the expression of this miR.

One-way ANOVA analysis revealed that miRNA-124a level in striatum and hippocampus were reduced in both of them in optically stimulated rats in comparison with corresponding stroke (Fig.5a and b). Anyway, revealed that miR-124a transcripts in both regions of the MCAO group was significantly increased compared with the sham rats (Fig 5a and b, $p<0.05$ and $p<0.01$ respectively). Moreover, the pretreatment with optogenetic stimulation decreased the expression level of miR-124a in hippocampus compared to the stroke (Fig 5b. $p<0.05$), while the changes of miRNA-124a expression were not statistically significant in striatum of optically stimulated group compared to the stroke (Fig. 5a).

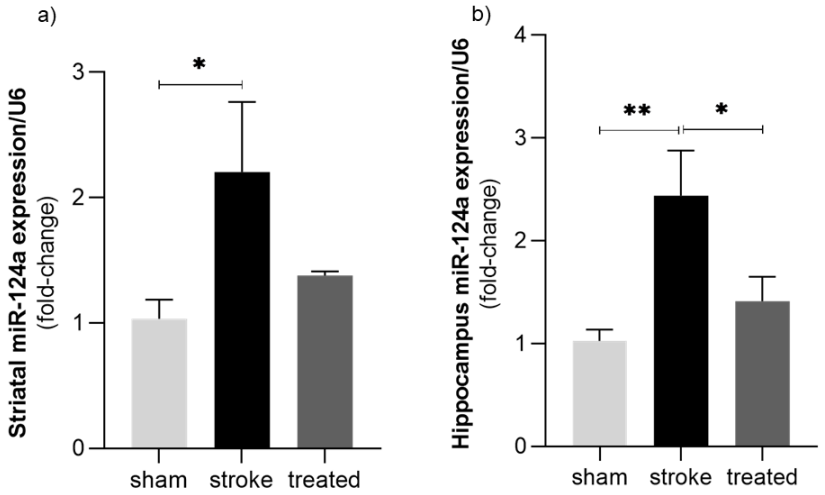

Fig 5: miR-124a expression by quantitative polymerase chain reaction (qPCR) (n=4/group). a) In the striatum level expression of miR-124a in the treated group decreased compared to the stroke and shame groups. But this decrease was not significant. b) In the hippocampus expression level of miR-124a in the treated group decreased compared to the stroke and sham groups. All data are presented as mean ± SEM. (*p<0.05, **p<0.01)

Discussion

In the current study, we established that an animal model of ischemic stroke was caused to develop ischemia tolerance when glutamatergic neurons in the striatum were optogenetically stimulated.

The evaluation of sensory-motor capabilities to assess the severity of the stroke or the stroke recovery process following therapy is one of the stroke research's most scientific assessments. According to the current study, glutamatergic neurons can be stimulated optogenetically to reduce neurological impairments. Taking into account earlier research,

it was shown that optogenetic stimulation has a considerable impact on improving neurological impairments (Chavoshinezhad et al., 2021; Safial Hosseini, Bigdeli, Khaksar, & Aliaghaei, 2020; Shah et al., 2017). To activate helpful pathways that aid in recovery, only optogenetic stimulation is required (Cheng et al., 2014). An approach for maintaining protective neurons in the striatum and primary motor regions is optogenetic activation, as has been shown (R. Chen et al., 2017; Pendharkar et al., 2021).

Previous research has also demonstrated a correlation between the recovery of motor function and the migration of DCX+ neuroblasts from the SVZ to the peri-infarct region (Song et al., 2017). The forepaw sensorimotor ability and somatosensory cortical circuit function are both enhanced by optogenetic activation. These findings imply that an optogenetic strategy can rewire thalamocortical circuits and repair dysfunctional brain activity (Tennant, Taylor, White, & Brown, 2017). Thus, the activation of glutamatergic neurons and axons in the striatum may constitute the initial result of brain plasticity on stimulation of striatal cells. It has been suggested that the striatum and the sensory-motor regions of the cortex and the hippocampus are anatomically connected indirectly, highlighting the importance of glutamatergic neurons in these interactions. Through the central nucleus of the amygdala, Wang et al. (2016) shown that the striatum and the hippocampus are anatomically connected. The cortico-striatal-thalamo-cortical (CSTC) network is one of the major pathways that have been shown to connect various brain regions (Rădulescu, Herron, Kennedy, & Scimemi, 2017). Researchers looked at the glutamatergic circuits that mediate the indirect connection between the cortex and the striatum. They demonstrated that in these circuits, the prefrontal cortex activates the

striatum by transmitting glutamatergic neurons, and the thalamus communicates with areas of the sensory-motor and prefrontal cortex as well (Buschman & Miller, 2014). These results may provide a possible explanation for how activating glutamatergic neurons in the striatum may promote neurogenesis in the cortical or hippocampus's neurogenic niche. The neurological function ratings in the pretreatment group likewise showed a significant decline. Recovery from neurological deficiencies is likely linked to lessening of damage to nearby brain regions.

Interestingly, we found that light-induced activation of the striatum had significant results in addition to having an additive effect on the diminution of infarct volume. When compared to those not receiving light stimulation, we saw that the infarct volume in the striatum area was much smaller in the pretreatment group. According to Bo, Li, Li, Wang, and Tong (2018) and Y. Lu et al. (2017), optogenetic activation of certain neurons during a stroke is neuroprotective and shrinks the infarct size.

Increasing the activity of the glutamate neurotransmitter and its various receptors through the optogenetic technique trigger neuroprotective molecules (Lerchundi et al., 2015) which are established to protect neurons in ischemia and hypoxia, eventually cell destruction in the striatum should be reduced (Cheng et al., 2014; Monteiro et al., 2021). These molecules are known to protect neurons in ischemia and hypoxia. The rate of cell death in the hippocampus decreased as a result of the infarct volume, and because the striatum and the hippocampus are indirectly related, the glutamate accumulation in the

hippocampus as a neurological niche is effective in inducing the effect of neuroprotection on neurogenesis as well. The infarct volume caused by optogenetic pretreatment of the striatum and hippocampus has been greatly reduced, most likely as a result of this.

MicroRNAs are one of the main epigenetic elements influencing many nervous system systems (Rink & Khanna, 2011). The potential of miRNAs as biomarkers for brain damage in ischemic stroke has been investigated (Eyileten et al., 2018; Mirzaei et al., 2018). MiRNAs are thought to be important in many cellular alterations that occur after an ischemic stroke, according to a large body of research (Vilar-Bergua et al., 2016). MiR-21, which is often raised in many forms of stroke and engaged in anti-apoptotic pathways, is regarded as a significant miRNA among those found in ischemic stroke (Y. Chen et al., 2008; Xu et al., 2014).

In specific, miR-21 has been demonstrated to be a strong antiapoptotic factor (Chan, Krichevsky, & Kosik, 2005). Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells (Papagiannakopoulos, Shapiro, & Kosik, 2008). The most important target genes for miR-21 are PDCD4, FASLG and PTEN (MirTarBase database)[1], studies have shown that all of them are highly effective in exacerbating apoptosis and inflammation (Buller et al., 2010; Gaudet, Fonken, Watkins, Nelson, & Popovich, 2018; Young, Lafourcade, Platel, Lin, & Bordey, 2014). A study by Buller et al. Showed that miR-21 plays a definitive role in decrease of ischemic

[1] http://mirtarbase.mbc.nctu.edu.tw/php/search.php

cell death by targeting FASLG 3′-UTR that is a main cell death-inducing ligand of the TNF-α family of ligands (Buller et al., 2010). [Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury.] It can act as a neuroprotective against stroke, and nerve and heart stem cells can be protected from stressful stimuli such as hypoxia and apoptosis (R. Chen et al., 2017; Shi et al., 2017)

In the current work, optogenetic stimulation of glutamatergic neurons in the striatum area elevated the expression of miR-21 in the pretreatment group compared to the stroke group. It is possible to conclude that up-regulation of miR-21 in the striatum brought on by optogenetic stimulation results in an increase in neuroprotective processes and ultimately prevents cell death brought on by ischemia based on the results of neurological severity score and infarct volume in the striatum region. Even while miR-21 expression in the hippocampus region of the pretreatment group increased, the results did not indicate any relevance.

A bulk of studies show that miR-124 is the most extremely expressed miRNA in the CNS that is affiliated with the development of ischemic stroke (C. Wang, Wei, Jiang, Liu, & Medicine, 2017). plasma level of miR124a can be utilized to diagnose ischemic brain damage (Laterza et al., 2009). After ischemia in rats' plasma level of miR-124 was found to be elevated that offer its capacity as biomarker for ischemic stroke (Weng et al., 2011). Some studies acknowledged that cell proliferation and promote cell differentiation can

inhibit via miR-124 (Cai et al., 2012; Lang, Ling, & communications, 2012; Makeyev, Zhang, Carrasco, & Maniatis, 2007).

According to findings of Liu et al, brain level of inhibitory member of the apoptosis-stimulating proteins of p53 family (iASPP) as a possible target of miR-124, reduced after ischemic stroke in vivo condition. And additionally, inhibition of miR-124 increased level of iASPP and significantly decreased infarction in ischemia. Also, their study indicates that after ischemic stroke neural cell death mediated by p53 can be nontranscriptionally regulated by suppressing mechanism of endogenous cell death inhibitors by miR-124 (X. Liu et al., 2013). Based on the findings of this study, we observed that the expression of miR-124a in neural progenitor cells in the hippocampus decreased significantly one day after ischemic stroke. In other words, due to the fact that the hippocampal region is a neurogenic niche and neural progenitor cells differentiate in this region, pretreatment by optogenetic technique caused that miR-124a was down regulated. The observed improvement in disease status was consistent with the results of stroke volume measurements. Understanding the interaction between miRs and the regulatory mechanisms in adult brain after stroke could potentially provide new therapies to preventing neural cell death after ischemic stroke.

Conclusion

With the aid of light-induced proteins, optogenetics has become a potent method for controlling intracellular signaling cascades. Through the assessment of microRNA 21 and 124a expression levels, we used this method to excite glutamatergic neurons in an animal model of ischemic stroke in order to look into the potential pretreatment effect of the optogenetic technology. The present study's findings suggest that up-regulation of miR-21, a neuroprotection marker, improves neurological abnormalities and decreases infarct volume in the striatum and hippocampus. Furthermore, it can be deduced from the downregulation of miR-124a that this gene contributes to neurogenesis, neuroprotection, and the differentiation of neural progenitor cells into neurons, all of which alleviate ischemia conditions. Because compulsive ischemia may raise the likelihood of blood clot formation or conditions needing surgery, such as carotid aneurysms, our finding shows that neuroprotection induced by optogenetics can be useful to produce ischemic tolerance. We therefore believed that using optogenetic techniques in the clinical setting could be helpful in reducing brain damage brought on by cerebral ischemia in situations of compulsive ischemia based on our findings and those of earlier investigations.

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Conflict of interest

Hereby the authors confirm that there are no conflicts of interest in the present research.

Ethical Approval

All the experimental protocols were approved by the Animal Research Ethics Committee at Shahid Beheshti University.

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